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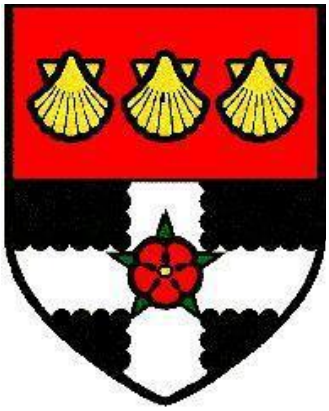

Effect of Diversity of Plant Variety and Domestic Processing on the Flavour Chemistry of Cabbage and Their Impact on Sensory Profile and Consumer Acceptance

A thesis submitted in partial fulfilment of the requirement for the degree of Doctor of Philosophy (PhD)

Department of Food and Nutritional Sciences

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To Ademola, Ikedolapo, Osisienemo & Oluwafunmito

To Him "*….whose Grace is Sufficient for me*"

- 2 Cor. 12:9

Declaration

I confirm that this is my own work and the use of all material from other sources has been properly and fully acknowledged.

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June 2018

Abstract

Increased consumption of *Brassica vegetables* such as cabbage (*Brassica oleracea*) reduces the risk of cardiovascular diseases (CVD) and cancer mainly because of the bioactive compounds they contain. Cabbage contains high amounts of glucosinolates (GSLs) which, when hydrolysed, yield several products (such as isothiocyanates, thiocyanates, nitriles and epithionitriles), depending on the conditions of the hydrolysis process. Isothiocyanates (ITCs), one group of hydrolysis products formed from myrosinase enzyme action on glucosinolates (GLSs), are responsible for many of the health promoting properties of cabbage. In addition, GSLs, ITCs and other sulfur-containing compounds are also responsible for the bitter taste and sulfurous aromas of cabbage, which is reported to reduce consumer liking and consumption of cabbage. This thesis investigates the effect of variety and domestic cooking methods on phytochemical and volatile composition of cabbage with subsequent impact on sensory profile and consumer acceptability.

The effect of variety, growing conditions and domestic cooking on the GSL-myrosinase system of cabbage was investigated. The results presented highlight significant differences in the myrosinase activity and stability, GSLs and glucosinolate hydrolysis products (GHPs) of the different cabbage types and varieties studied. Field grown cabbages with lower growing temperatures resulted in higher myrosinase activity and GSLs. The severity of the cooking method influenced the types and amounts of GHPs formed. Steaming led to denaturation of epithiospecifier protein (responsible for GSL hydrolysis to nitriles and epithionitriles) but retention of active myrosinase resulting in the formation of more beneficial ITCs than nitriles or epithionitriles. The highest concentrations of beneficial ITCs were observed in steamed white (WC1) and red (RC3) cabbage varieties.

In addition to cabbage GSL-myrosinase system, other non-volatiles (amino acids, sugars and organic acids) and flavour volatiles were also analysed and their influence on sensory profile and consumer acceptability explored. Cabbage type/variety and domestic cooking influenced the types and amounts of phytochemical compounds formed. Sulfides, responsible for undesirable sulfurous aromas of cabbage were the main volatile compounds identified in raw cabbage. Cooking reduced the perception of bitter taste, and amounts of sulfurous volatiles produced, with consequent increase in consumer liking and acceptance of cabbage. Black kale was perceived to be more bitter than red cabbage even though red cabbage contained twice the amount of GSLs found in black kale. The difference in bitterness perception was related to the ratio of bitter-tasting GSLs to sweet-tasting compounds such as

sugars and amino acids. The GSL-sugar/amino acid ratio for black kale was 1:4 and that of red cabbage was 1:8. The results suggest that higher amounts of sweet-tasting compounds have a masking effect on bitterness perception.

To understand individual differences in bitter taste perception and consumer liking of cabbage, consumers were genotyped for their *TAS2R38* and gustin *rs2274333* genes which influence taster status. *TAS2R38* had a significant effect on bitter taste perception and liking but the effect was not as expected and was mostly driven by the *TAS2R38* rare genotype group. Gustin *rs2274333* influenced bitter taste perception and liking in black kale varieties but differences were not clearly defined. Overall, it was observed that, irrespective of bitter taste genotype, cooking was the main driver of bitter taste perception as all genotypes perceived cooked cabbage significantly less bitter than raw cabbage.

In conclusion, the results of this study provide helpful insights into the relationship between cabbage phytochemical composition and sensory characteristics. Breeding of cabbage varieties with high amounts of sweet-tasting compounds such as sugars without reduction in the concentration of beneficial GSLs may be a viable way of improving cabbage consumption. The study demonstrates that not only does mild cooking of cabbage enhance formation of beneficial ITCs, it also improves consumer liking and acceptability regardless of variety or bitter taste genotype.

Acknowledgement

I am indebted to God for strength and grace throughout the period of my studies.

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List of Abbreviations

A – Appearance

AA – Amino Acids

AAI – Alanine-Alanine-Isoleucine

AAV -Alanine Alanine-Valine

AE – Aftereffects

AEDA – Aroma extract dilution analysis

AHC – Agglomerative Hierarchical Cluster

AITC – Allyl isothiocyanate

ANOVA – Analysis of variance

AVI – Alanine-Valine-Isoleucine

BK – Black Kale

CVD – Cardiovascular diseases

DNS – Did not survive

EPT – Epithionitrile

ESP – Epithiospecifier proteins

F – Flavour

GC – Gas chromatography

GH – Glasshouse

GHP – Glucosinolate hydrolysis product

GSL – Glucosinolates

HPLC- High performance liquid chromatography

HS-SPME – Headspace solid-phase micro extraction

ITC – Isothiocyanate

I3C – indole-3-carbinol

LC – Liquid chromatography

LMS – Labelled magnitude scale

MF – Mouthfeel

MFA – Multifactor analysis

Min – Minutes

MAP – Modified atmospheric packaging

MS – Mass spectrometry

MW – Microwaved

ND – Not detected

NS – No sample

O – Odour

OA – Organic acid

OAV – Odour activity values

PAV – Proline-Alanine-Valine

PC – Principal component

PCA – Principal component analysis

PEITC – 2-phenyethyl isothiocyanate

PROP – 6-n-propylthiouracil

PVDF -Durapore polyvinylidene

RC- Red cabbage

RFA – Rotated factor analysis

SBSE – Sorptive extraction

SC- Savoy cabbage

Sec - Seconds

SF – Stir-fried

SFN – Sulforaphane nitrile

SFP – Sulforaphane

SMCSO – S-methyl-L-cysteine sulfoxide

ST – Steamed

T – Taste

TC – Tronchuda cabbage

WC – White cabbage

WD – Wild cabbage

Chapter 1: Literature Review

1.1 Introduction

Diet related diseases – such as cardiovascular diseases (CVD) and cancer, for which obesity is a primary risk factor has led to different health campaigns encouraging changes in unhealthy dietary behaviours. Increased vegetable consumption has many health benefits in combatting diet related diseases, due to a number of factors including fibre, phytochemicals and low energy density. Epidemiological studies have shown that the consumption of *Brassica* vegetables proffers several health benefits to consumers such as reduced risk of cardiovascular diseases (CVD) and cancer (Herr & Buchler, 2010).

Brassica vegetables are unique when compared to other vegetables because they contain a group of thioglucosides called glucosinolates (GSLs). These GSLs can be hydrolysed by an endogenous enzyme, myrosinase, to yield various hydrolysis products, some of which possess health promoting characteristics (Mithen et al., 2000). These hydrolysis products and GSLs alongside other sulphur containing compounds are responsible for the bitter taste and pungent flavour of *Brassica* vegetables which limits consumer acceptance and liking of *Brassica* vegetables (Baik et al., 2003). Epithiospecifier protein (ESP) is responsible for an alternative pathway following the hydrolysis of GLSs which leads to the formation of simple nitriles and epithionitriles instead of the more beneficial isothiocyanates (Lambrix et al., 2001). *Brassica* vegetables are mostly subjected to one form of thermal processing/cooking before consumption. These processes modify the GSL-myrosinase system as well as types and concentrations of phytochemical compounds formed. However, the effect of these processes varies between and within *Brassica* species. Other factors influencing GSLs and myrosinase activity include plant genotype and growing conditions. *Brassica* vegetables contain the bitter tasting thiourea group (N-C=S), the sensitivity to which may impact consumer acceptance and consumption of *Brassica* vegetables (Sandell & Breslin, 2006).

In this review, the GSL-myrosinase system and flavour volatile compounds will be discussed. Potential health benefits derived from *Brassica* consumption will be explored and factors influencing phytochemical content and myrosinase activity will be highlighted to gain major understanding on the differences between *B. oleracea* species. Sensory characteristics and effect of bitter taste receptor genotype on *Brassica* consumption will also be reviewed. It should be noted that the review is focused mainly on *B. oleracea* species with references made to other *Brassica* vegetables where appropriate.

1.2 *Brassica* vegetables

Brassica vegetables, also known as cruciferous vegetables, are an important and highly diversified group of crops belonging to the *Brassicaceae* family, commonly called the mustard family (Ciju, 2014). *Brassica* vegetables have flowers with four equal-sized petals in the shape of a 'crucifer' cross. "*Brassica*" is a Latin word meaning cabbage. Common vegetables from the *Brassica* genus being consumed include cabbage, Chinese cabbage, broccoli, Brussels sprouts, cauliflower and kale. The *Brassicaceae* family is comprised of about 350 genera and 3000 species with *Brassica* being the most economically important genera and comprising of about 100 species (Fahey et al., 2001; Latté et al., 2011). *Brassica* vegetables are reported to have originated from Western Europe, the Mediterranean region and temperate regions of Asia (Ciju, 2014). Although *Brassica* crops are cultivated under different climatic conditions, they are mostly cool weather crops with optimum temperatures ranging between 14 °C and 21 °C and minimum and maximum growing temperatures of 4 °C and 30 °C (Wurr et al., 1996). *Brassicas* are grown in both temperate and tropical regions and generally prefer deep, well-drained, fertile silty loam soil with neutral pH (Bjorkman et al., 2011). *Brassica* vegetables are biennials but are being grown annually for commercial purposes. Almost all parts of some *Brassica* vegetables are edible (e.g. broccoli) while only the leaves, modified stems and flower form the edible part of others (e.g. cabbage and cauliflower). *Brassica* vegetables play an important role in maintaining good dietary health. They are low in calories and fat but contain essential nutrients and phytochemicals such as flavonoids, vitamins C and A, folic acid, calcium, potassium and dietary fibre (West et al., 2004) and are reported to be the richest source of plant-based antioxidants in human diet (Ciju, 2014). However, interest in *Brassica* vegetables stems from the high amounts of GSLs they contain.

1.3 Phytochemicals in *Brassica oleracea*

1.3.1 Glucosinolate- myrosinase system

1.3.1.1 Glucosinolate

Glucosinolates (β-thioglucoside N-hydrosulphates) are sulphur and nitrogen-containing biologically active secondary metabolites found in plants of the order Capparales which includes the *Brassicaceae* family and other economically important agricultural crops (Mithen et al., 2000; Mithen, 2001; Redovnikovi et al., 2008). Glucosinolates (GSLs) are produced in these crops as a defence mechanism against herbivores and pests. There are

about 200 GSLs identified to date with a common chemical structure containing a β-D-thioglucose group linked to a sulfonated aldoxime moiety and a variable R-group obtained from amino acids, as shown in Table 1.1 (Fahey et al., 2001; Redovnikovi et al., 2008; Ishida et al., 2014).

GSLs have been grouped into three main classes based on the structure of their different amino acid precursors; these groups are aliphatic, aromatic and indole GSLs. The structure and grouping of GSLs identified in *B. oleracea* species to-date is presented in Table 1.1. Aliphatic GSLs are derived from alanine, leucine, isoleucine, methionine, or valine; aromatic GSLs from phenylalanine or tyrosine while tryptophan derived GSLs are called indole GSLs (Wittstock & Halkier, 2002; Halkier & Gershenzon, 2006).

GSLs are biosynthesized from amino acids and the biosynthetic pathway has mostly been explained through *Arabidopsis*. The GSL pathway is comprised of three major stages: the chain elongation, formation of the core GSL structure, and finally the secondary modification (Fahey et al., 2001). Firstly, an aldoxime is formed from the elongation of amino acids such as methionine and phenylalanine, a process which is regulated by the activity of the CYP79 gene family, each of which have substrate specificity for different amino acid precursors. The aldoxime is then reconfigured to form the core structure of the parent GSL and, finally, the side chain of the parent GSL formed can undergo secondary modifications to determine the final structure of the GSL (Ishida et al., 2014). These modifications are important because the physicochemical properties and biological activity of GSL degradation products are mainly due to the structure of the GSL side chain.

Over 200 GSLs have been identified to date in glucosinolate producing families (such as Brassicaceae, Akaniaceae, Moringaceae and Resedaceae) of the order Brassicales but the average number of GSLs found in individual cultivars is less than 23 and normally distributed across various parts of the plant (Fahey et al., 2001).

The GSL profiles and concentration are influenced by several factors which include, plant species and genotype, plant age, growing conditions, the part of plant, storage and processing (Herr & Buchler, 2010; Pérez-Balibrea et al., 2011).

1.3.1.2 Myrosinase enzyme

Myrosinase (thioglucoside glucohydrolase EC 3. 2. 3.147, formerly EC 3.2.3.1) is the enzyme responsible for GSL hydrolysis and is found in all cruciferous vegetables, most of which are consumed as part of the human diet (Ludikhuyze et al., 2000). Myrosinase structure characterized in white mustard seed (*Sinapis alba*) is described as a glycosylated dimer

stabilized by Zn^{2+} where the enzyme folds into a $(\beta/\alpha)_8$ barrel structure similar to that of β-glucosidase found in white clover (Burmeister et al., 1997). The structure possesses hydrophobic pockets which are suited to bind to different hydrophobic side chains of GSLs, and two arginine residues which interact with the sulfate group of the substrate. Furthermore, myrosinase structure contains a number of salt bridges and hydrogen bonds between charged and neutral atoms which enhances myrosinase stability and limits denaturation (Burmeister et al., 1997). All myrosinase enzymes found in plants are reported to be glycosylated with a carbohydrate content of between 9-23 % and present as myrosin grains in myrosin cells of seeds, seedlings and mature tissues of the plant (Bones & Rossiter, 1996; Andreasson & Jorgensen, 2003).

There are different types of myrosinase isoenzymes and they vary between *Brassica* vegetables and differ to some extent in characteristics and activity (Yen & Wei, 1993; Bones & Rossiter, 1996). Myrosinase isoenzyme distribution in plants appear to be both organ and species specific (Bones & Rossiter, 1996). However, little is known of their substrate specificity. In a previous study on *Brassica napus*, the two types of myrosinase isoenzymes studied degraded different GSLs at different rates, but the highest activity for both isoenzymes was reported in aliphatic GSL degradation and the least in indole GSLs (James & Rossiter, 1991).

Myrosinase activity is affected by several intrinsic factors (ascorbic acid, Magnesium Chloride ($MgCl_2$), ferrous ions, presence of epithiospecifier protein) and extrinsic factors (pH, temperature, pressure, plant growth conditions) with optimal pH and temperature of myrosinase activity varying among plant species (Travers-Martin et al., 2008; Wei et al., 2011). Ascorbic acid and $MgCl_2$ has been shown to increase myrosinase activity at certain concentrations, pH and temperatures (Bones & Rossiter, 1996; Ludikhuyze et al., 2000). Myrosinase is mostly accompanied by one or more GSLs.

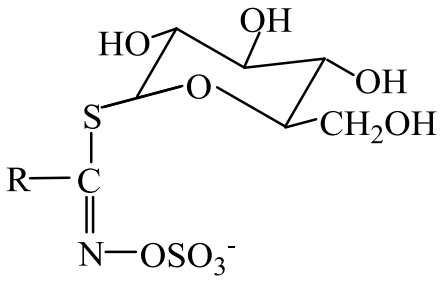
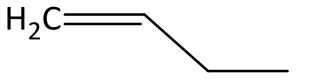
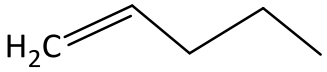
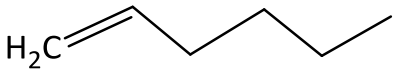
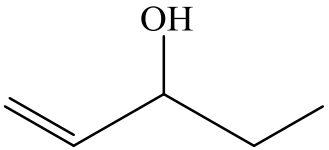
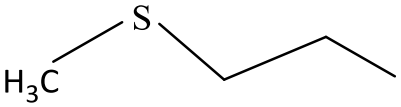
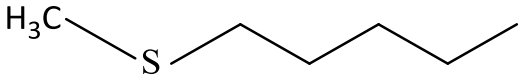
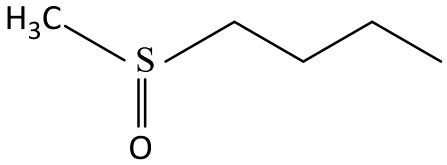
Several bacterial strains (such as *Escherichia coli*, *Bacteroides thetaiotaomicron*, *Enterococcus faecalis*, *Enterococcus faecium*, and *Lactobacillus agilis*) present in the gut microflora of humans are reported to produce myrosinase and have been linked with GSL hydrolysis in the gut (Traka & Mithen, 2009; Tian et al., 2017). In broccoli extracts, lactic acid bacteria, *Lactobacillus plantarum* KW30 and *Lactococcuslactis subsp lactis* KF147 hydrolysed 30- 33 % of glucoraphanin (GRPN), glucoerucin (GER), glucoiberin (GIBN) and glucoiberverin (GIBVN) into their various nitriles and other unknown metabolites (Mullaney et al., 2013). Variation in individual microbiota may influence the rate and extent of GSL hydrolysis.

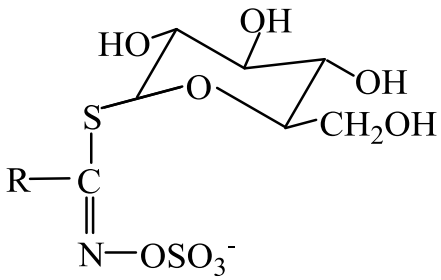
Antibiotic treatment and mechanical cleansing, which lowers the amounts of bowel microflora, have been shown to virtually prevent GSL hydrolysis in the gut (Tian et al., 2017).

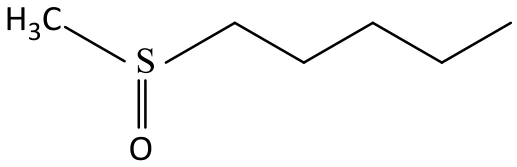
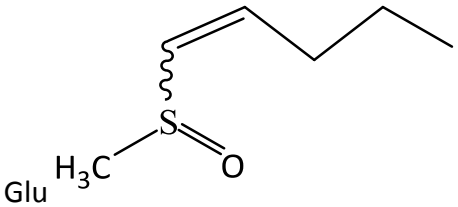
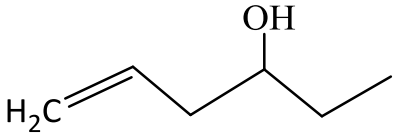
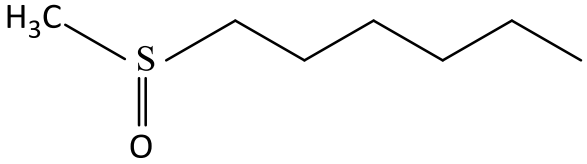
1.3.1.3 Epithiospecifier protein

Some proteins reported to interact with myrosinase include myrosinase-binding proteins, myrosinase associated proteins, thiocyanate forming proteins (TFP) and epithiospecifier proteins (ESP) (Bones & Rossiter, 1996; Martinez-Ballesta & Carvajal, 2015). ESP is however, the most studied and considered to be the most important when discussing interactions with myrosinase activity. ESP, a small protein with a molar mass of 30 – 40 kDa was first isolated in *Crambe abyssinica* seeds and is found in the cytoplasm and nucleus of plants (Tookey, 1973). ESP, a myrosinase co-factor, is responsible for the alternative pathway of glucosinolate hydrolysis to yield epithionitriles and nitriles and is only active in the presence of ferrous ions and myrosinase enzyme (Lambrix et al., 2001). ESP hydrolyses non-alkenyl GSLs to nitriles and alkenyl GSLs to epithionitriles (EPTs) by transferring the sulphur atom of the basic glucosinolate backbone to the terminal alkene residue of the side chain (Lambrix et al., 2001; Halkier & Gershenzon, 2006). However, ESP is unable to hydrolyse some GSLs and is also absent in some *Brassica* such as horseradish and *Sinapis alba* (Bones & Rossiter, 1996).

Table 1.1: Structure of individual glucosinolates identified in various *B. oleracea* species

Basic GSL structure	R-Group structure	Chemical name	Common name
		Aliphatic GSLs	
		2-Propenyl	Sinigrin
		3-Butenyl	Gluconapin
		4-Pentenyl	Glucobrassicanapin
		2(R)-2-Hydroxy-3-butenyl	EPI/Progoitrin
		3-Methylthiopropyl	Glucoibeverin
		4-Methylthiobutyl	Glucoerucin
		3-Methylsulphinylpropyl	Glucoiberin



	4-Methylsulphinylbutyl	Glucoraphanin
	4-Methylsulphinyl-3-butenyl	Glucoraphenin
	2-Hydroxy-4-pentenyl	Gluconapoleiferin
	5-Methylsulphinylpentenyl	Glucoalyssin
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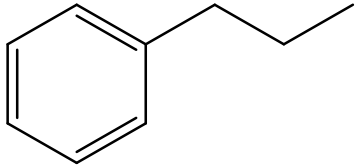	2-Phenylethyl	Gluconasturtiin
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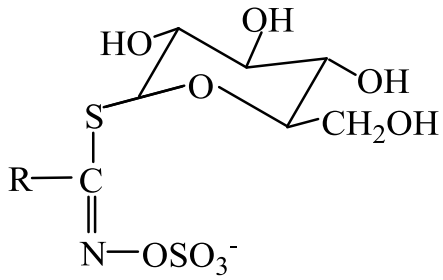
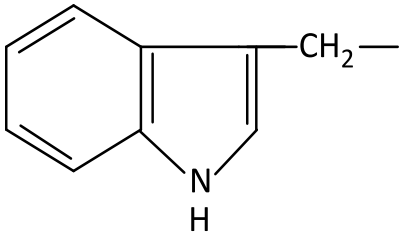
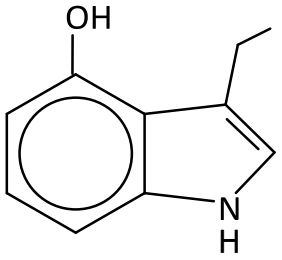
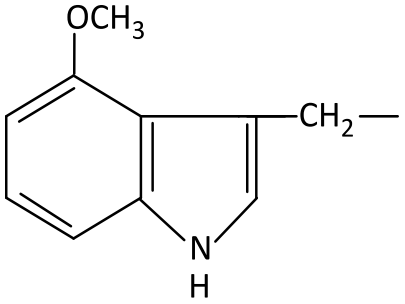


	3-Indolylmethyl	Glucobrassicin
	4-Hydroxy-3-indolylmethyl	4-hydroxyglucobrassicin
	4-Methoxy-3-indolylmethyl	4-methoxyglucobrassicin

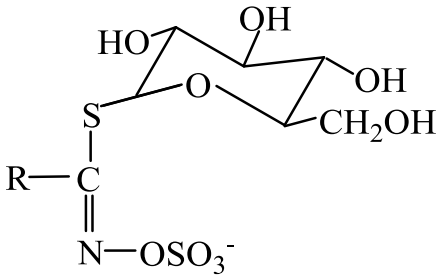
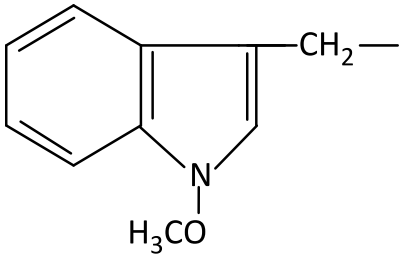
		1-Methoxy-3-indolylmethyl	Neoglucobrassicin

Table 1.2: Glucosinolate profiles of various *B. oleracea* species identified to-date

Species	Common name	Sinigrin	Gluconapin	Glucobrassicanapin	Epi/progoitrin	Glucoibeverin	Glucoerucin	Glucoiberin	Glucoraphanin	Glucoraphenin	Gluconapoleiferin	Glucoalyssin	Gluconasturtiin	Glucobrassicin	4-hydroxyglucobrassicin	4-methoxyglucobrassicin	Neoglucobrassicin	References
Brassica oleracea var. *capitata* f. *alba*	White cabbage	X	X	nd	X	X	nd	X	X	nd	nd	nd	X	X	X	X	X	1,
Brassica oleracea var. *capitata*	Green cabbage	X	nd	nd	nd	nd	nd	nd	X	X	nd	X	nd	X	X	X	X	2,
Brassica oleracea var. *capitata* f. *sabauda*	Savoy cabbage	X	X	nd	X	X	X	X	X	nd	nd	nd	X	X	X	X	X	1,
Brassica oleracea var. *capitata* f. *rubra*	Red cabbage	X	X	nd	X	X	X	X	X	nd	nd	nd	X	X	X	X	X	1,
Brassica oleracea var. *costata*	Tronchuda cabbage	X	X	X	X	X	nd	X	X	nd	nd	X	X	X	X	X	X	4,
Brassica oleracea var. *acephala*	Black kale	X	X	X	X	X	nd	nd	X	nd	nd	nd	X	X	X	X	X	3,4
Brassica oleracea var. *alboglabra*	Chinese kale	X	X	nd	X	nd	X	X	X	nd	X	X	X	X	X	X	X	5
Brassica oleracea var. *italica*	Broccoli	X	X	X	X	nd	nd	X	X	nd	X	X	X	X	X	X	X	3,
Brassica oleracea var. *botrytis*	Cauliflower	X	X	X	X	X	X	X	X	X	X	nd	X	X	X	X	X	1,3
Brassica oleracea var. *gemmifera*	Brussels sprouts	X	X	X	X	X	X	X	X	nd	X	X	X	X	X	X	X	1,3
Brassica oleracea var. *viridis*	Collard greens	X	X	X	X	X	X	X	X	nd	nd	nd	X	X	nd	nd	nd	6
Brassica oleracea var. *gongylodes*	Kohlrabi	X	X	nd	X	X	X	X	X	X	nd	nd	X	X	X	X	X	1,

Key: nd = not detected; X = glucosinolate present in species. References: 1 = Ciska et al. (2000); 2 = Park et al. (2014c); 3 = Kushad et al. (1999); 4 = Cartea et al. (2008); 5 = Sun et al. (2011); 6 = Carlson et al. (1987).

1.3.1.4 Glucosinolate hydrolysis products

GSLs and myrosinase enzyme coexist in separate compartments in the plants, while GSLs exists in the vacuoles of various cells, myrosinase enzymes are localised inside the myrosin cells (Kelly et al., 1998; Mithen, 2001).

When plant tissue is damaged as a result of autolysis, chewing or processing, GSLs are exposed to, and hydrolysed by, myrosinase. Upon hydrolysis, glucose and an unstable aglycone (thiohydroxamate-*O*-sulfonate) are produced. The unstable aglycone (thiohydroxamate-*O*-sulfonate) immediately rearranges to form different hydrolysis products such as isothiocyanates (ITCs), thiocyanates, nitriles, EPTs and oxazolidine-2-thiones (Figure 1.1). The extent of glucosinolate hydrolysis and the type of hydrolysis compound produced is dependent on a number of factors which include; coexisting myrosinase enzyme, ascorbic acid, Fe^{2+}, $MgCl_2$, structure of the glucosinolate side chain, plant species as well as reaction conditions such as pH and temperature (Bones & Rossiter, 1996; Ludikhuyze et al., 2000; Wittstock & Halkier, 2002). At neutral pH, GSLs are hydrolysed to ITCs by myrosinase. Oxazolidine-2-thiones such as goitrin (5-vinyloxazolidine-2-thione) are formed from progoitrin hydrolysis, while 2-propenyl, benzyl and 4-Methylthiobutyl GSLs can be degraded to thiocyanates by TFP (Bones & Rossiter, 1996). Thiocyanates are thought to be formed from the rearrangement of *E*-aglycones in GSLs with stable cations. In the presence of ESP, alkenyl glucosinolates are hydrolysed to epithionitriles and other glucosinolates to nitriles instead of isothiocyanates (Galletti et al., 2000; Lambrix et al., 2001). The concentration of ITCs, nitriles or epithionitriles formed during glucosinolate hydrolysis is dependent on the ratio of myrosinase to ESP activity. Glucosinolate hydrolysis products (GHPs) contribute both positively and negatively to the sensory and health characteristics of *Brassica* vegetables.

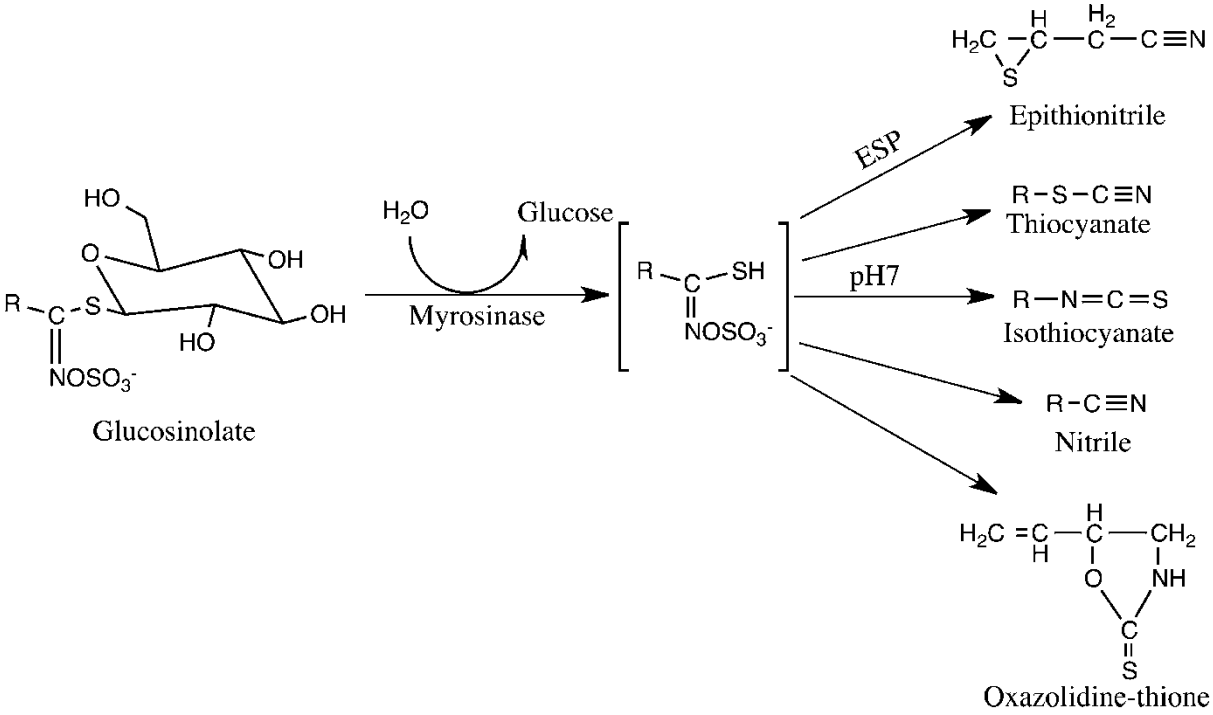


Figure 1.1. The glucosinolate- myrosinase system showing hydrolysis products produced under different conditions
(Adapted from Bell et al. (2015))

1.3.2 Flavour volatiles in B. oleracea species

Several types of flavour volatiles have been identified from different *B. oleracea* species. Sulfur- containing compounds such as methanethiol, hydrogen sulfide, dimethyl sulfide (DMS) dimethyl disulfide (DMDS), dimethyl trisulfide (DMTS), dimethyl tetrasulfide (DMTTS) and methyl (methylthio) methyl sulfide; MMMS) and GHPs (ITCs) are reported to be the major flavour volatiles present *B. oleracea* tissues (Chin & Lindsay, 1993; Chin et al., 1996; Kubec et al., 1998; Engel et al., 2002b; Baik et al., 2003; Valette et al., 2003) and reviewed recently by Bell et al. (2018). Sulfides are secondary metabolites formed from the thermal degradation of naturally occurring non-protein sulfur-containing S-methylcysteine and its sulfoxide (SMCSO) by the endogenous enzyme cysteine sulfoxide lyase (C-S lyase) (Chin & Lindsay, 1994a; Kubec et al., 1998). When C-S lyase hydrolyses SMCSO, unstable methanesulfenic acid which readily converts to methyl methanethiosulfenic acid is formed. Methyl methanethiosulfenic acid then undergoes nonenzymic chemical reactions to yield methanethiol and sulfides. Tissue moisture content, heating time and temperature all influence the type and amounts of degradation products during SMCSO hydrolysis. Methanethiol and hydrogen sulfide although important contributors to flavour of *Brassica* vegetables, are highly reactive, unstable and readily oxidized

to DMDS and DMTS (Chin & Lindsay, 1993; Chin & Lindsay, 1994b). These compounds are of importance to *B. oleracea* species mainly because they can be detected at very low concentrations (0.02 ppb).

In addition to sulfides and GHPs, acids, alcohols, aldehydes, alkanes, ketones, terpenes and esters are also present in *B. oleracea* species (Macleod & Macleod, 1968; MacLeod & MacLeod, 1970b; Chin & Lindsay, 1993; Hansen et al., 1997; Engel et al., 2002b; Baik et al., 2003; Valette et al., 2003; Spadone et al., 2006; de Pinho et al., 2009; Fernandes et al., 2009). Two metabolic pathways; β-oxidation and the lipoxygenase pathway are responsible for the formation of aliphatic saturated and unsaturated alcohols, aldehydes, ketones and acids and esters. However, the lipoxygenase pathway is generally considered to occur when plant tissue is damaged and oxygen is introduced into the system, for example during processing or mastication of *B. oleracea* (Siegmund, 2015). C6 and C9 aldehydes and alcohols such as 3-hexenal and 3-hexen-1-ol are important odour compounds in *B. oleracea*. They are formed from the action of lipoxygenase enzymes on linoleic or linolenic fatty acids and are responsible for green leafy/grassy notes of *Brassica* (Raffo et al., 2018).

Different analytical methods such as GC-O, aroma extract dilution analysis (AEDA), stir-bar sorptive extraction (SBSE) and headspace-solid phase micro extraction (HS-SPME) have been used to extract and determine the profile and concentration of volatile compounds in *Brassica* vegetables. A recent study suggested that to achieve the best results in terms of flavour volatile identification and quantification, various analytical methods should be used to determine the profile and intensities of odour compounds as extraction methods and duration can influence the concentrations and probable impact of specific compounds on the flavour profile of *Brassica* vegetables (Raffo et al., 2018). These volatile compounds can have both desirable and undesirable impacts on sensory characteristics of *B. oleracea* species but for the compounds to be perceived by consumers, they must be present in levels above their detection thresholds. However, to determine the impact of volatile compounds on the odour profile of the vegetables, odour activity values (OAVs) must be calculated. OAV is defined as the ratio between the concentration of the aroma compound and its odour threshold; an OAV value >1 suggests that the odour compound is likely to contribute to the flavour profile of the food product (Parker, 2015). Compounds which define the flavour of a food product are called character impact compounds. High concentrations of a compound in a food matrix does not necessarily translate to its contribution to the flavour profile of that product; compounds low in concentration with low detection thresholds can contribute more to the flavour profile than

compounds with higher thresholds though present in high concentrations. DMS accounted for 34 % of volatiles present in cooked cabbage, but it is thought that its contribution to cabbage flavour may be low because of its high detection threshold (up to 15 ppb) (Macleod & Macleod, 1968; Chin & Lindsay, 1993). Volatile compounds can also vary in their characteristic odour notes; for e.g., aldehydes with C7 chain lengths and above can have both fruity/floral and fatty odour notes depending on their concentrations and the sensitivity of the individual perceiving them (Parker, 2015).

The factors affecting the type and concentrations of flavour volatiles present in *B. oleracea* include plant species and variety, plant cultivation conditions as well as storage and processing. These factors, as well as the impact of volatiles on sensory characteristics, will be discussed in detail in section 1.6. Non-volatile compounds such as flavonoids (flavonols and anthocyanins), amino acids, sugars, organic acids, minerals and vitamins also contribute to the nutritional and organoleptic properties of *B. oleracea* species.

1.4 Health promoting properties of *B. oleracea* species

B. oleracea vegetables are rich in flavonoids, vitamins, protein, minerals, amino acids and sugars, all of which contribute to the health and nutritional benefits obtained from their consumption (Kim et al., 2004; Ayaz et al., 2006; Padilla et al., 2007; Ayaz et al., 2008; Park et al., 2014a). Flavonoids possess a variety of biological activities which contributes to protecting the human body against chronic diseases such as cancer (Schmidt et al., 2010). In addition, some flavonoids when consumed in high amounts possess strong antioxidant characteristics lowering the risk of coronary heart diseases (Ayaz et al., 2008).

However, for some years now, interest in the health promoting properties of *Brassica* vegetables have been on the increase mainly due to the presence of ITCs and indoles, shown to possess several health benefits which include action against neurodegenerative diseases, anti-carcinogenic, cardioprotective, radioprotective and antimicrobial activities (Kala et al., 2018). Most of these studies were conducted in animals and human cells and have focused on activities of sulforaphane (SFP), 2-phenyethyl ITC (PEITC), erucin, allyl ITC (AITC), iberin and indole-3-carbinol (I3C) hydrolysis products of glucoraphanin, gluconasturtiin, glucoerucin, sinigrin, glucoiberin and glucobrassicin respectively (Vaughn & Berhow, 2005; Jadhav et al., 2007; Geng et al., 2011). ITCs have been shown to have protective effects against various types of cancer by inducing phase II detoxification enzymes while inhibiting phase I enzymes which are responsible for the bio-activation of carcinogens (Dekker et al., 2000; Mithen, 2001).

The anticancer activity of SFP alone and in combination with other anti-proliferative agents against Barrett esophageal adenocarcinoma (BEAC) was studied both in cancer cells and in tumour induced mice (Qazi et al., 2010). Effects of SFP on drug resistance by Rhodamine efflux assay and induction of apoptosis using annexin V labelling and Western blot analysis of poly (ADP-Ribose) were evaluated. The study showed treatment with SFP resulted in both time and dose-dependent decline in cell survival, cell cycle arrest and apoptosis with a significant reduction in tumour volume observed in a subcutaneous tumour model of BEAC. SFP also reduced activities of multidrug resistance proteins, reduced drug efflux while increasing the activity of other anti-proliferative. The anticancer potency of SFP was attributed to the induction of caspase 8 and p21 and down-regulation of hsp90, a heat-shock protein required for the activity of several proteins associated with cancer proliferation. Another study evaluating the effect of purified SFP extracted from *Brassica oleraceae var rubra* (red cabbage) against human epithelial carcinoma HEp-2 and Vero cells found SFP prevented proliferation of HEp-2 and Vero cells by preventing the expression of antiapoptotic bcl-2 and inducing p53, proapoptotic (bax) protein and caspase-3 (Devi & Thangam, 2012). The potency of SFP has been demonstrated against human and mouse ovarian cancer cells (Chaudhuri et al., 2007), pancreatic cancer (Li et al., 2012) and A549 lung cancer cells (Farag & Motaal, 2010).

AITC a common ITC present in cabbages has been found to prohibit the development in cultured human cell lines and animal tumour models (Zhang, 2010). A study using rat models and human bladder cells by Bhattacharya et al. (2010) showed that AITC, inhibits the development of rat AY-27 cancer cells and human bladder UM-UC-3 cancer cells when consumed in low amounts, making it a possible bladder cancer chemo-preventive/therapeutic agent. Another study showed that AITC can arrest human bladder cancer cells preventing mitosis and also induce apoptosis by increasing the ubiquitination and degradation of α- and β-tubulin (Geng et al., 2011). AITC was also reported to be potent against human breast cancer cells (Tsai et al., 2012), human erythroleukemic K562 cells (Leoni et al., 1997), and more potent on human A549 and H1299 non-small cell lung cancer cells *in vitro* than PEITC (Tripathi et al., 2015). Erucin has also been reported to be potent against prostate PC3, BPH-1 and LnCap cancer cells lines, leukaemia cells, colon cancer cells and HepG2 hepatoma cells (Melchini et al., 2009). I3C is known to have anti-cancerous activities on reproductive organs, reducing the proliferation of cancer cells in the breast, prostrate, cervical and colon cell lines and also preventing tumour development in rodents (Cashman et al., 1999; Bonnesen et al., 2001; Kim et al., 1997). Though ITCs and indoles both have anticancer activities, their mechanism of action

differ; while ITCs induce cytotoxicity resulting in apoptosis within cancer cells, indoles prevent cell proliferation in a cytostatic way implying that the activity of both compounds could be effective at different stages of cancer development (Verhoeven et al., 1997; Pappa et al., 2006).

Other potent activities of SFP include antioxidant properties by reducing antioxidative stress and preventing tissue damage in *in-vivo* and *in-vitro* experiments (Guerrero-Beltran et al., 2012), neuroprotective activities in neurodegenerative diseases such as Alzheimer's and Parkinson's disease (Tarozzi et al., 2013) and anti-microbial properties against a wide range of bacteria and fungi. Fahey et al. (2001) reported that SFP inhibited the growth of *Helicobacter pylori*, the micro-organism responsible for helicobacter pylori infection which induces the development of gastric cancer. Despite the several health benefits reported, the doses required to achieve these beneficial effects is unclear and not well defined.

Some adverse effects of *Brassica* consumption have also been reported. Goitrin and thiocyanates formed from epi/progoitrin hydrolysis are reported to have goitrogenic properties, adversely affecting thyroid metabolism and resulting in a condition known as goitre (Verhoeven et al., 1997). There is however, little evidence of its goitrogenic activities in healthy individuals, and average intake of these compounds in *Brassica* vegetables are lower than doses required to produce adverse effects (Steinmetz & Potter, 1991; Han & Kwon, 2009). In high doses, ITCs are also reported to have toxic effects *in vitro* with conflicting results reported and action not clearly defined. SFP dose of 64 mg/kg was found to induce hepatotoxicity in mouse with other studies contradicting such results (Fofaria et al., 2015). However, toxicity is unlikely to occur in humans because toxicities reported are all in animals fed very high doses of ITCs in excess of what is normally available in the diet and consumed by humans.

The health benefits derived from consuming *Brassica* vegetables will be dependent on the presence of genes involved in ITC metabolism as well as ITC absorption and bioavailability after consumption. SFP when absorbed is conjugated with glutathione and metabolized through the mercapturic acid pathway with activities of glutathione S-transferases (GSTs) thought to be responsible for these reactions (Zhang et al., 1995). After consuming 100 g serving of broccoli, individuals with the null glutathione S-transferase M1 (*GSTM1*) allele excreted about 99 % of sulforaphane metabolites in the urine via the mercapturic acid pathway while those with functional *GSTM1* allele excreted only about 70 % of ingested sulforaphane (Gasper et al., 2005). The authors suggested that those with a functional *GSTM1* allele may have retained some of the sulforaphane in the body and metabolised it in a different way which may explain why the GSTM1- positive individuals gain more protection from broccoli consumption

than GSTM1- null individuals. Consumption of larger portions of standard broccoli or normal portions of super- broccoli (Beneforté broccoli with three times more glucoraphanin than a standard variety) was proposed as a way of reducing the effect of genotype in *GSTM1*-null individuals. Up to 90 % of AITC was absorbed when orally administered indicating its high bioavailability (Zhang, 2010). However, low absorption of ITCs from cooked *Brassica* vegetables have been reported due to inactivation of myrosinase during cooking. GSL hydrolysis on consuming cooked vegetables is then limited by the efficiency of conversion by the gut microflora. In order to ensure potential health benefits from *Brassica* consumption it is therefore important to prevent myrosinase inactivation during thermal treatment. To further improve benefits derived from *Brassica* vegetables and mitigate for losses as a result of processing, breeders are beginning to seek ways of increasing phytochemical contents of the vegetables to try and make up for low consumption of these vegetables by consumers (Kopsell et al., 2007). These has been demonstrated in the breeding of hybrid broccoli (Beneforté broccoli; discussed above) with two to three times more glucoraphanin than regular broccoli varieties (Traka et al., 2013).

1.5 Sensory characteristics of *B. oleracea* species

Despite the several health promoting properties of *Brassica* vegetables, consumer acceptance and consumption is low. This might be attributed to the bitter taste and pungent flavour/aroma of *Brassica* vegetables. Cox et al. (2012) reported that *Brassica* acceptance was low in adults due to the sensory characteristics. GSLs (sinigrin, progoitrin, gluconapin, glucobrassicin and neoglucobrassicin) and goitrin (hydrolysis product of progoitrin) are reported to be responsible for the bitter taste of *Brassica* vegetables (Fenwick et al., 1983; Doorn et al., 1998; Drewnowski & Gomez-Carneros, 2000a; Beck et al., 2014).

A study conducted by Doorn et al. (1998) reported that sinigrin and progoitrin were the main contributors to the bitter taste of Brussels sprouts with consequent effect on consumer acceptance. Another study showed bitterness was strongly correlated with total GSL content in raw cabbage (Beck et al., 2014). On the other hand, a study carried out on nineteen broccoli cultivars found no correlation between GSLs and bitterness of the cultivars (Baik et al., 2003). This might have been due to low amounts of bitter- taste producing glucosinolates in the cultivars studied as broccoli is known to be high in glucoraphanin which does not impact bitter taste. Detection thresholds for most GSLs are unavailable but low detection thresholds have been reported for sinigrin and goitrin; 106 $mg.L^{-1}$ and 12 $mg.L^{-1}$ respectively (Fenwick et al.,

1983). In a different study focused on extracts of *B. oleracea* species, total and individual GSL concentrations alone did not explain the perceived bitterness reported (Zabaras et al., 2013). The authors suggested that phenols, flavonoids and other compounds associated with bitterness present in the vegetables contribute to bitterness of *B. oleracea* vegetables.

ITCs and sulfides present in *B. oleracea* species are responsible for the undesirable sulfurous and overcooked aromas of *B. oleracea* vegetables (Chin et al., 1996; Kubec et al., 1998). Methanethiol, DMDS and DMTS are the main off-flavour notes of overcooked *Brassica* vegetables (Kubec et al., 1998). Chin et al. (1996) reported that AITC was responsible for the sharp, black mustard-like pungent aroma in fresh cabbage. AITC is an important contributor to fresh cabbage flavour and is generally considered to be a desirable flavour component in cabbage (Chin & Lindsay, 1993). 3-butenyl ITC (3BITC) is another important compound contributing a pungent wasabi-like flavour and heat to cabbage flavour (Depree et al., 1999). However, the high odour thresholds of AITC and 3BITC (375 and 380 ppm in water respectively) may influence the degree to which these pungent aromas are detected during cabbage consumption and their impact on cabbage flavour (Buttery et al., 1976). Erucin and PEITC at low concentrations are described as having a radish-like flavour, but their contributions to flavour is not very clear (Raffo et al., 2018). Their low odour thresholds (3.4 and 6 ppm in water respectively) implies that they may be major contributors to *Brassica* aromas even when present in low concentrations (Buttery et al., 1976). Aldehydes and alcohols (C6), especially hexenal and hexanol, possessing a green/grassy note are also important contributors to the flavour and odour of *B. oleracea* vegetables. Hedonic test for liking performed on five broccoli cultivars showed that cultivars abundant in green/grassy odour was described as having a fresh green odour and more preferred than cultivars with stronger sulfurous aromas (Hansen et al., 1997). The interpretation of this result however might not represent the average consumer as ten trained panellists were used to perform the liking test.

Other factors affecting the sensory properties of *Brassica* vegetables are the presence of sugars (glucose, fructose and sucrose), organic acids and amino acids in the food matrix (Vale et al., 2015). Perceived bitterness can be reduced by high concentrations of sugars and sweet tasting amino acids such as alanine, glycine, proline and glutamine. In white cabbage juice, cauliflower and Brussels sprouts, sweet tasting compounds like sucrose were found to reduce and mask bitter taste of sinigrin and goitrin (van Doorn, 1999; Beck et al., 2014). Consumers also preferred broccoli and cauliflower with high sucrose content and lower bitter tasting GSL content (Schonhof et al., 2004). High sugar-GSL ratios have been shown to reduce bitterness

perception in salad rocket (*Eruca sativa*), inferring that with increase in sugar concentrations, GSL concentrations can been maintained and bitter taste perception decreased. Most studies have focused on the GSL and volatile contents of *B. oleracea* vegetables without considering the resulting impact on the sensory perception and in cases where sensory perception has been carried out, it has focused mainly on bitter taste perception mostly in extracts or pure compounds. However, the contents of GSLs, ITCs and sulfides in *B. oleracea* vegetables alone does not provide a clear picture of the sensory characteristics of *B. oleracea* vegetables as other compounds in the plant matrix can influence and modulate the sensory perception of these vegetables. It has been reported that high sugar content in the *Brassica* matrix can have a masking effect on bitterness (Jones et al., 2006). This can be a way of reducing bitter taste perception in *Brassica* varieties making them more acceptable to consumers. Further research on the interactions of these compounds within their food matrix is therefore necessary to fully understand the sensory characteristics of *B. oleracea* vegetables perceived by the consumers. Some GSLs (e.g. glucoraphanin and glucoiberin) and their hydrolysis compounds are not known to contribute to the sensory characteristics of *Brassica* vegetables (Traka & Mithen, 2009). Processing, growing conditions and differences in types and concentration of GSLs among *B. oleracea* vegetables will affect the types of ITCs formed and consequently influence the taste and flavour of the vegetables.

Familiarity and frequency of *B. oleracea* consumption can influence consumer sensitivity and perception. Non- consumers of cauliflower were more sensitive to sinigrin and AITC perceiving them more intensely than medium and high consumers. The intense perception of these compounds may be responsible for cauliflower rejection by non-consumers (Engel et al., 2002b). Genotypic variations in consumer sensitivity to bitter taste and flavour perception can also influence consumer sensory perception and acceptance of *B. oleracea* vegetables. An example of a variation in flavour perception is the ability to detect green aroma from 3-hexen-1-ol which is affected by the genotype of OR2J3 (McRae et al., 2012). However, there are conflicting reports on the influence of these variations on flavour perception with some authors suggesting that the impact is minimal and others stating that the effect maybe be more than currently reported (Hasin et al., 2008; Reed & Knaapila, 2010). Keller et al. (2007) reported that genetic variations in human odour receptor, OR7D4 partly accounted for the variation and perception of androstenone and androstadienone odours in individuals. Odour receptors for ITCs are not reported and it is unknown whether these genetic variations exist for ITCs (Bell et

al., 2018). GSLs are known to contain the genetic bitter taste group (N-C=S), the sensitivity of which is known to differ between individuals and will be discussed in section 1.7.

1.6 Factors affecting the glucosinolate-myrosinase system and flavour profile of *B. oleracea* species

B. oleracea undergo several stages from sowing to consumption. During the stages, several factors can result in variations in phytochemical content and influence activities occurring in the GSL-myrosinase system of the plant with consequent effect on sensory and potential health characteristics. Some of these factors include plant species and variety, growing conditions, postharvest and storage processes, industrial and domestic processing (Figure 1.2).

1.6.1 Brassica oleracea species and variety

1.6.1.1 Effect of B. oleracea species /variety on glucosinolate content

B. oleracea species are known and reported to contain high concentrations of GSLs. However, genetic variations between species and between varieties within a species in types and abundance of these GSLs can be significant and is considered to be the most important factor influencing GSL contents of *B. oleracea* vegetables (Verkerk et al., 2009). It is difficult to compare GSL concentrations between *B. oleracea* species as the variety being studied has a greater influence on GSL content than the species itself. Out of the 200 individual GSLs identified to date, sixteen (16) have been identified in various *B. oleracea* species; 11 aliphatic GSLs, four indoles and one aromatic GSL. The greatest number (15) of individual GSLs identified to-date in *B. oleracea* species were in cauliflower (*Brassica oleracea* var. *botrytis*) and Brussels sprout (*Brassica oleracea* var. *gemmifera*) while white cabbage (*Brassica oleracea* var. *capitata*) with eight GSLs had the least. Sinigrin, glucoraphanin and glucobrassicin were the only GSLs present in all species, with variations in GSL profiles observed across all species as illustrated in Table 1.2 (Carlson et al., 1987; Kushad et al., 1999; Ciska et al., 2000; Cartea et al., 2008; Park et al., 2014b).

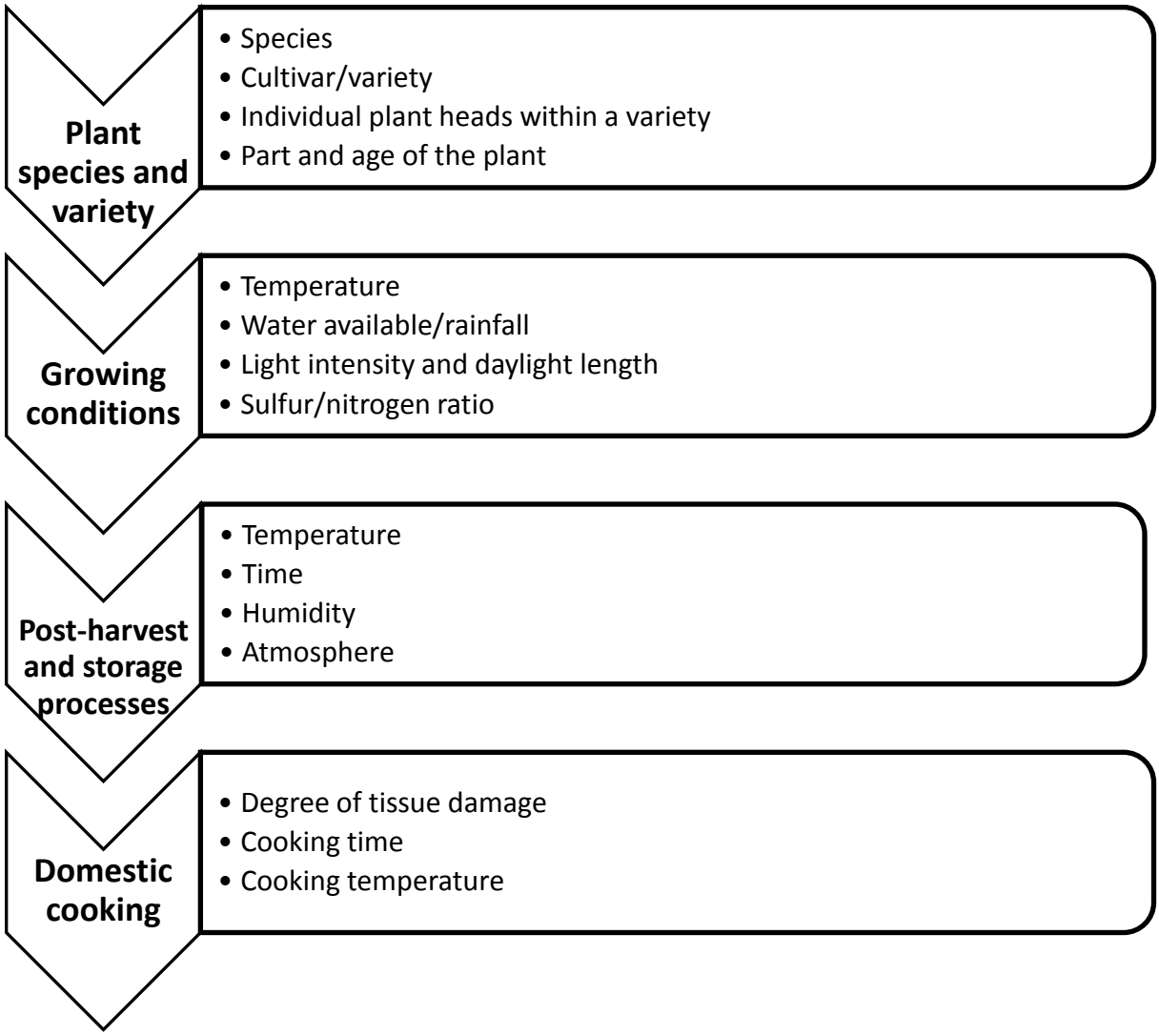


Figure 1.2. Factors affecting GSL-myrosinase system and flavour profile of *Brassica oleracea* species

With the exception of kale, aliphatic GSLs are the most abundant GSLs in *B. oleracea* species (Ciska et al., 2000). Indole GSLs were however reported as the most abundant GSLs in a particular broccoli cultivar (*B. oleracea* L. *Alef. convar. botrytis* [L.] var. *italica Plenck cv. Marathon*) (Aires et al., 2012). When GSL concentrations in different *B. oleracea* species (broccoli, Brussels sprouts, cauliflower, cabbage and kale) were studied, one study found total GSL content was highest in broccoli (Song & Thornalley, 2007), while another study reported highest concentrations in Brussels sprout (Kushad et al., 1999). A further study reported kale to contain significantly higher concentrations of total GSL compared to the other *B. oleracea* species studied, and Kohlrabi to contain significantly lower (Ciska et al., 2000). Cabbage GSL has been widely studied and similar concentrations and profiles are reported (Mithen et al., 2000; Dekker et al., 2000; Ciska et al., 2000). However, higher GSL concentrations are reported for red cabbage compared to other cabbage types. In inbred lines of green and red cabbage,

significantly higher total GSL concentrations were observed in red cabbage compared to green cabbage though their GSL profile was similar (Park et al., 2014b). The variations observed in the different studies between *B. oleracea* species can be explained by the different varieties of the species studied. These differences make it difficult to conclude possible benefits that can be derived from consumption of these vegetables, as the variety being consumed would have a significant influence on the potential benefit of the vegetable.

Concentrations of individual GSLs also vary between *B. oleracea* species. Red cabbages contain less sinigrin and more glucoraphanin and gluconapin than other cabbage types (Ciska et al., 2000; Verkerk & Dekker, 2004; Oerlemans et al., 2006; Volden et al., 2008). Glucobrassicin is the major GSL found in Brussels sprouts and cauliflower, while glucoraphanin has been identified as the most abundant GSL in broccoli and kale where it is present in higher concentrations than those found in Brussels sprout and cauliflower (Kushad et al., 1999; Ciska & Kozłowska, 2001; Song & Thornalley, 2007). Studies on Collard GSLs are limited but concentrations are proposed to be relatively high, containing mainly sinigrin, progoitrin, glucoiberin and glucobrassicin GSL in its profile (Carlson et al., 1987; Deng et al., 2015; Kim et al., 2017).

A few studies have reported significant differences in total and individual GSL contents between cultivars of the same *B. oleracea* species grown under the same condition (Brown et al., 2002; Kushad et al., 2004; Park et al., 2012; Park et al., 2014b; Park et al., 2014c; Kim et al., 2017). Variation in GSL concentrations have also been reported between heads of the same *Marathon* broccoli cultivar (Winkler et al., 2007). To combat these differences, the authors suggested several replicates be analysed and multiple heads be combined and analysed together as a single sample to ensure representative samples of plants are analysed but, in so doing, the inherent differences in individual plants within a variety can be lost, giving a false sense of uniformity in the plant (Bell & Wagstaff, 2017). This is particularly important if plants have not been bred for uniform GSLs concentrations (Fukuda et al., 2015). The part and age of the plant being studied can also affect the type and concentrations of GSLs present. The effect of cultivar and developmental stage on concentration of GSLs and their hydrolysis products in different *B. oleracea* species (broccoli, cauliflower, white, savoy and red cabbage) was studied (Hanschen & Schreiner, 2017). GSL and hydrolysis products profile and concentrations differed significantly between cultivars, and between sprouts and matured heads. Sprouts possessed up to ten times more GSL and hydrolysis products than matured heads.

Some of the differences in type and concentrations of individual GSLs are the result of breeding activities targeted at selecting cultivars for disease resistance, taste and flavour as well as breeding for improved health benefits. Standard broccoli cultivars have been bred for higher levels of glucoraphanin and glucoiberin achieved by crossing broccoli cultivar with *B. villosa*, a wild *B. oleracea* high in glucoiberin (Faulkner et al., 1998; Mithen et al., 2003; Sarikamis et al., 2006). Gene interactions between the two parent plants resulted in higher glucoraphanin contents in the hybrid broccoli. Human intervention trials conducted on the cultivars showed three times more SFP (hydrolysis product of glucoraphanin) was present in the plasma in the cultivars than standard cultivars (Gasper et al., 2005). Enhanced amounts of the GSLs and their ITCs are not expected to have a negative effect on taste or flavour since these GSLs and their ITCs contribute little or nothing to taste and flavour. However, breeding for increased concentrations of certain GSLs like sinigrin (bitter tasting GSL) might result in more bitter and pungent cultivars which maybe undesirable to consumers.

1.6.1.2 Effect of B. oleracea species /variety on myrosinase activity

GSLs are hydrolysed by myrosinase enzyme, however, the rate of hydrolysis is largely dependent on the activity of myrosinase in the plant matrix. The myrosinase activity of *B. oleracea* is known to vary between and within species. Similar to GSLs, the differences observed between species was dependent on the variety being studied. Brussels sprouts and white cabbage myrosinase were reported to be more active (up to 75 %) than sprouting broccoli, cauliflower, kohlrabi, red and savoy cabbage myrosinase (Wilkinson et al., 1984). The same study reported no significant difference in the myrosinase activity of cauliflower, kohlrabi, red and white cabbage. In two different studies conducted by Yen & Wei (1993) and Travers-Martin et al. (2008), highest myrosinase activity was observed in broccoli and white cabbage when compared to the other *B. oleracea* species studied. Similar results were observed by Singh et al. (2007), but in their study the highest myrosinase activity was reported in broccoli and Brussels sprout. The studies also show that white cabbage myrosinase is significantly more active than red and savoy cabbage. However, in another study, no significant difference was observed in the myrosinase activities of broccoli, white cabbage and tronchuda cabbage (Aires et al., 2012). Despite the differences in myrosinase activity between *B. oleracea* species, studies suggest that average myrosinase activity is highest in broccoli and Brussels sprouts. Most studies on myrosinase activity are focused on single cultivars of B. *oleracea* species and studies on myrosinase activity of different varieties within a species are limited. However, few authors have reported differences in myrosinase activity between varieties within a *B. oleracea* species.

Variations were reported in myrosinase activities of different varieties of Brussels sprouts, broccoli, cauliflower, Chinese cabbage and white cabbage (Singh et al., 2007). The authors found a two-fold difference in the myrosinase activities of five broccoli varieties, two cauliflower and Chinese cabbage varieties. The myrosinase activity of five white cabbage varieties grown in the eastern part of Spain was studied (Penas et al., 2011). Significantly higher myrosinase activity was observed in two out of the five varieties studied.

The differences observed in myrosinase activity between and within *B. oleracea* species maybe due to different myrosinase isoenzymes with varying activities and action present within the plant tissue (James & Rossiter, 1991; Bones & Rossiter, 1996). The differences in myrosinase activity can influence the amounts of beneficial GHPs produced. Selection and breeding of varieties with high myrosinase activity will potentially improve potential benefits from *B. oleracea* consumption. More studies on myrosinase activity of different varieties of *B. oleracea* vegetables are needed to ensure that varieties with high myrosinase activity are selected for commercial breeding and consumption. In addition, studies on the stability of these enzymes should also be considered as high myrosinase activity might not necessarily translate to high stability after processing. This is important as most *B. oleracea* vegetables are consumed after one form of post-harvest processing or another.

1.6.1.3 Effect of B. oleracea species /variety on flavour profile

Similar flavour volatile compounds have been identified in *B. oleracea* species; however, differences in their abundance can affect and influence the flavour profile of the vegetables. Sulfides, ITCs, aldehydes and alcohols are the main volatiles identified in B. oleracea species (Buttery et al., 1976; Chin & Lindsay, 1993; Engel et al., 2002b; Valette et al., 2003). The volatile profile of the three *B. oleracea* species (broccoli, cabbage and cauliflower) studied were generally similar but variations in the abundance of individual compounds were observed (Buttery et al., 1976). Sulfides (DMDS, DMTS and MMMS) were detected in all samples. AITC, PEITC and 2BITC were the predominant volatiles identified in cabbage, erucin and erucin nitrile in broccoli and iberverin and its nitrile in cauliflower. AITC and 3BITC were not detected in broccoli. The types of ITC volatiles detected could be related to the GSLs present in the samples, although GSL contents of vegetables were not analysed in the study. Relatively high concentrations of nonanal (11 % of total volatiles) was also detected in cauliflower and broccoli and might contribute a fruity/floral aroma to the vegetables, especially because of its detection threshold (1 ppm in water). In a separate study aldehydes were detected as the main volatile

constituent of cauliflower, whereas ITCs and nitriles were the major volatiles in Brussels sprouts (Van Langenhove et al., 1991)

A study by Chin & Lindsay (1993) on volatiles in 38 cabbage cultivars showed that some cultivars produced more unpleasant sulfides volatiles than others, but most cultivars produced concentrations above detection thresholds. AITC was not detected in some cultivars. Volatiles in leaves and inflorescence of Romanesco cauliflower were extracted by steam distillation. More volatiles were present in fresh leaves (61) than in the inflorescence samples (35). (Z)-3-Hexenol was the predominant volatile in fresh leaves (61 %) while, in addition to (Z)-3-Hexenol, DMDS and DMTS were identified as the main volatiles in inflorescence tissues (30.3, 24.2 and 21.7 % respectively) (Valette et al., 2003).

de Pinho et al. (2009) studied the volatile constituents of internal and external leaves of tronchuda cabbage. Internal leaves, which were pale yellow and tender, contained more aldehydes and sulfur volatiles and less ketones, terpenes and norisopreniod compounds compared to external leaves which were dark green in colour. The higher levels of ketones and norisopreniod compounds, with corresponding lower amounts of aldehydes and sulfur compounds in the external leaves, is likely to result in more desirable floral and fruity odour characteristics in external leaves than the undesirable sulfurous aromas internal leaves might potentially possess. The effect of fertilizer regimes was also studied and the authors found that fertilizers with higher sulfur produced cabbage leaves with more sulfur volatiles and lower concentrations of terpenes and norisoprenoid compounds. In kale, ITCs were the dominant volatiles in seeds; sulfides and ITCs in sprouts; aldehydes, alcohols, ketones and norisopreniod compounds in fully developed leaves (Fernandes et al., 2009). AITC and PEITC were the only sulfur volatiles detected in matured leaves with concentrations significantly lower than present in seeds and sprouts. The authors suggested that the absence of sulfur compounds in fully developed leaves may be related to their importance in kale development rather than being produced during growth. Based on the results of this study, commercialization and consumption of kale sprouts rather than the matured leaves may be beneficial to health, but consumer acceptance of the flavour characteristics needs to be considered.

1.6.2 Cultivation conditions of Brassica oleracea species/varieties

1.6.2.1 Effect of growing conditions on glucosinolate content of B. oleracea species/varieties

In addition to species and varietal differences, conditions under which *B. oleracea* species/varieties are grown can greatly influence accumulation of GSLs. *B. oleracea* species and

varieties growing in spring/summer time are generally reported to have higher GSL concentrations compared to those grown in the autumn/winter months (Rosa & Heaney, 1996; Vallejo et al., 2003b; Charron et al., 2005a; Velasco et al., 2007; Cartea et al., 2008; Martinez-Villaluenga et al., 2009). The authors suggested that higher GSL accumulation in spring time may be due to the higher temperatures in the spring/summer season when compared to the autumn/winter months. White cabbages grown in the eastern part of Spain with higher temperatures and radiation, and lower mean rainfall had higher GSLs concentrations and more individual GSLs detected than those grown in the northern part of Spain with lower temperatures and radiation, and higher mean rainfall (Penas et al., 2011). Increased GSL accumulation was observed in *B. oleracea* species grown at higher environmental temperatures with lower rainfall than those grown at lower temperatures and higher rainfall, with up to two-fold increases reported in some cases (Ciska et al., 2000; Steindal et al., 2013). Higher GSL accumulations in low rainfall conditions may be due to increased concentration of GSL per unit dry weight due to lower moisture content in the plant tissue. It can also be a defensive response to stressful conditions or increased accumulation of GSL precursors such as amino acids, sugars and sulfur during drought and high temperature conditions (Bjorkman et al., 2011). Reduced water supply or drought conditions favours increased phytochemical accumulation (Schreiner, 2005). The higher GSL concentrations accumulated in spring/summer grown plants can also be related to interactions between higher environmental temperatures, high light intensity and longer daylight hours during the spring/summer months as some studies have shown that GSL accumulation is enhanced with higher light intensity and longer daylight exposure. Increase in light levels was found to enhance GSL accumulation in cabbage (Rosa & Rodrigues, 1998), broccoli and cauliflower (Schreiner et al., 2006; del Carmen Martínez-Ballesta et al., 2013). In a recent study by Moreira-Rodríguez et al. (2017), broccoli sprouts exposed to high ultraviolet (UV) rays for 24 hours increased in GSL concentrations with up to 170 % recorded in 4-methoxyglucobrassicin after exposure to ultraviolet B rays (UVB). The authors explained that UV treatment induced the expression of genes in GSL biosynthetic pathways.

The differences in GSL concentrations due to temperature can, however, differ depending on the type of GSL; in broccoli and cabbage varieties, low temperatures increased contents of aliphatic GSLs and high temperatures increased indole GSLs (Schonhof et al., 2007a; Choi et al., 2014). Steindal et al. (2015), also found higher sinigrin accumulated in kale in low temperatures. The authors suggested that cold temperature stress may be beneficial for GSL accumulation but can also be organ and species dependent. On the contrary, Martinez-

Villaluenga et al. (2009) found higher indole GSL concentrations in cabbage varieties cultivated in the winter (colder months) than those grown in the summer months. Differences observed may be due to different temperature and radiation requirement for GSL synthesis in different species and varieties.

Sulfur and nitrogen application during plant growth can also affect GSL accumulation. Sulfur application during cultivation increases GSL concentrations while GSL is reduced with increase in nitrogen. Park et al. (2017), found application of sulfur (1 mM and 2 mM S) enhanced GSL concentration in kale. In a kohlrabi pot experiment, amounts of GSL hydrolysis products formed decreased with increased nitrogen supply and reduced sulfur application (Gerendás et al., 2008). These reductions can be explained by reduced GSL formed with increased nitrogen application (Schreiner, 2005). However, the effect of increased nitrogen on GSL accumulation can be countered with increase in sulfur. When sulfur levels were increased, increase in nitrogen applied did not reduce GSL accumulation in turnip but at low sulfur levels, increased nitrogen resulted in reduced GSL accumulation (Li et al., 2007).

Other ways of increasing GSL accumulation in *B. oleracea* crops is through the application of amino acids such as methionine. Applying methionine (the precursor of aliphatic GSLs such as glucoraphanin) at low concentrations (5mM – 10 mM) to broccoli sprouts increased total GSL concentrations by about 19 % with 28 % increase observed for indolic GSLs (Baenas et al., 2014). Another study recorded 28 % increase in broccoli GSL when 200 mM methionine was infused into the leafstalk (Scheuner et al., 2005). Exposure to pests can also increase GSL concentrations. *B. oleracea* crops exposed to generalists herbivores had higher aliphatic GSLs than those grown in environments where these herbivores were absent (Mithen et al., 1995). Addition of CO_2 has provided conflicting results with decreases in GSL observed in some species (e.g. mustard); no effect in radish or turnip and increases detected in broccoli which was mainly due to increase in aliphatic GSL though levels of indole GSL decreased (Karowe et al., 1997; Schonhof et al., 2007b). During elevated CO_2 conditions, because nitrogen levels will decrease, plants will apportion less nitrogen to plant defence which in turn reduces the amounts of nitrogen-containing compounds like GSL produced (Karowe et al., 1997). However, this effect might be species-specific or GSL type-specific as shown in the results of the various studies.

1.6.2.2 Effect of growing conditions on myrosinase activity of B. oleracea species/varieties

Low temperature conditions are reported to increase myrosinase activity of B. *oleracea* species (Brussels sprout, broccoli, cauliflower, cabbage and kale) grown in the autumn season

(Charron et al., 2005b). White cabbage varieties grown in the northern part of Spain with low temperatures had higher myrosinase activity than those grown in the spring season and eastern part of Spain with warmer temperatures (Penas et al., 2011). Charron & Sams (2004) grew rapid-cycling *Brassica oleracea* (RCBO) leaves in different temperatures with 24-hour photoperiod and in simulated spring and autumn conditions. In the temperature experiment, myrosinase activity increased more in RCBO leaves grown at 12 and 32 °C than those grown at 22 °C. In the spring and autumn simulations, myrosinase activity was highest in the spring season leaves than the autumn season leaves. The higher myrosinase activity observed at 12 and 32 °C and in spring simulated leaves may be due to exposure of the plants to longer daylight hours (24 hours). Increase in myrosinase activity due to light exposure may be the result of increase in myrosin cell production which influences the induction of different myrosinase isoenzymes in plants grown in the light when compared to those grown in the dark (Bones & Iversen, 1985). Another possible reason for higher enzyme activity at 12 and 32 °C may be the optimisation of the myrosinase isoenzyme for relatively cold or hot temperatures rather than intermediate growth temperature of 22 °C. Myrosinase isoenzymes in *B. oleracea* species/varieties differ in their requirement for temperature and light (Charron & Sams, 2004). Also, the plant organ and developmental stage may contribute to variations in myrosinase activity. In the Charron & Sams (2004) study, myrosinase activity of the plant roots and stems of the plants were studied. The authors found similar results in the stems as was observed in the leaves. However, in the roots higher myrosinase activity was reported at 22 °C with no differences observed in spring and autumn simulations. Myrosinase enzymes are present in a single plant in several isoforms which are expressed at different developmental stages and tissues, giving a temporal-spatial tissue specificity which may influence overall activity (Martinez-Ballesta & Carvajal, 2015).

In drought conditions, increased concentrations of the plant hormone, abscisic acid (ABA), enhanced myrosinase activity in Arabidopsis plants (Zhao et al., 2008). Down-regulation of encoding genes involved in myrosinase enzyme synthesis resulted in lower myrosinase activity in the plants, in turn enabling storage of extra sulfur and nitrogen as GSLs in leaf tissues of *A. thaliana* when it was grown in nitrogen or nitrogen/sulphur limiting conditions (Hirai et al., 2004). In conditions where only sulfur was deficient, the expression of genes encoding myrosinase was induced. In two broccoli cultivars, salt stress decreased myrosinase activity both in ambient and elevated CO_2 concentrations (Rodríguez-Hernández et al., 2014). In broccoli sprouts treated with varying levels of NaCl 80-100 mmol/L NaCl application increased

myrosinase activity in 5-day old sprouts but significant reductions were observed in 7-day old sprouts. The results suggest that effects of NaCl addition may be dependent on the age of the plant and concentrations used (Guo et al., 2013).

Influence of growing conditions on myrosinase activity needs to be further explored as most studies available have focused on cultivation temperature and photoperiod and are limited to few *Brassica* species and varieties, making it difficult to fully ascertain the impact of these factors on myrosinase activity and if these impacts will be similar across various species. However, based on the results of the various studies highlighted, it is obvious that growing conditions have significant effect on the GSL-myrosinase system which will in turn affect the type and amounts of GHPs formed. Care must be taken to ensure that *B. oleracea* crops are grown under conditions that best favour increased GSL accumulation and myrosinase activity to enhance their potential health beneficial properties.

1.6.3 Brassica oleracea post-harvest and domestic processing

Most *B. oleracea* species undergo one form of storage, post-harvest and/or thermal processing after harvest and before consumption. These processes can affect the phytochemicals and GSL-myrosinase system of the plant in several ways. The rate and extent is dependent on the conditions under which these processes occur. Some of the factors that can influence these processes include extent of tissue damage, texture of tissue, humidity, as well as time and temperature conditions during storage and processing.

1.6.3.1 Effect of post-harvest methods and storage on GSL-myrosinase system

Time, temperature, relative humidity (RH) and atmospheric conditions under which *B. oleracea* are transported and stored after harvest all influence phytochemical composition and physical quality of the vegetables. For post-harvest quality to be maintained in vegetables, high RH (98-100 %) should be maintained. However, effect of RH on GSL content is influenced by the storage temperature. Under low RH, broccoli stored at 20 °C for five days resulted in >80 % glucoraphanin loss (Rodrigues & Rosa, 1999). About 50 % loss in glucoraphanin was also observed in broccoli stored at low RH in open boxes for three days at 20 °C while significant losses were not recorded in heads stored in plastic bags at high RH (90 %) (Rangkadilok et al., 2002). In the same study, when broccoli heads were stored at 4 °C in open boxes or plastic bags with varying RH, there was no significant loss in glucoraphanin content in both conditions though RH was low. Losses observed at low RH, correlated with visible deterioration (senescence/yellowing) of the heads signifying cell wall breakdown and myrosinase

degradation of GSL. Loss of GSL concentration during low RH storage can be countered if accompanied by low temperature storage maintaining visual qualities and preventing senescence and GSL degradation or loss.

In a storage experiment conducted by Aires et al. (2012), broccoli, white cabbage and tronchuda cabbage were stored at 4 and 22 °C for 72 hours. The results showed that broccoli and white cabbage were more stable at 4 °C and tronchuda at 22 °C. In some *Brassica* species, plant metabolism and GSL synthesis increases at high temperatures and decreases at lower temperatures (Rosa et al., 2007). Average GSL concentrations for both storage conditions showed losses in GSL for broccoli and white cabbage while increase in indole GSL was observed in tronchuda. GSL losses in broccoli and white cabbage coincided with loss in visual quality which was less pronounced in tronchuda cabbage. Since GSLs have the capacity for new biosynthesis ("de novo" synthesis) in plant tissue (Chen & Andreasson, 2001), the authors suggested that young leaves in the interior of tronchuda cabbage maybe responsible for new synthesis and mobilization of indole GSLs. There was no change in GSL concentrations of Brussels sprouts, cauliflower and green cabbage stored at 4-8 °C for three days, however about 26 % loss was observed after seven days of storage (Song & Thornalley, 2007). In a broccoli study simulating current domestic and international transport, storage and market/shelf conditions, no difference was observed in glucoraphanin and flavonoid concentrations at transport and storage conditions (1-4 °C for 28 days) and in shelf life conditions (8, 15 or 20 °C) suggesting that current market practices do not have negative effects on GSL concentrations (Winkler et al., 2007). Apart from storage temperature, the storage time equally influences GSL concentration but time-temperature requirement varies between species.

Controlled atmosphere (CA) and modified atmospheric packaging (MAP) (treatments) have been shown to preserve GSL in plants during storage, but with conflicting results. Storage of broccoli at 4 °C under CA (1.5 % O_2 and 6 % CO_2) for 25 days or MAP (0.2 % O_2 and 15 % CO_2) for 10 days retained GSL concentration and tissue quality compared to storage at ambient conditions (Rangkadilok et al., 2002). On the contrary, total GSL increased by 21 % in broccoli stored at 10 °C for 7 days under CA (0.5 % O_2 and 20 % CO_2), whereas 58 % loss of indole GSL was observed in the presence of 20 % CO_2 and absence O_2 (Hansen et al., 1995). In another study, 71- 80 % of GSL was lost in low-density polyethlene film wrapped broccoli stored at 1 °C for seven days under CA (15 % O_2 and 2 % CO_2) (Vallejo et al., 2003a). Mini heads of broccoli and cauliflower had different MAP requirement for storage under the same temperature (8 °C). While mini broccoli heads retained GSL concentrations under MAP treatment of 1 % O_2 and 21

% CO_2, cauliflower retained GSL under 8 % O_2 and 14 % CO_2 MAP treatment (Schreiner et al., 2006). While the GSL preservative effects of CA and MAP cannot be denied, treatment requirements are unclear and differ between species and varieties and the developmental stage of the tissue being sold.

Chopping and shredding of *B. oleracea* vegetables is common before processing or cooking and will alter the food matrix. Finely shredded (5 mm cubes or 5 mm leaf squares) Brussels sprouts, cauliflower and green cabbage stored at 23 °C led to 75 %, 75 % and 60 % loss in total GSL respectively over a 6-hour period but larger cuts resulted in <10 % loss in GSL (Song & Thornalley, 2007). In a different study by Verkerk et al. (2001), broccoli, red and white cabbage were chopped (1 cm^2) or homogenized in a blender. Limited losses of aliphatic GSL occurred in chopped samples but a surprising 15-fold concentration increase in white cabbage and 3.5-fold in broccoli was observed for indole GSLs. When *B. napus* plants were punctured by needles, to simulate tissue damage, a 3-fold increase in indole GSL was also observed (Bodnaryk, 1992). The mechanism for indole GSL increase in both studies is unclear but may be due to "de novo" synthesis as previously explained. Major losses were however, observed in homogenized samples in the Verkerk et al. (2001) study and agrees with another study where GSL of homogenized Brussels sprouts was completed hydrolysed to ITCs and nitrile (Smith et al., 2003). The losses observed in homogenized and finely shredded samples compared to chopped samples can be attributed to greater GSL hydrolysis due to smaller surface area after homogenization. On the other hand, increased levels of indole GSL was attributed to stress induced responses by the plants as a defence mechanism from attack.

Conditions for transport and storage are normally optimized for individual crops mainly to prevent visual damage and degradation and not phytochemical losses (Schouten et al., 2009). There is, however, a need to consider the effects of this conditions on the phytochemical content to preserve the health protective properties of *Brassica* vegetables.

1.6.3.2 Effect of domestic processing on the glucosinolate content of B. oleracea

Traditionally, *B. oleracea* vegetables are mostly blanched, boiled, steamed, microwaved, stir-fried and in some cases baked and pickled. Blanching is usually carried out as a pre-treatment process to soften the vegetables, inactivate enzymes and increase shelf life before freezing or canning. In a study conducted by Cieślik et al. (2007), blanching at 80 °C for 8 min (vegetable-water ratio 1:1) led to reduction in GSL concentrations (2- 30 %) of the various *Brassica* vegetables studied. Korus et al. (2014) reported a loss in kale GSL of 30 % after blanching for 2.5 mins at 98 °C (vegetable-water ratio 1:5). Volden et al. (2008) also recorded a

60 % loss in total GSL after blanching small pieces (1 cm) of red cabbage in water (vegetable-water ratio 1:10) for 3 min. Similar results (30- 52 % loss in total GSL) was observed after blanching 5 cauliflower cultivars under similar condition. After blanching two white cabbage varieties for 5 min, GSL concentrations reduced substantially by 50 and 74 % (Wennberg et al., 2006). However, no change in total GSL content was observed in broccoli blanched at 92 ˚C for 105 sec (Rungapamestry et al., 2008b). Most of the losses due to blanching occurred when vegetables were blanched for longer periods and in the presence of large amounts of water which would have led to leaching of GSLs into blanching water.

When *B. oleracea* vegetables are boiled, it leads to leaching of GSLs into the boiling water leading to significant loss in GSL levels (Shapiro et al., 2001). The rate of GSL leaching is dependent on type of *Brassica* vegetable, degree of shredding, ratio of water to vegetable, cooking time and type of GSL (Dekker et al., 2000; Ciska & Kozłowska, 2001; Volden et al., 2008). Oerlemans et al. (2006) reported that during boiling of red cabbage, GSLs were lost majorly due to leaching as GSLs are generally stable at boiling temperatures (100 ˚C). Song & Thornalley (2007) after boiling for 30 min reported progressive decrease in GSL concentrations of 58 % in Brussels sprout, 66 % in green cabbage, 75 % in cauliflower and 77 % in broccoli. Differences observed in GSL losses between species can be attributed to differences in leaching rate due to tissue structure and texture. For example, the thicker and firmer tissue of green cabbage will prevent leaching better than the softer texture of broccoli. A study comparing high pressure boiling of broccoli florets to conventional/domestic boiling showed significant loss of GSL in both methods (33 and 55 % respectively), however, loss of indole GSLs was higher than aliphatic GSL (Vallejo et al., 2002). Similar results were observed in boiled white cabbage with variation in diffusivity of GSLs the probably explanation for higher loss of aliphatic GSLs (Ciska & Kozłowska, 2001; Wennberg et al., 2006).

Steaming is regarded as a better way to cook vegetables in general because there is no direct contact of the vegetable with water, thereby preventing losses due to leaching as obtained in boiling. However, there are conflicting results on steaming of *B. oleracea* vegetables. Rungapamestry et al. (2006) reported no loss to sinigrin or total GSLs when cabbages were steamed for 0 - 7 min. Red cabbage and five cauliflower cultivars steamed for 10 min resulted in 19 % and 18-22 % loss in total GSL respectively (Volden et al., 2008; Volden et al., 2009). On the contrary, Vallejo et al. (2002) reported only a 2 % loss in broccoli steamed for 3.5 min while no significant loss in GSL concentration was observed when Brussels sprout, green cabbage, cauliflower and broccoli were steamed for 20 min (Song & Thornalley, 2007).

However, Giallourou et al. (2016) found a significant increase in watercress gluconasturtiin concentration after steaming for 15 min. Similarly, 1.4-1.6 fold increase in aliphatic and indole GSLs respectively was reported when broccoli was steamed for 10 min (Gliszczyńska-Świgło et al., 2006). However, in a later study, the authors found no increase in GSL content after steaming for 3.5 min and hypothesized that increase in GSL during steaming was time-dependent. It is hypothesized that increase in GSL after steaming for long periods could be the result of increased extractability of otherwise bound GSL being released from the cell wall due to steaming temperatures, translating into better bioavailability which benefits the consumer as has been demonstrated in a simulated *in vitro* sinigrin bioavailability study (Ciska & Kozłowska, 2001; Mahn & Reyes, 2012; Girgin & El, 2015). The differences in degree of losses suggests that there is an optimum time for steaming as shorter steaming time tends to retain GSL more than steaming for longer periods. Another possible reason for variation in losses might be the size of the vegetable cuts before steaming as larger cuts (large wedges; Rungapamestry et al. (2006)) retained more GSL than smaller cuts (1 cm cubes; Volden et al. (2008)), relating to differences in heat transfer and core temperature of the vegetable.

As with steaming, there are conflicting reports on GSL concentrations after microwaving. Substantial loss of total GSL (up to 74 %) was observed in broccoli microwaved for 5 mins at 1000 W resulted (Vallejo et al., 2002). Microwaving of cabbage for 3 mins at 900 W showed no significant loss in total GSL content but losses due to leaching when large volumes of water was used during the heating process was reported (Song & Thornalley, 2007). Similarly, minor losses in GSL content was observed when watercress was microwaved for 3 mins (Giallourou et al., 2016). Differences in the results observed between broccoli and cabbage could be related to the time and temperature combinations during microwaving as well as the difference in thickness of the vegetables. On the hand, Verkerk & Dekker (2004) reported a significant increase in GSL content of red cabbage above that present in untreated cabbage when microwaved under different time and energy combinations. This increase was attributed to increased extraction of chemical compounds because of cell disintegration. Losses were not recorded probably because water was not used during the process which prevented leaching of GSLs.

Stir-fry, a method of cooking in Asian countries has become more popular in western countries and other parts of the world. Broccoli florets were stir-fried using different oils (125 – 140 ˚C) for 3.5 min with no significant loss in GSL content observed in all cases (Moreno et al., 2007). Stir-frying green cabbage, cauliflower and Brussels sprouts for 3-5 min over preheated

oil (200 ˚C) had no significant effect on GSL content and the authors concluded that myrosinase must have been inactivated which allowed GSLs to remain intact or the heat was unable to penetrate throughout the tissue under the cooking conditions (Song & Thornalley, 2007). Another possible reason for GSL retention might be due to rapid drop in cooking oil temperature after addition of the vegetables, which consequently reduced the core temperature of the vegetables. In contrast, stir-frying red cabbages (3 cm pieces) at 130 ˚C for 5 min led to 77 % loss in total GSL concentrations (Xu et al., 2014). The authors did not provide a reason for the losses observed but moisture loss due to evaporation during stir-frying may have been responsible for loss in GSL concentrations.

Based on the literatures reviewed, steaming, microwaving, and to some extent stir-frying, seem the most preferable way to cook *B. oleracea* vegetables as they retain more GSL and in some cases increased GSL concentrations. However, GSL retention will be of little importance if myrosinase enzyme is inactivated during the cooking processes.

1.6.3.3 Effect of domestic processing on the myrosinase activity of B. oleracea

Though myrosinase can be hydrolysed in the gut, studies have shown that low levels of ITCs are formed in the process compared to when endogenous myrosinase hydrolyses GSL (Shapiro et al., 2001; Van Eylen et al., 2007). It is therefore important to ensure endogenous myrosinase is still active during consumption of *Brassica* vegetables. Myrosinase enzyme is thermolabile and can, therefore, be lost during domestic cooking processes, however, thermal stability of the enzyme in *B. oleracea* varies depending on the species and cooking condition.

Myrosinase is totally denatured during blanching, boiling and blanch-freezing (Shapiro et al., 2001; Sarikamis et al., 2006; Rungapamestry et al., 2008b). Matusheski et al. (2004) reported that there was a significant decrease in myrosinase activity after broccoli florets and sprouts were heated in water at 60 ˚C for 5 and 10 mins respectively. Steaming cabbages (core temperature 68 ˚C) for up to 420 secs resulted in 90 % loss in myrosinase activity while microwaving for 2 min at 750 W resulted in total inactivation of myrosinase with core temperatures rising up to 91 ˚C (Rungapamestry et al., 2006). In cabbages, myrosinase activity is enhanced at temperatures up to 60 ˚C. At higher temperatures denaturation of the enzyme will occur both in the cabbage and after leaching into the cooking water (Dekker et al., 2000). A study conducted on stir-fried broccoli by Rungapamestry et al. (2008a) showed that myrosinase enzyme was 17 % active after stir-frying for 2 min.

Myrosinase activity of chopped cabbage was studied under varying microwave time and power levels (Verkerk & Dekker, 2004). Significant myrosinase activity was retained at low

power levels (180 W for 24 min), and intermediate power levels (540 W for 8 min). However, at high power levels, (900 W for 4.8 min equivalent of 259.2 kJ energy input) complete denaturation of myrosinase occurred. The authors explained that the higher retention of myrosinase activity at low microwave power despite the longer time was due to slower rate at which cabbage core temperature increased. At 180 W, it took 25 min for cabbage core temperature to get to 90 °C while at 900 W, cabbage core temperature was 100 °C after 2.8 min. Oliviero et al. (2014), studied the effect of moisture content and temperature on myrosinase enzyme inactivation in broccoli. Freeze-dried broccoli with between 10 and 90 % moisture content were incubated at varying temperatures of 40 – 70 °C. Results showed that the driest samples had the most myrosinase stability while samples with 31 % moisture content and above did not differ in myrosinase stability.

Yen and Wei (1993) investigated crude myrosinase extract from red and white cabbage, finding increase in activity up to 60 °C for 30 min, followed by a dramatic decrease with rising temperatures above 60 °C. At 70 °C, 90 % of myrosinase was lost in both samples. The authors also found that red cabbage myrosinase was more stable than white cabbage myrosinase at 60 °C though white cabbage myrosinase activity was higher in untreated samples. Crude myrosinase extract from broccoli is reported to be the least stable myrosinase being inactivated at temperatures above 30 °C (Ludikhuyze et al., 2000) whereas broccoli juice myrosinase was stable at 40 °C with 90 % activity lost after heating for 10 mins at 60 °C (Van Eylen et al., 2007). Similarly, green cabbage myrosinase was reported to be thermally stable at temperatures below 35 °C (Ghawi et al., 2012).

Myrosinase enzyme, when present within plant tissue, is more thermal stable than its crude extract. However, limited studies are available on myrosinase stability in various *B. oleracea* species and within varieties of the same species. Studies on myrosinase activity in different varieties of a *B. oleracea* species, should be encouraged as myrosinase stability will most likely vary between varieties which will have consequent effect on GSL hydrolysis and the type of hydrolysis products produced. Breeding programmes should also focus on breeding and selecting varieties with more stable myrosinase for commercial production.

1.6.3.4 Effect of thermal processing on the glucosinolate hydrolysis products of B. oleracea

The type and amount of hydrolysis products formed during GSL hydrolysis is dependent on the GSL present and ratio of myrosinase to ESP present within the plant matrix. ESP activity results in the unstable GSL degradation products derived from myrosinase activity, undergoing a rearrangement to form EPTs and nitriles, as opposed to ITCs. It is important to prevent ESP

activity during GSL hydrolysis so that more beneficial ITCs are formed rather than the unbeneficial EPTs and nitriles. ESP is more thermolabile than myrosinase and is rapidly degraded upon heating (Matusheski et al., 2004). Thus, mild cooking and heating can destroy ESP and prevent its activity resulting in more ITCs formed. However, further cooking can denature myrosinase and thus reduce ITC formation.

Four broccoli cultivars were boiled, steamed and microwaved under vary time-temperature combinations (Wang et al., 2012). Sulforaphane nitrile (SFN) was the predominant hydrolysis product formed in raw untreated samples; boiling and microwave heating caused initial loss in SFN formation with increase in sulforaphane (SFP) production at the early stages of heating with consequent loss in SFP with continuous heating for one minute. In steamed samples however, SFP formation was enhanced in all but one cultivar after three minutes of steaming. The rate of SFP to SFN formation varied across varieties for all the heating methods. Previous work on cabbage also found the conversion of sinigrin yielded predominantly the inactive 1-cyano-2-epithiopropane after short cooking times and greater conversion to AITC after longer cooking time: however, conversion to the ITCs diminished above 60 °C (Rungapamestry et al., 2006). A decrease in ESP activity accompanied with increase in SFP production and decrease in SFN formation was observed in broccoli florets and sprouts heated for 5 and 10 min respectively.

In a recent study, broccoli was stir-fried directly and soaked for 90 min at 37 ˚C before stir-frying to allow for GSL hydrolysis (Wu et al., 2018). The authors found that soaking before stir-frying increased the amounts of ITCs formed compared to direct stir-frying. This may be a more efficient way of stir-frying broccoli, however, the broccoli florets had to be cut into 2mm pieces which may be a drawback of the method as consumers may not be willing to cut their broccoli into such tiny pieces before stir-frying. Jones et al. (2010) in their study of SFP and SFN in cooked broccoli florets, demonstrated that higher SFP was formed in steamed samples while SFP production was limited in boiled and microwaved samples. The authors suggested that loss of SFP was due to both leaching in water and thermal degradation of both ESP and myrosinase as core temperature of florets was above 70 °C. In a bioavailability study of raw and steamed broccoli using an *in vitro* digestion model, SFP concentration after digestion was higher in samples steamed for one minute than in raw and samples steamed for longer (Sarvan et al., 2017). It is also worth mentioning that SFP was higher in the raw sample than in samples steamed for 2 min and above.

It is apparent a higher proportion of SFP was formed in *B. oleracea* vegetables when thermal processes inactivated ESP while retaining myrosinase activity. However, temperature requirements to achieve this differs between species and varieties. Optimum heating conditions that denature ESP but retain myrosinase must therefore be established for individual species/varieties to ensure myrosinase is still active when vegetables are consumed. Most of the studies to date have focused on SFP formation in broccoli with little or no literature on other ITCs and *B. oleracea*. However, recent epidemiological studies show the potency of other ITCs like PEITC and erucin to proffer health benefits similar to SFN.

1.6.3.5 Effect of thermal processing on volatile and non-volatile flavour compounds of B. oleracea

When *B. oleracea* vegetables are heated, several reactions occur within the plant changing the profile and concentrations of volatile and non-volatile compounds present with the plant matrix. The moisture content, heating time and temperature as well as other compounds within the plant all have an influence on the compounds produced.

Chin & Lindsay (1993) reported that concentrations of AITC, DMDS and DMTS increased with time when cabbages were heated at 30 °C for 100 mins with rapid formation and disappearance of methanethiol and hydrogen sulfide in the heat-treated cabbages. The rapid oxidation of methanethiol to DMDS may explain the disappearance of this compound during prolonged heat treatment. DMS was the most abundant compound (34 % of total volatiles) in boiled cabbage (30 min) with lower amounts of AITC (6 %), DMDS (0.5 %), alcohols, aldehydes and ketones formed (Macleod & Macleod, 1968). In another study, the effect of variation in cooking methods (boiling and microwave radiation) and different parts of cabbage were investigated (MacLeod & MacLeod, 1970a). Saturated aldehydes and sulfides increased over the 90-min boiling period with new sulfide compounds such as dipropyl sulfide formed after 60 mins of heating. Hydrolysis products of sinigrin (AITC and allyl cyanide) increased within 20 mins and then decreased beyond that time. During microwave radiation, AITC and allyl cyanide formation was faster in the absence of water with more nitriles than AITC formed, suggesting that dry cooking conditions favour cyanide formation. In the same study, a taste test was conducted on AITC perception and liking. The authors found that the amount of AITC (6.5 %) produced within 10 mins of boiling was desirable to consumers but level produced at 20 mins (15 %) was undesirable to consumers and suggested that this was the main difference between properly cooked and over cooked cabbage. Sulfur compounds were the predominant volatiles in cooked inner/young cabbage leaves and alcohols and aldehydes the main volatiles in

outer/older leaves. The results suggest that young cabbage leaves are the main contributors to undesirable sulfur volatiles in cabbage.

Aldehydes and alcohols were the most abundant volatiles (90-95 % of total volatiles) identified in steamed and blanched broccoli varieties. A trained sensory panel described the odour of the broccoli samples as hay (overcooked vegetable) and green (freshly cut grass) with descriptions correlating with concentrations of saturated aldehydes and alcohols in the samples (Hansen et al., 1997). Van Langenhove et al. (1991) studied the effect of blanching on Brussels sprouts and cauliflower. Results obtained showed that while ITCs and nitriles were the major volatiles produced in Brussels sprout, aldehydes and sulfides (mainly DMDS) were the major volatiles in cauliflower. A study on the sensory and flavour profile of 11 cooked cauliflower varieties showed that AITC, DMTS and to a smaller extent DMS and methanethiol were the major contributors to undesirable sulfur aromas of cauliflower flavour and are determinants for cauliflower rejection by consumers (Engel et al., 2002b). A major drawback of these studies is that raw samples were not analysed so comparisons between volatiles produced in raw and cooked samples and consequent effect on sensory characteristics cannot be made.

During thermal processing, concentrations of other non-volatiles such as phenolics, amino acids and sugars present in *B. oleracea* vegetables are changed. Concentrations of soluble sugars and phenolics were significantly decreased after boiling, steaming and stir-frying of red cabbage (Xu et al., 2014). Similar results were reported for blanched red cabbage (Volden et al., 2008) and boiled and stir-fried broccoli (Yuan et al., 2009). In blanched and cooked kale, significant reduction in amino acid content was reported with longer blanching time resulting in greater losses (Murcia et al., 2001; Korus, 2012). Similar results were observed in cooked Brussels sprouts (Lisiewska et al., 2008) but no significant loss in amino acid content was observed in cooked green cauliflower (Słupski et al., 2010). The reduction in sugars and amino acids can be attributed to Maillard reactions taking place during heating or losses due to leaching into cooking water. These also contribute to the nutrient content of *Brassicas* and losses during thermal processing can reduce the nutritional value of the vegetables being consumed. Cooking methods that best retain these nutrients should be employed.

The changes occurring in volatile and non-volatile compounds during thermal processing will modify taste and flavour profiles and consequently influence taste and flavour characteristics of cooked *B. oleracea* vegetables.

1.7 Bitter taste receptor and genotypes

Taste is one of the five traditional senses which include smell, touch, sight and hearing delivering different sensations to consumers. Taste is used as a way of identifying the nutritional value of a food constituent before ingestion or absorption (Behrens & Meyerhof, 2013). It is widely accepted that there are five basic taste namely sweet, sour, salty, umami and bitter. Bitter taste is perceived as unpleasant and signals the presence of a toxic or poisonous substance (Behrens et al., 2013). Bitter taste perception normally occurs on the tongue and is mediated by interaction of tastants with G protein-coupled TAS2R receptors. There are 25 functional TAS2R genomes encoded in humans residing in three chromosomes; 1 gene on chromosome 5p, 9 on chromosome 7q and 15 genes on chromosome 12p (Shi et al., 2003). TAS2R genes exhibit extensive coding sequence diversity which is responsible for the unusually high levels of allelic variations found within the TAS2R loci and the appreciable difference in human TAS2R allele frequencies (Kim et al., 2005). Genetically, humans vary in their ability to detect certain compounds such as the synthetic bitter compounds phenylthiocarbamide (PTC) and 6-*n*-propylthiouracil (PROP), which contain a thiourea group "N-C=S" (Tepper et al., 2009). Because of the genetic difference in individual sensitivity to PROP, it has been recommended as a way of measuring human sensitivity to bitter taste. Individuals have been classified into three groups based on PROP sensitivity; super-tasters, medium-tasters and non-tasters (Calò et al., 2011). TAS2R38 gene, one of the most widely studied TAS2R gene is found on the 7q chromosome and specifically detects the bitter thiourea group found in PTC and PROP ((Kim et al., 2003; Meyerhof et al., 2010).

Gustin (CA6) gene (*rs2274333*), is a zinc dependent salivary protein secreted by the parotid, submandibular and con Ebner glands (Padiglia et al., 2010). It is a trophic factor that promotes growth and development of taste buds by acting on taste-bud stem cells (Henkin et al., 1999). Decreased secretion of gustin has been linked with reduced taste function. Precious studies have shown a relationship between genetic gustin polymorphism and PROP sensitivity (Padiglia et al., 2010).

1.7.1 Influence of TAS3R38 genotype on sensitivity to PROP

The ability to taste PROP is associated with three single-nucleotide polymorphisms (SNPs) which form haplotypes within the *TAS2R38* gene and are associated with differences in individual ability to taste PROP (Duffy et al., 2004). These SNPs result in amino acid substitutions at different positions; at position 49 (proline/alanine, Pro49Ala - *rs713598*), 262 (alanine/valine,

Ala262Val - *rs1726866*) and 296 (valine/isoleucine, Val296Ile - *rs10246939*) (Kim et al., 2003). There are two common haplotypes of the *TAS2R38* gene; the dominant/sensitive variant, PAV (Pro-Ala-Val) and the recessive/insensitive variant, AVI (Ala-Val-Ile) (Calò et al., 2011). Individuals with at least one copy of the sensitive PAV allele can detect bitter taste in PROP while individuals with homozygous insensitive AVI allele are unable to taste PROP (Carrai et al., 2011). Two rare haplotypes (AAI and AAV) and two extremely rare haplotypes (PAI and PVI) have also been identified (Hayes et al., 2008; Risso et al., 2016). See Table 1.3 for a summary of the SNPs and haplotype frequencies. Several studies have shown significant differences in PROP detection thresholds for the different *TAS2R38* genotypes. Individuals with AVI/AVI genotype have significantly higher PROP detection thresholds than PAV/PAV and PAV/AVI individuals, but no significant difference in threshold was found between PAV/PAV and PAV/AVI genotype (Duffy et al., 2004; Hayes et al., 2008; Calò et al., 2011). At suprathreshold levels, *TAS2R38* genotype did not fully explain PROP bitterness as PAV homozygotes were not always PROP supertasters and some AVI homozygotes were able to taste PROP. Based on these results, Hayes et al. (2008) and Calò et al. (2011) stated that *TAS2R38* alone cannot explain bitterness at suprathreshold levels suggesting that other polymorphisms and mechanisms, such as fungiform papillae density and gustin, may partially explain bitterness sensitivity at suprathresholds. As expected, individuals with one of the rare PAI or PVI haplotypes generally had lower PROP thresholds than those with homozygous AAV or AVI (Hayes et al., 2008).

Additional factors influencing bitter taste sensitivity in individuals include age (children being more sensitive than adults), gender, and cultural differences (Keller et al., 2010; Mennella et al., 2010). A previous study found that the fungiform papillae density of children (aged 8-9 years) was one-third greater than that observed in adults (aged 18-30 years) (Segovia et al., 2002). The higher fungiform papillae density observed in children may influence bitter taste sensitivity.

Table 1.3: *TAS2R38* SNPs and haplotype frequency

	Position						
	Nucleotide			Amino acid			
Haplotype	145	785	886	49	262	296	Frequency
AAI	G	C	A	A	A	I	Rare
AAV	G	C	G	A	A	V	Rare
AVI	G	T	A	A	V	I	Common
PAI	C	C	A	P	A	I	Rare
PAV	C	C	G	P	A	V	Common
PVI	C	C	A	P	V	I	Rare

The table shows the composition of each haplotype with respect to nucleotide positions 145, 785 and 886 and composition with respect to amino acid positions 49, 262 and 296. The last column summaries the haplotype frequencies.
(Adapted from Wooding et al. (2010)).

1.7.2 Influence of gustin (CA6) on sensitivity to PROP

Gustin polymorphism *rs2274333* (A/G) influences zinc binding to gustin and its activity is dependent on the presence of zinc at the active site. Individuals with the AA genotype (associated with the fully functional protein) are more liking to be PROP supertasters, those of GG genotype (associated with disruption in the protein) tend to be PROP nontasters and individuals with one A allele (A/G) are considered medium tasters (Padiglia et al., 2010). Previous studies have shown association of gustin genotype with PROP sensitivity but in most cases this association is in combination with *TAS2R38* and fungiform papillae density (Calò et al., 2011; Melis et al., 2013). Melis et al. (2013) found that treatment of isolated cells with saliva from AA genotype individuals or application of an active iso-form of gustin protein increase fungiform cell proliferation and metabolic activity. The authors suggested that gustin gene influences PROP sensitivity by acting on fungiform papillae development and maintenance. In a different study by Feeney & Hayes (2014), PROP bitterness perception did not differ between individuals due to gustin genotype. The authors suggested that the larger sample size and ethnic diversity of the study group compared to other studies might be responsible for the results obtained.

1.7.3 Influence of bitter taste genotype on Brassica taste perception, liking and intake

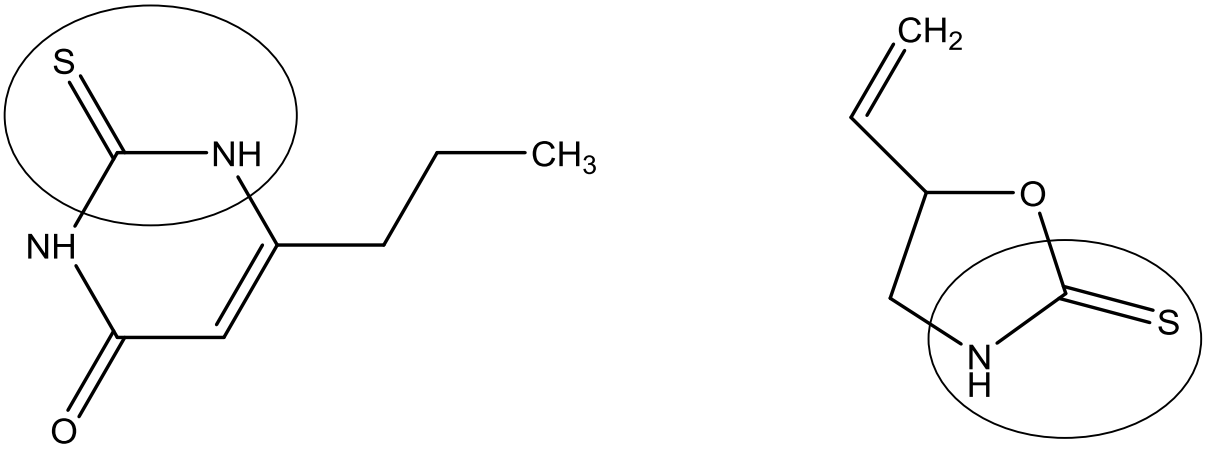
There is substantial evidence that liking is the main determinant of food choice and taste is often reported as the main driver of liking (Schätzer et al., 2009). The thiourea group “N-C=S” contained in the chemical structure of the synthetic product, PROP, is also found in GSLs and

some oxazolidine-thiones (goitrin) present in *Brassica* vegetables (Figure 1.3). This may influence individual bitter taste perception and preference of *Brassica* vegetables.

Wooding et al. (2010) investigated the effect of *TAS2R38* genotype on bitter taste perception of goitrin, a bitter tasting ITC. The results demonstrated variations in taste response to goitrin, those of PAV/PAV genotype perceived goitrin significantly more bitter than AVI/AVI though thresholds for goitrin perception were higher than that of PROP. In another study, all *TAS2R38* genotypes differed in their perception of bitter taste in *Brassica* vegetables with PAV/PAV genotyping perceiving *Brassica* vegetables 60 % more bitter than AVI/AVI genotype (Sandell & Breslin, 2006). To corroborate this result, Sacerdote et al. (2007) and Sandell et al. (2014) (n= 634 and 1,903 respectively) found PAV/PAV individuals consumed less *Brassica* vegetables than AVI/AVI. On the other hand, Feeney et al. (2014) examined vegetable intake in 525 Irish children aged 7-13 years old. The authors found that although supertasters were less likely to have tried or tasted *Brassica* vegetables, the *TAS2R38* genotype was not significantly associated with intake and liking of *Brassica* vegetables. Instead, the study found that preference of *Brassica* vegetable was influenced more by interactions between the environment, social and physical factors. A study of elderly British women (n= 3,383) also showed that *TAS2R38* genotype did not determine choice or consumption of *Brassica* vegetables (Timpson et al., 2005).

In the only study investigating the effect of *TAS2R38* genotype and gustin on bitter taste, intake and liking of *Brassica* vegetables (n= 136), Shen et al. (2016) reported that PAV/PAV subjects perceived a stronger bitter intensity than the other two *TAS2R38* genotypes while AVI/AVI liked *Brassica* vegetables more. However, in terms of intake, PAV/PAV and AVI/AVI consumed more vegetables than PAV/AVI. The authors did not find a significant effect of gustin on bitterness sensitivity but gustin was significantly associated with *Brassica* vegetable intake. Similar to Feeney et al. (2014), the authors found that taste genotype and phenotype alone could not adequately predict vegetable liking as demographics (gender and ethnicity) had a considerable influence on vegetable preference and intake, suggesting that these factors play an important role in vegetable liking.

The conflicting results show several factors influence *Brassica* intake and liking and more studies are required to validate current findings as well as provide better understanding of the influence of taste genotypes on *Brassica* intake and liking.



a) 6-*n*-propylthiouracil (PROP) b) Goitrin

Figure 1.3. Chemical structures of 6-*n*-propylthiouracil (PROP) and goitrin highlighting the thiourea group "N-C=S".

1.8 Summary

B. oleracea species are a good source of GSLs which produce health beneficial ITCs when hydrolysed by myrosinase. These compounds alongside other compounds confer the undesirable bitter taste and sulfurous aromas on *Brassica* vegetables. However, the GSL-myrosinase system varies between and within *B. oleracea* species and differences alongside other flavour volatile and non-volatile compounds influence both the sensory and health promoting properties of the vegetables.

Previous research has focused mostly on the GSL concentration and myrosinase activity of *B. oleracea* vegetables. In most cases, inferences are made on possible ITCs that will be produced based on the GSLs present within the plant matrix. However, GSLs concentrations are not necessarily a good indicator of the type and abundance of hydrolysis products that will be produced, as conditions under which the hydrolysis occurs has a major influence on the types and amounts of hydrolysis products formed. Also, in some cases, some GHPs have been identified in the absence of their intact GSL. Plant genotype, growing conditions and thermal processing influence the type and amounts of volatile compounds and GSL formed, as well as myrosinase enzyme stability of the plant, all of which differ between species and varieties. These factors will impact the taste and flavour characteristics of the plant. Studies have shown that more beneficial ITCs are formed from mildly heated vegetables because myrosinase activity is retained while ESP is denatured. Most thermal processes investigated to-date do not however reflect standard domestic cooking methods and cover a limited number of species and

varieties. They do not, therefore, reflect what the consumer experiences when *B. oleracea* vegetables are consumed.

Most taste and volatile studies have not taken into consideration other compounds present within the food matrix that could influence the perception of bitterness and sulfurous aromas. Bitter taste is considered the main driver of low *Brassica* consumption. However, limited and conflicting evidence exists on the influence of bitter taste genotypes on *Brassica* intake and liking. Finally, studies on the correlation between phytochemical data and sensory studies of *B. oleracea* are lacking.

This study therefore aims to answer the following questions:

- How do plant variety, growing conditions and domestic cooking methods affect myrosinase activity and stability in cabbage accessions/varieties?
- Does plant variety and growing conditions influence the profile and concentrations of GSLs and GHPs in different cabbage accessions/varieties?
- To what extent do domestic cooking methods influence the profile and concentrations of GSLs and GHPs in different cabbage accessions/varieties?
- Do plant type/variety and domestic cooking methods play a role in the types and amounts of volatiles produced in different cabbage accessions/varieties?
- Can bitter taste and sulfurous aromas be reduced and consumer acceptability improved through domestic cooking?
- Does bitter taste sensitivity influence bitter taste perception and liking of cabbage?
- Is there a relationship between phytochemical composition, sensory attributes and consumer perception and liking of cabbage varieties?

To answer the questions enumerated above, the following hypotheses were proposed:

1.8.1 Study hypotheses

1) Primary hypothesis: By variety selection and optimised processing conditions, it is hypothesised that ESP can be minimized and myrosinase activity maximised to:

- Increase health-beneficial GSL hydrolysis products at point of consumption
- Minimise bitter taste and sulfurous aromas

- Improve consumer acceptability

2) Secondary hypothesis: whilst human bitter taste receptor genotype will influence bitter taste perception, it is hypothesised that consumer liking of cabbage will be increased through variety selection and optimised processing condition, irrespective of genotype.

1.8.2 Objectives of the study

To examine these hypothesis, the research objectives have been set out in 5 chapters:

- Chapter 2: Effect of growing conditions, cabbage variety and cooking methods on myrosinase activity and stability of cabbage (Hypothesis 1)
- Chapter 3: The influence of growing conditions and cabbage (*Brassica oleracea*) variety on glucosinolates and their hydrolysis products (Hypothesis 1)
- Chapter 4: The impact of domestic cooking methods on glucosinolates and their hydrolysis products in different cabbage (*Brassica oleracea*) varieties (Hypothesis 1)
- Chapter 5: The effects of variety and cooking method on phytochemical and volatile composition of black kale (*Brassica oleracea* var. *acephala*) subsequent sensory profile and acceptability by consumers varying in bitter taste sensitivity (Hypothesis 1 and 2)
- Chapter 6: The effects of cultivar and cooking method on phytochemical and volatile composition of red cabbage (*Brassica oleracea* var. *capitata* f. *rubra*) and subsequent sensory profile and acceptability by consumers varying in bitter taste sensitivity (Hypothesis 1 and 2)

Chapter 2: The effects of growing conditions, variety and domestic cooking methods on the activity and stability of cabbage (*Brassica oleracea*)

Status: This chapter has been written in the style of a research paper and will be submitted to Food Chemistry.

Abstract

Myrosinase enzyme, present in *Brassica* vegetables, is responsible for the hydrolysis of glucosinolates to yield health promoting isothiocyanate compounds. The effects of growth conditions, domestic cooking methods and different cabbage accessions/varieties on myrosinase activity were investigated. 18 cabbage accessions made up of six different types of cabbages were selected and planted under identical growing conditions. Cabbages were steamed, microwaved and stir-fried before analysis of the activity and stability of the extracted myrosinase. Myrosinase activity was analysed using the coupled enzyme method. Growing conditions, thermal processing and cabbage accession all significantly affected myrosinase activity; between and within different cabbage types. One savoy cabbage accession (SC-PW) had the highest myrosinase activity (154.8 U/g DW) but was the least stable, while black kale and red cabbage accessions had the most stable myrosinase across all domestic cooking methods. Steaming and microwaving resulted in over 90 % loss of myrosinase activity in some accessions. Myrosinase was most stable after stir-frying with up to 65 % activity left. The results obtained help provide information on the optimum cooking methods for cabbage in order to enhance beneficial isothiocyanate production.

2.1 Introduction

Cabbage (*Brassica oleracea*) belongs to the *Brassicaceae* family and comprises of different types, such as red, white and savoy cabbage. Epidemiological studies have shown that the consumption of *Brassica* vegetables reduces the risks of cardiovascular diseases, cancer (Herr & Buchler, 2010) and is more recently reported to have a cytoprotective effect against tissue damage associated with oxidative stress as well as antimicrobial activity against bacterial and fungal pathogens (Verkerk et al., 2001; Guerrero-Beltran et al., 2012).

Brassica vegetables are unique in comparison to other vegetables because they contain a group of thioglucosides called glucosinolates. These glucosinolates when hydrolysed by an endogenous enzyme, myrosinase, yields various hydrolysis products some of which are responsible for the health promoting characteristics of *Brassica*s (Mithen et al., 2000). Glucosinolates (GLSs) and myrosinase enzymes coexist in separate compartments in the plants; while glucosinolates exists in the vacuoles of various cells (Mithen, 2001), myrosinase enzymes are localised inside the myrosin cells. Glucosinolates are hydrolysed by myrosinase upon plant tissue damage as a result of processing, plant injury, chewing etc (Bones & Rossiter, 1996).

Alternatively, glucosinolate hydrolysis can occur as a result of microbial activities in the gastrointestinal tract of humans. However, despite the ability of microorganisms in the human gut to hydrolyse glucosinolates, the conversion is three times less efficient when compared to glucosinolate hydrolysis by myrosinase (Conaway et al., 2000). It is therefore important to ensure that myrosinase enzyme remains active during consumption of *Brassica* vegetables. Myrosinase activity is affected by plant growth conditions (Wei et al., 2011) and by reaction conditions including pH, temperature and metal ions; with the optimal pH and temperature of myrosinase activity varying among plant species (Travers-Martin et al., 2008).

Cabbages, like other *Brassica* vegetables, are mostly subjected to some form of thermal processing/cooking before consumption. Several studies have shown that myrosinase is inactivated during thermal processing, or domestic cooking, of cabbage leading to decreased production of beneficial hydrolytic compounds (Verkerk & Dekker, 2004; Oerlemans et al., 2006; Rungapamestry et al., 2006; Ghawi et al., 2012). However, some of these studies processed the cabbages for much longer time- temperature combinations than would normally be applicable during domestic cooking of cabbage. Furthermore, some of these studies focused on particular cabbage varieties types (mostly red and white cabbage) and on crude myrosinase extracts or cabbage juice.

In this study, 18 cabbage accessions across six different cabbage types were selected from a genetic resources unit and grown under two different conditions. Activity and stability of the myrosinase from the cabbage accessions was analysed using simulated common domestic cooking methods. It was hypothesised that growth conditions, cabbage type and variety would all affect myrosinase activity. It was also hypothesised that through controlled thermal processing, myrosinase activity and stability would be enhanced, hence improving the health benefits associated with cabbage consumption.

2.2 Materials and methods

2.2.1 Plant material

Cabbage accessions (variety) were selected from the University of Warwick Crop Centre Genetic Resources Unit (Wellesbourne, UK). 18 cabbage accessions comprising of six cabbage types (red, savoy, white, black kale, wild and tronchuda) were used for the experiment. Cabbages were selected based on their geographical origin, whether or not they were of hybrid descent, and morphology of head formation (closed heart or open leaf) as shown in Table 2.1 and Appendix VI.

Cabbages were grown from 7th March to 25th November 2014 in the plant growth facilities, Whiteknights campus of the University of Reading, UK. Fifteen (15) biological replicates of each variety were germinated in seedling trays using potting compost under controlled environmental conditions (Saxcil cabinets). A 16-hour photo period was used (16 hours light, 8 hours dark); humidity was 60 % day with day and night temperatures 22 ^{o}C and 16 ^{o}C respectively. Seedlings were allowed to grow in seedling trays until the appearance of 3-4 true leaves, before being transplanted to individual 2.5 L pots containing loam-based compost (7th – 8th May 2014) and left to grow in the glasshouse (minimum night temperature 13 ^{o}C). After 50 days (26th - 27th June 2014), five replicates of each accession were transplanted to larger pots (10 L) containing loam-based compost and allowed to grow till commercial maturity in the glasshouse while seven replicates of each accession were transplanted to the field and allowed to grow to commercial maturity. On the field, each accession was planted on 7 metre beds with 0.6 metre between plants and rows. Both glasshouse and field cabbages were fertilized twice weekly with nitrogen phosphate potassium (NPK) (100 kg/ha N, 100 kg/ha P and 200 kg/ha K) fertilizer. Insecticides and pesticides were sprayed before and after planting to prevent insect attack. Standard agricultural practices were employed in the cultivation of the

cabbages. Cabbages were grown between 7th March to 25th November 2014 in the plant growth facilities, Whiteknights campus of the University of Reading, UK.

Cabbages were harvested over a period of two days upon reaching commercial maturity based on visual inspection. Though some varieties attained commercial maturity earlier than others, they were assumed stable on the field and were therefore left until all varieties were mature before harvesting. Harvested plants were placed on ice in freezer bags and immediately stored in a cold room at 4 ^{o}C for 24 hours before processing. Average weight of each field cabbage head per plant was 700 g (closed heart) and 300 g (open leaf) while the glasshouse cabbages were smaller (400 g for closed heart and 250 g for open leaf cabbages). Climatic data for both growing conditions is presented in Appendix IV (Table S2a).

2.2.2 Reagents and chemicals

Sinigrin standard was purchased from Santa Cruz (Germany) and D-glucose determination kit from Boehringer Mannheim (Germany). All other chemicals used were purchased from Sigma–Aldrich (UK)

2.2.3 Cabbage thermal processing

The outer leaves and central core of 4 -5 cabbage heads (biological replicates) were removed and discarded in order to remove older leaves and achieve a representative sample. Cabbages were chopped into pieces of approximately 1 cm in width using a kitchen knife (representing how cabbages would normally be sliced by consumers), mixed together, washed under running tap water and excess water drained using a salad spinner (OXO Good Grips Clear Manual Salad Spinner). Cabbages were subjected to steaming, microwave or stir-fry cooking. Unprocessed (raw) cabbage samples were used as controls. Cooking methods were chosen to represent common ways of cooking cabbage. Time and temperature combinations used for each method was based on preliminary consumer study with 60 participants to determine consumer acceptability of the samples as steamed, microwaved and stirfried cabbage (data not shown). These conditions were deemed acceptable with a mean score of between 2.7 to 3.3 on a 5-point degree of cooking scale, where “3” would represent ‘just about right’. Only field grown cabbages were processed.

Table 2.1: Origin, botanical and common names of cabbage accessions planted

Genus/Type	Accession name	Common name	Origin	Head formation
Black kale				
Brassica oleracea var. *acephala*	BK-CNDTP	Fodder black kale	Italy	Open leaf
Brassica oleracea var. *acephala*	BK-CPNT	Black kale	Italy	Open leaf
Brassica oleracea var. *acephala*	BK-CNDTT	Fodder black kale	Italy	Open leaf
Wild cabbage				
Brassica oleracea	WLD-8707	Wild cabbage	Great Britain	Open leaf
Brassica oleracea	WLD-GRU	Wild cabbage	New Zealand	Open leaf
Brassica oleracea	WLD-8714	Wild cabbage	Great Britain	Open leaf
Tronchuda cabbage				
Brassica oleracea var. *tronchuda*	TC-PCM	Tronchuda cabbage	Portugal	Open leaf
Brassica oleracea var. *tronchuda*	TC-CPDP	Tronchuda cabbage	Portugal	Open leaf
Brassica oleracea var. *tronchuda*	TC-T	Tronchuda cabbage	Portugal	Open leaf
Savoy cabbage				
Brassica oleracea var. *capitata*	SC-HSC	Hybrid savoy cabbage	Great Britain	Closed heart
Brassica oleracea var. *capitata*	SC-PW	Savoy cabbage	Great Britain	Closed heart
Brassica oleracea var. *capitata*	SC-SDG	Savoy cabbage	Italy	Closed heart
Red cabbage				
Brassica oleracea var. *capitata*	RC-RL	Red cabbage	Great Britain	Closed heart
Brassica oleracea var. *capitata*	RC-RM	Hybrid red cabbage	Great Britain	Closed heart
Brassica oleracea var. *capitata*	RC-RD	Red cabbage	Netherlands	Closed heart
White cabbage				
Brassica oleracea var. *capitata*	WC-FEM	White spring cabbage	Great Britain	Closed heart
Brassica oleracea var. *capitata*	WC-CRB	White cabbage	Portugal	Closed heart
Brassica oleracea var. *capitata*	WC-DLI	Hybrid white cabbage	Great Britain	Closed heart

Key: BK-CNDTP: Cavolo nero di toscana o senza palla; BK-CPNT: Cavolo palmizio; BK-CNDTT: Cavolo nero di toscana o senza testa; WLD-8707: Wild cabbage; WLD-GRU: Wild cabbage; WLD-8714: Wild cabbage; TC-PCM: Penca mistura; TC-CPDP: Penca povoa; TC-T: Tronchuda; SC-HSC: Hybrid savoy wirosa cabbage; SC-PW: Pointed winter; SC-SDG: Dark green; RC-RL: Red langendijker; RC-RM: Rocco marner (Hybrid); RC-RD: Red Danish; WC-FEM: Early market; WC-CRB: Couve repolho; WC-DLI: De louviers.

2.2.3.1 Steaming

The method of Rungapamestry et al. (2006) was adopted with slight modifications. 120 g cabbage was placed in the topmost layer of a 3 tier 18 cm stainless steel steamer (Kitchen craft, Birmingham UK) containing already boiling water (in the lowest layer) and allowed to steam for 2 min. Core temperature of cabbage during steaming ranged between 75 – 80 °C and was measured using a temperature probe.

2.2.3.2 Microwaving

The method described by Rungapamestry et al. (2006) was adopted. 120 g of cabbage was put into 1-pint Pyrex glass jug, 16 mL water was added and the jug covered with a PVC cooking film pierced with 9 holes. Cabbages were microwaved for 3 minutes. Microwaving was carried out using a 900 W microwave at 60 % power output (SANYO microwave oven EM-S355AW/AS, Japan). A microwave thermometer was used to measure the core temperature of the cabbage during processing. Core temperature during processing ranged between 88 – 95 °C.

2.2.3.3 Stir-fry

Cabbage samples were stir-fried as described by Rungapamestry et al. (2008b) with modifications. 120 g cabbage was stir-fried in a frying pan for 90 seconds in 5mL of preheated olive oil (100 °C) (Asda, UK) with continuous stirring using a wooden spatula. Core temperature of cabbage during stir-frying ranged between 65 – 70 °C and was measured using a temperature probe.

Samples were put into sterile sterilin tubes immediately after cooking, placed on ice and transferred to a -80 °C freezer. Frozen samples were freeze-dried (Stokes freeze drier, Philadelphia USA), ground using a tissue grinder (Thomas Wiley® Mini-Mill, Thomas Scientific, USA) and stored at -20 °C.

2.2.4 Myrosinase enzyme extraction

Myrosinase enzyme was extracted using the method described by Ghawi et al. (2012). 0.1 g sample was suspended in 0.15 g polyvinylpolypyrrolidone (PVPP) and 10 mL of Tris- HCL buffer (200mM, pH 7.5) containing 0.5 mM ethylenediaminetetracetic acid (EDTA) and 1.5 mM dithiothreitol (DTT). The mixture was stirred for 15 mins at 5 °C and centrifuged at 15, 000 rpm for 15 mins at 5 °C. The final volume of supernatant was made up to 10 mL using the Tris- HCL buffer. 6.2 g ammonium sulfate was added to the supernatant to achieve 90 % saturation and

stirred at 5 °C for 30 min. The samples were then centrifuged at 17,500 rpm for 15 min at 5 °C. The resulting pellet was suspended in 2 mL Tris-HCl buffer (10mM, pH 7.5) and assayed for myrosinase activity.

2.2.5 Myrosinase enzyme assay

Myrosinase activity was measured using the coupled enzyme method described by Gatfield & Sand (1983) and Wilkinson et al. (1984) with slight modifications. The procedure depends on the glucose released from the reaction between myrosinase enzyme and the substrate (sinigrin). The mixture for the reaction consisted of 0.9 mL of 5mM ascorbic acid, 0.5 mL ATP/ NADP+ solution, 10 µL hexokinase/ glucose-6-phosphate dehydrogenase and 50 µL crude enzyme extract. The mixture was homogenized, allowed to stand for 3 mins and 50 µL sinigrin substrate (0.6 M) added. The change in absorbance due to NADP formation was read on a spectrophotometer at 340 nm. Myrosinase enzyme activity was determined by taking the slope of the linear part of the curve of absorbance versus time of reaction. One unit of myrosinase activity is defined as the amount of enzyme that produces 1 µmol of glucose from sinigrin substrate per minute at pH 7.5.

2.2.6 Protein assay

Protein content was measured using the Bradford method (Bradford, 1976). The procedure is based on formation of a complex between dye (brilliant Blue G, Sigma- Aldrich) and the protein present in the sample and absorbance read at 595 nm using a spectrophotometer. 50 µL crude enzyme extract was added to 1.5 mL of concentrated dye reagent, vortexed and allowed to stand for 20 minutes before taking the absorbance reading. Bovine serum albumin (BSA), (Sigma- Aldrich, UK) was used to construct a standard curve and the protein concentration of sample calculated from the standard curve obtained. Protein content was used to calculate the specific activity of myrosinase enzyme (U/mg protein).

2.2.7 Statistical analysis

Results are the averages of three processing replicates and two analytical replicates (n= 6). Data obtained were analysed using 2- way ANOVA and Turkey's HSD multiple pair wise comparison test performed in XLSTAT (Addinsoft, Paris, France).

2.3 Results and discussion

2.3.1 Effect of growing conditions on myrosinase activity

Myrosinase activity of cabbages grown on the field and in a glass house is as shown in Figure 2.1 with the ANOVA table showing significant differences presented in Table 2.2. Out of the 18 accessions planted, WC-DLI (white cabbage) did not survive either on the field or in the glasshouse while RC-RM (red cabbage) and SC-SDG (savoy cabbage) did not grow in the glass house. Myrosinase activity ranged from 12.2 U/g DW (BK-CPNT) to 127.4 U/g DW (SC-PW) in glasshouse samples and from 31.5 U/g DW (BK-CPNT and RC-RL) to 154.8 U/g DW (SC-PW) in field samples. Growing condition, cabbage type, cabbage accession and the interactions between these parameters significantly ($p<0.0001$) affected myrosinase activity (Table 2.2). There were significant differences in the myrosinase activity of field and glass house grown cabbages across most of the accessions studied. Field grown cabbages had significantly higher myrosinase activity than glass house cabbages. Myrosinase activity of TC-PCM, RC-RL, WC-FEM and WC-CRB accessions did not differ significantly between field and glasshouse grown cabbages. Authors have previously reported that growing/environmental conditions affect myrosinase activity (Charron & Sams, 2004; Charron et al., 2005a; Penas et al., 2011; Wei et al., 2011) and results obtained from this study agree with their reports. The lower myrosinase activity of glasshouse cabbages might have been due to higher growth temperatures than those grown on the field. Minimum and maximum glasshouse temperatures were 14 and 43 °C respectively while minimum and maximum field temperatures were 6 and 24 °C respectively (Appendix IV; Table S2a). Penas et al. (2011) in their study of cabbages grown in different parts of Spain reported that myrosinase activity was lower in cabbages grown in eastern Spain that were exposed to a higher growing temperature when compared to those grown in northern Spain with lower growing temperature.

Another possible reason for significantly lower enzyme activity in glasshouse cabbages could be due to stress factors during growth. Glasshouse cabbages were grown in pots which may have reduced the amounts of nutrients available to them and limited space available for their roots to spread, leading to plant stress. The Hirai et al. (2004) found that under nitrogen and/or sulfur limiting growth conditions genes encoding myrosinase enzyme synthesis were down-regulated in *Arabidopsis* in order to facilitate storage of these elements in the form of glucosinolates in the leaf tissue. Yuan et al. (2010) showed that salt stress reduced myrosinase activity in radish sprouts. Cabbage grown in the glasshouse achieved a lower above ground

biomass than the field grown ones, indicating some form of stress. This was also evident in the differences in size of the closed heart cabbage heads, with the glasshouse plants having smaller heads than the field plants as reported in section 2.2.1. Accessions that did not show significantly different myrosinase activity between the two growing environments might have been able to tolerate the glasshouse conditions while accessions that did not survive in the glasshouse may have found the conditions too harsh.

The result of this study shows that cabbages differ in their requirements for growth and it is important to plant cabbage accessions in growing conditions that are best suited for their maximum development.

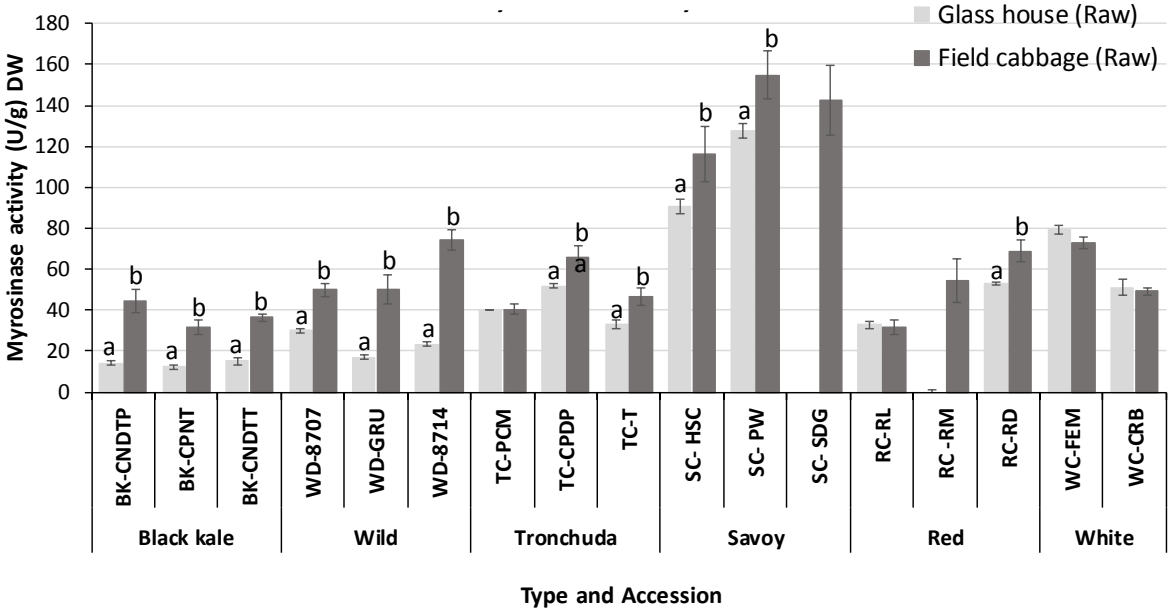


Figure 2.1. Myrosinase activity of field and glass house grown cabbage.
Values are means of three processing replicates and two technical replicates (n=6). Error bars represent standard deviation from the mean. Data points missing implies cabbage variety that did not grow under that condition. Differing letters at the top of each bar indicates significant differences ($P < 0.0001$) within accessions between the two growing conditions. Absence of letters indicates no significant differences were observed.
Key: BK-CNDTP: Cavolo nero di toscana o senza palla, BK-CPNT: Cavolo palmizio, BK-CNDTT: Cavolo nero di toscana o senza testa, WD-8707: Wild cabbage 8707, WD-GRU:Wild cabbage 7338, WD-8714:Wild cabbge 8714, TC-PCM: Penca mistura, TC-CPDP: Penca povoa, TC-T: Tronchuda, SC-HSC: Hybrid savoy wirosa cabbage, SC-PW: Pointed winter, SC-SDG: Dark green, RC-RL: red langendijker, RC-RM: Rocco marner (Hybrid), RC-RD: Red danish WC-FEM: Early market, WC-CRB: Couve repolho, WC-DLI: De louviers.

Table 2.2: ANOVA table showing the influence of cabbage type, accession and growing conditions on myrosinase activity of cabbage

Source	DF	Sum of squares	Mean squares	F	Significance (Pr > F)
Cabbage type	5	189452	37890	229	< 0.0001
Cabbage accession	16	212061	13254	416	< 0.0001
Growing condition	1	13675	13675	429	< 0.0001
Cabbage type*Growing condition	5	7836	1567	9	< 0.0001
Cabbage accession*Growing condition	14	9963	712	22	< 0.0001

*Indicates an interaction tested between two factors

2.3.2 Effect of domestic cooking on residual myrosinase enzyme activity

Myrosinase stability of cabbage accessions after domestic cooking was studied and the result is presented in Table 2.4. Domestic cooking affected the stability of myrosinase enzyme. Relative activity is defined as the ratio of myrosinase activity of processed cabbage to unprocessed (raw) cabbage (A/A_0). Myrosinase stability differed significantly ($p<0.0001$) between domestic cooking processes. Myrosinase stability after steaming and microwaving was not significantly different from each other, but differed significantly from stir-frying. Myrosinase was most stable after stir-frying, retaining up to 65 % (i.e. $A/A_0 = 0.65$) of its activity in some accessions studied. Steaming and microwaving resulted in loss of myrosinase activity of up to 98 and 99 % respectively with highest stability of 15 and 13 % respectively. Rungapamestry et al. (2008b), in their study of broccoli florets reported that stir-frying retained the highest myrosinase activity (17 %) compared to boiling (14 %).

The effect of domestic cooking processes on myrosinase stability varied among cabbage accessions and cabbage types and will be discussed in detail later. The stability of myrosinase in different *Brassica* vegetables and under different processing conditions has been discussed by several authors (Yen & Wei, 1993; Matusheski et al., 2004; Verkerk & Dekker, 2004; Rungapamestry et al., 2006; Rungapamestry et al., 2008b; Ghawi et al., 2012). Differences in myrosinase stability on cooking can be attributed to the maximum core temperature of the vegetable during heating. In our study stir-fry had the lowest core temperature (65 – 70 °C) compared to steaming (75 – 80 °C) and microwave (88 – 95 °C). Core temperatures during cooking of cabbage required to prevent myrosinase inactivation was reported to be between 50 and 60 °C which can be achieved by steaming for 7 min and microwaving (700 W) for 120 sec (Rungapamestry et al., 2006). However, in their study, cabbage was cut into wedges, which is not representative of how cabbages are generally prepared before cooking. Verkerk & Dekker

(2004) reported that inactivation of myrosinase enzyme during microwave cooking is affected by the time- energy output combination. Their study showed that a considerable amount of myrosinase activity was retained when red cabbage was microwaved at 180 W for 24 min and 540 W for 8 min; while microwaving for 4.8 min at 900 W resulted in total loss of myrosinase activity despite the fact that the total energy output of all three processes was the same (259.2 KJ). The authors explained the resulting effect as a function of the time it takes for the cabbage to reach its maximum core temperature with the higher energy output and shorter time reaching a high (100 $^{\circ}$C) core temperature faster and maintaining that core temperature for the remaining cooking time, while the lower energy output with longer cooking time resulted in a maximum core temperature of 90 $^{\circ}$C at a much slower rate.

Physical examination of the cooked cabbage samples showed that the stir-fried cabbage looked firmer than steamed and microwaved cabbage, and can also be used to assess the severity of the thermal process. The intense heat during stir-frying can lead to drying out of the surface area thereby resulting in a firmer texture, which reduces the rate of heat penetration as a result of less damage to the cell wall (Adler-Nissen, 2002; Rungapamestry et al., 2008b)

It is important to determine myrosinase stability after processing or cooking as inactivation of myrosinase results in a decrease in formation of beneficial hydrolysis products.

2.3.3 Effect of domestic cooking and cabbage accession on myrosinase enzyme activity and stability of field grown cabbages.

Figure 2.2 and Table 2.5 show the myrosinase activity and thermal stability of 17 cabbage accessions studied. Significant differences ($p<0.0001$) were observed in myrosinase activity and stability of field grown cabbages as a result of cabbage type, cabbage variety, cooking method, and the interactions between parameters (Table 2.3). Myrosinase activity within cabbage types was significantly different for most of the cabbages studied. For example, myrosinase activity for the three red cabbage accessions studied differed significantly between one another, while black kale accessions did not differ significantly in their myrosinase activity. This agrees with previous reports that myrosinase activity varies within varieties and plant species (Travers-Martin et al., 2008). Singh et al. (2007) and Penas et al. (2011) also reported variations in myrosinase activity of different cabbage varieties within and between cabbage types.

There was no relationship between myrosinase activity and myrosinase stability; indeed, some accessions which had high activity had the lowest stability. Savoy cabbage accessions had the highest myrosinase activity in all accessions studied (116.3, 142.6 and 154.8

U/g DW) while black kale accessions had the lowest myrosinase activity (31.5, 36.3 and 44.4 U/g DW). However, black kale, tronchuda and red cabbage had the highest enzyme stability, while savoy and white cabbage, which had the highest myrosinase activity, were the least stable after domestic processing. As discussed earlier, steaming and microwaving resulted in lower myrosinase stability overall with up to 99 % inactivation occurring in some cases. However, a critical look at the stability of myrosinase in steamed and microwaved cabbages (Figure 2.2) shows that some accessions had relatively higher myrosinase stability when compared to others. Red cabbage accessions RC-RM and RC-RL were the most stable retaining up to 15 % after steaming (RC-RM) and 13 % after microwaving (RC-RL). The result obtained is in agreement with the result of Yen & Wei (1993) who stated that red cabbage myrosinase was more stable than white cabbage myrosinase after thermal processing.

A possible reason for the difference in myrosinase stability across accessions might be due to difference in myrosinase isoenzymes found in each accession with the red cabbage accessions having more thermally stable myrosinase isoenzyme. Rask et al. (2000) reported that different isoforms of myrosinase existed and some of these isoforms form complexes by interacting with myrosinase- binding proteins and, hence, may enhance their stability.

Myrosinase activity values obtained in this study were higher in most cases than those reported by other authors (Charron & Sams, 2004; Rungapamestry et al., 2006), except in the case of white cabbage accessions where values were similar to those obtained by Penas et al. (2011). This might be because in most previous studies, cabbages were obtained from the supermarkets, while in this study and the study conducted by Penas et al. (2011) the cabbages were grown for the experiment and transferred into cold conditions immediately after harvest. Such minimal transfer and storage time reduces postharvest effects unlike in the supermarket samples.

There was no relationship between accession origin, physical characteristics (open leaf or heart forming) and whether hybrid or not on myrosinase activity and stability.

Table 2.3: ANOVA table showing the influence of cabbage type, accession and domestic cooking methods on cabbage myrosinase activity

Source	DF	Sum of squares	Mean squares	F	Pr > F
Cabbage type	5	25310	5062	83	< 0.0001
Cabbage accession	16	30038	1877	117	< 0.0001
Cooking	3	275334	91778	5703	< 0.0001
Cabbage type*Cooking	15	94394	6293	103	< 0.0001
Cabbage type*Cooking	48	107599	2242	139	< 0.0001

Table 2.4: Relative activity (A/A_0±SD) of myrosinase after domestic cooking of field grown cabbage

		Relative activity (A/A_0)		
		Treatment		
Type	Accession	Steamed	Microwaved	Stir-fried
Black kale	BK-CNDTP	0.05±0.01^{ab}	0.05±0.01^{ab}	0.65±0.014^{l}
	K-CPNT	0.11±0.014^{a-e}	0.11±0.05^{a-e}	0.52±0.11^{k}
	BK-CNDTT	0.08±0.05^{a-e}	0.09±0.05^{a-e}	0.56±0.13^{kl}
Wild	WD-8707	0.05±0.02^{ab}	0.05±<0.01^{ab}	0.41±0.03^{hij}
	WD-GRU	0.08±0.03^{a-d}	0.05±0.01^{ab}	0.46±0.12^{h-k}
	WD-8714	0.03±<0.01^{ab}	0.03±<0.01^{ab}	0.38±0.03^{hi}
Tronchuda	TC-PCM	0.08±0.03^{a-e}	0.08±0.03^{a-e}	0.34±0.06^{gh}
	TC-CPDP	0.08±0.03^{a-d}	0.07±0.02^{a-d}	0.38±0.05^{ghi}
	TC-T	0.07±0.03^{a-d}	0.06±0.02^{a-d}	0.20±0.05^{ef}
Savoy	SC- HSC	0.04±0.01^{ab}	0.02±0.01^{a}	0.19±0.03^{def}
	SC- PW	0.02±0.01^{a}	0.01±<0.01^{a}	0.03±<0.01^{ab}
	SC- SDG	0.02±0.01^{a}	0.02±0.01^{a}	0.09±0.01^{a-e}
Red	RC-RL	0.13±0.04^{a-e}	0.13±0.04^{a-e}	0.49±0.18^{ijk}
	RC -RM	0.15±0.03^{b-f}	0.10±0.04^{a-e}	0.37±0.08^{ghi}
	RC-RD	0.04±0.01^{ab}	0.05±0.02^{ab}	0.26±0.02^{fg}
White	WC-FEM	0.04±0.02^{ab}	0.05±0.02^{ab}	0.09±0.02^{a-e}
	WC-CRB	0.06±0.03^{abc}	0.05±0.02^{ab}	0.18±0.03^{c-f}

Values are means of three processing replicates and two analytical replicates (n=6) ± standard deviation (SD). Values not sharing similar letters are significantly different ($p \leq 0.0001$) between accessions and treatment. A/A_0 = residual activity, defined as the ratio of myrosinase activity of processed cabbage to unprocessed (raw) cabbage. Key: BK-CNDTP: Cavolo nero di toscana o senza palla, BK-CPNT: Cavolo palmizio, BK-CNDTT: Cavolo nero di toscana o senza testa, WD-8707: Wild cabbage 8707, WD-GRU: Wild cabbage 7338, WD-8714: Wild cabbge 8714, TC-PCM: Penca mistura, TC-CPDP: Penca povoa, TC-T: Tronchuda, SC-HSC: Hybrid savoy wirosa cabbage, SC-PW: Pointed winter, SC-SDG: Dark green, RC-RL: red langendijker, RC-RM: Rocco marner (Hybrid), RC-RD: Red danish WC-FEM: Early market, WC-CRB: Couve repolho, WC-DLI: De louviers

2.3.4 Protein content and specific activity of cabbage

The protein content and specific activity of myrosinase for all accessions and cooking methods is presented in Table 2.5. There were significant differences in the protein content and specific activity of all accessions both cooked and raw. Protein content decreased with processing, with the rate of reduction corresponding to the severity of the cooking process. This can be attributed to denaturation of protein into free amino acids during cooking. Protein content of untreated samples did not correspond with myrosinase activity. Savoy and white cabbage accessions which had the highest myrosinase activity had the lowest protein contents. Just like myrosinase activity, protein content of glasshouse samples was significantly lower than the raw field samples. This might be as a result of plant stress during growth which prevents the plant from producing more nutrients than required or using up its stored nutrients in order to survive. Plant proteins have been reported to react to environmental stress (Charron & Sams, 2004). Results obtained are in agreement with Rosa & Heaney (1996) who reported higher protein contents in Portuguese cabbage grown in lower environmental temperatures compared to those grown in higher temperatures.

Specific activity of the cabbages was similar to myrosinase activity, with field grown cabbages generally having higher specific activity than the glasshouse cabbages. Savoy and white cabbage accessions had significantly higher specific activities than other cabbage types. White cabbage has previously been reported to have higher specific activity than red cabbage (Yen & Wei, 1993), which is in agreement with the results of this study. However, a study conducted by Singh et al. (2007) showed red cabbage with a higher specific activity than white and savoy cabbage. This might have been due to the differences in varieties studied or protein content of the cabbages, which was not reported in their study.

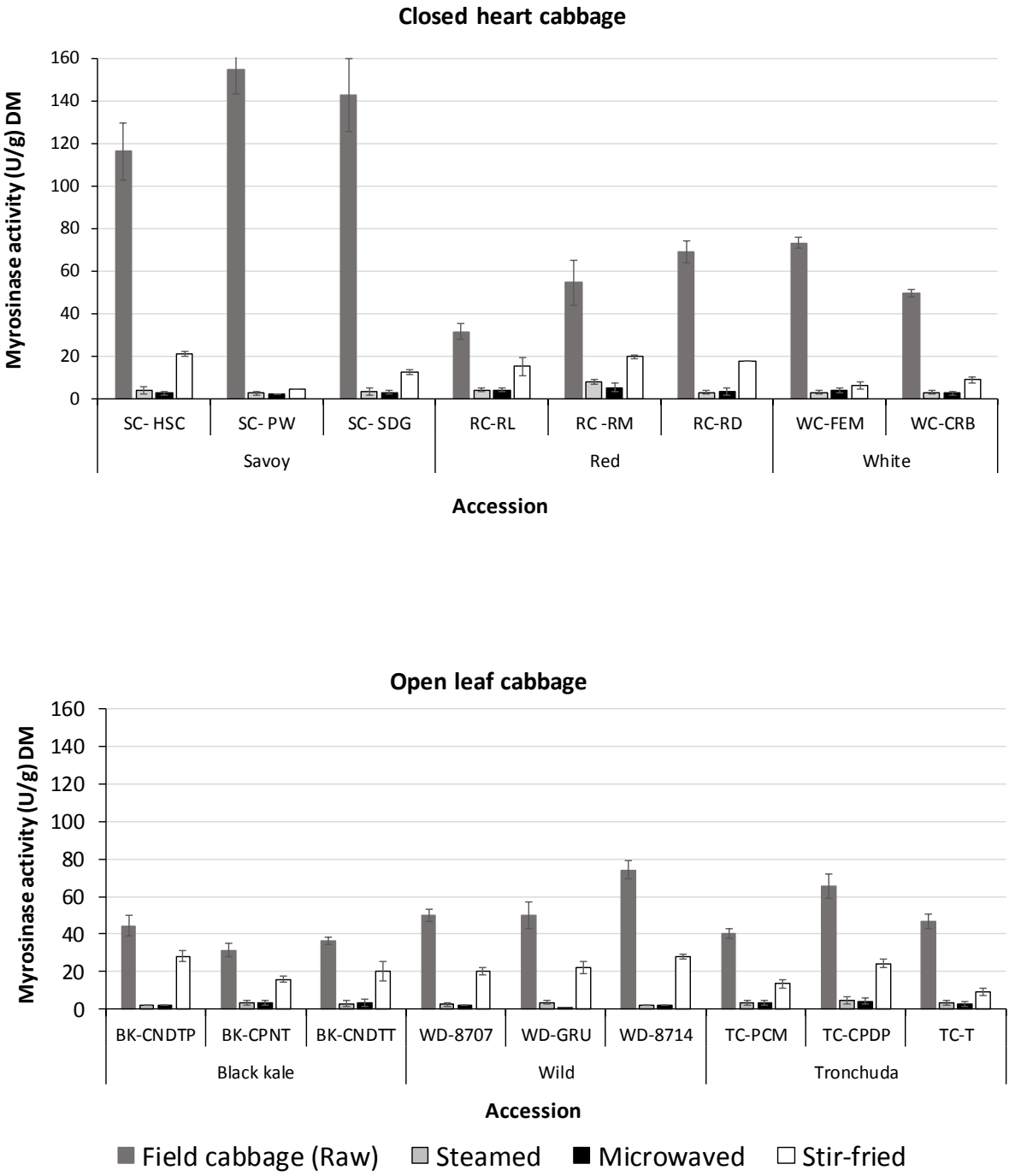


Figure 2.2. Effect of domestic cooking and cabbage accession on myrosinase activity and stability of field grown cabbage (U/g DW)

Values are means of three processing replicates and two technical replicates (n=6). Error bars represent standard deviation from the mean.

Key: SC-PW: Pointed winter, SC-SDG: Dark green, RC-RL: red langendijker, RC-RM: Rocco marner (Hybrid), RC-RD: Red danish WC-FEM: Early market, WC-CRB: Couve repolho, WC-DLI: De louviers, BK-CNDTP: Cavolo nero di toscana o senza palla, BK-CPNT: Cavolo palmizio, BK-CNDTT: Cavolo nero di toscana o senza testa, WD-8707: Wild cabbage 8707, WD-GRU:Wild cabbage 7338, WD-8714:Wild cabbge 8714, TC-PCM: Penca mistura, TC-CPDP: Penca povoa, TC-T: Tronchuda.

Table 2.5: Myrosinase activity ((U/g±SD) DW), protein content ((mg/g±SD) DW) and specific activity ((U/mg soluble protein±SD) DW) of cabbage accessions grown in either glasshouse or field and after domestic processing

Type	Accession	Treatment	*Myrosinase activity (U/g±SE) DW*	*Protein content (mg/g±SE) DW*	*Specific activity (U/mg soluble protein±SE) DW*
Black kale	BK-CNDTP	Glasshouse (Raw)	14.1±1.1^{-j}	29.1±0.4^{qrs}	0.5±0.1^{a-h}
		Field (Raw)	44.4±5.4^{s-v}	33.7±0.6^{vw}	1.3±0.1^{g-r}
		Steamed	2.2±<0.1^{a}	11.0±0.3^{abc}	0.2±<0.1^{a}
		Microwaved	2.2±<0.1^{a}	11.2±0.4^{abc}	0.2±<0.1^{a}
		Stir-fried	28.1±3.0^{m-q}	29.0±0.7^{qrs}	1.0±0.1^{a-o}
	BK-CPNT	Glasshouse (Raw)	12.2±1.2^{b-i}	24.5±0.1^{mn}	0.5±0.1^{a-h}
		Field (Raw)	31.5±3.6^{o-r}	35.4±1.0^{wx}	0.9±0.1^{a-o}
		Steamed	3.3±1.2^{ab}	11.7±0.6^{bc}	0.3±0.1^{abc}
		Microwaved	3.3±1.2^{ab}	11.9±1.4^{bc}	0.3±0.1^{abc}
		Stir-fried	15.9±1.7^{g-l}	21.6±1.9^{jkl}	0.7±0.1^{a-m}
	BK-CNDTT	Glasshouse (Raw)	15.2±1.7^{f-k}	25.4±3.9^{nop}	0.6±0.1^{a-j}
		Field (Raw)	36.3±1.8^{q-s}	36.7±0.7^{x}	1.0±0.1^{a-p}
		Steamed	3.0±1.6^{a}	12.7±0.1^{cd}	0.2±0.1^{a-c}
		Microwaved	3.3±1.9^{ab}	12.5±0.1^{bcd}	0.3±0.1^{a-c}
		Stir-fried	20.4±5.1^{i-m}	24.9±0.1^{mno}	0.8±0.1^{a-n}
Wild cabbage	WD-8707	Glasshouse (Raw)	30.0±1.2^{n-q}	29.9±0.8^{opq}	1.1±0.1^{b-p}
		Field (Raw)	50.0±3.1^{vw}	31.4±1.2^{s-v}	1.6±0.1^{m-u}
		Steamed	2.6±0.9^{a}	11.1±0.1^{abc}	0.2±0.1^{abc}
		Microwaved	2.2±0.0^{a}	10.9±0.1^{abc}	0.2±<0.1^{a}
		Stir-fried	20.4±1.7^{i-m}	27.4±0.4^{ghi}	1.1±0.1^{a-p}
	WD-GRU	Glasshouse (Raw)	17.0±1.1^{h-m}	25.3±0.1^{m-p}	0.7±<0.1^{a-k}
		Field (Raw)	50±7.0^{vw}	29.9±0.6^{rs}	1.7±0.1^{n-u}
		Steamed	3.7±1.1^{a-c}	10.7±0.4^{abc}	0.3±0.1^{a-e}
		Microwaved	0.5±0.0^{a}	10.6±0.1^{abc}	0.2±0.1^{ab}
		Stir-fried	22.2±3.4^{j-n}	18.1±1.1^{fgh}	1.2±0.1^{e-q}
	WD-8714	Glasshouse (Raw)	23.3±1.2^{k-o}	18.4±0.1^{gh}	1.3±0.1^{f-q}
		Field (Raw)	74.1±5.0^{xy}	30.6±0.8^{stu}	2.4±0.2^{tu}

		Steamed	2.2±0.0^{a}	10.9±0.1^{abc}	0.2±<0.1^{a}
		Microwaved	2.2±0.0^{a}	11.0±0.2^{abc}	0.2±<0.1^{a}
		Stir-fried	24.1±1.1^{m-q}	16.9±0.5^{efg}	1.7±0.1^{n-u}
	TC-PCM	Glasshouse (Raw)	40.0±0.0^{r-t}	32.8±0.1^{tuv}	1.2±<0.1^{e-q}
		Field (Raw)	40.4±2.6^{r-u}	33.6±0.2^{vw}	1.2±0.1^{d-p}
		Steamed	3.3±1.2^{ab}	11.1±0.3^{abc}	0.3±0.1^{a-c}
		Microwaved	3.3±1.2^{ab}	11.1±0.1^{abc}	0.3±0.1^{a-c}
		Stir-fried	13.7±2.2^{d-j}	19.9±1.52^{hij}	0.7±0.1^{a-l}
Tronchuda cabbage	TC-CPDP	Glasshouse (Raw)	51.9±1.1^{vw}	21.2±0.2^{ijk}	2.4±0.1^{u}
		Field (Raw)	65.6±6.2^{x}	27.8±0.6^{pqr}	2.4±0.2^{tu}
		Steamed	4.8±1.7^{abcd}	11.0±0.3^{abc}	0.4±0.2^{a-g}
		Microwaved	4.4±1.4^{abc}	11.0±0.3^{abc}	0.4±0.1^{a-f}
		Stir-fried	24.4±2.4^{l-p}	18.1±0.8^{fgh}	1.4±0.2^{h-s}
	TC-T	Glasshouse (Raw)	33.0±2.2^{pqr}	30.5±0.2st	1.1±0.1^{a-p}
		Field (Raw)	46.7±4.0^{t-w}	33.1±0.8^{uvw}	1.4±0.1^{i-s}
		Steamed	3.3±1.2^{ab}	10.9±0.2^{abc}	0.3±0.1^{a-c}
		Microwaved	3.0±1.1^{a}	10.8±0.2^{abc}	0.3±0.1^{a-c}
		Stir-fried	9.3±1.7^{a-h}	15.7±0.9^{ef}	0.6±0.1^{a-j}
	SC- HSC	Glasshouse (Raw)	90.7±3.8^{z}	24.5±0.9^{mn}	1.0±0.1^{v}
		Field (Raw)	116.3±13.4$^{a"}$	24.6±1.4^{mn}	4.7±0.3^{w}
		Steamed	4.1±1.7^{a-c}	10.7±0.4^{abc}	0.4±0.1^{a-e}
		Microwaved	2.6±0.9^{a}	10.6±0.3^{abc}	0.2±0.1^{a-c}
		Stir-fried	21.1±1.2^{-n}	12.0±1.1^{bc}	1.8±0.2^{o-u}
	SC- PW	Glasshouse (Raw)	127.4±3.6$^{b"}$	24.1±0.1^{lmn}	5.3±0.1^{wx}
Savoy cabbage		Field (Raw)	154.8±11.6$^{d"}$	24.3±0.3^{mn}	6.4±0.5^{y}
		Steamed	2.6±0.9^{a}	12.0±1.2^{bc}	0.2±0.1^{a-c}
		Microwaved	2.2±0.0^{a}	10.1±0.2^{ab}	0.2±0.1^{a-c}
		Stir-fried	4.4±0.0^{a-c}	14.8±0.3^{de}	0.3±0.1^{a-c}
	SC- SDG	Glasshouse (Raw)	DNG	DNG	DNG
		Field (Raw)	142.6±16.9$^{c"}$	24.4±0.5^{mn}	5.8±0.7^{xy}
		Steamed	3.3±1.9^{ab}	10.3±0.4^{abc}	0.3±0.2^{a-d}

		Microwaved	3.0±1.1^{a}	10.6±0.1^{abc}	1.5±0.1^{j-s}
		Stir-fried	12.6±1.1^{c-i}	11.4±0.3^{abc}	1.1±0.1^{c-p}
	RC-RL	Glasshouse (Raw)	33.0±1.7^{p-r}	21.0±0.5^{ijk}	1.6±0.1^{l-u}
		Field (Raw)	31.5±3.6^{o-r}	33.6±0.6^{vw}	0.9±0.1^{a-o}
		Steamed	4.1±0.9^{a-c}	11.0±0.3^{abc}	0.4±0.1^{a-e}
		Microwaved	4.1±0.9^{a-c}	11.2±0.4^{abc}	0.4±0.1^{a-e}
		Stir-fried	15.2±4.3^{f-k}	29.0±0.7^{qrs}	0.5±0.1^{a-i}
Red cabbage	RC -RM	Glasshouse (Raw)	DNG	DNG	DNG
		Field (Raw)	54.4±10.6^{w}	35.4±1.0^{wx}	1.5±0.3^{k-t}
		Steamed	7.8±1.2^{a-g}	11.7±0.6^{bc}	0.7±0.1^{a-k}
		Microwaved	5.2±1.8^{a-e}	11.9±1.4^{bc}	0.4±0.2^{a-g}
		Stir-fried	19.6±0.9^{i-m}	21.6±1.9^{jkl}	0.9±0.1^{a-o}
	RC-RD	Glasshouse (Raw)	53.0±0.9^{vw}	25.3±0.1^{nop}	2.1±0.1^{q-u}
		Field (Raw)	68.9±5.1^{x}	36.7±0.7^{x}	1.9±0.1^{p-u}
		Steamed	3.0±1.1^{a}	12.7±0.1^{cd}	0.2±0.1^{a-c}
		Microwaved	3.3±1.9^{ab}	12.5±0.1^{bcd}	0.3±0.1^{a-c}
		Stir-fried	17.8±0.0^{h-l}	24.9±3.9^{mno}	0.7±0.1^{a-m}
White cabbage	WC-FEM	Glasshouse (Raw)	79.3±2.3^{y}	21.2±0.9^{ijk}	3.8±0.2^{v}
		Field (Raw)	73.0±2.6^{xy}	21.3±0.4^{ijk}	3.4±0.2^{v}
		Steamed	3.0±1.1^{a}	10.1±0.2^{ab}	0.3±0.1^{a-c}
		Microwaved	3.7±1.1^{a-c}	10.1±0.1^{ab}	0.4±0.1^{a-e}
		Stir-fried	6.3±1.7^{a-f}	10.9±0.2^{abc}	0.6±0.2^{a-i}
	WC-CRB	Glasshouse (Raw)	51.1±3.7^{vw}	22.8±0.6^{klm}	2.2±0.2^{s-u}
		Field (Raw)	49.3±1.7^{u-w}	23.0±1.1^{k-n}	2.1±0.1^{r-u}
		Steamed	3.0±1.1^{a}	10.2±0.1^{ab}	0.3±0.1^{a-c}
		Microwaved	2.6±0.9^{a}	10.2±0.1^{a-c}	0.3±0.1^{a-c}
		Stir-fried	8.9±1.4^{a-h}	12.1±0.7^{bc}	0.7±0.2^{a-m}

Values are means of three processing replicates and two technical replicates (n=6±SD). SD: standard deviation from mean. Values not sharing the same superscript in the same column are significantly different (p≤0.05) from each other. DNS: Did not grow
Key: BK-CNDTP: Cavolo nero di toscana o senza palla, BK-CPNT: Cavolo palmizio, BK-CNDTT: Cavolo nero di toscana o senza testa, WD-8707: Wild cabbage 8707, WD-GRU:Wild cabbage 7338, WD-8714:Wild cabbge 8714, TC-PCM: Penca mistura, TC-CPDP: Penca povoa, TC-T: Tronchuda, SC-HSC: Hybrid savoy wirosa cabbage, SC-PW: Pointed winter, SC-SDG: Dark green, RC-RL: red langendijker, RC-RM: Rocco marner (Hybrid), RC-RD: Red danish WC-FEM: Early market, WC-CRB: Couve repolho, WC-DLI: De louviers

2.4 Conclusion

This study analysed the effect of domestic cooking methods, cabbage variety/accessions and growing conditions on myrosinase activity and stability. The study demonstrated that planting cabbages in high growth temperatures and stressful conditions resulted in lower myrosinase activity. Myrosinase activity differed between accessions and cabbage types. Savoy cabbage accessions had the highest myrosinase activity while black kale accessions had the lowest myrosinase activity.

Domestic cooking resulted in significant loss of myrosinase activity with stir-frying having the highest residual activity compared to the other two cooking methods. Microwave cooking was the most severe heat treatment resulting in the highest loss of myrosinase activity of up to 99 % in some cases. The result of this study showed that mild cooking prevents complete inactivation of myrosinase activity. Myrosinase enzyme stability was also significantly different in cabbage accessions between and within cabbage types. Black kale myrosinase was the most stable after stir-frying while red cabbage accessions were most stable after steaming and microwaving. Some of the limitations of the cooking protocol include reproducibility, differences in surface area of the cabbage cut and differences in stirring during stir-frying.

However, despite the significant loss of activity due to the cooking methods, our study of isothiocyanate formation in the cabbage accessions showed that thermal processing enhanced the health beneficial isothiocyanate production when compared to unprocessed cabbage (see Chapter 4). This implies that residual myrosinase activity was enough to initiate the glucosinolate hydrolysis process and could potentially have a positive impact on the intake of beneficial isothiocyantes from cabbage consumption. Mild cooking of cabbage might be helpful in improving uptake of isothiocyanates from cabbages.

Chapter 3: Influence of growing conditions and cabbage (*Brassica oleracea*) variety on glucosinolates and their hydrolysis products

Status: This chapter has been written in the style of a paper and will be submitted to Food Chemistry.

Abstract

Glucosinolates are secondary plant metabolites present in *Brassicas* such as cabbage. When hydrolysed, glucosinolates produce different hydrolysis products of which some have health promoting properties. The influence of growing conditions and cabbage variety on glucosinolates and their hydrolysis products of 18 gene-bank *B. oleracea* varieties was studied. Nine glucosinolates and 22 hydrolysis products were identified. Glucosinolate and glucosinolate hydrolysis profiles differed across varieties studied. Growing conditions, cabbage type and variety all significantly affected concentrations of glucosinolates and the hydrolysis products. The study highlights factors responsible for differences in concentration of glucosinolates and their hydrolysis products. Aliphatic glucosinolates accounted for more than 60 % of total glucosinolates in the samples. Nitriles and epithionitriles were the most abundant hydrolysis products formed. The results show that consumption of raw cabbages might reduce amounts of beneficial hydrolysis products available to the consumer as more nitriles were produced on hydrolysis compared to beneficial isothiocyanates. However, red and white cabbages secreted high concentrations of glucoraphanin and its isothiocyanate hydrolysis product, sulforaphane. This implies that careful selection of varieties high in certain glucosinolates can improve benefits derived from raw cabbage consumption.

3.1 Introduction

Glucosinolates (GSLs) are sulfur-containing secondary plant metabolites found in members of the *Brassicaceae* family. In plants, GSLs act as plant defense mechanisms against stress, insect and pest attack (Bjorkman et al., 2011). When plant tissue is disrupted, GSLs are hydrolysed by plant myrosinase enzyme resulting in the formation of various hydrolysis products such as isothiocyanates (ITCs), thiocyanates, nitriles and epithionitriles (Mithen et al., 2000). The type of glucosinolate hydrolysis products (GHPs) formed is dependent on the conditions under which the process takes place. ITCs, the primary products of GSL hydrolysis from myrosinase, are responsible for the well documented health-promoting properties of *Brassica* vegetables (Mithen et al., 2000). However, in the presence of epithiospecifier proteins (ESPs), nitriles and epithionitriles, which have not been shown to proffer any beneficial characteristics are formed (Lambrix et al., 2001). GSLs and ITCs are also partly responsible for the bitter taste and pungent aromas of *Brassica* vegetables (Baik et al., 2003).

There are several factors that affect the profile and concentration of GSLs in *Brassicas*; these factors include climatic factors, location and growing conditions, type and variety of plant (Rosa & Rodrigues, 1998). There are several studies on the formation GSLs and GHPs in cabbages, some of which focus on cabbages grown under different conditions - mostly focused on different locations or different seasons (Charron et al., 2005a; Velasco et al., 2007; Cartea et al., 2008; Ciska et al., 2000; Penas et al., 2011). However, none of the studies analysed the GHPs of the cabbages. Little is known of the GHPs in cabbages as most studies have focused on a specific cabbage variety (Daxenbichler et al., 1977) or have focused on specific ITCs in other *B. oleracea* such as broccoli (Van Eylen et al., 2009). A recent study analysed the GSLs and GHPs of cabbages, but its focus was on red, white and savoy cabbages only (Hanschen & Schreiner, 2017). However, to fully understand the health benefits that can be derived from cabbage consumption, there is a need to characterize the GHPs produced from GSL hydrolysis and the factors affecting the type and concentrations of GHPs formed.

The purpose of this study was to investigate the influence of growing conditions and variety on the GSL and GHP content of cabbage. 18 cabbage accessions/varieties comprising of black kale, wild, tronchuda, savoy, red and white cabbage types were selected from a genetic resources unit and grown under two different conditions. The primary hypothesis of the study was that cabbage growth conditions will affect GSL and GHP contents of cabbage. The secondary hypothesis was that cabbage type rather than variety will affect the profile and concentrations of GSLs and GHPs formed.

3.2 Materials and methods

3.2.1 Plant material

Cabbage accessions/varieties (n=18) comprising of six cabbage types (red, savoy, white, black kale, wild and tronchuda) were selected from the University of Warwick Crop Centre Genetic Resources Unit (Wellesbourne, UK) and grown between 7th March to 25th November 2014, in the plant growth facilities, Whiteknights campus of the University of Reading, UK. Detailed growing protocol is as previously described in Chapter 2. Table 3.1 shows the names and description of the varieties used for the study.

Cabbages were harvested upon reaching commercial maturity based on visual inspection, placed on ice in freezer bags and immediately stored in a cold room at 4 $^{\circ}$C for 24 hours before processing. Average weight of each field cabbage head per plant was 700 g (closed heart) and 300 g (open leaf) while the glasshouse cabbages were smaller (400 g for closed heart and 250 g for open leaf cabbages).

3.2.2 Reagents and chemicals

All chemicals used were purchased from Sigma–Aldrich (UK) unless stated otherwise.

3.2.3 Cabbage processing

The outer leaves and central core of 4-5 cabbage heads (biological replicates) were removed and discarded. Cabbages were chopped into pieces of approximately 1 cm in width using a kitchen knife (representing how cabbages would normally be sliced by consumers), mixed together, washed under running tap water and excess water drained using a salad spinner (OXO Good Grips Clear Manual Salad Spinner). 120 g cabbage samples were put into sterile sterilin tubes, immediately placed on ice and transferred to a -80 $^{\circ}$C freezer. Frozen samples were freeze-dried (Stokes freeze drier, Philadelphia USA), ground using a tissue grinder (Thomas Wiley® Mini-Mill, Thomas Scientific, USA) and stored at -20 $^{\circ}$C till further analysis.

Table 3.1: Origin, botanical and common names of cabbage accessions planted

Genus/Type	Accession name	Code	Common name	Origin	Head formation
Black kale					
Brassica oleracea var. *acephala*	BK-CNDTP	BK1	Fodder black kale	Italy	Open leaf
Brassica oleracea var. *acephala*	BK-CPNT	BK2	Black kale	Italy	Open leaf
Brassica oleracea var. *acephala*	BK-CNDTT	BK3	Fodder black kale	Italy	Open leaf
Wild cabbage					
Brassica oleracea	WD-8707	WD1	Wild cabbage	Great Britain	Open leaf
Brassica oleracea	WD-GRU	WD2	Wild cabbage	New Zealand	Open leaf
Brassica oleracea	WD-8714	WD3	Wild cabbage	Great Britain	Open leaf
Tronchuda cabbage					
Brassica oleracea var. *tronchuda*	TC-PCM	TC1	Tronchuda cabbage	Portugal	Open leaf
Brassica oleracea var. *tronchuda*	TC-CPDP	TC2	Tronchuda cabbage	Portugal	Open leaf
Brassica oleracea var. *tronchuda*	TC-T	TC3	Tronchuda cabbage	Portugal	Open leaf
Savoy cabbage					
Brassica oleracea var. *capitata*	SC-HSC	SC1	Hybrid savoy cabbage	Great Britain	Closed heart
Brassica oleracea var. *capitata*	SC-PW	SC2	Savoy cabbage	Great Britain	Closed heart
Brassica oleracea var. *capitata*	SC-SDG	SC3	Savoy cabbage	Italy	Closed heart
Red cabbage					
Brassica oleracea var. *capitata*	RC-RL	RC1	Red cabbage	Great Britain	Closed heart
Brassica oleracea var. *capitata*	RC-RM	RC2	Hybrid red cabbage	Great Britain	Closed heart
Brassica oleracea var. *capitata*	RC-RD	RC3	Red cabbage	Netherlands	Closed heart
White cabbage					
Brassica oleracea var. *capitata*	WC-FEM	WC1	White spring cabbage	Great Britain	Closed heart
Brassica oleracea var. *capitata*	WC-CRB	WC2	White cabbage	Portugal	Closed heart
Brassica oleracea var. *capitata*	WC-DLI	WC3	Hybrid white cabbage	Great Britain	Closed heart

Key: BK-CNDTP: Cavolo nero di toscana o senza palla; BK-CPNT: Cavolo palmizio; BK-CNDTT: Cavolo nero di toscana o senza testa; WLD-8707: Wild cabbage; WLD-GRU: Wild cabbage; WLD-8714: Wild cabbage; TC-PCM: Penca mistura; TC-CPDP: Penca povoa; TC-T: Tronchuda; SC-HSC: Hybrid savoy wirosa cabbage; SC-PW: Pointed winter; SC-SDG: Dark green; RC-RL: Red langendijker; RC-RM: Rocco marner (Hybrid); RC-RD: Red Danish; WC-FEM: Early market; WC-CRB: Couve repolho; WC-DLI: De louviers

3.2.4 Glucosinolate extraction

The method used for GSL extraction is as described by Bell et al. (2015) with modifications. Briefly, 40 mg ground cabbage powder was heated in a dry-block at 75 °C for two minutes. 1 mL 70 % (v/v) methanol preheated to 70 °C was added to each sample, vortexed and placed in a preheated (70 °C) water bath for 20 minutes. Samples were centrifuged for five minutes (6000 rpm, 18 °C) and supernatant collected in fresh Eppendorf tubes. The volume was adjusted to 1 mL with 70 % (v/v) methanol and frozen at -80 °C until analysis further analysis.

3.2.5 LC-MS^2 Analysis

Samples were filtered using 0.22 μm Millex syringe filters with a low protein binding Durapore polyvinylidene fluoride (PVDF) membrane (Fisher scientific, UK) and diluted with 9 mL HPLC-grade water. LC-MS analysis of GSL extracts was performed in negative ion mode on an Agilent 1200 Series LC system (Agilent, Stockport, UK) equipped with a variable wavelength detector and coupled to a Bruker HCT ion trap (Bruker, Coventry, UK). Sample separation was achieved on a Gemini 3 μm C_{18} 110 Å (150 x 4.6 mm) column (with Security Guard column, C_{18}; 4mm x 3mm; Phenomenex, Macclesfield, UK). GSLs were separated during a 40-minute chromatographic run, with 5-minute post-run sequence. Mobile phases consisted of ammonium formate (0.1 %) and acetonitrile with an isocratic gradient of 95 % and 5 % respectively. The flow rate was optimised for the system at 0.4 mL/min, with a column temperature of 30 °C, with 5 μl of sample injected into the system. GSLs were quantified at a wavelength of 229 nm.

MS analysis settings were as follows: electrospray ionization (ESI) was carried out at atmospheric pressure in negative ion mode (scan range m/z 100–1500 Da). Nebulizer pressure was set at 50 psi, gas-drying temperature at 350 °C, and capillary voltage at 2,000 V. GSLs were quantified using sinigrin hydrate standard. Five concentrations of sinigrin hydrate (0.22-3.5 mg/mL) was prepared with 70 % methanol and used to prepare an external calibration curve (r^2 = 0.942). Compounds were identified using their parent mass ion and characteristic ion fragments as well as comparing with literature ion data (Table 3.2). Compounds were quantified using Bruker Daltonics HyStar software (Bruker). Relative response factors (RRFs) were used in the calculation of GSL concentrations where available (Clarke, 2010). Where such data could not be found for intact GSLs, RRFs were assumed to be 1.0.

3.2.6 Extraction of glucosinolate hydrolysis products

Glucosinolate hydrolysis products (GHPs) were extracted and analysed following the method described by Bell et al. (2017c). 0.5 g of lyophilized cabbage was mixed with 10 mL deionized water, vortexed and allowed to incubate for three hours at 30 °C. The mixture was then centrifuged at 9500 rpm (18 °C) for ten minutes and the supernatant collected. The pellet was extracted two more times with 10 mL deionized water and the supernatants combined and filtered (0.45 µm syringe filters, Epsom, UK) into glass centrifuge tubes. GHPs were extracted by adding equal volume of dichloromethane (DCM) to the supernatant, vortexed for a minute and centrifuged at 3500 rpm for ten minutes. After centrifugation, the organic phase was collected and the extraction step repeated twice. The organic phase collected was combined, 2 g sodium sulphate salt added to remove any excess liquid present and filtered into a round-bottom flask. Filtrate was dried using a rotatory evaporator (37 °C), re-dissolved in 1 mL DCM and filtered (0.22 µm filter; Fisher scientific, UK) in GC-MS glass vials for GC-MC analysis.

3.2.7 GC-MS Analysis

GC–MS analysis was performed on an Agilent 7693/5975 GC–MS autosampler system (Agilent, Manchester, UK). The sample was injected onto a HP-5MS 15 m non-polar column DB-5MS (J and W scientific, USA) (0.25-µm film thickness, 0.25mm I.D.). The injection temperature was 250 °C in split mode (1:20). The oven temperature was programmed from 40 to 320 °C at a rate of 5 °C/min till 250 °C. The carrier gas was helium with flow rate of 1.1 mL/min and pressure of 7.1 psi. Mass spectra were obtained by electron ionization at 70 eV, and mass scan from 35 to 500 amu. 1 µl of sample was injected and compounds separated during a 42-min run. Compounds were identified using National Institute of Standards and Technology (NIST) library and from literature ion data (Table 3.3) and quantified based on an external standard calibration curve. Five concentrations (0.25–2 mg/mL) of sulforaphane standard (Sigma Aldrich, UK) were prepared in DCM (r^2 = 0.99). Data analysis was performed using ChemStation for GC-MS (Agilent).

3.2.8 Statistical analysis

Results are the average of three processing replicates and two analytical replicates (n= 6). Data obtained were analysed using 2- way ANOVA (with Tukey's HSD multiple pair wise comparison test) and multifactor analysis (MFA) performed in XLSTAT (Addinsoft, Paris, France) to visualise the data in a minimum number of dimensions (two or three).

Table 3.2: Intact glucosinolates identified in cabbage varieties

Common name	Chemical name	Abbreviation	Mass parent ion	MS^2 spectrum ion (base ion in bold)	Reference
Sinigrin	2-propenyl (allyl) GS	SIN	358	278, 275, 259, 227, 195, 180, 162	Rochfort et al. (2008), Lelario et al. (2012)
Gluconapin	3-butenyl GSL	GPN	372	292, 275, **259**, 195, 194, 176	Bennett et al. (2004), Rochfort et al. (2008)
Epi/progoitrin	(R, S)-2-hydroxy-3-butenyl GSL	PROG	388	332, 308, 301, 275, **259**, 210, 195, 146, 136	Bennett et al. (2004), Rochfort et al. (2008), Lelario et al. (2012)
Glucoibeverin	3-(methylthio) propyl GSL	GIBVN	406	326, 275, **259**, 288, 228,195	Rochfort et al. (2008), Lelario et al. (2012), Bell et al. (2015)
Glucoerucin	4-(methylthio) butyl GSL	GER	420	340, 291, 275, **259**, 227, 195, 178, 163	Rochfort et al. (2008), Lelario et al. (2012), Bell et al. (2015)
Glucoiberin	3-(methylsulfinyl) propyl GSL	GIBN	422	407, **358**, 259	Bennett et al. (2004), Rochfort et al. (2008), Lelario et al. (2012)
Glucoraphanin	4-(methylsulfinyl) butyl GSL	GRPN	436	422, **372**, 291, 259, 194	Bennett et al. (2004), Rochfort et al. (2008), Bell et al. (2015)
Glucobrassicin	3-indolylmethyl GSL	GBSN	447	275, **259**, 251, 205	Bennett et al. (2004), Rochfort et al. (2008), Lelario et al. (2012)
4-hydroxyglucobrassicin	4-hydroxy-3-indolylmethyl GSL	4-HOH	463	383, **285**, 267, 259, 240, 195	Bennett et al. (2004), Rochfort et al. (2008), Lelario et al. (2012)

Key: GSL- glucosinolate

Table 3.3: Glucosinolate hydrolysis products identified in cabbage varieties

Precursor GSL	*Common name*	*Chemical name*	*Abbreviation*	*LRI[a,b]*	*MS² spectrum ion (base ion in bold)*	*Reference*
Sinigrin	Allyl thiocyanate	2-propenyl thiocyanate	ATC	871	99, 72, 45, 44, **41**, 39	Al-Gendy & Lockwood (2003)
	Allyl-ITC	2-propenyl isothiocyanate	AITC	884	**99**, 72, 71, 45, 41, 39	Al-Gendy & Lockwood (2003), Arora et al. (2014)
	1-cyano-2,3-epithiopropane	3,4-epithiobutane nitrile	CETP	1004	**99**, 72, 66, 59, 45, 41, 39	Al-Gendy & Lockwood (2003)
Gluconapin	3-Butenyl-ITC	1-butene, 4-isothiocyanate	3BITC	983	113, 85, **72**, 64, 55, 46, 45, 41	Al-Gendy & Lockwood (2003), Hong & Kim (2013), Arora et al. (2014)
	4,5-epithiovaleronitrile	1-cyano-3,4-epithiobutane	EVN	1121	**113**, 86, 80, 73, 60, 45	Hong & Kim (2013)
Progoitrin	Goitrin	5-vinyloxazolidin-2-thione	GN	1545	**129**, 86, 85, 68, 57, 45, 43, 41, 39	Spencer & Daxenbichler (1980)
	1-cyano-2-hydroxy-3,4-epithiobutane isomer 1	2-hydroxy-3,4-epithiobutylcyanide diastereomer-1	CHETB-1	1225	129, 111, 89, 84, 68, **61**, 58, 55, 45	Spencer & Daxenbichler (1980)
	1-cyano-2-hydroxy-3,4-epithiobutane isomer 2	2-hydroxy-3,4-epithiobutylcyanide diastereomer-2	CHETB -2	1245	129, 111, 89, 84, 68, **61**, 58, 55, 45	Spencer & Daxenbichler (1980)
Glucoibeverin	Iberverin	3-methylthiopropyl-ITC	IBVN	1307	147, **101**, 86, 73, 72, 61, 47, 46, 41	Al-Gendy & Lockwood (2003)
	4-methylthiobutyl nitrile	4-methylthio butanenitrile	4MBN	1085	115, 74, 68, **61**, 54, 47, 41	Al-Gendy & Lockwood (2003)

Glucoerucin	Erucin	4-(methylthio)-butyl-ITC	ER	1427	161, 146, **115**, 85, 72, 61, 55	Al-Gendy & Lockwood (2003), Arora et al. (2014)
	Erucin nitrile	1-cyano-4-(methylthio) butane	ERN	1200	129, 87, 82, **61**, 55, 48, 41, 47	Al-Gendy & Lockwood (2003), Arora et al. (2014)
Glucoiberin	Iberin	3-methylsulfinylpropyl-ITC	IB	1617	163, 130, 116, 102, 100, 86, **72**, 63, 61,41	Al-Gendy & Lockwood (2003)
	Iberin nitrile	4-methylsulfinylbutanenitrile	IBN	1384	**131**, 78, 64, 47, 41	Al-Gendy & Lockwood (2003)
Gluconasturtin	2-phenylethyl-ITC	2-isothiocyanatoethyl benzene	PEITC	1458	163, 105, **91**, 65, 51, 40	Al-Gendy & Lockwood (2003)
	Benzenepropanenitrile	2-phenylethyl cyanide	BPN	1238	131, **91**, 85, 65, 63, 57, 44, 51	Hong & Kim (2013)
Glucoraphanin	Sulforaphane	4-methylsulfinylbutyl-ITC	SFP	1757	160, 114, 85, **72**, 64, 63, 61, 55. 41, 39	Arora et al. (2014),Bell et al. (2017c)
	Sulforaphane nitrile	5-(methylsulfinyl) pentanenitrile	SFN	1526	145, 128, 82, 64, **55**, 41	Arora et al. (2014), Bell et al. (2017c)
Glucobrassiccin	Indole-3-carbinol	1H-Indole-3-methanol	I3C	1801	**144**, 145, 116, 108, 89	Spencer & Daxenbichler (1980)
	Indoleacetonitrile	1H-Indole-3-acetonitrile	1IAN	1796	**155**, 145, 144, 130, 116, 89, 101, 63	Hanschen et al. (2017)
Pentyl GSL	Pentyl-ITC	1-isothiocyanato-pentane	PITC	1165	129, 114, 101, 96, 72, 55, **43**, 41, 39	de Pinho et al. (2009)
Indole	1H-Indole	Indole (8CI)	1H-I	1290	**117**, 90, 89, 63, 58	Vaughn et al. (2017)
Glucotropaeolin	Benzeneacetonitrile	2-Phenylacetonitrile	BAN	1137[c]	**117**, 90, 89, 77, 63, 51	Vaughn et al. (2017)

Key: ITC- isothiocyanate. [a] Linear retention index on a HP-5MS non-polar column. [b]Mass spectrum agrees with reference spectrum in the NIST/NIH mass spectra database and those in literature. [c]Mass spectrum and LRI agree with those of authentic compound.

3.3 Results and discussion

3.3.1 Effect of cabbage type and variety on GSL profile and concentration

GLS profiles across cabbage varieties are presented in Figure 3.1; statistical output of significant differences within and between cabbage types documented in Appendix VIII (Table S3a). In total, nine different GSLs were identified across all varieties tested (Table 3.2); seven aliphatic GSLs namely sinigrin (SIN), gluconapin (GPN) and epi/progoitrin (PROG), Glucoibeverin (GIBVN), glucoerucin (GER), glucoiberin (GIBN) and glucoraphanin (GRPN) and two indole GSLs: glucobrassicin (GBSN) and 4-hydroxyglucobrassicin (4-HOH). PROG, GIBN and GRPN were the most abundant GSLs across varieties studied with 4-HOH, GIBVN and GER being the least abundant. 4-HOH was present in negligible amounts (<1.0 $mg.g^{-1}$ DW) in all varieties, contributing not more than 1 % to the total GSL content of the cabbages.

GSL profiles and concentrations varied across cabbage varieties and differed significantly in some cases between and within cabbage types and varieties. Only five of the nine individual GSLs identified in cabbages studied were found in black kale varieties; GIBN, GRPN, GBSN, 4-HOH, and GER, the last of which was present in B3 alone. GRPN was the major GSL present in black kale varieties consisting of over 50 % on average of the total GSL content of Black kale. The proportion of GRPN is similar to those previously reported by Kushad et al. (2004), but much higher than those reported by Cartea et al. (2008). Previous studies detected SIN and PROG in kale and reported SIN as the main GSL in kale varieties (Kushad et al., 1999; Velasco et al., 2007; Cartea et al., 2008); however, SIN and PROG were not detected in this study. There was a significant difference in total and individual GSL concentrations within black kale varieties except 4-HOH, which did not differ significantly ($p=0.401$). B2 had the highest total GSL content (48.6 $mg.g^{-1}$ DW).

GIBVN and GER were identified in some but not all wild and tronchuda cabbage varieties, while GIBN and GRPN were not identified in the WD1 variety. Concentration of individual GSLs differed significantly ($p<0.0001$) across all wild and tronchuda cabbages. PROG and GPN were the most abundant GSLs in WD1 and WD3, while PROG and GRPN were the most abundant in WD2. In tronchuda cabbages, SIN, GIBN and GBSN were at the highest concentrations, with GIBN comprising of up to 45 % in TC3. A previous study (Cartea et al., 2008) on GSL profile and concentrations in tronchuda cabbage identified 14 GSLs compared to seven found in this study. However, in both studies, GER was not identified and proportions of the individual GSLs reported were similar to those found in this study. The total GSL content

of wild and tronchuda varieties differed significantly ($p<0.01$ and $p<0.0001$ respectively) between varieties within each cabbage type.

The most abundant GLSs in savoy cabbages were GIBN, SIN and GBSN, with GIBN concentrations as high as 60.6 $mg.g^{-1}$ DW (58 % of the total GLSs) in SC1. GER was not identified in savoy varieties and GPN was present in very low amounts in SC3 only. Similar proportions of GSLs were reported by Ciska et al. (2000) and Hanschen & Schreiner (2017) but in both studies more individual GSLs were identified than those reported in this study. For example, both studies identified GER in savoy cabbages, although present in trace amounts in the Ciska et al. (2000) study. Total GSL content of savoy cabbages ranged from 45.9 mg. g^{-1} DW to 104.4 $mg.g^{-1}$ DW. The SC3 variety had significantly higher ($p<0.0001$) total GSLs than SC1 and SC2, with SC1 having significantly lower total GSLs than the other two varieties.

In red and white cabbages, PROG, GIBN and GRPN were the most abundant GSLs. GBSN was also abundant in WC2 and RC1 varieties while GER was not identified in either variety. The concentrations of GRPN, GIBVN and GER did not differ significantly between red cabbage varieties. WC2 had significantly higher amounts of SIN, GIBN, GBSN and total GSL compared to WC1, but differences in PROG and GRPN content were not significant. The total GSL content of RC1 was significantly ($p<0.0001$) higher than the other two red cabbage varieties. Results obtained agree with those previously reported (Ciska et al., 2000; Volden et al., 2008; Beck et al., 2014; Hanschen & Schreiner, 2017). However, a few studies disagree with the findings of this study; a previous study conducted by Park et al. (2014b) quantifying red cabbage GSL reported SIN absent in red cabbage, while Zabaras et al. (2013) found GPN as the most abundant GSL in red cabbage.

Individual GSLs and total average GSL concentrations differed significantly ($p<0.0001$) across all varieties, irrespective of cabbage type. Total average GSL concentrations of varieties studied ranged from 19.3 $mg.g^{-1}$ DW (BK3) to 149.8 $mg.g^{-1}$ DW (WD3). These differences are due to variations in GSL profiles and concentrations of individual GSLs. Wild cabbages generally had higher total GSL concentrations (Figure 3.1b) than other cabbage types, and these high concentrations were driven by significantly higher amounts of PROG in wild cabbages. Lower concentrations of total GSL observed in black kale varieties (19.3 $mg.g^{-1}$ DW to 48.6 $mg.g^{-1}$ DW) are due to lower numbers and concentrations of individual GSLs compared to the other cabbage types studied (Figure 3.1a). The variability in GSL concentrations between and within cabbage types and varieties is in agreement with previous reports that GSL profiles and concentrations vary between *Brassica* species and varieties (Mithen et al.,

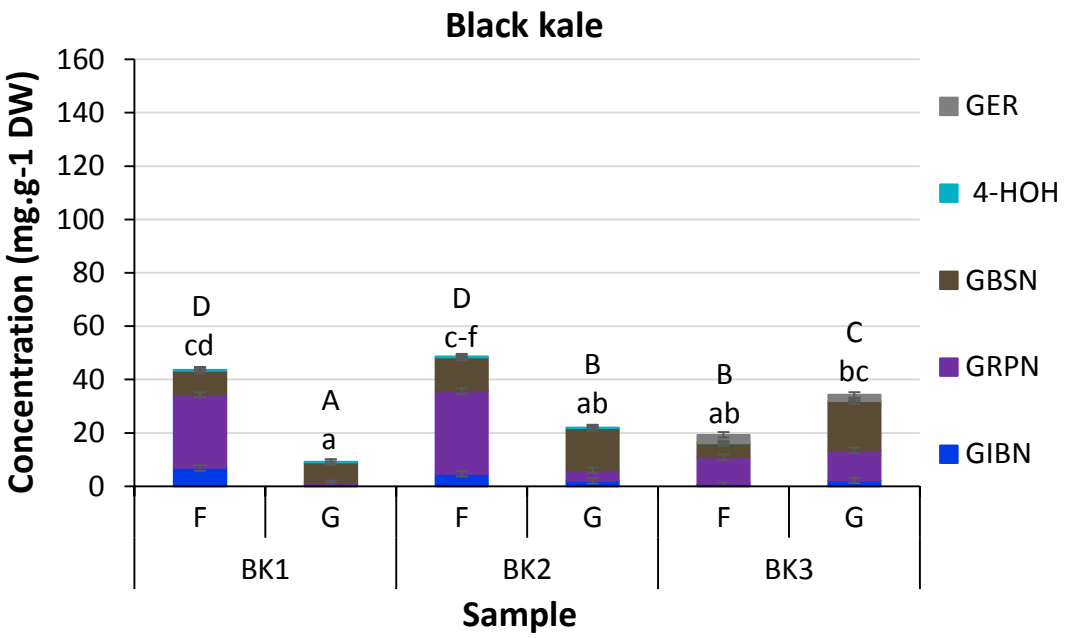
2000; Fahey et al., 2001; Charron et al., 2005a; Cartea et al., 2008; Hanschen & Schreiner, 2017; Penas et al., 2011; Bell et al., 2015). The difference in GSL profiles of *Brassica* vegetables has been linked to genetic factors while interactions between environmental and genetic factors are largely responsible for differences in GSL concentrations (Bjorkman et al., 2011). In general, concentrations of individual and total GSL of the gene bank cabbages reported in this study are much higher than those reported for commercial and gene bank cabbage varieties in literature (Kushad et al., 1999; Ciska et al., 2000; Cartea et al., 2008; Penas et al., 2011; Hanschen & Schreiner, 2017). This may be due to the different varieties studied implying that the gene bank may indeed be a useful source from which to select varieties with higher GSL. Another possible reason may be differences in the conditions under which the plants were grown and/or harvested. The higher GSL concentrations in the present study can enhance potential health benefits that may be derived from their consumption.

The differences in GSL profiles and concentrations of the varieties studied can potentially influence the sensory and health properties of the cabbages. For example, the absence of SIN and PROG in black kale varieties may potentially influence the sensory characteristics of these cabbages as SIN and PROG have been linked with bitter taste in *Brassica* vegetables (Drewnowski & Gomez-Carneros, 2000b). On the other hand, higher amounts of GRPN in kale, red and white cabbages could enhance the potential health benefits derived from their consumption (Vaughn & Berhow, 2005). The differences in cabbage varieties, growing conditions and location, as well as environmental factors during cabbage cultivation, all play a vital role in GSL profile and concentration and therefore make comparing results between different studies difficult.

3.3.2 Effect of growing conditions on GSL concentrations in cabbage varieties.

The effect of growing conditions on GSL concentration is as presented in Figure 3.1 with significant differences within and between cabbage types presented in Appendix VIII (Table S3a). White cabbage variety WC3 did not survive both on the field and in the glasshouse, while varieties SC3 and RC2 did not grow in the glasshouse. The GSL profile of cabbage varieties studied did not differ between growing conditions. Total GSL concentrations in field grown samples ranged from 19.3 $mg.g^{-1}$ DW (BK3) to 149.8 $mg.g^{-1}$ DW (WD3) and glasshouse samples from 9.2 $mg.g^{-1}$ DW (BK1) to 93.9 $mg.g^{-1}$ DW (WD3). WD3 had significantly higher concentrations of total GSLs compared to all other varieties and this was largely due to the abundance of PROG and GPN making up 83 % and 69 % of total GSLs in field and glasshouse samples respectively.

(a)



(b)

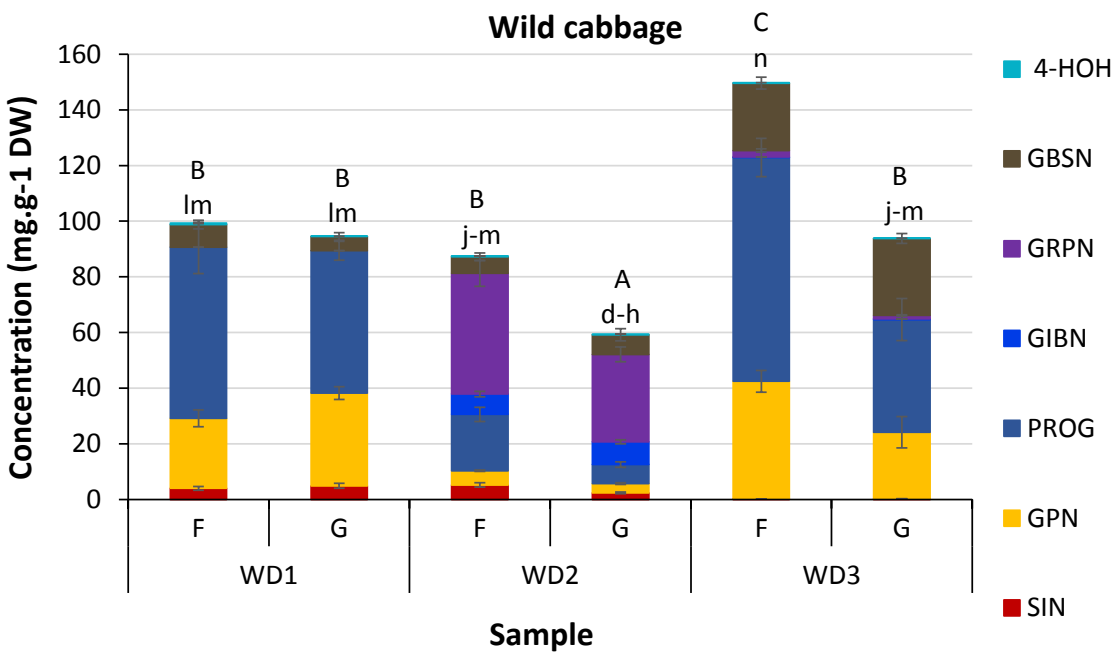
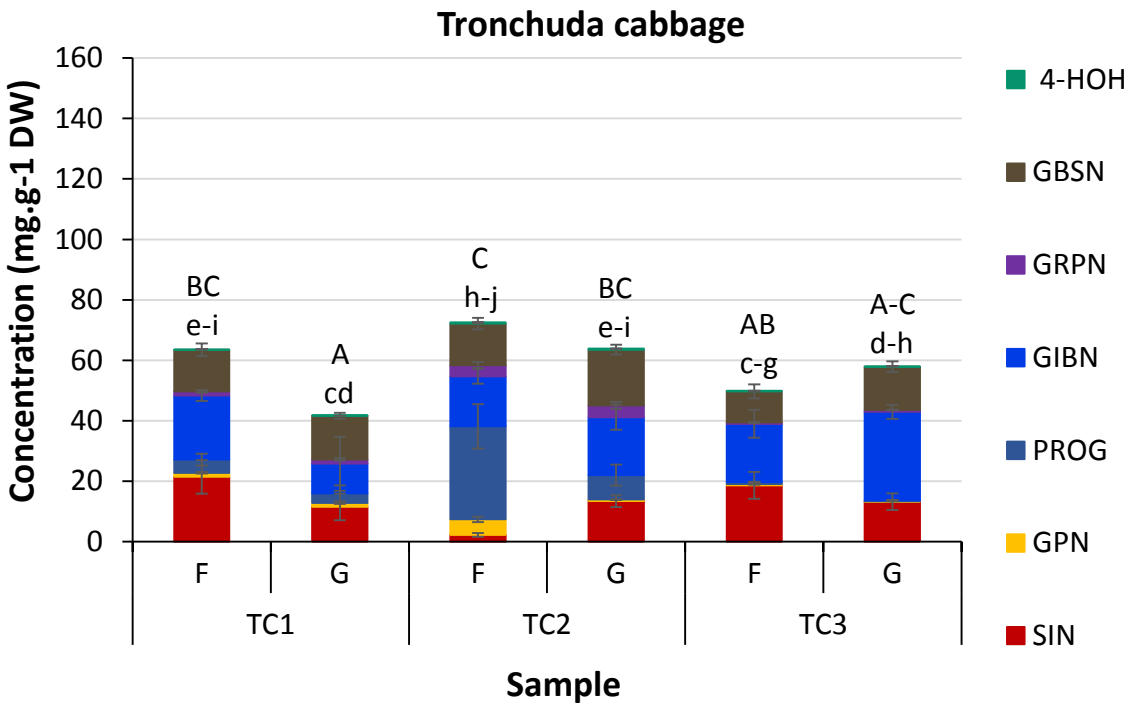


Figure 3.1. Glucosinolate (GSL) concentrations (mg.g^{-1} DW) of field and glasshouse cabbages. Error bars represent standard deviation from mean values. Letters above bars refer to differences in total GSL concentration. Letters 'ABC': bars not sharing a common letter differ significantly ($p<0.05$) between varieties and growing conditions within a cabbage type (i.e. within each separate graph). Letters 'abc': bars not sharing a common letter differ significantly ($p<0.0001$) between cabbage types, varieties and growing conditions (i.e. between the separate graphs). Abbreviations: F = Field, G = glasshouse; DNG, did not grow; SIN, sinigrin; GPN, gluconapin; PROG, epi/progoitrin; GIBVN, Glucoibeverin; GER, glucoerucin; GIBN, glucoiberin; GRPN, glucoraphanin; GBSN, glucobrassicin; 4- HOH, 4-hydroxyglucobrassicin. For full names of cabbage varieties see Table 3.1.

(C)



(d)

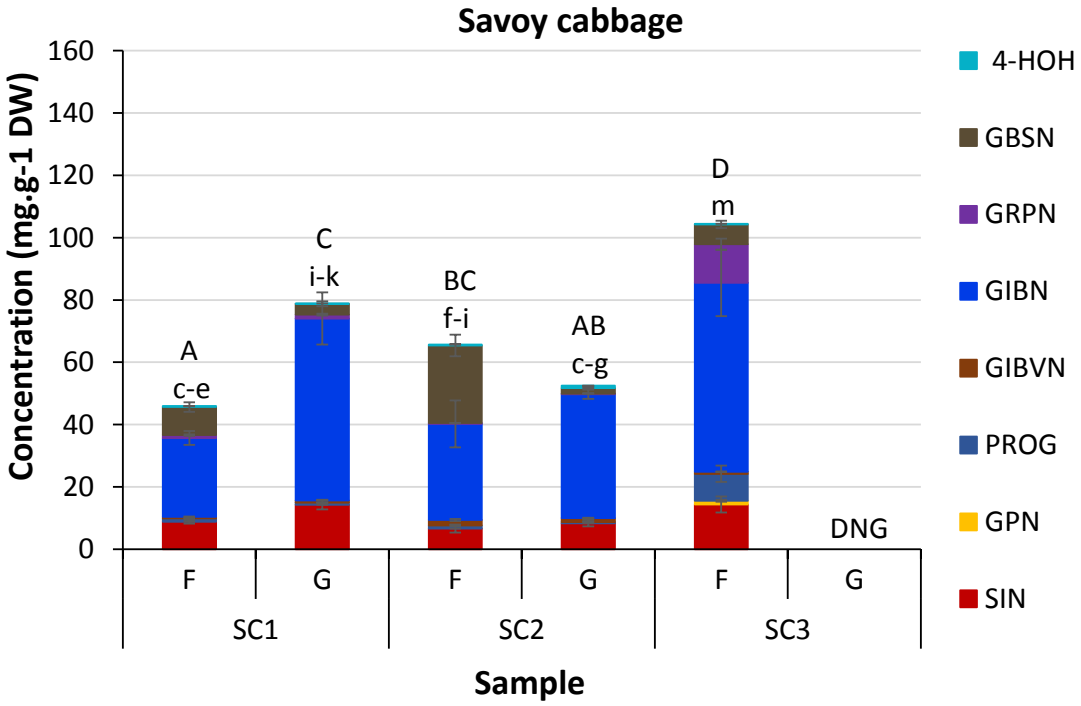
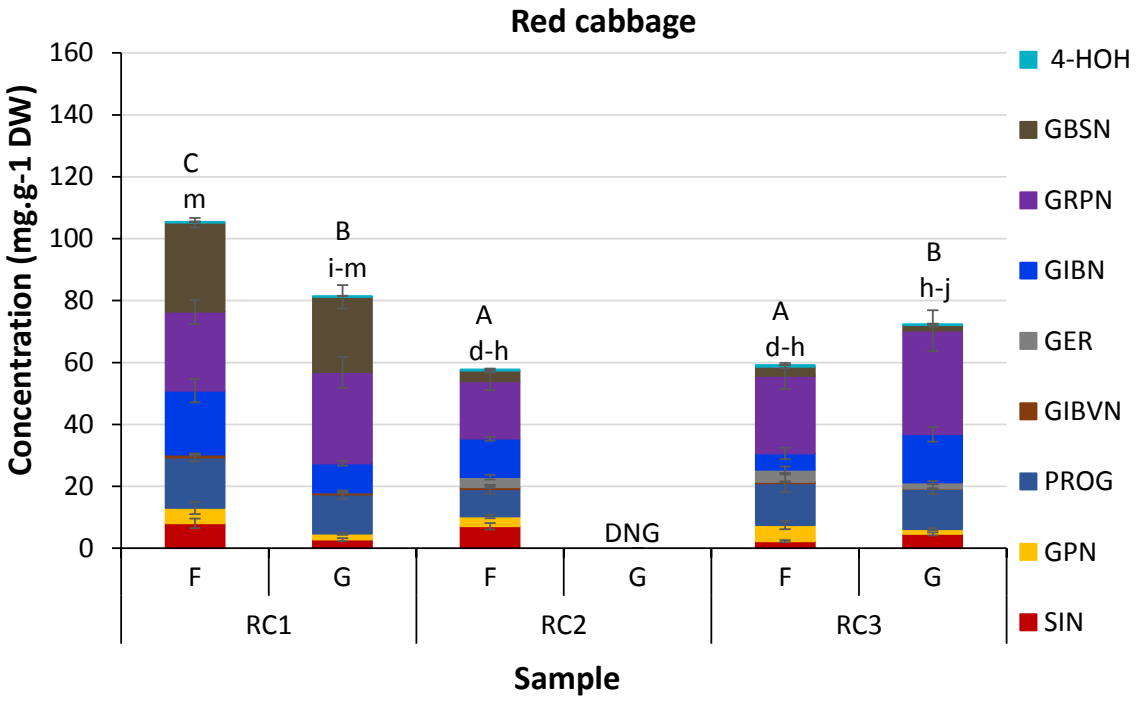


Figure 3.1 - continued.

(e)



(f)

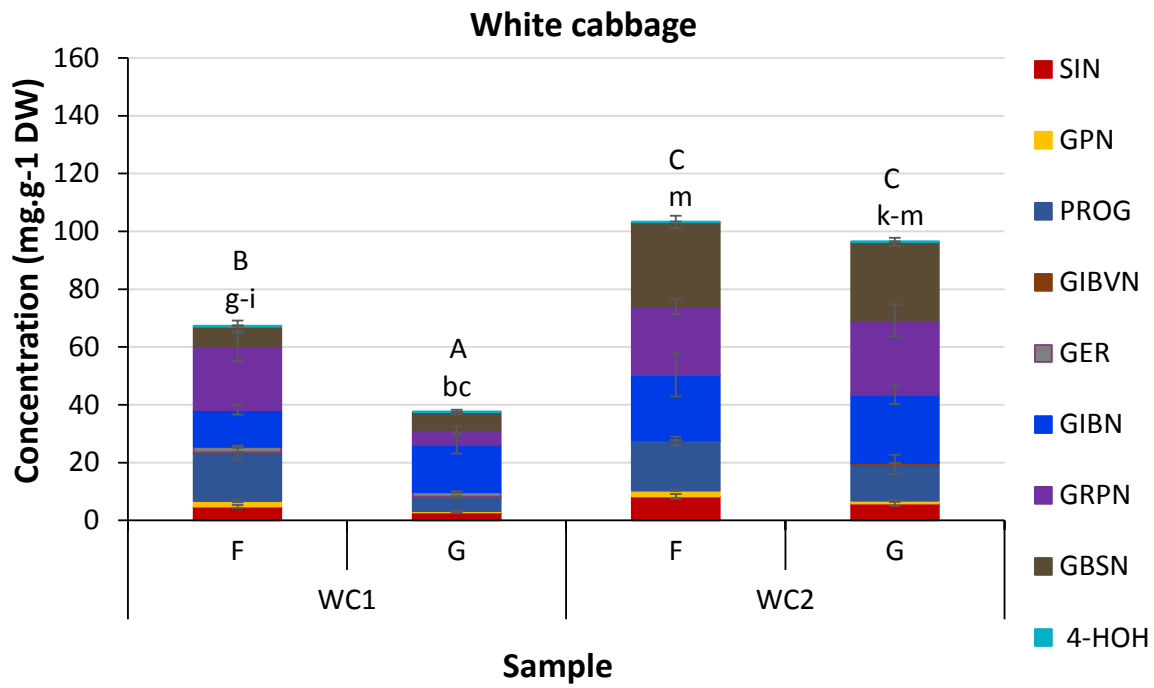


Figure 3.1 – continued.

Cabbages grown in the field had higher total GSL concentrations than glasshouse samples across most varieties studied, with a few exceptions (BK3, TC3, SC1 and RC3) where total GSL concentrations were higher in glasshouse samples. These differences were significant in some, but not all cases. Growing conditions significantly affected individual GSL concentrations between and within types and varieties. Both field and glasshouse cabbages were predominantly abundant in aliphatic GSLs (82 and 78 % respectively) while indole GSLs comprised of only 18 and 22 % of individual GSLs in field and glasshouse samples.

There was no clear pattern for the abundance of individual GSLs as some GSLs were significantly higher in glasshouse samples for some varieties, but lower or not significantly different in others. PROG and GRPN were either significantly higher in field samples or did not significantly differ from glasshouse samples within varieties, except for RC3 variety where GRPN was significantly higher ($p<1.0001$) when glasshouse grown. GRPN abundance in BK1 and BK2 field grown varieties was up to 90 % more than the corresponding glasshouse grown cabbages. GBSN was the most stable GSL across growing conditions as there was no significant difference ($p=0.101$) in GBSN between field and glasshouse cabbages.

There are several possible reasons for the differences observed in GSL concentrations in the different growing conditions. The higher total GSLs content reported in most field samples could be due to production of higher amounts of GLSs by the plant in response to insect and pest attack on the field when compared to glasshouse samples. GLS compounds are plant metabolites produced by plants for defence against stress and attack from insect and pests (Bjorkman et al., 2011; Rohr et al., 2006). In addition, the higher amount of GSLs in field samples could also be due to the lower average temperatures during growth (6 to 24 °C) compared to the higher temperatures in the glasshouse (14 and 43 °C) (Appendix IV; Table S2a). Growth temperatures have been reported to influence GSL concentrations in *Brassica* vegetables. *Brassica* vegetables are generally thought to be cool weather crops with average growing temperatures between 4 – 30 °C (Wurr et al., 1996). The optimum temperature for growth varies between different types of *Brassica* and going below or above that temperature could affect concentrations of GSL. However, literature studies have generally reported higher GSLs at higher growing temperatures; Rosa & Rodrigues (1998) reported a higher GSL content in young cabbage plants when grown at 30 °C compared to 20 °C. Several authors have reported higher GSL concentrations in spring/summer grown cabbages (average temperatures between 25 - 30 °C) compared to autumn grown plants (temperatures < 20 °C) (Ciska et al., 2000; Charron et al., 2005a; Cartea et al., 2008; Penas et al., 2011). The lower

amounts of GSL accumulated in glasshouse plants could also be the result of plant growing conditions. Glasshouse samples were grown in pots with drainage holes to allow excess water to seep out. However, this could have also led to sulfur leaching, leading to sulfur deficiency in the soil and plants were not fed with sulfur fertilizers. Sulfur is a major precursor for GSL biosynthesis and its deficiency has been reported to reduce GSL concentrations in *Brassica* plants, especially aliphatic GSLs as sulfur deficiency limits methionine synthesis (basic substrate for aliphatic GLS biosynthesis) as opposed to tryptophan; a non-sulfur amino acid and precursor for indole GSL biosynthesis (Zhao et al., 1994). On average, reduced amounts of aliphatic GSLs were accumulated in glasshouse plants compared to field plants, while glasshouse samples accumulated higher amounts of indole GSLs than field samples. Sulfur was reported to influence the aliphatic GSL concentrations in rapeseed more than indole GSL (Zhao et al., 1994). However, glasshouse plants which had significantly higher GSLs concentrations compared to their field counterparts may have found the glasshouse conditions more favorable than other varieties which resulted in enhanced GSL production. This study highlights the importance of finding the optimal growing conditions for different cabbage varieties for enhanced GSL production as individual plants respond differently under different environmental conditions.

3.3.3 Effect of cabbage type and variety on GHP profile and concentrations

A total of 22 glucosinolate hydrolysis products (GHPs) were identified and quantified from cabbage varieties studied, comprising of 11 ITCs and 11 nitriles and epithionitriles (Table 3.3). Concentrations of GHPs are presented in Figure 3.2 with significant differences between and within cabbage types and varieties presented in in Appendix VII (Table S3b). Results are expressed as sulforaphane equivalents. The type and concentration of GHPs formed differed between cabbage varieties. Predominant GHPs did not differentiate between varieties within a cabbage type but varied across cabbage types. There was a significant difference in the concentrations of individual and total GHPs formed within and between cabbage types and varieties (Appendix VIII; Table S3b). Wild cabbage varieties had the highest levels of GHPs formed (526.4 $\mu g.g^{-1}$ DW – 1186.9 $\mu g.g^{-1}$ DW; Figure 3.2b) and tronchuda varieties the lowest (64.9 $\mu g.g^{-1}$ DW – 210.7 $\mu g.g^{-1}$ DW; Figure 3.2c).

GHPs of GRPN and GBRN were the main GHPs detected in black kale varieties with nitrile concentrations accounting for 74-89 % of the total GHPs. BK2 varieties had significantly lower total GHPs than BK1. Isomers of CHETB, nitriles of PROG hydrolysis were the most abundant GHPs formed in wild cabbages except for WD2 which had higher amounts of GN

(PROG ITC) compared to nitriles formed. This was unexpected and it is unclear why this happened because more nitriles than ITCs were formed for other GSLs present in the same sample. GN have been associated with bitter taste (Fenwick et al., 1983) and adverse effects on thyroid metabolism leading to goiter formation. However, the reports on goitre formation are limited and based on animal studies which show that average daily intake is not enough to produce adverse effects in humans (Bjorkman et al., 2011). However, to limit the health risks, genetic manipulation and selective breeding methods used to increase GRPN contents by threefold in '*Beneforte*' broccoli (Traka et al., 2013) could be employed to reduce PROG contents in the wild varieties. The main GHPs of tronchuda varieties were CETP and IBN; nitriles of SIN and GIBN respectively. Total GHPs of TC2 was significantly higher than TC3. IBN and IB (GIBN hydrolysis products) were the most abundant GHPs in savoy cabbages and SFP and SFN (hydrolysis products of GRPN) the most abundant in red and white cabbages. In savoy, SC1 varied significantly from SC2 and SC3 varieties, containing up to 60 % more GHPs than the other two varieties. The much lower concentrations of GHPs in SC2 compared to SC1 was unexpected due to similar concentrations of GSLs in both varieties. A similar trend was noticed between WC2 and WC1 varieties where much lower GHPs were formed in WC2 varieties with significantly higher GSLs than WC1. This might be related to variation in myrosinase and ESP activity in the samples. WC1 was found to have higher myrosinase activity than WC2 (see Chapter 2), which explains the higher concentrations of GHPs formed. However, this is not the case in savoy cabbages, as SC2 had the highest myrosinase activity. It is hypothesized that myrosinase isoenzyme and ESP of SC2 variety may be less stable than the other varieties and therefore denatured before permitting full hydrolysis. Several GHPs were identified in cabbage varieties where their GSLs were not detected: tiny amounts of 3BITC (GPN hydrolysis product) was formed in B3; 4MBN (nitrile of GIBVN) in tronchuda; EVN (GPN nitrile) in savoy cabbages and ER and ERN (GER GHPs) in red and white cabbages. PEITC and BPN (GHPs of gluconasturtiin), PITC and BAN was also formed in most varieties. These could have been due to very low amounts of these GSLs in the samples that were not detected during analysis, or the analytical method was not robust enough to identify them. A previous study in turnips detected GHPs of glucoberteroin though the intact GLS was not present (Klopsch et al., 2017). The profile of GHPs is this study is in agreement with the study of Hanschen & Schreiner (2017). However, in their study, they found CETP (nitrile from SIN hydrolysis) as the main GHPs in savoy, red and white cabbages, which is inconsistent with this

study where GIBN GHPs (IB and IBN) and GRPN GHPs (SFP and SFN) were the main compounds detected. This difference can be attributed to the different varieties studied.

The relationship between GSLs and GHPs was as expected where the most abundant GHPs was a reflection of GSLs concentrations, which is helpful in confirming the efficiency or accuracy of the GHPs extraction method. Overall, nitriles and epithionitriles were the major hydrolysis products formed across all cabbage varieties as has been reported previously (Matusheski et al., 2006; Hanschen et al., 2017). This is due to the activity of ESP and other nitrile forming proteins present in the samples, which hydrolyse GSLs to epithionitriles and nitriles instead of the more beneficial ITCs (Matusheski et al., 2006).

3.3.4 Effect of growing condition on GHP concentrations

GHPs profile and concentration in the two different growing conditions studied is presented in Figure 3.2, with the significant differences between growing conditions reported in Appendix VIII (Table S3b). The profile of the GHPs detected were similar between growing conditions with a few exceptions. For example, BPN was identified in black kale field samples but not detected in glasshouse samples. GHP concentrations in field and glasshouse ranged from 64.9 $\mu g.g^{-1}$ DW (TC3) to 1186.9 $\mu g.g^{-1}$ DW (W1) and 44.3 $\mu g.g^{-1}$ DW (B1) to 981.7 $\mu g.g^{-1}$ DW (WD1) respectively. Within varieties, total GHP accumulation was significantly higher in field plants than glasshouse; except for wild cabbage varieties, TC1 and WC2, where total GHPs were higher in field samples (except in WC2) but the differences were not significant. Generally, total GHP concentrations followed a similar pattern as total GSLs with a few exceptions. For example, BK3 glasshouse sample had significantly lower total GHPs compared to the field sample (Figure 3.2a) despite the significantly higher total GSL in the glasshouse sample (Figure 3.1a). Significantly higher myrosinase activity (see Chapter 2) and possibly ESP activity in the BK3 field compared to glasshouse sample may have led to formation of more GHPs.

In summary, the result of this study shows the importance of GSL accumulation during plant growth as it has a direct impact on the hydrolysis compounds formed. It is there important to ensure that cabbages are planted under conditions that favour high GSL accumulation.

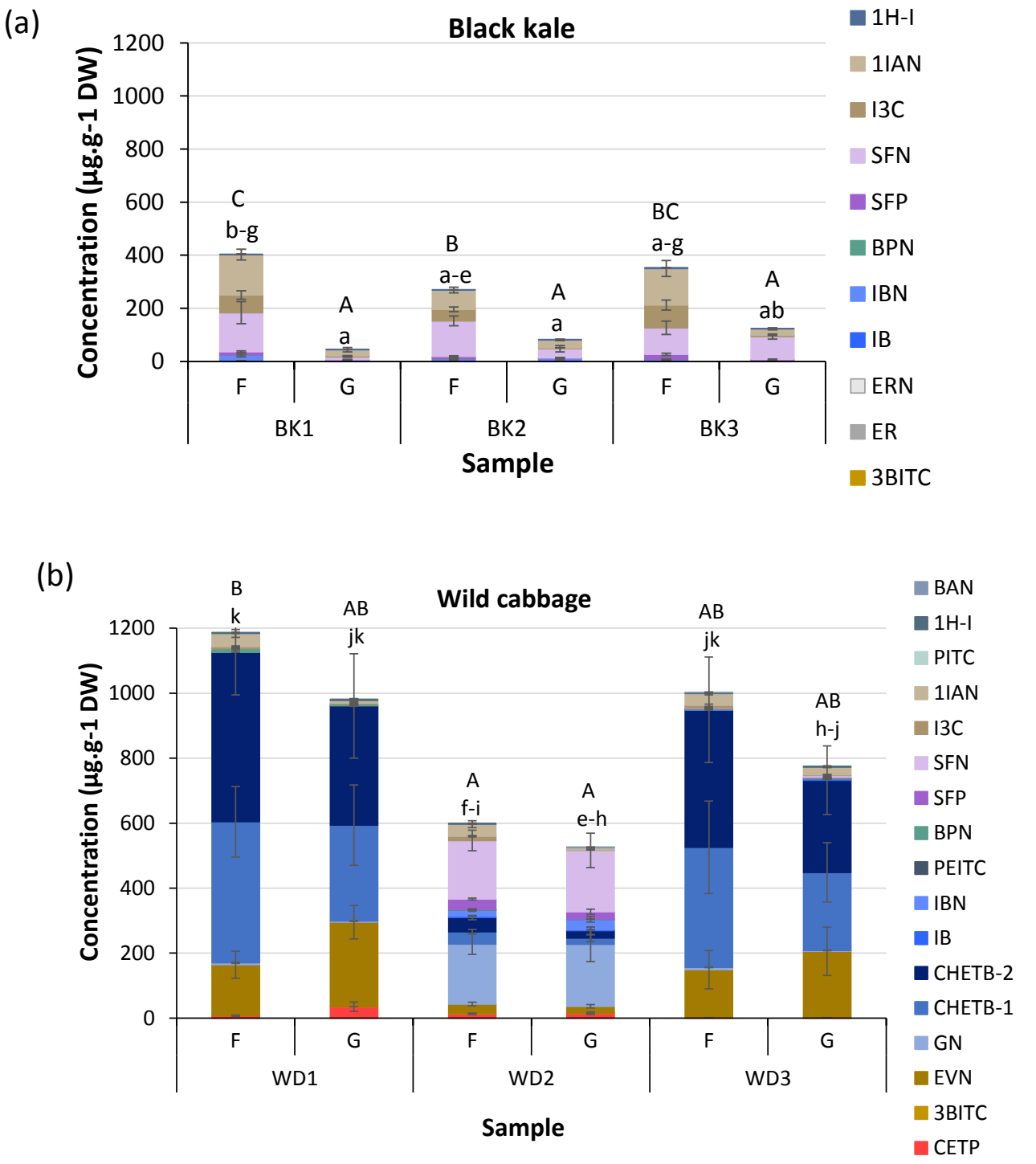
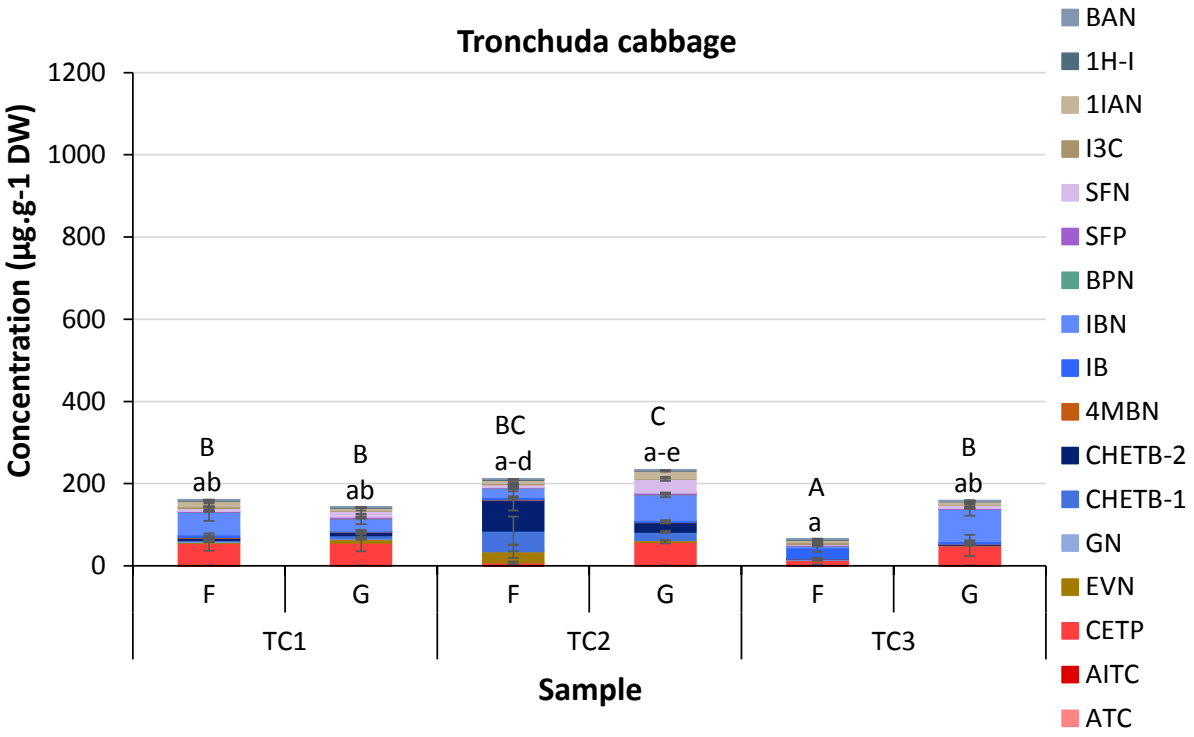


Figure 3.2. Glucosinolate hydrolysis products (GHPs) (µg.g^{-1} DW) of field and glasshouse

Results expressed as sulforaphane equivalents. Error bars represent standard devation from mean values. Letters above bars refer to differences in total GHP concentration. Letters 'ABC': bars not sharing a common letter differ significantly ($p<0.05$) between varieties and growing conditions within a cabbage type (i.e. within each separate graph). Letters 'abc': bars not sharing a common letter differ significantly ($p<0.0001$) between cabbage types, varieties and growing conditions (i.e. between the separate graphs). Compounds with colour shades similar to one another are GHPs of corresponding GSL in Fig 3.1. Abbreviations: F = Field, G = glasshouse; DNG, did not grow. ATC, allyl thiocyanate; AITC, allyl isothiocyanate; CEPT, 1-cyano-2,3-epithiopropane; 3BITC, 3-Butenyl-ITC; EVN, 4,5-epithiovaleronitrile; GN, goitrin; CHETB-1, 1-cyano-2-hydroxy-3,4-epithiobutane isomer 1; CHETB-2, 1-cyano-2-hydroxy-3,4-epithiobutane isomer 2; 4MBN, 4-methylthiobutyl nitrile; ER, erucin; ERN, erucin nitrile; IB, iberin; IBN, iberin nitrile; PIETC, 2-phenylethyl-ITC; BPN, benzenepropanenitrile; SFP, sulforaphane; SFN, sulforaphane nitrile; I3C, indole-3-carbinol; 1IAN, indoleacetonitrile; PITC, Pentyl-ITC; 1H-I, 1H-Indole; BAN, benzeneacetonitrile. For full names of cabbage varieties see Table 3.2.

(c)



(d)

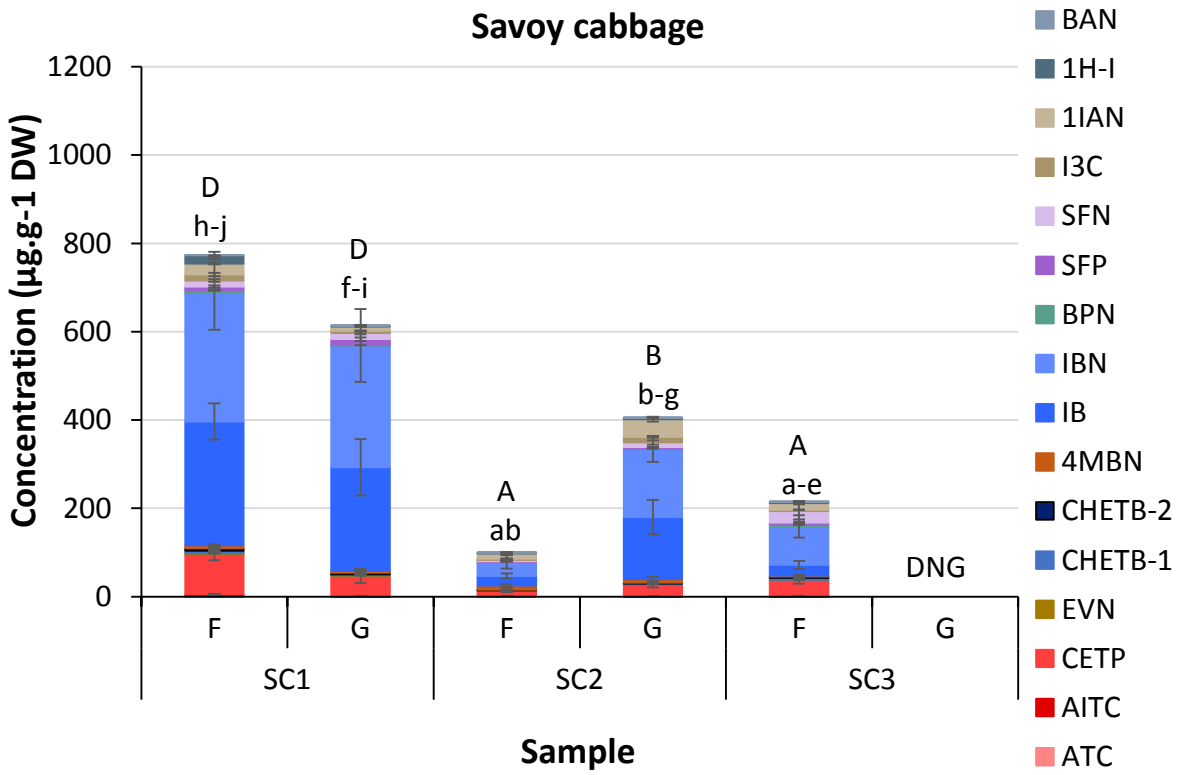
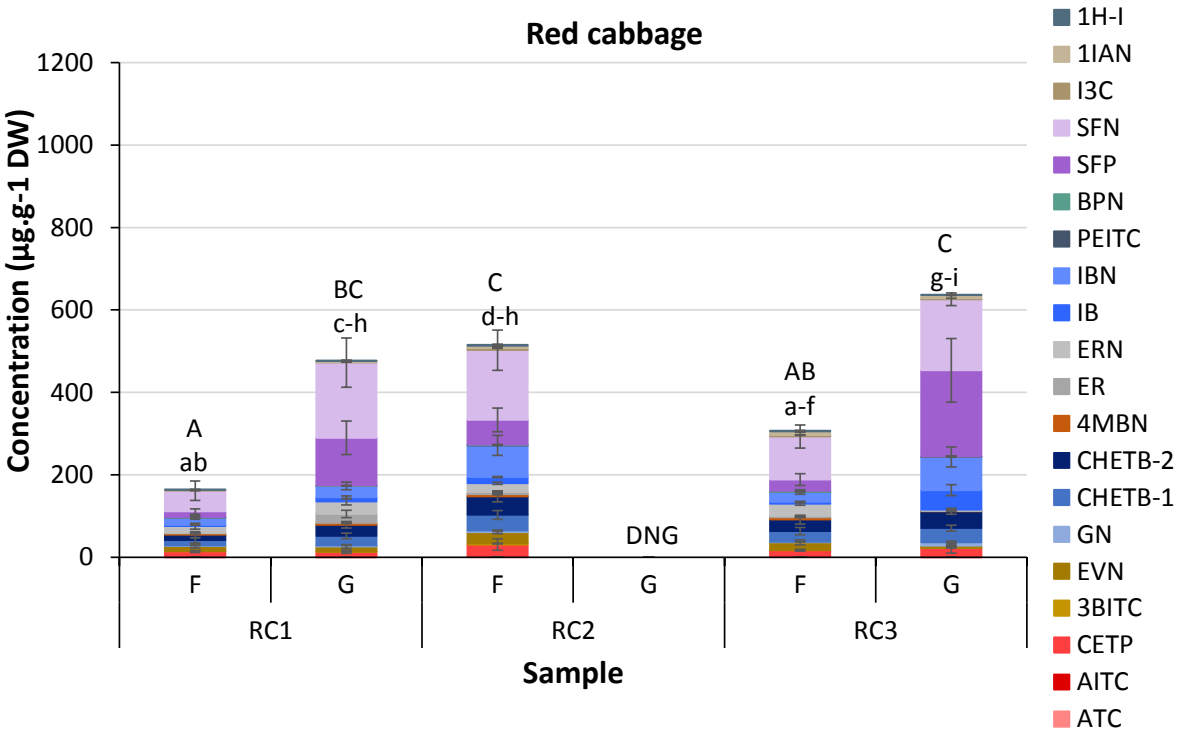


Figure 3.2 – continued

(e)



(f)

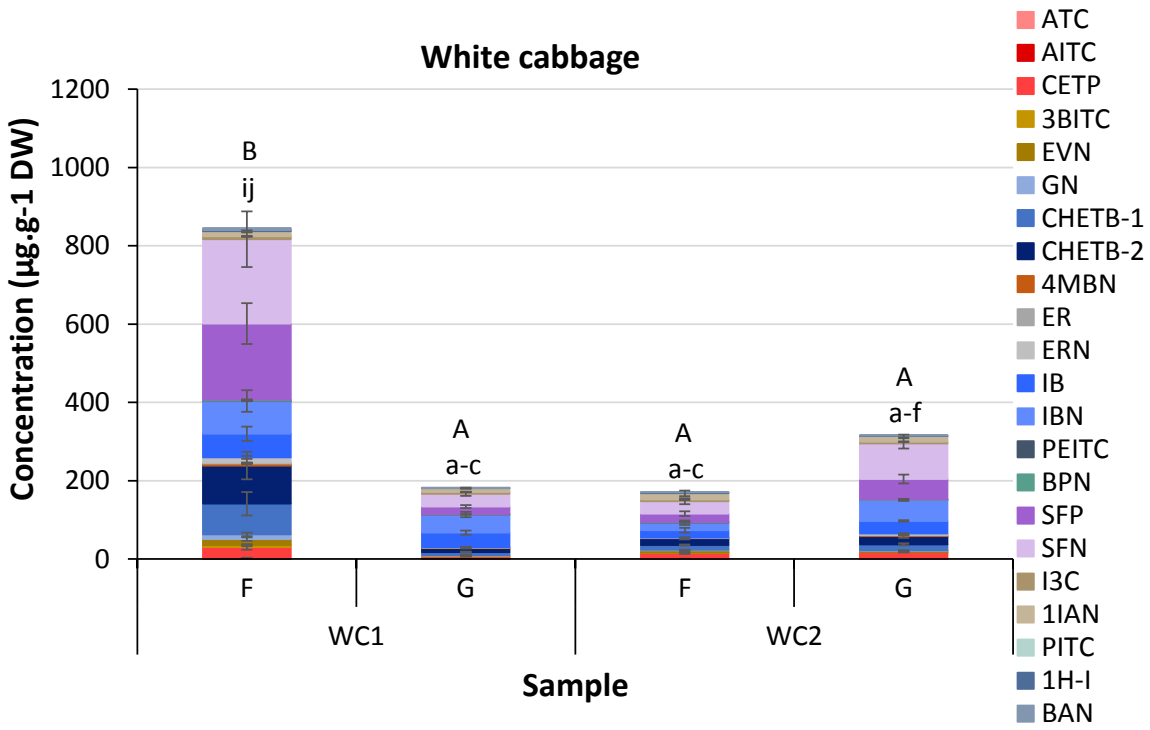


Figure 3.2 – continued

3.3.5 Multifactor analysis (MFA) of GSLs and GHPs in cabbages

Figure 3.3 shows distribution of the cabbage varieties as well as the scores and loadings of MFA performed on the mean data of GSLs and GHPs. PC1 and PC2 explained 42 % of the variance in the data but other PCs did not provide any new information, therefore, only PC1 and PC2 are presented and discussed. The plot demonstrates that individual GSLs were positively correlated with their corresponding GHPs. From the plot, cabbages were mostly distinguished based on type rather than varieties or growing conditions, except for wild cabbage varieties where there was a clear separation of WD2 from WD1 and WD3. Based on the MFA, samples were grouped into three distinct clusters; one cluster comprised of BK, RC, WC and WD1 varieties, another TC and SC varieties and the final cluster WD1 and WD3 varieties. BK, RC, WC and WD2 correlated positively with GRPN, GER, 4-HOH and their hydrolysis products. TC and SC correlated positively with GIBN, GIBVN, SIN and their hydrolysis products. WD1 and WD2 correlated positively with GPN, PROG and their nitriles, as well as total GSLs and GHPs, but was negatively correlated with BK, RC, WC and WD2 varieties. An additional pearson correlation demonstrated significant correlations ($p<0.05$) between various GSLs and GHPs is presented in Appendix VIII (Table S3c). GIBN correlated negatively ($r^2>-0.3$; $p<0.01$) with PROG and its hydrolysis products, GPN and its hydrolysis products and PITC. On the contrary, GPN was strongly positively correlated ($r^2>0.6$; $p<0.0001$) with PROG and its hydrolysis products, EVN, PITC, total GSL and total GHPS. Total GSLs positively correlated significantly ($r^2=0.5$; $p<0.01$) with total GHPs. Strong significant positive correlations ($r^2>0.5$; $p<0.05$) was observed between individual GSLs and their corresponding GHPs. For example, GRPN was positively correlated with SFP and SFN ($r^2>0.5$ and 0.8; $p<0.01$ and $p<0.0001$ respectively).

It is obvious that the separations observed between samples are mainly driven by differences in GSLs and GHPs most accumulated in the samples: GN, GRPN, GER, 4-HOH and their GHPs in BK, RC WC and WD2 varieties; GIBN, SIN, GIBVN and their GHPs in TC and SC varieties; and lastly PROG, GPN and their GHPs in WD1 and WD3 varieties. WD1 and WD3 had the highest concentration of total GSLs and GHPs and this was responsible for the positive correlation of these varieties to total GSLs and GHPs observed. It is worth mentioning that PROG and CHETB, which were largely responsible for the high concentrations of total GSLs and GHPs in these varieties also correlated positively with total GSLs and GHPs. The result obtained provide a clear picture of the similarities and differences in GSL and GHP profile and concentrations of the different cabbage types and varieties studied.

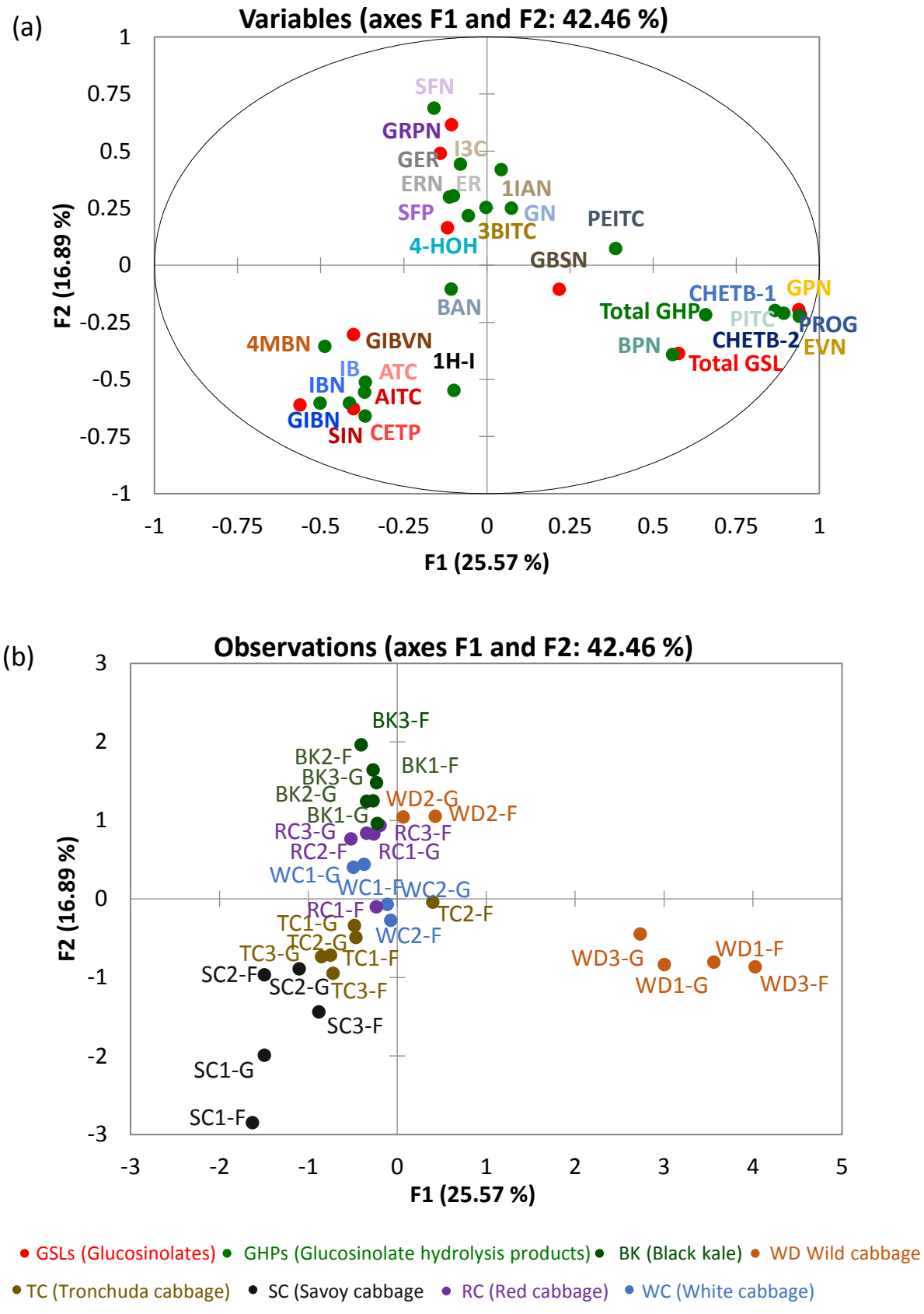


Figure 3.3. MFA map of glucosinolates and glucosinolate hydrolysis products (a) distribution of variables and (b) sample distribution.
For codes and distribution on plot refer to Table 3.1 (varieties) and Table 3.2 and Table 3.3 (compounds). Compounds with different shades of the same colour in Fig 3.3(a) refer to the GSL and corresponding GHP.

3.4 Conclusion

The profile and concentrations of GSLs and GHPs was influenced by growing conditions, cabbage types and varieties. The amounts and types of compounds accumulated differed between varieties, within and across cabbage types. While genetic factors had more influence on the GSL profile of cabbage, differences in the GSL concentration were more affected by environmental factors during growth. Variations in the GSL and GHP contents imply differences in the health-promoting characteristics of the cabbages studied. Field grown cabbages had much higher GLSs and GHPs than glasshouse plants with a few exceptions (SC1 and RC3). However, the major differences observed was between cabbage types irrespective of the conditions under which they were grown. The result of this study suggest that cabbage type and variety might be a more important factor for GSLs and GHPs accumulation by plants rather than the conditions under which they are grown. Verkerk et al. (2009) stated that variations due to genetic differences is most important factor determining GSL concentrations. The difference in GSL and GHP concentrations could not be linked to morphology of head formation (closed heart or open leaf). All GSLs and their corresponding GHPs were identified in the varieties studied and a correlation between GSLs and GHPs was found.

Aliphatic GSLs, nitriles and epithionitriles were the most abundant compounds identified. The results suggest that consumption of raw cabbages may provide limited health benefits as more nitriles and epithionitriles are formed than the more beneficial ITCs. It is therefore recommended to process the cabbages in ways that ensure hydrolysis of GSL to ITCs rather than nitriles. Despite the high amounts of nitriles and epithionitriles formed overall, high amounts of health beneficial SFP was detected in some red and white cabbages. The result suggests that some gene bank varieties can be a good source of beneficial compounds and could be used in breeding programmes to introgress areas of the genome from the gene bank varieties that regulate these compounds into elite commercial cultivars. This can also be helpful for selection of more beneficial varieties for commercial cultivation and production.

Chapter 4: The impact of domestic cooking methods on glucosinolates and their hydrolysis products in different cabbage (*Brassica oleracea*) varieties

Status: This chapter has been written in the style of a research paper and will be submitted to Food Chemistry.

Abstract

Glucosinolate hydrolysis products are responsible for the health promoting properties of *Brassica* vegetables. The impact of domestic cooking on glucosinolates and its hydrolysis products in 18 cabbage varieties was investigated. Cabbages were steamed, microwaved and stir-fried. Cooking significantly affected the concentrations of glucosinolates in cabbage. Stir-frying resulted in the greatest decrease in glucosinolate concentration resulting in up to 70 % loss. Steamed cabbages retained the most glucosinolates after cooking. The types and amounts of glucosinolate hydrolysis products detected varied across all cooking methods studied. Cooking reduced the amounts of nitriles and epithionitriles formed. Steaming led to a significant increase in the concentration of beneficial isothiocyanates present in the cabbage and a significantly lower level of nitriles compared to other samples. Microwaving led to reduction in concentrations of both nitriles and isothiocyanates. The level of glucosinolate loss and resulting levels of glucosinolate hydrolysis products varied across cabbage types and varieties. The result obtained in this study suggest that mild cooking of cabbage may lead to the he most desirable nutritional profile of cabbage.

4.1 Introduction

Consumption of *Brassica* or cruciferous vegetables such as cabbage (*Brassica oleracea*) is reported to result in chemo-protective effects (Herr & Buchler, 2010). This has been attributed to the high amounts of the glucosinolates (GSLs) they contain. GSLs are hydrolysed by endogenous myrosinase into isothiocyanates (ITCs), thiocyanates, nitriles or epithionitriles (EPTs), depending on the conditions of the reaction. Nitriles and EPTs are formed in the presence of epithiospecifier proteins (ESP) instead of the more beneficial ITCs (Matusheski et al., 2006). ITCs such as sulforaphane (SFP) and erucin (ER) are particularly reported to be responsible for the health promoting properties of *Brassica*s (Mithen et al., 2000).

Cooking cabbage can result in total or partial ESP and myrosinase inactivation, which in turn influences the type of glucosinolate hydrolysis products (GHPs) formed. The time and temperature of cooking, vegetable matrix and degree of tissue damage all influence the changes observed during cooking (Dekker et al., 2000). Previous studies on GSL concentrations in cooked cabbage showed conflicting results. Some authors have reported loss of myrosinase activity as a result of domestic cooking leading to lower levels of ITCs detected (Verkerk & Dekker, 2004; Oerlemans et al., 2006). The same authors also reported increase in GSL content after microwaving cabbage. Rungapamestry et al. (2006); Song & Thornalley (2007) and Xu et al. (2014) reported minimal losses or no change in GSL concentration after steaming and microwaving cabbage. Xu et al. (2014) recorded 77 % loss in GSL concentration after stir-frying. The variation in results can be attributed to different cooking conditions and size of cut cabbage pieces and in most cases, do not represent standard domestic ways of cooking cabbage.

There are very few studies on the effect of cooking on GHP formation in cabbages and the studies have focused on single GSLs and GHPs, or just ITCs (Rungapamestry et al., 2006; Song & Thornalley, 2007; Ghawi et al., 2013). However, because of the changes that occur in the GSL-myrosinase system during cooking, it is important to analyse the effect of cooking on both GSL and GHPs to get a better picture of the reactions that occur during cooking.

In this study, the effect of steaming, microwaving and stir-frying on the GSLs and GHPs on 18 cabbage varieties was investigated. Cooking times were chosen to represent standard domestic practices. It was hypothesised that mild cooking conditions will result in minimal GSL loss while increasing production of health beneficial ITCs. It was also hypothesised that

the stability of GSLs and production of GHPs would vary across different cabbage varieties and types.

4.2 Materials and methods

4.2.1 Plant material

The cabbages used for the study were sourced from the University of Warwick Crop Centre Genetic Resources Unit (Wellesbourne, UK) and grown as previously described and present in Chapter 3. Only field grown cabbages were used for this study. See Table 3.1 (Chapter 3) for full list of varieties.

4.2.2 Reagents and chemicals

All chemicals used were purchased from Sigma–Aldrich (UK) unless stated otherwise.

4.2.3 Cabbage thermal processing

Cabbages were cleaned and prepared as previously described in Chapter 2. Briefly, central core and outer leaves of 4-5 cabbage heads were removed and discarded. Cabbages were chopped into pieces of approximately 1 cm in width using a kitchen knife (representing how cabbages will normally be sliced by consumers), mixed and washed under running tap water with excess water drained using a salad spinner (OXO Good Grips Clear Manual Salad Spinner).

Cabbages were either steamed, microwaved or stir-fried using the methods described by Rungapamestry et al. (2006) and Rungapamestry et al. (2008b) with slight modifications as previously described in Chapter 2. Unprocessed cabbage samples served as control. Cooking methods were chosen to represent common ways of cooking cabbage. Time and temperature combinations used for each method was based on a preliminary consumer study with 60 participants to determine consumer acceptability of the samples as steamed, microwaved and stirfried cabbage (data not shown). These conditions were deemed acceptable with a mean score of between 2.7 to 3.3 on a 5-point 'just about right' scale.

Samples were put into sterile sterilin tubes immediately after cooking, placed on ice and transferred to a -80 $^{\circ}$C freezer. Frozen samples were freeze-dried (Stokes freeze drier, Philadelphia USA), ground using a tissue grinder (Thomas Wiley® Mini-Mill, Thomas Scientific, USA) and stored at -20 $^{\circ}$C until further analysis.

4.2.4 Glucosinolate and glucosinolate hydrolysis products analysis

GSLs and GHPs were extracted following the method described by Bell et al. (2015) and Bell et al. (2017c) respectively as described in Chapter 3. GSLs extracted with 70 % methanol, analysed by LC-MS/MS and quantified using sinigrin hydrate standard. Five concentrations of sinigrin hydrate (0.22-3.5 mg/mL) was prepared with 70 % methanol and used to prepare an external calibration curve (r^2 = 0.942). Compounds were identified using their mass parent ion, characteristic ion fragments as well as comparing with ion data from literature (Table 3.2; Chapter 3).

GHPs were extracted using dichloromethane and analysed by GC-MS. Compounds were identified using literature ion data (Table 3.3; Chapter 3) and quantified based on an external standard calibration curve. Five concentrations (0.25–2 mg/mL) of sulforaphane standard (Sigma Aldrich, UK) were prepared in DCM (r^2 = 0.99). Data analysis was performed using ChemStation for GC-MS (Agilent).

4.2.5 Statistical analysis

Results are the averages of three processing replicates and two technical replicates (n= 6). Data obtained were analysed using 2- way ANOVA (with Tukey's HSD multiple pair wise comparison test) and principal component analysis (PCA) and multifactor analysis (MFA) performed in XLSTAT (Addinsoft, Paris, France) to visualise the data in a minimum number of dimensions (two or three).

4.3 Results and discussion

4.3.1 Effect of domestic cooking GSL profile and concentration

GSL concentrations for all samples before and after cooking are presented in Figure 4.1 with significant differences within and between cabbage types presented in Appendix IX (Table S4a). White cabbage variety, WC3 did not survive on the field. GSL type and concentrations varied across varieties within and between cabbage types. Five to nine individual GSLs were identified within all cabbages studied; seven aliphatic GSLs namely sinigrin (SIN), gluconapin (GPN) and epi/progoitrin (PROG), Glucoibeverin (GIBVN), glucoerucin (GER), glucoiberin (GIBN) and glucoraphanin (GRPN) and two indole GSLs: glucobrassicin (GBSN) and 4-hydroxyglucobrassicin (4-HOH) (Table 3.2). As discussed in chapter 3, Black kale (BK) varieties had the least number of GSLs identified (five) while nine GSLs were identified in red (RC) and white (WC) cabbages. GBSN and 4-HOH where the only GSLs that occurred in all varieties and types. Total GSLs differed significantly between varieties

($p<0.0001$), between cooking method ($p<0.0001$) and the interaction between the two was significant ($p<0.0001$). Aliphatic GSLs were the most abundant GSLs in all varieties, making up about 80 % of total GSLs.

Cooking significantly reduced GSL concentration in all cabbage samples. GSL stability varied across varieties and cooking methods studied. GIBN was the least stable GSL resulting in an average loss of 59 % across all varieties. However, GIBN loss varied largely between varieties with tronchuda variety TC1 recording a loss of up to 83 % and savoy SC1 as low as 14 %. Results agree with those reported by Oerlemans et al. (2006) and Dekker et al. (2009) who report variation in GSL stability between GSLs and variations in stability of the same GSL across different *Brassica* vegetables. In a previous study, concentrations of GIBN (aliphatic GSL) and GBSN in white cabbage were found to decrease significantly during cooking due to their high leaching potential into the cooking water (Rosa & Heaney, 1993; Ciska & Kozłowska, 2001).

Total GSLs in steamed cabbage ranged between 16.9 $mg.g^{-1}$ DW (BK3) to 136 $mg.g^{-1}$ DW (WD3). There was a significant difference in GSL concentrations of steamed cabbages across varieties and between varieties of the same cabbage type. The differences observed were mostly due to initial GSL concentration of the raw samples rather than the steaming process. In relation to residual GSL content of cabbage samples after steaming, steamed WC1 had the most stable total GSL retaining up to 97 % GSL concentration while the biggest loss of total GSL was in steamed SC3 where up to 56 % loss was recorded. In some varieties, steaming did not affect the concentrations of some individual GSLs; for e.g., SIN and PROG in WD3 and WC1 respectively. There was a significant ($p<0.0001$) reduction in individual and total GSL content for all samples, except for GPN which did not differ significantly ($p=0.32$) from raw to cooked samples. Stability of individual GSLs varied greatly between varieties within and between cabbage types. For example, in BK samples, loss of GRPN did not differ in all three varieties (8 – 10 %) while in TC samples, steaming led to between 45 % (TC2) to <1 % (TC1) loss of GRPN content. Previous studies reported no loss (Jones et al., 2006; Rungapamestry et al., 2006; Song & Thornalley, 2007; Gliszczyńska-Świgło et al., 2006) or minimal losses (Vallejo et al., 2002; Francisco et al., 2010; Xu et al., 2014) in broccoli, turnip and cabbages. Xu et al. (2014) reported a loss of about 15 % in steamed red cabbage, however the large sample size (3 cm cubes) may have caused lower losses in comparison to the present study. Similar to the current study, Vallejo et al. (2002) reported losses in some individual GSL (GRPN) and no loss in others (GIBN) after steaming for 3.5 minutes. The minimal losses

reported in steamed samples has been attributed to low levels of leaching into cooking water that are normally reported under boiling conditions (Dekker et al., 2000).

In microwaved samples, total GSL varied between 11. 4 $mg.g^{-1}$ DW (BK3) to 120 $mg.g^{-1}$ DW (WD3). Microwaving significantly affected the amount of GSLs in cabbage samples with reductions up to 76 % of GRPN in TC1 and residual total GSL varying between 50 % to 93 %. Microwaving led to significantly lower GSL concentrations when compared to raw cabbages. As in steamed samples, effect of microwaving differed between varieties and individual GSLs. Some GSLs were more stable than others in certain varieties within and between cabbage types. High core temperatures (85-95 °C) of microwaved samples led to myrosinase inactivation (see chapter 2) which could have prevented GLS hydrolysis during the microwave process and can account for high retention of GSL concentrations of some microwaved cabbages. There are several conflicting reports on the effect of microwaving on GSL contents in *Brassica* vegetables. Song & Thornalley (2007) and Xu et al. (2014) reported no significant difference in GSL concentration after microwaving green and red cabbage samples for three and five minutes respectively. The authors stated that the stability of GSL might be due to myrosinase inactivation and absence of water during microwaving prevented GSL leaching into cooking water. The large size of the shredded cabbage pieces in the two studies may also have reduced loss of GSLs. A study on broccoli resulted in 74 % decrease of total GSL content after microwaving and was attributed to leaching in water and more intense microwave conditions (150 g broccoli to 150 g water and microwaving for 5 min at 1000 W power) (Vallejo et al., 2002). However, a contrary result was observed by Verkerk & Dekker (2004) and Oerlemans et al. (2006) who reported an increase of up to 78 % and 35 % respectively in GSL concentrations after microwaving of red cabbage though the increase was not significant in the Oerlemans et al. (2006) study due to large sample variability. The authors attributed the increase to enhanced extractability of GSL after microwaving which can be more of an analytical artefact than an actual increase in GSL concentration.

Stir-frying led to significant decrease in total and individual GSL content of cabbages. Total GSL ranged between 10.5 $mg.g^{-1}$ DW (BK3) to 101 $mg.g^{-1}$ DW (WD3). There was a significant difference in GSL concentrations between varieties, within and between cabbage types. Residual total GSL varied between 27 % (SC3) – 82 % (SC1). The highest loss of aliphatic individual GSLs concentration was recorded in stir-fried TC1 where there was a decrease of between 79 – 83 %. Indole GLSs, GBSN and 4-HOH were the most stable GSL in stir-fried cabbages. Relative thermostability of individual GSLs (if under the same conditions of

myrosinase level and stability) can be influenced by their chemical structure and has been reported to vary with heating temperature (Wathelet et al., 1996; Oerlemans et al., 2006). Among all the cooking methods studied, stir-frying resulted in significantly greater losses of GSL than steaming or microwaving which is in agreement with previous reports. A study on the effect of different types of cooking oil on GSLs in stir-fried broccoli resulted in up to 49 % loss irrespective of the cooking oil used (Moreno et al., 2007). Xu et al. (2014) also reported 77 % loss in GSL concentration after stir-frying of red cabbage while there was no significant loss in GSL content when green cabbage was stir-fried for 5 mins (Song & Thornalley, 2007). The difference in leaf structure may have influenced GSL stability in green cabbage. Green cabbage can have thicker leaves with more uneven surface texture that may create microclimates around the leaf during the cooking process. It is hypothesized that losses due to stir-frying can be attributed to substantial moisture evaporation. During stir-frying, cabbage loses moisture and GSLs are leached into the moisture which evaporates during the cooking process. A study conducted by Adler-Nissen (2002) showed that when carrot cubes were stir-fried, despite temperatures only reaching 70 °C, a high evaporation loss was observed. Another possible reason for lower GSL amounts in stir-fried cabbages can be attributed to GSL hydrolysis by myrosinase. The lower core temperatures (65 °C - 70 °C) of stir-fried cabbages, resulted in higher myrosinase stability of the samples compared to steamed and microwaved cabbages (Chapter 2). The relative stability of aliphatic GSLs to indoles varied between varieties, but generally indole GSLs were more stable than aliphatics.

In summary, WD1 variety had the most stable individual and total GSLs while GSLs of SC3 were the most thermolabile across all cooking methods despite having one of the highest GSL concentration in the raw sample. Different varieties of the same cabbage type can vary in their GSL stability during cooking resulting in large differences in GSL content lost between species. The rate and extent of loss is dependent on the type of cabbage, cooking time and temperature, amount of moisture, and initial concentration of GSL (Ciska & Kozłowska, 2001; Jones et al., 2006). The variation of residual GSL in the cabbages will have an impact on the amounts of GHPs produced.

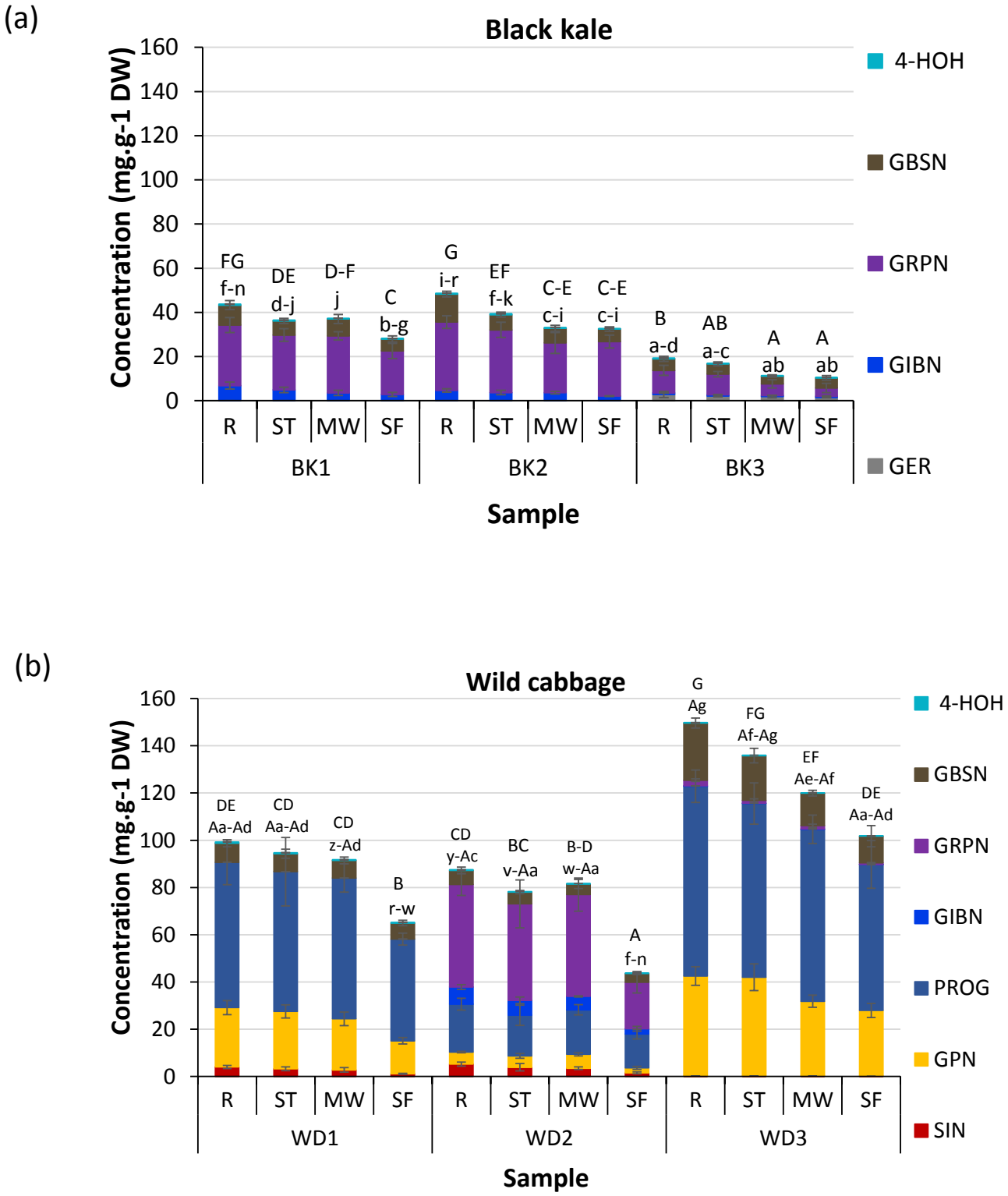
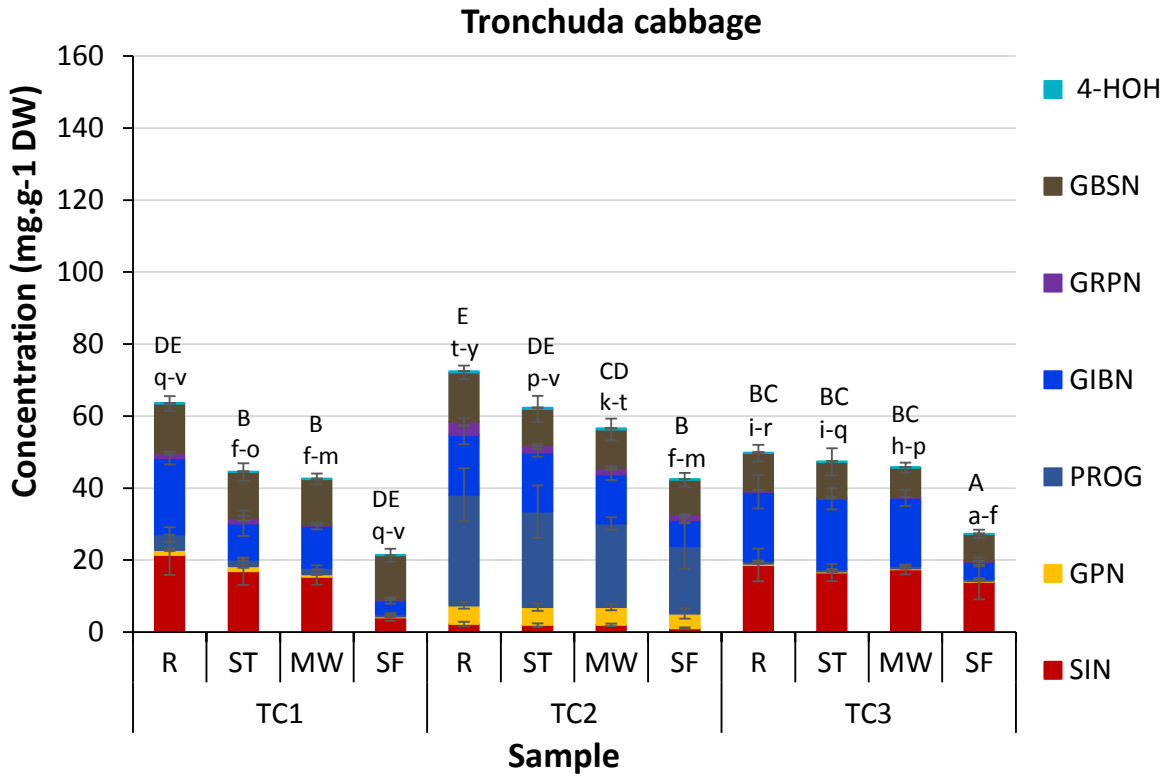


Figure 4.1. Glucosinolate (GSL) concentrations (mg.g^{-1} DW) of cabbages.
Error bars represent standard deviation from mean values. Letters above bars refer to differences in total GSL concentration. Letters 'ABC': bars not sharing a common letter differ significantly ($p<0.05$) between varieties and cooking conditions within a cabbage type (i.e. within each separate graph). Letters 'a,b,c – Ag': bars not sharing a common letter differ significantly ($p<0.0001$) between cabbage types, varieties and cooking conditions (i.e. between the separate graphs). Abbreviations: R = raw, ST = steamed, MW = microwave, SF = stir-fried; SIN, sinigrin; GPN, gluconapin; PROG, epi/progoitrin; GIBVN, Glucoibeverin; GER, glucoerucin; GIBN, glucoiberin; GRPN, glucoraphanin; GBSN, glucobrassicin; 4- HOH, 4-hydroxyglucobrassicin. For full names of cabbage varieties see Table 3.1.

(C)



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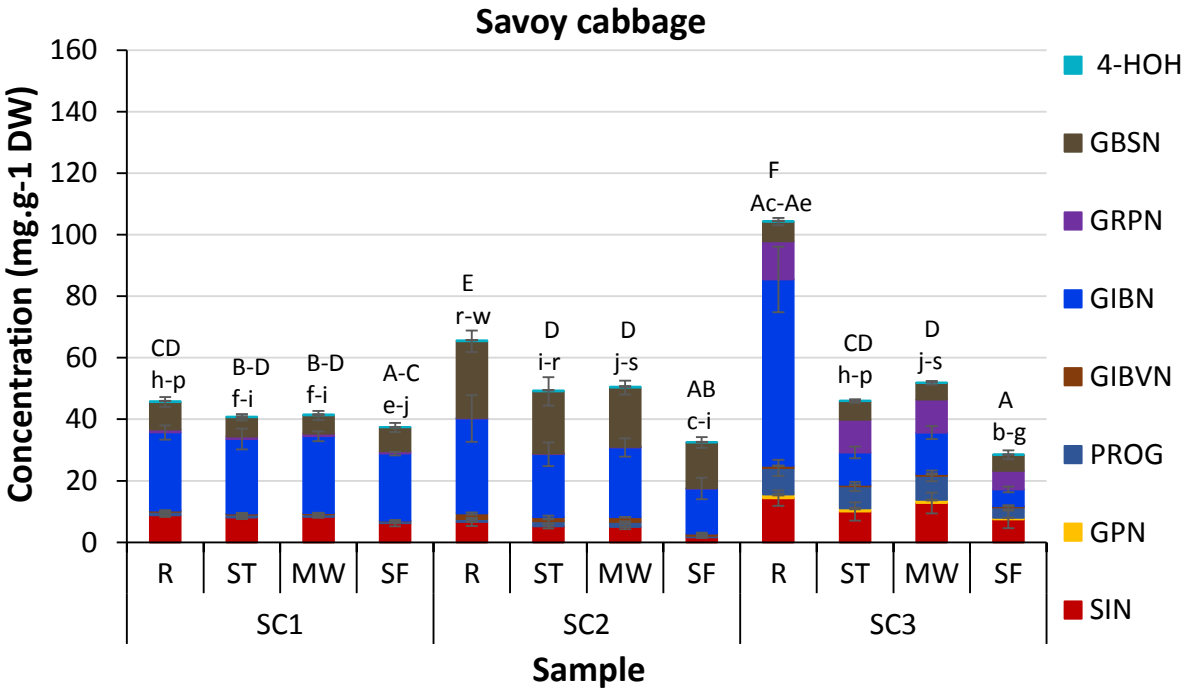
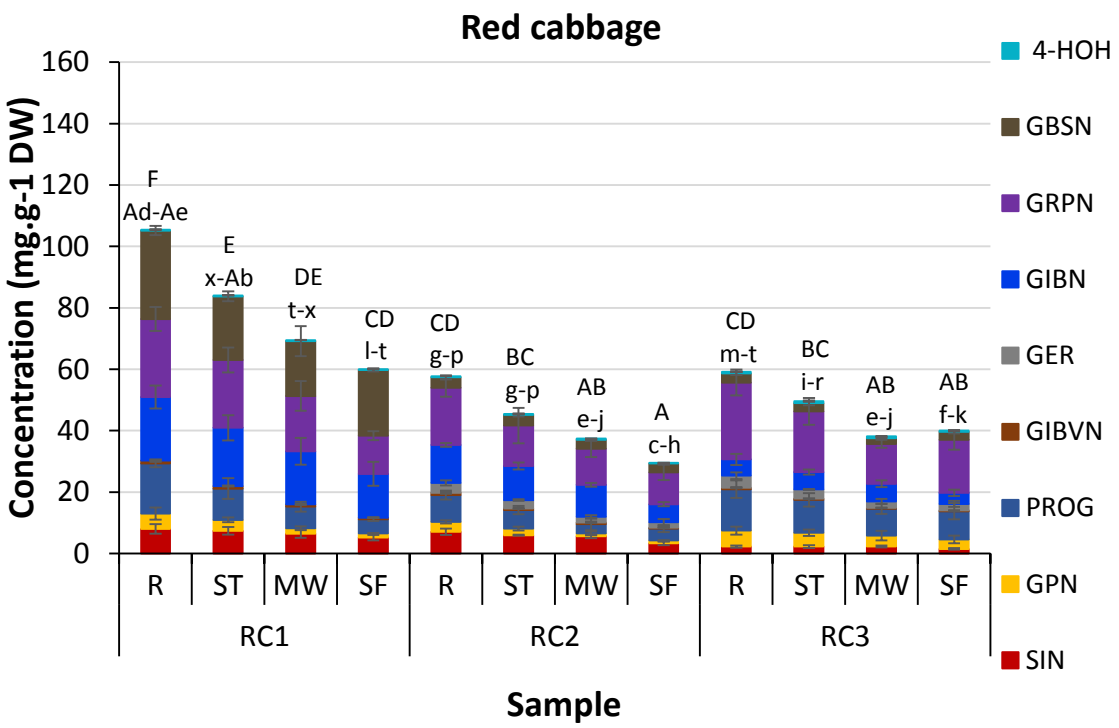


Figure 4.1 – continued.

(e)



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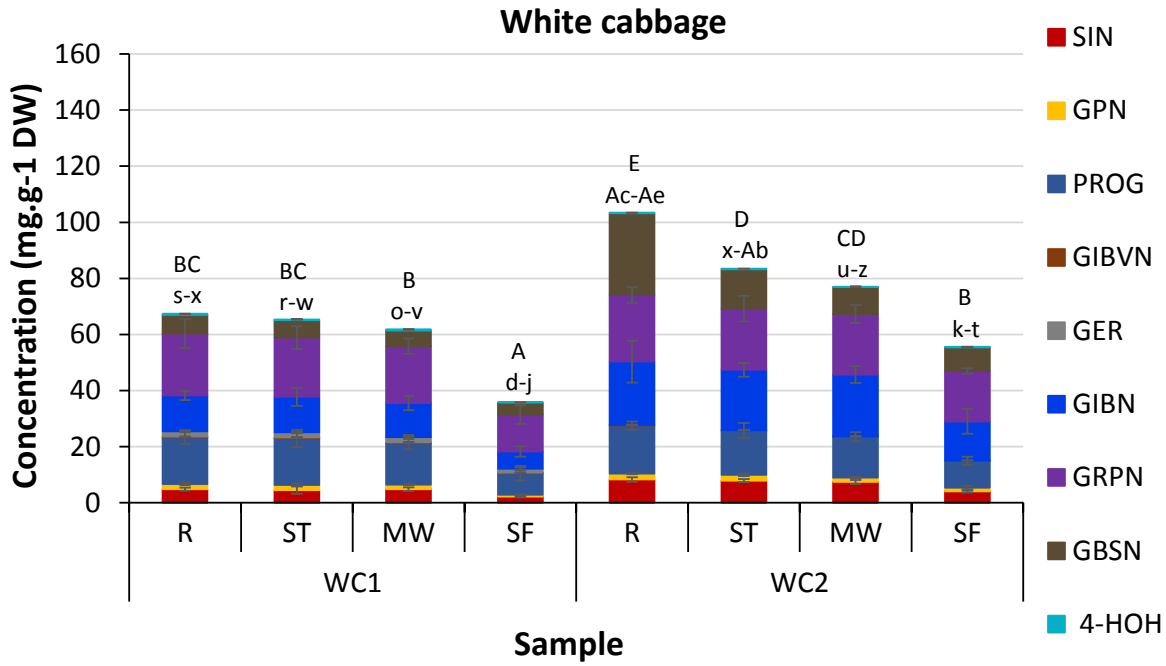


Figure 4.1 – continued

4.3.2 Effect of domestic cooking on GHP profile and concentration

The profile and concentration of GHPs resulting from cooking cabbage is presented in Figure 4.2 and statistics in Appendix IX (Table S4b). 24 different GHPs were detected as a result of GSL hydrolysis during cooking. Variety, cooking method and interaction between the two significantly influenced the type and concentration of GHPs. Total GHPs across all varieties and cooking methods ranged between 25.3 $\mu g.g^{-1}$DW (TC3-MW) to 1186.9 $\mu g.g^{-1}$ DW (WD1-R). In raw samples, GSL hydrolysis led to production of majorly nitriles and epithionitriles. Matusheski & Jeffery (2001) and Mithen et al. (2003) in their studies of fresh and freeze-dried raw broccoli found that GRPN hydrolysis led to formation of primarily SFN than its ITC, SFP. In most varieties, raw (R) and stir-fried (SF) cabbages had the highest total GHPs in all samples; apart from red and white cabbage varieties where the highest total GHPs was recorded in steamed (ST) cabbages. BK samples had the lowest GHPs identified which can be related to the lower number of individual GSL present in the variety. However, some GHPs were identified where intact GSL was not detected and this occurred across all varieties tested. In BK and SC varieties, 3BITC was detected in cooked samples though intact GPN was not present. A similar trend was noticed by Bell et al. (2017c) who found 3BITC in rocket samples in the absence of GPN. The presence of 3BITC might be the result of SFP degradation. A study conducted on broccoli showed standard SFP solution was degraded to 3BITC under thermal conditions (Chiang et al., 1998). PEITC and BPN, hydrolysis products of gluconasturtiin were detected in low amounts across all varieties though intact gluconasturtiin was not detected in samples. The small amounts detected suggest that that the GSL was present in low amounts in the sample and may have been hydrolysed during sample preparation, or amounts present was too low for the analytical procedure to pick up.

Cooking significantly reduced the amount of nitriles and EPTs formed, and increased the amount of ITCs present. GN, IB and SFP were the major GHPs in cooked cabbage. Of all the cooking methods, microwaved samples had the lowest levels of GHPs; little or no nitriles and EPTs were detected while very low amounts of ITCs were also formed. Though in most cases, more ITCs were still formed in microwaved samples than raw ones. On the contrary, highest concentrations of ITCs were formed in steamed samples across all varieties, with increase in ITCs formed as much as 23-fold more than raw samples (SFP in RC3-ST) with none or small amounts of nitriles present. In most samples, total and individual GHPs did not differ significantly between stir-fried and raw samples, though higher amounts of ITCs were formed in stir-fried samples. The pattern of GHP formation did not differ across varieties.

Differences in severity of cooking methods which may have influenced residual myrosinase activity in relation to ESP activity, can account for the difference in types and concentration of GHPs present. ESP promotes formation of nitriles and EPTs from GSL hydrolysis instead of ITCs from myrosinase (Matusheski et al., 2006). Stir-frying cooking temperature was the least severe, leading to formation of EPTs, nitriles and ITCs - as ESP and myrosinase would have still been active in the samples. Lower amounts of GSL detected in stir-fried cabbages did not seem to affect total GHPs but might have be partly responsible for the higher amounts of nitriles formed as GSL was hydrolysed by ESP present in samples during the stir-frying process. Microwave cooking was the most severe cooking method employed and is responsible for the tiny amounts or absence of nitriles and low amounts of ITCs in microwaved cabbages. High core temperatures during microwaving (85 – 95 °C) would have led to complete denaturation of ESP and almost total myrosinase inactivation (see Chapter 2). On the other hand, the steaming temperature was just enough to denature ESP while still retaining substantial myrosinase activity, as reported in Chapter 2. The nitriles detected in both microwaved and steamed samples may have been formed with residual ESP present during the cooking process, while ITCs present in microwaved samples could be the result of residual myrosinase activity. In cooked broccoli, ESP was found to be denatured at temperatures above 50 °C with corresponding reduction in SFN production (Matusheski et al., 2006). Rungapamestry et al. (2006) in their study of SIN hydrolysis products in cooked cabbage, found that microwaving for 120 secs resulted in reduction of nitriles, allyl cyanide and CEP (about 87 %) with increase in AITC formation (about 88 %). The authors found that steaming cabbages for seven minutes resulted in increase in AITC of up to 578 %. The authors also found that AITC was formed in cabbages with no residual myrosinase activity and attributed it to formation during the hydrolysis and cooking process, which may have been bound to the cell membranes but released during processing.

The results obtained in this study are similar to that observed by several authors during thermal processing of *Brassica* vegetables (Matusheski et al., 2004; Rungapamestry et al., 2006; Song & Thornalley, 2007; Jones et al., 2010; Ghawi et al., 2013). This study adds to the findings of previous researches; however, the study is particularly conclusive as it demonstrates similar findings across cabbage types and varieties. The increased ITCs formed in steamed cabbages can improve the health benefits derived from steamed cabbage consumption.

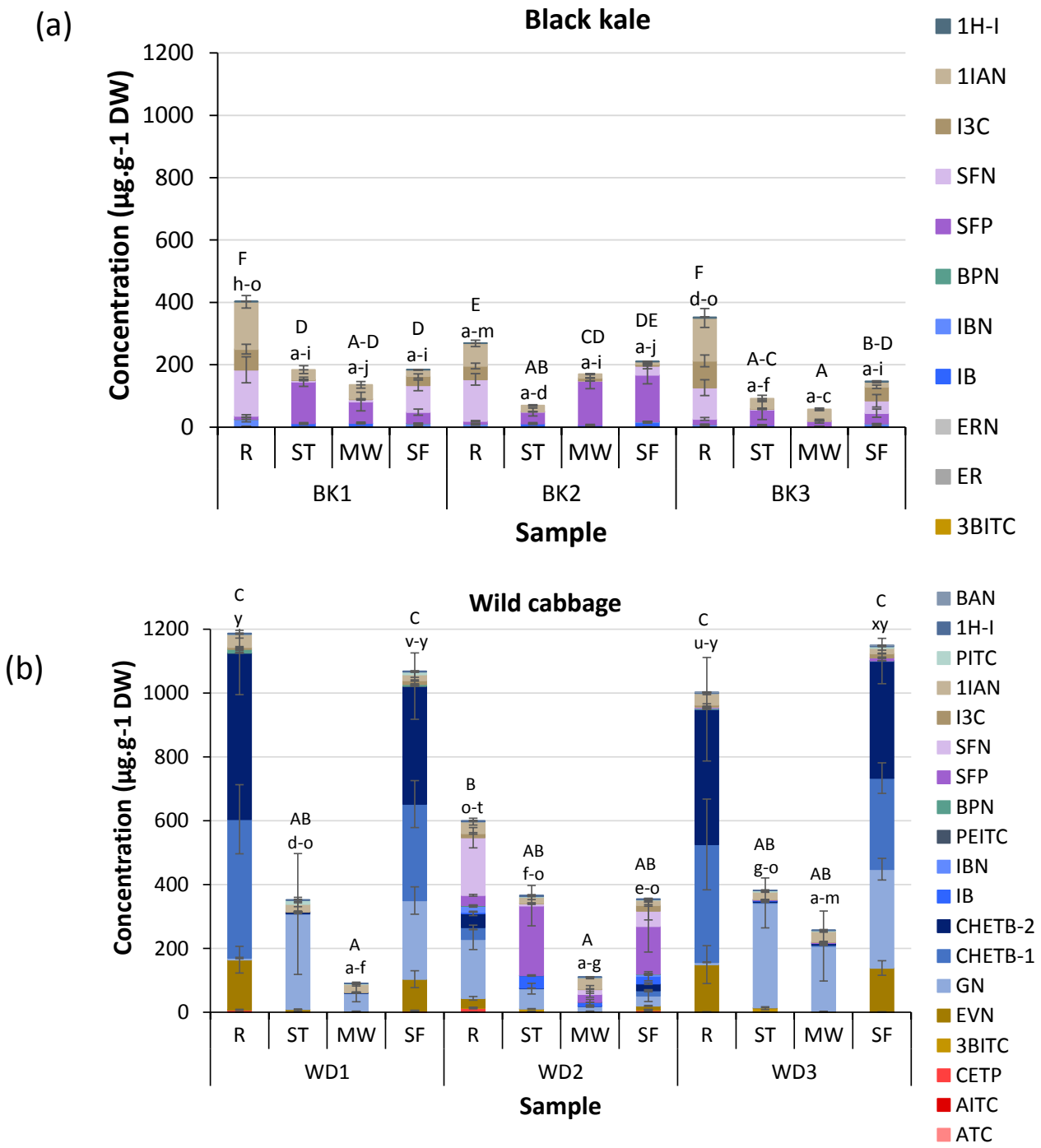
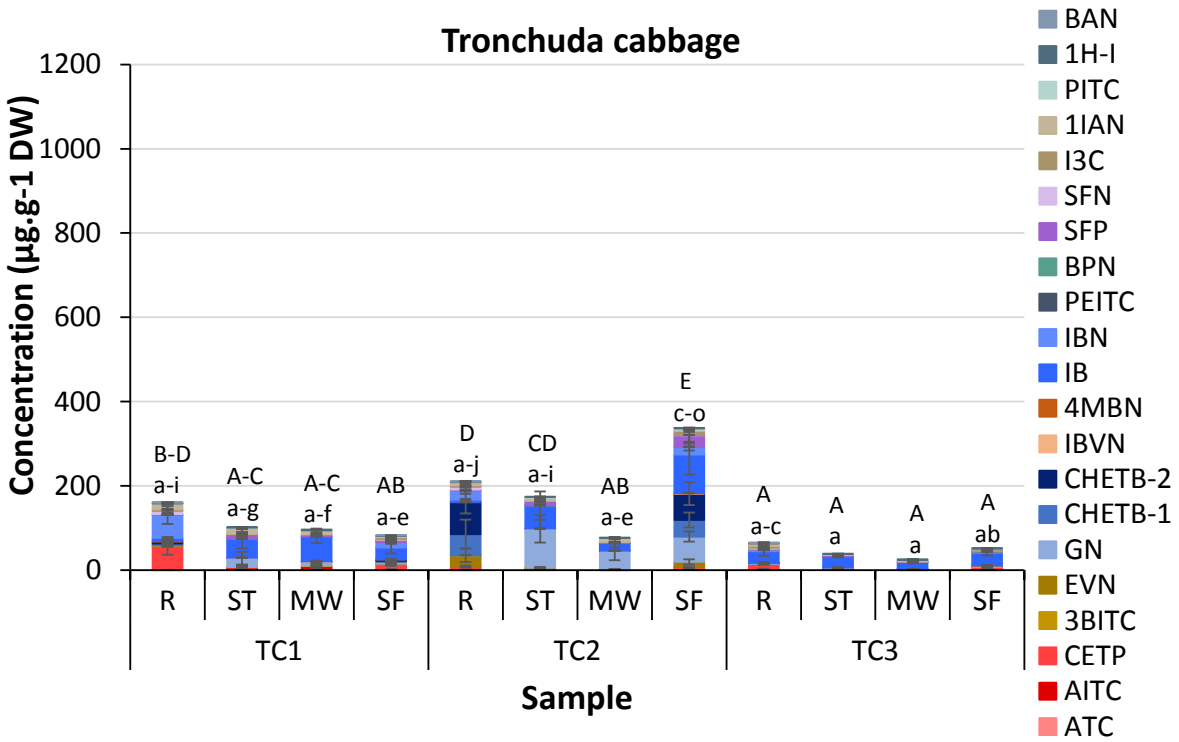


Figure 4.2. Glucosinolate hydrolysis products (GHPs) (µg.g-1 DW) of cabbages.
Error bars represent standard deviation from mean values. Letters above bars refer to differences in total GSL concentration. Letters 'ABC': bars not sharing a common letter differ significantly ($p<0.05$) between varieties and cooking conditions within a cabbage type (i.e. within each separate graph). Letters 'a,b,c – Ag': bars not sharing a common letter differ significantly ($p<0.0001$) between cabbage types, varieties and cooking conditions (i.e. between the separate graphs). Compounds with colour shades similar to one another are GHPs of corresponding GSL in Fig 4.1. Abbreviations: R = raw, ST = steamed, MW = microwave, SF = stir-fried. ATC, allyl thiocyanate; AITC, allyl isothiocyanate; CEPT, 1-cyano-2,3-epithiopropane; 3BITC, 3-Butenyl-ITC; EVN, 4,5-epithiovaleronitrile; GN, goitrin; CHETB-1, 1-cyano-2-hydroxy-3,4-epithiobutane isomer 1; CHETB-2, 1-cyano-2-hydroxy-3,4-epithiobutane isomer 2; IBVN, Iberverin; 4MBN, 4-methylthiobutyl nitrile; ER, erucin; ERN, erucin nitrile; IB, iberin; IBN, iberin nitrile; PIETC, 2-phenylethyl-ITC; BPN, benzenepropanenitrile; SFP, sulforaphane; SFN, sulforaphane nitrile; I3C, indole-3-carbinol; 1IAN, indoleacetonitrile; PITC, Pentyl-ITC ; 1H-I, 1H-Indole; BAN, benzeneacetonitrile. For full names of cabbage varieties see Table 3.2 (Chapter 3).

(c)



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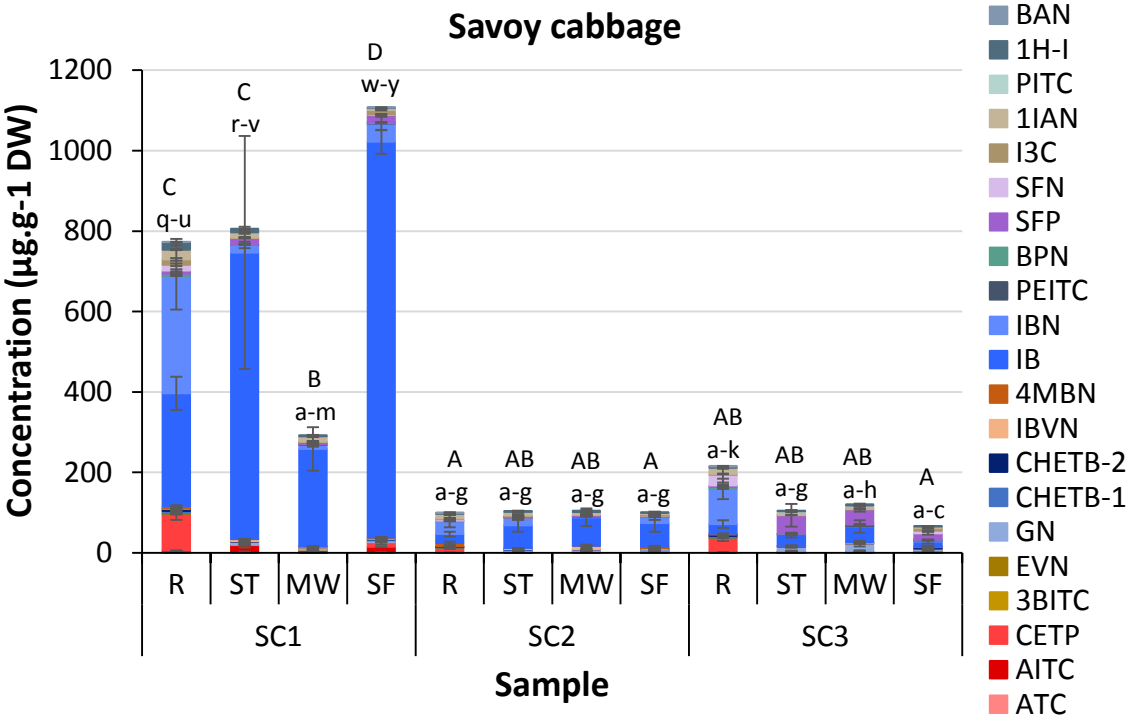
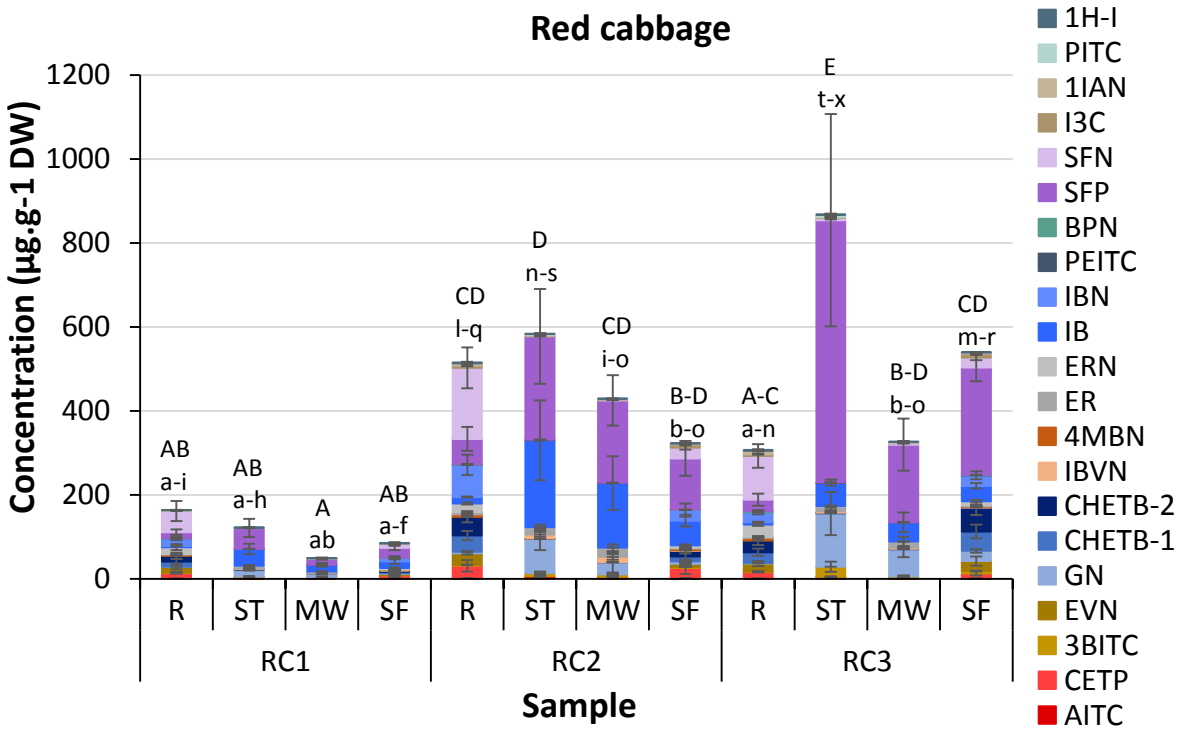


Figure 4.2 – continued.

(e)



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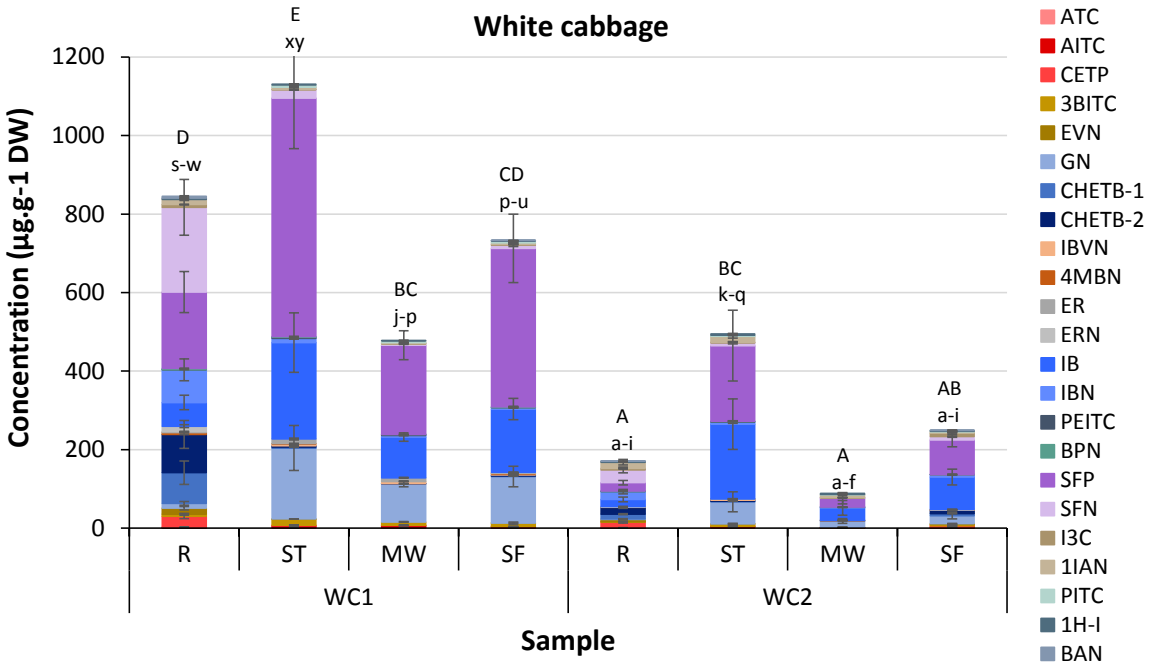


Figure 4.2 – continued

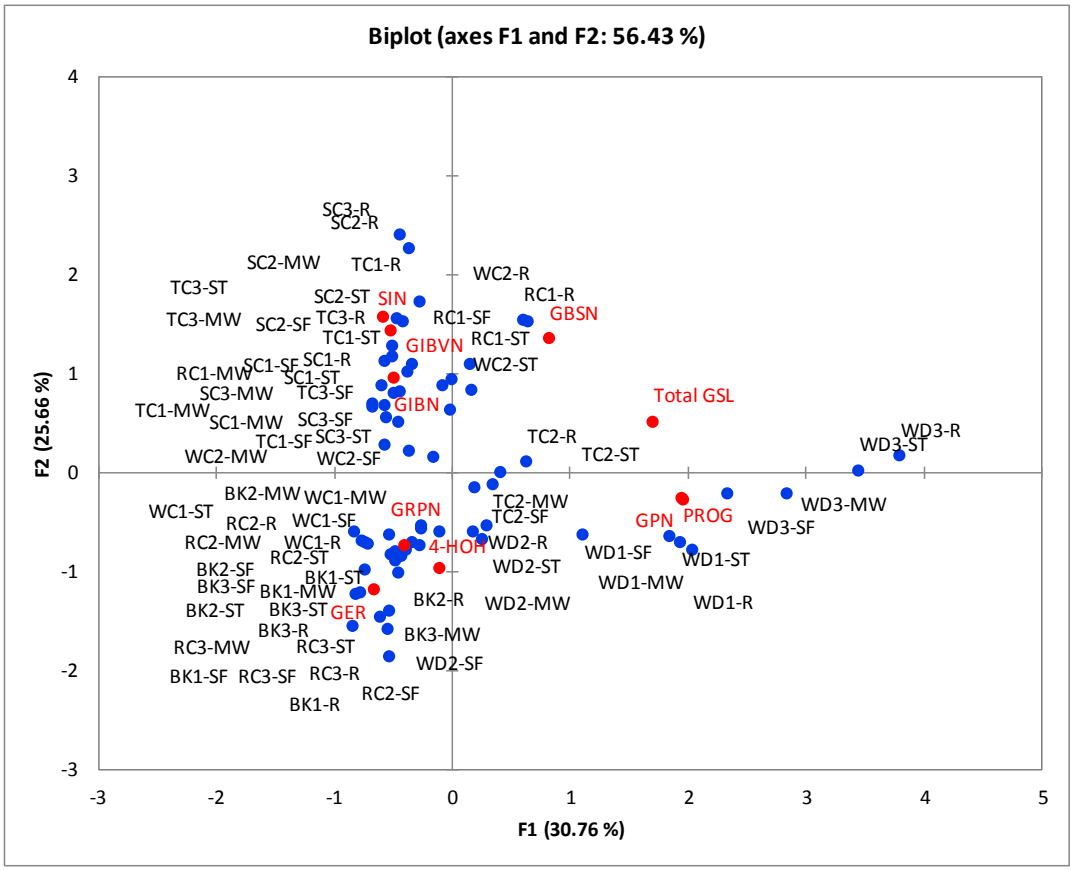
4.3.3 PCA and MFA analysis of GSLs and GHPs in cooked cabbage

To differentiate samples based on their GSLs and GHPs content, PCA analysis was conducted as shown in Figure 4.3. Figure 4.3a shows the biplot for GSL distribution in samples, where PCs 1 and 2 account for 56.4 % of the observed variation. The plot shows TC2 and Wild cabbage varieties were characterized by high PROG and GPN contents, black kale and most red cabbages except for RC1 had a higher tendency to accumulate GRPN and GER. Savoy cabbages, RC1, TC1 and TC3 correlated positively with one another and was characterized by the amounts of SIN, GIBVN and GIBN they contain. Samples were separated based on cabbage type and variety rather than cooking methods. On the contrary, the PCA biplot for GHPs (Figure 4.3b) shows differentiations in samples based on cooking. PC1 and 2 explain only 39.6 % of the variations, however, other plots did not provide any new information. Steamed and stir-fried cabbages correlated positively with ITCs while nitriles correlated mostly with raw cabbages. There was no correlation observed in microwaved samples with GHPs probably due to the low amounts of nitriles and ITCs present in the samples. Samples were separated on the type and amounts of GHP they contained.

To get a better understanding of the results, MF was performed on the varieties in relation to their GSL and GHP concentrations as shown in Figure 4.4. PC1 and 2 represents only 34.30 % of the variations but other PCs did not provide additional information. The result observed is similar to that observed in the biplot of GSL. Samples were separated in the same pattern as with GSLs and was based on type and variety rather than cooking. Individual GSLs correlated with their corresponding GHPs.

The results show that cooking has a greater effect on GHPs than GSLs but when combined, samples were differentiated on their GSL content and the type of GHP present.

(a)



(b)

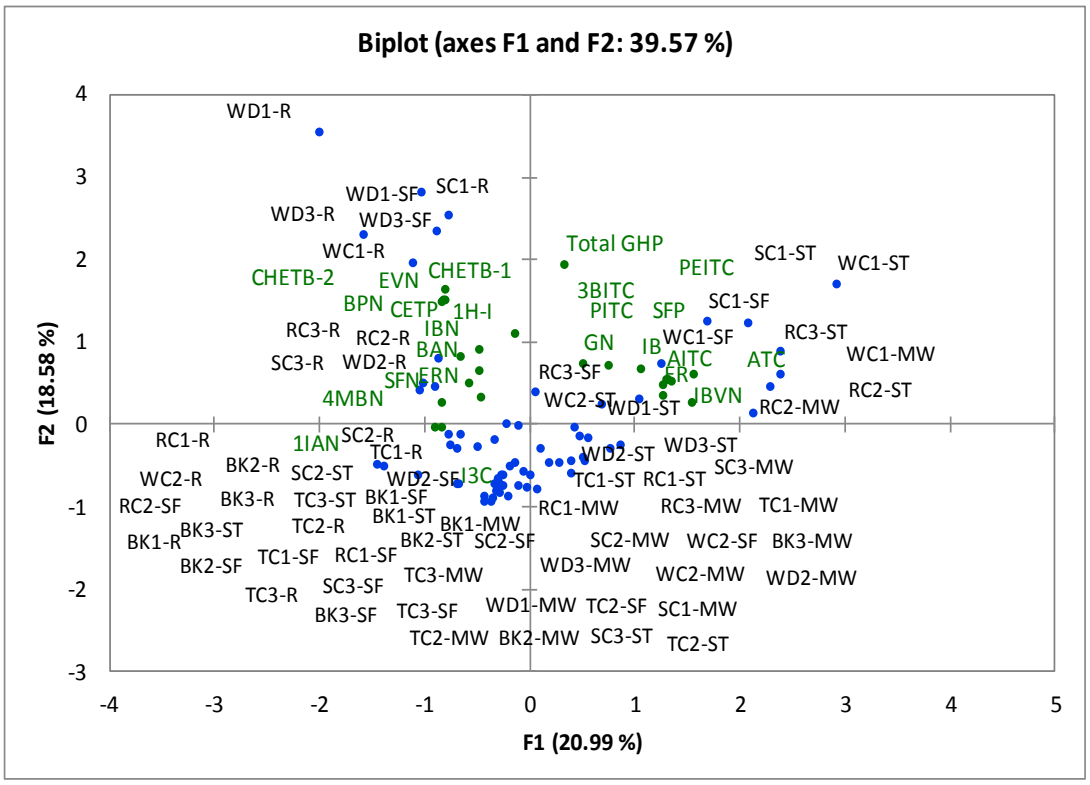
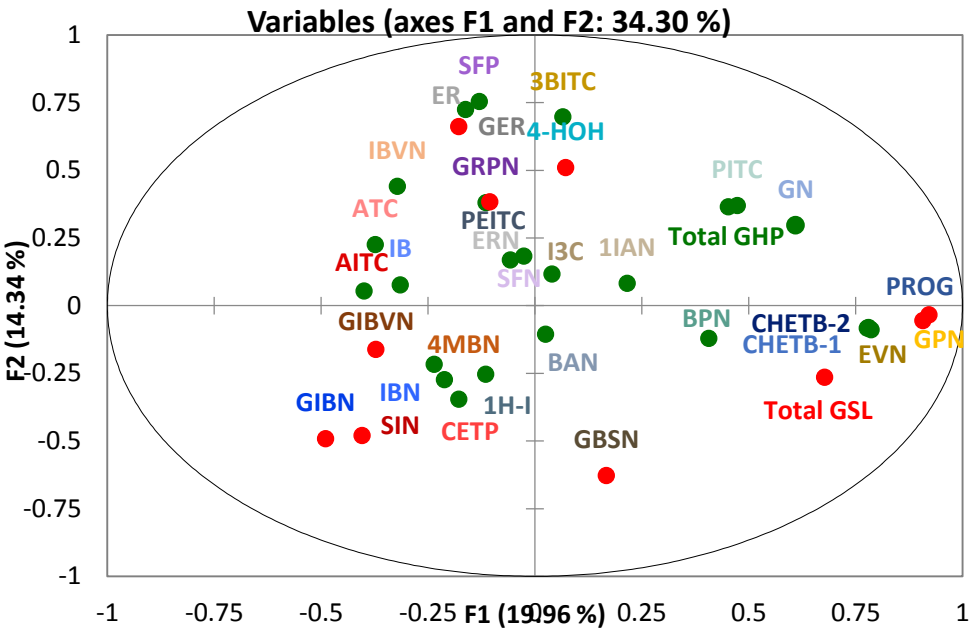


Figure 4.3. (a) PCA plot for samples tested and their relative distributions in relation to GSL concentrations. **(b)** PCA plot for samples tested and their relative distributions in relation to GHP concentrations.

For full names of samples and compounds see Table 3.1 and Table 3.2 and Table 3.3 respectively. Red = GSLs; Green = GHPs; Blue = Samples

(a)



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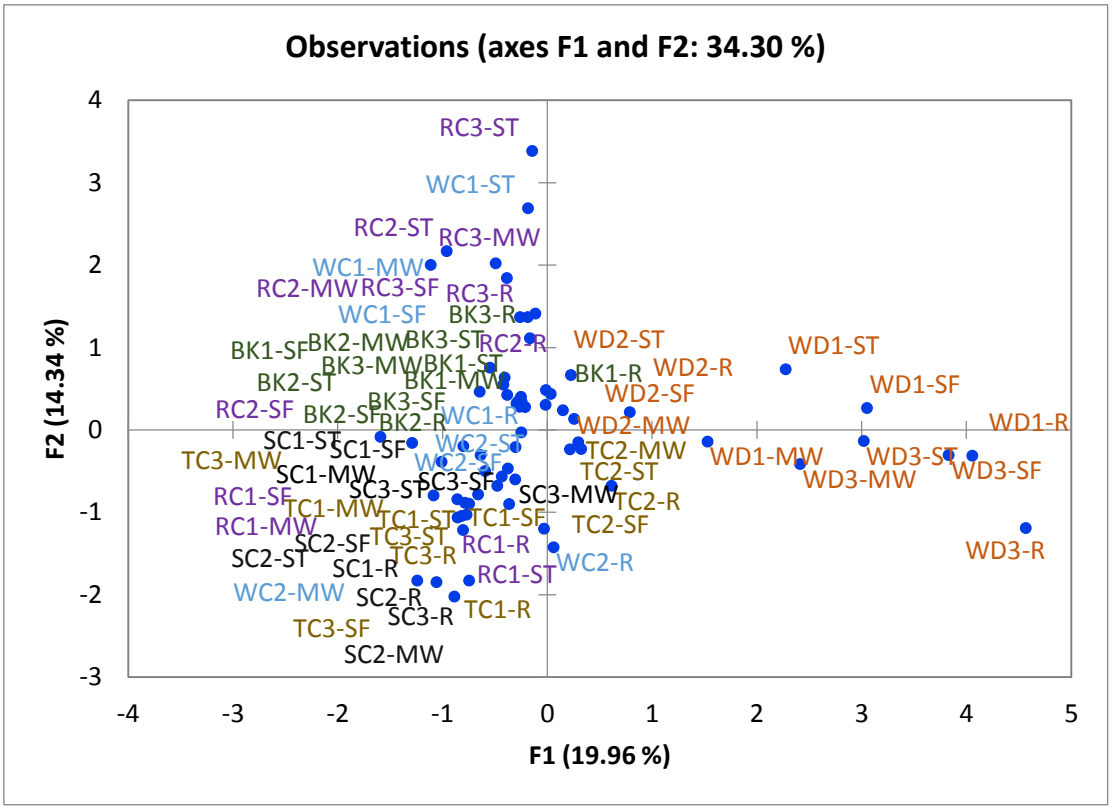


● GSLs (Glucosinolates) ● GHPs (Glucosinolate hydrolysis products) ● BK (Black kale) ● WD Wild cabbage
● TC (Tronchuda cabbage) ● SC (Savoy cabbage ● RC (Red cabbage) ● WC (White cabbage)

Figure 4.4. MFA map of glucosinolates and glucosinolate hydrolysis products (a) distribution of variables and (b) sample distribution.
For codes and distribution on plot refer to Table 3.1 (varieties) and Table 3.2 and Table 3.3 (compounds). Compounds with different shades of the same colour in Fig 3.3(a) refer to the GSL and corresponding GHP. **Blue bullets = Samples**

4.4 Conclusion

The result of this study concludes that domestic cooking has an effect on GSLs and GHPS. Cooking led to reduction in GSL concentrations with stir-frying having the greatest effect compared to the other two cooking methods. The rate of loss in GSL and GHP concentrations due to cooking varied between varieties with some more stable than the others, although the trend was generally the same. Considering that cabbages are consumed cooked, it important for breeders to work alongside nutritionists to select varieties with more thermally stable GSL and myrosinase to ensure that the benefit of cabbage consumption is not lost. The study found a relationship between ESP, myrosinase and GSL for GHPs formation. ESP and myrosinase were the main factors influencing the type and amounts of GHPs formed. GHPs of raw cabbages were mainly nitriles and EPTs because of the presence of ESP in the samples. The severity of the cooking method influenced the types and amounts of GHPs formed in the cabbages. Cooking led to a reduction in the amount of nitriles and EPTs formed with levels differing between cooking methods; optimal cooking conditions led to degradation of ESP but retention of active myrosinase. Microwaving resulted in significantly lower amounts of nitriles, EPTs and ITCs formation while steaming of cabbages led to production of significantly higher amounts of ITCs. However, the study showed that low residual myrosinase activity can still result in ITC formation.

The study concludes that consumption of raw or severely heat-treated cabbage can reduce possible health benefits while mild thermal processing of cabbages such as mild steaming, which enhances ITC formation especially IB and SFP in the cabbages studied, could improve possible health benefits derived from their consumption.

Foreword to Chapter 5

At the start of the research, the objective was to examine the effect of domestic cooking methods on myrosinase activity and stability, glucosinolate and glucosinolate hydrolysis concentrations of 18 gene bank cabbage accessions/varieties. The aim was to use the result obtained to screen and select varieties with extreme myrosinase activity and stability, GSL and hydrolysis product content for flavour and sensory studies. However, due to time constraints only myrosinase activity and stability analysis was completed before commencement of the field experiment for the second-year cabbages. Based on the myrosinase activity and stability results, two varieties each of red cabbage (RL and RD) and black kale (CNDTP and CPNT), varying in myrosinase stability were selected for further studies. One red cabbage and black kale commercial variety (RM and BM respectively) also were grown alongside the gene bank accessions. Based on the results of the cooking methods, only steaming and stir-frying methods were selected as cooking treatments to proceed with, as microwaving led to almost complete inactivation of myrosinase enzymes in cabbage samples.

Chapter 5: The effects of cultivar and cooking method on phytochemical and volatile composition of black kale (*Brassica oleracea* L. *acephala*) and subsequent sensory profile and acceptability by consumers varying in bitter taste sensitivity

Status: This paper has been written in the style of a research paper but will be subdivided into two and submitted to two different journals; Food Composition and Analysis and Food Chemistry

Abstract

Kale is a popular vegetable with many health beneficial compounds present within its leaves. Glucosinolates, volatiles, sugars, amino acids and organic acids interact to influence its taste and flavour, but it is unknown how this influences consumer preference and perceptions. The aim was to conduct comprehensive phytochemical and volatile analysis of three black kale cultivars (raw and cooked), determine sensory characteristics, and conduct consumer analysis with individuals of varying *TAS2R38* and Gustin genotypes. Significant differences were observed between raw and cooked samples for phytochemical and volatile components. Steaming and stir-frying influenced the abundance of glucosinolate hydrolysis products, with stir-frying preserving residual myrosinase activity better than steamed. *TAS2R38* and Gustin genotypes played only a limited role in determining consumer liking. The effect of rare *TAS2R38* genotypes influenced bitter perception more than has been previously reported. Stir-frying may be an optimal way of cooking black kale, as important isothiocyanates are preserved, due to the inactivation of nitrile specifier proteins and preservation of myrosinase. Consumers prefer sweeter tasting kale leaves where bitter perception is masked by higher sugar content. The data presented are encouraging for the preservation of health promoting compounds by stir-frying.

5.1 Introduction

Black kale (*Brassica oleracea* L. *acephala*) a member of the *Brassica*ceae family, is a headless leafy cabbage commonly consumed raw or as soup in some parts of Europe (Ayaz et al., 2006; Fonseca et al., 2002). Black kale (BK) like other *Brassica* vegetables contains a group of thioglucosides called glucosinolates (GSLs) and myrosinase enzymes (thioglucoside glucohydrolase EC 3.2.3.1) (Mithen, 2001). Hydrolysis of GSL by myrosinase enzyme leads to production of various hydrolysis products, some of which possess health promoting characteristics (Mithen et al., 2000). Isothiocyanates (ITCs) have been shown to have protective effects against various cancers and some cardiovascular diseases (Dekker et al., 2000; Mithen, 2001; Fahey et al., 2001; Herr & Buchler, 2010).

GSLs, glucosinolate hydrolysis products (GHPs) alongside other sulfur containing compounds are thought to be primarily responsible for the bitter taste and pungent aromas of *Brassica* vegetables (Baik et al., 2003; Kubec et al., 1998); these can reduce consumer acceptability when perceived strongly. Bitterness has been reported as one of the main reasons for low consumption and rejection of *Brassica* vegetables (Drewnowski & Gomez-Carneros, 2000a).

The *hTAS2R38* receptor detects compounds containing a thiourea moiety (N-C=S), such as propylthiouracil (PROP) (Kim et al., 2003). GSLs also contain the thiourea moiety and bind to the *hTAS2R38* bitter receptor, of which there is a known genetic difference in bitter taste sensitivity (Sandell et al., 2014). Generally, three diplotypes are common in humans; PAV/PAV (supertasters), PAV/AVI (medium-tasters) and AVI/AVI (non-tasters) (Calò et al., 2011). Additionally, gustin (CA6), a trophic factor responsible for taste bud develop development has been associated with sensitivity to PROP. Individuals who carry the A/A genotype on the *rs2274333* SNP are thought to have higher PROP sensitivity than those with the G/G (Calò et al., 2011).

The GSL- myrosinase system in plants is affected by species and genotypes, growing conditions and processing (Pérez-Balibrea et al., 2011). Several studies have shown that myrosinase is inactivated during thermal processing or domestic cooking of cabbage, leading to decreased production of beneficial hydrolytic compounds (Verkerk & Dekker, 2004; Rungapamestry et al., 2006; Ghawi et al., 2012). GSLs are generally thermostable up to ~100 °C, with losses mostly related to leaching into processing water (Verkerk & Dekker, 2004).

Other compounds contributing to kale sensory characteristics include free amino acids, sugars and organic acids. Amino acids can be sweet (e.g. alanine), bitter (e.g. leucine)

and savoury (e.g. glutamic acid); sucrose has been shown to mask bitter taste perception of GSL compounds (Beck et al., 2014).

There are several studies on the volatile composition of different species of *Brassica* (Kubec et al., 1998; Chin & Lindsay, 1993; Bell et al., 2016; de Pinho et al., 2009; Kato et al., 2011; Akpolat & Barringer, 2015). These studies have mainly assessed ITCs and other sulfur-containing compounds. Previous studies on kale have focused on its GSL (Kushad et al., 2004) and nutrient content (Ayaz et al., 2006); studies on BK have focused on volatiles produced when fed to butterflies (Fernandes et al., 2010) and those produced throughout germination (Fernandes et al., 2009), which is of limited use in providing information about human sensory perceptions. To date, there is no study relating kale flavour profile to its sensory characteristics.

The aim of this study was to investigate the effect of variety and domestic cooking on phytochemical and volatile composition of BK, the subsequent effect on sensory profiles, and acceptability by consumers with varying bitter taste sensitivity. It was hypothesised that through plant breeding, selection and improved processing, bitter taste can be minimised, and bioactive compounds maximized. It was hypothesised that consumer acceptability will be improved with cooking and not relate to human bitter taste receptor genotype, as the volatile profile will be more directly linked to liking than the bitter taste.

5.2 Materials and methods

5.2.1 Plant material

Three BK cultivars were sourced: two gene bank accessions (Cavolo nero di toscana O senza palla, CNDTP; Cavolo palmizio nero di Toscana, CPNT) from University of Warwick Crop Centre Genetic Resources Unit (Wellesbourne, UK), and one commercial variety (Black magic, BM) from Tozer Seeds Ltd (Cobham, Surrey, UK).

Cabbage seeds were sown on the 1st of June 2015 in 84 cell modular trays using multipurpose compost. After 30 days (2nd July 2015), plants were transplanted to the open field in a replicated block design. Before transplanting, the field was prepared using plough, harrow and ring roll for final bed formation. Plants were planted across three parallel beds approximately 12 m in length. 30 plants of each accession/variety were planted as paired rows of 15 plants each in two center rows with RM variety sown surrounding each trial block as a guard crop. Based on standard agricultural practices, blank beds were sprayed with glyphosate before planting to control weeds. Beds were fertilized with NPK (100 kg/ha N, 100

kg/ha P and 200 kg/ha K) before planting and 100 kg/ha N six weeks after planting. Insecticides, fungicides and herbicides were sprayed at different times before and after planting to prevent insect attack. Beds were also covered with cultivation nets to prevent insect and pest attack. The trial was hand weeded in early September and plants were watered as needed throughout the growth period. Climatic data for the growth period is given in Appendix IV (Table S2b). Plants were grown at Tozer Seeds Ltd (Cobham, Surrey, UK) from the 1st of June to 2nd October 2015.

Upon attaining commercial maturity based on visual inspection, plants were harvested on the morning of 2nd October 2015, loaded into crates and transported in a van immediately to the University of Reading, UK (< 1 hour) and stored in a cold rom at 4 ^{o}C until further processing.

5.2.2 Black kale thermal processing

Outer and older leaves from four plants were removed, chopped into small pieces of approximately one cm (representing domestic cutting) and mixed together. Chopped leaves were washed carefully under running tap water and excess water drained using a manual salad spinner. Leaves were either steamed (ST) or stir-fried (SF) with raw samples used as control.

Cooking methods were selected to represent common ways of cooking cabbages. Time and temperature combinations used for each method were based on a preliminary consumer study with 60 participants, to determine consumer acceptability of the samples as steamed and stir-fried cabbage. The method described by Rungapamestry et al. (2006) was adopted with slight modifications for cabbage steaming and Rungapamestry et al. (2008b) for stir-frying. Full protocol is presented in Chapter 2.

For sensory analysis, samples were served immediately after cooking to panellists and consumers. Samples used for phytochemical analysis were put into freezer bags, placed on ice and transferred to -80 $^{\circ}$C. Frozen samples were lyophilized, milled using a Mini Cutting Mill (Mini-Mill, Thomas Scientific, USA), and stored at -20 $^{\circ}$C.

5.2.3 Phytochemical analyses

5.2.3.1 Myrosinase enzyme extraction and assay

Myrosinase enzyme was extracted using the method described by Ghawi et al. (2012) as modified by Oloyede et al. (2014). Myrosinase activity was measured using the coupled enzyme method (Wilkinson et al., 1984) as modified by Ghawi et al. (2012). One unit of

myrosinase activity is defined the amount of enzyme that produces 1 µmol of glucose from sinigrin substrate per minute at pH 7.5. Full methodology for myrosinase enzyme extraction and assay is as described in Chapter 2.

Protein content of the crude enzyme extract was determined using the Bradford method (Bradford, 1976). Brilliant Blue G (Sigma- Aldrich) was used as a dye and Bovine serum albumin (BSA) (Sigma- Aldrich, UK) was used to construct a standard curve from which protein concentration in crude extracts were calculated and used to determine specific activity ($U.mg^{-1}$ protein).

5.2.3.2 Free sugars and Organic acid analyses

Lyophilized BK powder (40 mg) was suspended in 10 mL 0.01 M hydrochloric acid, stirred for 30 minutes at room temperature and left to stand for one hour. 1.5 mL of the supernatant was centrifuged at 13,200 rpm for 30 min, filtered through 0.22 µm Millex Millipore filter unit and analysed by HPLC. Standard curves were constructed using external standards ranging from 0.05-2.50 $mg.L^{-1}$ and 10-100 $mg.L^{-1}$ for sugars (glucose, fructose and sucrose) and organic acids (OAs) (citric, malic and succinic acids) in order to quantify compounds of interest.

The HPLC method was adopted from Zeppa et al. (2001) with slight modifications. Briefly, HPLC analysis was performed on an Agilent 1100 series HPLC (Agilent Waldbronn, Germany) equipped with a line degasser, isocratic pump, auto injector, Hewlett Packard series 1050 DAD and series ERC-7515A refractive index (RI) from Polymer laboratories (Shropshire, UK). Sample separation was performed using Aminex HPX-87H column (300 x 7.8 mm, 9µm particle size) from Bio-Rad (Hertfordshire, UK). Sulfuric acid (5 mM) was used as the mobile phase with an isocratic gradient at a flow rate of 0.6 mL/min with column temperature maintained at 45 °C. Sample injection volume was 50 µL. OAs were quantified at a wavelength of 210 nm, whereas RI detector was used to quantify sugarFree amino acid analysis

BK powder (0.1 g) was suspended in 2 mL of 25 % acetonitrile in 0.01 M hydrochloric acid. Samples were vortexed for five minutes and left to stand for one hour at room temperature (~20 °C), and then centrifuged at 14, 000 rpm for 30 minutes. Supernatant was decanted and filtered through a 0.22 µm filter disc with low protein binding Durapore polyvinylidene (PVDF) membrane (Millex, USA).

The EZ-Faast free amino acid analysis kit was used to prepare derivatized amino acids from 100 µL aliquot of the filtrate for analysis by GC-MS as described by Elmore et al. (2005). To prepare sample for GC-MS analysis, 200 nmol of norvaline was added to the sample as

internal standard. This was followed by a solid-phase extraction and a two-step derivatization process at room temperature. Derivatized amino acids were extracted into isooctane/chloroform (100 µL) and analyzed on an Agilent 7890A/5795C GC-MS instrument in electron impact mode. An aliquot of the derivatized AA solution (1 µL) was injected at 280 °C in split mode (40:1) onto a Zebron ZB-AAA capillary column (10 m x 0.25 mm; 0.25 µm film thickness). Oven temperature was held at the 110 °C for one minute and then increased at 30 °C/min to 310 °C. The transfer line and ion source were maintained at 320 and 230 °C, respectively; carrier gas flow rate was kept constant throughout the run at 1.5 mL/min.

Free amino acid standards in 0.1 M hydrochloric acid were prepared and analysed using the same method. A calibration curve was plotted for each AA and used to quantify the amount of each amino acid in the sample. A specific mass spectral fragment ion was chosen for quantification of each amino acid. The area of this ion in the peak of each amino acid was measured relative to the area of the m/z 158 ion of norvaline. Arginine cannot be determined using the EZ-Faast method and therefore was not identified in the samples.

5.2.3.3 Glucosinolate and glucosinolate hydrolysis products analysis

GSLs were extracted and analysed as described by Bell et al. (2015) and as outlined in Chapter 3 with the following modifications: LC-MS analysis of GSL extracts was performed in negative ion mode on an Agilent 1260 Infinity Series LC system (Stockport, UK) equipped with a variable wavelength detector, and coupled to an Agilent 6120 Series single quadrupole mass spectrometer. Sample separation was achieved on a Gemini 3 µm C_{18} 110 Å (150 x 4.6 mm) column (with Security Guard column, C_{18}; 4mm x 3mm; Phenomenex, Macclesfield, UK). GSLs were separated during a 40-minute chromatographic run, with 5-minute post-run sequence. Mobile phases consisted of ammonium formate (0.1 %; A) and acetonitrile (B) with the following gradient: (i) 0 min (A-B, 95:5, v/v); (ii) 0-13 min (A-B, 95:5, v/v); (iii) 13-18 min (A-B, 40:60, v/v); (iv) 18-26 min (A-B, 40:60, v/v); 26-30 min (A-B, 95:5, v/v); (v) 30-40 min (A-B, 95:5, v/v). The flow rate was optimised for the system at 0.4 mL/min, with a column temperature of 30°C, with 25 µl of sample injected into the system. MS analysis settings were as follows: ESI was carried out at atmospheric pressure in negative ion mode (scan range m/z 100–1500 Da). Nebulizer pressure was set at 50 psi, gas-drying temperature at 350 °C, and capillary voltage at 2,000 V. Five-point sinigrin hydrate calibration curve was constructed (concentrations 0.22-3.5 mg/mL; $r^2 = 0.99$). GSLs were identified using literature ion data and quantified at a wavelength of 229 nm. Data was analysed using Agilent OpenLAB CDS ChemStation software (Agilent).

Glucosinolate hydrolysis products (GHPs) were extracted, identified and quantified according to the protocol in Bell et al. (2017c) as described in Chapter 3. Quantification was based on an external standard calibration curve of sulforaphane (concentrations 0.25–2 mg/mL; r^2 = 0.99). Table 5.1 and 5.2 shows the literature ion data of all GSL and GHP compounds identified in BK.

5.2.4 Volatile compounds analysis

Volatile compounds were extracted using automated headspace solid-phase microextraction (HS-SPME) system as described by Morales-Soto et al. (2015) with modifications. Freshly cooked sample (10 g) was blended for 30 secs using a hand blender with 5 g of the sample subsequently placed in a 15 mL SPME vial with a fitted screw cap. The vial was placed in an automated GC Sampler 120 (Agilent) and incubated for 10 min at 37 °C to achieve equilibrium. A 50/30 µm DVB/CAR/PDMS SPME fibre (Supelco, Bellefonte, USA) was exposed to the sample headspace of the sample for 30 min. The sample was stirred constantly at 37 °C and desorbed for 20 min in the GC injector at 250 °C in splitless mode.

After extraction, the fibre was inserted to the injection port of an Agilent 7890A gas chromatography system coupled to an Agilent 5975C inert MSD triple axis detection system. A Stabilwax®-DA (30 m × 0.25 mm × 0.5 µm film thickness; Restek) column was used for chromatographic separation. The temperature programme started at 40 °C for 5 min, then increased at a rate of 4 °C/min to 260 °C with the final temperature held for 5 min. Helium was used as the carrier gas at a flow rate of 0.9 mL/min. Mass spectra were measured in electron-impact mode at an ionization voltage of 70 eV, source temperature of 230 °C with a scan range of 20 to 280 m/z and scan rate of 5.3 scans/s. Volatile compounds were identified by comparison with mass spectra from National Institute of Standards and Technology (NIST) database or spectra published in literature. To confirm identification, the linear retention index (LRI) of each compound was calculated using the retention times of a homologous series of C_6 – C_{25} *n*-alkanes and compared to the LRI values of authentic compounds. Semi-quantitative results were expressed as relative amounts of total peak areas for each sample. Four replicates were analysed per sample.

5.2.5 Sensory analysis

Fresh BK samples were assessed by a trained sensory panel (n= 11); a consensus vocabulary on the sensory attributes of the products (appearance, odour, mouthfeel, taste,

flavour and after effect) was developed over three training sessions. Reference standards were used where necessary. Samples were rated using unstructured line scales over four sessions in duplicate. Samples were presented in monadic balanced order, in well-ventilated isolated booths, under artificial daylight and controlled temperature (23 ˚C). Water and frozen natural yoghurt (Yeo Valley Farms, Bristol, UK) were used as palate cleansers between samples. Data were collected using Compusense at-hand (version 8.6, Compusense Inc., Canada).

5.2.6 Consumer study

The primary outcome measure for the study was to determine whether there were significant differences between samples. For this, the aim was to recruit about 100 participants. The outcome measure is a liking score on a 9-point category scale. There is an 80 % chance of detecting a mean difference of size 0.8 on a 9-point hedonic scale between two sample means at the 95 % confidence interval with 84 participants, allowing a conservative RMSL (root mean square divided by scale length) of 0.23. Aiming to recruit 100 allows for a 10% drop out rate. The secondary output measure was to compare between consumers of different genotypes. It was estimated that approximately 25 participants of PAV/PAV and AVI/AVI TAS2R38 genotype and 50 of PAV/AVI were required. Using power calculations there is 80 % chance of detecting a difference of size 1.0 (on a 9-point hedonic scale) between two means at the 95 % confidence interval with 23 participants, allowing a standard deviation of 1.5.

Healthy consumers (n=105, aged 18 – 65 years) were recruited within Reading (UK); the study was approved by the School of Chemistry, Food and Pharmacy Research Ethics committee (study number: 37/15). 105 participants were recruited based on the calculation of sample size. Consumers gave their prior consent and attended a single tasting session. Consumers rated samples for liking (9-point hedonic scale; dislike extremely to like extremely), taste perception (using labelled magnitude scale (LMS)) and consumption intent (5-point category scale). During the visit, consumers were asked to complete a demographic questionnaire (Table 5.8); samples were presented as for the sensory panel. At the end of the visit, consumers provided buccal samples (in duplicate) using Isohelix sterile swabs (Cell Projects Ltd, Kent, UK). The swabs were stored at room temperature in a tube with dri-capsule inserts.

Table 5.1: Intact glucosinolates identified in black kale varieties, by LC-MS

Common name	Chemical name	Abbreviation	Mass parent ion	Reference
Glucoiberin	3-(methylsulfinyl) propyl GSL	GIBN	422	Bennett et al. (2004), Rochfort et al. (2008), Lelario et al. (2012)
Glucoraphanin	4-(methylsulfinyl) butyl GSL	GRPN	436	Bennett et al. (2004), Rochfort et al. (2008), Bell et al. (2015)
Glucobrassicin	3-indolylmethyl GSL	GBSN	447	Bennett et al. (2004), Rochfort et al. (2008), Lelario et al. (2012)
4-hydroxyglucobrassicin	4-hydroxy-3-indolylmethyl GSL	4-HOH	463	Bennett et al. (2004), Rochfort et al. (2008), Lelario et al. (2012)
4-methoxyglucobrassicin	4-Methoxy-3-indolylmethyl-GLS	4-MeOH	477	Rochfort et al. (2008), Lelario et al. (2012), Bell et al. (2015)
Neoglucobrassicin	N-Methoxy-3-indolylmethyl-GLS	NEO	477	Bennett et al. (2004), Rochfort et al. (2008)

Key: GSL, glucosinolate

Table 5.2: Glucosinolate hydrolysis products identified in black kale varieties by GC-MS respectively

Precursor GSL	*Common name*	*Chemical name*	*Abbreviation*	*LRI[a,b]*	*MS² spectrum ion (base ion in bold)*	*Reference*
Gluconapin	3-Butenyl-ITC	1-butene, 4-isothiocyanate	3BITC	983	113, 85, **72**, 64, 55, 46, 45, 41	Al-Gendy & Lockwood (2003), Hong & Kim (2013), Arora et al. (2014)
Glucoerucin	Erucin	4-(methylthio)-butyl-ITC	ER	1427	161, 146, **115**, 85, 72, 61, 55	Al-Gendy & Lockwood (2003), Arora et al. (2014)
	Erucin nitrile	1-cyano-4-(methylthio) butane	ERN	1200	129, 87, 82, **61**, 55, 48, 41, 47	Al-Gendy & Lockwood (2003), Arora et al. (2014)

Glucoiberin	Iberin	3-methylsulfinylpropyl-ITC	IB	1617	163, 130, 116, 102, 100, 86, **72**, 63, 61,41	Al-Gendy & Lockwood (2003)
	Iberin nitrile	4-methylsulfinylbutanenitrile	IBN	1384	**131**, 78, 64, 47, 41	Al-Gendy & Lockwood (2003)
	Benzenepropanenitrile	2-phenylethyl cyanide	BPN	1238	131, **91**, 85, 65, 63, 57, 44, 51	Hong & Kim (2013)
Glucoraphanin	Sulforaphane	4-methylsulfinylbutyl-ITC	SFP	1757	160, 114, 85, **72**, 64, 63, 61, 55. 41, 39	Arora et al. (2014),Bell et al. (2017c)
	Sulforaphane nitrile	5-(methylsulfinyl) pentanenitrile	SFN	1526	145, 128, 82, 64, **55**, 41	Arora et al. (2014), Bell et al. (2017c)
Glucobrassiccin	Indole-3-carbinol	1H-Indole-3-methanol	I3C	1801	**144**, 145, 116, 108, 89	Spencer & Daxenbichler (1980)
	Indoleacetonitrile	1H-Indole-3-acetonitrile	1IAN	1796	**155**, 145, 144, 130, 116, 89, 101, 63	Hanschen et al. (2017)
Glucotropaeolin	Benzeneacetonitrile	2-Phenylacetonitrile	BAN	1137[c]	**117**, 90, 89, 77, 63, 51	Vaughn et al. (2017)

Key: ITC, isothiocyanate. [a] Linear retention index on a HP-5MS non-polar column. [b]Mass spectrum agrees with reference spectrum in the NIST/NIH mass spectra database and those in literature. [c]Mass spectrum and LRI agree with those of authentic compound.

5.2.7 DNA extraction

DNA analysis was carried out by iDNA genetics (Peterborough, UK). DNA taken from buccal swabs was extracted using the Isohelix Buccalyse DNA extraction kit (Cell Projects Ltd, Kent, UK) according to manufacturer's instructions. Before analysis, samples were diluted with water in a ratio of 1:8. SNPs were analysed using KASP genotyping (LGC Group, Teddington, Middlesex UK). The h*TAS2R38* (Ala49Pro (*rs713598*), Val262Ala (*rs1726866*) and Ile296Val (*rs10246939*)) and CA6 (*rs2274333*) polymorphisms were analysed.

5.2.8 Statistical analysis

Results of all phytochemical data (except HS-SPME) were averages of three processing replicates and two technical replicates (n = 6). All phytochemical, consumer and genotyping data were analysed individually using both one- and two- way ANOVA with Tukey's HSD multiple pair wise comparison test (XLSTAT; Addinsoft, Paris, France). Agglomerative Hierarchical Cluster (AHC) analysis was carried out on consumer overall liking scores to group consumers with similar liking scores into clusters. Cluster means were subsequently analysed by ANOVA. A mixed model ANOVA (with Tukey's HSD multiple pair wise comparison test) and principal component analysis (PCA) were carried out in Senpaq (version 4.2, Qi Statistics, UK) and used to analyse sensory profiling data. A mixed model ANOVA tests the main effects (i.e. samples and assessors) against their interaction.

Rotated factor analysis (RFA) and multiple factor analysis (MFA) were carried out on the means of all datasets to analyse for relationships between phytochemical, sensory and consumer data using XLSTAT.

5.3 Results and discussion

5.3.1 Phytochemical analysis

5.3.1.1 Myrosinase activity and stability

The results of myrosinase activity and stability are presented in and Appendix X (Table S5a). Myrosinase activity and stability were significantly affected by BK variety ($p<0.0001$), cooking ($p<0.0001$) and their interaction ($p<0.0001$). Raw CNDTP had the highest myrosinase activity (22.6 $U.g^{-1}$ DW) while raw BM has the lowest myrosinase activity (15.2 $U.g^{-1}$ DW). This is in agreement with previous authors, reporting differences in myrosinase activity between varieties of the same type of *Brassica* (Penas et al., 2011; Travers-Martin et al., 2008; Okunade et al., 2015).

The stability of myrosinase varied significantly ($p<0.0001$) across samples. Residual activity of myrosinase is defined as the ratio of processed to raw (A/A_0) (Figure 5.1). Myrosinase in ST BK was significantly ($p<0.0001$) lower than SF, while CNDTP was significantly ($p<0.0001$) more stable after cooking, with SF CNDTP retaining up to 68 % of its myrosinase activity. ST-CPNT had the lowest myrosinase stability (17 %) after cooking. Thermal processing has been previously reported to influence myrosinase stability (Yen & Wei, 1993; Verkerk & Dekker, 2004; Rungapamestry et al., 2008c). The difference between cooking methods can be attributed to the rate of heat transfer and core leaf temperature, which is influenced by time and temperature during cooking. The core temperature required to inactivate myrosinase has been reported to vary between varieties but ranges between 50 and 60 ˚C (Rungapamestry et al., 2006). In this study, the core temperature of the ST-BK was higher (75 – 80 ˚C) when compared to SF (65 – 70 ˚C). The time taken to achieve the required core temperature for myrosinase inactivation is also dependent on the thickness of the shredded vegetable, as the rate of heat transfer in thinner slices will be faster than thicker cuts. The difference in myrosinase stability between accessions might be due to different myrosinase isoenzymes present in the varieties, with some being more stable than others (Rask et al., 2000). Overall, myrosinase activity and stability of gene bank samples (CNDTP and CPNT) was higher than that of BM (commercial variety). This could therefore influence the production of potential health beneficial compounds such as ITCs and indoles.

Protein content and specific activity is presented in . Cooking significantly reduced the protein content of myrosinase which might be due to breakdown in proteins to amino acids during cooking. No significant differences in protein content were found between cooking methods. Differences in protein content due to variety, though significant, were small (and Appendix X; Table S5a). Specific activity differed between varieties with CNDTP, which had the lowest myrosinase activity (10.5 $U.mg^{-1}$ protein DW), having the highest specific activity (0.7 $U.mg^{-1}$ protein DW; Appendix X - Table S5a). SF-BK had the highest specific activity with ST BK the lowest.

The study of myrosinase activity and its stability after cooking is important as the presence of myrosinase is crucial in the production of health beneficial compounds from glucosinolate hydrolysis, as will be discussed in section 5.3.1.5.

5.3.1.2 Free amino acids

Table 5.4 shows the result of 18 free amino acids (AAs) identified and quantified in BK samples. AAs were significantly different between cooking methods and variety (except α -

aminobutyric acid; which did not differ across cooking). Glutamine was the most abundant AA, with CPNT being significantly ($p<0.0001$) lower than CNDTP and BM; Methionine and α-aminobutyric acid were the least abundant, with cooking leading to a significant reduction in total AAs. Lisiewska et al. (2008) reported a decrease in kale AAs after cooking and attributed it to leaching into the cooking water; whereas this seemingly supports the ST BK results in this study, it does not account for the similar reduction on stir frying.

Concentrations of AAs in kale samples found in previous studies vary considerably between varieties, but were higher in most cases than those found in this study (Ayaz et al., 2006; Lisiewska et al., 2008; Eppendorfer, 1996). Similar trends were observed in terms of highest and lowest amounts of individual AAs across previous studies. The commercial variety BM, had the highest total free AAs (38.7 $\mu g.g^{-1}$ DW) but did not differ significantly from the CNDTP cultivar. Apart from some AAs being involved in GSL synthesis, AAs might be important taste contributors; alanine and glycine for sweetness, valine and leucine for bitterness, and aspartic acid and glutamic acid sour taste (Park et al., 2014c). Lower concentration of sweet-tasting AAs as a result of variety and cooking may affect the perception of bitter taste in kale but might depend on the ratio of GSL to AAs.

Table 5.3: Myrosinase activity (U.g^{-1} DW), protein content (mg.g^{-1} DW) and specific activity (U.mg^{-1} protein DW) of black kale samples

Cultivars	Treatment	Myrosinase activity (*U.g^{-1} DW*)	Protein content (*mg.g^{-1} DW*)	Specific activity (*U.mg^{-1} protein DW*)
BM	Raw	15.2^{c}	33.5^{b}	0.5^{ab}
	ST	3.7^{a}	11.2^{a}	0.3^{a}
	SF	7.0^{ab}	11.9^{a}	0.6^{bc}
CNDTP	Raw	22.6^{d}	36.1^{b}	0.6^{bc}
	ST	4.8^{ab}	11.2^{a}	0.4^{ab}
	SF	15.2^{c}	13.2^{a}	1.1^{d}
CPNT	Raw	20.0^{d}	36.0^{b}	0.6^{bc}
	ST	3.3^{a}	11.1^{a}	0.3^{a}
	SF	8.1^{b}	11.3^{a}	0.7^{c}
	P-value	< 0.0001	< 0.0001	< 0.0001

Mean values with different superscripts in the same column significantly different at p<0.05
Abbreviations: ST = steamed, SF = stir fried

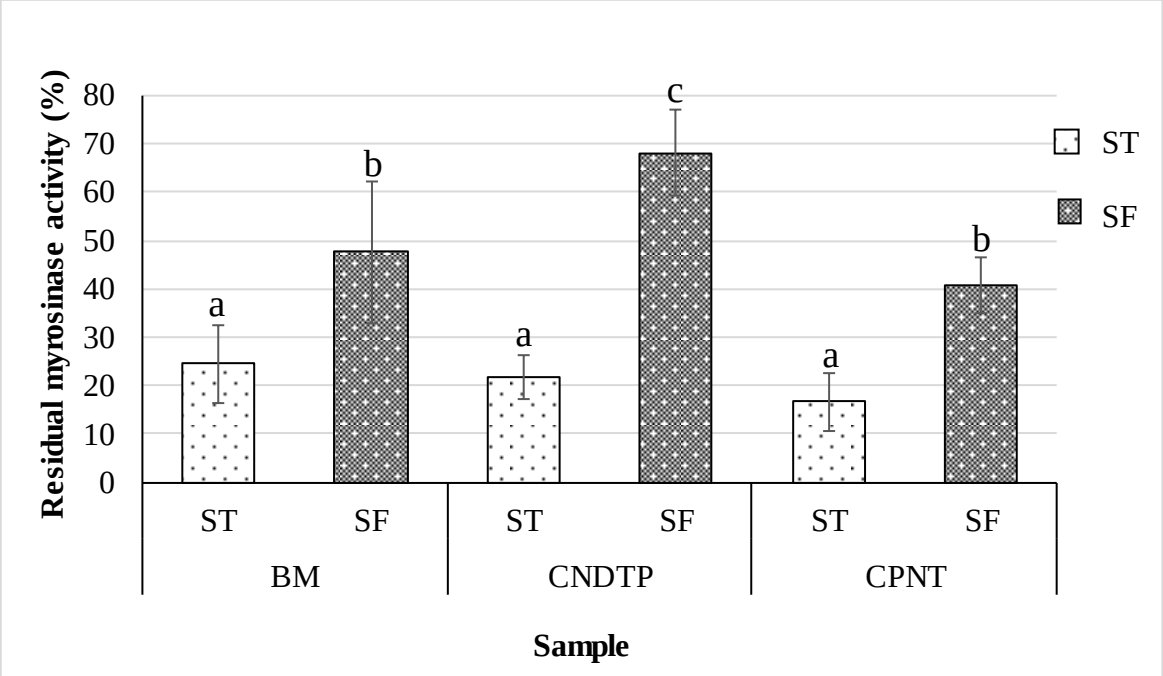


Figure 5.1. Residual myrosinase activity (%) of black kale samples (varieties BM, CNDTP and CPNT) after cooking.
Bars with differing letters indicates significant differences (p<0.0001) between samples. Error bars represent standard deviation from mean values. Abbreviations: ST = steamed, SF = stir fried

Table 5.4: Amino acid (µg.g^{-1} DW), sugars (mg.g^{-1} DW) and organic acid (mg.g^{-1} DW) concentrations of black kale (ST = steamed, SF = stir fried)

		BM			CNDTP			CPNT			Significance
Code	Compound	Raw	ST	SF	Raw	ST	SF	Raw	ST	SF	(*P-value*)
	Amino acids (µg/g DW)										
Ala	Alanine	3.12^{c}	2.46^{abc}	2.09^{ab}	2.82^{bc}	2.60^{abc}	2.05^{a}	2.09^{ab}	2.41^{abc}	2.20^{ab}	< 0.001
Gly	Glycine	0.10^{c}	0.03^{ab}	0.04^{ab}	0.05^{b}	0.04^{ab}	0.04^{ab}	0.10^{c}	0.03^{a}	0.03^{ab}	< 0.0001
Val	Valine	0.79^{d}	0.65^{bc}	0.51^{a}	0.63^{abc}	0.68^{bcd}	0.56^{ab}	0.70^{cd}	0.64^{bc}	0.57^{ab}	< 0.0001
Leu	Leucine	0.43^{c}	0.15^{a}	0.15^{a}	0.22^{b}	0.17^{ab}	0.18^{ab}	0.38^{c}	0.15^{a}	0.16^{a}	< 0.0001
Iso	Isoleucine	0.37^{c}	0.26^{ab}	0.22^{a}	0.26^{ab}	0.29^{b}	0.25^{ab}	0.30^{b}	0.26^{ab}	0.24^{ab}	< 0.0001
Thr	Threonine	0.99^{e}	0.79^{cd}	0.57^{ab}	0.75^{bcd}	0.71^{abcd}	0.52^{a}	0.86^{de}	0.65^{abc}	0.54^{a}	< 0.0001
Ser	Serine	3.60^{f}	1.80^{abc}	2.23^{cd}	2.60^{de}	1.74^{ab}	2.01^{abc}	2.95^{e}	1.61^{a}	2.06^{bc}	< 0.0001
Pro	Proline	3.24^{bcd}	2.57^{abc}	2.40^{ab}	3.52^{cd}	3.68^{d}	2.49^{ab}	3.00^{bcd}	1.84^{a}	1.66^{a}	< 0.0001
Asp	Asparagine	3.77^{d}	1.79^{b}	1.79^{b}	2.45^{c}	1.83^{b}	1.60^{b}	1.59^{b}	0.64^{a}	0.95^{a}	< 0.0001
Asp.acid	Aspartic acid	3.24^{d}	2.82^{bcd}	2.17^{ab}	2.77^{bcd}	2.34^{abc}	1.75^{a}	2.69^{bcd}	3.06^{cd}	1.80^{a}	< 0.0001
Met	Methionine	0.06^{c}	0.01^{a}	0.01^{a}	0.03^{b}	0.01^{a}	0.02^{ab}	0.06^{c}	0.02^{ab}	0.01^{a}	< 0.0001
Glu.acid	Glutamic acid	1.05^{a}	2.70^{c}	1.04^{a}	1.20^{a}	1.96^{b}	0.68^{a}	0.78^{a}	1.76^{b}	0.70^{a}	< 0.0001
Phy	Phenylalanine	0.51^{e}	0.40^{bcd}	0.29^{a}	0.33^{ab}	0.39^{bc}	0.33^{ab}	0.48^{de}	0.42^{cd}	0.37^{abc}	< 0.0001
Glu	Glutamine	14.7^{d}	9.78^{c}	7.82^{bc}	15.2^{d}	9.35^{c}	8.11^{bc}	8.87^{c}	3.52^{a}	4.10^{ab}	< 0.0001
Lys	Lysine	0.69^{e}	0.30^{c}	0.21^{ab}	0.30^{c}	0.28^{bc}	0.26^{abc}	0.45^{d}	0.18^{a}	0.19^{a}	< 0.0001
His	Histidine	1.56^{d}	0.27^{ab}	0.23^{ab}	0.78^{c}	0.48^{bc}	0.43^{b}	0.37^{ab}	0.09^{a}	0.14^{ab}	< 0.0001
Tyr	Tyrosine	0.26^{c}	0.14^{b}	0.13^{ab}	0.11^{ab}	0.12^{ab}	0.10^{ab}	0.15^{b}	0.12^{ab}	0.09^{a}	< 0.0001
Tryp	Tryptophan	0.13^{ab}	0.13^{ab}	0.10^{a}	0.11^{a}	0.15^{b}	0.13^{ab}	0.11^{a}	0.11^{ab}	0.10^{a}	< 0.001
TAA	Total Amino acids	38.7^{c}	27.1^{b}	22.0^{ab}	34.1^{c}	26.8^{b}	21.5^{ab}	26.0^{b}	17.5^{a}	15.9^{a}	< 0.0001

Sugars (mg.g^{-1} DW)										
Sucrose	15.4^{abc}	16.0^{abc}	12.1^{a}	19.6^{c}	17.4^{bc}	16.2^{abc}	15.0^{abc}	12.1^{a}	12.7^{ab}	< 0.001
Glucose	7.9^{ab}	10.4^{cde}	8.4^{abc}	11.6^{e}	10.7^{de}	8.0^{ab}	8.8^{bcd}	9.0^{bcd}	6.5^{a}	< 0.0001
Fructose	8.8^{bcd}	9.6^{cd}	7.6^{ab}	10.0^{d}	9.8^{d}	8.1^{abc}	9.6^{cd}	9.2^{bcd}	7.1^{a}	< 0.0001
Total sugars	32.1^{abcd}	36.1^{cde}	28.2^{ab}	41.2^{e}	38.0^{de}	32.3^{abcd}	33.3^{bcd}	30.4^{abc}	26.3^{a}	< 0.0001
Organic acids (OAs) (mg.g^{-1} DW)										
Citric	25.3^{ab}	21.1^{a}	16.0^{a}	16.0^{a}	21.9^{a}	15.6^{a}	25.1^{ab}	37.8^{b}	25.2^{ab}	< 0.0001
Malic	29.5^{abc}	33.5^{abc}	20.8^{a}	23.3^{ab}	28.8^{abc}	25.6^{abc}	36.8^{bc}	40.6^{c}	27.9^{abc}	0.002
Succinic	53.4	32.5	33.8	36.5	27.4	38.3	54.6	45.9	34.7	0.224
Total organic acids	108.2^{abc}	87.2^{abc}	70.6^{a}	75.8^{ab}	77.9^{ab}	79.5^{ab}	116.6^{bc}	124.3^{c}	87.8^{abc}	0.001

Mean values with different superscripts in the same row significantly different at $p<0.05$

5.3.1.3 Sugars and organic acids

The results of sugars and organic acids are presented in Table 5.4. Sucrose, fructose and glucose were the soluble sugars found in kale samples. Significant differences were observed in the sugar content of BK due to variety and cooking. CNDTP had significantly higher sugar content than the other two varieties. SF significantly reduced sugar concentration of BK samples while ST had no significant effect. This is in agreement with the results of a previous study on red cabbage which showed significant reduction in sugar concentrations as a result of SF with no effect after ST (Xu et al., 2014). Decrease in sugar concentration on SF could be attributed to Maillard reactions, which perhaps explains the loss of some AAs on SF and leads to production of Maillard-derived volatiles Table 5.5. Sucrose was the most abundant sugar found in kale, which did not agree with the results observed by Ayaz et al. (2006) who found fructose to be the most abundant sugar in kale leaves. High sugar concentrations in kale might be helpful in masking the taste of bitter-tasting GSLs (Beck et al., 2014). Bell et al. (2017a) reported that high concentrations of sugar did not necessarily reduce bitter taste of rocket but the ratio of sugars to GSL was an important determinant of bitter taste and pungency.

OAs have been reported to influence astringency and sourness in foods (Hufnagel & Hofmann, 2008a; Hufnagel & Hofmann, 2008b). Citric, malic and succinic acids were the organic acids (OAs) detected in kale samples. Ayaz et al. (2006) detected only citric and malic acids in their study of kale. The type and profile of OAs detected in plants depends on the species, age and type of plant (López-Bucio et al., 2000); succinic acid was the most abundant OA in the sample. Succinic acid concentration did not differ between varieties but there was a significant ($p<0.02$) reduction in concentration as a result of cooking. CPNT had significantly higher citric and malic acids than the other two cultivars, while SF significantly lowered citric and malic acid when compared to ST.

5.3.1.4 Glucosinolates

Figure 5.2 shows the results of GSL concentrations in kale samples with significant differences between varieties and cooking methods (Appendix X; Tables S5b and S5c). Six GSLs were identified and quantified. Concentrations differed significantly due to interactions between variety and cooking methods for all individual and total GSLs. No significant differences were found for some GSLs as a result of variety or cooking alone. Cooking did not significantly affect concentrations of glucoraphanin (GRPN), glucobrassicin (GBSN) and 4-methoxyglucobrassicin (4-MeOH), but the concentrations of other GSLs identified

(glucoiberin, GIBN; 4-hydroxyglucobrassicin, 4-HOH; and Neoglucobrassicin, NEO) were significantly reduced.

Between the two cooking methods employed, ST led to more GSLs losses. Some authors have also reported stability or minimal losses (about 2 %) of some GSLs, and significant losses of others after ST, which is comparable to the findings of this study (Rungapamestry et al., 2006; Vallejo et al., 2002; Jones et al., 2010). One study reported 41 % loss of GIBN, but no loss of GRPN after 3.5 min of ST broccoli florets (Vallejo et al., 2002). Conversely, some studies have reported increases in GSL concentrations after ST of some *Brassicas*, and have attributed the increase to enhanced extractability of the compounds as a result of broken down cell walls during heating (Dekker et al., 2000; Ciska & Kozłowska, 2001; Jones et al., 2010). With the exception of 4-HOH, SF did not result in significant losses of GSL (Appendix X; Tables S5c) and this can be attributed to the lower core temperatures during SF compared to ST. The findings of this study are contrary to that reported by Yuan et al. (2009) where up to 55 % of GSLs were lost in broccoli florets after SF for 5 min. The effect of cooking on GSL composition and concentration of *Brassica* vegetables is dependent on the cooking method and time, type of vegetable, and degree of tissue damage during sample preparation (Yuan et al., 2009).

BM had the highest total GSL (17.6 $mg.g^{-1}$ DW) and CNDPT the lowest (11.0 $mg.g^{-1}$ DW). In general, ST samples had the lowest amount of individual GSL. NEO did not differ significantly in cultivars studied. Previous studies (Park et al., 2014c; Kushad et al., 2004; Korus et al., 2014; Sun et al., 2011) found other GSLs such as sinigrin and progoitrin in BK cultivars which were not found in this study. GRPN and GBSN were the most abundant GSLs in kale samples, which agrees with previous findings of Kushad et al. (Kushad et al., 2004) but disagrees with other studies where GRPN was not detected or present in low concentrations (Park et al., 2014c; Sun et al., 2011; Cartea et al., 2008). GSLs concentrations in BK cultivars were higher than those previously reported (Bell & Wagstaff, 2017). It has been previously reported that GSL profiles and concentrations differ across varieties and species (Kushad et al., 1999; Mithen, 2001). High GRPN and GBSN content could enhance the health benefits associated with consuming BK (Fahey et al., 2001; Mithen, 2001).

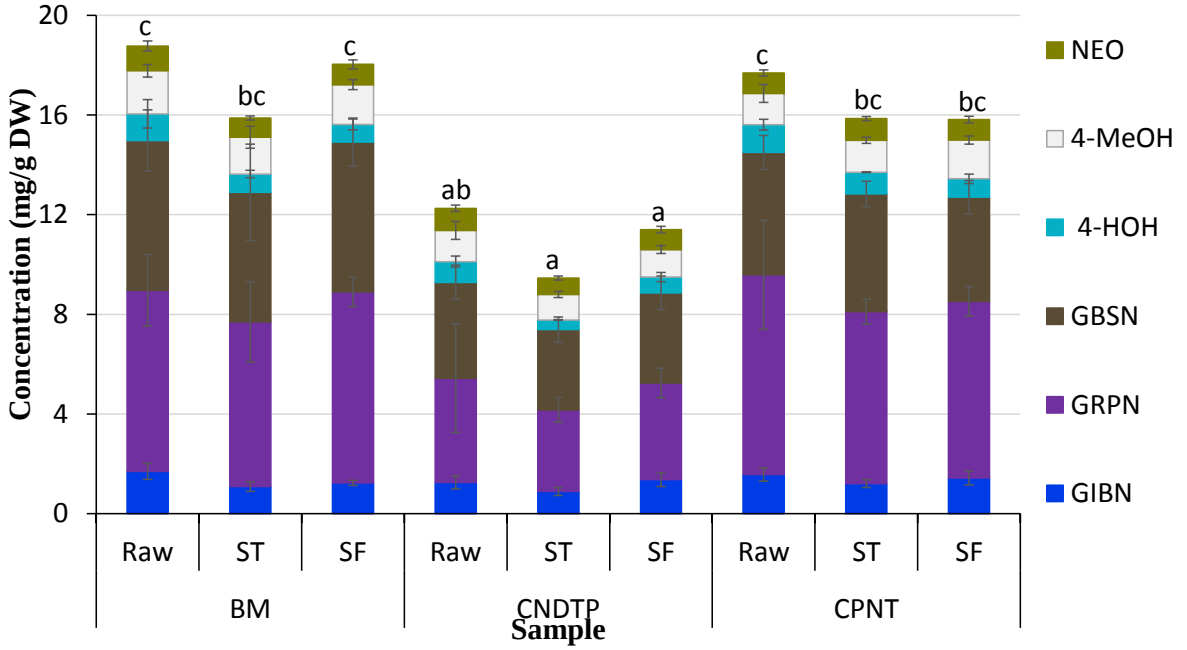


Figure 5.2. Glucosinolate (GLS) concentrations (mg.g^{-1} DW) of black kale samples (varieties BM, CNDTP, CPNT).
Error bars represent standard deviation from mean values. Bars not sharing a common letter differ significantly ($p<0.0001$) between samples. Letters above bars refer to differences in total GSL concentration. Abbreviations: ST = steamed, SF = stir fried. Abbreviations: NEO, neoglucobrassicin; 4-MeOH, 4-methoxyglucobrassicin; 4-HOH, 4-hydroxyglucobrassicin; GBSN, glucobrassicin; GRPN, glucoraphanin; GIBN, glucoiberin.

5.3.1.5 Glucosinolate hydrolysis products (GHPs)

The results for GHPs are presented in Figure 5.3 with significant differences due to variety and cooking methods (Appendix X; Tables S5b and S5c). Concentrations are expressed as sulforaphane (SFP) equivalents. In total, 12 GHPs were identified and quantified in kale samples. ITCs and nitriles of GRPN and GBSN were the predominant hydrolysis products formed. This was expected, as GRPN and GBSN were the most abundant GSL detected in the BK samples. A relationship could be seen between concentrations of GSLs and GHPs formed in varieties. For example, CNDTP which had the highest concentration of GRPN, also had the highest amount of SFP and sulforaphane nitrile (SFN), while CPNT, with significantly lower GRPN concentrations, produced the lowest amounts of GRPN hydrolysis products. Some GHPs such as 3-butenyl ITC (3BITC), benzeneacetonitrile, benzenepropanenitrile, erucin and erucin nitrile were also found, even though their intact GSLs (gluconapin, glucotropaeolin, gluconasturtiin and glucoerucin) were not. The inability to detect these GSLs could be due to very low concentrations present in the samples, which might have been hydrolysed during sample preparation and processing, as their GHPs were present in very low concentrations.

Previous study of turnips, for example, have found that breakdown products of glucoberteroin were detected though the intact GSL was not (Klopsch et al., 2017).

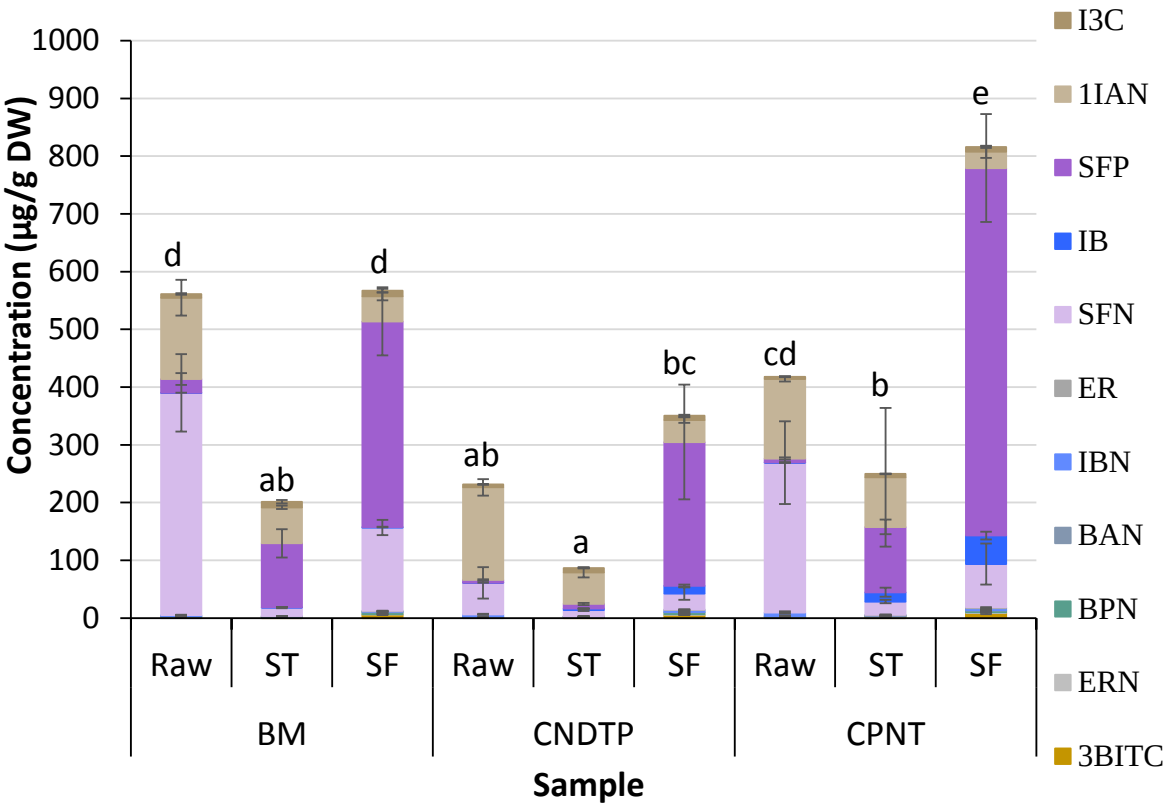


Figure 5.3. Glucosinolate hydrolysis products (GHP) concentration (µg/g DW; sulforaphane equivalent) in black kale samples (varieties BM, CNDTP, CPNT).
Error bars represent standard deviation from the mean. Bars not sharing a common letter differ significantly ($p<0.0001$) between samples. Letters above bars refer to differences in total GHP concentration. Compounds with similar colour shades refer to GHPs from corresponding GSL in Figure 5.2. Abbreviations: ST = steamed, SF = stir fried. Abbreviations: 13C, indole-3-carbinol; 1HIC, indoleacetonitrile; SFP, sulforaphane; IB, iberin; SFN, sulforaphane nitrile; ER, erucin; IBN, iberin nitrile; BAN, benzeneacetonitrile; BPN, Benzenepropanenitrile; ERN, erucin nitrile; 3BITC, 3-butenyl isothiocyanate.

Total GHPs varied greatly in concentration and types between varieties and cooking methods. Total GHPs were significantly higher in CPNT and BM varieties as well as SF samples (Appendix X; Tables S5c). SF-CNDTP has the highest total GHPs formed. The predominant hydrolysis products of raw samples were nitriles, while ITCs were predominantly formed in cooked samples. For example, 3BITC was not detected in raw samples, and erucin nitrile was not detected in ST samples. Significantly lower amounts of nitriles were formed in ST samples in comparison to SF samples. SF led to formation of significantly higher concentrations of ITCs than ST. The low concentrations of nitriles formed in ST samples could be linked to the denaturation of epithiospecifier proteins (ESP) and retention of myrosinase activity during ST

which prevents the ESP hydrolytic process, and enhances myrosinase breakdown of GSLs into ITCs. ESP leads to nitrile formation from GSL hydrolysis rather than ITCs, but it is more heat labile than myrosinase and will be denatured at temperatures above 50 °C (Lambrix et al., 2001; Matusheski et al., 2004). A study on ESP activity in broccoli florets showed significant reduction in ESP activity when the florets where heated above 50 °C for 10 min (Matusheski et al., 2004). Higher concentrations of ITCs formed in SF samples can be attributed to higher (up to 65 %) residual myrosinase activity after SF (Figure 5.1 and Table 5.3). A study on broccoli showed that mild cooking increased the conversion of GRPN to SFP rather than SFN (Ghawi et al., 2013). The result of this present study shows a relationship between ESP, myrosinase activity, ITC and nitrile formation, and is in agreement with previous studies (Rungapamestry et al., 2006; Jones et al., 2010; Matusheski et al., 2006; Lambrix et al., 2001). Other GHPs found included iberin (IB) and iberin nitrile (IBN) (hydrolysis products of GIBN), and indoleacetonitrile (1HIC) and indole-3-carbinol (I3C), the hydrolysis products of GBSN; these were the second most abundant GHPs formed. Overall, BK samples had much lower GHPs formed when compared to other *Brassica oleracea* species reported in literature such as red cabbage (Ciska & Pathak, 2004; Klopsch et al., 2017; Hanschen et al., 2017).

The findings of this study demonstrate that mild cooking/ heat treatment of kale before consumption is important as it leads to the formation of more health beneficial ITCs, rather than nitriles. Nitrile formation has been reported to reduce the health benefits of *Brassicas* consumed (Matusheski & Jeffery, 2001). The findings of this study, therefore suggest that though consumption of mildly heat-treated BK might lead to nitrile formation, more ITCs are still formed compared to when BK is severely heat treated; thus, potentially proffering more health benefits to the consumer.

5.3.2 Volatile compounds

In addition to ITCs, other compounds such as sulfides, aldehydes and alcohols also contribute to the flavour and aroma characteristics of *B. oleracea* vegetables. However, the profile and concentration of these volatile compounds may differ due to variety and/or domestic cooking which may in turn affect the sensory characteristics of the samples. More than 70 compounds were identified in the headspace of raw and cooked (ST or SF) samples in the three cultivars, and the most abundant compounds are listed in Table 5.5. These included 14 alcohols, 19 aldehydes, eight sulfur-containing compounds, two nitriles, six esters, five terpenes, ten hydrocarbons, four ketones and eight other compounds. Significant

quantitative differences were observed between the three different cultivars and between raw and cooked samples.

Alcohols comprised more than 40 % of the total volatiles collected in all raw samples. Similar percentages were observed for the BM and CPNT SF samples, however, this percentage decreased in the ST samples (down to 4 %). The most abundant alcohol was (Z)-3-hexen-1-ol, followed by (E)-2-hexenol and 1-hexanol. Longer chain saturated and unsaturated alcohols were present only in the SF samples. Aldehydes comprised less than 11 % of the total volatiles collected in raw and ST samples, a percentage that increased to more than 49 % in SF samples. The most abundant aldehyde was (E)-2-hexenal, present mainly in the SF samples. C6 alcohols and aldehydes, compounds formed from fatty acids via the lipoxygenase pathway, are known to impart green and grass-like aromas (Lignou et al., 2015). These compounds have been identified in other *Brassica* species such as rocket leaves (Raffo et al., 2018; Bell et al., 2016; Jirovetz et al., 2002), cabbage (Akpolat & Barringer, 2015), radish (Blazevic & Mastelic, 2009a), Wasabi (Depree et al., 1999), cauliflower (Engel et al., 2002a) and broccoli (Spadone et al., 2006). A number of long-chain alkenals such as (E)-2-octenal, (E)-2-nonenal, (E)-2-decenal, but also alkadienals such as (2,4-decadienal and (E,E)-2,4,-decadienal), imparting fatty odour notes, were present only in the SF samples. These compounds were generated during the heating of cooking oil. 2-Methypropanal, 2-methylbutanal and 3-methylbutanal, the products of Strecker degradation between a reactive dicarbonyl and an AA (in this case of valine, leucine and isoleucine respectively), impart a malty aroma, and were present only in SF samples. Similarly, phenylacetaldhyde from phenylalaninine, has a floral, honey, or rose aroma (Lignou et al., 2015), and was present only in SF samples.

Eight sulfur-containing compounds were identified in the headspace of the samples. Dimethyl disulfide and trisulfide, both known to have a sulfurous cabbage-like odour, were amongst the most abundant. In *Brassica* species, these compounds can either be formed from (+)-S-methyl-L-cysteine sulfoxide (SMCSO) or by degradation of volatiles derived from GSL break down (Banerjee et al., 2014). 2-Ethylthiophene was only present in raw samples, whereas, methyl thiocyanate, a GHP, was present in raw and stir-fried samples. Engel et al. (2002b) reported that this compound had a sulfury odour in cooked cauliflower. 3BITC, a gluconapin hydrolysis product, was found in both ST and SF samples in all three cultivars. This compound was found in wasabi (Depree et al., 1999) and rocket leaves imparts a vegetable leaf, cabbage odour (Raffo et al., 2018).

Six esters with a base of 6 carbon atoms were present mainly in the raw samples; these included hexyl acetate, (E)-3-hexenyl acetate, (Z)-3-hexenyl acetate, (Z)-2-hexenyl acetate, hexyl butanoate and (Z)-3-hexenyl butanoate. Hexyl acetate is known to have a fruity and sweet odour, whereas (Z)-3-hexenyl acetate imparts, a green and fresh odour (Conde-Martínez et al., 2014). This compound which is formed from the esterification of (Z)-3-hexenol (Hatanaka, 1993), was sometimes reduced more than 100-fold in the ST and SF samples.

Terpenes were also identified in the headspace of the samples with D-limonene being the most abundant in all three varieties. The levels of this compound increased consistently in ST samples and decreased in SF samples. p-Cymene was present only in the ST samples.

Besides alcohols, aldehydes, sulfur-containing compounds, esters and terpenes, some hydrocarbons and ketones were identified. The abundance of most hydrocarbons was high in raw samples, and negligible in ST or SF samples. 2,4-Dimethyl-heptane and 2,2,4,6,6-pentamethyl-heptane were only present in cooked samples. 3-Pentanone (a ketone) was present in all three cultivars in raw samples; it was not detected in ST samples, and was reduced by more than half in SF samples. Acetophenone was present only in ST samples, whereas 1-penten-3-one was only in the SF. Raffo et al. (2018) associated the latter compound with the trigeminal sensation of pungency, but also described it as sulfurous, gas-like and truffle-like in rocket leaves.

It is worth noting that a number of compounds not present in raw samples were formed during SF in all three cultivars as a result of the Maillard reaction. These compounds were 5-methyl-2-furancarboxaldehyde, furfural, 2-furanmethanol, methylpyrazine, and 1-(1H-1-pyrrolyl)-2-propanone, potentially contributing to cooked and burnt notes.

Table 5.5: Relative amounts of volatiles identified and quantified in black kale samples (ST = steamed, SF = stir fried)

Code	Compound	LRI[b]	ID[c]	BM			CNDTP			CPNT			*P-value*
				Raw	ST	SF	Raw	ST	SF	Raw	ST	SF	
	Alcohol												
a01	1-penten-3-ol (1pent3)	1173	A	92^{c}	ND	74^{bc}	79^{bc}	ND	40^{a}	53^{ab}	ND	79^{bc}	< 0.0001
a02	1-pentanol (1-pent)	1261	A	10^{abc}	4^{a}	20^{c}	5^{a}	9^{abc}	17^{bc}	6^{ab}	14^{abc}	11^{abc}	0.001
a03	(E)-2-penten-1-ol (2pent (E))	1325	A	30^{c}	ND	6^{ab}	12^{b}	ND	2^{a}	11^{b}	ND	5^{ab}	< 0.0001
a04	(Z)-2-penten-1-ol (2pent (Z))	1332	A	ND	ND	45^{ab}	ND	ND	32^{a}	ND	ND	57^{b}	< 0.0001
a05	1-hexanol (1hex)	1363	A	490^{c}	ND	169^{b}	468^{c}	ND	7^{a}	423^{c}	ND	156^{b}	< 0.0001
a06	(E)-3-hexen-1-ol (3hex(E))	1374	A	84^{b}	ND	20^{a}	76^{b}	ND	14^{a}	63^{b}	ND	19^{a}	< 0.0001
a07	(Z)-3-hexen-1-ol (3hex(Z))	1395	A	2744^{d}	17^{a}	868^{c}	2497^{d}	17^{a}	203^{ab}	2393^{d}	26^{a}	537^{bc}	< 0.0001
a08	(E)-2-hexen-1-ol (2hex(E))	1417	A	516^{c}	ND	413^{bc}	466^{c}	ND	17^{a}	369^{bc}	ND	295^{b}	< 0.0001
a09	1-octen-3-ol (1-octen-3-ol)	1459	A	ND	ND	6^{b}	ND	ND	5^{ab}	ND	ND	4^{a}	< 0.0001
a10	1-heptanol (1hep)	1464	A	ND	ND	3^{a}	ND	ND	2^{a}	ND	ND	3^{a}	< 0.0001
a11	2-ethyl-1-hexanol (1hex2ethyl)	1497	A	9^{a}	ND	ND	18^{b}	ND	ND	18^{b}	ND	ND	< 0.0001
a12	1-octanol (1oct)	1567	A	ND	3^{a}	11^{c}	ND	3^{a}	5^{ab}	ND	5^{ab}	7^{b}	< 0.0001
a13	(E)-2-octen-1-ol (2-oct(E))	1626	A	ND	ND	1^{a}	ND	ND	1^{a}	ND	ND	3^{b}	< 0.0001
a14	phenylethanol (phenyethyl)	1933	B	ND	ND	2^{b}	ND	ND	1^{a}	ND	ND	2^{b}	< 0.0001
	Aldehyde												
ald01	2-methylpropanal (prop)	823	A	ND	ND	3^{ab}	ND	ND	4^{b}	ND	ND	2^{a}	< 0.0001
ald02	2-methylbutanal (but2)	927	A	ND	ND	3^{a}	ND	ND	15^{b}	ND	ND	11^{b}	< 0.0001
ald03	3-methylbutanal (but3)	931	A	ND	ND	3^{a}	ND	ND	15^{c}	ND	ND	8^{b}	< 0.0001
ald04	pentanal (pent)	997	A	ND	12^{ab}	ND	ND	10^{a}	ND	ND	15^{b}	ND	< 0.0001

ald05	hexanal (hex)	1198	A	40^{ab}	15^{ab}	474^{d}	28^{ab}	20^{ab}	124^{b}	25^{ab}	2^{a}	257^{c}	< 0.0001
ald06	(E)-2-pentenal (2pent)	1147	A	20^{d}	ND	14^{cd}	18^{d}	ND	5^{ab}	5^{ab}	ND	9^{bc}	< 0.0001
ald07	(Z)-2-hexenal (2hex (Z))	1218	B	ND	ND	47^{b}	ND	ND	18^{a}	ND	ND	33^{ab}	< 0.0001
ald08	(E)-2-hexenal (2hex (E))	1236	A	438^{bc}	ND	1451^{e}	560^{bc}	ND	837^{cd}	171^{ab}	ND	1038^{d}	< 0.0001
ald09	octanal (oct)	1302	A	ND	7^{bc}	2^{a}	ND	9^{c}	2^{a}	ND	6^{b}	2^{a}	< 0.0001
ald10	(E)-2-heptenal (2hep)	1343	A	ND	ND	14^{b}	ND	ND	7^{a}	ND	ND	6^{a}	< 0.0001
ald11	nonanal (Non)	1408	A	ND	28^{cd}	17^{bc}	ND	19^{bc}	14^{bc}	ND	38^{d}	13^{ab}	< 0.0001
ald12	(E,E)-2,4-hexadienal (2,4Dec(E,E))	1426	B	12^{bc}	ND	45^{e}	14^{bc}	ND	18^{c}	8^{ab}	ND	34^{d}	< 0.0001
ald13	(E)-2-octenal (2Octe)	1448	B	ND	ND	6^{b}	ND	ND	6^{b}	ND	ND	4^{a}	< 0.0001
ald14	(E,E)-2,4-heptadienal (2Hep(E,E))	1488	A	13^{c}	ND	4^{a}	7^{ab}	ND	7^{ab}	7^{b}	ND	5^{ab}	< 0.0001
ald15	(E)-2-nonenal (2Non)	1555	A	ND	ND	2^{b}	ND	ND	1^{a}	ND	ND	2^{b}	< 0.0001
ald16	(E)-2-decenal (2Dec)	1664	A	ND	ND	2^{b}	ND	ND	1^{a}	ND	ND	2^{b}	< 0.0001
ald17	phenylacetaldehyde (Phe)	1670	A	ND	ND	7^{b}	ND	ND	7^{b}	ND	ND	4^{a}	< 0.0001
ald18	2,4-decadienal (2,3Dec)	1747	B	ND	ND	5^{b}	ND	ND	2^{a}	ND	ND	3^{a}	< 0.0001
ald19	(E,E)-2,4-decadienal (2,3Dec (E,E))	1835	A	ND	ND	4^{ab}	ND	ND	4^{b}	ND	ND	2^{a}	< 0.0001
	Sulfur-containing compounds												
s01	carbon disulfide (Car)	740	A	7^{d}	3^{ab}	4^{abc}	6^{cd}	2^{a}	3^{ab}	5^{bc}	3^{ab}	4^{abc}	< 0.0001
s02	dimethyl sulfide (DMS)	764	A	ND	3^{b}	2^{ab}	ND	2^{b}	3^{b}	ND	7^{c}	2^{ab}	< 0.0001
s03	dimethyl disulfide (DMDS)	1087	A	311^{d}	21^{a}	139^{bc}	82^{ab}	13^{a}	49^{a}	158^{c}	13^{a}	53^{a}	< 0.0001
s04	2-ethylthiophene (ThioEster)	1184	B	51^{a}	ND	ND	50^{a}	ND	ND	67^{a}	ND	ND	< 0.0001
s05	methyl thiocyanate (Thio)	1292	B	66^{b}	ND	20^{a}	89^{b}	ND	3^{a}	71^{b}	ND	15^{a}	< 0.0001
s06	dimethyl trisulfide (DMTS)	1400	A	52^{b}	3^{a}	43^{b}	69^{b}	4^{a}	49^{b}	63^{b}	3^{a}	41^{b}	< 0.0001
s07	3-butenyl isothiocyanate (3BITC)	1476	B	ND	2^{a}	4^{c}	ND	4^{bc}	5^{c}	ND	2^{ab}	3^{abc}	< 0.0001

s08	methyl (methylthio)methyl disulfide (DMMM)	1689	B	2^{bc}	ND	4^{d}	2^{ab}	ND	2^{c}	1^{a}	ND	2^{bc}	< 0.0001
	Nitriles												
n01	benzyl nitrile (Benz)	1957	B	2^{b}	ND	ND	1^{a}	ND	ND	2^{ab}	ND	ND	< 0.0001
n02	benzenepropanenitrile (Benzprop)	2082	B	2^{abc}	ND	3^{bcd}	7^{e}	ND	1^{ab}	5de	ND	4^{cd}	< 0.0001
	Ester												
e01	hexyl acetate	1283	B	227^{b}	ND	ND	296^{c}	ND	ND	191^{a}	ND	ND	< 0.0001
e02	(E)-3-hexenyl acetate (3hex(E))	1320	A	47^{b}	ND	2^{a}	64^{b}	ND	2^{a}	44^{b}	ND	2^{a}	< 0.0001
e03	(Z)-3-hexenyl acetate (3hex(Z))	1331	A	2797^{b}	23^{a}	71^{a}	2661^{b}	15^{a}	2^{a}	2152^{b}	31^{a}	8^{a}	< 0.0001
e04	(Z)-2-hexenyl acetate (32hex(Z))	1346	A	154^{b}	ND	ND	153^{b}	ND	ND	86^{a}	ND	ND	< 0.0001
e05	hexyl butanoate (But acid)	1428	B	3^{a}	ND	ND	4^{a}	ND	ND	7^{b}	ND	ND	< 0.0001
e06	(Z)-3-hexenyl butanoate (But acid 3hex)	1476	B	33^{a}	ND	7^{a}	112^{b}	ND	6^{a}	90^{b}	ND	2^{a}	< 0.0001
	Terpene												
t01	β-myrcene (Myr)	1171	A	9^{d}	6^{c}	ND	7^{c}	2^{b}	ND	1^{ab}	2^{ab}	ND	< 0.0001
t02	D-limonene (Limon)	1202	A	215^{ab}	401^{b}	47^{a}	462^{b}	975^{c}	59^{a}	87^{a}	928^{c}	78^{a}	< 0.0001
t03	p-cymene (Cym)	1284	A	ND	6^{ab}	ND	ND	4^{a}	ND	ND	7^{b}	ND	< 0.0001
t04	D-carvone (D-carv)	1761	B	3^{b}	ND	ND	4^{c}	ND	ND	1^{a}	ND	ND	< 0.0001
t05	(E)-β-ionone (Ion)	1963	A	10^{b}	ND	ND	7^{ab}	ND	ND	5^{a}	ND	ND	< 0.0001
	Hydrocarbon												
h01	2,4-dimethyl-heptane ((Hep)	809	B	ND	10^{d}	6^{c}	ND	4^{bc}	6^{c}	ND	6^{c}	2^{ab}	< 0.0001
h02	2,2,4,6,6-pentamethyl-heptane (Hep 2,2)	952	A	ND	2^{sb}	2^{bc}	ND	1^{a}	2^{abc}	ND	2^{abc}	3^{c}	< 0.0001
h03	3-ethyl-1,5-octadiene isomer [d] (3Ethyl 1)	1018	B	16^{b}	ND	ND	13^{b}	ND	ND	5^{a}	ND	ND	< 0.0001

h04	3-ethyl-1,5-octadiene isomer [d] (3Ethyl 2)	1034	B	127[b]	3[a]	4[a]	128[b]	3[a]	1[a]	33[a]	1[a]	1[a]	< 0.0001
h05	3-ethyl-1,5-octadiene isomer [d] (3Ethyl 3)	1091	B	158[b]	ND	ND	144[b]	ND	ND	56[a]	ND	ND	< 0.0001
h06	1,4-dimethylbenzene (p-Xylene)	1155	B	ND	ND	5[a]	ND	ND	6[a]	ND	ND	6[a]	< 0.0001
h07	1-dodecene (1Dod)	1244	A	7[b]	ND	ND	8[b]	ND	ND	6[a]	ND	ND	< 0.0001
h08	1-tridecene (Trid)	1346	A	5[d]	2[ab]	ND	4[cd]	4[cd]	ND	4[cd]	3[bc]	ND	< 0.0001
h09	octadecane (Oct)	1797	A	2[b]	ND	ND	4[c]	ND	ND	1[a]	ND	ND	< 0.0001
h10	nonadecane (Nona)	1900	A	3[a]	ND	ND	3[a]	ND	ND	2[a]	ND	ND	< 0.0001
	Ketone												
k01	3-pentanone (3Pent)	994	A	75[e]	ND	21[bc]	54[d]	ND	10[ab]	41[cd]	ND	19[ab]	< 0.0001
k02	1-penten-3-one (1pent)	1038	A	ND	ND	29[b]	ND	ND	14[a]	ND	ND	13[a]	< 0.0001
k03	2,3-octanedione (2,3Oct)	1338	B	10[d]	ND	3[a]	6[c]	ND	4[abc]	5[bc]	ND	3[ab]	< 0.0001
k04	acetophenone (Acet)	1678	A	ND	0.4[a]	ND	ND	0.4[a]	ND	ND	1[b]	ND	< 0.0001
	Other												
o01	hexanoic acid (Hex)	1862	A	4[d]	1[a]	2[bc]	2[bc]	1[ab]	1[ab]	2[c]	1[a]	1[ab]	< 0.0001
o02	octanoic acid (Oct)	2085	A	3[bc]	2[abc]	2[abc]	2[abc]	3[bc]	1[a]	3[c]	2[abc]	2[ab]	< 0.0001
o03	2-pentylfuran (Fur)	1250	A	ND	ND	4[b]	ND	ND	2[a]	ND	ND	5[b]	< 0.0001
o04	5-methyl-2-furancarboxaldehyde (2Fur)	1598	B	ND	ND	4[a]	ND	ND	7[b]	ND	ND	6[ab]	< 0.0001
o05	furfural	1485	B	ND	ND	5[a]	ND	ND	16[b]	ND	ND	13[b]	< 0.0001
o06	2-furanmethanol (2Fumet)	1676	B	ND	ND	4[a]	ND	ND	8[b]	ND	ND	6[ab]	< 0.0001
o07	methylpyrazine (Pyr)	1284	A	ND	ND	3[a]	ND	ND	12[b]	ND	ND	9[b]	< 0.0001
o08	1-(1H-1-pyrrolyl)-2-propanone	1743	B	ND	ND	1[a]	ND	ND	1[a]	ND	ND	2[b]	0.001

[a] Values are peak area means of four replicates divide by 10^5. [b] Linear retention index on a Stabilwax-DA column. [c] A, mass spectrum and LRI agree with those of authentic compound; B, mass spectrum agrees with reference spectrum in the NIST/NIH mass spectra database and LRI agree with those in literature. [d] Pair of stereoisomers. ND = Not detected. Mean values with different superscripts in the same row significantly different at $p<0.05$.

5.3.3 Sensory attributes

The trained panel described the kale samples using 42 attributes. Mean scores with significant differences for all attributes are presented in Table 5.6. Assessor-sample interactions, discrimination and repeatability were checked for all assessors. The panel found 42 of the 45 attributes to be significantly different between samples. Cooking significantly enhanced moist, green and shiny appearance of BK samples.

Significant differences in odour attributes were mostly due to cooking rather than cultivar. Leafy green and stalky odour/flavour attributes were significantly higher in raw samples than cooked samples which confirms the results of HS-SPME analysis where higher amounts of six-carbon aldehydes and alcohols (especially (Z)-3-hexen-1-ol) were found in raw BK samples. As expected, only SF samples were reported to have burnt and sesame odours/flavours. This can be attributed to the Maillard reactions taking place during SF, which resulted in the production of furans and pyrazines that were detected only in SF samples (Table 5.5). Warming mouthfeel, sulfurous and swede odours/flavours were higher in raw samples than cooked samples; though the difference was not significant in ST samples. This is reflected in the high abundance of sulfur-containing compounds present in raw samples. Though ST samples had significantly lower amounts of sulfur compounds formed than SF samples, ST samples were perceived to be more sulfurous than stir-fried samples which might be due to the binding effect of the oil used for SF and prevents release in the mouth. This could also be due to the change in perceived flavour when these compounds are in combination with other volatiles, such as Maillard derived volatiles. Cooked samples were perceived to have sweeter odours than raw samples, though it was only significant in SF CPNT sample.

In terms of mouthfeel, raw samples were rated as more crunchy, tough, fibrous and having more residue (bits-in-mouth) aftereffects. These differences are due to the harder texture of the raw samples, which makes it more difficult to chew. Raw samples were rated significantly more bitter in taste and aftertaste than cooked samples, with cooked samples having higher sweet taste scores although this difference was not significant in most cases. This could be due to the ratio between sweet and bitter compounds present in the samples and how cooking affects the balance of this ratios. For example, ST samples with lower GSLs contents had higher sugar content compared to other samples. Raw samples were rated to have more throat catching aftereffects, bitter and lingering aftertaste which are all related to the higher bitter taste and sulfurous aromas perceived during eating. High sulfurous and

pungent aromas have been reported to be undesirable characteristics of *Brassica* vegetables, and can influence consumer liking and acceptability (Kubec et al., 1998; Baik et al., 2003).

5.3.4 Consumer study

5.3.4.1 Consumer demographics and black kale consumption

Summarized results of demographic characteristics for the 105 consumers who took part in the study are presented in Table 5.7. 62.9 % (n=66) participants were between the ages of 18 – 30 years. 50 % of participants were of white ethnicity and 74.3 % females. 61 %, 44.8 % and 74.3 % of consumers mentioned boiling, steaming and stir-frying (respectively) as their method of cooking any type of cabbage; only 24.8 % stated that they ate cabbages raw. 40 % (n= 42) of participants consumed any types of cabbage sometimes and 31.4 % frequently. Participants were asked about their consumption of kale and only 36.2 % (n=38) said they consumed it.

5.3.4.2 Consumer results for liking, taste perception and cluster analysis

Table 5.8 shows the mean values of consumer responses and cluster analysis. Cooking had significant effects on all parameters, while no significant effect due to cultivar was observed. Appearance liking differed significantly between raw and cooked samples. The preference for cooked samples might be due to the moist, shiny and greener appearance attributes (Table 5.6).

In terms of mouthfeel liking, cooked samples were liked significantly more than raw samples which might be due to the moist, softer, and less fibrous texture of the cooked BK leaves which made them easier to chew and swallow. Sensory profiling results showed that raw samples were tougher and more fibrous compared to cooked samples. The result for taste liking followed a similar trend to appearance and mouthfeel liking. Consumers preferred the taste of cooked samples to raw, and scored them significantly higher for taste liking.

There was a relationship between bitter and sweet taste perception of BK samples by consumers. Raw samples were perceived significantly more bitter and less sweet than cooked sample and *vice versa*. This result agrees with the findings of the sensory profiling data where trained panellists scored raw samples as being more bitter and less sweet. Bitterness perception has long been regarded as an important factor that can influence consumer liking and acceptance of *Brassica* vegetables. The findings of this study agrees with that hypothesis, as the results of taste liking shows consumers preferred the less bitter and sweeter samples. Mild steaming and cooking might therefore be a way of improving consumer liking and

acceptance of BK. This could consequently have a positive effect on the health benefits derived from BK consumption, as results from GHPs showed higher concentrations were formed in cooked samples.

Following this trend, and based on the results of taste perception and liking, cooked samples were significantly more liked and preferred in terms of overall liking. The ratings for consumption intent showed that consumers were significantly more likely to consume cooked BK than raw BK, which is in consonance with the taste perception and liking results. ST- CNDTP sample was the most preferred, and had the highest scores for almost all parameters. In some cases, it differed significantly to the ST and SF samples of the other two cultivars.

The results of agglomerative hierarchal cluster (AHC) analysis for consumer overall liking data is presented in Table 5.8. Two clusters were observed for liking patterns of BK samples. ANOVA analysis on the data revealed significant differences in the liking scores of the two clusters. Cluster 1 (39 %), was the smaller cluster, and scored all samples significantly higher than cluster 2 (61 %). From their liking scores, consumers in cluster 1 liked all samples while cluster 2 rated their liking of the cooked cabbages higher; but generally scored all samples lower than cluster 1. Consumers in both cluster 1 and 2 liked cooked BK more than raw.

5.3.5 Effect of genotype on taste perception and consumer liking

The number of consumers in the different genotype groups is presented in Table 5.8. Figures 5.4 and 5.4 show the results for bitter perception and taste liking according to genotype. For *TAS2R38*, 21 % (n=22) carried the AVI/AVI genotype, 48.5 % (n=51) PAV/AVI, 20 % (n=21) PAV/PAV and 10.5 % (n=11) the rare genotypes, which comprised AAI and AAV. There were 46 % (n=48) people with the A/A gustin genotype, 39 % (n=41) with A/G and 15 % (n=16) with the G/G genotype.

Table 5.6: Mean scores for sensory attributes of black kale samples (ST = steamed, SF = stir fried).

	BM			CNDTP			CPNT			
Attributes	Raw	ST	SF	Raw	ST	SF	Raw	ST	SF	*P-value*
Appearance										
Bubbly_A	46.4^{a}	43.4^{ab}	43.0^{ab}	31.3^{b}	44.7^{ab}	35.3^{ab}	45.0^{ab}	45.8^{a}	40.4^{ab}	0.011
Brown scorch marks_A	0.0^{c}	0.0^{c}	9.4^{b}	0.0^{c}	0.0^{c}	24.5^{a}	0.0^{c}	0.0^{c}	16.9^{ab}	<.0001
Prominence of veins_A	44.7	46.5	42.9	40.1	47.4	37.9	47.7	49.2	38.2	0.131
Green_A	53.6^{b}	75.2^{a}	73.2^{a}	49.8^{b}	71.0^{a}	65.9^{a}	53.7^{b}	67.3^{a}	67.7^{a}	<.0001
Shiny_A	7.5^{c}	46.4^{b}	62.5^{a}	4.3^{c}	43.1^{b}	54.2^{ab}	6.4^{c}	44.0^{b}	62.3^{a}	<.0001
Oily Surface_A	0.0^{b}	0.1^{b}	28.3^{a}	0.0^{b}	0.1^{b}	27.3^{a}	0.0^{b}	0.6^{b}	34.2^{a}	<.0001
Moist_A	7.9^{b}	57.0^{a}	57.4^{a}	7.6^{b}	52.8^{a}	49.0^{a}	10.5^{b}	57.3^{a}	52.7^{a}	<.0001
Cooked_A	0.0^{c}	63.2^{ab}	69.6^{ab}	0.0^{c}	59.2^{b}	73.5^{a}	0.0^{c}	61.9^{b}	74.4^{a}	<.0001
Odour										
Sweet_O	24.6^{b}	35.4^{ab}	34.9^{ab}	23.2^{b}	36.4^{ab}	35.3^{ab}	20.9^{b}	35.5^{ab}	40.7^{a}	0.0004
Stalky_O	34.6^{a}	17.0^{b}	10.6^{b}	32.7^{a}	14.5^{b}	4.8^{b}	35.0^{a}	16.4^{b}	6.6^{b}	<.0001
Leafy Green_O	52.1^{a}	39.1^{abc}	28.3^{cde}	45.1^{ab}	35.4^{bcd}	19.3^{e}	51.7^{a}	40.6^{abc}	24.1^{de}	<.0001
Sesame_O	0.0^{b}	0.0^{b}	28.8^{a}	0.0^{b}	0.0^{b}	36.8^{a}	0.0^{b}	0.0^{b}	32.9^{a}	<.0001
Metallic_O	13.3^{a}	6.2^{ab}	3.5^{b}	10.9^{ab}	3.8^{b}	4.4^{b}	10.9^{ab}	5.5^{ab}	4.1^{b}	0.0008
Swede_O	16.3^{abc}	25.8^{a}	10.1^{bc}	16.0^{abc}	21.9^{ab}	4.4^{c}	15.9^{abc}	24.6^{a}	8.2^{bc}	<.0001

Sulfurous_O	22.0^{a}	16.4^{ab}	6.0^{bc}	23.7^{a}	16.3^{ab}	1.8^{c}	22.3^{a}	14.6^{ab}	2.8^{c}	<.0001
Burnt_O	0.0^{b}	0.0^{b}	9.6^{ab}	0.0^{b}	0.0^{b}	21.2^{a}	0.0^{b}	0.0^{b}	18.7^{a}	<.0001
Cooked_O	0.0^{d}	62.4^{bc}	74.0^{ab}	0.0^{d}	58.5^{c}	76.7^{a}	0.0^{d}	62.9^{bc}	73.2^{ab}	<.0001
Mouthfeel										
Crunchy_MF	47.9^{a}	29.2^{b}	37.3^{ab}	48.7^{a}	31.5^{b}	32.4^{b}	48.3^{a}	26.7^{b}	37.2^{ab}	<.0001
Moist_MF	19.7^{b}	52.0^{a}	47.6^{a}	22.7^{b}	50.1^{a}	45.2^{a}	20.8^{b}	51.4^{a}	43.2^{a}	<.0001
Warming_MF	13.8^{a}	3.8^{bc}	6.3^{abc}	11.0^{abc}	4.4^{abc}	3.2^{c}	13.2^{ab}	6.2^{abc}	5.8^{abc}	0.001
Fibrous_MF	41.9^{a}	30.1^{b}	28.3^{b}	35.5^{ab}	29.8^{b}	29.5^{b}	37.1^{ab}	31.2^{ab}	30.5^{b}	0.002
Toughness_MF	51.5^{a}	35.0^{cd}	35.9^{cd}	50.3^{ab}	34.3^{cd}	33.6^{d}	46.0^{abc}	38.4^{bcd}	34.9^{cd}	<.0001
OilyMouthfeel_MF	0.3^{b}	0.4^{b}	18.8^{a}	0.0^{b}	0.4^{b}	28.3^{a}	0.1^{b}	1.0^{b}	28.4^{a}	<.0001
Taste										
Stalk: Bitter_T	20.5^{a}	11.3^{ab}	13.5^{ab}	14.0^{ab}	12.4^{ab}	9.6^{b}	17.9^{ab}	11.9^{ab}	10.5^{b}	0.0084
Stalk: Sweet_T	31.6	36.7	28.9	38.8	39.0	36.5	34.7	35.4	32.6	0.231
Leaf: Bitter_T	41.0^{a}	21.4^{bcd}	21.4^{bcd}	33.8^{abc}	20.5^{cd}	16.3^{d}	34.9^{ab}	24.5^{bcd}	16.1^{d}	<.0001
Leaf: Sweet_T	17.6^{ab}	28.5^{a}	24.5^{ab}	19.5^{ab}	28.6^{a}	28.4^{a}	16.0^{b}	28.3^{a}	28.7^{a}	0.0004
Leaf: Salthy_T	2.3^{cde}	6.7^{abc}	8.2^{ab}	1.6^{e}	5.4^{bcde}	10.1^{a}	2.2^{de}	6.3^{abcd}	8.5^{ab}	<.0001
Leaf: Savoury_T	18.2^{de}	28.3^{bc}	34.1^{ab}	17.0^{e}	26.8^{bcd}	39.1^{a}	19.4^{cde}	28.0^{bc}	40.0^{a}	<.0001
Leaf: Metallic_T	17.6^{a}	5.5^{c}	7.7^{bc}	14.4^{abc}	6.0^{c}	6.4^{c}	16.4^{ab}	6.1^{c}	6.0^{c}	<.0001
Flavour										

Leafy Green_F	57.2^{ab}	51.3^{abc}	36.5^{def}	56.7^{abc}	44.5^{cde}	30.0^{f}	60.2^{a}	47.2^{bcd}	32.3^{ef}	<.0001
Stalky_F	32.3^{a}	13.6^{bc}	8.5^{c}	26.2^{ab}	12.6^{bc}	6.1^{c}	28.1^{a}	11.5^{c}	7.5^{c}	<.0001
Peppery_F	10.7^{a}	3.4^{bcd}	4.4^{bcd}	9.3^{ab}	1.7^{d}	3.1^{cd}	7.9^{abc}	2.6^{cd}	5.4^{abcd}	<.0001
Sesame_F	0.0^{b}	0.1^{b}	20.8^{a}	0.0^{b}	0.1^{b}	29.4^{a}	0.0^{b}	0.1^{b}	26.5^{a}	<.0001
Sulfury_F	25.7^{a}	14.1^{bc}	7.8^{cd}	23.5^{ab}	12.9^{cd}	3.9^{d}	23.1^{ab}	11.8^{cd}	4.6^{cd}	<.0001
Burnt_F	0.4^{c}	0.0^{c}	6.1^{bc}	0.1^{c}	0.0^{c}	14.9^{a}	0.2^{c}	0.0^{c}	10.2^{ab}	<.0001
Aftereffects										
Bitter_AE	29.0^{a}	8.8^{c}	11.2^{bc}	24.4^{a}	11.1^{bc}	7.1^{c}	22.1^{ab}	12.3^{bc}	9.4^{c}	<.0001
Throat catching_AE	3.7^{a}	1.3^{a}	1.5^{a}	2.2^{a}	0.8^{a}	2.1^{a}	2.4^{a}	0.8^{a}	1.4^{a}	0.233
Residue (Bits in mouth)_AE	25.6^{a}	13.8^{c}	16.5^{bc}	23.1^{a}	15.7^{c}	15.3^{c}	22.6^{ab}	16.0^{c}	15.1^{c}	<.0001
Oily mouthcoating_AE	0.0^{b}	0.2^{b}	17.0^{a}	0.0^{b}	0.6^{b}	27.5^{a}	0.1^{b}	0.7^{b}	25.4^{a}	<.0001
Lingering aftertaste_AE	39.9^{a}	27.1^{b}	33.9^{ab}	38.5^{ab}	26.8^{b}	34.0^{ab}	33.9^{ab}	28.2^{ab}	31.9^{ab}	0.004
Burnt_AE	0.4^{b}	0.0^{b}	5.7^{a}	0.3^{b}	0.0^{b}	9.6^{a}	0.2^{b}	0.0^{b}	7.6^{a}	<.0001
Salty_AE	1.8^{b}	4.7^{ab}	6.5^{a}	2.0^{b}	4.5^{ab}	7.2^{a}	2.3^{b}	5.7^{a}	6.5^{a}	<.0001
Nutty_AE	0.3^{b}	0.9^{b}	5.2^{ab}	0.2^{b}	1.0^{b}	7.0^{a}	0.4^{b}	0.1^{b}	6.7^{a}	<.0001
Metallic_AE	16.3^{a}	5.3^{c}	5.6^{bc}	12.2^{abc}	6.6^{bc}	5.4^{bc}	14.3^{ab}	6.4^{bc}	5.1^{c}	0.0001

Mean values with different superscripts in the same row are significantly different at $p<0.05$.

Table 5.7: Demographic characteristics of consumers (n=105)

Question	Number of individuals (%)
Age	
18-30	66 (62.9%)
31-45	19(18.1%)
46-61	20(19%)
Median age	28
Mean age	31
Ethnicity	
Arab	3 (2.9%)
Black African	14 (13.3%)
Carribbean	3 (2.9%)
Chinese	12 (11.4%)
Indian	2 (1.9 %)
White and Black Asian	4 (3.8%)
White British	39 (37.1%)
White Irish	1 (1%)
White Other	13 (12.4%)
Other ethnic group- any other	13 (12.4%)
Prefer not to declare	1 (1%)
Gender	
Male	27 (25.7%)
Female	78 (74.3%)
Cabbage cooking methods (consumers ticked all that applied)	
Raw	26 (24.8%)
Baked	9 (8.6%)
Boiled	64 (61.0%)
Microwaved	11 (10.5%)
Steamed	47 (44.8%)
Stir-fried	78 (74.3%)
Kale consumption	
Yes	38 (36.2%)
No	67 (63.8%)
Frequency of cabbage (any type) consumption	
Question: How often do to you consume cabbage?	
Never	7 (6.7%)
Rarely (less than once/month)	23 (21.9%)
Sometimes (approximately once/month)	42 (40.0%)
Frequently (approximately once/week)	33 (31.4%)

Table 5.8: Summary table of [x]consumer responses (n=105) and [x]cluster analysis results of mean overall liking scores

	BM			CNDTP			CPNT			
Attribute	Raw	ST	SF	Raw	ST	SF	Raw	ST	SF	*P-value*
Appearance liking	4.8^{a}	6.2^{cd}	6.0^{cd}	5.2^{ab}	6.6^{d}	5.7^{bc}	5.0^{ab}	6.1^{cd}	6.0^{cd}	< 0.0001
Mouthfeel liking	4.4^{a}	5.7^{b}	5.8^{b}	4.5^{a}	6.6^{c}	6.0^{bc}	4.2^{a}	6.2^{bc}	5.7^{b}	< 0.0001
Taste liking	4.3^{a}	6.0^{b}	6.3^{bc}	4.6^{a}	6.8^{c}	6.4^{bc}	4.3^{a}	6.4^{bc}	6.0^{bc}	< 0.0001
Overall liking	4.2^{a}	5.9^{b}	6.1^{bc}	4.4^{a}	6.8^{c}	6.3^{bc}	4.2^{a}	6.4^{bc}	6.0^{b}	< 0.0001
Bitter taste perception	33.5^{b}	12.8^{a}	11.6^{a}	30.8^{b}	8.5^{a}	9.9^{a}	28.7^{b}	9.7^{a}	13.0^{a}	< 0.0001
Sweet taste perception	5.6^{a}	18.1^{bc}	15.8^{b}	7.4^{a}	22.4^{c}	18.7^{bc}	7.4^{a}	20.1^{bc}	15.3^{b}	< 0.0001
Savoury taste perception	19.5	20.1	22.1	19.6	19.5	20.0	19.7	19.6	20.3	0.987
Consumption Intent	2.3^{a}	3.5^{b}	3.6^{b}	2.5^{a}	4.1^{c}	3.7^{bc}	2.4^{a}	3.7^{bc}	3.4^{b}	< 0.0001
Mean overall liking scores for two clusters of consumers										
Cluster 1 (*n*=41, 39%)	$5.9^{a,B}$	$6.9^{b,B}$	$7.1^{b,B}$	$5.7^{a,B}$	$7.6^{b,B}$	$7.0^{b,B}$	$5.3^{a,B}$	$7.1^{b,B}$	$7.2^{b,B}$	0.001
Cluster 2 (*n*=64, 61%)	$3.1^{a,A}$	$5.2^{b,A}$	$5.5^{bc,A}$	$3.5^{a,A}$	$6.3^{c,A}$	$5.8^{bc,A}$	$3.6^{a,A}$	$5.9^{bc,A}$	$5.1^{b,A}$	< 0.0001
P-value (cluster effect)[y]	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.001	< 0.0001	< 0.001	< 0.0001	

[x] Mean values with different superscripts 'abc' in the same row significantly different at p<0.05

[y] Mean values with different superscripts 'ABC' in the same column significantly different at p<0.05

TAS2R38 genotype had a significant effect on bitter taste perception (p=0.007), however the differences between the common diplotypes was not as direct as expected from the literature, where those expressing the sensitive PAV allele are expected to be more sensitive to bitter taste (Sandell et al., 2014). In the present study the PAV/PAV consumers did rate BK to be significantly more bitter than the PAV/AVI consumers (p=0.038), but there was no significant difference between PAV/PAV and AVI/AVI consumer ratings. The largest difference was between the high overall scores of the PAV/PAV group and the low overall scores of the rare genotype group (p=0.036). There were no significant interactions between *TAS2R38* genotype and BK cultivar or cooking method on bitter taste ratings (p=0.44; p=0.98 respectively). Considering raw BK, all four *TAS2R38* groups found bitterness to be significantly higher than in ST or SF BK, and there was no difference in ratings between the four groups.

TAS2R38 genotype had a significant effect on liking of BK taste (p=0.0004), but the differences were not driven by any difference between PAV/PAV and AVI/AVI. The PAV/AVI group rated their liking of BK taste to be significantly higher than the PAV/PAV group (a difference of 0.48 on 9-point scale; p = 0.018). As with bitter perception, the biggest difference in taste liking was driven by the *TAS2R38* rare genotype group who rated taste liking higher than both the PAV/PAV (p=0.0005) and the AVI/AVI (p=0.014) groups. Typically, the *TAS2R38* rare genotypes are removed from data sets prior to analysis; however, in this study where their proportion in the population was >10%, they were kept within the analysis and our results suggest that this group should not be ignored. There were no interactions between *TAS2R38* genotype and BK cultivar or cooking on taste liking (p = 0.97 and p = 0.92 respectively).

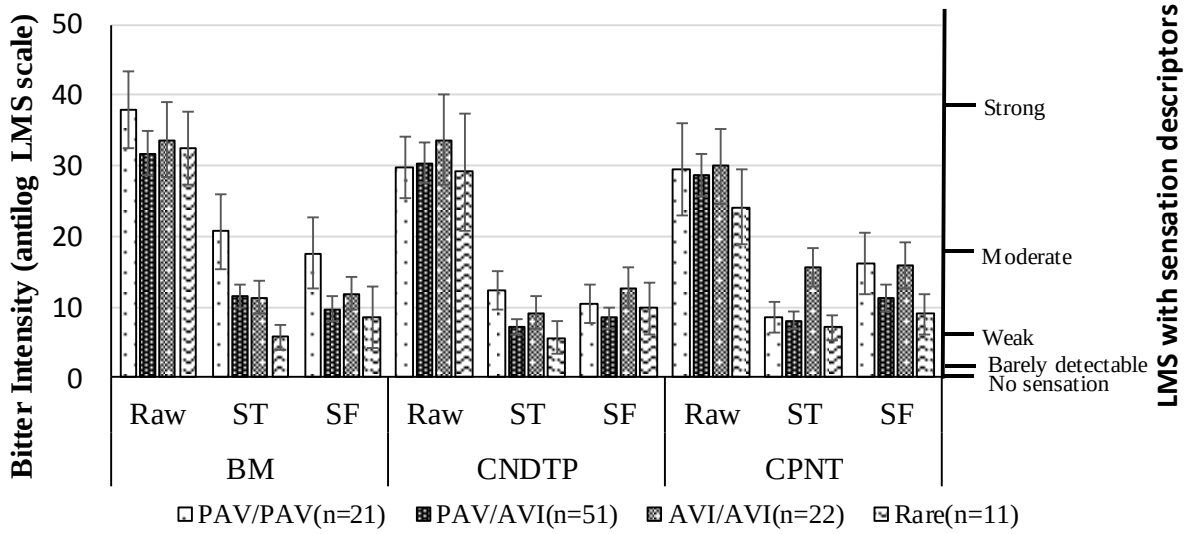
Gustin *rs2274333* genotype had a significant effect on bitter taste perception (p=0.033), however the differences between the GG group (that have been proposed to produce less taste cells), and others were not clearly defined. GG consumers did not rate bitterness of BK significantly differently to the AA or AG group (p = 0.76; p = 0.46 respectively) and the AA group (reported to produce the most taste cells) rated bitterness lower than the AG group (p=0.025). In liking of BK taste, the influence of gustin was significant (p = 0.021), however it was the GG group that rated liking lower than the AA and AG groups (p = 0.036; p = 0.02 respectively). There were no interactions between Gustin genotype and BK cultivar or cooking on taste perception or liking.

Although it would have been interesting to investigate any interaction between *TAS2R38* and Gustin on bitter perception and taste liking of BK, this was not justified due to the very low numbers of participants in some of the subgroups.

Table 5.9: Distribution of consumers based on genotype

Genotypes	Category	Number (%)
TAS2R38		
	AVI/AVI	22 (21.0%)
	PAV/AVI	51 (48.6%)
	PAV/PAV	21 (20.0%)
	Rare	11 (10.5%)
Gustin (CA6)		
	A/A	48 (45.7%)
	A/G	41 (39.0%)
	G/G	16 (15.2%)

(a)



(b)

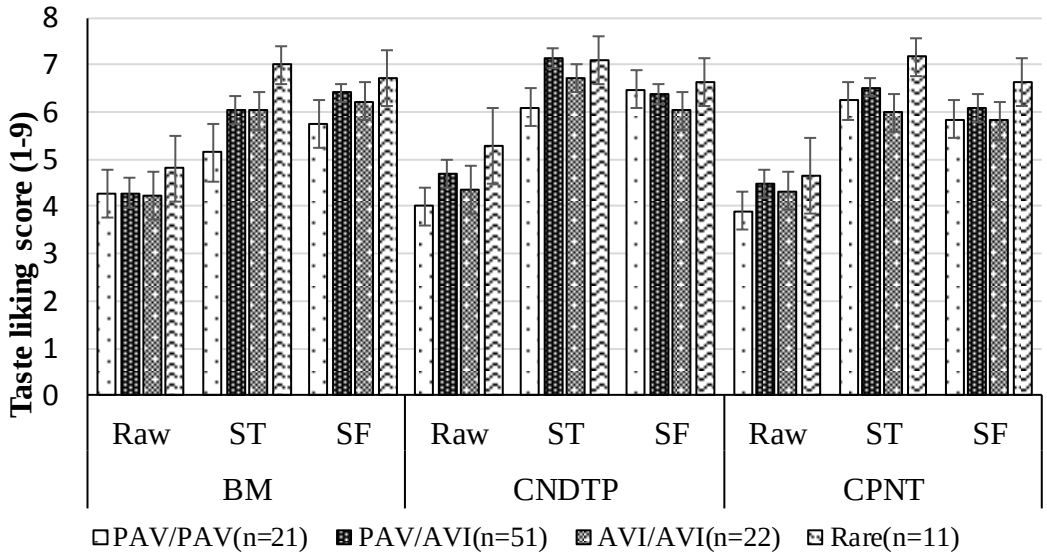
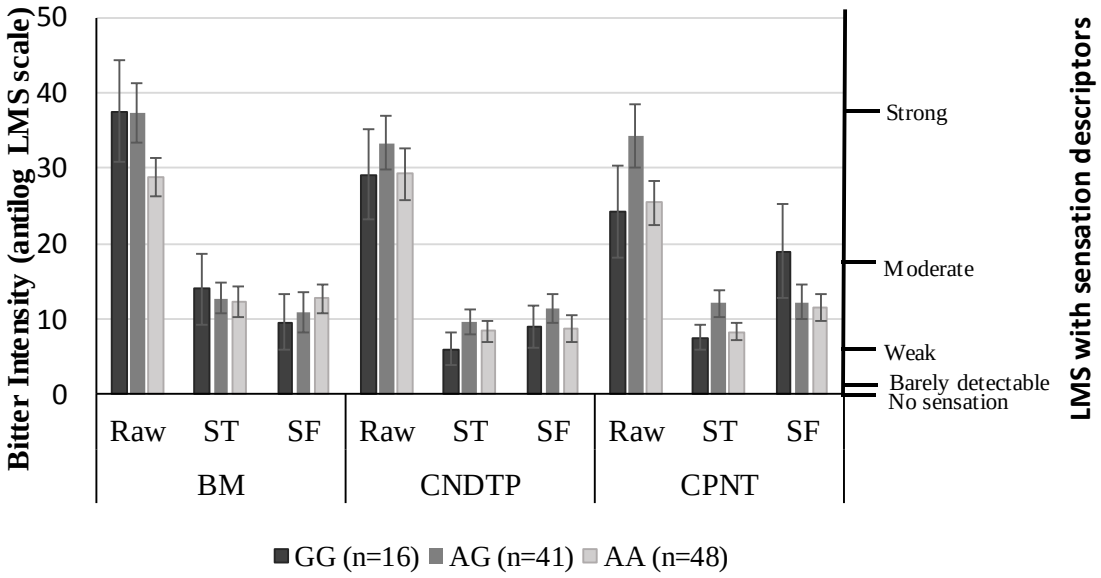


Figure 5.4. (a) Bitter intensity (BT) and (b) taste liking (TL) means scores of black kale samples (varieties BM, CNDTP, CPNT) according to TAS2R38 genotype.
Bitterness perception are given as antilog values. Error bars represent standard errors of mean values.

(a)



(b)

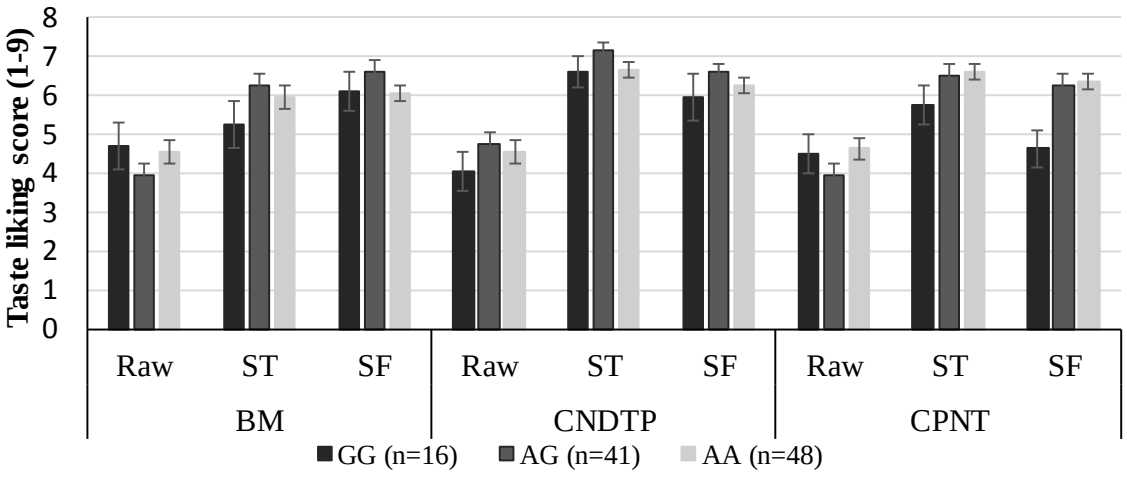


Figure 5.5. (a) Bitter intensity (BT) and (b) taste liking (TL) means scores of black kale samples (varieties BM, CNDTP, CPNT) according to Gustin (CA6) genotype.
Bitterness perception are given as antilog values. Error bars represent standard errors of mean values.

5.3.6 Rotated factor analysis (RFA) and multiple factor analysis (MFA)

Principal component analysis (PCA) with rotation was performed on each data subset (except for myrosinase activity). Rotated factor scores were collated and arranged based on the logical temporal order of data collection before running an MFA as shown in Figure 5.6. PC1 and PC2 explained 53 % of the data but other PCs did not provide any pertinent information, therefore only PC1 and PC2 are discussed.

Raw samples were positively correlated with sulfurous and stalky odours, warming, fibrous mouthfeel, bitter and metallic taste, sulfury and stalky flavour, and bitter and throat-catching aftereffects. These attributes were positively correlated with most of the volatiles (sulfur-containing compounds, and aldehydes), succinic acid, GSLs, GHPs (which are associated with undesirable bitter taste), sulfurous and green-grassy flavours. It was unexpected to see that sucrose and sweet tasting AAs were also positively correlated with bitter and sulfurous attributes. This might be due to low concentrations of these compounds which might therefore impact on sweet taste perception; or due to suppressing effects of the bitter tasting compounds (Beck et al., 2014; Bell et al., 2017b). Bitter taste perception for all consumer genotypes was positively correlated with raw samples, and negatively correlated to liking and sweet taste perception. This implies that consumers did not differ substantially in their bitter taste perception, irrespective of their genotype.

ST samples were positively correlated with glucose and fructose, sweet stalk taste, glutamic acid and tryptophan, taste liking for PAV/AVI, AVI/AVI and rare *TAS2R38* genotypes, appearance liking and consumption intent. ST samples were also positively correlated with sweet taste perception, but the correlation was not significant. SF samples were correlated with some GHPs (3BITC, erucin, IB and SFP), burnt and oil attributes, furans, aldehydes and alcohols. Stir-fried samples also correlated positively with taste liking for PAV/PAV genotypes and to some extent with liking factors and consumption intent.

Both consumer clusters 1 and 2 correlated positively with sweet taste perception, liking data and consumption intent; all of which were negatively correlated with bitter taste, GSLs, GHPs, bitter taste perception and sulfury and bitter attributes. The results show that bitter taste and sulfurous attributes of raw BK samples are undesirable characteristics for all consumers in the study, and that they would be more likely to consume cooked than raw kale.

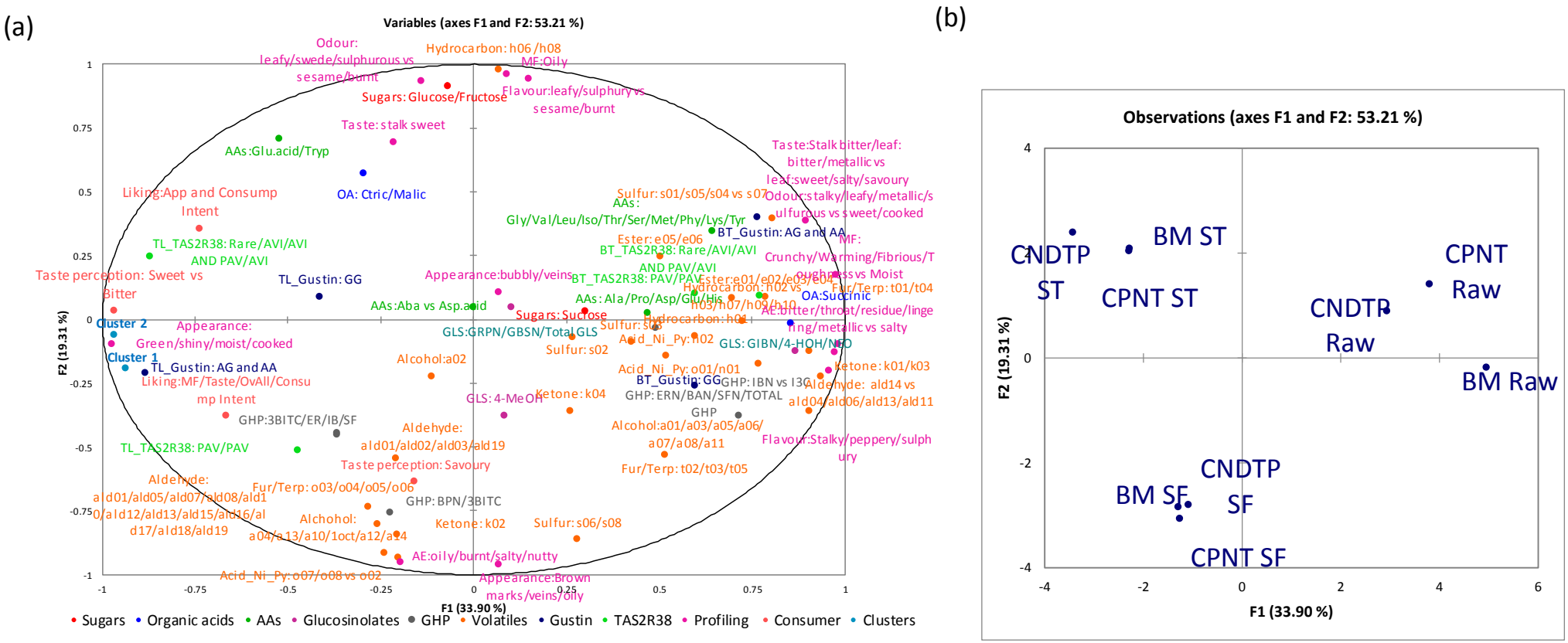


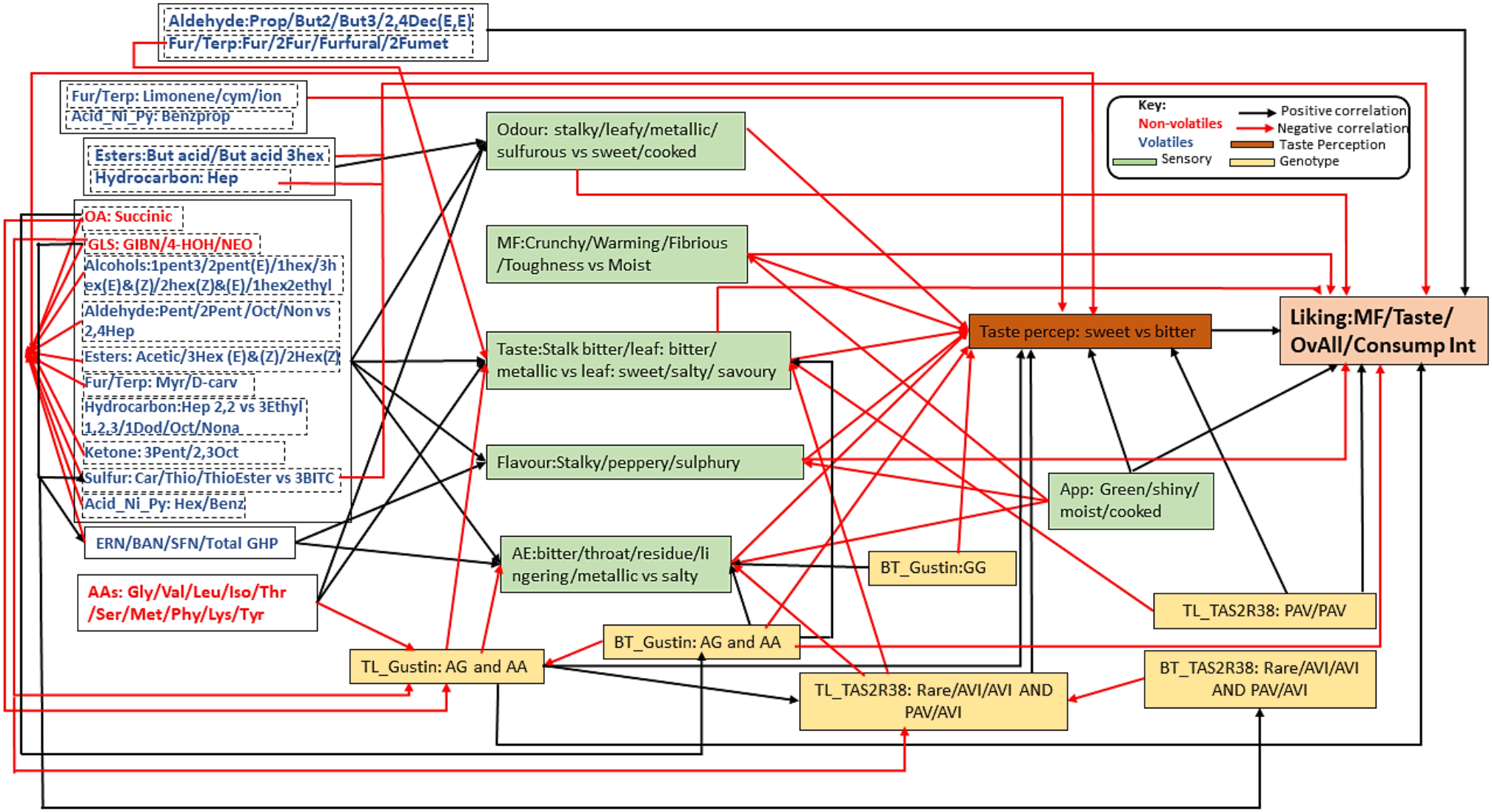
Figure 5.6. MFA map of rotated factors for phytochemical and sensory attributes (a) distribution of variables and (b) sample distribution in map. Codes and abbreviations on plot refer to compound codes in Tables 6.1, 6.3 and 6.4.

5.3.7 Correlation map showing drivers of liking

To fully understand the drivers of liking and consumption intent, factors that correlated (positively and negatively) with liking and consumption intent directly or indirectly from the MFA correlation result were extracted and used to produce a map as presented in Figures 8a and 8b. Correlation values are presented in Appendix X (Table S5d). Cluster results were not included in the correlation because both clusters leaned towards the same preferences and did not discriminate between consumers. Only correlations above r = 0.6 were included in the map. Figure 8a shows the factors driving consumption intent, mouthfeel, taste and overall liking.

GSL positively correlated with sulfur compounds (which were mostly GHPs), bitter taste perception of 'medium' and 'super-tasters', and negatively correlated with genotype taste liking groups. GSL, GHPs, succinic acid, alcohols, aldehydes, esters, hydrocarbons, ketones, sulfur-containing and acidic compounds were positively correlated and drivers of undesirable sensory attributes (stalky and sulfurous odours and flavours, bitter taste, and throat-catching aftereffects). All the sensory attributes mentioned were negatively correlated to sweet taste perception, genotype liking data, liking and consumption intent. Aldehydes impacting sweet odour (such as 2- and 3- methylbutanals) correlated negatively with bitter taste and positively with liking and consumption intent. Sweet taste perception drove taste liking for all genotypes. Green/shiny and moist appearance was a positive driver of liking and correlated negatively with undesirable sensory attributes. The map for appearance liking and consumption intent (Figure 8b) showed similar patterns to Figure 8a.

(a)


Aldehyde:Prop/But2/But3/2,4Dec(E,E)
Fur/Terp:Fur/2Fur/Furfural/2Fumet
Fur/Terp: Limonene/cym/ion
Acid_Ni_Py: Benzprop
Esters:But acid/But acid 3hex
Hydrocarbon: Hep
OA: Succinic
GLS: GIBN/4-HOH/NEO
Alcohols:1pent3/2pent(E)/1hex/3hex(E)&(Z)/2hex(Z)&(E)/1hex2ethyl
Aldehyde:Pent/2Pent/Oct/Non vs 2,4Hep
Esters: Acetic/3Hex (E)&(Z)/2Hex(Z)
Fur/Terp: Myr/D-carv
Hydrocarbon:Hep 2,2 vs 3Ethyl 1,2,3/1Dod/Oct/Nona
Ketone: 3Pent/2,3Oct
Sulfur: Car/Thio/ThioEster vs 3BITC
Acid_Ni_Py: Hex/Benz
ERN/BAN/SFN/Total GHP
AAs: Gly/Val/Leu/Iso/Thr /Ser/Met/Phy/Lys/Tyr
Odour: stalky/leafy/metallic/ sulfurous vs sweet/cooked
MF:Crunchy/Warming/Fibrious /Toughness vs Moist
Taste:Stalk bitter/leaf: bitter/ metallic vs leaf: sweet/salty/ savoury
Flavour:Stalky/peppery/sulphury
AE:bitter/throat/residue/lingering/metallic vs salty
TL_Gustin: AG and AA
BT_Gustin: AG and AA
BT_Gustin:GG
Taste percep: sweet vs bitter
App: Green/shiny/ moist/cooked
TL_TAS2R38: PAV/PAV
BT_TAS2R38: Rare/AVI/AVI AND PAV/AVI
TL_TAS2R38: Rare/AVI/AVI AND PAV/AVI
Liking:MF/Taste/ OvAll/Consump Int
Key:
Non-volatiles
Volatiles
Sensory
Positive correlation
Negative correlation
Taste Perception
Genotype

(b)

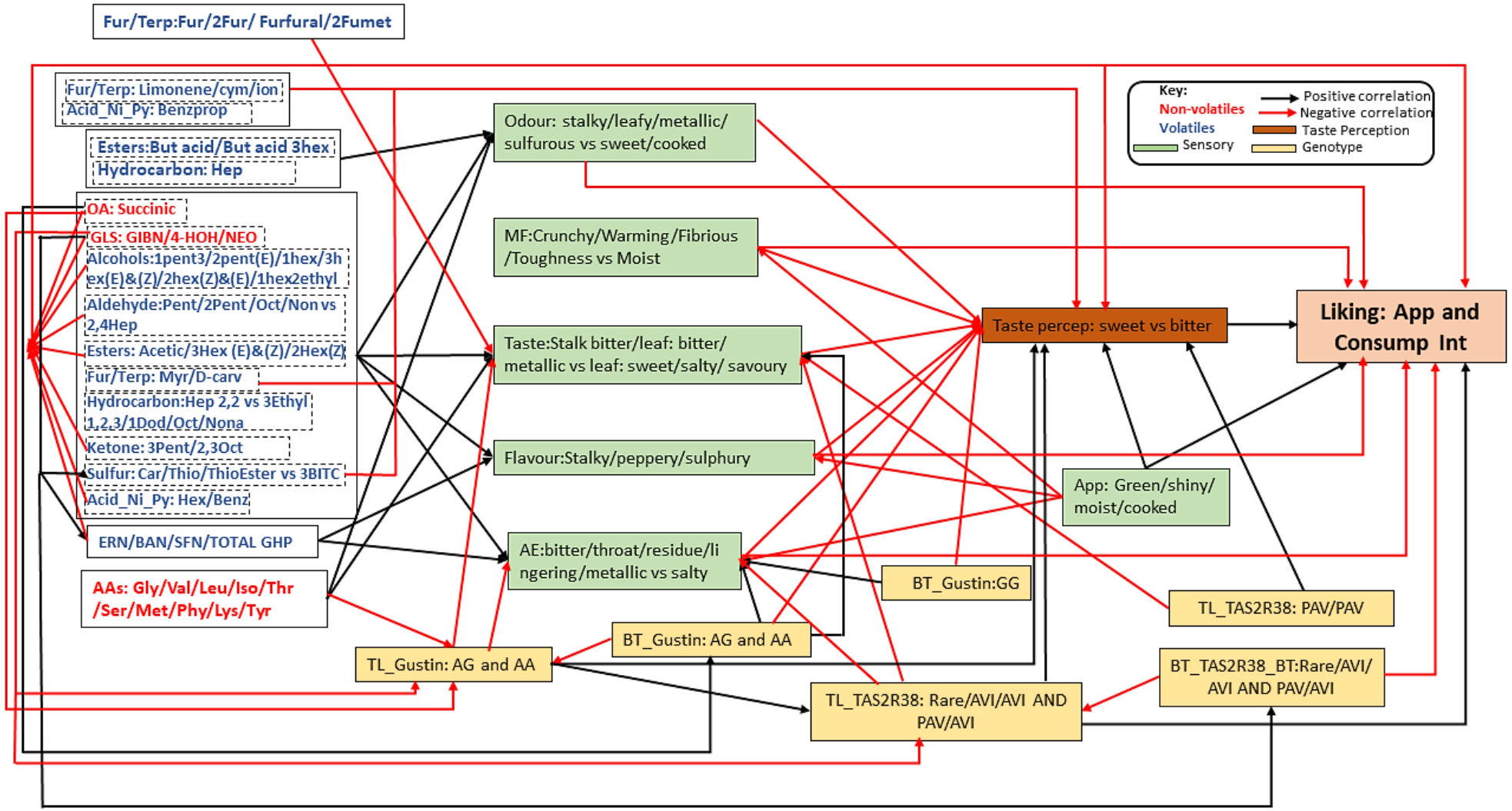


Figure 5.7. (a) Correlation map for drivers of consumption intent, mouthfeel, taste and overall liking. (b) Correlation map for drivers of appearance and overall liking.

'vs' refers to negative correlations between factors in a group. Codes and abbreviations on plot refer to compound codes in Tables 5.1, 5.3 and 5.4. All correlations > r = 0.6.

5.4 Conclusion

This is the first study that has tried to demonstrate the relationship between phytochemical and sensory data and its consequent influence on bitter taste sensitivity and perception in black kale. The results of this study show that mild cooking can retain residual myrosinase activity and enhance the production of health beneficial ITCs from GSL hydrolysis. The relatively high amounts of GRPN present in BK can be potentially beneficial to consumer health. The study also shows that amounts of ITCs produced are more directly linked to GSL concentrations than myrosinase activity, as varieties with higher myrosinase activity and stability did not necessarily yield the highest concentrations of GHPs. Cultivars with higher GSL content resulted in higher amounts of GHPs. This implies that minimal myrosinase activity is enough to hydrolyse GSLs.

From the results obtained, it can be concluded that cooking rather than cultivar, mostly drove differences in BK samples. The results show that most of the phytochemical compounds imparted undesirable taste and sulfurous aromas, which were not acceptable to consumers. Consumers, irrespective of their bitter taste sensitivity found the cooked samples to be more desirable and liked because they were less bitter and sulfurous.

These results support the hypothesis that cooking reduces production of undesirable compounds, improves consumer acceptability and that preference is not significantly related to bitter taste sensitivity. BK cooking can therefore be a method of improving consumer liking and consumption, and can in turn proffer health benefits to consumers.

Chapter 6: The effects of cultivar and cooking method on phytochemical and volatile composition of red cabbage (*Brassica oleracea* var. *capitata* f. *rubra*) and subsequent sensory profile and acceptability by consumers varying in bitter taste sensitivity

Status: This paper has been written in the style of a research paper but will be subdivided into two and submitted to two different journals; Food Composition and Analysis and Food Chemistry

Abstract

Red cabbage is commonly consumed raw in salads/coleslaw, pickled, stir-fried or baked in vinegar in the UK. However, during thermal processing myrosinase activity, phytochemical and volatile profiles of *Brassica* vegetables are affected. In this study, three red cabbage varieties were subjected to domestic cooking processes. Samples were analysed for myrosinase activity and stability, phytochemical and volatile concentrations. The sensory profile of the samples was determined by a trained panel and a consumer study was conducted. Consumers were genotyped for their TAS2R38 and gustin taster status to determine the effect of bitter taste sensitivity on bitter taste perception. Residual myrosinase activity was retained in steamed and stir-fried cabbages. Cooking influenced the types and concentrations of phytochemicals and volatiles detected. Isothiocyanates were the dominant glucosinolate hydrolysis products in cooked cabbage, especially steamed cabbage; sulfides however, were the main volatiles of raw cabbage. Consumer preference of cabbage samples varied between individuals but was not related to bitter taste genotype. The study suggests that steaming may be an ideal way of preparing red cabbages as higher concentrations of beneficial isothiocyanates were produced after steaming and steamed samples also correlated positively with sweet taste and consumer liking.

6.1 Introduction

Consumption and consumer acceptability of *Brassica* vegetables, including red cabbage (*Brassica oleracea var. capitata f. rubra*), is limited despite their chemoprotective properties and maybe due to their bitter taste and sulfurous or pungent odours (Herr & Buchler, 2010). Glucosinolates (GSLs), their myrosinase hydrolysis products and other sulfur-containing compounds are reported to be largely responsible for the bitter taste and sulfurous aromas (Kubec et al., 1998; Drewnowski & Gomez-Carneros, 2000a). In the presence of epithiospecifier protein (ESP), GSLs are hydrolysed to nitriles and epithionitriles (EPTs; with no health properties) instead of the more beneficial isothiocyanates (ITCs) (Lambrix et al., 2001).

As discussed in chapter 5, GSLs have been linked with the *hTAS2R38* gene and thiourea moiety (N-C=S) also found in propylthiouracil (PROP) which is known to influence bitter taste sensitivity in individuals (Sandell & Breslin, 2006). Generally, the *TAS2R38* gene has two main haplotypes: PAV (sensitive) and AVI (insensitive) haplotype (Kim et al., 2003). Individuals are primarily classified into three groups based on their diplotypes; PAV/PAV (supertasters), PAV/AVI (medium-tasters) and AVI/AVI (non-tasters) (Hayes et al., 2008). Studies have also shown strong associations of PROP with gustin (CA6), a trophic factor responsible for taste bud development. Individuals with higher PROP sensitivity are thought to carry the A/A genotype of CA6 on the *rs2274333* SNP and less sensitivity individuals the G/G genotype (Calò et al., 2011).

Red cabbage is commonly eaten in a number of different forms such as raw in salads, or subjected to thermal processing/cooking such as stir-frying, baking, boiling or steaming before consumption. Several studies have shown that GSLs are generally stable during thermal processing, however, myrosinase is mostly inactivated during thermal processing with differences in myrosinase stability influenced by variety and severity of the thermal process (Verkerk & Dekker, 2004; Oerlemans et al., 2006; Rungapamestry et al., 2006; Ghawi et al., 2012). ESP on the other hand, is more thermal labile than myrosinase and requires myrosinase to still be active for it to be effective (Matusheski et al., 2004). The interaction of GSL-myrosinase system with other phytochemical compounds (such as amino acids (AAs), sugars, organic acids (OAs) and volatiles) is thought to influence the sensory characteristics of cabbage but the corresponding effect on consumer perception and liking is not clear.

Previous studies on cabbage has focused mainly on myrosinase activity and stability (Yen & Wei, 1993), GSL concentration due to growth conditions, variety and processing (Ciska

& Kozłowska, 2001; Oerlemans et al., 2006; Penas et al., 2011), glucosinolate hydrolysis products (GHPs; mostly in raw samples) (Hanschen & Schreiner, 2017), flavonols, sugars, organic acids, vitamins and amino acids (Park et al., 2014a; Park et al., 2014b; Xu et al., 2014). Studies on cabbage volatiles are limited and are typically on sulfur volatiles produced in raw cabbage, with studies on red cabbage volatiles rare (Chin & Lindsay, 1993; Akpolat & Barringer, 2015). A previous study investigated the effect of taste-active extracts from key phytochemicals (GSLs, sugars and phenolics) on the taste profile of raw red cabbage as determined by a trained panel (Zabaras et al., 2013). The study showed sugar had a masking effect on bitterness and that GSL did not correlate with bitterness perception. However, the study was conducted on extracts and not intact plant tissues.

This study aims to (a) investigate the effect of variety and domestic cooking methods on myrosinase activity, phytochemical and volatile concentration on red cabbage, and (b) determine the effect of the phytochemical and volatile contents on red cabbage sensory profile and the subsequent influence on taste perception and liking of consumers with varying bitter taste sensitivity. It is hypothesized that cooking will minimize bitter taste and sulfurous odours while enhancing production of bioactive flavour compounds. It is also hypothesized that consumer liking and acceptability of red cabbage will be related to consumer preference, but not directly linked to bitter taste sensitivity.

6.2 Materials and methods

6.2.1 Plant material and growing

Three red cabbage (RC) cultivars were used for this study; two non-commercial accessions (Red Danish, RD and Red langendijker, RL) sourced from the University of Warwick Crop Center Genetic Resource Unit (Wellesbourne, UK) and one commercial variety (Red meteor) from Tozer Seeds Ltd (Cobham, Surrey, UK).

Plants were grown in open field at Tozer seeds Ltd (Cobham, Surrey, UK) from 1st June to 6th November 2015. Standard UK agricultural practices were employed in the cultivation. Pesticides and insecticides were sprayed before and during planting and fertilizer (NPK; 100 kg/ha N, 100 kg/ha P and 200 kg/ha K) applied at intervals before and after planting. Plants were harvested on the morning of 6th November 2015 upon attaining commercial maturity, based on visual inspection, and transported immediately to the University of Reading, (Reading, UK) where they were stored in a cold room for three days at 4 $^{\circ}$C for further

processing. Detailed planting protocol is as presented in Chapter 5. See Appendix IX (Table S2b) for climatic data of the field experiment.

6.2.2 Red cabbage thermal processing

Damaged leaves from five heads were removed and discarded, headed were chopped into small pieces of approximately one centimeter (representing domestic cutting) and mixed together. Chopped leaves were washed carefully under running tap water and excess water drained using a manual salad spinner. Leaves were either steamed (ST) or stir-fried (SF) with raw samples used as control.

Cooking methods were selected to represent common ways of cooking cabbages. Cabbages were steamed and stir-fried using the methods described of Rungapamestry et al. (2006) and Rungapamestry et al. (2008b) respectively with slight modifications as described in Chapter 2.

Immediately after cooking, samples used for phytochemical analyses were put in freezer bags, placed on ice and transferred to a -80 ˚C freezer. Frozen samples were freeze-dried and milled using a Mini Cutting Mill (Mini-Mill, Thomas Scientific, USA), and stored at -20 ˚C prior to further analysis. Samples used for sensory analysis were served to panellists and consumers freshly prepared, immediately after cooking.

6.2.3 Phytochemical analyses

6.2.3.1 Myrosinase enzyme extraction and assay

The method described by Ghawi et al. (2012) as modified by Oloyede et al. (2014) was used in the extraction of myrosinase enzyme. Myrosinase activity was measured using the coupled enzyme method outlined by Wilkinson et al. (1984) and modified by Ghawi et al. (2012). One unit of myrosinase activity is defined the amount of enzyme that produces 1 µmol of glucose from sinigrin substrate per minute at pH 7.5.

Protein content of the crude enzyme extract was determined using the Bradford method (Bradford, 1976). Protein concentration in crude extract was calculated and used to determine specific activity ($U.mg^{-1}$ protein). Full protocol for myrosinase and protein analysis is as described in Chapter 2.

6.2.3.2 Free amino acids, sugars and organic acids analysis

Free amino acids were extracted using 25 % acetonitrile in 0.01 M hydrochloric acid and analysed using the EZfaast free amino acid derivation by GC-MS kit as described by Elmore

et al. (2005). Multifactor analysis (MFA) was performed in XLSTAT (Addinsoft, Paris, France) to visualise the data in a minimum number of dimensions.

Sugars and organic acids were extracted from 40 mg sample using 0.01 M hydrochloric acid and analysed by HPLC, as described by Zeppa et al. (2001) with slight modifications. Detailed methodology for free amino acids, sugars and organic acids is presented in Chapter 5.

6.2.3.3 Glucosinolate and glucosinolate hydrolysis products analysis

GSLs and GHPs were extracted and analysed as described in Chapter 5 and Chapter 3 respectively. GSLs were extracted with 70 % methanol and analysed using LC-MS as described by Bell et al. (2015) with a few alterations as detailed in Chapter 5. A five-point sinigrin hydrate external calibration curve was constructed (r^2 = 0.99) and used to quantify GSLs in cabbage samples.

Identification and quantification of GHPs was carried out using GC-MS as described by Bell et al. (2017c). Compounds were extracted using dichloromethane and identified by comparing mass fragments with NIST database as well as literature ion data. Quantification was based on an external standard calibration curve of sulforaphane (concentrations 0.25–2 mg/mL; r^2 = 0.99). and 6.2 shows the literature ion data of all GSL and GHP compounds identified in red cabbage.

6.2.4 Volatile compounds analysis

Volatile compounds from freshly prepared blended red cabbage samples (5 g; n = 4) representing chewing in the mouth were extracted and analysed using headspace solid-phase microextraction (HS-SPME) as described by Morales-Soto et al. (2015) with a few modifications as outlined in Chapter 5.

Table 6.1: Intact glucosinolates identified in red cabbage varieties by LC-MS

Common name	Chemical name	Abbreviation	Mass parent ion	Reference
Sinigrin	2-propenyl (allyl) GS	SIN	358	Rochfort et al. (2008), Lelario et al. (2012)
Gluconapin	3-butenyl GSL	GPN	372	Bennett et al. (2004), Rochfort et al. (2008)
Epi/progoitrin	(R, S)-2-hydroxy-3-butenyl GSL	PROG	388	Bennett et al. (2004), Rochfort et al. (2008), Lelario et al. (2012)
Glucoerucin	4-(methylthio) butyl GSL	GER	420	Rochfort et al. (2008), Lelario et al. (2012), Bell et al. (2015)
Glucoiberin	3-(methylsulfinyl) propyl GSL	GIBN	422	Bennett et al. (2004), Rochfort et al. (2008), Lelario et al. (2012)
Gluconasturtiin	2-phenylethyl GSL	GNAS	422	Bennett et al. (2004), Lelario et al. (2012), Bell et al. (2015)
Glucoraphanin	4-(methylsulfinyl) butyl GSL	GRPN	436	Bennett et al. (2004), Rochfort et al. (2008), Bell et al. (2015)
Glucobrassicin	3-indolylmethyl GSL	GBSN	447	Bennett et al. (2004), Rochfort et al. (2008), Lelario et al. (2012)
4-hydroxyglucobrassicin	4-hydroxy-3-indolylmethyl GSL	4-HOH	463	Bennett et al. (2004), Rochfort et al. (2008), Lelario et al. (2012)
4-methoxyglucobrassicin	4-Methoxy-3-indolylmethyl-GLS	4-MeOH	477	Rochfort et al. (2008), Lelario et al. (2012), Bell et al. (2015)
Neoglucobrassicin	N-Methoxy-3-indolylmethyl-GLS	NEO	477	Bennett et al. (2004), Rochfort et al. (2008)

Key: GSL, glucosinolate

Table 6.2: Glucosinolate hydrolysis products identified in red cabbage varieties GC-MS respectively

Precursor GSL	*Common name*	*Chemical name*	*Abbreviation*	*LRI[a,b]*	*MS^2 spectrum ion (base ion in bold)*	*Reference*
Sinigrin	Allyl thiocyanate	2-propenyl thiocyanate	ATC	871	99, 72, 45, 44, **41**, 39	Al-Gendy & Lockwood (2003)
	Allyl-ITC	2-propenyl isothiocyanate	AITC	884	**99**, 72, 71, 45, 41, 39	Al-Gendy & Lockwood (2003), Arora et al. (2014)
	1-cyano-2,3-epithiopropane	3,4-epithiobutane nitrile	CETP	1004	**99**, 72, 66, 59, 45, 41, 39	Al-Gendy & Lockwood (2003)
Gluconapin	3-Butenyl-ITC	1-butene, 4-isothiocyanate	3BITC	983	113, 85, **72**, 64, 55, 46, 45, 41	Al-Gendy & Lockwood (2003), Hong & Kim (2013), Arora et al. (2014)
	4,5-epithiovaleronitrile	1-cyano-3,4-epithiobutane	EVN	1121	**113**, 86, 80, 73, 60, 45	Hong & Kim (2013)
Progoitrin	Goitrin	5-vinyloxazolidin-2-thione	GN	1545	**129**, 86, 85, 68, 57, 45, 43, 41, 39	Spencer & Daxenbichler (1980)
	1-cyano-2-hydroxy-3,4-epithiobutane isomer 1	2-hydroxy-3,4-epithiobutylcyanide diastereomer-1	CHETB-1	1225	129, 111, 89, 84, 68, **61**, 58, 55, 45	Spencer & Daxenbichler (1980)
	1-cyano-2-hydroxy-3,4-epithiobutane isomer 2	2-hydroxy-3,4-epithiobutylcyanide diastereomer-2	CHETB -2	1245	129, 111, 89, 84, 68, **61**, 58, 55, 45	Spencer & Daxenbichler (1980)
Glucoibeverin	Iberverin	3-methylthiopropyl-ITC	IBVN	1307	147, **101**, 86, 73, 72, 61, 47, 46, 41	Al-Gendy & Lockwood (2003)
	4-methylthiobutyl nitrile	4-methylthio butanenitrile	4MBN	1085	115, 74, 68, **61**, 54, 47, 41	Al-Gendy & Lockwood (2003)

Glucoerucin	Erucin	4-(methylthio)-butyl-ITC	ER	1427	161, 146, **115**, 85, 72, 61, 55	Al-Gendy & Lockwood (2003), Arora et al. (2014)
	Erucin nitrile	1-cyano-4-(methylthio) butane	ERN	1200	129, 87, 82, **61**, 55, 48, 41, 47	Al-Gendy & Lockwood (2003), Arora et al. (2014)
Glucoiberin	Iberin	3-methylsulfinylpropyl-ITC	IB	1617	163, 130, 116, 102, 100, 86, **72**, 63, 61,41	Al-Gendy & Lockwood (2003)
	Iberin nitrile	4-methylsulfinylbutanenitrile	IBN	1384	**131**, 78, 64, 47, 41	Al-Gendy & Lockwood (2003)
Gluconasturtin	2-phenylethyl-ITC	2-isothiocyanatoethyl benzene	PEITC	1458	163, 105, **91**, 65, 51, 40	Al-Gendy & Lockwood (2003)
	Benzenepropanenitrile	2-phenylethyl cyanide	BPN	1238	131, **91**, 85, 65, 63, 57, 44, 51	Hong & Kim (2013)
Glucoraphanin	Sulforaphane	4-methylsulfinylbutyl-ITC	SFP	1757	160, 114, 85, **72**, 64, 63, 61, 55. 41, 39	Arora et al. (2014),Bell et al. (2017c)
	Sulforaphane nitrile	5-(methylsulfinyl) pentanenitrile	SFN	1526	145, 128, 82, 64, **55**, 41	Arora et al. (2014), Bell et al. (2017c)
Glucobrassiccin	Indole-3-carbinol	1H-Indole-3-methanol	I3C	1801	**144**, 145, 116, 108, 89	Spencer & Daxenbichler (1980)
	Indoleacetonitrile	1H-Indole-3-acetonitrile	1IAN	1796	**155**, 145, 144, 130, 116, 89, 101, 63	Hanschen et al. (2017)
Pentyl GSL	Pentyl-ITC	1-isothiocyanato-pentane	PITC	1165	129, 114, 101, 96, 72, 55, **43**, 41, 39	de Pinho et al. (2009)
Indole	1H-Indole	Indole (8CI)	1H-I	1290	**117**, 90, 89, 63, 58	Vaughn et al. (2017)
Glucotropaeolin	Benzeneacetonitrile	2-Phenylacetonitrile	BAN	1137[c]	**117**, 90, 89, 77, 63, 51	Vaughn et al. (2017)

Key: ITC, isothiocyanate. [a] Linear retention index on a HP-5MS non-polar column. [b]Mass spectrum agrees with reference spectrum in the NIST/NIH mass spectra database and those in literature. [c]Mass spectrum and LRI agree with those of authentic compound.

6.2.5 Sensory analysis, consumer study and DNA extraction

A consensus vocabulary accurately describing the sensory attributes of freshly prepared red cabbage was developed by a trained sensory panel (n = 12). For the consumer study, 112 healthy and consenting individuals aged 18 – 65 years were recruited within Reading (UK) and rated samples on taste perception and liking. During the visit, volunteers provided buccal swab samples (in duplicates) for DNA extraction to determine their bitter-taste genotype.

The full protocol for sensory analysis, consumer study and DNA extraction is as presented in Chapter 5.

6.2.6 Statistical analysis

Results for all phytochemical data except for HS-SPME were averages of three processing replicates and two analytical replicates (n = 6). All statistical analyses performed using XLSTAT (Addinsoft, Paris, France).

ANOVA followed by Tukey's HSD multiple pair wise comparison test to determine significant differences was conducted on all phytochemical, consumer and genotyping data. Agglomerative Hierarchical Cluster (AHC) analysis was carried out on consumer overall liking scores and cluster means analysed by ANOVA. A mixed model ANOVA (with Tukey's HSD multiple pair wise comparison test) and principal component analysis (PCA) were carried out in Senpaq (version 4.2, Qi Statistics, UK) and used to analyse sensory profiling data. A mixed model ANOVA tests the main effects (i.e. samples and assessors) against their interaction.

Rotated factor analysis (RFA) and multiple factor analysis (MFA) were carried out on the means of all datasets to analyse for relationships between phytochemical, sensory and consumer data using XLSTAT.

6.3 Results and discussion

6.3.1 Phytochemical analysis

6.3.1.1 Myrosinase activity and stability

The activity and stability of myrosinase enzyme was significantly influenced by cooking ($p<0.0001$), variety ($p<0.0001$) and an interaction between the two factors ($p<0.0001$) (Table 6.2 and Appendix XI; Table S6a). Myrosinase activity differed significantly across the three varieties studied. Raw RD had significantly higher myrosinase activity (56.7 $U.g^{-1}$ DW) than RM and RL. The lowest myrosinase activity was observed in raw RL variety (19.3 $U.g^{-1}$ DW).

Previous authors have reported differences in myrosinase activity between cabbage varieties (Singh et al., 2007; Penas et al., 2011).

Table 6.3: Myrosinase activity (U.g^{-1} DW), protein content (mg.g^{-1} DW) and specific activity (U.mg^{-1} protein DW) of red cabbage samples

Varieties	Treatment	Myrosinase activity (*U.g^{-1} DW*)	Protein content (*mg.g^{-1} DW*)	Specific activity (*U.mg-1 protein DW*)
RM	Raw	37.0^{d}	22.8^{e}	1.6^{d}
	ST	5.2^{a}	10.5^{a}	0.5^{a}
	SF	12.6^{b}	11.1^{ab}	1.1^{c}
RD	Raw	56.7^{e}	20.3^{c}	2.8^{f}
	ST	7.8^{ab}	10.6^{a}	0.7^{ab}
	SF	23.3^{c}	11.2^{ab}	2.1^{e}
RL	Raw	19.3^{c}	21.4^{d}	0.9^{bc}
	ST	5.2^{a}	10.9^{ab}	0.5^{a}
	SF	12.2^{b}	11.6^{b}	1.1^{bc}
	P-value	< 0.0001	< 0.0001	< 0.0001

Mean values with different superscripts in the same column significantly different at p<0.0001). Abbreviations: ST = steamed, SF = stir fried

The residual myrosinase stability after cooking is presented in Figure 6.1. Residual activity is defined as the ratio of processed to unprocessed (raw) myrosinase activity. Cooking resulted in a significant loss of myrosinase activity with the degree of myrosinase stability varying between cooking methods and varieties. Steaming led to significantly (p<0.0001) lower myrosinase stability (up to 14 %) than stir-frying (up to 34 %). There was no significant difference in the stability of myrosinase in steamed cabbages between the three varieties studied. However, a significant difference in myrosinase stability was observed after stir-frying with SF-RL myrosinase significantly (p<0.0001) more stable (68 %) than the other two varieties though absolute myrosinase activity was still higher in SF-RD variety because of the higher myrosinase activity of raw RD. RL variety had the most stable myrosinase enzyme after cooking while the lowest stability was observed in RM variety (commercial variety). ST-RL did not differ significantly between both ST and SF samples for RM and RD. The difference in myrosinase stability between red cabbage varieties can be attributed to difference in

myrosinase isoenzymes. Myrosinase isoenzymes are reported to be both plant and species-specific and can influence both myrosinase activity and stability (Bones & Rossiter, 2006).

The higher myrosinase stability observed after stir-frying could be attributed to the slower rate of heat transfer to the cabbage core when compared to the steaming process. Adler-Nissen (2002), in their study of stir-fried vegetables stated that during stir-frying, the intense heat results in drying of the surface area which reduces cell wall damage and subsequently slower heat penetration to the core. This was evident in the present study as the core temperature after stir-frying was lower (60 – 65 °C) than steaming (73 -78 °C). Similar results have been reported by Rungapamestry et al. (2008b) in stir-fried and steamed broccoli, and in this thesis on studying Kale (Chapter 5).

The protein content of myrosinase extracts and myrosinase specific activity is presented in Table 6.2 and Appendix XI (Table S6a). Protein content and specific activity differed significantly as a result of variety ($p<0.0001$), cooking ($p<0.0001$) and the interaction ($p<0.0001$) between the two. Highest protein content was observed in RM and lowest in RD. A breakdown of proteins into amino acids as a result of cooking might be responsible for the significantly lower protein contents observed in the samples resulting in losses of up to 50 %. The difference in severity of heat treatment might have influenced protein stability as protein content was higher after stir-frying than steaming (Appendix XI; Table S6a). The results of specific activity followed a similar pattern like myrosinase activity. RD variety had significantly higher specific activity and RL the lowest specific activity. Cooking also led to significantly lower specific activity; it being significantly higher after stir frying (1.1 $U.mg^{-1}$ protein DW) than after steaming (0.5 $U.mg^{-1}$ protein DW).

Microbial myrosinase in the gut has been found to hydrolyse GSLs, however, the bioavailability of the compounds produced is lower than that produced from GSL hydrolysis by plant myrosinase (Conaway et al., 2000; Traka & Mithen, 2009). The study of myrosinase activity and stability after cooking is therefore important as plant myrosinase is necessary for the hydrolysis of GSLs into beneficial ITCs. The difference in myrosinase stability between varieties and cooking methods can be important from a health standpoint as it can influence the types and amounts of beneficial ITCs that will be produced as will be discussed in section 6.3.1.5.

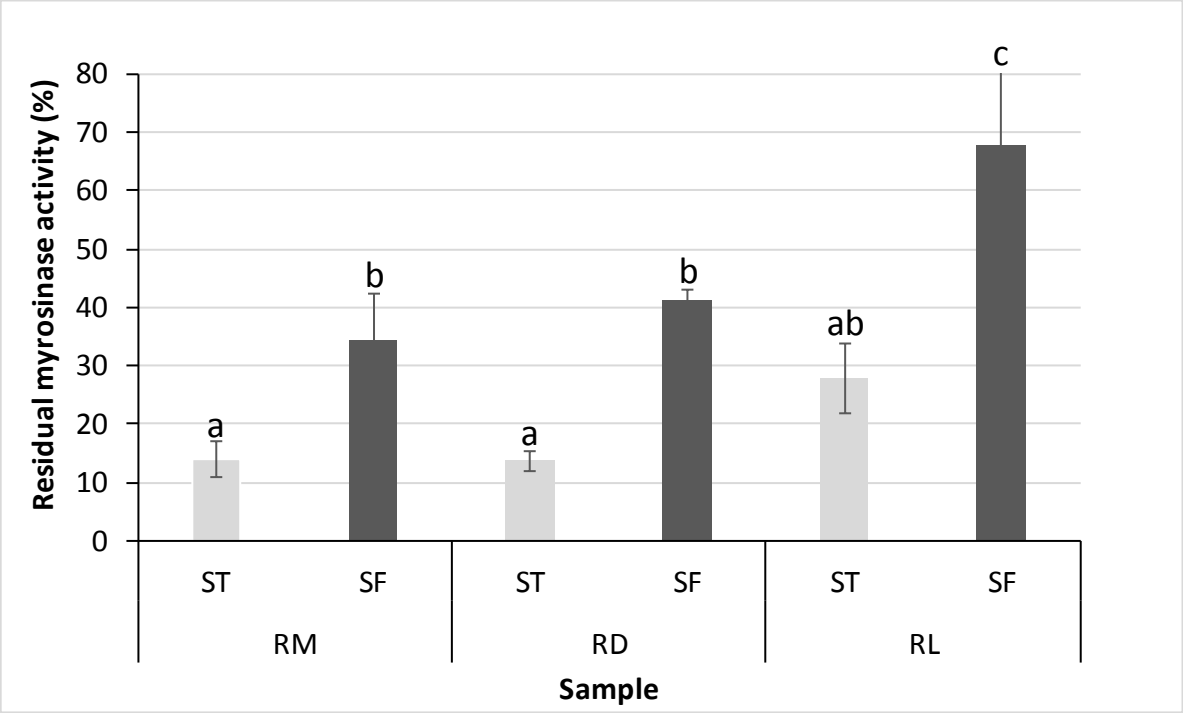


Figure 6.1. Residual myrosinase activity (%) of red cabbage samples (varieties RM, RD and RL) after cooking.
Bars with differing letters indicates significant differences ($p<0.0001$) between samples. Error bars represent standard deviation from mean values. Abbreviations: ST = steamed, SF = stir fried.

6.3.1.2 Free amino acids

Free amino acids (AAs) are known to contribute to sensory perceptions of food. In red cabbage samples, 19 free amino acids (AAs) were detected and quantified with concentrations presented in Table 6.3 and Appendix XI (Table S6b). Significant differences were observed in the abundance of most AAs as a result of cooking, variety and an interaction between both factors; with the exception of proline, which did not differ across all samples studied (Table 6.4). Levels of total AAs observed in red cabbage samples were substantially higher than reported for black kale (Chapter 5 section 5.3.1.2). Total AAs in samples ranged from 43.5- 92.9 $\mu g.g^{-1}$ DW with the highest amounts observed in RM samples and significantly higher than RL varieties for most AAs detected (Appendix XI; Table S6a). There was no significant change in the concentrations of most individual and total AAs after steaming; however, an increase in glutamic acid (48 %) was observed (Appendix XI; Table S6a). In stir-fried cabbages, there was a significant reduction in individual and total AAs except for tyrosine where no significant difference was observed after cooking. Very little is known on the influence of cooking on amino acids in cabbage or other *Brassica* vegetables. The result

obtained is in agreement with the findings of Zhang et al. (2011) where the authors found no difference in amounts of AAs after steaming but a loss in concentrations after stir-frying.

Glutamine was the most abundant AA identified in all samples making up over 50 % of total AA concentration. Higher amounts of glutamine could be due to leaf senescence as a result of protein breakdown and enzymatic conversion to increase efficiency of nitrogen transport (Buchanan-Wollaston et al., 2003). Park et al. (2014b) also found glutamine as the highest accumulated AA (> 60 % of total AAs) in inbred lines of green and red cabbage. RM had significantly higher glutamine concentrations than RD and RL (Appendix XI; Table S6a). Glutamine is reported to be a sweet tasting AA (Nelson et al., 2002). The higher glutamine concentration in RM can have a significant impact on sweet taste perception as it can mask bitter taste which may in turn influence consumer acceptance of the variety in comparison to the other two varieties.

It is worthy of note to mention the low amounts of free methionine, tryptophan and phenylalanine observed in the cabbage samples. These AAs were not found in inbred red and green cabbage lines (Park et al., 2014b) while higher concentrations of phenylalanine were found in black kale samples previously studied though levels were low and below 0.5 µg/g DW (Chapter 5; Table 5.4). Eppendorfer & Bille (1996) also found low amounts of phenylalanine in kale but the authors did not provide a possible reason for this. These AAs are however, important because they are the AAs mainly associated with the synthesis of aliphatic, aromatic and indole GSLs (Mithen, 2001). It is hypothesised that the low amounts of methionine, tryptophan and phenylalanine observed may be due to their role in GSL synthesis which makes them unavailable in their free form in the samples.

6.3.1.3 Sugars and organic acids

The content of soluble sugars (glucose, fructose and sucrose) and organic acids (citric, malic and succinic) in cooked red cabbages are presented in Table 6.4 and Appendix XI (Table S6b). Glucose was, on average, the major sugar present in the cabbages followed by fructose. Significant differences were observed in the individual and total sugars content of cabbage varieties except for fructose, where concentrations did not differ significantly ($p= 0.12$) in varieties studied. Rosa et al. (2001) reported differences in sugar content between cabbage varieties and found glucose and fructose to be the most dominant sugars, which is in agreement with the current study. Compared with raw samples, stir-frying was the only cooking method that led to significant losses in individual and total sugar concentrations. However, cooking had no effect ($p = 0.28$) on sucrose content. The lower concentrations in

stir-fried samples might be due to loss of sugars with evaporated water during stir-frying. Another possible reason, might be formation of Maillard-derived volatiles as a result of Maillard reactions of sugars with AAs which might also explain the high loss of AAs in stir-fried cabbages. Previous authors have also reported drastic loss in sugar concentrations due to stir-frying in red cabbage and broccoli (Yuan et al., 2009; Xu et al., 2014). RM contained significantly higher total sugars (155.6 $mg.g^{-1}$ DW) than RD (138.2 $mg.g^{-1}$ DW) and RL (136.5 $mg.g^{-1}$ DW) (Appendix XI; Table S6b).

Citric acid was the most abundant organic acid (OA) in cabbage samples which is in agreement with previous studies on black kale and red cabbage (Ayaz et al., 2006; Vale et al., 2015). Variety and cooking significantly affected the amount of individual and total OAs in cabbage samples. The concentrations of citric and malic acids were not affect by steaming, while stir-frying led to a significant decrease in contents of both individual and total OAs. The largest decrease in succinic acid content was observed in steamed samples with up to 50 % reduction in succinic acid content. RM variety had significantly lower amounts of individual and total OAs compared to the other varieties studied. Vale et al. (2015) also reported variation in OA contents in different red cabbage varieties. OAs are known to influence organoleptic properties of vegetables with some OAs linked to sourness and astringency (Hufnagel & Hofmann, 2008a).

Table 6.4: Amino acid (µg.g^{-1} DW), sugars (mg.g^{-1} DW) and organic acid (mg.g^{-1} DW) concentrations of red cabbage (ST = steamed, SF = stir fried)

		RM			RD			RL			Significance
Code	Compound	Raw	ST	SF	Raw	ST	SF	Raw	ST	SF	(P-*value*)
	*Amino acids (*µg.g^{-1} DW*)*										
Ala	Alanine	4.89^{b}	4.32^{ab}	4.10^{ab}	4.71^{b}	3.97^{ab}	3.31^{a}	4.91^{b}	4.81^{b}	4.75^{b}	0.002
Gly	Glycine	0.23^{c}	0.21^{bc}	0.19^{bc}	0.22^{bc}	0.18^{abc}	0.13^{a}	0.21^{bc}	0.19^{bc}	0.17^{ab}	< 0.0001
AAA	α-Aminobutyric acid	0.05^{ab}	0.04^{ab}	0.03^{a}	0.06^{bc}	0.06^{bc}	0.04^{ab}	0.06^{bc}	0.08^{c}	0.05^{ab}	< 0.0001
Val	Valine	1.37^{bc}	1.27^{bc}	1.02^{ab}	1.48^{c}	1.03^{ab}	0.82^{a}	1.09^{ab}	1.11^{abc}	0.87^{a}	< 0.0001
Leu	Leucine	0.29^{bcd}	0.30^{cd}	0.23^{abc}	0.35^{d}	0.21^{a}	0.20^{a}	0.20^{a}	0.22^{ab}	0.17^{a}	< 0.0001
Iso	Isoleucine	0.90^{cd}	0.75^{bcd}	0.70^{bc}	0.98^{d}	0.57^{ab}	0.42^{a}	0.65^{b}	0.64^{ab}	0.52^{ab}	< 0.0001
Thr	Threonine	0.98^{cd}	0.89^{bcd}	0.77^{abc}	1.06^{d}	0.72^{ab}	0.59^{a}	0.67^{ab}	0.69^{ab}	0.56^{a}	< 0.0001
Ser	Serine	5.71^{d}	3.21^{ab}	4.19^{bc}	4.60^{cd}	2.69^{a}	2.81^{a}	4.14^{bc}	2.94^{ab}	3.75^{abc}	< 0.0001
Pro	Proline	1.37	1.22	1.31	1.69	1.77	1.23	1.70	1.58	1.08	0.097
Asp	Asparagine	3.22^{b}	2.85^{ab}	2.62^{ab}	3.10^{b}	2.60^{ab}	1.87^{a}	2.55^{ab}	2.39^{ab}	2.24^{ab}	0.010
Asp.A	Aspartic acid	5.43^{bc}	6.00^{c}	4.49^{abc}	5.97^{c}	5.82^{c}	3.37^{a}	4.94^{abc}	5.06^{bc}	4.02^{ab}	< 0.0001
Met	Methionine	0.06^{b}	0.05^{ab}	0.04^{ab}	0.05^{ab}	0.05^{ab}	0.03^{a}	0.06^{b}	0.05^{ab}	0.05^{ab}	0.017
Glu.A	Glutamic acid	3.96^{bc}	6.35^{d}	1.89^{a}	2.52^{ab}	5.44^{cd}	1.29^{a}	1.95^{a}	5.58^{cd}	1.76^{a}	< 0.0001
Phy	Phenylalanine	0.15^{c}	0.15^{c}	0.11^{ab}	0.14^{bc}	0.11^{ab}	0.11^{ab}	0.11^{ab}	0.13^{abc}	0.10^{a}	< 0.0001
Glu	Glutamine	62.5^{d}	54.0^{cd}	45.8^{bcd}	53.4^{cd}	41.1^{abc}	26.2^{a}	40.5^{abc}	38.1^{abc}	35.0^{ab}	< 0.0001
Lys	Lysine	0.29^{d}	0.20^{abcd}	0.21^{bcd}	0.23^{cd}	0.13^{abc}	0.10^{a}	0.10^{a}	0.11^{ab}	0.10^{a}	< 0.0001
His	Histidine	1.16^{d}	0.89^{cd}	0.88^{cd}	0.78^{bcd}	0.99^{cd}	0.78^{bcd}	0.48^{ab}	0.70^{abc}	0.32^{a}	< 0.0001
Tyr	Tyrosine	0.17^{d}	0.14^{cd}	0.16^{cd}	0.11^{bc}	0.07^{ab}	0.06^{ab}	0.05^{ab}	0.05^{ab}	0.04^{a}	< 0.0001
		RM			RD			RL			

Code	Compound	Raw	ST	SF	Raw	ST	SF	Raw	ST	SF	*P-value*
Tryp	Tryptophan	0.17^{de}	0.14^{de}	0.12^{cde}	0.16^{e}	0.10^{bcd}	0.08^{abc}	0.06^{ab}	0.06^{ab}	0.04^{a}	< 0.0001
T_AAs	Total Amino acids	92.9^{d}	83.0^{cd}	68.8^{bc}	81.6^{cd}	67.6^{bc}	43.5^{a}	64.5^{abc}	64.5^{abc}	55.6^{ab}	< 0.0001
	*Sugars (*mg.g^{-1} DW*)*										
Suc	Sucrose	40.6^{c}	40.9^{c}	38.8^{c}	34.2^{bc}	31.7^{bc}	35.7^{bc}	26.3^{ab}	26.6^{ab}	18.3^{a}	< 0.001
Glu	Glucose	68.6^{cd}	67.4^{cd}	52.9^{ab}	63.7^{cd}	58.9^{bc}	43.3^{a}	71.1^{d}	67.1^{cd}	48.0^{a}	< 0.0001
Fru	Fructose	59.9^{b}	58.2^{b}	39.2^{a}	55.3^{b}	53.1^{b}	38.6^{a}	56.6^{b}	54.6^{b}	40.7^{a}	< 0.0001
T_Sugars	Total sugars	169.1^{e}	166.5^{e}	131.0^{bc}	153.3^{de}	143.7^{cd}	117.6^{ab}	154.0^{de}	148.3^{d}	107.2^{a}	< 0.0001
	Organic acids (mg.g^{-1} DW)										
	Citric	43.0^{abc}	41.5^{ab}	32.9^{a}	67.1^{d}	74.6^{d}	52.2^{abcd}	65.7^{cd}	70.5^{d}	58.4^{bcd}	< 0.0001
	Malic	58.2^{bc}	50.6^{abc}	40.7^{a}	57.5^{bc}	60.5^{bc}	45.4^{ab}	60.1^{bc}	65.2^{c}	50.0^{ab}	< 0.0001
	Succinic	44.5^{c}	21.0^{a}	39.7^{bc}	52.3^{cd}	24.8^{a}	44.6^{c}	62.4^{d}	28.3^{ab}	52.1^{cd}	< 0.0001
	Total organic acids	145.6^{ab}	113.2^{a}	113.3^{a}	177.0^{bc}	160.0^{bc}	142.2^{ab}	188.2^{c}	164.0^{bc}	160.5^{bc}	< 0.0001

Mean values with different superscripts in the same row significantly different at p<0.05

The low OA concentrations and high sugars contents in RM could influence the perception of sweet and bitter taste. The nature and amount of sugars have been reported to play a role in taste perception with studies suggesting that bitter and sweet taste are closely related (Walters, 1996; Schonhof et al., 2004). The ratio between sweet (such as glutamine, alanine, serine and sugars) and bitter tasting or astringent compounds like OAs, leucine, valine and GSLs, may be more important when discussing the intensity of sweet and bitter taste perceptions in cabbage (see section 6.3.4.2).

6.3.1.4 Glucosinolates

The GSL concentrations for red cabbage samples are presented in Figure 6.2 with significant differences presented in Appendix XI (Table S6c). Significant differences were observed in individual GSLs due to variety, cooking and an interaction between the two in the samples studied (Appendix XI (Tables S6c and S6d)). In total, 11 individual GSL were identified and quantified in the red cabbage sample. Total and individual GSL concentrations differed significantly in samples except for GRPN where no significant (p= 0.71) difference was observed. Highest total GL content was in raw RD (51.7 $mg.g^{-1}$ DW) and the lowest was in SF RM and RL (26.2 $mg.g^{-1}$ DW). GSL concentrations were substantially higher in red cabbage than in black kale previously discussed in Chapter 5 (see Figure 5.2). Red cabbage variety and cooking significantly affected GSL concentrations. Highest average total GSL content was observed in RD variety (44.5 $mg.g^{-1}$ DW) and lowest in RM (28.7 $mg.g^{-1}$ DW) (Appendix XI; Table S6d). Cooking resulted in significant losses, with up to 32 % loss recorded in stir-fried samples. GRPN was the most stable GSL as no significant difference was observed after cooking (p= 0.076). Some aliphatic GSLs (SIN, PROG, GIBN and GRPN) were relatively stable after steaming as concentrations did not differ significantly from raw samples. The major individual GSLs present in the samples differed between varieties (GBSN in RM, GNAS in RD and 4-MeOH in RL) while 4-HOH was the GSL at lowest concentration in all varieties. RL-GSL was the least stable as higher significant losses were observed after steaming (38 %) and stir-frying (45 %) when compared to the other two varieties.

The GSL profile and difference in concentrations of individual GSL obtained in red cabbage samples is similar to those reported in red cabbage varieties by Ciska et al. (2000) and Hanschen & Schreiner (2017) with Park et al. (2014b) mentioning 4-HOH as the least abundant GSL in the red cabbage varieties studied. Previous authors have reported aliphatic GSLs to be more stable that indole GSLs during thermal processing (Ciska & Kozłowska, 2001; Oerlemans et al., 2006; Dekker et al., 2009). Xu et al. (2014) also reported higher losses in GSL

concentrations after stir-frying than steaming. Lower GSL concentrations during stir-frying might be due to lower core temperatures during cooking resulting in higher enzymatic degradation of GSL by residual active myrosinase as previously discussed. Rungapamestry et al. (2006) found the GSL in raw cabbage stable after steaming of 420 sec while Volden et al. (2008) recorded a 19 % loss in GSL after steaming for 10 min. Differences observed GSL stability in the different studies can be due to the different varieties analysed as well as cut size and cooking time of the cabbage during processing.

GSLs have been previously associated with bitter taste (Drewnowski & Gomez-Carneros, 2000a); hence, higher amounts of GSLs, though important for improved production of beneficial ITC, may enhance bitter taste characteristics which can negatively influence consumer liking and acceptability. In order to reduce the effect of variation in GSL thermal stability, selection of varieties with more stable GSL through planting breeding should be considered to enhance possible health benefits derived from red cabbage consumption.

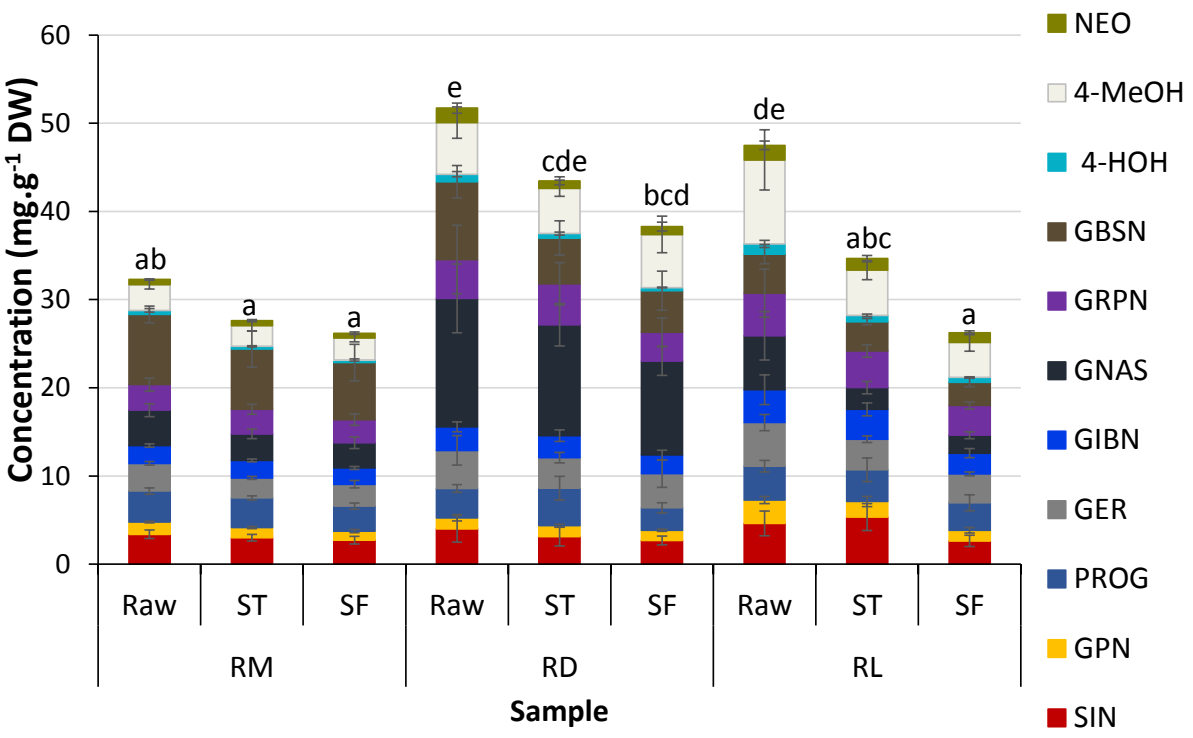


Figure 6.2. Glucosinolate (GLS) concentrations (mg.g^{-1} DW) of red cabbage samples (varieties RM, RD, RL).

Bars not sharing a common letter differ significantly ($p<0.0001$) between samples. Error bars represent standard deviation from mean values. Abbreviations: ST = steamed; SF = stir fried; NEO, neoglucobrassicin; 4-MeOH, 4- methoxyglucobrassicin; 4- HOH, 4-hydroxyglucobrassicin; GBSN, glucobrassicin; GRPN, glucoraphanin; GNAS, gluconasturtiin; GIBN, glucoiberin; GER, glucoerucin; PROG, progoitrin; GPN, gluconapin; SIN, sinigrin.

6.3.1.5 Glucosinolate hydrolysis products

The result of GHP concentrations is presented in Figure 6.3, with significant differences presented in Appendix XI (Table S6c). Concentrations are expressed as sulforaphane equivalents. A total of 23 GHPs were detected across all samples. Low concentrations of GHPs of glucoiberverin, pentyl GSL and glucotropaeolin were found despite their intact GSLs not being detected. The low concentrations found may explain the inability to detect their intact GSL in the samples. Previous authors have also reported identifying certain GHPs in rockets and turnips where their intact GSL was not detected (Bell et al., 2017c; Klopsch et al., 2017); this was also reported previously for black kale (Chapter 5; section 5.3.1.5).

Total and individual GHPs varied significantly across samples, varieties and cooking methods. Total GHPs ranged from 462.3 $\mu g.g^{-1}$DW in SF-RL to 1132.6 $\mu g.g^{-1}$DW in ST-RD. GHPs from GRPN, GIBN and PROG were the most abundant GHPs detected in cabbage samples (about 80 % of total GHPs) though their GSLs were not the most abundant in the samples. This might be due to the relative stability of these GHPs compared to other GHPs which are easily lost by evaporation during the extraction and analytical process. Some GHPs are reported to be more volatile; for example AITC has been found to be very volatile leading to loss by evaporation during sample processing (Song & Thornalley, 2007). Bell et al. (2017c) also found SFP (ITC of GRPN) as the most abundant GHP in rocket though GRPN was not the most abundant GSL in the rocket samples. Type and concentrations of GHPs was significantly influenced by cooking. Nitriles and EPTs were the predominant GHPs in raw cabbages with more ITCs formed in cooked cabbages. Nitriles and EPTs are reported to be the main hydrolysis products of raw cabbage (Kyung et al., 1995; Rungapamestry et al., 2006; Song & Thornalley, 2007). Significantly higher concentration of ITCs and no or very low amounts of nitriles and EPTs were formed after steaming compared to stir-frying, supporting previous reports that the severity of the thermal process influences the types and amounts of GHPs produced.

Myrosinase and ESP activities are important determinant factors in the types and concentrations of GHPs formed. ESP which is responsible for the formation of nitriles and EPTs from GSL hydrolysis is reported to be more heat labile than myrosinase, being denatured at temperatures above 50 °C while myrosinase remains active (Matusheski et al., 2004). The higher cooking temperatures during steaming may have resulted in almost total denaturisation of ESP with stir-frying retaining more ESP activity. However, the steaming

temperatures were not enough to result in complete denaturation of residual myrosinase as previously discussed (section 6.3.1.1), resulting in the conversion of more GSLs to ITCs rather than nitriles and EPTs. There are limited studies on the effect of cooking on GHPs in cabbages and where available specific GHPs are targeted. Steamed cabbage was found to contain more AITC than its nitrile (hydrolysis product of SIN) with concentrations increasing with increase in steaming time (Rungapamestry et al., 2006). In heat-treated broccoli florets, SFP rather than SFN was the dominant GHP of GRPN formed (Matusheski et al., 2004).

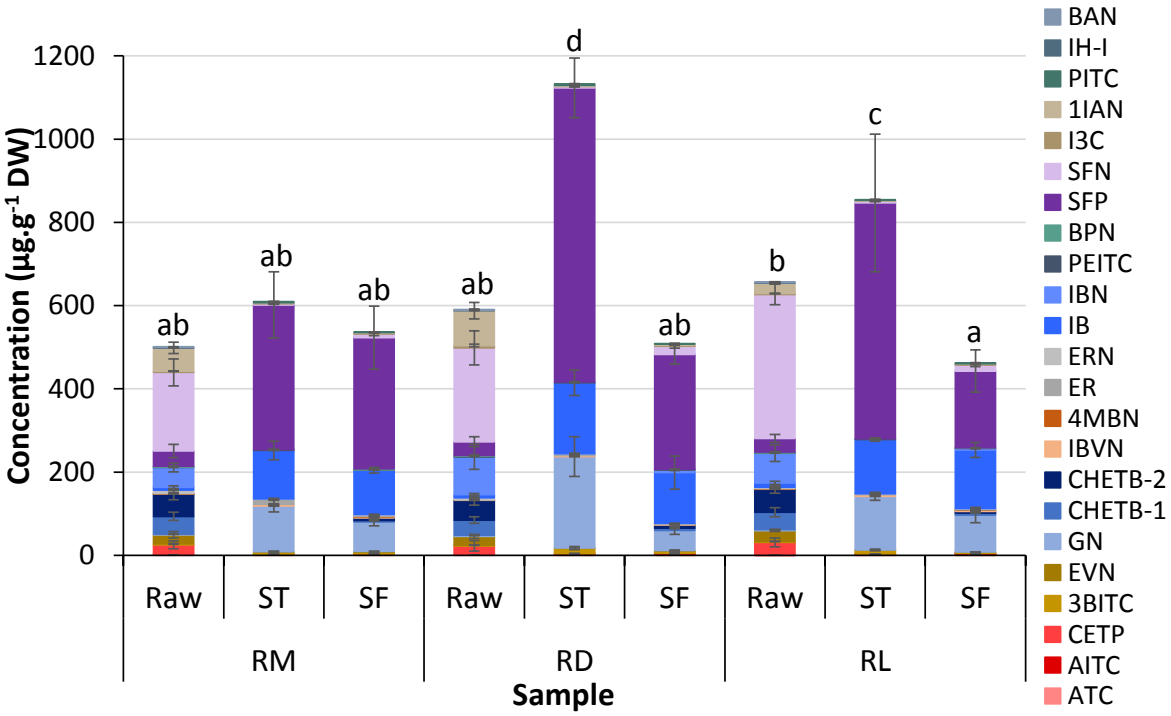


Figure 6.3. Glucosinolate hydrolysis products (GHP) concentrations (mg.g^{-1} DW) of red cabbage samples (varieties RM, RD, RL).

Bars not sharing a common letter differ significantly ($p<0.0001$) between samples. Error bars represent standard deviation from mean values. Compounds with similar colour shades refer to GHPs from corresponding GSL in Figure 6.2. Abbreviations: ST = steamed; SF = stir fried; ATC, allyl thiocyanate; AITC, allyl isothiocyanate; CEPT, 1-cyano-2,3-epithiopropane; 3BITC, 3-Butenyl-ITC; EVN, 4,5-epithiovaleronitrile; GN, goitrin; CHETB-1, 1-cyano-2-hydroxy-3,4-epithiobutane isomer 1; CHETB-2, 1-cyano-2-hydroxy-3,4-epithiobutane isomer 2; IBVN, Iberverin; 4MBN, 4-methylthiobutyl nitrile; ER, erucin; ERN, erucin nitrile; IB, iberin; IBN, iberin nitrile; PIETC, 2-phenylethyl-ITC; BPN, benzenepropanenitrile; SFP, sulforaphane; SFN, sulforaphane nitrile; I3C, indole-3-carbinol; 1IAN, indoleacetonitrile; PITC, Pentyl-ITC ; 1H-I, 1H-Indole; BAN, benzeneacetonitrile.

Highest average GHPs concentration was observed in RD variety and lowest in RM. This correlates with the results of GSL concentrations, where highest and lowest GSLs was found in RD and RM respectively. Myrosinase activity however, did not follow the same trend as cooked RL, which had the most stable myrosinase, did not accumulate the most GHPs, while ST-RD which had the least residual myrosinase resulted in the highest concentration of GHPs, most of which were ITCs. This may be related to differences in the rate of the hydrolysis process. The results obtained show that light steaming of red cabbage might be the most preferred way to consume such cabbage because SFP, which has been widely reported has possessing several health benefits with no contribution to cabbage flavour (Sultana et al., 2003), was the most abundant GHP in steamed red cabbages.

6.3.2 Volatile compounds

Volatile compounds such as sulfides are important to cabbage flavour as they contribute to the undesirable sulfurous flavour and aroma of *B. oleracea* vegetables. Table 6.5 shows the relative abundance of 58 volatile compounds identified in the headspace of freshly blended raw and cooked red cabbage samples. Volatiles identified include 11 alcohols, 10 aldehydes, 22 sulfur-containing compounds (sulfides and ITCs), nine nitriles and six others. Types and relative amounts of volatile compounds differed significantly between varieties and as a result of cooking and may influence the overall flavour characteristics of the samples.

The highest abundance of alcohols was detected in raw cabbage samples, though varying significantly between varieties. Alcohols comprised of 58, 46 and 29 % of total volatiles in raw RM, RD and RL respectively. (Z)-3-hexen-1-ol was the most abundant alcohol in the samples and did not differ significantly across varieties. The second most abundant alcohol was 1-hexanol in RM and RL varieties and (E)-2-penten-1-ol in RD variety. Cooking led to about 99 % loss in alcohol abundance, with ST-RM retaining the highest amounts (6 %). Only four out of the 11 alcohols identified were present in ST samples. Aldehydes comprised of not more than 6 % in all samples, except in ST-RM where it comprised 11 % of total volatiles. The higher percentage however was largely due to the relative abundance of other volatiles, rather than higher amounts of aldehydes present in the sample. Hexanal and (E)-2-hexenal which were majorly present in SF samples were the most abundant aldehydes detected. The C6 alcohols and aldehydes, derived from metabolism of polyunsaturated fatty acids through lipoxygenase pathway is responsible for imparting the green and leafy-like aromas in vegetables (Raffo et al., 2018). Several authors have also identified similar alcohols and aldehydes in various *Brassica* vegetables (Blazevic & Mastelic, 2009b; de Pinho et al., 2009;

Akpolat & Barringer, 2015; Bell et al., 2016; Raffo et al., 2018). (E)-2-octenal, benzaldehyde and nonanal present in SF samples are known to impart fatty odours and were mostly present in cooked samples.

The most abundant volatiles identified in cabbage samples were the sulfur-containing volatiles which comprised of sulfides and ITCs. Twenty-two (22) sulfur-containing volatiles, comprising of 97 % of total volatiles, were identified. In raw cabbages, dimethyl disulfide and dimethyl trisulfide were the most abundant sulfur-containing compounds formed making up about 43 % of total volatiles, with the percentage dropping to about 1 % after cooking. Other sulfides identified include dimethyl tetrasulfide, methanethiol and carbon sulfide (in raw samples) and dimethyl sulfide in steamed samples only. Amounts of sulfides formed in raw RM were significantly lower than that present in RD and RL varieties. These compounds which are mainly formed from degradation of S-methyl-L-cysteine sulfoxide (SMCSO) by cysteine sulfoxide lyase (C-S lyase) or degradation of GHPs are known to be responsible for the largely undesirable sulfurous and 'rotten cabbage' off odours in cabbage (Chin & Lindsay, 1993; Kubec et al., 1998; Banerjee et al., 2014) . These compounds are of important to cabbage flavour because of their low detection thresholds (0.01- 12 ppb) (Buttery et al., 1976). The lower amounts of dimethyl di- and trisulfides in the samples can be due to denaturation of the C-S lyase during cooking which prevents SMCSO breakdown. In Allium species, optimum temperature for C-S lyase activity was found to be between 36 and 40 °C (Krest et al., 2000).

Of the 22 sulfur-containing compounds detected, 17 were ITCs. AITC and 3BITC were the most abundant volatile compounds in cooked cabbage samples comprising about 63 and 69 % of total volatiles respectively. Cabbage variety and cooking affected the amounts of AITC and 3BITC. Significantly higher amounts of AITC and 3BITC were detected in SF samples while AITC was significantly higher in cooked RL variety and 3BITC in RD variety. ATC was the third most abundant ITC in SF samples with less than 6 % of AITC and BITC detected in raw samples. ATC is said to possess a musty, sulfurous, garlic-like note, while 3BITC is known to have a pungent, wasabi-like, cabbage-like aroma (Chin et al., 1996; Sultana et al., 2003). AITC is known for its pungent and lacrymatory aroma and is described as the characteristic aroma compound in cabbage with a detection threshold of 375 ppb (Akpolat & Barringer, 2015). The mild pungency of AITC is considered a desirable flavour in cabbages and is thought to impart 'freshness' to cabbage flavour (Chin et al., 1996; Akpolat & Barringer, 2015). It is hypothesized that the lower levels of sulfides and higher levels of AITC in cooked cabbages especially SF samples may improve consumer liking and acceptance, which will be discussed later in section

6.3.4.2. Other ITCs such as iberverin, erucin and PEITC identified are characterized as possessing radish-like odours (Sultana et al., 2003; Raffo et al., 2018). However, because of the low levels detected they are unlikely to contribute significantly to cabbage flavour if present below their detection thresholds. The higher amounts of ITCs formed in cooked sample is the result of GSL hydrolysis to ITCs by myrosinase as previously discussed. Sulfides, AITC, and CETP has been reported as the main hydrolysis products of fresh cabbage (Chin & Lindsay, 1993; Chin et al., 1996; Akpolat & Barringer, 2015).

Nitrile compounds were mostly identified in raw samples (about 14 % of total volatiles), mainly due to the activity of ESP in GSL hydrolysis. The most abundant nitrile was CETP which has a weak, musty sulfurous odour and is barely perceivable at thresholds of 200 ppm (Chin et al., 1996). The impact of nitriles on *Brassica* flavour is not fully known, but nitrile compounds have been associated with bitter and pungent attributes in a Japanese leafy vegetable (*B. rapa* cv. *nakajimana*) (Kato et al., 2011). 5-methylthiopentyl nitrile has been associated with broccoli and cabbage flavour (Jirovetz et al., 2002). The six other volatiles identified in cabbage samples did not exceed 1 % of total volatiles detected and include furfural and 2-ethylfuran formed in SF samples as a result of Maillard reactions which may contribute to burnt notes of SF samples. In conclusion, some of the GHPs identified in the headspace above the freshly prepared samples had been previously detected during fully quantitative GHP analysis which used a solvent extract of the dried samples. Indeed, the ratio between raw and cooked samples followed a similar pattern, however, the headspace analysis also detected other GHPs not previously found by the solvent extraction, such as isobutyl isothiocyanate (IBITC) and hexyl isothiocyanate (HITC) which can also contribute to the green/grassy note of cabbage.

Table 6.5: [a] Relative amounts of volatiles identified and quantified in red cabbage samples (ST = steamed, SF = stir fried)

				RM			RD			RL			
Code	Compound	LRI	[b]ID	Raw	ST	SF	Raw	ST	SF	Raw	ST	SF	*P-value*
	Alcohols												
a01	1-penten-3-ol (1pent3)	1097	A	16^{b}	ND	16^{b}	24^{c}	ND	3^{a}	14^{b}	ND	16^{b}	< 0.0001
a02	1-pentanol (1pent)	1200	A	12^{bc}	39^{d}	7^{ab}	13^{bc}	12^{bc}	6^{ab}	15^{c}	8^{abc}	4^{a}	< 0.0001
a03	1-hexanol (1hex)	1226	A	249^{c}	ND	43^{ab}	28^{a}	ND	5^{a}	315^{c}	ND	127^{b}	< 0.0001
a04	(E)-2-penten-1-ol (2pent(E))	1305	A	17^{a}	ND	15^{a}	431^{b}	ND	14^{a}	23^{a}	ND	11^{a}	< 0.0001
a05	(E)-3-hexen-1-ol (3hex(E))	1306	A	22^{bc}	ND	10^{ab}	29^{c}	ND	3^{a}	33^{c}	ND	36^{c}	< 0.0001
a06	(Z)-3-hexen-1-ol (3hex(Z))	1312	A	1951^{bc}	2^{a}	249^{a}	2443^{c}	5^{a}	23^{a}	1690^{b}	ND	131^{a}	< 0.0001
a07	(E)-2-hexen-1-ol (2hex(E))	1324	A	16^{b}	ND	14^{b}	20^{b}	ND	19^{b}	14^{b}	ND	41^{c}	< 0.0001
a08	2-ethyl-1-hexanol (2hex2ethyl)	1429	A	2^{b}	ND	ND	4^{c}	ND	ND	4^{c}	ND	ND	< 0.0001
a09	(E)-2-hepten-1-ol (2hep(E))	1452	B	ND	ND	30^{c}	ND	ND	16^{b}	ND	ND	15^{b}	< 0.0001
a10	1-octanol (1oct)	1481	A	5^{a}	13^{bcd}	13^{bcd}	8^{ab}	16^{d}	15^{cd}	12^{bcd}	9^{ab}	9^{abc}	< 0.0001
a11	1-nonanol (1non)	1608	A	6^{ab}	8^{ab}	17^{b}	6^{ab}	9^{ab}	2^{a}	11^{ab}	66^{c}	6^{ab}	< 0.0001
	Aldehydes												
ald01	3-hexenal (3hex)	1138	A	35^{b}	ND	153^{c}	36^{b}	ND	44^{b}	30^{b}	ND	43^{b}	< 0.0001
ald02	hexanal (Hex)	1140	A	66^{a}	43^{a}	307^{b}	46^{a}	40^{a}	53^{a}	48^{a}	43^{a}	61^{a}	< 0.0001
ald03	(Z)-2-hexenal (2hexe(Z))	1198	B	6^{b}	ND	15^{c}	5^{b}	ND	6^{b}	4^{b}	ND	5^{b}	< 0.0001
ald04	(E)-2-hexenal (2hexe(Z))	1208	A	19^{a}	ND	146^{c}	32^{a}	ND	113^{bc}	40^{ab}	ND	168^{c}	< 0.0001
ald05	heptanal (Hep)	1268	A	ND	5^{b}	10^{c}	ND	8^{c}	8^{c}	ND	9^{c}	9^{c}	< 0.0001
ald06	octanal (Oct)	1307	A	ND	7^{b}	ND	ND	13^{c}	ND	ND	15^{c}	ND	< 0.0001

				RM			RD			RL			
Code	Compound	LRI	[b]ID	Raw	ST	SF	Raw	ST	SF	Raw	ST	SF	*P-value*
ald07	(E,E)-2,4-hexadienal (2hep(E,E))	1381	A	16^{d}	ND	17^{d}	16^{d}	ND	14^{cd}	8^{bc}	ND	5^{ab}	< 0.0001
ald08	nonanal (Non)	1426	A	10^{a}	57^{cd}	36^{bc}	8^{a}	63^{d}	35^{bc}	15^{ab}	137^{e}	26^{ab}	< 0.0001
ald09	benzaldehyde (Benz)	1451	A	ND	4^{b}	7^{d}	ND	ND	6^{cd}	5^{bc}	ND	5^{bcd}	< 0.0001
ald10	(E)-2-octenal (2octe)	1469	A	ND	ND	14^{c}	ND	ND	6^{b}	ND	ND	5^{b}	< 0.0001
	Sulfur-containing compounds												
s01	methanethiol (Met)	690	B	16^{b}	2^{a}	ND	59^{c}	3^{a}	ND	23^{b}	3^{a}	ND	< 0.0001
s02	dimethyl sulfide (DMS)	805	A	ND	19^{b}	ND	ND	25^{b}	ND	ND	4^{a}	ND	< 0.0001
s03	carbon disulfide (CDS)	826	A	1^{a}	ND	ND	8^{b}	ND	ND	10^{b}	ND	ND	< 0.0001
s04	dimethyl disulfide (DMDS)	1075	A	588^{b}	5^{a}	4^{a}	1843^{c}	4^{a}	4^{a}	1551^{c}	11^{a}	8^{a}	< 0.0001
s05	methyl thiocyanate (Thio)	1234	B	39^{bc}	ND	4^{a}	59^{c}	ND	2^{a}	52^{c}	13^{ab}	12^{a}	< 0.0001
s06	isothiocyanatocyclopropane isomer (ITCP-1)	1328	B	ND	5^{a}	57^{cd}	ND	16^{ab}	51^{c}	ND	34^{b}	72^{d}	< 0.0001
s07	allyl thiocyanate (ATC)	1331	B	12^{a}	19^{a}	628^{b}	23^{a}	143^{a}	1101^{c}	61^{a}	224^{a}	1063^{c}	< 0.0001
s08	isobutyl isothiocyanate (IBITC)	1338	B	ND	7^{ab}	22^{cd}	ND	13^{bc}	29^{d}	ND	55^{e}	23^{cd}	< 0.0001
s09	allyl isothiocyanate (AITC)	1350	B	31^{a}	170^{a}	5719^{bc}	148^{a}	1395^{a}	7590^{c}	443^{a}	3216^{ab}	11129^{d}	< 0.0001
s10	Isothiocyanatocyclopropane isomer (ITCP-2)	1362	B	ND	8^{a}	46^{cd}	ND	18^{ab}	58^{d}	ND	35^{bc}	66^{d}	< 0.0001
s11	dimethyl trisulfide (DMTS)	1383	A	255^{a}	5^{a}	16^{a}	735^{b}	6^{a}	21^{a}	1610^{c}	2^{a}	17^{a}	< 0.0001
s12	butyl isothiocyanate (BITC)	1395	B	ND	3^{b}	ND	ND	6^{c}	ND	ND	15^{d}	ND	< 0.0001
s13	3-butenyl isothiocyanate (3BITC)	1449	B	15^{a}	475^{a}	4823^{b}	24^{a}	5307^{b}	5654^{b}	66^{a}	377^{a}	4475^{b}	< 0.0001
s14	3-methylbutyl isothiocyanate (3MBITC)	1478	B	ND	10^{a}	19^{ab}	ND	48^{c}	51^{c}	ND	143^{d}	40^{bc}	< 0.0001
s15	pentyl isothiocyanate (PITC)	1524	B	ND	5^{a}	10^{a}	ND	24^{a}	16^{a}	ND	232^{b}	12^{a}	< 0.0001

				RM			RD			RL			
Code	Compound	LRI	[b]ID	Raw	ST	SF	Raw	ST	SF	Raw	ST	SF	*P-value*
s16	methyl (methylthio)methyl disulfide (DMMM)	1569	B	14^{b}	ND	ND	28^{c}	ND	ND	16^{b}	ND	ND	< 0.0001
s17	4-methylpentyl isothiocyanate (4MPITC)	1606	B	ND	4^{a}	2^{a}	ND	6^{a}	3^{a}	ND	66^{b}	9^{a}	< 0.0001
s18	hexyl isothiocyanate (HITC)	1654	B	ND	5^{ab}	26^{c}	ND	16^{bc}	15^{bc}	ND	53^{d}	24^{c}	< 0.0001
s19	dimethyl tetrasulfide (DMTT)	1691	B	14^{a}	ND	ND	54^{b}	ND	ND	119^{C}	ND	ND	< 0.0001
s20	iberverin (IBVN)	1803	B	ND	44^{c}	27^{bc}	ND	29^{bc}	16^{ab}	ND	384^{d}	19^{abc}	< 0.0001
s21	erucin (ER)	1960	B	ND	31^{a}	38^{a}	ND	404^{b}	34^{a}	ND	1065^{c}	46^{a}	< 0.0001
s22	2-phenylethyl isothiocyanate (PEITC)	2005	B	ND	19^{a}	3^{a}	ND	29^{a}	11^{a}	ND	346^{b}	3^{a}	< 0.0001
	Nitriles												
n01	butanenitrile (But)	966	A	35^{c}	ND	15^{ab}	27^{bc}	ND	13^{ab}	65^{d}	ND	18^{abc}	< 0.0001
n02	3-methylbutyronitrile (3MBN)	1053	A	2^{b}	ND	ND	3^{c}	ND	ND	2^{b}	ND	ND	< 0.0001
n03	3-butenenitrile (3but)	1095	B	32^{bc}	3^{a}	6^{a}	25^{abc}	4^{a}	3^{a}	40^{c}	5^{ab}	4^{a}	< 0.001
n04	1-cyano-2,3-epithiopropane (CETP)	1500	B	204^{b}	ND	22^{a}	215^{b}	ND	15^{a}	618^{c}	ND	93^{ab}	< 0.0001
n05	4-methylthiobutyl nitrile (4MBN)	1503	B	20^{c}	ND	6^{a}	6^{a}	ND	4^{a}	16^{bc}	ND	8^{ab}	< 0.0001
n06	4,5-epithiovaleronitrile (EVN)	1557	B	91^{b}	ND	15^{a}	54^{ab}	ND	7^{a}	202^{c}	ND	12^{a}	< 0.0001
n07	5-methylthiopentyl nitrile (5MPN)	1655	B	90^{d}	ND	6^{ab}	52^{c}	ND	8^{ab}	20^{b}	ND	3^{ab}	< 0.0001
n08	benzeneacetonitrile (BAN)	1776	A	5^{b}	ND	ND	11^{c}	ND	ND	17^{d}	ND	ND	< 0.0001
n09	benzenepropanenitrile (BPN)	2007	B	12^{c}	ND	ND	8^{b}	ND	ND	15^{d}	ND	ND	< 0.0001
	Other												
o01	2,2,4,6,6-pentamethyl-heptane (Hep2,2)	1386	A	18^{b}	6^{a}	20^{b}	22^{b}	15^{ab}	23^{b}	15^{ab}	16^{ab}	17^{ab}	0.002
o02	2-ethylfuran (2fur)	1022	A	ND	ND	15^{c}	ND	ND	12^{c}	ND	ND	4^{b}	< 0.0001

o03	1-penten-3-one (1pent)	1002	A	ND	ND	ND	ND	ND	ND	ND	ND	6^{b}	< 0.0001
o04	D-limonene (Limon)	1238	A	16^{c}	4^{ab}	ND	5^{ab}	7^{b}	ND	25^{d}	14^{c}	ND	< 0.0001
o05	hexyl acetate (Hexace)	1308	B	6^{bc}	ND	ND	6^{c}	ND	ND	4^{b}	ND	ND	< 0.0001
o06	furfural (Fur)	1386	A	ND	ND	13^{c}	ND	ND	5^{b}	ND	ND	4^{b}	< 0.0001

[a] Values are peak area means of four replicates divide by 10^{4}. [b] Linear retention index on a Stabilwax-DA column. [c] A, mass spectrum and LRI agree with those of authentic compound; B, mass spectrum agrees with reference spectrum in the NIST/NIH mass spectra database and literature. [d] Pair of stereoisomers. ND = Not detected. Mean values with different superscripts in the same row significantly different at $p<0.05$

6.3.3 Sensory attributes

The mean sensory scores of red cabbage samples alongside significant differences are presented in Table 6.6. A total of 44 attributes were agreed upon and used to describe the samples. Discrimination, repeatability and assessor-sample interactions were checked for all assessors. There was no significant difference found between samples in four out of the 44 attributes. All appearance attributes differed significantly between samples with an increase in purple colour, moistness and rubbery feeling after cooking. SF led to increase in shiny appearance.

Cooking significantly reduced swede, sulfurous and stalky odours/flavours in red cabbage, supporting the volatiles result where the levels of sulfides and C6 aldehydes and alcohols responsible for sulfurous and stalky odours were significantly lower in cooked cabbages (Table 6.5). Significantly higher warming mouthfeel and peppery flavour observed in raw cabbages can also be attributed to higher amounts of sulfides present in the samples. A strong correlation was observed in rocket samples between warming and peppery attributes and sulfur volatiles (Bell et al., 2017a). On the contrary, cooking led to a significant increase in sweet odour with a decrease in bitter taste (leaf and stalk), as supported by the lower levels of GSLs detected in cooked samples resulting in enhanced perception of sugars and other sweet-tasting compounds; though sugars were also reduced with cooking (Figure 6.2 and Table 6.4). Though cooked samples were rated higher than raw samples in sweet taste perception of leaf and stalk, scores were not significant.

Raw samples were found to have crunchier and tougher mouthfeel than cooked samples. This might be the result of cell wall and pectic breakdown leading to moisture loss during cooking which results in softer tissues. Burnt and sesame odours/flavours and aftereffects were perceived in SF samples which is in agreement with the furan compounds detected in SF samples (Table 6.5). Earthy flavour and aftereffect significantly reduced after cooking and was much lower in SF samples. Significantly higher scores for cooked odour observed in cooked samples may be attributed to the higher concentrations of AITC in the headspace of cooked cabbages, indeed AITC has been described as the characteristic flavour of cooked cabbage (Table 6.5). The tougher and crunchier mouthfeel of raw samples led to more residues left in the mouth after chewing. Overall, differences observed in samples were mostly due to cooking not variety, implying that cooking may be more important than variety when considering the sensory profile of red cabbage.

Table 6.6: Mean scores for sensory attributes of red cabbage samples (ST = steamed, SF = stir fried).

	RM			RD			RL			
Attributes	Raw	ST	SF	Raw	ST	SF	Raw	ST	SF	*P-value*
Appearance										
Brown scorch marks_A	0.0^{b}	0.0^{b}	12.0^{a}	0.0^{b}	0.0^{b}	12.8^{a}	0.0^{b}	0.0^{b}	15.7^{a}	<0.0001
Darkness of colour_A	31.8^{e}	50.5^{cd}	45.6^{d}	42.6^{de}	67.3^{ab}	59.9^{bc}	43.6^{de}	74.5^{a}	61.5^{abc}	<0.0001
Purple colour_A	29.7^{e}	56.6^{abc}	39.5^{de}	29.0^{e}	61.4^{ab}	46.6^{cd}	29.1^{e}	65.3^{a}	49.3^{bcd}	<0.0001
Shiny_A	14.4^{c}	24.9^{bc}	72.1^{a}	16.2^{bc}	23.3^{bc}	72.1^{a}	20.4^{bc}	30.2^{b}	72.0^{a}	<0.0001
Oily Surface_A	2.1^{b}	1.5^{b}	73.9^{a}	0.0^{b}	0.3^{b}	69.0^{a}	0.0^{b}	5.1^{b}	73.2^{a}	<0.0001
Moist_A	21.1^{b}	50.6^{a}	59.4^{a}	22.9^{b}	52.1^{a}	57.7^{a}	21.5^{b}	57.0^{a}	57.7^{a}	<0.0001
Feels rubbery on fingers_A	20.6^{d}	51.4^{ab}	34.9^{bcd}	23.8^{cd}	54.1^{a}	39.0^{abc}	22.0^{d}	51.9^{b}	32.3^{cd}	<0.0001
Amount of coloured liquid released_A	0.1^{b}	13.7^{a}	8.6^{ab}	0.2^{b}	13.5^{a}	7.5^{ab}	0.0^{b}	16.3^{a}	10.3^{a}	<0.0001
Cooked_A	0.0^{b}	62.1^{a}	69.2^{a}	0.5^{b}	60.0^{a}	69.5^{a}	0.5^{b}	60.8^{a}	73.1^{a}	<0.0001
Odour										
Sweet_O	20.9^{bcd}	31.7^{abcd}	40.4^{a}	17.5^{cd}	32.9^{ab}	34.5^{ab}	17.2^{d}	34.5^{ab}	32.0^{abc}	<0.0001
Stalky_O	37.2^{a}	21.3^{b}	10.2^{c}	28.7^{ab}	21.2^{b}	9.0^{c}	35.4^{a}	24.8^{b}	9.8^{c}	<0.0001
Sesame_O	0.0^{b}	3.4^{b}	45.8^{a}	0.0^{b}	1.9^{b}	51.7^{a}	0.1^{b}	5.6^{b}	46.2^{a}	<0.0001
Metallic_O	3.8	1.1	0.8	3.4	0.6	2.2	4.0	0.7	0.9	0.050
Swede_O	23.8^{a}	20.6^{abc}	6.5^{bc}	21.5^{ab}	21.0^{abc}	5.5^{c}	26.1^{a}	21.4^{abc}	6.0^{bc}	<0.0001
Sulphurous_O	16.6^{a}	6.7^{b}	1.9^{b}	16.8^{a}	5.3^{b}	3.0^{b}	19.6^{a}	6.9^{b}	3.4^{b}	<0.0001
Burnt_O	0.0^{b}	0.0^{b}	8.3^{a}	0.1^{b}	0.1^{b}	10.9^{a}	0.0^{b}	0.4^{b}	9.9^{a}	<0.0001

	RM			RD			RL			
Attributes	Raw	ST	SF	Raw	ST	SF	Raw	ST	SF	*P-value*
Cooked_O	0.1^{c}	64.7^{ab}	72.9^{a}	0.2^{c}	58.6^{b}	69.8^{ab}	0.0^{c}	63.8^{ab}	71.9^{ab}	<0.0001
Mouthfeel										
Crunchy_MF	77.0^{a}	48.9^{d}	64.5^{bc}	74.9^{ab}	47.4^{d}	56.5^{cd}	76.7^{a}	45.3^{d}	50.7^{d}	<0.0001
Moist_MF	35.8^{b}	59.0^{a}	48.9^{a}	28.3^{b}	55.1^{a}	49.0^{a}	28.2^{b}	54.8^{a}	49.6^{a}	<0.0001
Warming_MF	14.3^{a}	1.0^{b}	1.6^{b}	13.4^{a}	2.2^{b}	2.0^{b}	14.1^{a}	1.3^{b}	2.3^{b}	<0.0001
Fibrous_MF	1.9	0.2	1.0	3.1	1.3	0.4	3.4	1.4	0.5	0.083
Toughness_MF	31.3^{abc}	18.8^{d}	21.8^{bcd}	33.5^{ab}	19.2^{d}	23.4^{bcd}	36.7^{a}	14.8^{d}	20.8^{cd}	<0.0001
Oily_MF	0.1^{b}	1.9^{b}	44.9^{a}	0.0^{b}	0.2^{b}	48.1^{a}	0.0^{b}	1.3^{b}	48.1^{a}	<0.0001
Taste										
Stalk: Bitter_T	12.0^{ab}	6.5^{b}	6.5^{b}	12.9^{ab}	7.7^{b}	6.9^{b}	15.8^{a}	7.7^{b}	8.7^{ab}	0.0006
Stalk: Sweet_T	29.6	27.3	29.2	24.5	24.4	26.7	25.4	25.7	27.6	0.967
Leaf: Bitter_T	12.8^{abc}	8.4^{bc}	6.4^{c}	15.3^{ab}	8.2^{c}	6.3^{c}	18.1^{a}	7.9^{c}	7.9^{c}	<0.0001
Leaf: Sweet_T	25.6	28.8	27.6	22.2	27.0	31.3	22.4	24.1	30.1	0.030
Leaf: Salthy_T	2.5^{c}	3.7^{bc}	6.6^{ab}	2.3^{c}	4.5^{bc}	7.2^{ab}	1.7^{c}	3.7^{bc}	8.8^{a}	<0.0001
Leaf: Savoury_T	28.5^{ab}	36.2^{ab}	40.2^{a}	26.6^{b}	29.7^{ab}	35.6^{ab}	29.4^{ab}	26.5^{b}	39.2^{a}	0.0003
Leaf: Metallic_T	2.9^{ab}	1.1^{b}	1.3^{b}	3.8^{ab}	0.5^{b}	1.2^{b}	6.1^{a}	0.4^{b}	2.7^{ab}	<0.0001
Flavour										
Sesame_F	0.0^{b}	0.8^{b}	36.0^{a}	0.0^{b}	0.7^{b}	39.3^{a}	0.0^{b}	2.9^{b}	37.4^{a}	<0.0001
Stalky_F	29.3^{ab}	19.2^{bc}	8.5^{c}	32.5^{a}	19.2^{bc}	12.0^{c}	30.2^{ab}	18.9^{bc}	7.8^{c}	<0.0001

	RM			RD			RL			
Attributes	Raw	ST	SF	Raw	ST	SF	Raw	ST	SF	*P-value*
Earthy_F	21.1^{ab}	11.7^{bcd}	6.4^{d}	23.7^{a}	15.8^{abcd}	5.9^{d}	25.3^{a}	16.7^{abc}	7.2^{cd}	<0.0001
Sulphury_F	12.6^{a}	4.1^{b}	2.1^{b}	14.0^{a}	3.8^{b}	3.2^{b}	15.6^{a}	4.7^{b}	3.7^{b}	<0.0001
Burnt_F	0.0^{b}	0.1^{b}	6.2^{ab}	0.0^{b}	0.0^{b}	8.4^{a}	0.0^{b}	0.0^{b}	8.3^{a}	<0.0001
Peppery_F	13.7^{a}	2.2^{b}	2.5^{b}	14.2^{a}	1.7^{b}	3.6^{b}	17.3^{a}	1.8^{b}	3.3^{b}	<0.0001
Aftereffects										
Bitter_AE	9.3^{ab}	5.4^{b}	4.4^{b}	13.5^{a}	6.9^{ab}	5.1^{b}	12.4^{a}	4.7^{b}	4.6^{b}	<0.0001
Residue (Bits in mouth) _AE	14.5^{abc}	8.8^{cd}	13.6^{abc}	15.8^{a}	7.5^{d}	13.2^{abcd}	15.0^{ab}	9.8^{bcd}	13.3^{abcd}	<0.0001
Oily mouthcoating_AE	0.1^{b}	0.7^{b}	29.4^{a}	0.0^{b}	0.4^{b}	34.5^{a}	0.1^{b}	1.3^{b}	38.6^{a}	<0.0001
Lingering aftertaste_AE	29.9^{ab}	21.3^{bc}	26.2^{ab}	30.1^{a}	20.9^{bc}	28.7^{ab}	32.5^{a}	17.3^{c}	28.3^{ab}	<0.0001
Burnt_AE	0.0^{b}	0.0^{b}	4.7^{a}	0.0^{b}	0.0^{b}	6.2^{a}	0.0^{b}	0.1^{b}	6.8^{a}	<0.0001
Salty_AE	2.1^{cd}	2.8^{cd}	6.8^{a}	1.2^{b}	2.0^{cd}	5.4^{abc}	1.7^{d}	3.0^{bcd}	6.5^{ab}	<0.0001
Nutty_AE	2.6^{abc}	1.5^{bc}	9.9^{abc}	1.6^{bc}	0.5^{c}	12.1^{ab}	2.1^{bc}	1.3^{c}	12.9^{a}	0.0002
Earthy_AE	16.9^{ab}	10.3^{bcd}	5.1^{cd}	17.4^{ab}	11.8^{abc}	3.6^{d}	19.4^{a}	12.8^{abc}	4.9^{cd}	<0.0001

Mean values with different superscripts in the same row are significantly different at $p<0.05$

6.3.4 Consumer study

6.3.4.1 Consumer demographics and cabbage consumption

Table 6.7 shows the demographic profile of the 118 consumers who participated in the study. 80 % of the participants also took part in the black kale study (Chapter 5). The majority of the consumers were between the ages of 18-30 years (65 %) with 18 % and 17 % aged 31-45 and 46-61 years respectively. The mean and median ages were 31 and 26 respectively. Approximately 50 % were of white ethnicity, 12 % black African and 14 % of Chinese descent. More females (73.7 %) than males (26.3 %) took park in the study. The majority of consumers usually consumed cabbage stir-fried (76.3 %), boiled (60.2 %) and steamed (44.9 %). Only 10.2 % and 25.4 % consumed cabbage microwaved and raw respectively. Consumers were asked about frequency of cabbage (any type) consumption and 30.5 % stated that they consumed cabbages frequently (approximately once a week), 40.7 % sometimes, 22.0 % rarely and only 6.8 % said never. In terms of red cabbage consumption, only 30.5 % (n= 36) stated that they consumed red cabbage.

6.3.4.2 Consumer results for liking, taste perception and cluster analysis

The mean scores of consumer responses and cluster analysis are presented in Table 6.8. Significant differences were observed for all parameters except mouthfeel liking where mean scores did not differ ($p= 0.063$) between samples. Significant differences observed were mostly due to cooking rather than variety. Similar to the sensory profile results, raw samples were perceived significantly ($p < 0.0001$) more bitter than cooked cabbages. However, although the trained panel did not find any significant difference in the sweet taste attribute, consumers perceived the cooked cabbages to be significantly ($p < 0.0001$) sweeter than raw samples. ST-RM was significantly sweeter than ST-RL while raw RM did not differ significantly between raw and cooked RD and RL samples. SF cabbages were perceived to have more savoury (umami) taste which might have been enhanced during the stir-frying process.

Consumers preferred the appearance of raw samples which might have been due to lower 'amount of liquid released' (as described by the sensory panel) and moistness of the raw samples. The difference in mouthfeel characteristics (Table 6.6) of the raw and cooked samples did not affect the mean consumer liking of mouthfeel. Cooking significantly ($p < 0.0001$) improved taste liking of red cabbages which followed the increase is sweet taste perception. Taste liking of Raw RM did not differ significantly ($p=0.075$) between raw and

cooked RD and RL samples; and taste liking of raw and cooked RD samples did not differ significantly between raw RM and RL samples.

Overall liking results showed that there was no significant difference in the overall liking of samples except for raw RD which was significantly less liked to SF-RL. Consumption intent followed a similar trend as taste liking with consumers more likely to consume cooked rather than raw cabbage. The results showed that consumers were least likely to consume raw RD. We hypothesize that the significantly sweeter and less bitter taste of RM samples might be due to the significantly lower total GSL content (Figure 6.2) and higher total sugars content (Table 6.4) detected which in turn improved consumer taste liking of the samples. On the contrary, the significantly higher contents of GSLs (Figure 6.2) and lower sugar concentrations (Table 6.4) in cooked RD samples compared to other cooked samples may be responsible for the lower (although not significantly different) taste liking of the sample. Bitterness has been generally reported has one of the major reasons for consumer rejection of *Brassica* vegetables and the results obtained showed that GSL-sugar/amino acid ratio may be an important factor in taste liking and bitter and sweet taste perceptions.

Results of cluster analysis for consumer overall liking scores is presented in Table 6.8. Three clusters explained the liking pattern of consumers. There were significant differences ($p<0.0001$) in the liking scores of the three clusters. Consumers in cluster 1 (30 %) gave generally low liking scores, disliked stir fried cabbage and had a tendency to like raw more than steamed. Cluster 2 (30 %) consumers liked cooked cabbages and disliked raw ones, although they scored raw RM notably higher than the other two raw samples. Consumers in cluster 3 (40 %), which was the largest cluster, they generally liked everything and did not discriminate between samples. Consumers in this cluster gave significantly higher overall liking scores for all samples except raw RM and SF-RM where scores did not differ from cluster 1 and cluster 2 respectively. The differences observed in consumers liking between clusters may have been due to differences in consumer preference for cabbage texture, bitter taste and sulfurous odours and flavours. Some consumers prefer the bitter taste and sulfurous flavour of raw cabbages (cluster 1), others prefer the sweeter taste and less sulfurous notes of cooked samples (cluster 2) while the last group of consumers like both raw and cooked cabbages. Some of these differences might be due to differences in perception of bitter taste and/or sulfurous aroma.

Table 6.7: Demographic characteristics of consumers (n=118)

Question	Number of individuals (%)
Age	
18-30	77 (65 %)
31-45	21(17.8 %)
46-61	20(17 %)
Median age	26
Mean age	31
Ethnicity	
Arab	4 (3.4 %)
Black African	14 (11.8 %)
Caribbean	3 (2.5 %)
Chinese	17 (14.4 %)
Indian	2 (1.7 %)
White and Black Asian	4 (3.4 %)
White British	41 (34.7 %)
White Irish	1 (0.8 %)
White Other	13 (11.1 %)
Other ethnic group- any other	18 (15.2 %)
Prefer not to declare	1 (0.8 %)
Gender	
Male	31 (26.3 %)
Female	87 (73.7 %)
Cabbage cooking methods (consumers ticked all that applied)	
Raw	30 (25.4 %)
Baked	10 (8.5 %)
Boiled	71 (60.2 %)
Microwaved	12 (10.2 %)
Steamed	53 (44.9 %)
Stir-fried	90 (76.3 %)
Red cabbage consumption	
Yes	36 (30.5 %)
No	82 (69.5 %)
Frequency of cabbage (any type) consumption	
Question: How often do to you consume cabbage?	
Never	8 (6.8 %)
Rarely (less than once/month)	26 (22.0 %)
Sometimes (approximately once/month)	48 (40.7 %)
Frequently (approximately once/week)	36 (30.5 %)

Table 6.8: Summary table of [x]consumer responses (n=118) and [x]cluster analysis results of mean overall liking scores

	RM			RD			RL			
Attribute	Raw	ST	SF	Raw	ST	SF	Raw	ST	SF	*P-value*
Bitter taste perception	12.1^{b}	5.5^{a}	6.0^{a}	15.9^{b}	7.5^{a}	6.6^{a}	15.7^{b}	7.5^{a}	6.2^{a}	< 0.0001
Sweet taste perception	17.5^{abcd}	22.0^{d}	19.0^{bcd}	12.9^{a}	19.6^{cd}	16.9^{abcd}	14.0^{ab}	16.3^{abc}	19.0^{bcd}	< 0.0001
Savoury taste perception	14.0^{a}	14.4^{ab}	20.7^{cd}	14.2^{ab}	13.2^{a}	21.8^{d}	14.0^{ab}	15.1^{abc}	20.3^{bcd}	< 0.0001
Appearance liking	6.4^{bc}	5.1^{a}	5.0^{a}	6.5^{c}	5.5^{a}	5.5^{a}	6.6^{c}	5.7^{ab}	5.4^{a}	< 0.0001
Mouthfeel liking	6.2	6.2	6.2	5.6	6.1	6.0	5.7	6.1	6.1	0.063
Taste liking	6.0^{abc}	6.3^{bc}	6.4^{c}	5.4^{a}	6.1^{abc}	6.2^{abc}	5.6^{ab}	6.3^{c}	6.5^{c}	< 0.0001
Overall liking	6.1^{ab}	6.2^{ab}	6.2^{ab}	5.6^{a}	6.2^{ab}	6.1^{ab}	5.7^{ab}	6.2^{ab}	6.4^{b}	0.008
Consumption Intent	3.5^{abc}	3.6^{bc}	3.8^{c}	3.1^{a}	3.6^{bc}	3.6^{bc}	3.3^{ab}	3.4^{abc}	3.8^{c}	< 0.0001
Mean overall liking scores for two clusters of consumers										
Cluster 1 (*n*=35, 30 %)	$6.2^{d,B}$	$5.3^{abcd,A}$	$4.1^{a,A}$	$5.7^{cd,B}$	$5.3^{abcd,A}$	$4.3^{ab,A}$	$6.0^{cd,B}$	$5.5^{bcd,A}$	$4.9^{abc,A}$	0.001
Cluster 2 (*n*=36, 30 %)	$5.0^{bc,A}$	$5.7^{cd,A}$	$6.9^{f,B}$	$3.8^{a,A}$	$6.0^{de,A}$	$6.2^{def,B}$	$4.1^{ab,A}$	$6.0^{def,A}$	$6.7^{ef,B}$	< 0.0001
Cluster 3 (*n*=47, 40 %)	6.9^{B}	7.3^{B}	7.3^{B}	6.9^{C}	7.0^{B}	7.4^{C}	6.8^{C}	6.8^{B}	7.4^{C}	0.491
P-value (cluster effect)[y]	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.001	< 0.0001	

[x] Mean values with different superscripts 'abc' in the same row significantly different at p<0.05

[y]Mean values with different superscripts 'ABC' in the same column significantly different at p<0.05

The differences in consumer liking of red cabbage may influence how cabbages are consumed (raw or cooked) and the general acceptability of red cabbage. A previous study on biscuits treated with varying levels of 4-methylthio-3-trans-butenyl isothiocyanate (the main flavour compound of white radish and known for its pungent characteristics) showed differences in liking of pungency based on cultural exposures. Consumers of Japanese descent preferred the more pungent biscuits while Australians and Koreans preferred the less pungent biscuits (Wills & Coogan, 2003). The authors attributed the differences observed to difference in how Japanese and Koreans use white radish and the unfamiliarity of Australians to white radish. In the present study, representation of different ethnic groups was too low to analyse within clusters.

6.3.5 Impact of genotype on taste perception and consumer liking

Table 6.9 presents the number of consumers in the different genotype groups while the results of difference in bitterness perception and taste liking are presented in Figure 6.4 (TAS2R38) and Figure 6.5 (gustin). For TAS2R38, 20.3 % (n= 24) of consumers carried the AVI/AVI genotype, 50 % (59) PAV/AVI, 20.3 % (24) PAV/PAV genotype and 9.3 % (n= 11) the rare genotype comprised of AAI/AAV. Based on gustin genotypes, 43.2 % (n= 51) of participants had the A/A genotype, 39.8 % (n= 47) A/G and 17 % (n= 20) G/G genotype.

TAS2R38 genotype did not significantly affect bitter taste perception (p=0.20). Though not significant, rare genotypes gave the lowest scores for bitter intensity while AVI/AVI and PAV/AVI consumers had higher bitter intensity scores than PAV/PAV consumers. The result did not follow a similar trend as in black kale previously discussed in Chapter 5 (section 5.3.4). Hayes et al. (2008) reported that rare AAA/AVI had higher PROP thresholds than PAV haplotypes and behaved similarly to the less sensitive AVI homozygotes. The result obtained is contrary to reports in literature where PAV/PAV individuals rated *Brassica* vegetables 60 % more bitter than AVI/AVI individuals (Sandell & Breslin, 2006). A similar result was observed in another study, where PAV/PAV consumers perceived significantly stronger bitter intensity for white cabbage and broccoli than other genotypes (Shen et al., 2016). No significant interaction in bitter taste intensity was found between TAS2R38 genotype and red cabbage variety (p= 0.87) or as a result of cooking (p= 0.92). All genotype groups found ST and SF samples to be significantly less bitter compared to raw samples, except rare genotype consumers who did not rate raw samples significantly different from cooked samples.

Taste liking was significantly influenced (p<0.0001) by TAS2R38 genotype. However, this difference was driven by rare genotypes. The rare genotype group rated the cabbages

significantly higher than the other genotypes but no significant effect was found in taste liking ratings of PAV/PAV, PAV/AVI and AVI/AVI genotypes; though PAV/PAV gave the lowest liking scores (a maximum difference of 0.23 on a 9-point scale). Though the rare genotype group, which are not normally considered, consisted of only 9 % of the total population, the results obtained show that they might be an important group and detailed studies on their relevance is needed. There were no significant interactions between TAS2R38 genotype and variety or cooking for taste liking ($p= 0.97$ and $p= 0.12$ respectively).

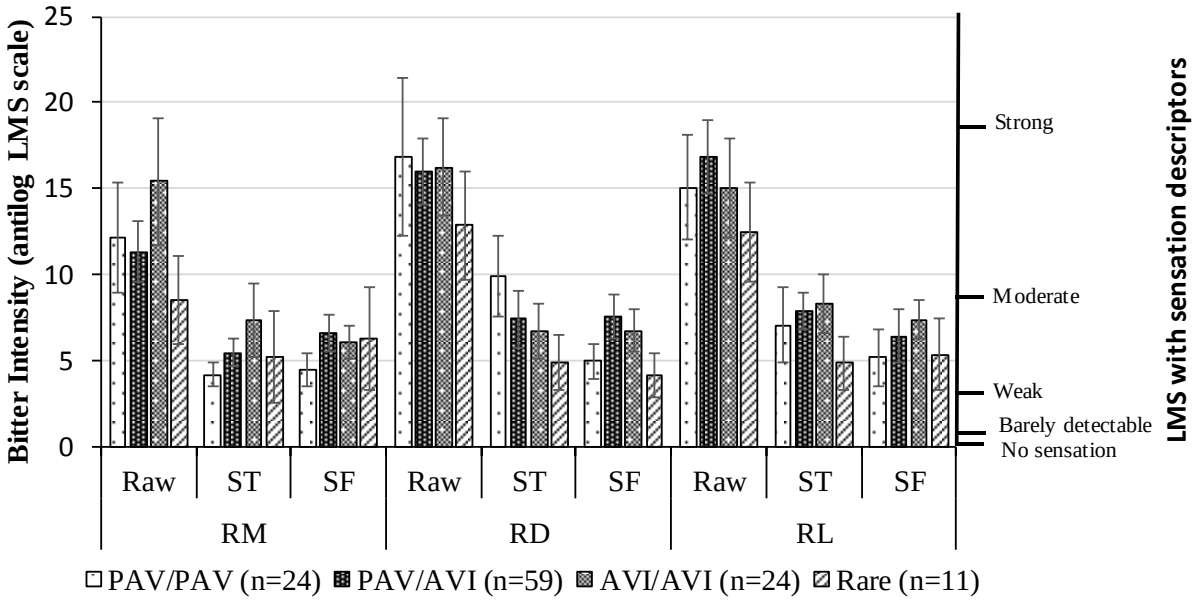
Gustin (CA6) did not have a significant effect on perception of bitter taste ($p= 0.22$). However, the GG group reported to produce less taste cells (Padiglia et al., 2010) gave the lowest scores for bitter taste while the AG group rated samples more bitter than the AA group (reported to produce the most taste cells). Interactions between gustin and variety was not significant for bitter taste ($p= 0.93$) while significant interaction was found between gustin and cooking ($p= 0.003$). All genotypes found cooked samples significantly less bitter than raw samples. However, the AA genotype group did not find significant difference in bitterness intensity between raw and steamed samples ($p= 0.06$), whereas the AG group found raw samples significantly more bitter than the AA group ($p= 0.007$). A significant effect ($p<0.001$) of gustin was found in taste liking with the AG group liking the cabbage significantly more than the GG group. The AA group did not rate liking scores differently from AG and GG ($p= 0.07$ and $p= 0.06$ respectively). There were no significant interactions between gustin and variety or cooking on taste liking.

Interactions between TAS2R38 and gustin on bitter taste intensity and taste liking were not studied because of the low numbers of consumers in some of the groups.

Table 6.9: Distribution of consumers based on genotype

Genotypes	Category	Number (%)
TAS2R38		
	AVI/AVI	24 (20.3 %)
	PAV/AVI	59 (50.0 %)
	PAV/PAV	24 (20.3%)
	Rare	11 (9.3 %)
Gustin (CA6)		
	A/A	51 (43.2 %)
	A/G	47 (39.8 %)
	G/G	20 (17.0 %)

(a)



(b)

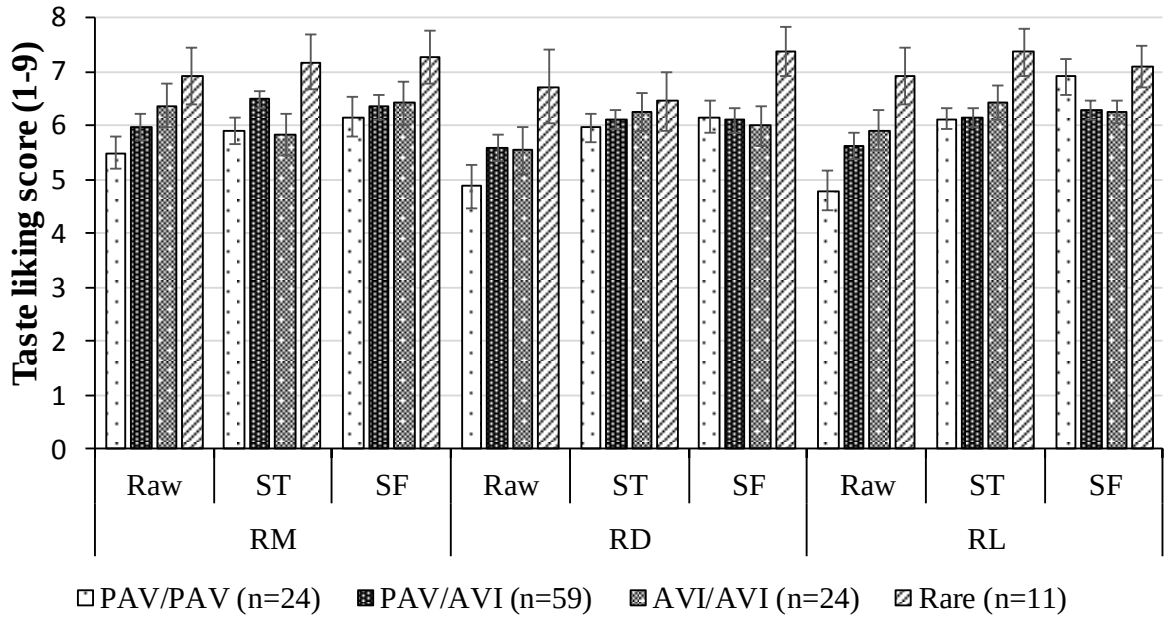
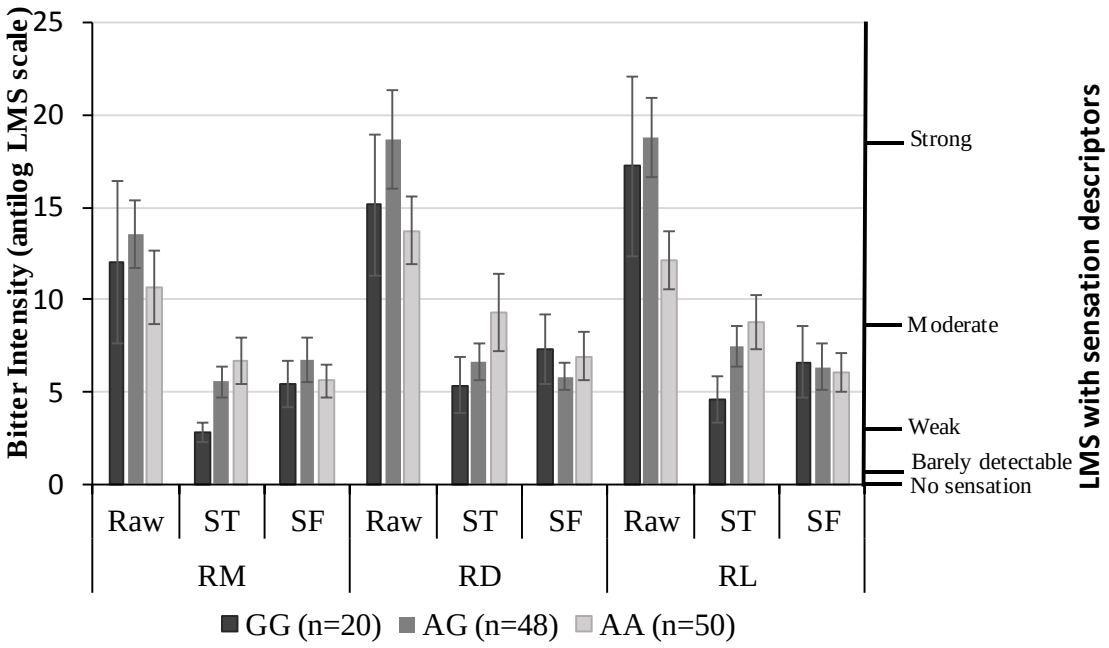


Figure 6.4. (a) Bitter intensity (BT) and (b) taste liking (TL) means scores of red cabbage samples (varieties RM, RD and RL) according to TAS2R38 genotype.
Bitterness perception are given as antilog values. Error bars represent standard errors of mean values.

(a)



(b)

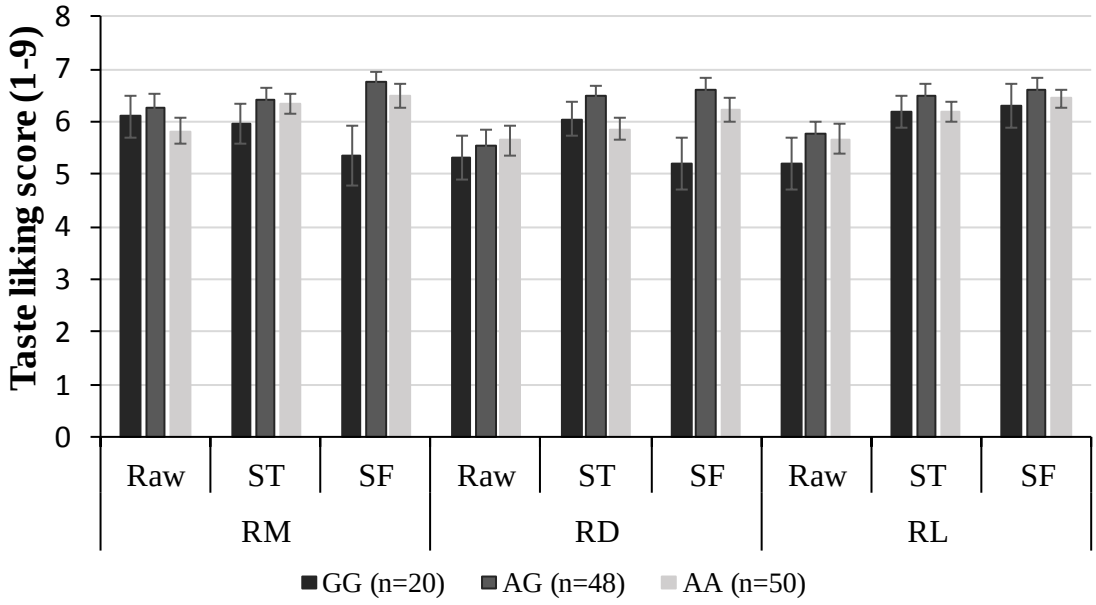


Figure 6.5. (a) Bitter intensity (BT) and (b) taste liking (TL) means scores of red cabbage samples (varieties RM, RD and RL) according to Gustin (CA6) genotype.
Bitterness perception are given as antilog values. Error bars represent standard errors of mean values.

6.3.6 Rotated factor analysis (RFA) and multiple factor analysis (MFA)

To study the relationship between all data collected, principal component analysis (PCA) with rotation was performed on individual data sets (except myrosinase activity). Rotated factor scores where then arranged in a logical temporal order based on data collection (from phytochemical results through to sensory and consumer data) and multiple component analysis (MFA) was used to simultaneously analyse for relationships between all rotated factor groups as presented in Figure 6.6. PC1 and PC2 explained 56 % of variation in the data. Discriminations were strongly based on cooking methods more than cabbage variety with three distinct groups describing the variations.

Stalky, earthy, peppery and sulfurous odours and flavours (see brown text in Fig 6.6, coordinates F1 -0.7, F2 -0.75) correlated positively with succinic acid (blue text) and volatile sulfides and alcohols (grey text), and were all associated with astringent, sulfurous and grassy flavour (see brown text in Fig 6.6, coordinates F1 -0.1, F2 -0.75). The same odour and flavour sensory attributes also correlated positively with bitter taste and aftereffects as well as with warming and crunchy mouthfeel (brown text); and all of these attributes correlating positively with raw samples. As expected raw samples correlated positively with GSLs (see purple text in Fig 6.6, coordinates F1 -0.25 to -0.7, F2 0 to -0.3). The group of data described above was negatively correlated with sweet taste, liking and consumption intent (all positioned to right of the Fig 6.6 plot, coordinates F1 +0.5 to +0.8; F2 +0.1 to + 0.75).

ITCs excluding ITCs from SIN hydrolysis (grey text), taste liking based on gustin GG genotype and TAS2R38 PAV/PAV, PAV/AVI and AVI/AVI (green text) correlated positively with ST samples which was also positively correlated with liking and consumer sweet taste perception (see pink text in Fig 6.6, coordinates F1 0 to 0.75, F2 0 to 0.75). However, unexpectedly, PROG, known to be a bitter tasting GSL, correlated positively with ST samples (see purple text in Fig 6.6, coordinates F1 -0.5, F2 0.75) but the bitter taste effects may have been suppressed and not pronounced in the samples due to low concentrations present (Bell et al., 2017b). ST samples also correlated with glucose, fructose and total sugars to some extent and may have suppressed the potential bitter taste from PROG (see red text in Fig 6.6, coordinates F1 -0.75, F2 0.50). Appearance attributes (purple colour, running liquid, moistness and cooked) all correlated positively with ST samples.

SF samples correlated positively with volatile AITC and ATC (see grey text in Fig 6.6, coordinates F1 0.5, F2 -0.75), sesame/burnt flavour and odour, shiny and oily appearance/aftereffects as well as burnt and nutty aftereffects (see grey text in Fig 6.6,

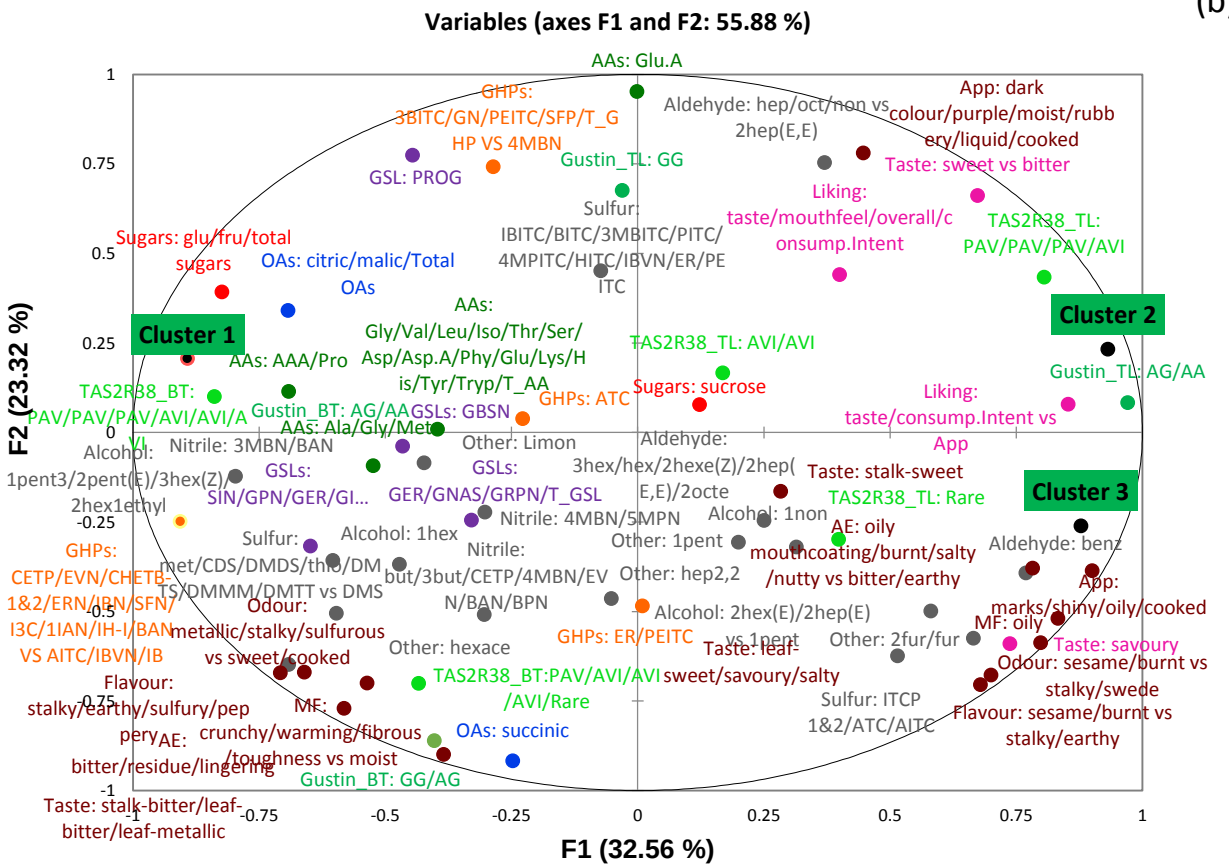
coordinates F1 0.5 to 0.75, F2 -0.50 to -0.75). These attributes were also positively correlated with benzaldehyde, furans and nonanol all known to be responsible for almond, burnt and fatty flavour/odour characteristics (Lignou et al., 2015). Taste liking of the TAS2R38 rare genotype group and gustin AG/AA group also correlated positively with SF samples and with sweet taste attribute, taste liking and consumption intent (see Fig 6.6, coordinates F1 0.2 to 1, F2 -0.25 to 0.25).

Clusters 2 and 3 correlated positively with sweet taste liking, consumption intent and cooked samples, and negatively with bitter taste perception and sulfurous and stalky characteristics and compounds. Both clusters 2 and 3 were also negatively correlated with cluster 1, which correlated positively with raw samples with their characteristic bitter taste, sulfurous and stalky flavour and compounds.

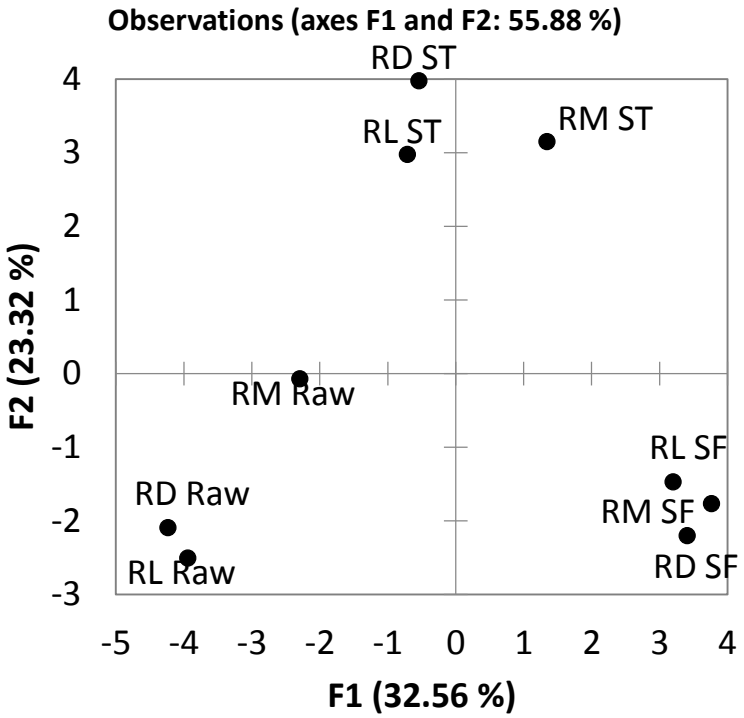
PC3 and PC4 (data not shown) explained 15 and 11 % of variations respectively splitting the data across varieties. PC3 separated out the RM variety mainly due to its low GSL content, high contents of AAs and sugars and sweet taste of its stalk. On the other hand, PC4 separated the RD variety from others because of its high GSLs concentrations as previously discussed in earlier sections (Table 6.4 and Figure 6.2); therefore, these PC figures are not presented.

Finally, correlations observed accurately reflected results obtained. The results showed that consumer preference of red cabbages differ and, contrary to previous reports, bitter taste and sulfurous odours are not considered undesirable sensory characterises by all consumers.

(a)



(b)



● Sugars ● Organic acids (OAs) ● Amino acids (AAs) ● Glucosinolates (GSLs) ● Glucosinolate hydrolysis products (GHPs) ● Volatiles ● Profiling ● Gustin ● TAS2R38 ● Consumer ● Clusters

Figure 6.6. MFA map of rotated factors for phytochemical and sensory attributes (a) distribution of variables and (b) sample distribution in map. Codes and abbreviations on plot refer to compound codes in Tables 6.1, 6.3 and 6.4.

6.3.7 Correlation map showing drivers of liking

For a better understanding of the key factors driving liking and consumption intent, a map showing factors with direct and indirect positive or negative correlations to liking and consumption intent was produced and presented in Figure 6.7 with correlations values presented in Appendix XI (Table S6e). Only correlations above r>0.6 were included in the map. Clusters were not included in the map; however as discussed above cluster 1 was negatively correlated to liking and consumption intent, while clusters 2 and 3 correlated positively with sweet taste, liking and consumption intent. Two rotated liking groups (from the RFA) were found and have been drawn separately in Figure 6.7. The first group incorporated taste, mouthfeel and overall liking as well as consumption intent (Figure 6.7a) and the second group incorporated taste liking and consumption intent and appearance liking (where appearance liking was negatively correlated with the other two factors and represented as 'vs' on Figure 6.7b). Black arrows in Figure 6.7 indicate directions of positive correlation, whereas red lines denote negative correlation.

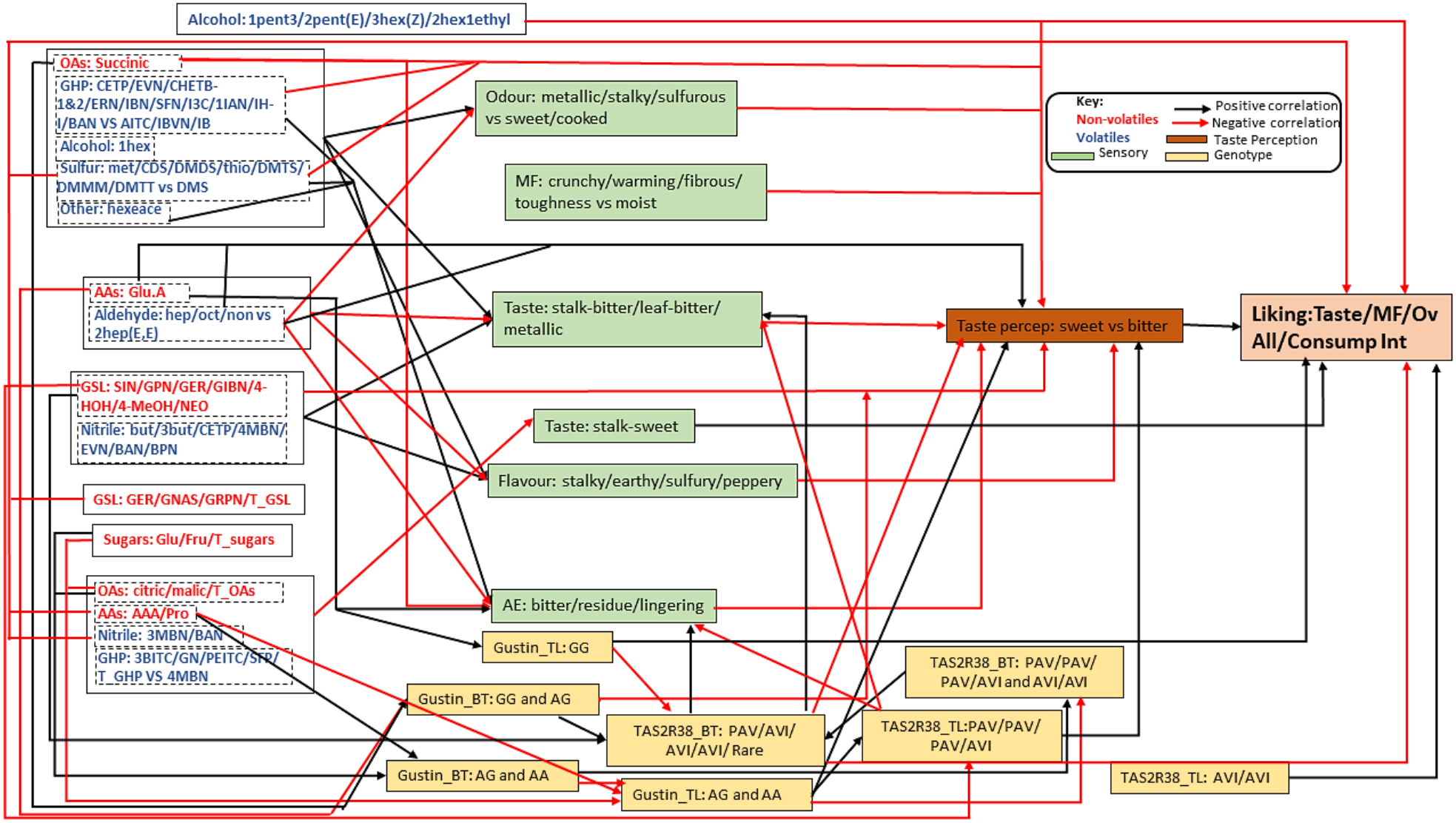
Succinic acid, GSLs, nitriles and EPTs, sulfides, where drivers of metallic, bitter, stalky and sulfurous sensory attributes which correlated negatively with sweet taste perception. Organic acids, ITCs, proline and α-aminobutyric acid (AAA) were negatively correlated with sweet stalk taste (Figure 6.7a). Bitter tasting GSL (SIN, GPN, GBSN and NEO) and alcohols (1pent3/2pent(E)/3hex(Z)/2hex1ethyl) all had a direct negative correlation to sweet taste perception. Alcohols, benzaldehyde, volatiles from SIN hydrolysis (ITCP, ATC and AITC) and furans correlated positively with sweet and savoury leaf taste and burnt, nutty and oily mouthcoating aftereffects but negatively with bitter and earthy aftereffects (Figure 6.7b). PROG, GHPs, organic acids, sugars, proline and AAA correlated positively to bitter and earthy aftereffects and positively to burnt and oily mouthcoating (Figure 6.7b). We propose that the unexpected positive correlation of sugars to bitter/earthy aftereffects might be due to masking effects of other factors such as the oily and bitter characteristics.

Bitter taste perception of the PAV/AVI, AVI/AVI and rare *TAS2R38* genotypes correlated positively with the sensory profiling panel scores of bitter taste and lingering aftereffect attributes Bitter taste perception was positively correlated with 5 genotype groups (gustin GG and AG; *TAS2R38* PAV/PAV, PAV/AVI and AVI/AVI consumers) and this was negatively correlated with sweet taste perception for the *TAS2R38* PAV/AVI, AVI/AVI and rare genotypes. Bitter taste perception within gustin genotypes correlated negatively with taste liking within these groups. GSLs were negatively correlated with taste liking for all of the

TAS2R38 genotype groups; and taste liking of the PAV/PAV and PAV/AVI groups correlating positively with sweet taste perception. *TAS2R38* PAV/AVI and AVI/AVI genotype drove negative liking for bitter and metallic tastes and aftereffects.

Sulfides, GSLs, GHPs and AAs (proline and AAA) were all directly negatively correlated to liking and consumption intent. Burnt marks, shiny, oily and cooked appearance positively drove liking and consumption intent but were negatively correlated to appearance liking (Figure 6.7b), indicating that although most consumers preferred the flavour and taste of cooked samples, they did not like the appearance of these samples. Bitter taste perception due to *TAS2R38* and gustin genotypes all correlated negatively with liking and consumption intent (Figure 6.7b).

(a)


Alcohol: 1pent3/2pent(E)/3hex(Z)/2hex1ethyl
OAs: Succinic
GHP: CETP/EVN/CHETB-1&2/ERN/IBN/SFN/I3C/1IAN/IH-I/BAN VS AITC/IBVN/IB
Alcohol: 1hex
Sulfur: met/CDS/DMDS/thio/DMTS/DMMM/DMTT vs DMS
Other: hexeace
Odour: metallic/stalky/sulfurous vs sweet/cooked
MF: crunchy/warming/fibrous/toughness vs moist
Key:
Non-volatiles
Volatiles
Sensory
Positive correlation
Negative correlation
Taste Perception
Genotype
AAs: Glu.A
Aldehyde: hep/oct/non vs 2hep(E,E)
Taste: stalk-bitter/leaf-bitter/metallic
Taste percep: sweet vs bitter
Liking:Taste/MF/Ov All/Consump Int
GSL: SIN/GPN/GER/GIBN/4-HOH/4-MeOH/NEO
Nitrile: but/3but/CETP/4MBN/EVN/BAN/BPN
Taste: stalk-sweet
Flavour: stalky/earthy/sulfury/peppery
GSL: GER/GNAS/GRPN/T_GSL
Sugars: Glu/Fru/T_sugars
OAs: citric/malic/T_OAs
AAs: AAA/Pro
Nitrile: 3MBN/BAN
GHP: 3BITC/GN/PEITC/SFP/T_GHP VS 4MBN
AE: bitter/residue/lingering
Gustin_TL: GG
TAS2R38_BT: PAV/PAV/PAV/AVI and AVI/AVI
Gustin_BT: GG and AG
TAS2R38_BT: PAV/AVI/AVI/AVI/ Rare
TAS2R38_TL:PAV/PAV/PAV/AVI
Gustin_BT: AG and AA
Gustin_TL: AG and AA
TAS2R38_TL: AVI/AVI

(b)

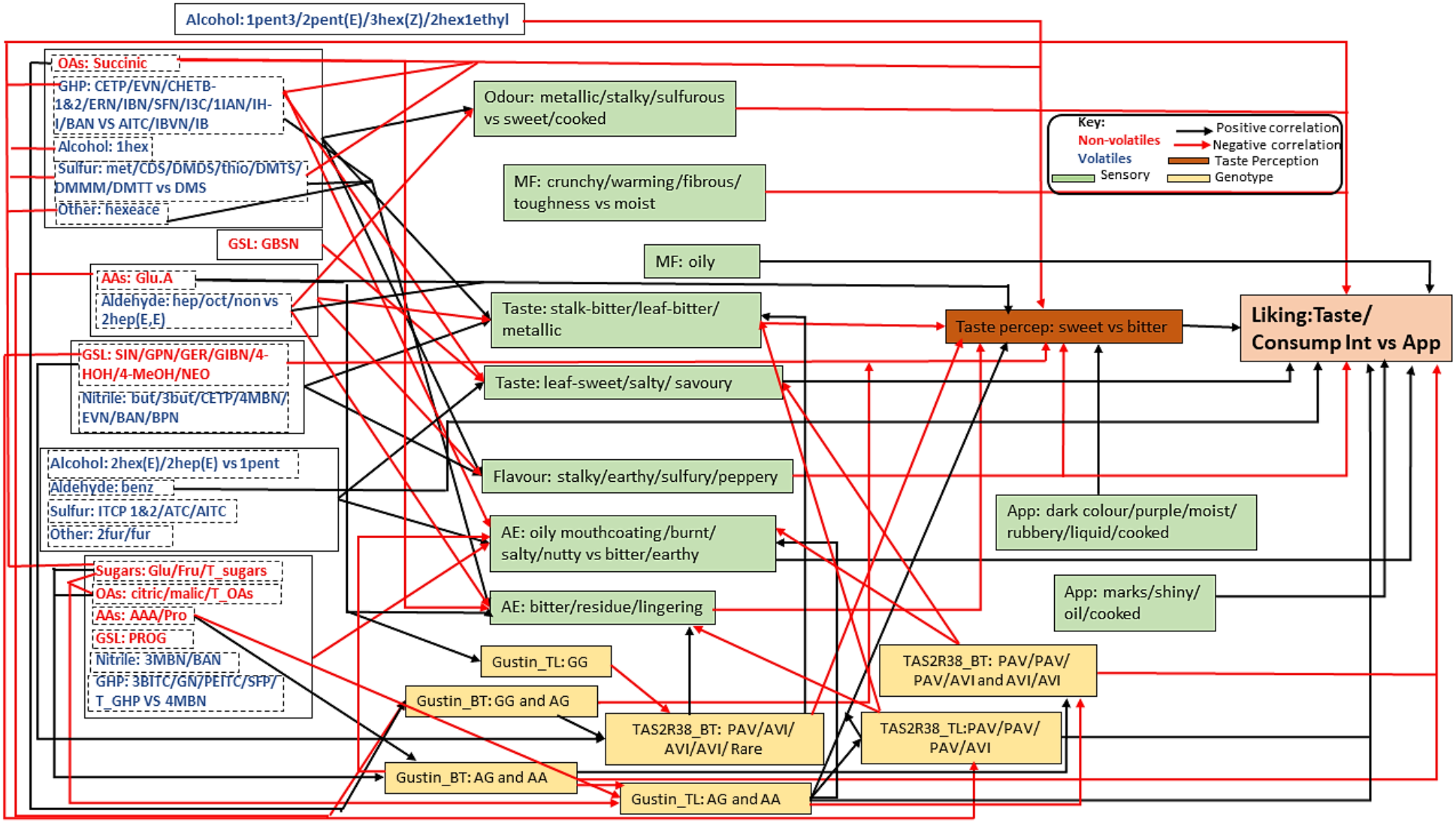


Figure 6.7. (a) Correlation map for drivers of taste, mouthfeel, overall liking and consumption intent (b) Correlation map for drivers of taste liking and consumption intent vs appearance liking.

'vs' refers to negative correlations between factors in a group. Codes and abbreviations on plot refer to compound codes in Tables 6.1, 6.3 and 6.4. All correlations > r = 0.6

6.4 Conclusion

This study has tried to understand the complex relationship between phytochemicals and sensory characteristics in red cabbage while linking them to effects of bitter taste sensitivity of consumers. The findings of the study show that stir-frying led to significant loss in GSL concentrations. Mild steaming, on the other hand, retained myrosinase activity while denaturing ESP resulting in significantly higher concentrations of ITCs formed. SFP was the dominant GHP produced in cooked cabbages with significantly higher amounts produced in the RM variety. This is particularly interesting as SFP is interest for its health beneficial properties while not affecting the taste and flavour of *Brassicas* (Sultana et al., 2003). The study found GSL concentration a more important factor in final product concentration than level of residual myrosinase as GHPs were directly linked to GSL content even where samples did not have the most stable myrosinase enzyme. The study suggests that mild cooking might be a good way of enhancing formation of beneficial ITCs while preventing nitrile formation.

The results showed that cooking, while enhancing amounts of desirable AITC formed in cabbage headspace, led to significant reduction in undesirable sulfide and stalky flavours and odours with consequent effects on sensory characteristics. The hypothesis that consumers consider bitter taste, sulfurous and stalky flavours undesirable was not fully supported in this study as some consumers (30 %) preferred the more bitter, sulfurous and stalky flavours of raw red cabbage to cooked samples with less bitter and sulfurous characteristics. It is therefore a need for breeders and nutritionists to consider ways to satisfy both groups; one way can be production of several varieties varying in different sensory characteristics for consumers to select their preferred choice.

The results do not support the hypothesis that consumers with PAV/PAV genotype perceive cabbages to be more bitter than consumers of other bitter taste genotypes as no difference was found in their bitter taste perception. However, results obtained support the hypothesis of the study that red cabbage liking was related to consumer preference and not associated with consumer bitter taste genotype. The study also confirmed the hypothesis that cooking reduces production of undesirable sulfur compounds and improves consumer liking and consumption intent of red cabbage as liking and consumption intent were related to cooked samples that were low in sulfurous compounds and flavours.

Chapter 7: General discussion, limitations and future work

7.1 General discussion

The purpose of the final chapter of this thesis is to highlight the key findings of the research and provide recommendations for future work. The first part of the research focused on understanding the glucosinolate-myrosinase system of the cabbage varieties studied. Results of the myrosinase activity and stability of the varieties were then used to determine the selection of varieties to plant for the flavour and sensory studies which were the second part of the research.

The study addressed the following hypotheses:

Primary hypothesis: By variety selection and optimised processing conditions, it was hypothesised that ESP activity could be minimized and myrosinase activity maximised to:

- Increase health-beneficial GSL hydrolysis products at point of consumption
- Minimise bitter taste and sulfurous aromas
- Improve consumer acceptability

Secondary hypothesis: whilst human bitter taste receptor genotype will influence bitter taste perception, it was hypothesised that consumer liking of cabbage would be increased through variety selection and optimised processing condition, irrespective of human genotype.

7.1.1 Study findings

7.1.1.1 Through variety selection and optimised processing conditions, nitrile and epithionitrile formation was minimized and beneficial isothiocyanate formation maximized.

Myrosinase enzyme activity and stability is known to vary between *Brassica* types and varieties (Yen & Wei, 1993; Rungapamestry et al., 2006). In the present investigation there were variations in the myrosinase activity and stability, type and concentrations of glucosinolates (GSL) and glucosinolate hydrolysis products (GHPs) formed in the cabbages studied. The results of this work support previous findings that cabbage variety, growing and environmental conditions all affect the myrosinase activity and GSL concentrations (Charron & Sams, 2004; Charron et al., 2005a; Penas et al., 2011; Wei et al., 2011; Hanschen & Schreiner, 2017). Generally, the high growth temperatures and potentially stressful growing conditions in the glasshouse, compared to field conditions, resulted in lower myrosinase activity and GSL content. This was probably due to glasshouse growth temperatures being above the optimal growth temperature requirements of the plants (Chapter 2 and 3). GSL

concentration in salad rocket was found to be higher when grown at colder temperatures in a controlled environment than in hotter temperatures on the field (Bell et al., 2015; Bell et al., 2017c). It is possible that higher temperatures produce higher GSL turnover to GHP during growth. It is possible that kinetics of the GSL hydrolysis reaction increase in warmer temperatures and the GSL substrates are rapidly hydrolysed and volatilised so they are lost from the plant. *Brassica* vegetables are generally thought to be cool weather crops with average growing temperatures between 4 – 30 °C (Wurr et al., 1996).

An additional factor that may be responsible for lower GSL concentrations in glasshouse samples maybe nutrient deficiency. Though, additional fertilizer was supplied throughout the growing period in both growth conditions, because glasshouse plants were grown in pots with drainage holes, sulfur leaching may have occurred when plants were watered. GSLs, especially aliphatic GSLs, are synthesized from methionine, a sulfur-containing amino acid which requires sulfur for its synthesis (Zhao et al., 1994). However, because the sulfur contents in the soil were not determined, it is not clear whether sulfur leaching actually occurred. *Brassica* vegetables are generally thought to be cool weather crops with average growing temperatures between 4 – 30 °C (Wurr et al., 1996). In addition, glasshouse grown cabbages achieved a lower above ground biomass than the field grown ones, indicating some form of stress – either due to nutrient limitation or root growth restriction because of the pot they were sown in.

Larger variations were observed in the myrosinase activity and stability, GSL and GHP profile between cabbage types than between varieties within a cabbage type. Savoy cabbage myrosinase was the most active, but least stable, while red cabbage and black kale myrosinase was the most stable under the cooking conditions studied. Most varieties within a cabbage type had similar GSL profiles with a few exceptions. For example, GER was identified in red cabbage varieties RC2 and RC3 but not in RC1 (Chapter 3). The highest concentrations of GSL and GHP was observed in wild cabbage varieties which was mostly due to the high amounts (up to 60 % of total GSL and GHP) of PROG and its hydrolysis products. Conversely, black kale varieties had the least complex profile of GSLs and GHPs while the lowest concentrations of GHP was observed in tronchuda cabbage varieties.

In chapter 4, it was noted that more beneficial ITCs were formed in cooked cabbage samples compared to raw samples. However, the severity of the cooking methods affected the amounts of ITCs formed. Microwaving, with higher core temperatures (88 – 95 °C) compared to the other processes, did not accumulate as much ITCs as steamed or stir-fried

samples which was mostly due to myrosinase inactivation during the microwave process, though GSLs were relatively stable under those conditions. Steaming accumulated significantly higher ITCs than stir-frying despite myrosinase activity being the most stable after stir-frying (Chapter 2). In addition to the lower GSL contents in stir-fried cabbage (up to 70 % loss in GSL content; see chapter 4) which could have reduced amounts of GHPs formed, the lower core temperatures during stir-frying (65 – 70 °C) may have resulted in GSL hydrolysis to nitriles and EPTs due to ESP activity rather than complete conversion of GSL to ITCs by myrosinase. Steamed red and white cabbage varieties accumulated the highest amount of beneficial ITCs especially SFP.

Some previous studies suggested that because myrosinase is thermosensitive, the temperatures under which domestic cooking occurs will result in total inactivation of myrosinase, thus preventing conversion of GSLs to beneficial ITCs, particularly in cabbage where myrosinase is inactivated at temperatures above 60 °C (Yen & Wei, 1993; Verkerk & Dekker, 2004; Ghawi et al., 2012). The result of the present study is contrary to these findings as low residual myrosinase activity in the absence of ESP, as obtained mostly in steamed samples, was enough to hydrolyse GSLs into beneficial ITCs. The findings of this study suggest that mild cooking processes can be sufficient to denature ESP but retain some myrosinase activity, and hence enhance ITC formation; which is in agreement with Matusheski et al. (2004) where heating broccoli sprouts and florets at 60 °C increased SFP formation and decreased amount of SFN formed.

In the literature, most studies use GSL abundance and/or myrosinase activity to predict concentrations of GHPs that will be formed, without analytically determining GHPs in the samples. However, the results of this study suggest this might not be an accurate way to predict types and concentrations of GHPs formed in samples. In this study, some GHPs were identified in the cabbage samples where intact GSLs were not detected. Also, the high GSL concentrations did not always translate into high GHPs formed as was observed in black kale variety BK2 and red cabbage variety RC3 (Chapter 4). Both samples had similar myrosinase activity after steaming, but the BK2 variety with significantly higher glucoraphanin content than RC3 had lower sulforaphane formed. This implies that different myrosinase isoenzymes present in different varieties may vary in their ability to hydrolyse specific GSLs into ITCs (James & Rossiter, 1991). Therefore, GSL contents and/or myrosinase activity may not always predict the amounts of ITCs that will be formed.

This result of this study demonstrates that cooking methods can be optimized to prevent complete myrosinase inactivation, ensuring that consumption of cooked cabbages will result in production of higher amounts of beneficial ITCs. However, optimized cooking conditions may differ between cabbage varieties. For example, stir-frying may be the optimal way of cooking black kale as more beneficial ITCs were formed compared to when samples were steamed, whereas red cabbages accumulated the highest amounts of ITCs after steaming.

7.1.1.2 Influence of growing cabbage varieties at two different sites in two different years

Red cabbage varieties (RL and RD) and black kale varieties (CNDTP and CPNT) were grown in two different sites in two years. Plants were grown in the plant growth facilities, Whiteknights campus of the University of Reading in the first-year and at Tozer seeds Ltd (Cobham, Surrey, UK) in the second year. The study found that plants grown in Reading (first year plants; Chapter 2- 4) had higher myrosinase activity and GSL content than plants grown in Surrey (second year plants; Chapter 5 and 6) with first year plants accumulating twice the amount of GSL detected in second year plants in some varieties. This might be due to less average extreme temperatures (10.1 – 18.7 °C) and longer sunlight hours (160 hours) in the first-year cabbage compared to the second-year cabbage (temperature, 3.6 – 24.6 °C; sunshine hours, 120 hours). Higher GSL concentrations have been reported in *Brassica* vegetables grown at lower temperatures and longer daylight exposure (Rosa & Rodrigues, 1998; Choi et al., 2014). Differences in 'degree days' experienced may have also been a factor in the differences observed. Degree days is a calculation of how many hours a crop has been exposed to temperatures suitable for their growth. First year cabbages may have had more degree days than second year cabbages since they were grown for a longer period on the field (seven months) than second-year cabbages (five months) possibly exposing them to longer optimal growing temperatures than the second-year cabbages (Appendix IV; Tables S2a and S2b).

Another possible reason could have been exposure to insect and pest attack. The plants grown in the second year in Surrey, were covered netting to prevent insect and pest attack while first year plants were not covered with netting and were therefore exposed to insect and pest attack. This could have triggered production of high amounts of GSLs as a defence mechanism by the plants in response to pest attack (Bjorkman et al., 2011). In addition to the factors mentioned, differences in soil characteristics in the two locations may also have been responsible for the differences observed. It is worth mentioning that the result

obtained is a snapshot of both plants at the point of harvest. The level of fluctuations in myrosinase activity and GSLs between or within days during the growing cycle is unknown and can vary a lot even within a single variety.

Despite lower myrosinase activity and GSL content in second year plants, GHPs, were higher in the second-year plants grown in Surrey compared to those grown in Reading. This may have been the result of higher myrosinase stability observed in the second-year plants than in first year plants after processing. The reason for the difference in myrosinase stability is however unclear but may be related to the growing conditions of the plants. The results suggest that growing the same cabbage varieties at two different locations in different years under different environmental conditions may have an impact on myrosinase activity and stability and proportions of GSL and GHPs produced.

7.1.1.3 Influence of cabbage variety and domestic cooking on bitter taste and sulfurous aromas with consequent implications on consumer acceptability

Bitter taste and sulfurous aromas are considered reasons for rejection of *Brassica* vegetables (Kubec et al., 1998; Baik et al., 2003). Previous studies identified dimethyl disulfide (DMDS) and dimethyl trisulfide (DMTS) as the key compounds responsible for undesirable sulfurous aromas in *Brassica* vegetables with concentrations increasing during thermal processing (Chin & Lindsay, 1993). There is however, little evidence in the literature of what happens under domestic cooking conditions. In this study, cooking significantly reduced concentrations of undesirable sulfurous compounds in the samples, which was reflected in the reduced sulfury aromas perceived by consumers in cooked samples. On the contrary, higher concentrations of AITC, which has been described as a desirable flavour compound in cabbage, was detected in cooked red cabbage samples.

Cooking also significantly reduced bitter taste perception in both red cabbage and black kale samples with an increase in sweet taste perception. The reduction in bitter taste perception was mostly due to the ratio of GSL to sweet tasting compounds like sugars and sweet-amino acids in the cooked samples. While cooking significantly reduced GSL contents, concentrations of sugars and amino acids were not as affected by cooking, allowing the relatively higher sweet tasting compounds mask the bitter taste of GSLs in cooked samples. This effect was also observed between black kale and red cabbage samples. Consumers gave higher scores for bitter taste perception in kale samples compared to red cabbage samples, with much lower scores for sweet taste perception in raw black kale than raw red cabbage samples even though red cabbages contained almost twice the concentration of GSLs than

black kale. The ratio of GSL-sugar/amino acid in red cabbage was about 1:8 while that of kale was 1:4 suggesting that the higher amounts of sweet tasting compounds in relation to GSLs in red cabbage would mask the bitter taste of GSL in red cabbage more than would occur in black kale. Sucrose has been previously reported to mask the bitter taste of sinigrin and goitrin in some *B. oleracea* vegetables (van Doorn, 1999; Beck et al., 2014). However, it is worth mentioning that only 88 % of consumers assessed the both red cabbage and black kale samples and this could have biased the results to some extent.

Cooking improved consumer acceptability of cabbage with consumers preferring cooked samples with less sulfurous aromas and bitter taste than raw samples. This was especially true for black kale samples as taste liking, overall liking and consumption intent results showed that consumers preferred cooked samples and would probably consume them in preference to raw samples (Chapter 5; Table 5.7). In contrast, although consumers scored cooked red cabbage samples higher for taste liking, overall liking and consumption intent, the scores were not too different from raw samples, implying that consumers generally liked both raw and cooked red cabbages (Chapter 6; Table 6.7). Cluster analysis of consumer overall liking for red cabbage also showed that while some consumers preferred the cooked samples, others preferred raw cabbage which was found to be more bitter with sulfurous aromas. The difference in consumer liking results for red cabbage and black kale might be related to lower bitter taste perceived in raw red cabbage due to higher levels of sweet tasting compounds. Though there were slight differences in amounts of taste and flavour compounds between cabbage varieties within a cabbage type, differences were mainly driven by cooking and not variety. The results show that breeding of *B. oleracea* varieties with high sweet-tasting compounds may be a way of improving cabbage consumption and yet maintaining the health beneficial GSLs.

7.1.1.4 Influence of bitter taste genotype on bitter taste perception and consumer liking

This study examined the effects of *TAS2R38* and gustin *rs2274333* genotype on bitter taste perception and consumer liking of cabbage. The results obtained were unexpected as individuals with the *TAS2R38* PAV/PAV genotype (the more bitter sensitive group) did not rate the cabbages as more bitter than the AVI/AVI genotype (the less bitter sensitive group) individuals. While there was no significant difference in bitter taste perception due to all *TAS2R38* genotypes (including the rare genotype) in red cabbage, PAV/PAV individuals rated black kale samples significantly more bitter than PAV/AVI and rare genotypes. The results contradicted previous reports where PAV/PAV individuals perceived *Brassica* vegetables

more bitter than other genotypes (Sandell et al., 2014; Shen et al., 2016). Individuals with the rare *TAS2R38* genotype were the only consumers who gave significantly higher taste liking scores for cabbages. The small percentage of rare genotypes (10 %) identified in this study may not be enough to make conclusions on their importance but the results suggest that rare genotypes maybe more different than previously assumed. More studies are therefore required to verify these findings. Gustin *rs2274333* genotype had no effect on bitter taste perception and liking in red cabbage, while in black kale gustin influenced bitter taste perception and liking but the differences were unexpected and not clearly defined. The result of this study shows that the main factor driving perception of bitterness was cooking as all genotypes perceived raw samples significantly more bitter than cooked samples. This confirms our hypothesis that cooking will reduce bitter taste perception irrespective of genotype. However, ethnicity and gender must also be considered as they have been reported to be important factors when discussing bitter taste perception in relation to genotypes.

7.2 Limitations of the study

Like any other study, some limitations were encountered in this study:

7.2.1 Variation in cabbage samples

Cabbage seeds used for these study, except for the two commercial varieties, were obtained from a gene bank. This means they have not been bred for uniformity in abundance of phytochemical compounds. In addition, breeding programmes are mostly focused on developing disease and environmentally resistant crops with less emphasis on the phytochemical compounds. This implies that there may be large variations in phytochemical compounds between cabbage heads/plants of the same variety, as has been observed in *Marathon* broccoli heads, and this may have influenced the results obtained (Winkler et al., 2007). To reduce the effects of possible variation between plant heads, four to five heads were mixed together to obtain a representative sample. However, considering the amounts of heads used during the study, some variations may still have existed in the samples.

7.2.2 Sample analysis

The method used to determine concentration of glucosinolate hydrolysis products (GHPs) was long and required several steps to ensure that all GHPs present in the sample could be identified. However, most GHPs are very volatile and unstable compounds and, therefore, can easily be lost during analysis. Though care was taken during the analysis to

prevent losses, the rigorous analytical method may have resulted in loss of some of the more volatile compounds. Therefore, results obtained for GHPs analysis may not be the exact representation of what is obtainable in the samples.

7.2.3 Familiarity with red cabbage and black kale

Though 70 % of consumers who took part in the study said they consumed one form of cabbage at least once a month, only 30 % and 36 % of consumers consumed red cabbage and black kale respectively. This means that most of the consumers in the study were not familiar with the taste of the cabbages and their scores may have been influenced by their feelings or their general idea of how cabbage should taste and not necessarily how red cabbage or black kale actually tasted. This could have biased the average means scores of the consumer data. In addition, only 25 % of consumers consumed cabbage raw while most consumed cabbage after one form of cooking. Their liking for cooked cabbage as observed in the study may have been biased towards greater familiarity to cooked cabbage than raw cabbage. There is a possibility that If the consumer test was conducted in an environment or culture where consumers ate more raw than cooked cabbage, they result obtained may be different.

7.2.4 Unbalanced number of participants in demographic subgroups

Ethnicity and gender have been shown to affect bitterness perception based on genotype. However, in this study, due to insufficient participants in different ethnic groups, effect of ethnicity on bitter taste genotypes could not be studied. In addition, more females (74 %) than males took part in the study and most of the participants (65 %) were aged between 18-30 years. All of these factors may have biased the results obtained in one way or another as both gender and age have been shown to affect bitter taste perception with females being more sensitive to bitter taste than men (Beardsworth et al., 2002). The higher ratio of females to males may not have had significant impact on the results of this study since significant differences due to PAV/PAV genotype or the more taste sensitive gustin genotype was not observed. However, there may have been a gender (or age) effect on cabbage familiarity which could have biased the result.

7.3 Recommendations for future work

Based on the results of this study the following recommendations are made:

- From the study, individual GSL and GHP profiles of black kale varieties were very different and lower in number compared to other cabbage types like red cabbage. The

result suggests that breeding programmes focused on increasing types of individual GSLs present in cabbage may be more important for some cabbage types (in this case black kale varieties) than for others. This will help to enhance their GSL and GHP profiles with possible consequences on health benefits derived from their consumption and in the process reducing likely disadvantages that may result from consuming certain types of cabbage.

- Several volatile ITCs were found in the headspace of red cabbage and it is generally accepted that volatile ITCs contribute to cabbage flavour in various ways. However, most of their odour detection thresholds are unknown and their contributions to flavour are therefore not fully understood and will need to be investigated further. Volatile ITCs in the cabbage headspace can be identified using headspace-solid phase microextraction (HS-SPME) analysis but to determine the aroma of individual ITCs and which compounds are likely contributors to cabbage flavour, gas chromatography-olfactory (GC-O) analysis will need to be conducted. In GC-O analysis, trained assessors describe and estimate the intensity of the aroma compound (Parker, 2015). To identify and estimate the most dominant and odour-active compounds in cabbage, aroma extract dilution analysis (AEDA) can be carried out. This involves repeat analysis of several serial dilutions of the cabbage aroma extract by GC-O until only the most potent aroma compounds are detected in the extract; these compounds are then regarded as the most important contributors to cabbage aroma (Parker, 2015). Recombinants, replicating cabbage flavour, can also be prepared and analysed by a sensory panel alongside an original cabbage extract to determine if all compounds contributing to cabbage flavour have been identified. This technique can also be potentially used to determine what could be the most favourable aroma profile of cabbage with possibility of breeding cabbage varieties with high concentrations of these compounds as a way of improving consumer acceptability of cabbage.
- The result of this study has shown that in the presence of high GSL-sugars/amino acids ratio, bitter taste is masked in raw cabbages and consumer liking and acceptability is improved. Consumers have also been shown to prefer broccoli and cauliflower with high sucrose content (Schonhof et al., 2004). Therefore, instead of breeding programmes directed at reducing GSL concentrations to reduce bitter taste of *Brassicas*, breeding programmes can focus on breeding sweet-tasting cabbages with high sweet-tasting compounds such as sugars and amino-acids, yet maintaining high

GSLs, in order to develop less bitter varieties with high GSL-sugars/amino acids ratio which can be consumed raw in salads and still maintain high levels of the health promoting GSLs. There are few reports on breeding programmes aimed at increasing sugar content and identifying molecular markers linked to sugar content in fruits and vegetables (Nookaraju et al., 2010). Sweet and taste-modifying proteins have also been used to increase sweet taste in lettuce (Sun et al., 2006). Monellin, a sweet protein, 100,000 times sweeter than sucrose has been used to enhance the sweetness and flavour of tomato and lettuce (Peñarrubia et al., 1992). To achieve this, the single-chain monellin gene was placed under the control of fruit ripening promoters resulting in the expression of these genes, which led to the accumulation of monellin protein in the fruit and leaf of the tomato and lettuce.

- Since mild cooking processes significantly reduces undesirable bitter taste and sulfurous aromas while increasing the amounts of beneficial ITCs formed, public health campaigns should focus on encouraging consumers to steam or stir-fry their cabbages mildly as a way of getting many people to eat more of these vegetables in their diets. UK National Diet and Nutrition Survey (2012 to 2014), showed that only 8 % of children aged 11-18 years meet up with the 5-A-Day requirement for fruit and vegetable consumption compared to 27 % and 35 % in adults aged 19- 64 years and older adults (64 and over) respectively (NDNS, 2016). The report also found that daily intake of fruits and vegetables for children aged 11-18 years was 2.8 portions/day of fruits and 4.0 and 4.2 portions/day for adults and older adults respectively. A study on intake and liking of turnip in UK children aged 3 to 5 years (n = 132), showed that mean vegetable intake was 3.8 portions/day with only 0.9 portion/day of *Brassica* vegetables recorded (Mohd Nor et al., 2018). Repeated taste exposure studies, which involve repeated tastings of a particular food over a period of time to improve familiarity, have been reported to be an effective way of increasing liking and acceptance for *Brassica* and non-*Brassica* vegetables in children of various ages (Wardle et al., 2003; Anzman-Frasca et al., 2012; Mohd Nor et al., 2018). Since children are more sensitive to bitter taste and generally consume fewer vegetables compared to adults, starting children off with lightly steamed or stir-fried cabbages might be a better option to get children familiar with consuming cabbages. However, studies need to be conducted to determine if cooking cabbage will improve cabbage liking and acceptability in children.

- Cooking led to formation of high amounts of beneficial ITCs in cabbage samples. However, this was under laboratory conditions and not in the human system. A study exploring the bioavailability of beneficial ITCs in the body after consuming the cooked cabbages with active residual myrosinase will be interesting. It is expected that since myrosinase is still active in the samples and ESP denatured, more beneficial ITCs will be available on consumption and levels of excretion could be compared. A study by Conaway et al. (2000) showed that bioavailability of SFP was three times more in raw broccoli with active myrosinase compared to broccoli steam cooked for 15 min where myrosinase has been inactivated; however, mild cooking was not considered in the study. Based the results of the present study, where mild cooking led to enhanced formation of ITCs and denaturation of ESP, it is hypothesized, that bioavailability of ITCs in mildly cooked *Brassica* will be higher than that observed in raw *Brassica*. A different study found that soaking broccoli in water for 90 min (at 37 °C) to allow for GSL hydrolysis before stir-frying increased ITC concentrations about three times more compared to direct stir-frying but concentrations in soaked broccoli did not differ significantly from raw broccoli (Wu et al., 2018). Because the hydrolysis process prior to stir-frying was conducted at 37 °C, high amounts of nitriles were formed in the soaked/stir-fried sample than in the direct stir-fry sample though amounts were significantly lower than was present in the raw sample. However, a draw back to the study was that to achieve this, broccoli was chopped into tiny 2mm pieces and soaked for 90 min which may not be acceptable to consumers or as convenient compared to mild steaming/stir-frying. In a recent study by Okunade et al. (in press), addition of brown mustard as active myrosinase to cooked broccoli increased SFN bioavailability over four times more than in cooked broccoli without added myrosinase. Though, the study shows increased ITC bioavailability with mustard addition, consumers may prefer mildly steamed or stir-fried *Brassicas* to *Brassicas* with added mustard because of the pungent and peppery aroma of mustard.

7.4 Conclusion

The results obtained from this study have advanced our understanding of the relationship between phytochemical composition, sensory profile and resulting effects on consumer acceptability of cabbage. The study supports previous findings that mild cooking increases formation of beneficial compounds as residual myrosinase is still active while ESP

activity is decreased. Therefore, consumption of mildly cooked cabbages will improve potential health benefits derived from cabbage consumption. The study also found that cooking improved liking and acceptability and did not depend on variety or human bitter taste genotype.

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Appendix I: Published paper

Appendix II: Published conference paper

This paper was reviewed and published in the book of Weurman 2014 proceedings

Appendix III: Titles of oral and poster presentations in conferences

Oloyede, O.O., Wagstaff, C. and Methven, L. (2017). The effect of plant variety and domestic cooking on flavour volatiles and their impact on sensory profile and consumer liking of cabbage. In Fifteenth Weurman Flavour Research Symposium, Graz, Austria (Poster presentation)

Oloyede, O.O., Senna, E., Wagstaff, C. and Methven, L. (2017). The influence of domestic cooking methods, genotypic variations in bitter taste sensitivity and glucosinolates on consumer perception and liking of cabbage. In Twelfth Pangborn Sensory Science, Rhodes Island, USA (Poster presentation)

Oloyede, O.O., Senna, E., Wagstaff, C. and Methven, L. (2016). Impact of Domestic Cooking Methods and Genotypic Variations in Bitter Taste Sensitivity on Consumer Perception and Liking of Cabbage. In Seventh European Conference on Sensory and Consumer Research (Eurosense), Dijon, France (Poster presentation)

Oloyede, O.O., Wagstaff, C. and Methven, L. (2016). The Impact of Plant Variety and Domestic Cooking Methods on the Flavour Profile of Red Cabbage. In IFST Sensory Science Group Conference, London, UK (Poster presentation)

Oloyede, O.O., Wagstaff, C. and Methven, L. (2016). The Impact of Plant Variety and Domestic Cooking Methods on the Flavour Profile of Red Cabbage. In IFST Sensory Science Group Conference, London, UK (Poster presentation)

Oloyede, O.O., Wagstaff, C. and Methven, L. (2016). The Impact of Plant Variety and Domestic Cooking Methods on the Flavour Profile of Cabbage. In Fourth Nursten Flavour Research Symposium, Reading, UK (Oral presentation)

Oloyede, O.O., Wagstaff, C. and Methven, L. (2015). The Diversity and Processing Stability of Myrosinase in Cabbage Varieties and Subsequent Implications for Flavour and Bioactivity. In Third Nursten Flavour Research Symposium, Northumbria, UK (Oral presentation)

Oloyede, O.O., Wagstaff, C. and Methven, L. (2014). Myrosinase Activity of Different Cabbage Varieties Grown Under Controlled Environment. In Third International Glucosinolate Conference, Wageningen, Netherlands (Poster presentation)

Oloyede, O.O., Wagstaff, C. and Methven, L. (2014). Effect of Plant Variety on Cabbage Myrosinase Activity, the Enzyme Key to Cabbage Flavour. In Fourteenth Weurman Flavour Research Symposium, Cambridge, UK (Poster presentation)

Oloyede, O.O., Wagstaff, C. and Methven, L. (2014). Effect of Diversity of Plant Varieties and Controlled Processing on the Flavour Chemistry of Cabbage and Their Impact on Sensory Profile and Consumer Acceptance. In Second Nursten Flavour Research Symposium, Nottingham, UK (Flash poster presentation)

Appendix IV: Climatic data during cabbage growth

Table S2a: Climatic data of field and glasshouse cabbages (2014 growing season)

		Mean temperature (°C)			
	Month	Minimum	Maximum	Total rainfall (mm)	Total sunshine (hours)
Field	May	8.2	16.8	79.8	183.8
	June	10.9	20.7	55.4	224.3
	July	13.4	24.31	35.3	257.4
	August	11.3	20.29	83.6	193.1
	September	10.9	20.63	8.4	123.7
	October	10.1	16.5	94.6	99.3
	November	5.9	11.9	95.5	38.2
Glasshouse	May	17.5	33.7		
	June	18.2	38.7		
	July	19.0	43.1		
	August	18.0	34.5		
	September	18.3	40.1		
	October	17.4	31.6		
	November	13.9	30.1		

Source: The University of Reading Atmospheric Observatory

Table S2b: Climatic data of field grown cabbages (2015 growing season)

	Mean temperature (°C)			
Month	Minimum	Maximum	Total rainfall (mm)	Total sunshine (hours)
July	4.3	34.8	43.2	198.5
August	5.5	29.5	76.6	147.5
September	2.1	22.1	67.6	154.6
October	0.9	19.3	37.8	93.4
November	5.0	18.5	9.6	8.6

Source: RHS Garden Wesley, Surrey, UK

Appendix V: Cross-section of cabbage grown in the first year (2014)

Controlled environment

Glasshouse cabbage

Appendix VI: Example of cabbage heads harvested at maturity

(a)

(b)

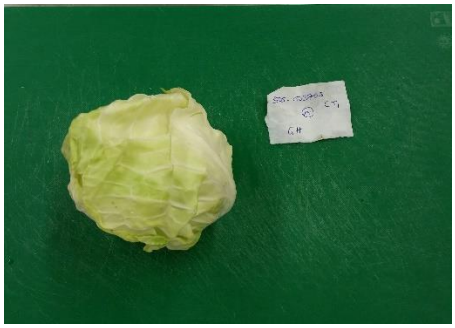
(c)

(d)

(e)

(f)

(g)



(h)

Cross-section of planted cabbage types (a) Field savoy (b) Field red cabbage (c) Field white cabbage (d) Field black kale (e) Field wild cabbage (f) Field tronchuda (g) Glasshouse white cabbage (h) Glasshouse savoy

Appendix VII: Cross section of cabbages grown in the second year (2015)

Appendix VIII: Glucosinolate (mg/g DW) and glucosinolate hydrolysis concentration (µg/g DW sulforaphane equivalent) of cabbage grown under different conditions

Table S3a: Glucosinolate concentration (mg/g DW) in cabbage grown under different conditions

			Glucosinolate content (mg/g DW)									
Type	Accession	Treatment	SIN	GPN	PROG	GIBVN	GER	GIBN	GRPN	GBSN	4-HOH	Total GSL
Black kale	BK1	F	ND	ND	ND	ND	ND	$6.9^{a\text{-}d\ E}$	$27.4^{f\text{-}j\ C}$	$9.0^{a\text{-}g\ AB}$	$0.4^{j\ B}$	$43.7^{cd\ D}$
		G	ND	ND	ND	ND	ND	$0.5^{a\ A}$	$0.7^{a\ A}$	$7.9^{a\text{-}f\ AB}$	$0.2^{a\text{-}d\ A}$	$9.2^{a\ A}$
	BK2	F	ND	ND	ND	ND	ND	$4.7^{a\text{-}d\ D}$	$30.9^{h\text{-}j\ C}$	$12.6^{c\text{-}h\ A\text{-}C}$	$0.4^{j\ B}$	$48.6^{c\text{-}f\ D}$
		G	ND	ND	ND	ND	ND	$2.1^{a\text{-}c\ BC}$	$3.9^{a\ A}$	$15.8^{gh\ BC}$	$0.2^{c\text{-}g\ A}$	$22.0^{ab\ B}$
	BK3	F	ND	ND	ND	ND	2.9^{cd}	$0.6^{ab\ AB}$	$10.2^{bc\ B}$	$5.2^{a\text{-}c\ A}$	$0.3^{ij\ B}$	$19.3^{ab\ B}$
		G	ND	ND	ND	ND	2.2^{bc}	$2.2^{a\text{-}c\ C}$	$11.3^{bc\ B}$	$18.4^{hi\ C}$	$0.2^{c\text{-}g\ A}$	$34.3^{bc\ C}$
Wild	WD1	F	$4.0^{b\text{-}e\ C}$	$25.1^{e\ B}$	$61.6^{j\ D}$	ND	ND	ND	ND	$8.0^{a\text{-}f\ A}$	$0.5^{k\ C}$	$99.3^{lm\ B}$
		G	$4.9^{c\text{-}g\ CD}$	$33.3^{f\ C}$	$51.2^{i\ D}$	ND	ND	ND	ND	$5.2^{a\text{-}c\ A}$	$0.1^{ab\ A}$	$94.6^{lm\ B}$
	WD2	F	$5.2^{c\text{-}g\ D}$	$5.1^{cd\ A}$	$20.3^{f\ B}$	ND	ND	$7.3^{a\text{-}e\ B}$	$43.4^{k\ C}$	$6.0^{a\text{-}d\ A}$	$0.2^{d\text{-}i\ B}$	$87.5^{j\text{-}m\ B}$
		G	$2.3^{a\text{-}c\ B}$	$3.3^{b\text{-}d\ A}$	$6.9^{a\text{-}d\ A}$	ND	ND	$8.2^{a\text{-}f\ C}$	$31.4^{ij\ B}$	$7.1^{a\text{-}f\ A}$	$0.2^{c\text{-}h\ B}$	$59.4^{d\text{-}h\ A}$
	WD3	F	$0.1^{ab\ A}$	$42.3^{g\ D}$	$80.4^{k\ E}$	ND	ND	$0.2^{a\ A}$	$2.4^{a\ A}$	$24.2^{ij\ B}$	$0.2^{a\text{-}e\ B}$	$149.8^{n\ C}$
		G	$0.2^{ab\ A}$	$23.9^{e\ B}$	$40.5^{h\ C}$	ND	ND	$0.2^{a\ A}$	$1.4^{a\ A}$	$27.7^{j\ B}$	$0.1^{ab\ A}$	$93.9^{j\text{-}m\ B}$
Tronchuda	TC1	F	$21.4^{j\ D}$	$1.4^{ab\ B}$	$4.3^{a\text{-}c\ AB}$	ND	ND	$21.2^{h\text{-}k\ BC}$	$1.4^{a\ A}$	$13.8^{e\text{-}h\ B}$	$0.1^{a\text{-}c\ AB}$	$63.6^{e\text{-}i\ BC}$

		G	11.5$^{hi\ B}$	1.3$^{ab\ B}$	3.1$^{a\text{-}c\ AB}$	ND	ND	9.8$^{c\text{-}f\ A}$	1.5$^{a\ A}$	14.4$^{f\text{-}h\ B}$	0.1$^{a\ A}$	41.7$^{cd\ A}$
	TC2	F	2.2$^{a\text{-}c\ A}$	5.1$^{cd\ C}$	30.8$^{g\ C}$	ND	ND	16.6$^{f\text{-}i\ AB}$	3.7$^{a\ B}$	13.7$^{d\text{-}h\ B}$	0.4$^{ij\ C}$	72.5$^{h\text{-}j\ C}$
		G	13.4$^{i\ BC}$	0.5$^{ab\ A}$	8.1$^{b\text{-}d\ B}$	ND	ND	19.2$^{g\text{-}j\ B}$	3.9$^{a\ B}$	18.4$^{hi\ C}$	0.3$^{h\text{-}j\ C}$	63.8$^{e\text{-}i\ BC}$
	TC3	F	18.6$^{j\ CD}$	0.4$^{ab\ A}$	0.6$^{a\ A}$	ND	ND	19.3$^{g\text{-}j\ B}$	0.4$^{a\ A}$	10.3$^{b\text{-}f\ A}$	0.2$^{a\text{-}d\ B}$	49.9$^{c\text{-}g\ AB}$
		G	13.3$^{i\ BC}$	0.1$^{ab\ A}$	0.3$^{a\ A}$	ND	ND	29.3$^{kl\ C}$	0.6$^{a\ A}$	14.3$^{f\text{-}h\ B}$	0.1$^{a\text{-}c\ AB}$	58.0$^{d\text{-}h\ A\text{-}C}$
	SC1	F	8.8$^{gh\ A}$	ND	1.0$^{ab\ A}$	0.5$^{cd\ A}$	ND	25.4$^{j\text{-}l\ A}$	0.9$^{a\ A}$	9.0$^{a\text{-}g\ C}$	0.3$^{g\text{-}j\ B}$	45.9$^{c\text{-}e\ A}$
		G	14.3$^{i\ B}$	ND	0.6$^{a\ A}$	0.7$^{ef\ A}$	ND	58.4$^{n\ C}$	1.2$^{a\ A}$	3.5$^{ab\ AB}$	0.2$^{a\text{-}d\ A}$	78.9$^{i\text{-}k\ C}$
Savoy	SC2	F	6.6$^{d\text{-}g\ A}$	ND	1.0$^{ab\ A}$	1.7$^{h\ C}$	ND	30.9$^{l\ AB}$	0.3$^{a\ A}$	24.9$^{ij\ D}$	0.3$^{f\text{-}j\ B}$	65.7$^{f\text{-}i\ BC}$
		G	8.3$^{f\text{-}h\ A}$	ND	0.4$^{a\ A}$	1.2$^{g\ B}$	ND	39.8$^{m\ B}$	0.2$^{a\ A}$	1.8$^{a\ A}$	0.7$^{l\ C}$	52.5$^{c\text{-}g\ AB}$
	SC3	F	14.4$^{i\ B}$	1.2$^{ab\ A}$	8.7$^{cd\ B}$	0.6$^{d\text{-}f\ A}$	ND	60.6$^{n\ C}$	12.4$^{cd\ B}$	6.3$^{a\text{-}e\ BC}$	0.2$^{c\text{-}g\ A}$	104.4$^{m\ D}$
		G	Did not grow									
	RC1	F	8.0$^{f\text{-}h\ C}$	5.0$^{cd\ BC}$	16.3$^{ef\ C}$	0.9$^{fg\ B}$	ND	20.7$^{g\text{-}k\ D}$	25.5$^{f\text{-}i\ AB}$	28.8$^{j\ C}$	0.3$^{ij\ AB}$	105.5$^{m\ C}$
		G	2.8$^{a\text{-}d\ A}$	1.9$^{a\text{-}d\ A}$	12.6$^{de\ B}$	0.7$^{d\text{-}f\ B}$	ND	9.3$^{b\text{-}f\ B}$	29.5$^{g\text{-}j\ BC}$	27.7$^{j\ B}$	0.3$^{e\text{-}j\ AB}$	81.5$^{i\text{-}m\ B}$
Red	RC2	F	7.1$^{e\text{-}g\ C}$	3.2$^{a\text{-}d\ AB}$	8.8$^{cd\ A}$	0.7$^{d\text{-}f\ B}$	3.2$^{de\ C}$	12.4$^{d\text{-}g\ BC}$	18.6$^{de\ A}$	3.4$^{ab\ A}$	0.3$^{ij\ B}$	57.7$^{d\text{-}h\ A}$
		G	Did not grow									
	RC3	F	2.2$^{a\text{-}c\ A}$	5.2$^{d\ C}$	13.5$^{d\text{-}f\ BC}$	0.5$^{c\text{-}e\ AB}$	3.9$^{e\ C}$	5.3$^{a\text{-}d\ A}$	25.0$^{f\text{-}h\ AB}$	3.0$^{ab\ A}$	0.6$^{k\ C}$	59.2$^{d\text{-}h\ A}$
		G	4.6$^{c\text{-}f\ B}$	1.6$^{ab\ A}$	13.0$^{de\ B}$	0.2$^{ab\ A}$	1.9$^{b\ B}$	15.6$^{e\text{-}i\ C}$	33.4$^{j\ C}$	1.9$^{a\ A}$	0.2$^{d\text{-}i\ A}$	72.4$^{h\text{-}j\ B}$

White	WC1	F	4.8$^{c\text{-}f\ B}$	1.9$^{a\text{-}c\ C}$	16.5$^{ef\ C}$	0.3$^{bc\ BC}$	1.9$^{b\ B}$	12.7$^{d\text{-}h\ A}$	22.1$^{ef\ B}$	6.7$^{a\text{-}f\ A}$	0.3$^{ij\ C}$	67.4$^{g\text{-}i\ B}$
		G	2.8$^{a\text{-}d\ A}$	0.4$^{ab\ A}$	4.9$^{a\text{-}c\ A}$	0.2$^{ab\ A}$	1.6$^{b\ B}$	16.4$^{f\text{-}i\ AB}$	5.2$^{ab\ A}$	6.2$^{a\text{-}e\ A}$	0.1$^{a\text{-}c\ A}$	37.6$^{bc\ A}$
	WC2	F	8.3$^{f\text{-}h\ C}$	2.1$^{a\text{-}d\ C}$	17.2$^{ef\ C}$	0.3$^{a\text{-}c\ AB}$	ND	22.5$^{i\text{-}l\ BC}$	23.7$^{e\text{-}g\ B}$	29.2$^{j\ B}$	0.2$^{b\text{-}f\ B}$	103.4$^{m\ C}$
		G	5.9$^{c\text{-}g\ D}$	0.9$^{ab\ B}$	12.6$^{de\ B}$	0.4$^{b\text{-}d\ C}$	ND	23.6$^{i\text{-}l\ C}$	25.8$^{f\text{-}i\ B}$	27.1$^{j\ B}$	0.1$^{a\text{-}c\ A}$	96.5$^{k\text{-}m\ C}$
	P-value		< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

Letters 'ABC': mean values with different superscripts in the same column are significantly different (P<0.0001) across varieties and growing conditions within a cabbage type. Letters 'abc': mean values with different superscripts in the same column are significantly different (P<0.05) across varieties and growing conditions. Abbreviations: F = Field, G = glasshouse; DNG, did not grow; SIN, sinigrin; GPN, gluconapin; PROG, epi/progoitrin; GIBVN, Glucoibeverin; GER, glucoerucin; GIBN, glucoiberin; GRPN, glucoraphanin; GBSN, glucobrassicin; 4- HOH, 4-hydroxyglucobrassicin. For full names of cabbage varieties see Table 3.1

Table S3b: Glucosinolate hydrolysis concentration (µg/g DW sulforaphane equivalent) in cabbage grown under different conditions

			Glucosinolate hydrolysis products (µg/g DW sulforaphane equivalent)																					
Type	Accession	Treatment	ATC	AITC	CETP	3BITC	EVN	GN	CHETB-1	CHETB-2	4MBN	ER	ERN	IB	IBN	PEITC	BPN	SFP	SFN	I3C	1IAN	PITC	1H-I	BAN	Total GHP
Black kale	BK1	F	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	1.5$^{ab\ D}$	22.6$^{a\ D}$	ND	1.0$^{a-c\ B}$	11.0$^{ab\ C}$	147.5$^{ef\ D}$	66.5$^{f\ C}$	152.1$^{h\ C}$	ND	1.3$^{a\ B}$	ND	403.4$^{b-g\ C}$
		G	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.3$^{a\ AB}$	3.5$^{a\ AB}$	ND	ND	2.2$^{a\ A}$	11.2$^{a\ A}$	3.5$^{a-d\ A}$	23.1$^{b-f\ A}$	ND	0.5$^{a\ A}$	ND	44.3$^{a\ A}$
	BK2	F	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.7$^{a\ BC}$	8.5$^{a\ BC}$	ND	1.0$^{a-c\ B}$	8.7$^{a\ BC}$	133.9$^{d-f\ CD}$	43.3$^{e\ B}$	72.5$^{g\ B}$	ND	0.6$^{a\ A}$	ND	269.3$^{a-e\ B}$
		G	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.3$^{a\ AB}$	10.5$^{a\ C}$	ND	ND	2.2$^{a\ A}$	34.8$^{ab\ A}$	3.4$^{a-d\ A}$	29.6$^{d-f\ A}$	ND	0.6$^{a\ A}$	ND	81.4$^{a\ A}$
	BK3	F	ND	ND	ND	1.0$^{ab\ A}$	ND	ND	ND	ND	ND	<0.1$^{a\ A}$	1.0$^{a\ B}$	0.9$^{a\ C}$	4.6$^{a\ A-C}$	ND	0.9$^{a-c\ B}$	17.8$^{ab\ D}$	100.2$^{c-e\ BC}$	85.8$^{g\ D}$	137.6$^{h\ C}$	ND	2.7$^{a\ C}$	ND	352.6$^{a-g\ BC}$
		G	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.7$^{a\ A}$	<0.1$^{a\ A}$	1.2$^{a\ A}$	ND	ND	5.6$^{a\ AB}$	85.7$^{b-d\ B}$	5.1$^{a-d\ A}$	24.4$^{c-f\ A}$	ND	0.5$^{a\ A}$	ND	123.2$^{ab\ A}$
Wild	WD1	F	ND	ND	7.6$^{ab\ AB}$	0.3$^{ab\ AB}$	156.7$^{b\ B}$	5.5$^{a\ A}$	434.3$^{d\ C}$	521.0$^{d\ C}$	ND	ND	ND	ND	ND	0.4$^{c\ B}$	11.2$^{g\ B}$	ND	ND	6.5$^{a-d\ BC}$	40.3$^{f\ C}$	0.3$^{b\ B}$	2.8$^{a\ B}$	ND	1186.9$^{k\ B}$
		G	ND	ND	35.0$^{d-h\ C}$	0.3$^{ab\ AB}$	260.0$^{d\ C}$	3.5$^{a\ A}$	295.1$^{bc\ BC}$	366.8$^{bc\ BC}$	ND	ND	ND	ND	ND	1.2$^{e\ C}$	4.5$^{ef\ A}$	ND	ND	3.8$^{a-d\ A-C}$	7.6$^{a-c\ A}$	<0.1$^{a\ A}$	3.8$^{a\ B}$	ND	981.7$^{jk\ AB}$
	WD2	F	ND	ND	13.1$^{a-c\ B}$	1.0$^{ab\ C}$	29.2$^{a\ A}$	184.9$^{b\ B}$	37.6$^{a\ A}$	43.7$^{a\ A}$	ND	ND	ND	4.3$^{ab\ C}$	18.8$^{a\ B}$	0.1$^{ab\ A}$	2.1$^{a-d\ A}$	31.7$^{a-c\ C}$	180.3$^{fg\ B}$	13.3$^{cd\ D}$	37.0$^{f\ C}$	<0.1$^{a\ A}$	3.0$^{a\ B}$	ND	600.0$^{f-i\ A}$
		G	ND	ND	15.0$^{a-d\ B}$	0.5$^{ab\ B}$	20.9$^{a\ A}$	191.0$^{b\ B}$	19.0$^{a\ A}$	22.6$^{a\ A}$	ND	ND	ND	2.8$^{ab\ B}$	32.5$^{a-h\ C}$	<0.1$^{ab\ A}$	0.9$^{a-c\ A}$	22.3$^{a-c\ B}$	188.8$^{fg\ B}$	1.8$^{ab\ A}$	5.9$^{ab\ A}$	<0.1$^{a\ A}$	1.0$^{a\ A}$	1.3$^{d\ B}$	526.4$^{e-h\ A}$
	WD3	F	ND	ND	0.8$^{a\ A}$	0.3$^{ab\ AB}$	148.1$^{b\ B}$	7.2$^{a\ A}$	369.2$^{cd\ BC}$	423.5$^{cd\ BC}$	ND	ND	ND	<0.1$^{a\ A}$	0.6$^{a\ A}$	<0.1$^{ab\ A}$	3.0$^{c-e\ A}$	1.8$^{a\ A}$	1.7$^{a\ A}$	6.7$^{a-d\ C}$	36.1$^{ef\ C}$	0.3$^{b\ B}$	2.9$^{a\ B}$	0.7$^{c\ A}$	1002.9$^{jk\ AB}$
		G	ND	ND	1.0$^{a\ A}$	0.2$^{a\ A}$	204.7$^{c\ BC}$	2.1$^{a\ A}$	240.7$^{b\ B}$	283.5$^{b\ B}$	ND	ND	ND	3.7$^{ab\ BC}$	0.5$^{a\ A}$	0.2$^{a-c\ AB}$	3.4$^{de\ A}$	2.2$^{a\ A}$	4.4$^{a\ A}$	2.5$^{ab\ AB}$	23.1$^{b-f\ B}$	0.3$^{b\ B}$	2.8$^{a\ B}$	ND	775.2$^{h-j\ AB}$
Tronchuda	TC1	F	0.1$^{a-e\ AB}$	0.4$^{a-c\ A}$	55.5$^{hi\ B}$	ND	3.8$^{a\ A}$	0.5$^{a\ A}$	3.6$^{a\ A}$	4.7$^{a\ AB}$	1.9$^{a-c\ C}$	ND	ND	6.5$^{ab\ A}$	54.6$^{c-i\ C}$	ND^{a}	0.6$^{ab\ AB}$	1.8$^{a\ A}$	7.0$^{a\ AB}$	4.1$^{a-d\ B}$	13.7$^{a-d\ B}$	ND	2.2^{a}	0.2$^{a-c\ C}$	160.3$^{ab\ B}$
		G	0.1$^{b-e\ B}$	0.1$^{ab\ A}$	55.4$^{hi\ B}$	ND	9.5$^{a\ A}$	1.3$^{a\ B}$	7.5$^{a\ A}$	8.4$^{a\ AB}$	0.7$^{ab\ AB}$	ND	ND	2.3$^{ab\ A}$	29.0$^{a-g\ B}$	ND^{a}	0.8$^{a-c\ B}$	4.1$^{a\ B}$	14.8$^{a\ B}$	0.6$^{a\ A}$	6.1$^{ab\ A}$	ND	1.2^{a}	0.2$^{a-c\ BC}$	142.3$^{ab\ B}$
	TC2	F	0.1$^{ab\ A}$	0.3$^{a-c\ A}$	7.7$^{ab\ A}$	ND	27.0$^{a\ B}$	0.3$^{a\ A}$	49.7$^{a\ B}$	75.9$^{a\ C}$	0.6$^{ab\ AB}$	ND	ND	5.5$^{ab\ A}$	21.4$^{a-d\ AB}$	ND^{a}	1.4$^{a-d\ C}$	1.3$^{a\ A}$	6.6$^{a\ A}$	3.4$^{a-d\ B}$	7.5$^{a-c\ A}$	ND	2.0^{a}	0.1$^{ab\ AB}$	210.7$^{a-d\ BC}$
		G	0.1$^{ab\ A}$	0.2$^{a-c\ A}$	57.4$^{i\ B}$	ND	4.5$^{a\ A}$	0.6$^{a\ A}$	19.7$^{a\ A}$	23.4$^{a\ B}$	0.8$^{ab\ AB}$	ND	ND	3.8$^{ab\ A}$	62.3$^{d-i\ CD}$	ND^{a}	0.5$^{ab\ AB}$	4.1$^{a\ B}$	34.1$^{ab\ C}$	0.7$^{a\ A}$	18.7$^{a-e\ C}$	ND	1.5^{a}	0.2$^{a-c\ BC}$	232.5$^{a-e\ C}$
	TC3	F	0.2$^{d-f\ C}$	2.7$^{f\ B}$	11.4$^{a-c\ A}$	ND	0.3$^{a\ A}$	1.1$^{a\ B}$	0.7$^{a\ A}$	0.8$^{a\ A}$	0.5$^{ab\ A}$	ND	ND	26.6$^{a-d\ B}$	3.6$^{a-c\ A}$	ND^{a}	0.4$^{ab\ A}$	2.3$^{a\ A}$	2.1$^{a\ A}$	4.6$^{a-d\ B}$	5.5$^{a\ A}$	ND	2.0^{a}	<0.1$^{ab\ A}$	64.9$^{a\ A}$
		G	0.1$^{a-e\ AB}$	0.2$^{a-c\ A}$	49.2$^{g-i\ B}$	ND	0.4$^{a\ A}$	0.4$^{a\ A}$	0.7$^{a\ A}$	1.3$^{a\ A}$	1.3$^{ab\ BC}$	ND	ND	6.3$^{ab\ A}$	77.8$^{f-i\ D}$	ND^{a}	0.6$^{ab\ AB}$	2.3$^{a\ A}$	7.3$^{a\ AB}$	0.8$^{a\ A}$	8.0$^{a-c\ A}$	ND	1.3^{a}	0.4$^{ab\ D}$	158.3$^{ab\ B}$
Savoy	SC1	F	0.8$^{g\ B}$	4.9$^{g\ C}$	89.9$^{j\ D}$	ND	2.9$^{a\ B}$	ND$^{a\ A}$	5.4$^{a\ C}$	6.1$^{a\ C}$	6.1$^{fg\ AB}$	ND	ND	280.5$^{g\ C}$	291.4$^{k\ C}$	ND	6.1$^{f\ B}$	8.0$^{a\ B}$	13.8$^{a\ AB}$	13.6$^{d\ B}$	24.3$^{c-f\ C}$	ND	19.1$^{b\ B}$	0.2$^{a-c\ B}$	773.1$^{h-j\ D}$
		G	<0.1$^{ab\ A}$	0.8$^{cd\ AB}$	46.1$^{f-i\ C}$	ND	1.3$^{a\ A}$	ND$^{a\ A}$	1.9$^{a\ AB}$	3.7$^{a\ B}$	5.3$^{d-f\ AB}$	ND	ND	233.9$^{f\ C}$	275.8$^{d\ C}$	ND	0.8$^{a-c\ A}$	13.5$^{ab\ C}$	13.8$^{a\ AB}$	5.5$^{a-d\ A}$	9.2$^{a-c\ A}$	ND	2.2$^{a\ A}$	0.5$^{a-c\ AB}$	614.2$^{f-i\ C}$
	SC2	F	0.1$^{a-c\ A}$	0.6$^{a-c\ A}$	12.4$^{a-c\ A}$	ND	0.6$^{a\ A}$	ND$^{a\ A}$	0.8$^{a\ A}$	1.1$^{a\ A}$	9.3$^{h\ B}$	ND	ND	21.6$^{a-c\ A}$	31.8$^{a\ A}$	ND	0.3$^{ab\ A}$	1.5$^{a\ A}$	3.0$^{a\ A}$	2.9$^{a-c\ A}$	11.4$^{a-c\ AB}$	ND	2.2$^{a\ A}$	0.7$^{c\ B}$	100.3$^{ab\ A}$
		G	0.1$^{ab\ A}$	0.3$^{a-c\ A}$	27.2$^{b-f\ AB}$	ND	0.5$^{a\ A}$	ND$^{a\ A}$	1.1$^{a\ A}$	2.0$^{a\ AB}$	8.5$^{gh\ B}$	ND	ND	140.4$^{e\ B}$	154.3$^{bc\ A}$	ND	0.1$^{a\ A}$	3.6$^{a\ A}$	10.8$^{a\ A}$	12.5$^{b-d\ B}$	40.3$^{f\ D}$	ND	3.3$^{a\ A}$	0.6$^{bc\ AB}$	405.8$^{b-g\ B}$
	SC3	F	0.1$^{ab\ A}$	1.3$^{de\ B}$	35.2$^{e-h\ BC}$	ND	1.7$^{a\ AB}$	ND$^{a\ A}$	3.9$^{a\ BC}$	2.9$^{a\ AB}$	2.7$^{a-d\ A}$	ND	ND	23.9$^{a-c\ A}$	87.3$^{ab\ AB}$	ND	4.6$^{ef\ B}$	4.1$^{a\ AB}$	26.2$^{a\ B}$	2.7$^{a-c\ A}$	15.1$^{a-d\ B}$	ND	2.5$^{a\ A}$	1.8$^{d\ C}$	216.1$^{a-e\ A}$
		G	Did not grow																						
Red	RC1	F	<0.1^{ab}	0.1$^{a-c\ A}$	13.8$^{a-c\ A}$	0.5$^{ab\ A}$	13.4$^{a\ B}$	0.7$^{a\ A}$	12.4$^{a\ A}$	13.4$^{a\ A}$	3.0$^{b-e\ B}$	1.4$^{a\ A}$	16.3$^{b\ B}$	2.8$^{ab\ A}$	17.9$^{a-d\ A}$	0.2$^{a-c\ A}$	0.6$^{ab\ A}$	14.2$^{ab\ A}$	50.4$^{a-c\ A}$	0.7$^{a\ A}$	1.4$^{a\ A}$	ND	0.6$^{a\ AB}$	ND	164.0$^{ab\ A}$
		G	<0.1^{ab}	0.2$^{a-c\ A}$	12.8$^{a-c\ A}$	0.5$^{ab\ A}$	12.4$^{a\ B}$	2.4$^{a\ A}$	23.0$^{a\ AB}$	27.2$^{a\ AB}$	4.3$^{c-f\ BC}$	21.8$^{c\ B}$	29.9$^{d\ C}$	10.6$^{ab\ AB}$	27.7$^{a-g\ A}$	0.2$^{a-c\ A}$	0.5$^{ab\ A}$	116.1$^{d\ B}$	182.6$^{fg\ C}$	1.1$^{a\ A}$	3.4$^{a\ B}$	ND	0.4$^{a\ A}$	ND	477.2$^{c-h\ BC}$
	RC2	F	0.1^{ab}	0.5$^{a-c\ B}$	30.2$^{c-g\ B}$	2.3$^{c\ B}$	27.7$^{a\ C}$	3.6$^{a\ A}$	38.4$^{a\ C}$	44.7$^{a\ C}$	5.6$^{ef\ C}$	5.3$^{b\ A}$	21.1$^{c\ BC}$	15.2$^{a-c\ B}$	76.4$^{e-i\ B}$	0.8$^{d\ B}$	1.0$^{a-c\ A}$	60.4$^{c\ AB}$	169.1$^{fg\ BC}$	5.3$^{a-d\ C}$	5.3$^{a\ C}$	ND	2.6$^{a\ C}$	ND	515.6$^{d-h\ C}$
		G	Did not grow																						
	RC3	F	0.1^{ab}	0.2$^{a-c\ A}$	16.4$^{a-e\ AB}$	0.7$^{ab\ A}$	18.5$^{a\ B}$	1.5$^{a\ A}$	25.7$^{a\ A-C}$	27.9$^{a\ AB}$	5.9$^{fg\ C}$	3.0$^{ab\ A}$	29.4$^{d\ C}$	4.8$^{ab\ AB}$	24.1$^{a-f\ A}$	0.2$^{bc\ A}$	2.5$^{b-e\ B}$	27.2$^{a-c\ A}$	104.6$^{c-e\ AB}$	3.3$^{a-d\ B}$	8.6$^{a-c\ D}$		2.4$^{a\ BC}$	ND	307.2$^{a-f\ AB}$
		G	<0.1^{ab}	0.3$^{a-c\ A}$	21.7$^{b-e\ AB}$	1.0$^{ab\ A}$	4.4$^{a\ A}$	8.2$^{a\ B}$	34.8$^{a\ BC}$	40.9$^{a\ BC}$	1.4$^{ab\ A}$	0.6$^{a\ A}$	1.8$^{a\ A}$	47.3$^{cd\ C}$	80.9$^{g-i\ B}$	0.7$^{d\ B}$	0.4$^{ab\ A}$	209.1$^{e\ C}$	172.1$^{fg\ C}$	2.6$^{ab\ B}$	7.5$^{a-c\ D}$	ND	1.1$^{a\ A-C}$	ND	637.0$^{g-i\ C}$
White	WC1	F	0.3$^{f\ B}$	1.7$^{e\ B}$	28.3$^{c-f\ C}$	5.1$^{d\ B}$	15.5$^{a\ C}$	12.1$^{a\ B}$	78.4$^{a\ B}$	97.3$^{a\ B}$	5.0$^{d-f\ C}$	2.3$^{ab\ B}$	13.7$^{b\ C}$	60.3$^{d\ C}$	83.4$^{hi\ C}$	0.3$^{c\ C}$	3.0$^{c-e\ B}$	194.6$^{e\ B}$	215.8$^{g\ C}$	7.0$^{a-d\ B}$	12.8^{a-d}	ND	3.6$^{a\ B}$	4.6$^{e\ B}$	845.2$^{ij\ B}$
		G	0.1$^{a-d\ A}$	0.2$^{a-c\ A}$	7.2$^{ab\ A}$	<0.1$^{a\ A}$	1.4$^{a\ A}$	1.5$^{a\ A}$	7.4$^{a\ A}$	10.8$^{a\ A}$	1.2$^{ab\ A}$	0.1$^{a\ A}$	0.8$^{a\ A}$	36.9$^{b-d\ B}$	45.9$^{a-i\ B}$	0.2$^{a-c\ BC}$	0.4$^{ab\ A}$	19.8$^{a-c\ A}$	32.5$^{b\ AB}$	4.6$^{a-d\ A}$	9.7^{a-c}	ND	1.1$^{a\ A}$	0.1$^{ab\ A}$	182.1$^{a-c\ A}$
	WC2	F	0.3$^{ef\ AB}$	0.7$^{b-d\ A}$	14.3$^{a-c\ B}$	1.3$^{bc\ A}$	5.5$^{a\ B}$	1.5$^{a\ A}$	12.2$^{a\ A}$	17.2$^{a\ A}$	1.0$^{ab\ A}$	0.1$^{a\ A}$	0.8$^{a\ A}$	18.5$^{a-c\ A}$	19.8$^{a-d\ A}$	<0.1$^{ab\ A}$	1.3$^{a-d\ A}$	21.3$^{a-c\ A}$	32.1$^{ab\ A}$	4.0$^{a-d\ A}$	16.0^{a-d}	ND	2.2$^{a\ A}$	0.3$^{a-c\ A}$	170.4$^{a-c\ A}$
		G	0.2$^{c-f\ AB}$	0.2$^{a-c\ A}$	17.1$^{a-e\ B}$	0.4$^{ab\ A}$	3.4$^{a\ AB}$	0.9$^{a\ A}$	15.3$^{a\ A}$	20.7$^{a\ A}$	2.8$^{a-e\ B}$	0.2$^{a\ A}$	4.4$^{a\ B}$	31.6$^{a-d\ AB}$	54.1$^{b-i\ B}$	0.1$^{a-c\ AB}$	0.6$^{ab\ A}$	52.1$^{bc\ A}$	91.2$^{cd\ B}$	4.3$^{a-d\ A}$	14.6^{a-d}	ND	1.2$^{a\ A}$	0.5$^{a-c\ A}$	315.9$^{a-f\ A}$
	P-value		< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

Letters 'ABC': mean values with different superscripts in the same column are significantly different (P<0.0001) across varieties and growing conditions within a cabbage type. Letters 'abc': mean values with different superscripts in the same column are significantly different (P<0.05) across varieties and growing conditions. Abbreviations: F = Field, G = glasshouse; DNG, did not grow; For full names of cabbage varieties see Table 3.1 and Table 3.2 for compound names.

Table S3c: Pearson correlation matrix table showing correlations between glucosinolates and glucosinolate hydrolysis products identified in cabbage grown under two conditions

Variables	SIN	PROG	GIBN	GRPN	GPN	GBSN	4-HOH	GIBVN	GER	Total GLS	ATC	AITC	CETP	3BITC	EVN	GN	CHETB-1	CHETB-2	4MBN	ER	ERN	IB	IBN	PEITC	BPN	SFP	SFN	I3C	1IAN	PITC	1H-I	BAN	Total HPS
SIN	**1**	-0.263	**0.660**	-0.298	-0.263	-0.051	-0.215	0.175	-0.266	0.118	0.340	**0.399**	**0.690**	-0.113	-0.245	-0.117	-0.256	-0.255	0.245	-0.113	-0.128	0.282	**0.406**	-0.130	-0.051	-0.124	**-0.355**	-0.332	**-0.389**	-0.272	0.129	0.093	-0.238
PROG	-0.263	**1**	**-0.356**	-0.121	**0.947**	0.205	-0.022	-0.226	-0.139	**0.746**	-0.205	-0.210	-0.213	0.084	**0.840**	0.031	**0.938**	**0.937**	-0.276	-0.016	-0.016	-0.237	-0.311	**0.390**	**0.614**	-0.031	-0.198	-0.202	-0.099	**0.832**	0.035	0.011	**0.719**
GIBN	**0.660**	**-0.356**	**1**	-0.162	**-0.394**	-0.096	0.010	**0.578**	-0.239	0.177	0.258	**0.351**	**0.486**	-0.134	**-0.400**	-0.151	**-0.396**	**-0.395**	**0.543**	-0.097	-0.124	**0.560**	**0.677**	-0.252	-0.097	-0.051	-0.262	-0.226	-0.274	**-0.360**	0.142	0.251	-0.225
GRPN	-0.298	-0.121	-0.162	**1**	-0.238	-0.012	0.174	-0.007	0.235	0.144	-0.137	-0.190	-0.282	**0.380**	-0.265	**0.505**	-0.259	-0.264	-0.065	0.309	**0.417**	-0.221	-0.180	0.125	-0.179	**0.524**	**0.859**	0.190	0.140	-0.250	-0.220	0.104	0.027
GPN	-0.263	**0.947**	**-0.394**	-0.238	**1**	0.162	-0.112	-0.239	-0.147	**0.645**	-0.248	-0.213	-0.184	-0.028	**0.924**	0.002	**0.941**	**0.937**	-0.288	-0.073	-0.082	-0.214	-0.300	**0.421**	**0.559**	-0.160	-0.276	-0.142	-0.041	**0.833**	0.063	-0.063	**0.711**
GBSN	-0.051	0.205	-0.096	-0.012	0.162	**1**	-0.315	0.106	**-0.398**	**0.402**	0.042	-0.141	-0.188	-0.162	0.095	-0.195	0.087	0.079	-0.093	0.169	0.038	-0.286	-0.328	-0.297	-0.157	-0.169	-0.231	-0.229	-0.164	0.208	-0.170	-0.123	-0.208
4-HOH	-0.215	-0.022	0.010	0.174	-0.112	-0.315	**1**	**0.378**	0.285	-0.084	-0.040	0.015	-0.117	0.162	-0.158	-0.059	0.005	0.006	**0.463**	0.129	0.345	0.147	0.120	-0.023	0.205	0.084	0.216	0.261	0.288	0.001	0.122	0.042	0.143
GIBVN	0.175	-0.226	**0.578**	-0.007	-0.239	0.106	**0.378**	**1**	0.030	0.148	0.138	0.176	0.133	0.063	-0.245	-0.179	-0.248	-0.251	**0.900**	0.245	0.342	**0.375**	**0.389**	-0.010	-0.117	0.090	-0.038	-0.184	-0.234	-0.245	0.111	0.168	-0.109
GER	-0.266	-0.139	-0.239	0.235	-0.147	**-0.398**	0.285	0.030	**1**	-0.291	-0.061	-0.059	-0.139	**0.479**	-0.149	-0.112	-0.129	-0.133	0.190	0.112	**0.516**	-0.093	-0.060	**0.353**	-0.091	**0.390**	**0.440**	0.203	0.082	-0.183	-0.045	0.072	0.010
Total GLS	0.118	**0.746**	0.177	0.144	**0.645**	**0.402**	-0.084	0.148	-0.291	**1**	-0.070	-0.076	0.000	0.094	**0.517**	0.079	**0.589**	**0.583**	0.015	0.086	0.093	-0.063	-0.049	0.227	**0.407**	0.072	-0.101	-0.340	-0.282	**0.535**	-0.003	0.149	**0.506**
ATC	0.340	-0.205	0.258	-0.137	-0.248	0.042	-0.040	0.138	-0.061	-0.070	**1**	**0.896**	**0.620**	0.187	-0.239	-0.159	-0.212	-0.209	**0.364**	-0.049	-0.008	**0.626**	**0.572**	-0.116	0.207	0.126	-0.113	-0.123	-0.205	-0.231	**0.807**	0.240	0.047
AITC	**0.399**	-0.210	**0.351**	-0.190	-0.213	-0.141	0.015	0.176	-0.059	-0.076	**0.896**	**1**	**0.557**	0.133	-0.202	-0.131	-0.180	-0.181	**0.371**	-0.040	-0.029	**0.688**	**0.618**	-0.108	0.270	0.081	-0.123	-0.079	-0.164	-0.188	**0.816**	0.249	0.100
CETP	**0.690**	-0.213	**0.486**	-0.282	-0.184	-0.188	-0.117	0.133	-0.139	0.000	**0.620**	**0.557**	**1**	-0.016	-0.128	-0.090	-0.179	-0.175	**0.360**	-0.056	-0.052	**0.580**	**0.710**	0.042	0.160	-0.001	-0.214	-0.273	-0.339	-0.264	**0.595**	0.138	0.082
3BITC	-0.113	0.084	-0.134	**0.380**	-0.028	-0.162	0.162	0.063	**0.479**	0.094	0.187	0.133	-0.016	**1**	-0.023	0.112	0.050	0.052	0.209	0.177	**0.428**	-0.019	0.011	**0.361**	0.084	**0.705**	**0.599**	0.023	-0.061	-0.067	0.034	**0.712**	0.323
EVN	-0.245	**0.840**	**-0.400**	-0.265	**0.924**	0.095	-0.158	-0.245	-0.149	**0.517**	-0.239	-0.202	-0.128	-0.023	**1**	0.000	**0.897**	**0.902**	-0.284	-0.067	-0.099	-0.196	-0.283	**0.552**	**0.587**	-0.155	-0.267	-0.142	-0.063	**0.760**	0.086	-0.093	**0.707**
GN	-0.117	0.031	-0.151	**0.505**	0.002	-0.195	-0.059	-0.179	-0.112	0.079	-0.159	-0.131	-0.090	0.112	0.000	**1**	-0.029	-0.032	-0.207	-0.064	-0.101	-0.115	-0.098	-0.059	-0.007	0.044	**0.465**	-0.049	-0.046	0.040	-0.037	0.122	0.171
CHETB-1	-0.256	**0.938**	**-0.396**	-0.259	**0.941**	0.087	0.005	-0.248	-0.129	**0.589**	-0.212	-0.180	-0.179	0.050	**0.897**	-0.029	**1**	**0.999**	-0.272	-0.065	-0.096	-0.184	-0.269	**0.428**	**0.725**	-0.081	-0.245	-0.132	-0.012	**0.906**	0.077	-0.001	**0.789**
CHETB-2	-0.255	**0.937**	**-0.395**	-0.264	**0.937**	0.079	0.006	-0.251	-0.133	**0.583**	-0.209	-0.181	-0.175	0.052	**0.902**	-0.032	**0.999**	**1**	-0.274	-0.067	-0.100	-0.184	-0.269	**0.442**	**0.729**	-0.080	-0.248	-0.134	-0.017	**0.896**	0.077	-0.001	**0.788**
4MBN	0.245	-0.276	**0.543**	-0.065	-0.288	-0.093	**0.463**	**0.900**	0.190	0.015	**0.364**	**0.371**	**0.360**	0.209	-0.284	-0.207	-0.272	-0.274	**1**	0.257	**0.424**	**0.552**	**0.581**	0.025	-0.054	0.195	0.024	-0.195	-0.265	-0.294	0.331	0.270	-0.007
ER	-0.113	-0.016	-0.097	0.309	-0.073	0.169	0.129	0.245	0.112	0.086	-0.049	-0.040	-0.056	0.177	-0.067	-0.064	-0.065	-0.067	0.257	**1**	**0.748**	-0.072	-0.047	0.150	-0.099	**0.413**	**0.417**	-0.116	-0.172	-0.103	-0.115	-0.026	0.066
ERN	-0.128	-0.016	-0.124	**0.417**	-0.082	0.038	0.345	0.342	**0.516**	0.093	-0.008	-0.029	-0.052	**0.428**	-0.099	-0.101	-0.096	-0.100	**0.424**	**0.748**	**1**	-0.105	-0.057	0.294	-0.062	**0.432**	**0.482**	-0.150	-0.240	-0.164	-0.096	0.086	0.035
IB	0.282	-0.237	**0.560**	-0.221	-0.214	-0.286	0.147	**0.375**	-0.093	-0.063	**0.626**	**0.688**	**0.580**	-0.019	-0.196	-0.115	-0.184	-0.184	**0.552**	-0.072	-0.105	**1**	**0.951**	-0.111	0.150	0.059	-0.154	-0.043	-0.100	-0.170	**0.711**	0.133	0.239
IBN	**0.406**	-0.311	**0.677**	-0.180	-0.300	-0.328	0.120	**0.389**	-0.060	-0.049	**0.572**	**0.618**	**0.710**	0.011	-0.283	-0.098	-0.269	-0.269	**0.581**	-0.047	-0.057	**0.951**	**1**	-0.095	0.094	0.103	-0.094	-0.094	-0.154	-0.256	**0.635**	0.188	0.178
PEITC	-0.130	**0.390**	-0.252	0.125	**0.421**	-0.297	-0.023	-0.010	**0.353**	0.227	-0.116	-0.108	0.042	**0.361**	**0.552**	-0.059	**0.428**	**0.442**	0.025	0.150	0.294	-0.111	-0.095	**1**	0.276	**0.423**	0.222	-0.175	-0.233	0.125	0.012	-0.009	**0.511**
BPN	-0.051	**0.614**	-0.097	-0.179	**0.559**	-0.157	0.205	-0.117	-0.091	**0.407**	0.207	0.270	0.160	0.084	**0.587**	-0.007	**0.725**	**0.729**	-0.054	-0.099	-0.062	0.150	0.094	0.276	**1**	-0.087	-0.190	-0.048	0.026	**0.653**	**0.483**	0.119	**0.719**
SFP	-0.124	-0.031	-0.051	**0.524**	-0.160	-0.169	0.084	0.090	**0.390**	0.072	0.126	0.081	-0.001	**0.705**	-0.155	0.044	-0.081	-0.080	0.195	**0.413**	**0.432**	0.059	0.103	**0.423**	-0.087	**1**	**0.693**	-0.084	-0.163	-0.175	-0.065	**0.467**	0.270
SFN	**-0.355**	-0.198	-0.262	**0.859**	-0.276	-0.231	0.216	-0.038	**0.440**	-0.101	-0.113	-0.123	-0.214	**0.599**	-0.267	**0.465**	-0.245	-0.248	0.024	**0.417**	**0.482**	-0.154	-0.094	0.222	-0.190	**0.693**	**1**	0.263	0.190	-0.261	-0.167	0.305	0.148
I3C	-0.332	-0.202	-0.226	0.190	-0.142	-0.229	0.261	-0.184	0.203	-0.340	-0.123	-0.079	-0.273	0.023	-0.142	-0.049	-0.132	-0.134	-0.195	-0.116	-0.150	-0.043	-0.094	-0.175	-0.048	-0.084	0.263	**1**	**0.951**	-0.096	0.050	-0.119	0.014
1IAN	**-0.389**	-0.099	-0.274	0.140	-0.041	-0.164	0.288	-0.234	0.082	-0.282	-0.205	-0.164	-0.339	-0.061	-0.063	-0.046	-0.012	-0.017	-0.265	-0.172	-0.240	-0.100	-0.154	-0.233	0.026	-0.163	0.190	**0.951**	**1**	0.056	0.010	-0.146	0.063
PITC	-0.272	**0.832**	**-0.360**	-0.250	**0.833**	0.208	0.001	-0.245	-0.183	**0.535**	-0.231	-0.188	-0.264	-0.067	**0.760**	0.040	**0.906**	**0.896**	-0.294	-0.103	-0.164	-0.170	-0.256	0.125	**0.653**	-0.175	-0.261	-0.096	0.056	**1**	0.045	-0.056	**0.677**
1H-I	0.129	0.035	0.142	-0.220	0.063	-0.170	0.122	0.111	-0.045	-0.003	**0.807**	**0.816**	**0.595**	0.034	0.086	-0.037	0.077	0.077	0.331	-0.115	-0.096	**0.711**	**0.635**	0.012	**0.483**	-0.065	-0.167	0.050	0.010	0.045	**1**	0.065	**0.383**
BAN	0.093	0.011	0.251	0.104	-0.063	-0.123	0.042	0.168	0.072	0.149	0.240	0.249	0.138	**0.712**	-0.093	0.122	-0.001	-0.001	0.270	-0.026	0.086	0.133	0.188	-0.009	0.119	**0.467**	0.305	-0.119	-0.146	-0.056	0.065	**1**	0.215
Total HPS	-0.238	**0.719**	-0.225	0.027	**0.711**	-0.208	0.143	-0.109	0.010	**0.506**	0.047	0.100	0.082	0.323	**0.707**	0.171	**0.789**	**0.788**	-0.007	0.066	0.035	0.239	0.178	**0.511**	**0.719**	0.270	0.148	0.014	0.063	**0.677**	**0.383**	0.215	**1**
Total GLS	0.118	**0.746**	0.177	0.144	**0.645**	**0.402**	-0.084	0.148	-0.291	**1.000**	-0.070	-0.076	0.000	0.094	**0.517**	0.079	**0.589**	**0.583**	0.015	0.086	0.093	-0.063	-0.049	0.227	**0.407**	0.072	-0.101	-0.340	-0.282	**0.535**	-0.003	0.149	**0.506**
Total HPS	-0.238	**0.719**	-0.225	0.027	**0.711**	-0.208	0.143	-0.109	0.010	**0.506**	0.047	0.100	0.082	0.323	**0.707**	0.171	**0.789**	**0.788**	-0.007	0.066	0.035	0.239	0.178	**0.511**	**0.719**	0.270	0.148	0.014	0.063	**0.677**	**0.383**	0.215	**1.000**

Values in bold are significantly different from each other at $p<0.05$

Appendix IX: Glucosinolate (mg/g DW) and glucosinolate hydrolysis concentration (µg/g DW sulforaphane equivalent) of cooked

Table S4a: Glucosinolate concentration (mg/g DW) in cabbage

			Glucosinolate content (mg/g) DW									
Type	Accession	Treatment	SIN	GPN	PROG	GIBVN	GER	GIBN	GRPN	GBSN	4-HOH	Total GSL
Black kale	BK1	R	ND	ND	ND	ND	ND	6.9$^{b\text{-}l\ E}$	27.4$^{n\text{-}p\ D\text{-}F}$	9.0$^{h\text{-}r\ D}$	0.4$^{q\text{-}t\ DE}$	43.7$^{f\text{-}n\ FG}$
		ST	ND	ND	ND	ND	ND	4.9$^{a\text{-}e\ D}$	24.8$^{k\text{-}p\ C\text{-}E}$	6.3$^{a\text{-}m\ BC}$	0.4$^{o\text{-}s\ DE}$	36.5$^{d\text{-}j\ DE}$
		MW	ND	ND	ND	ND	ND	3.5$^{a\text{-}c\ CD}$	25.8$^{m\text{-}p\ D\text{-}F}$	7.6$^{e\text{-}n\ CD}$	0.3$^{k\text{-}s\ C\text{-}E}$	37.3$^{j\ D\text{-}F}$
		SF	ND	ND	ND	ND	ND	2.9$^{a\text{-}c\ C}$	19.7$^{i\text{-}m\ C}$	5.3$^{a\text{-}j\ A\text{-}C}$	0.3$^{f\text{-}r\ A\text{-}D}$	28.2$^{b\text{-}g\ C}$
	BK2	R	ND	ND	ND	ND	ND	4.7$^{a\text{-}e\ D}$	30.9$^{p\ F}$	12.6$^{p\text{-}t\ E}$	0.4$^{r\text{-}t\ DE}$	48.6$^{i\text{-}r\ G}$
		ST	ND	ND	ND	ND	ND	3.7$^{a\text{-}d\ CD}$	28.4$^{op\ EF}$	6.9$^{b\text{-}n\ B\text{-}D}$	0.4$^{r\text{-}u\ E}$	39.4$^{f\text{-}k\ EF}$
		MW	ND	ND	ND	ND	ND	3.6$^{a\text{-}d\ CD}$	22.5$^{j\text{-}o\ CD}$	6.7$^{b\text{-}n\ B\text{-}D}$	0.3$^{j\text{-}s\ B\text{-}E}$	33.1$^{c\text{-}i\ C\text{-}E}$
		SF	ND	ND	ND	ND	ND	2.1$^{a\text{-}c\ BC}$	24.7$^{k\text{-}p\ C\text{-}E}$	5.7$^{a\text{-}k\ A\text{-}C}$	0.2$^{c\text{-}o\ A\text{-}C}$	32.7$^{c\text{-}i\ CD}$
	BK3	R	ND	ND	ND	ND	2.9$^{d\text{-}f\ C}$	0.6$^{ab\ AB}$	10.2$^{c\text{-}g\ B}$	5.2$^{a\text{-}l\ A\text{-}C}$	0.3$^{l\text{-}s\ C\text{-}E}$	19.3$^{a\text{-}d\ B}$
		ST	ND	ND	ND	ND	2.2$^{b\text{-}e\ BC}$	0.6$^{ab\ AB}$	9.3$^{b\text{-}f\ AB}$	4.7$^{a\text{-}h\ AB}$	0.2$^{b\text{-}l\ AB}$	16.9$^{a\text{-}c\ AB}$
		MW	ND	ND	ND	ND	1.9$^{bc\ B}$	0.4$^{ab\ A}$	5.3$^{a\text{-}d\ AB}$	3.5$^{a\text{-}e\ A}$	0.2$^{d\text{-}p\ A\text{-}C}$	11.4$^{ab\ A}$
		SF	ND	ND	ND	ND	1.4$^{b\ B}$	0.6$^{ab\ AB}$	3.7$^{a\text{-}c\ A}$	4.6$^{a\text{-}h\ AB}$	0.2$^{a\text{-}j\ A}$	10.5$^{ab\ A}$
Wild	WD1	R	4.0$^{c\text{-}l\ DE}$	25.1$^{op\ CD}$	61.6$^{q\ CD}$	ND	ND	ND	ND	8.0$^{e\text{-}p\ BC}$	0.5$^{t\text{-}v\ F}$	99.3$^{Aa\text{-}Ad\ DE}$
		ST	3.2$^{a\text{-}j\ D}$	24.3$^{no\ CD}$	59.2$^{q\ C}$	ND	ND	ND	ND	7.6$^{d\text{-}n\ A\text{-}C}$	0.4$^{stu\ EF}$	94.7$^{Aa\text{-}Ad\ CD}$
		MW	2.8$^{a\text{-}l\ CD}$	21.7$^{n\ C}$	59.6$^{q\ CD}$	ND	ND	ND	ND	7.4$^{c\text{-}n\ A\text{-}C}$	0.4$^{qrs\ DE}$	91.8$^{z\text{-}Ad\ CD}$
		SF	1.1$^{a\text{-}d\ AB}$	13.9$^{m\ B}$	43.1$^{p\ B}$	ND	ND	ND	ND	6.7$^{b\text{-}n\ AB}$	0.3$^{g\text{-}r\ CD}$	65.2$^{r\text{-}w\ B}$

	WD2	R	5.2$^{f\text{-}o\ E}$	5.1$^{i\text{-}l\ A}$	20.3$^{l\text{-}n\ A}$	ND	ND	7.3$^{c\text{-}j\ D}$	43.4$^{q\ C}$	6.0$^{a\text{-}l\ AB}$	0.2$^{e\text{-}q\ B\text{-}D}$	87.5$^{y\text{-}Ac\ CD}$
		ST	3.9$^{b\text{-}l\ D}$	4.9$^{g\text{-}l\ A}$	17.2$^{j\text{-}m\ A}$	ND	ND	6.3$^{a\text{-}l\ CD}$	40.8$^{q\ C}$	5.0$^{a\text{-}h\ AB}$	0.2$^{b\text{-}m\ A\text{-}C}$	78.2$^{v\text{-}Aa\ BC}$
		MW	3.4$^{a\text{-}k\ D}$	5.9$^{l\ A}$	18.9$^{k\text{-}n\ A}$	ND	ND	5.7$^{a\text{-}g\ C}$	43.1$^{q\ C}$	4.5$^{a\text{-}h\ AB}$	0.2$^{b\text{-}m\ A\text{-}C}$	81.7$^{w\text{-}Aa\ B\text{-}D}$
		SF	1.5$^{a\text{-}f\ BC}$	2.1$^{a\text{-}j\ A}$	14.3$^{f\text{-}l\ A}$	ND	ND	2.2$^{a\text{-}c\ B}$	19.7$^{i\text{-}m\ B}$	3.7$^{a\text{-}f\ A}$	0.1$^{a\text{-}d\ A}$	43.7$^{f\text{-}n\ A}$
	WD3	R	0.1$^{a\ A}$	42.3$^{r\ F}$	80.4$^{r\ E}$	ND	ND	0.2$^{a\ A}$	2.4$^{ab\ A}$	24.2$^{xyz\ F}$	0.2$^{b\text{-}i\ A\text{-}C}$	149.8$^{Ag\ G}$
		ST	0.1$^{a\ A}$	41.9$^{r\ F}$	73.5$^{r\ DE}$	ND	ND	0.2$^{a\ A}$	1.2$^{a\ A}$	18.9$^{vw\ E}$	0.1$^{a\text{-}g\ AB}$	136.0$^{Af\text{-}Ag\ FG}$
		MW	0.1$^{a\ A}$	31.7$^{q\ E}$	72.8$^{r\ C\text{-}E}$	ND	ND	0.1$^{a\ A}$	1.4$^{a\ A}$	13.7$^{stu\ D}$	0.1$^{a\text{-}h\ AB}$	120.1$^{Ae\text{-}Af\ EF}$
		SF	0.1$^{a\ A}$	27.8$^{p\ DE}$	61.8$^{q\ CD}$	ND	ND	0.1$^{a\ A}$	0.7$^{a\ A}$	11.3$^{n\text{-}t\ CD}$	0.1$^{a\text{-}d\ A}$	101.8$^{Ac\text{-}Ad\ DE}$
	TC1	R	21.4$^{u\ C}$	1.4$^{a\text{-}e\ A}$	4.3$^{a\text{-}e\ A}$	ND	ND	21.2$^{rs\ F}$	1.4$^{a\ B}$	13.8$^{s\text{-}u\ C}$	0.1$^{a\text{-}d\ A\text{-}C}$	63.6$^{q\text{-}v\ DE}$
		ST	16.9$^{st\ BC}$	1.4$^{a\text{-}e\ A}$	1.8$^{a\text{-}d\ A}$	ND	ND	10.1$^{d\text{-}k\ BC}$	1.4$^{a\ B}$	12.9$^{r\text{-}t\ C}$	0.1$^{a\text{-}d\ A\text{-}C}$	44.6$^{f\text{-}o\ B}$
		MW	15.3$^{r\text{-}t\ B}$	0.7$^{a\text{-}d\ A}$	1.8$^{a\text{-}d\ A}$	ND	ND	11.6$^{f\text{-}l\ BC}$	0.3$^{a\ A}$	12.7$^{q\text{-}t\ BC}$	0.1$^{a\text{-}e\ A\text{-}C}$	42.6$^{f\text{-}m\ B}$
		SF	4.1$^{c\text{-}l\ A}$	0.2$^{ab\ A}$	0.7$^{ab\ A}$	ND	ND	3.7$^{a\text{-}d\ A}$	0.3$^{a\ A}$	12.2$^{o\text{-}t\ BC}$	<0.1$^{a\ A}$	21.3$^{a\text{-}e\ A}$
Tronchuda	TC2	R	2.2$^{a\text{-}g\ A}$	5.1$^{jkl\ B}$	30.8$^{o\ D}$	ND	ND	16.6$^{k\text{-}r\ DE}$	3.7$^{a\text{-}c\ C}$	13.7$^{s\text{-}u\ C}$	0.4$^{q\text{-}s\ D}$	72.5$^{t\text{-}y\ E}$
		ST	1.9$^{a\text{-}g\ A}$	5.0$^{h\text{-}l\ B}$	26.5$^{no\ CD}$	ND	ND	16.3$^{k\text{-}r\ DE}$	2.1$^{a\ B}$	10.1$^{k\text{-}s\ A\text{-}C}$	0.3$^{l\text{-}s\ D}$	62.3$^{p\text{-}v\ DE}$
		MW	2.0$^{a\text{-}g\ A}$	5.0$^{h\text{-}l\ B}$	23.2$^{m\text{-}o\ BC}$	ND	ND	13.7$^{j\text{-}o\ CD}$	1.7$^{a\ B}$	10.7$^{m\text{-}t\ A\text{-}C}$	0.4$^{q\text{-}s\ D}$	56.6$^{k\text{-}t\ CD}$
		SF	1.0$^{a\text{-}c\ A}$	4.1$^{e\text{-}l\ B}$	18.8$^{k\text{-}n\ B}$	ND	ND	7.2$^{c\text{-}j\ AB}$	1.4$^{a\ B}$	9.7$^{i\text{-}s\ A\text{-}C}$	0.2$^{a\text{-}k\ C}$	42.5$^{f\text{-}m\ B}$
	TC3	R	18.6$^{tu\ BC}$	0.4$^{ab\ A}$	0.6$^{ab\ A}$	ND	ND	19.3$^{o\text{-}s\ EF}$	0.4$^{a\ A}$	10.3$^{l\text{-}t\ A\text{-}C}$	0.2$^{a\text{-}l\ BC}$	49.9$^{i\text{-}r\ BC}$
		ST	16.5$^{st\ BC}$	0.3$^{ab\ A}$	0.7$^{ab\ A}$	ND	ND	19.6$^{o\text{-}s\ EF}$	0.3$^{a\ A}$	10.0$^{j\text{-}s\ A\text{-}C}$	0.1$^{a\text{-}h\ BC}$	47.4$^{i\text{-}q\ BC}$
		MW	17.4$^{st\ BC}$	0.3$^{ab\ A}$	0.7$^{ab\ A}$	ND	ND	18.9$^{m\text{-}s\ EF}$	0.2$^{a\ A}$	8.1$^{f\text{-}q\ AB}$	0.1$^{a\text{-}h\ BC}$	45.8$^{h\text{-}p\ BC}$
		SF	13.9$^{rs\ B}$	0.3$^{ab\ A}$	0.6$^{ab\ A}$	ND	ND	4.7$^{a\text{-}e\ A}$	0.2$^{a\ A}$	7.6$^{e\text{-}n\ A}$	0.1$^{a\text{-}c\ AB}$	27.3$^{a\text{-}f\ A}$

Savoy	SC1	R	8.8$^{op\ CD}$	ND	1.0$^{a-c\ A}$	0.5$^{c-l\ A-C}$	ND	25.4$^{st\ EF}$	0.9$^{a\ A}$	9.0$^{h-r\ A}$	0.3$^{j-s\ D}$	45.9$^{h-p\ CD}$
		ST	8.1$^{m-p\ B-D}$	ND	0.9$^{a-c\ A}$	0.4$^{b-h\ A-C}$	ND	24.2$^{s\ EF}$	0.8$^{a\ A}$	6.2$^{a-m\ A}$	0.3$^{h-r\ CD}$	40.9$^{f-i\ B-D}$
		MW	8.2$^{m-p\ B-D}$	ND	0.9$^{a-c\ A}$	0.3$^{b-f\ AB}$	ND	25.0$^{st\ EF}$	0.8$^{a\ A}$	5.9$^{a-l\ A}$	0.3$^{i-s\ CD}$	41.5$^{f-i\ B-D}$
		SF	6.2$^{i-o\ BC}$	ND	0.6$^{ab\ A}$	0.2$^{a-d\ A}$	ND	21.8$^{rs\ C-E}$	0.7$^{a\ A}$	7.8$^{e-o\ A}$	0.1$^{a-h\ AB}$	37.5$^{e-j\ A-C}$
	SC2	R	6.6$^{j-o\ B-D}$	ND	1.0$^{a-c\ A}$	1.7$^{n\ D}$	ND	30.9$^{t\ F}$	0.3$^{a\ A}$	24.9$^{y-Aa\ D}$	0.3$^{i-s\ D}$	65.7$^{r-w\ E}$
		ST	5.2$^{f-n\ B}$	ND	1.6$^{a-d\ AB}$	1.4$^{m\ D}$	ND	20.4$^{p-s\ C-E}$	0.2$^{a\ A}$	20.3$^{wx\ C}$	0.3$^{j-r\ CD}$	49.4$^{i-r\ D}$
		MW	5.2$^{f-n\ AB}$	ND	1.6$^{a-d\ AB}$	1.5$^{mn\ D}$	ND	22.7$^{rs\ D-F}$	0.2$^{a\ A}$	19.3$^{vw\ C}$	0.3$^{j-s\ D}$	50.6$^{j-s\ D}$
		SF	1.6$^{a-f\ A}$	ND	0.5$^{ab\ A}$	0.8$^{j-l\ C}$	ND	14.6$^{k-q\ C-D}$	0.1$^{a\ A}$	14.9$^{t-v\ B}$	0.1$^{a-f\ AB}$	32.6$^{c-i\ AB}$
	SC3	R	14.3$^{rs\ F}$	1.2$^{a-e\ C}$	8.7$^{a-j\ C}$	0.7$^{g-l\ BC}$	ND	60.6$^{u\ G}$	12.4$^{e-h\ C}$	6.3$^{a-m\ A}$	0.2$^{b-m\ BC}$	104.4$^{Ac-Ae\ F}$
		ST	10.0$^{pq\ DE}$	1.0$^{a-d\ C}$	7.2$^{a-g\ C}$	0.5$^{e-j\ A-C}$	ND	10.5$^{e-k\ AB}$	10.6$^{c-g\ C}$	6.1$^{a-l\ A}$	0.1$^{a-h\ AB}$	46.0$^{h-p\ CD}$
		MW	12.8$^{qr\ EF}$	1.0$^{a-d\ C}$	7.8$^{a-l\ C}$	0.6±$^{f-l\ BC}$	ND	13.5$^{j-o\ A-C}$	10.7$^{c-g\ C}$	5.4$^{a-j\ A}$	0.1$^{a-h\ AB}$	51.9$^{j-s\ D}$
		SF	7.4$^{l-p\ B-C}$	0.5$^{a-c\ D}$	3.4$^{a-e\ B}$	0.4$^{b-h\ A-C}$	ND	5.4$^{a-f\ A}$	6.0$^{a-e\ B}$	5.2$^{a-l\ A}$	0.1$^{ab\ A}$	28.5$^{b-g\ A}$
Red	RC1	R	8.0$^{m-p\ F}$	5.0$^{h-l\ DE}$	16.3$^{h-m\ F}$	0.9$^{l\ D}$	ND	20.7$^{q-s\ E}$	25.5$^{l-p\ F}$	28.8$^{z-Aa\ D}$	0.3$^{k-s\ A-D}$	105.5$^{Ad-Ae\ F}$
		ST	7.3$^{l-p\ EF}$	3.6$^{c-l\ C-E}$	10.2$^{d-k\ DE}$	0.8$^{kl\ CD}$	ND	18.9$^{n-s\ DE}$	22.1$^{j-o\ D-F}$	20.7$^{w-y\ BC}$	0.3$^{-s\ A-D}$	84.1$^{x-Ab\ E}$
		MW	6.4$^{i-p\ C-F}$	1.8$^{a-f\ A-C}$	7.0$^{a-f\ B-D}$	0.8$^{i-l\ B-D}$	ND	17.3$^{l-r\ DE}$	18.0$^{h-k\ B-E}$	17.9$^{u-w\ B}$	0.3$^{i-s\ A-C}$	69.5$^{t-x\ DE}$
		SF	5.1$^{e-o\ C}$	1.4$^{a-e\ AB}$	4.6$^{a-e\ AB}$	0.5$^{d-j\ A-C}$	ND	14.3$^{k-q\ CD}$	12.4$^{e-h\ AB}$	21.5$^{w-y\ C}$	0.2$^{b-n\ A}$	60.0$^{n-u\ CD}$
	RC2	R	7.1$^{k-p\ D-F}$	3.2$^{b-l\ B-D}$	8.8$^{b-j\ CD}$	0.7$^{h-l\ A-D}$	3.2$^{fg\ DE}$	12.4$^{h-m\ C}$	18.6$^{h-l\ B-F}$	3.4$^{a-e\ A}$	0.4$^{p-s\ CD}$	57.7$^{l-t\ CD}$
		ST	5.9$^{h-o\ C-E}$	2.1$^{a-k\ A-C}$	6.0$^{a-f\ A-C}$	0.6$^{e-k\ A-D}$	2.7$^{c-f\ B-E}$	11.1$^{e-l\ C}$	13.2$^{f-l\ A-C}$	3.4$^{a-e\ A}$	0.3$^{m-s\ B-D}$	45.4$^{g-p\ BC}$
		MW	5.6$^{g-o\ CD}$	0.9$^{a-d\ A}$	3.1$^{a-e\ A}$	0.5$^{e-j\ A-D}$	1.8$^{b\ BC}$	10.4$^{e-l\ BC}$	11.8$^{d-h\ AB}$	2.9$^{a-c\ A}$	0.4$^{q-s\ CD}$	37.4$^{e-j\ AB}$
		SF	3.3$^{a-j\ B}$	0.9$^{a-d\ A}$	3.9$^{a-e\ AB}$	0.3$^{b-f\ A}$	1.6$^{b\ B}$	5.9$^{a-h\ AB}$	10.4$^{c-g\ A}$	2.9$^{a-c\ A}$	0.2$^{d-p\ AB}$	29.5$^{c-h\ A}$

White	RC3	R	2.2$^{a\text{-}h\ AB}$	5.1$^{k\text{-}l\ E}$	13.5$^{f\text{-}l\ EF}$	0.5$^{d\text{-}j\ A\text{-}C}$	3.9$^{g\ E}$	5.3$^{a\text{-}f\ A}$	25.0$^{l\text{-}p\ EF}$	3.0$^{a\text{-}d\ A}$	0.6$^{v\ F}$	59.2$^{m\text{-}t\ CD}$
		ST	2.2$^{a\text{-}h\ AB}$	4.5$^{f\text{-}l\ DE}$	10.7$^{e\text{-}k\ DE}$	0.4$^{b\text{-}h\ AB}$	3.0$^{ef\ C\text{-}E}$	5.6$^{a\text{-}g\ AB}$	19.8$^{i\text{-}m\ C\text{-}F}$	2.7$^{ab\ A}$	0.5$^{uv\ EF}$	49.5$^{i\text{-}r\ BC}$
		MW	2.3$^{a\text{-}h\ AB}$	3.6$^{d\text{-}l\ C\text{-}E}$	8.6$^{a\text{-}j\ CD}$	0.4$^{b\text{-}g\ AB}$	2.1$^{b\text{-}d\ B\text{-}D}$	5.7$^{a\text{-}g\ AB}$	13.0$^{e\text{-}i\ A\text{-}C}$	1.8$^{a\ A}$	0.6$^{v\ F}$	38.1$^{e\text{-}j\ AB}$
		SF	1.5$^{a\text{-}e\ A}$	3.1$^{a\text{-}l\ B\text{-}D}$	9.2$^{b\text{-}j\ CD}$	0.3$^{a\text{-}e\ A}$	2.2$^{b\text{-}e\ B\text{-}D}$	3.6$^{a\text{-}d\ A}$	17.2$^{g\text{-}j\ A\text{-}D}$	2.6$^{ab\ A}$	0.4$^{s\text{-}u\ DE}$	40.0$^{f\text{-}k\ AB}$
	WC1	R	4.8$^{d\text{-}n\ B}$	1.9$^{a\text{-}h\ B}$	16.5$^{i\text{-}m\ B}$	0.3$^{b\text{-}f\ D}$	1.9$^{bc\ B}$	12.7$^{i\text{-}n\ AB}$	22.1$^{j\text{-}o\ B}$	6.7$^{b\text{-}n\ A\text{-}C}$	0.3$^{n\text{-}s\ B}$	67.4$^{s\text{-}x\ BC}$
		ST	4.5$^{c\text{-}m\ B}$	1.8$^{a\text{-}g\ B}$	16.6$^{j\text{-}m\ B}$	0.3$^{b\text{-}f\ CD}$	1.8$^{b\ B}$	12.7$^{i\text{-}n\ AB}$	21.1$^{j\text{-}n\ B}$	6.1$^{a\text{-}m\ AB}$	0.3$^{i\text{-}s\ B}$	65.4$^{r\text{-}w\ BC}$
		MW	4.8$^{d\text{-}n\ B}$	1.7$^{a\text{-}f\ B}$	14.7$^{f\text{-}m\ B}$	0.3$^{a\text{-}e\ B\text{-}D}$	1.9$^{bc\ B}$	12.1$^{g\text{-}l\ AB}$	20.3$^{j\text{-}m\ B}$	5.6$^{a\text{-}k\ AB}$	0.4$^{q\text{-}s\ B}$	61.8$^{o\text{-}v\ B}$
		SF	2.2$^{a\text{-}h\ A}$	0.7$^{a\text{-}d\ A}$	7.6$^{a\text{-}h\ A}$	0.2$^{a\text{-}c\ A\text{-}C}$	1.4$^{b\ B}$	6.1$^{a\text{-}h\ A}$	13.2$^{f\text{-}l\ A}$	4.2$^{a\text{-}g\ A}$	0.1$^{a\text{-}h\ A}$	35.8$^{d\text{-}j\ A}$
	WC2	R	8.3$^{n\text{-}p\ C}$	2.1$^{a\text{-}j\ B}$	17.2$^{j\text{-}m\ B}$	0.3$^{a\text{-}e\ A\text{-}D}$	ND	22.5$^{rs\ C}$	23.7$^{j\text{-}o\ B}$	29.2$^{Aa\ E}$	0.2$^{b\text{-}l\ A}$	103.4$^{Ac\text{-}Ae\ E}$
		ST	7.9$^{m\text{-}p\ C}$	2.0$^{a\text{-}l\ B}$	15.8$^{g\text{-}m\ B}$	0.2$^{a\text{-}d\ A\text{-}C}$	ND	21.5$^{rs\ C}$	21.8$^{j\text{-}o\ B}$	14.1$^{s\text{-}u\ D}$	0.2$^{a\text{-}j\ A}$	83.5$^{x\text{-}Ab\ D}$
		MW	7.4$^{l\text{-}p\ C}$	1.6$^{a\text{-}f\ B}$	14.5$^{f\text{-}m\ B}$	0.2$^{a\text{-}c\ AB}$	ND	22.0$^{rs\ C}$	21.6$^{j\text{-}o\ B}$	9.5$^{i\text{-}s\ C}$	0.2$^{a\text{-}l\ A}$	77.0$^{u\text{-}z\ CD}$
		SF	4.0$^{c\text{-}l\ B}$	1.4$^{a\text{-}e\ B}$	9.5$^{c\text{-}j\ A}$	0.1$^{ab\ A}$	ND	13.9$^{k\text{-}p\ B}$	18.0$^{h\text{-}k\ AB}$	8.5$^{g\text{-}r\ BC}$	0.1$^{a\text{-}h\ A}$	55.6$^{k\text{-}t\ B}$
	P-value		< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.001	< 0.0001

Letters 'ABC': mean values with different superscripts in the same column are significantly different (P<0.0001) across varieties and growing conditions within a cabbage type. Letters 'abc': mean values with different superscripts in the same column are significantly different (P<0.05) across varieties and growing conditions. Abbreviations: R = raw, ST = steamed, MW = microwaved, SF = stir-fried; SIN, sinigrin; GPN, gluconapin; PROG, epi/progoitrin; GIBVN, Glucoibeverin; GER, glucoerucin; GIBN, glucoiberin; GRPN, glucoraphanin; GBSN, glucobrassicin; 4- HOH, 4-hydroxyglucobrassicin. For full names of cabbage varieties see Table 3.1 and Table 3.2 for compound names.

Table S4b: Glucosinolate hydrolysis concentration (µg/g DW sulforaphane equivalent) of cooked cabbage

			Glucosinolate hydrolysis products (µg/g) DW																				
Type	Accession	Treatment	ATC	AITC	CETP	3BITC	EVN	GN	CHETB-1	CHETB-2	IBVN	4MBN	ER	ERN	IB	IBN	PEITC	BPN	SFP	SFN	I3C	1IAN	PITC	1H-I	BAN	Total GHP
Black kale	BK1	R	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	1.5$^{ab\ A}$	22.6$^{a-c\ D}$	ND	1.0$^{a-e\ D}$	11.0$^{a\ A}$	147.5$^{hi\ D}$	66.5$^{i\ C}$	152.1$^{o\ E}$	ND	1.3$^{a-e\ C}$	ND	403.4$^{h-o\ F}$
		ST	ND	ND	ND	0.2$^{a\ A}$	ND	ND	ND	ND	ND	ND	ND	ND	11.5$^{ab\ C}$	ND	ND	ND	133.9$^{c-g\ D}$	5.0$^{ab\ A}$	1.4$^{a-e\ A}$	32.0$^{k-m\ A-C}$	ND	ND	ND	184.0$^{a-i\ D}$
		MW	ND	ND	ND	0.1$^{a\ A}$	ND	ND	ND	ND	ND	ND	ND	ND	12.7$^{ab\ C}$	ND	ND	ND	69.0$^{a-e\ C}$	6.7$^{ab\ AB}$	1.6$^{a-e\ A}$	45.5$^{m\ C}$	ND	ND	ND	135.7$^{a-h\ A-D}$
		SF	ND	ND	ND	0.3$^{a\ A}$	ND	ND	ND	ND	ND	ND	ND	ND	4.6$^{ab\ AB}$	6.3$^{ab\ BC}$	ND	0.6$^{a-d\ B}$	36.3$^{a-d\ A-C}$	85.9$^{ef\ C}$	29.5$^{j\ B}$	19.9$^{if-k\ AB}$	ND	0.6$^{a-c\ B}$	ND	184.0$^{a-i\ D}$
	BK2	R	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.7$^{a\ A}$	8.5$^{ab\ C}$	ND	1.0$^{a-e\ D}$	8.7$^{a\ A}$	133.9$^{gh\ D}$	43.3$^{k\ B}$	72.5$^{n\ D}$	ND	0.6$^{a-d\ B}$	ND	269.3$^{a-m\ E}$
		ST	ND	ND	ND	0.1$^{a\ A}$	ND	ND	ND	ND	ND	ND	ND	ND	11.4$^{ab\ C}$	ND	ND	ND	37.2$^{a-d\ A-C}$	0.5$^{a\ A}$	0.6$^{a-c\ A}$	19.3$^{f-k\ AB}$	ND	ND	ND	69.2$^{a-d\ AB}$
		MW	ND	ND	ND	0.2$^{a\ A}$	ND	ND	ND	ND	ND	ND	ND	ND	6.1$^{ab\ B}$	ND	ND	ND	141.2$^{d-h\ D}$	0.7$^{a\ A}$	9.1$^{d-i\ A}$	12.6$^{a-i\ AB}$	ND	ND	ND	170.0$^{a-i\ CD}$
		SF	ND	ND	ND	0.3$^{a\ AB}$	ND	ND	ND	ND	ND	ND	ND	ND	15.2$^{ab\ C}$	2.1$^{a\ AB}$	ND	0.2$^{a\ A}$	149.5$^{e-i\ D}$	28.5$^{a-d\ AB}$	6.3$^{a-h\ A}$	7.8$^{a-g\ A}$	ND	0.5$^{ab\ B}$	ND	210.4$^{a-j\ DE}$
	BK3	R	ND	ND	ND	1.0$^{a-c\ CD}$	ND	ND	ND	ND	ND	ND	<0.1$^{a\ A}$	1.0$^{a\ C}$	0.9$^{a\ A}$	4.6$^{ab\ A-C}$	ND	0.9$^{a-e\ CD}$	17.8$^{ab\ AB}$	100.2$^{fg\ C}$	85.8$^{m\ D}$	137.6$^{o\ E}$	ND	2.7$^{a-e\ D}$	ND	352.6$^{d-o\ F}$
		ST	ND	ND	ND	1.8$^{a-e\ E}$	ND	ND	ND	ND	ND	ND	0.3$^{a\ B}$	0.1$^{a\ A}$	3.6$^{ab\ AB}$	ND	ND	ND	49.5$^{a-e\ BC}$	2.2$^{a\ A}$	2.1$^{a-e\ A}$	31.8$^{j-m\ A-C}$	ND	ND	ND	91.4$^{a-f\ A-C}$
		MW	ND	ND	ND	0.7$^{ab\ BC}$	ND	ND	ND	ND	ND	ND	0.3$^{a\ B}$	ND	1.4$^{ab\ A}$	ND	ND	ND	16.4$^{ab\ AB}$	0.6$^{a\ A}$	1.3$^{a-e\ A}$	36.4$^{lm\ BC}$	ND	ND	ND	57.1$^{a-c\ A}$
		SF	ND	ND	ND	1.0$^{a-d\ D}$	ND	ND	ND	ND	ND	ND	0.1$^{a\ A}$	0.6$^{a\ B}$	4.2$^{ab\ AB}$	4.2$^{ab\ A-C}$	ND	0.6$^{a-e\ BC}$	33.5$^{a-d\ A-C}$	40.1$^{b-d\ B}$	44.7$^{k\ B}$	16.0$^{b-i\ AB}$	ND	1.4$^{a-e\ C}$	ND	146.7$^{a-i\ B-D}$
Wild	WD1	R	ND	ND	7.6$^{a-e\ C}$	0.3$^{a\ A}$	156.7$^{f\ C}$	5.5$^{ab\ A}$	434.3$^{e\ C}$	521.0$^{e\ C}$	ND	ND	ND	ND	ND	ND	0.4$^{a-d\ AB}$	11.2$^{i\ C}$	ND	ND	6.5$^{a-h\ B}$	40.3$^{m\ E}$	0.3$^{a\ A}$	2.8$^{a-e\ CD}$	ND	1186.9$^{y\ C}$
		ST	0.1$^{ab\ B}$	0.5$^{a-c\ A}$	ND	5.4$^{c-h\ BC}$	3.4$^{a-c\ A}$	298.1$^{ij\ CD}$	2.1$^{a\ A}$	4.3$^{ab\ A}$	ND	ND	ND	ND	ND	ND	1.3$^{i\ C}$	ND	ND	ND	0.7$^{a-d\ A}$	22.5$^{g-l\ A-C}$	13.0$^{h\ C}$	0.8$^{a-d\ AB}$	ND	352.3$^{d-o\ AB}$
		MW	<0.1$^{a\ AB}$	0.1$^{ab\ A}$	ND	0.7$^{ab\ A}$	2.5$^{a\ A}$	56.3$^{a-f\ AB}$	0.8$^{a\ A}$	1.7$^{a\ A}$	ND	ND	ND	ND	ND	ND	<0.1$^{ab\ A}$	ND	ND	ND	0.8$^{a-d\ A}$	25.3$^{i-l\ A-D}$	2.1$^{a-d\ A}$	0.2$^{a\ A}$	ND	90.4$^{a-f\ A}$
		SF	<0.1$^{a\ AB}$	0.2$^{ab\ A}$	3.8$^{a-c\ B}$	1.9$^{a-f\ A}$	97.9$^{e\ B}$	246.2$^{hi\ CD}$	301.8$^{c\ B}$	369.7$^{d\ B}$	ND	ND	ND	ND	ND	ND	0.4$^{a-e\ B}$	4.7$^{gh\ B}$	ND	ND	12.1$^{g-i\ C}$	18.8$^{e-k\ AB}$	8.8$^{g\ BC}$	2.3$^{a-e\ CD}$	ND	1068.6$^{v-y\ C}$
	WD2	R	ND	ND	13.1$^{c-e\ D}$	1.0$^{a-c\ A}$	29.2$^{d\ A}$	184.9$^{gh\ BC}$	37.6$^{ab\ A}$	43.7$^{a-c\ A}$	ND	ND	ND	ND	4.3$^{a\ AB}$	18.8$^{a-c\ C}$	0.1$^{ab\ AB}$	2.1$^{b-f\ AB}$	31.7$^{a-d\ A}$	180.3$^{ij\ C}$	13.3$^{hi\ C}$	37.0$^{lm\ DE}$	<0.1$^{a\ A}$	3.0$^{a-e\ CD}$	ND	600.0$^{o-t\ B}$
		ST	0.3$^{a-e\ C}$	1.9$^{a-g\ B}$	ND	8.1$^{hi\ C}$	0.1$^{a\ A}$	63.7$^{a-f\ AB}$	0.5$^{a\ A}$	0.8$^{a\ A}$	ND	ND	ND	ND	39.7$^{a\ D}$	ND	0.3$^{a-d\ AB}$	ND	218.3$^{g-i\ C}$	6.3$^{ab\ A}$	1.6$^{a-e\ A}$	18.9$^{e-k\ AB}$	2.5$^{a-f\ A}$	3.0$^{a-e\ D}$	ND	366.2$^{f-o\ AB}$
		MW	<0.1$^{ab\ AB}$	0.3$^{ab\ A}$	ND	2.1$^{a-f\ A}$	<0.1$^{a\ A}$	16.6$^{a-c\ A}$	<0.1$^{a\ A}$	0.4$^{a\ A}$	ND	ND	ND	ND	12.0$^{a\ B}$	ND	<0.1$^{ab\ A}$	ND	24.8$^{a-c\ A}$	15.3$^{a-d\ A}$	1.6$^{a-e\ A}$	35.1$^{lm\ C-E}$	0.8$^{a\ A}$	1.8$^{a-e\ BC}$	ND	110.9$^{a-g\ A}$
		SF	0.1$^{ab\ AB}$	0.4$^{a-c\ A}$	5.7$^{a-d\ BC}$	2.5$^{a-f\ AB}$	11.3$^{a-d\ A}$	31.2$^{a-d\ A}$	16.6$^{a\ A}$	21.8$^{ab\ A}$	ND	ND	ND	ND	23.4$^{a\ C}$	5.9$^{ab\ B}$	<0.1$^{a\ A}$	0.8$^{a-e\ A}$	149.6$^{e-i\ B}$	48.0$^{cd\ B}$	17.4$^{i\ D}$	16.4$^{c-i\ A}$	1.4$^{a-c\ A}$	2.3$^{a-e\ CD}$	ND	354.8$^{e-o\ AB}$
	WD3	R	ND	ND	0.8$^{a\ A}$	0.3$^{a\ A}$	148.1$^{f\ C}$	7.2$^{ab\ A}$	369.2$^{d\ BC}$	423.5$^{d\ BC}$	ND	ND	ND	ND	<0.1$^{a\ A}$	0.6$^{a\ A}$	<0.1$^{ab\ A}$	3.0$^{fg\ AB}$	1.8$^{a\ A}$	1.7$^{a\ A}$	6.7$^{a-h\ B}$	36.1$^{lm\ C-E}$	0.3$^{a\ A}$	2.9$^{a-e\ CD}$	0.7$^{bc\ B}$	1002.9$^{u-y\ C}$
		ST	<0.1$^{a\ A}$	<0.1$^{ab\ A}$	ND	12.5$^{ij\ D}$	1.8$^{a\ A}$	328.0$^{j\ D}$	2.3$^{a\ A}$	3.8$^{ab\ A}$	ND	ND	ND	ND	1.8$^{a\ A}$	ND	0.5$^{a-f\ B}$	ND	2.2$^{a\ A}$	<0.1$^{a\ A}$	0.6$^{a-c\ A}$	23.6$^{h-l\ A-D}$	4.0$^{b-f\ AB}$	0.6$^{a-d\ A}$	ND	381.8$^{g-o\ AB}$
		MW	<0.1$^{a\ AB}$	<0.1$^{ab\ A}$	ND	1.7$^{a-e\ A}$	0.4$^{a\ A}$	205.1$^{h\ CD}$	3.3$^{a\ A}$	5.3$^{ab\ A}$	ND	ND	ND	ND	0.4$^{a\ A}$	ND	0.1$^{ab\ AB}$	ND	3.2$^{a\ A}$	<0.1$^{a\ A}$	1.5$^{a-e\ A}$	32.7$^{k-m\ B-E}$	2.9$^{a-f\ A}$	0.5$^{ab\ A}$	ND	257.3$^{a-m\ AB}$
		SF	0.1$^{ab\ AB}$	<0.1$^{a\ A}$	0.6$^{a\ A}$	0.6$^{ab\ A}$	137.3$^{f\ BC}$	309.4$^{ij\ CD}$	285.4$^{c\ B}$	366.6$^{d\ B}$	ND	ND	ND	ND	0.2$^{a\ A}$	<0.1$^{a\ A}$	<0.1$^{ab\ A}$	1.1$^{a-e\ A}$	8.9$^{ab\ A}$	0.1$^{a\ A}$	13.2$^{hi\ C}$	16.9$^{d-j\ A}$	5.2$^{ef\ AB}$	3.0$^{a-e\ D}$	0.3$^{ab\ AB}$	1149.1$^{xy\ C}$
Tronchuda	TC1	R	0.1$^{ab\ AB}$	0.4$^{a-c\ A}$	54.5$^{i\ D}$	ND	3.8$^{a-c\ AB}$	0.5$^{a\ A}$	3.6$^{a\ A}$	4.7$^{ab\ A}$	ND	1.9$^{b-e\ C}$	ND	ND	6.5$^{ab\ AB}$	54.6$^{de\ C}$	ND	0.6$^{a-d\ CD}$	1.8$^{a\ A}$	7.0$^{ab\ C}$	4.1$^{a-g\ BC}$	13.7$^{a-i\ F}$	ND	2.2$^{a-e\ C}$	0.2$^{a\ C}$	160.3$^{a-i\ B-D}$
		ST	1.2$^{ij\ C}$	4.9$^{h-j\ BC}$	0.2$^{a\ A}$	0.9$^{a-c\ AB}$	<0.1$^{a\ A}$	20.9$^{a-d\ AB}$	0.4$^{a\ A}$	0.5$^{a\ A}$	0.4$^{ab\ B}$	ND	ND	ND	44.5$^{ab\ B-D}$	0.7$^{a\ A}$	<0.1$^{ab\ A}$	<0.1$^{a\ A}$	10.7$^{a\ A}$	ND	0.6$^{a-c\ A}$	13.2$^{a-i\ F}$	1.1$^{ab\ BC}$	1.7$^{a-e\ A-C}$	ND	102.1$^{a-g\ A-C}$
		MW	1.2$^{h-j\ C}$	7.9$^{k\ C}$	ND	1.5$^{a-d\ B}$	ND	8.7$^{ab\ A}$	0.3$^{a\ A}$	0.3$^{a\ A}$	0.5$^{ab\ BC}$	ND	ND	ND	58.6$^{a-c\ DE}$	ND	<0.1$^{ab\ A}$	ND	5.3$^{a\ A}$	ND	0.4$^{ab\ A}$	9.1$^{a-h\ E}$	0.4$^{a\ A-C}$	1.6$^{a-e\ A-C}$	ND	95.9$^{a-f\ A-C}$
		SF	0.4$^{a-f\ AB}$	0.9$^{a-e\ A}$	12.5$^{b-e\ C}$	0.3$^{a\ AB}$	1.3$^{a\ A}$	3.6$^{a\ A}$	2.6$^{a\ A}$	3.8$^{ab\ A}$	0.1$^{a\ A}$	0.2$^{a\ AB}$	ND	ND	28.1$^{ab\ A-D}$	11.9$^{ab\ AB}$	ND	0.2$^{a\ AB}$	5.9$^{a\ A}$	0.4$^{a\ A}$	5.4$^{a-h\ C}$	2.7$^{a-d\ AB}$	0.2$^{a\ AB}$	1.4$^{a-e\ A-C}$	0.1$^{a\ AB}$	82.0$^{a-e\ AB}$
	TC2	R	0.1$^{ab\ A}$	0.3$^{ab\ A}$	7.7$^{a-e\ A-C}$	ND	27.0$^{b-d\ C}$	0.3$^{a\ A}$	49.7$^{ab\ B}$	75.9$^{bc\ B}$	ND	0.6$^{a-c\ B}$	ND	ND	5.5$^{ab\ A}$	21.4$^{a-c\ B}$	ND	1.4$^{a-f\ E}$	1.3$^{a\ A}$	6.6$^{ab\ C}$	3.4$^{a-f\ B}$	7.4$^{a-f\ DE}$	ND	2.0$^{a-e\ BC}$	0.1$^{a\ B}$	210.7$^{a-j\ D}$
		ST	0.3$^{a-d\ AB}$	2.1$^{a-h\ AB}$	0.1$^{a\ A}$	3.0$^{a-g\ C}$	<0.1$^{a\ A}$	92.3$^{c-f\ D}$	0.3$^{a\ A}$	0.5$^{a\ A}$	0.7$^{ab\ CD}$	ND	ND	ND	53.8$^{a-c\ C-E}$	0.6$^{a\ A}$	0.3$^{a-d\ B}$	0.1$^{a\ AB}$	10.1$^{ab\ A}$	ND	0.3$^{ab\ A}$	6.5$^{a-f\ C-E}$	2.3$^{a-e\ DE}$	0.9$^{a-d\ AB}$	ND	174.3$^{a-i\ CD}$
		MW	0.1$^{ab\ A}$	0.5$^{a-c\ A}$	ND	1.1$^{a-c\ AB}$	ND	43.4$^{a-e\ BC}$	0.2$^{a\ A}$	0.4$^{a\ A}$	0.6$^{ab\ B-D}$	ND	ND	ND	17.5$^{ab\ A-C}$	ND	0.2$^{a-d\ B}$	ND	2.0$^{a\ A}$	ND	0.4$^{ab\ A}$	8.7$^{a-h\ E}$	1.3$^{a-c\ CD}$	0.8$^{a-d\ A}$	ND	77.4$^{a-e\ AB}$
		SF	0.3$^{a-d\ AB}$	2.2$^{a-h\ AB}$	3.6$^{a-c\ A-C}$	0.8$^{ab\ AB}$	12.6$^{a-d\ B}$	60.1$^{a-f\ C}$	39.5$^{ab\ B}$	62.4$^{a-c\ B}$	0.8$^{a-c\ D}$	0.6$^{a-c\ B}$	ND	ND	91.2$^{a-e\ E}$	18.2$^{a-c\ B}$	ND	0.6$^{a-d\ D}$	27.1$^{a-c\ B}$	0.8$^{a\ AB}$	9.5$^{e-i\ D}$	3.1$^{a-d\ A-C}$	2.7$^{a-f\ E}$	1.6$^{a-e\ A-C}$	ND	337.7$^{c-o\ E}$
	TC3	R	0.2$^{a-c\ AB}$	2.7$^{a-i\ AB}$	11.4$^{b-e\ BC}$	ND	0.3$^{a\ A}$	1.1$^{a\ A}$	0.7$^{a\ A}$	0.8$^{a\ A}$	ND	0.5$^{a-c\ B}$	ND	ND	26.6$^{ab\ A-D}$	3.6$^{ab\ A}$	ND	0.4$^{a-c\ B-D}$	2.3$^{a\ A}$	2.1$^{a\ B}$	4.6$^{a-g\ BC}$	5.5$^{a-f\ B-E}$	ND	2.0$^{a-e\ A-C}$	<0.1$^{a\ A}$	64.9$^{a-c\ A}$
		ST	0.4$^{a-f\ B}$	3.3$^{c-j\ AB}$	0.5$^{a\ AB}$	0.2$^{a\ A}$	0.1$^{a\ A}$	0.8$^{a\ A}$	<0.1$^{a\ A}$	<0.1$^{a\ A}$	0.6$^{ab\ B-D}$	ND	ND	ND	26.8$^{ab\ A-D}$	0.3$^{a\ A}$	<0.1$^{ab\ A}$	0.1$^{a\ AB}$	2.4$^{a\ A}$	ND	0.3$^{ab\ A}$	1.9$^{a-d\ AB}$	<0.1$^{a\ A}$	1.0$^{a-d\ AB}$	ND	38.9$^{a\ A}$
		MW	0.1$^{ab\ AB}$	1.3$^{a-f\ AB}$	ND	0.1$^{a\ A}$	ND	0.4$^{a\ A}$	<0.1$^{a\ A}$	<0.1$^{a\ A}$	0.6$^{ab\ B-D}$	ND	ND	ND	16.1$^{ab\ A-C}$	ND	<0.1$^{ab\ A}$	ND	1.4$^{a\ A}$	ND	0.4$^{ab\ A}$	3.7$^{a-d\ A-D}$	<0.1$^{a\ A}$	0.9$^{a-d\ AB}$	ND	25.3$^{a\ A}$
		SF	0.1$^{ab\ AB}$	1.2$^{a-f\ AB}$	5.5$^{a-d\ A-C}$	0.2$^{a\ A}$	0.3$^{a\ A}$	2.0$^{a\ A}$	0.5$^{a\ A}$	0.7$^{a\ A}$	0.7$^{ab\ CD}$	0.5$^{a-c\ B}$	ND	ND	28.9$^{ab\ A-D}$	2.8$^{ab\ A}$	ND	0.3$^{ab\ A-C}$	1.8$^{a\ A}$	<0.1$^{a\ A}$	3.7$^{a-g\ B}$	1.3$^{ab\ A}$	<0.1$^{a\ A}$	1.0$^{a-d\ AB}$	ND	51.7$^{ab\ A}$

Savoy	SC1	R	$0.8^{f\text{-}h\ BC}$	$4.9^{g\text{-}j\ BC}$	$89.9^{j\ D}$	ND	$2.9^{ab\ C}$	ND	$5.4^{a\ C}$	$6.1^{ab\ D}$	$<0.1^{a\ A}$	$6.1^{h\ C}$	ND	ND	$280.5^{g\ B}$	$291.4^{g\ C}$	ND	$6.1^{a\ D}$	$8.0^{a\ AB}$	$13.8^{a\text{-}c\ B}$	$13.6^{hi\ E}$	$24.3^{i\text{-}l\ F}$	ND	$19.1^{g\ C}$	$0.2^{a\ A}$	$773.1^{q\text{-}u\ C}$
		ST	$1.9^{k\ D}$	$17.1^{m\ E}$	$1.0^{a\ A}$	$0.9^{a\text{-}c\ B}$	ND	$6.4^{ab\ BC}$	$0.4^{a\ A}$	$0.6^{a\ A}$	$5.6^{ef\ C}$	$0.3^{ab\ A}$	ND	ND	$712.1^{h\ C}$	$19.1^{a\text{-}c\ A}$	$1.0^{e\text{-}i\ B\text{-}D}$	$0.5^{a\text{-}d\ AB}$	$16.1^{ab\ AB}$	ND	$1.0^{a\text{-}d\ A}$	$11.9^{a\text{-}i\ D}$	$0.3^{a\ BC}$	$10.0^{f\ B}$	ND	$806.2^{f\text{-}v\ C}$
		MW	$0.4^{a\text{-}f\ AB}$	$6.0^{jk\ C}$	$1.0^{a\ A}$	$0.3^{a\ AB}$	ND	$4.9^{ab\ B}$	$0.3^{a\ A}$	$0.3^{a\ A}$	$2.7^{a\text{-}e\ B}$	$0.4^{a\text{-}c\ A}$	ND	ND	$242.1^{fg\ B}$	$10.1^{ab\ A}$	$1.1^{g\text{-}i\ CD}$	ND	$6.4^{a\ AB}$	ND	$0.9^{a\text{-}d\ A}$	$11.8^{a\text{-}i\ D}$	$0.2^{a\ B}$	$3.7^{c\text{-}e\ A}$	ND	$292.8^{a\text{-}m\ B}$
		SF	$1.3^{ij\ CD}$	$13.5^{i\ D}$	$11.5^{b\text{-}e\ B}$	$0.6^{ab\ AB}$	$0.5^{a\ A}$	$3.7^{a\ AB}$	$2.1^{a\ B}$	$1.4^{a\ A\text{-}C}$	$2.8^{a\text{-}e\ B}$	$1.0^{a\text{-}c\ A}$	ND	ND	$983.4^{i\ D}$	$45.6^{cd\ AB}$	$1.3^{i\ D}$	$1.4^{a\text{-}f\ B}$	$18.4^{ab\ B}$	$1.3^{a\ A}$	$10.6^{f\text{-}i\ D}$	$4.9^{a\text{-}f\ A}$	$0.2^{a\ BC}$	$1.2^{a\text{-}d\ A}$	$0.1^{a\ A}$	$1107.1^{w\text{-}y\ D}$
	SC2	R	$0.1^{ab\ A}$	$0.6^{a\text{-}d\ A}$	$12.4^{b\text{-}e\ B}$	ND	$0.6^{a\ A}$	ND	$0.8^{a\ AB}$	$1.1^{a\ AB}$	ND	$9.3^{i\ D}$	ND	ND	$21.6^{ab\ A}$	$31.8^{b\text{-}d\ A}$	ND	$0.3^{ab\ AB}$	$1.5^{a\ A}$	$2.9^{a\ AB}$	$2.9^{a\text{-}f\ AB}$	$11.4^{a\text{-}i\ CD}$	ND	$2.2^{a\text{-}e\ A}$	$0.7^{c\ B}$	$100.3^{a\text{-}g\ A}$
		ST	$0.2^{a\text{-}c\ AB}$	$1.7^{a\text{-}f\ AB}$	$3.4^{a\text{-}c\ AB}$	$0.2^{a\ AB}$	ND	$2.9^{a\ AB}$	$0.5^{a\ A}$	$0.5^{a\ A}$	$2.3^{a\text{-}d\ AB}$	$0.3^{ab\ A}$	ND	ND	$56.1^{a\text{-}c\ A}$	$19.1^{a\text{-}c\ A}$	$0.2^{a\text{-}d\ AB}$	$0.2^{a\ A}$	$2.9^{a\ AB}$	ND	$2.6^{a\text{-}f\ AB}$	$8.2^{a\text{-}g\ B}$	$0.1^{a\ AB}$	$2.4^{a\text{-}e\ A}$	ND	$104.0^{a\text{-}g\ AB}$
		MW	$0.4^{a\text{-}f\ AB}$	$2.6^{a\text{-}i\ AB}$	$0.1^{a\ A}$	$0.2^{a\ AB}$	ND	$5.1^{ab\ B}$	$0.2^{a\ A}$	$0.1^{a\ A}$	$8.5^{fg\ D}$	$0.6^{a\text{-}c\ A}$	ND	ND	$70.8^{a\text{-}d\ A}$	$2.3^{ab\ A}$	$0.4^{a\text{-}e\ A\text{-}C}$	ND	$2.7^{a\ AB}$	ND	$0.8^{a\text{-}d\ A}$	$5.5^{a\text{-}f\ AB}$	$0.2^{a\ BC}$	$4.5^{e\ AB}$	ND	$105.2^{a\text{-}g\ AB}$
		SF	$0.3^{a\text{-}d\ AB}$	$2.1^{a\text{-}h\ AB}$	$3.0^{ab\ AB}$	$0.3^{a\ AB}$	$0.3^{a\ A}$	$3.1^{a\ AB}$	$0.5^{a\ A}$	$0.4^{a\ A}$	$1.5^{a\text{-}c\ AB}$	$3.9^{fg\ BC}$	ND	ND	$58.6^{a\text{-}c\ A}$	$15.1^{ab\ A}$	$<0.1^{ab\ A}$	$0.2^{a\ A}$	$3.0^{a\ AB}$	$0.3^{a\ A}$	$4.0^{a\text{-}g\ B}$	$2.6^{a\text{-}d\ A}$	$0.2^{a\ B}$	$1.8^{a\text{-}e\ A}$	ND	$101.2^{a\text{-}g\ A}$
	SC3	R	$0.1^{ab\ A}$	$1.3^{a\text{-}f\ A}$	$35.2^{h\ C}$	ND	$1.7^{a\ B}$	ND	$3.9^{a\ C}$	$2.9^{ab\ C}$	ND	$2.7^{d\text{-}f\ AB}$	ND	ND	$23.9^{ab\ A}$	$87.3^{f\ B}$	ND	$4.6^{gh\ C}$	$4.1^{a\ AB}$	$26.2^{a\text{-}d\ C}$	$2.7^{a\text{-}f\ AB}$	$15.1^{a\text{-}i\ E}$	ND	$2.5^{a\text{-}e\ A}$	$1.8^{d\ C}$	$216.1^{a\text{-}k\ AB}$
		ST	$0.3^{a\text{-}d\ AB}$	$2.0^{a\text{-}h\ AB}$	$0.3^{a\ A}$	$1.9^{a\text{-}f\ C}$	ND	$10.4^{ab\ C}$	$0.3^{a\ A}$	$0.4^{a\ A}$	$1.9^{a\text{-}c\ AB}$	$0.2^{a\ A}$	ND	ND	$26.7^{ab\ A}$	$1.2^{a\ A}$	$1.3^{hi\ D}$	$0.6^{a\text{-}d\ AB}$	$46.0^{a\text{-}e\ C}$	ND	$0.6^{a\text{-}c\ A}$	$8.5^{a\text{-}g\ BC}$	$0.5^{a\ D}$	$2.1^{a\text{-}e\ A}$	ND	$105.2^{a\text{-}g\ AB}$
		MW	$0.3^{a\text{-}e\ AB}$	$2.0^{a\text{-}h\ AB}$	$0.2^{a\ A}$	$2.3^{a\text{-}f\ C}$	ND	$18.0^{a\text{-}c\ D}$	$0.3^{a\ A}$	$0.3^{a\ A}$	$2.7^{a\text{-}e\ B}$	$<0.1^{a\ A}$	ND	ND	$39.5^{ab\ A}$	$1.1^{a\ A}$	$3.1^{i\ E}$	ND	$38.3^{a\text{-}d\ C}$	ND	$0.8^{a\text{-}d\ A}$	$8.4^{a\text{-}g\ BC}$	$0.3^{a\ C}$	$2.5^{a\text{-}e\ A}$	ND	$120.5^{a\text{-}h\ AB}$
		SF	$0.1^{ab\ A}$	$0.7^{a\text{-}e\ A}$	$2.9^{ab\ AB}$	$0.8^{ab\ AB}$	$1.7^{a\ B}$	$3.3^{a\ AB}$	$2.1^{a\ B}$	$2.5^{a\ BC}$	$0.5^{ab\ AB}$	$1.5^{a\text{-}d\ AB}$	ND	ND	$11.5^{ab\ A}$	$3.7^{ab\ A}$	$0.3^{a\text{-}d\ A\text{-}C}$	$0.6^{a\text{-}d\ AB}$	$16.3^{ab\ AB}$	$6.3^{ab\ AB}$	$7.0^{a\text{-}h\ C}$	$3.3^{a\text{-}d\ A}$	$0.2^{a\ BC}$	$1.1^{a\text{-}d\ A}$	ND	$66.6^{a\text{-}c\ A}$
Red	RC1	R	$<0.1^{ab\ A}$	$0.1^{ab\ A}$	$13.8^{d\text{-}f\ BC}$	$0.5^{a\ A}$	$13.4^{a\text{-}d\ BC}$	$0.7^{a\ A}$	$12.4^{a\ AB}$	$13.4^{ab\ AB}$	ND	$3.0^{d\text{-}f\ BC}$	$1.4^{ab\ A}$	$16.3^{d\ D}$	$2.8^{ab\ A}$	$17.9^{a\text{-}c\ B}$	$0.2^{a\text{-}c\ A}$	$0.6^{a\text{-}d\ AB}$	$14.2^{ab\ A}$	$50.4^{de\ B}$	$0.7^{a\text{-}d\ A}$	$1.4^{a\text{-}c\ A\text{-}C}$	ND	$0.6^{a\text{-}c\ A\text{-}C}$	ND	$164.0^{a\text{-}i\ AB}$
		ST	$0.3^{a\text{-}d\ C}$	$2.2^{a\text{-}h\ BC}$	ND	$2.8^{a\text{-}f\ A}$	ND	$16.6^{a\text{-}c\ A}$	$0.1^{a\ A}$	$0.2^{a\ A}$	$2.1^{a\text{-}d\ A}$	ND	$6.1^{cd\ A\text{-}C}$	$0.7^{a\ A}$	$39.7^{ab\ A}$	ND	$0.4^{a\text{-}d\ A\text{-}C}$	ND	$49.8^{a\text{-}e\ A\text{-}C}$	$1.0^{a\ A}$	$0.1^{a\ A}$	$0.3^{a\ AB}$	$0.3^{a\ AB}$	$0.4^{a\ AB}$	ND	$122.1^{a\text{-}h\ AB}$
		MW	$0.2^{ab\ A\text{-}C}$	$1.2^{a\text{-}e\ AB}$	ND	$1.8^{a\text{-}e\ A}$	ND	$9.2^{ab\ A}$	$<0.1^{a\ A}$	$<0.1^{a\ A}$	$1.2^{a\text{-}c\ A}$	ND	$3.4^{a\text{-}c\ AB}$	ND	$15.8^{ab\ A}$	ND	$0.2^{a\text{-}d\ AB}$	ND	$14.9^{ab\ A}$	$0.1^{a\ A}$	$0.1^{a\ A}$	$0.2^{a\ A}$	$0.3^{a\ AB}$	$0.1^{a\ A}$	ND	$48.7^{ab\ A}$
		SF	$0.1^{ab\ AB}$	$0.6^{a\text{-}e\ AB}$	$7.1^{a\text{-}e\ AB}$	$0.9^{ab\ A}$	$2.9^{ab\ A}$	$2.1^{a\ A}$	$3.0^{a\ A}$	$3.1^{a\ A}$	$0.3^{ab\ A}$	$1.4^{a\text{-}d\ AB}$	$1.1^{ab\ A}$	$2.9^{a\ AB}$	$16.0^{ab\ A}$	$8.3^{ab\ AB}$	$0.1^{a\text{-}c\ A}$	$0.2^{a\ AB}$	$23.6^{ab\ AB}$	$9.5^{ab\ A}$	$0.4^{ab\ A}$	$0.4^{a\ AB}$	ND	$0.1^{a\ A}$	ND	$84.1^{a\text{-}f\ AB}$
	RC2	R	$0.1^{ab\ AB}$	$0.5^{a\text{-}c\ AB}$	$30.2^{gh\ D}$	$2.3^{a\text{-}f\ A}$	$27.7^{cd\ E}$	$3.6^{a\ A}$	$38.4^{ab\ CD}$	$44.7^{a\text{-}c\ C}$	ND	$5.6^{h\ D}$	$5.3^{b\text{-}d\ AB}$	$21.1^{e\ D}$	$15.2^{ab\ A}$	$76.4^{ef\ C}$	$0.8^{d\text{-}i\ DE}$	$1.0^{a\text{-}e\ B}$	$60.4^{a\text{-}e\ A\text{-}C}$	$169.1^{hi\ D}$	$5.3^{a\text{-}h\ C}$	$5.3^{a\text{-}f\ F}$	ND	$2.6^{a\text{-}e\ D}$	ND	$515.6^{i\text{-}q\ CD}$
		ST	$1.0^{g\text{-}i\ E}$	$4.7^{g\text{-}j\ D}$	ND	$7.4^{gh\ A}$	ND	$81.4^{b\text{-}f\ C}$	$<0.1^{a\ A}$	$0.7^{a\ A}$	$10.5^{gh\ B}$	ND	$16.9^{g\ DE}$	$<0.1^{a\ A}$	$207.4^{fg\ B}$	ND	$1.0^{f\text{-}i\ E}$	ND	$246.5^{hi\ D}$	$0.3^{a\ A}$	$0.4^{ab\ A}$	$3.0^{a\text{-}d\ DE}$	$1.8^{a\text{-}d\ B}$	$1.1^{a\text{-}d\ BC}$	ND	$584.2^{n\text{-}s\ D}$
		MW	$0.7^{d\text{-}g\ D}$	$3.5^{e\text{-}j\ CD}$	ND	$6.2^{f\text{-}g\ A}$	ND	$28.8^{a\text{-}d\ AB}$	$<0.1^{a\ A}$	$0.1^{a\ A}$	$12.3^{h\ B}$	ND	$22.2^{h\ E}$	ND	$153.9^{c\text{-}f\ B}$	ND	$0.7^{c\text{-}h\ CD}$	ND	$196.7^{fi\ CD}$	$0.5^{a\ A}$	$0.8^{a\text{-}d\ A}$	$1.6^{a\text{-}c\ B\text{-}D}$	$0.7^{a\ AB}$	$0.4^{a\ AB}$	ND	$429.2^{i\text{-}o\ CD}$
		SF	$0.2^{ab\ A\text{-}C}$	$1.5^{a\text{-}f\ AB}$	$23.6^{fg\ CD}$	$3.1^{a\text{-}g\ A}$	$6.7^{a\text{-}d\ AB}$	$6.3^{ab\ A}$	$11.3^{a\ A}$	$13.9^{ab\ AB}$	$1.2^{a\text{-}c\ A}$	$3.6^{e\text{-}g\ C}$	$1.9^{a\text{-}c\ A}$	$6.0^{b\ BC}$	$58.9^{a\text{-}c\ A}$	$25.3^{a\text{-}d\ B}$	$0.1^{a\text{-}c\ A}$	$0.8^{a\text{-}e\ AB}$	$122.0^{b\text{-}g\ A\text{-}D}$	$25.9^{a\text{-}d\ AB}$	$7.5^{a\text{-}h\ D}$	$2.0^{a\text{-}d\ C\text{-}E}$	ND	$1.0^{a\text{-}d\ A\text{-}C}$	ND	$323.1^{b\text{-}o\ B\text{-}D}$
	RC3	R	$0.1^{ab\ AB}$	$0.2^{ab\ A}$	$16.4^{ef\ BC}$	$0.7^{ab\ A}$	$18.5^{a\text{-}d\ CD}$	$1.5^{a\ A}$	$25.7^{ab\ BC}$	$27.9^{a\text{-}c\ B}$	ND	$5.9^{h\ D}$	$3.0^{a\text{-}c\ AB}$	$29.4^{f\ E}$	$4.8^{ab\ A}$	$24.1^{a\text{-}c\ B}$	$0.2^{a\text{-}d\ AB}$	$2.5^{ef\ C}$	$27.2^{a\text{-}c\ AB}$	$104.6^{fg\ C}$	$3.3^{a\text{-}f\ B}$	$8.6^{a\text{-}h\ G}$	ND	$2.4^{a\text{-}e\ D}$	ND	$307.2^{a\text{-}n\ A\text{-}C}$
		ST	$0.6^{c\text{-}g\ D}$	$3.5^{d\text{-}j\ CD}$	ND	$24.2^{k\ B}$	ND	$127.2^{fg\ D}$	$0.6^{a\ A}$	$1.0^{a\ A}$	$3.7^{c\text{-}e\ A}$	ND	$11.3^{ef\ B\text{-}D}$	$1.1^{a\ AB}$	$55.6^{a\text{-}c\ A}$	ND	$0.5^{a\text{-}f\ B\text{-}D}$	ND	$625.0^{k\ E}$	$5.2^{ab\ A}$	$0.3^{ab\ A}$	$2.4^{a\text{-}d\ C\text{-}E}$	$4.9^{d\text{-}f\ C}$	$1.3^{a\text{-}e\ C}$	ND	$868.6^{t\text{-}x\ E}$
		MW	$0.3^{a\text{-}d\ BC}$	$1.1^{a\text{-}e\ AB}$	ND	$4.3^{a\text{-}h\ A}$	ND	$64.4^{a\text{-}f\ BC}$	$0.5^{a\ A}$	$0.4^{a\ A}$	$3.4^{b\text{-}e\ A}$	ND	$14.5^{fg\ C\text{-}E}$	ND	$45.3^{ab\ A}$	ND	$0.1^{ab\ A}$	ND	$185.2^{f\text{-}i\ B\text{-}D}$	$3.4^{a\ A}$	$0.4^{ab\ A}$	$1.8^{a\text{-}c\ C\text{-}E}$	$1.1^{ab\ AB}$	$0.8^{a\text{-}d\ A\text{-}C}$	ND	$327.0^{b\text{-}o\ B\text{-}D}$
		SF	$0.2^{ab\ A\text{-}C}$	$0.9^{a\text{-}e\ AB}$	$11.3^{b\text{-}e\ AB}$	$6.2^{e\text{-}h\ A}$	$23.4^{a\text{-}d\ DE}$	$24.3^{a\text{-}d\ A}$	$46.1^{ab\ D}$	$57.0^{a\text{-}c\ C}$	$1.1^{a\text{-}c\ A}$	$2.1^{c\text{-}e\ BC}$	$2.3^{a\text{-}c\ A}$	$10.0^{c\ C}$	$36.3^{ab\ A}$	$25.2^{a\text{-}d\ B}$	$0.6^{b\text{-}g\ CD}$	$0.7^{a\text{-}d\ AB}$	$255.8^{i\ D}$	$23.6^{a\text{-}d\ AB}$	$9.0^{c\text{-}i\ E}$	$3.0^{a\text{-}d\ E}$	ND	$0.7^{a\text{-}d\ A\text{-}C}$	ND	$539.8^{m\text{-}r\ CD}$
White	WC1	R	$0.3^{a\text{-}e\ AB}$	$1.7^{a\text{-}f\ AB}$	$28.3^{gh\ C}$	$5.1^{b\text{-}h\ B}$	$15.5^{a\text{-}d\ C}$	$12.1^{ab\ AB}$	$78.4^{b\ B}$	$97.3^{c\ B}$	ND	$5.0^{gh\ D}$	$2.3^{a\text{-}c\ B}$	$13.7^{d\ B}$	$60.3^{a\text{-}d\ AB}$	$83.4^{ef\ B}$	$0.3^{a\text{-}d\ A}$	$3.0^{fg\ C}$	$194.6^{f\text{-}i\ BC}$	$215.8^{j\ B}$	$7.0^{a\text{-}h\ C}$	$12.8^{a\text{-}i\ BC}$	ND	$3.6^{b\text{-}e\ CD}$	$4.6^{e\ B}$	$845.2^{s\text{-}w\ D}$
		ST	$1.2^{ij\ DE}$	$4.8^{g\text{-}j\ D}$	$1.2^{a\ A}$	$15.9^{i\ D}$	$0.2^{a\ A}$	$180.7^{gh\ E}$	$2.3^{a\ A}$	$3.5^{ab\ A}$	$4.9^{de\ C}$	$0.4^{a\text{-}c\ AB}$	$11.9^{ef\ D}$	ND	$245.8^{fg\ E}$	$10.6^{ab\ A}$	$2.6^{j\ C}$	$2.1^{c\text{-}f\ BC}$	$607.0^{k\ E}$	$21.4^{a\text{-}d\ A}$	$0.7^{a\text{-}d\ A}$	$5.9^{a\text{-}f\ A}$	$5.5^{f\ D}$	$2.1^{a\text{-}e\ BC}$	ND	$1130.7^{xy\ E}$
		MW	$1.5^{jk\ E}$	$5.5^{i\text{-}k\ D}$	$0.2^{a\ A}$	$8.5^{hi\ C}$	ND	$97.2^{d\text{-}f\ CD}$	$0.2^{a\ A}$	$0.2^{a\ A}$	$5.6^{ef\ C}$	ND	$9.4^{de\ C}$	ND	$103.4^{b\text{-}e\ BC}$	$4.0^{ab\ A}$	$2.7^{j\ C}$	$0.2^{a\ A}$	$226.9^{g\text{-}i\ C}$	$3.4^{a\ A}$	$0.5^{a\text{-}c\ A}$	$2.7^{a\text{-}d\ A}$	$5.2^{ef\ CD}$	$0.4^{a\ A}$	ND	$478.0^{j\text{-}p\ BC}$
		SF	$1.0^{g\text{-}i\ CD}$	$3.0^{b\text{-}i\ BC}$	$0.3^{a\ A}$	$8.6^{hi\ C}$	$0.3^{a\ A}$	$118.4^{e\text{-}g\ D}$	$1.4^{a\ A}$	$2.5^{a\ A}$	$2.9^{a\text{-}e\ B}$	$1.4^{a\text{-}d\ C}$	$1.8^{a\text{-}c\ AB}$	$0.5^{a\ A}$	$161.1^{d\text{-}f\ CD}$	$3.6^{ab\ A}$	$0.2^{a\text{-}d\ A}$	$2.2^{d\text{-}f\ BC}$	$403.1^{j\ D}$	$8.1^{ab\ A}$	$2.0^{a\text{-}e\ AB}$	$4.3^{a\text{-}e\ A}$	$4.3^{c\text{-}f\ C}$	$1.3^{a\text{-}e\ AB}$	$0.1^{a\ A}$	$732.6^{p\text{-}u\ CD}$
	WC2	R	$0.2^{a\text{-}c\ A}$	$0.7^{a\text{-}e\ A}$	$14.3^{d\text{-}f\ B}$	$1.3^{a\text{-}d\ A}$	$5.5^{a\text{-}d\ B}$	$1.5^{a\ A}$	$12.2^{a\ A}$	$17.2^{ab\ A}$	ND	$1.0^{a\text{-}c\ BC}$	$0.1^{a\ A}$	$0.8^{a\ A}$	$18.5^{ab\ A}$	$19.8^{a\text{-}c\ A}$	$<0.1^{ab\ A}$	$1.3^{a\text{-}f\ AB}$	$21.3^{ab\ A}$	$32.1^{a\text{-}d\ A}$	$4.0^{a\text{-}g\ B}$	$16.0^{b\text{-}i\ C}$	ND	$2.2^{a\text{-}e\ BC}$	$0.3^{ab\ A}$	$170.4^{a\text{-}i\ A}$
		ST	$0.7^{e\text{-}g\ BC}$	$4.1^{f\text{-}j\ CD}$	$0.2^{a\ A}$	$5.6^{d\text{-}h\ B}$	$0.5^{a\ A}$	$55.9^{a\text{-}f\ BC}$	$1.3^{a\ A}$	$2.5^{a\ A}$	$2.6^{a\text{-}d\ B}$	$0.1^{a\ A}$	$1.1^{ab\ AB}$	ND	$189.9^{e\text{-}g\ DE}$	$5.7^{ab\ A}$	$1.2^{hi\ B}$	$0.7^{a\text{-}d\ A}$	$192.6^{f\text{-}i\ BC}$	$7.2^{ab\ A}$	$1.4^{a\text{-}e\ A}$	$15.0^{a\text{-}i\ C}$	$2.6^{a\text{-}f\ B}$	$3.7^{de\ D}$	ND	$494.9^{k\text{-}q\ BC}$
		MW	$0.3^{a\text{-}e\ AB}$	$1.3^{a\text{-}f\ AB}$	$<0.1^{a\ A}$	$0.7^{ab\ A}$	ND	$16.1^{a\text{-}c\ AB}$	$0.1^{a\ A}$	$0.4^{a\ A}$	$0.8^{a\text{-}c\ A}$	ND	$0.2^{a\ A}$	ND	$31.5^{ab\ A}$	$1.5^{a\ A}$	$0.4^{a\text{-}d\ A}$	$0.2^{a\ A}$	$22.5^{ab\ A}$	$1.2^{a\ A}$	$0.7^{a\text{-}d\ A}$	$7.3^{a\text{-}f\ AB}$	$0.7^{a\ A}$	$2.1^{a\text{-}e\ BC}$	ND	$88.3^{a\text{-}f\ A}$
		SF	$0.5^{b\text{-}f\ AB}$	$2.2^{a\text{-}h\ AB}$	$3.6^{a\text{-}c\ A}$	$3.0^{a\text{-}g\ AB}$	$2.2^{a\ A}$	$20.4^{a\text{-}d\ AB}$	$5.4^{a\ A}$	$8.5^{ab\ A}$	$0.6^{ab\ A}$	$0.5^{a\text{-}c\ AB}$	$0.2^{a\ A}$	$0.7^{a\ A}$	$82.2^{a\text{-}d\ AB}$	$6.0^{ab\ A}$	$<0.1^{ab\ A}$	$0.5^{a\text{-}d\ A}$	$88.0^{a\text{-}f\ AB}$	$8.5^{ab\ A}$	$8.7^{b\text{-}h\ C}$	$3.7^{a\text{-}d\ A}$	$1.0^{ab\ A}$	$2.4^{a\text{-}e\ B\text{-}D}$	$0.1^{a\ A}$	$248.9^{a\text{-}i\ AB}$
	P-value		< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

Letters 'ABC': mean values with different superscripts in the same column are significantly different (P<0.0001) across varieties and growing conditions within a cabbage type. Letters 'abc': mean values with different superscripts in the same column are significantly different (P<0.05) across varieties and growing conditions. Abbreviations: R = raw, ST = steamed, MW = microwaved, SF = stir-fried; For full names of cabbage varieties see Table 3.1 and Table 3.2 for compound names.

Appendix X: Supplementary tables for Chapter 5

Table S5a: Effect of domestic cooking and black kale variety on myrosinase activity (U/g DW), protein content (mg/g DW) and specific activity (U/mg protein DW)

	Variety				Treatment			
	BM	CNDTP	CPNT	*P-value*	Raw	ST	SF	*P-value*
Myrosinase activity (*U/g DW*)	8.6^{a}	10.5^{b}	14.2^{c}	< 0.0001	19.2^{c}	4.0^{a}	10.1^{b}	< 0.0001
Protein content (*mg/g DW*)	18.9^{a}	20.2^{b}	19.5^{ab}	0.04	35.2^{b}	11.2^{a}	12.1^{a}	< 0.0001
Specific activity (*U/mg protein* DW	0.5^{a}	0.7^{b}	0.5^{a}	< 0.0001	0.5^{b}	0.3^{a}	0.8^{c}	< 0.0001

Mean values with different superscripts in the same row for each group (Variety and Treatment) significantly different at p<0.05

Table S5b: Glucosinolate (mg/g-1 DW) and glucosinolate hydrolysis products (µg/g-1 DW: sulforaphane equivalent) of kale samples

	BM			CNDTP			CPNT			Significance
Compound	Raw	ST	SF	Raw	ST	SF	Raw	ST	SF	*P-value*
Glucosinolates (mg/g-1 DW)										
Glucoiberin (GIBN)	1.7^{c}	1.1^{ab}	1.2^{abc}	1.3^{abc}	0.9^{a}	1.4^{abc}	1.6^{bc}	1.2^{abc}	1.4^{bc}	< 0.001
Glucoraphanin (GRPN)	7.3^{d}	6.6^{bcd}	7.7^{d}	4.2^{abc}	3.3^{a}	3.9^{ab}	8.0^{d}	6.9^{bcd}	7.1^{cd}	< 0.0001
Glucobrassicin (GBSN)	6.0^{c}	5.2^{bc}	6.0^{c}	3.8^{ab}	3.2^{a}	3.6^{ab}	4.9^{abc}	4.7^{abc}	4.2^{ab}	< 0.0001
4-hydroxyglucobrassicin (4-HOH)	1.1^{b}	0.7^{ab}	0.7^{ab}	0.8^{ab}	0.4^{a}	0.6^{ab}	1.1^{b}	0.9^{ab}	0.7^{ab}	0.001
4-methoxyglucobrassicin (4-MeOH)	1.7^{c}	1.5^{abc}	1.6^{bc}	1.3^{abc}	1.0^{a}	1.1^{ab}	1.2^{abc}	1.3^{abc}	1.5^{bc}	0.001
Neoglucobrassicin (NEO)	1.0^{b}	0.8^{ab}	0.8^{ab}	0.9^{ab}	0.7^{a}	0.8^{ab}	0.8^{ab}	0.9^{ab}	0.8^{ab}	0.02
Total glucosinolates (GLS)	18.8^{c}	15.9^{bc}	18.0^{c}	12.2^{ab}	9.4^{a}	11.4^{a}	17.7^{c}	15.8^{bc}	15.8^{bc}	< 0.0001
GLS hydrolysis products (µg/g^{-1} DW; sulforaphane equivalent)										
buten-3-yl ITC (3BITC)	ND	1.3^{ab}	6.0^{c}	ND	1.3^{ab}	5.9^{c}	ND	2.3^{b}	9.0^{d}	< 0.0001
Erucin nitrile (ERN)	1.1^{d}	ND	0.9^{cd}	0.6^{ab}	ND	0.6^{ab}	1.5^{e}	0.6^{a}	0.8^{bc}	< 0.0001
Benzenepropanenitrile (BPN)	1.1^{a}	1.0^{a}	3.2^{b}	0.8^{a}	0.8^{a}	3.4^{b}	1.1^{a}	0.8^{a}	2.6^{b}	< 0.0001
Benzeneacetonitrile (BAN)	1.4^{d}	0.5^{ab}	1.0^{c}	0.9^{c}	0.3^{a}	0.7^{bc}	0.9^{c}	0.8^{bc}	1.0^{c}	< 0.0001
Iberin nitrile (IBN)	1.6^{ab}	0.4^{a}	0.5^{a}	4.0^{c}	0.2^{a}	3.1^{bc}	6.0^{d}	0.1^{a}	3.3^{bc}	< 0.0001
Erucin (ER)	ND	0.4^{b}	0.9^{c}	ND^{a}	0.1^{ab}	0.9^{c}	ND	1.2^{c}	1.7^{d}	< 0.0001
Sulforaphane nitrile (SFN)	384.7^{d}	14.3^{a}	144.2^{b}	54.6^{a}	11.3^{a}	27.8^{a}	259.5^{c}	22.7^{a}	75.2^{a}	< 0.0001
Iberin (IB)	0.1^{a}	0.9^{a}	1.1^{a}	0.1^{a}	2.4^{a}	13.6^{b}	0.6^{a}	16.1^{b}	49.2^{c}	< 0.0001
Sulforaphane (SFP)	23.7^{ab}	110.6^{b}	355.9^{d}	4.8^{a}	8.0^{a}	248.8^{c}	6.8^{a}	113.1^{b}	636.4^{e}	< 0.0001

Indoleacetonitrile (1HIC)	140.8^{bc}	62.3^{ab}	43.4^{a}	160.6^{c}	54.8^{a}	38.3^{a}	137.9^{bc}	86.1^{abc}	28.3^{a}	< 0.0001
Indole-3-carbinol (I3C)	6.5^{abc}	9.7^{cd}	10.0^{d}	5.0^{ab}	7.2^{bcd}	7.2^{bcd}	3.4^{a}	5.9^{ab}	8.1^{bcd}	< 0.0001
Total hydrolysis products (HPS)	555.8^{d}	191.7^{ab}	557.1^{d}	226.4^{ab}	79.2^{a}	343.1^{bc}	414.2^{cd}	243.7^{b}	807.6^{e}	< 0.0001

Mean values with different superscripts in the same row significantly different at p<0.05

Table S5b: Effect of variety and cooking method on glucosinolate (mg/g-1 DW) and glucosinolate hydrolysis products (µg/g-1 DW; sulforaphane equivalent) of black kale

	Variety			Significance	Treatment			Significance
Compound	BM	CNDTP	CPNT	*P-value*	Raw	ST	SF	*P-value*
Glucosinolates (mg/g-1 DW)								
Glucoiberin (GIBN)	1.3^{ab}	1.2^{a}	1.4^{b}	0.03	1.5^{b}	1.1^{a}	1.3^{b}	< 0.0001
Glucoraphanin (GRPN)	7.2^{b}	3.8^{a}	7.3^{b}	< 0.0001	6.5	5.6	6.2	0.25
Glucobrassicin (GBSN)	5.7^{c}	3.6^{a}	4.6^{b}	< 0.0001	4.9	4.4	4.6	0.25
4-hydroxyglucobrassicin (4-HOH)	0.8^{b}	0.6^{a}	0.9^{b}	0.01	1.0^{b}	0.7^{a}	0.7^{a}	< 0.001
4-methoxyglucobrassicin (4-MeOH)	1.6^{c}	1.1^{a}	1.4^{b}	< 0.0001	1.4	1.3	1.4	0.17
Neoglucobrassicin (NEO)	0.9^{a}	0.8^{a}	0.8^{a}	0.22	0.9^{b}	0.8^{a}	0.8^{ab}	0.01
Total glucosinolates (GLS)	17.6^{b}	11.0^{a}	16.4^{b}	< 0.0001	16.2^{b}	13.7^{a}	15.1^{ab}	0.01
GLS hydrolysis products (µg/g^{-1} DW: sulforaphane equivalent)								
buten-3-yl ITC (3BITC)	2.4^{a}	2.4^{a}	3.8^{b}	< 0.0001	ND	1.6^{a}	7.0^{b}	< 0.0001
Erucin nitrile (ERN)	0.7^{b}	0.4^{a}	1.0^{c}	< 0.0001	1.1^{c}	0.2^{a}	0.8^{b}	< 0.0001
Benzenepropanenitrile (BPN)	1.7	1.6	1.5	0.59	1.0^{a}	0.8^{a}	3.0^{b}	< 0.0001

Benzeneacetonitrile (BAN)	1.0[b]	0.6[a]	0.9[b]	< 0.0001	1.0[c]	0.5[a]	0.9[b]	< 0.0001
Iberin nitrile (IBN)	0.8[a]	2.4[b]	3.1[b]	< 0.0001	3.7[c]	0.2[a]	2.3[b]	< 0.0001
Erucin (ER)	0.4[a]	0.3[a]	1.0[b]	< 0.0001	ND	0.6[a]	1.2[b]	< 0.0001
Sulforaphane nitrile (SFN)	181.1[c]	31.2[a]	119.1[b]	< 0.0001	232.9[c]	16.1[a]	82.4[b]	< 0.0001
Iberin (IB)	0.7[a]	5.3[b]	21.9[c]	< 0.0001	0.2[a]	6.4[b]	21.3[c]	< 0.0001
Sulforaphane (SFP)	163.4[b]	87.2[a]	252.1[c]	< 0.0001	11.8[a]	77.2[b]	413.7[c]	< 0.0001
Indoleacetonitrile (1HIC)	82.2	84.6	84.1	0.98	146.5[b]	67.7[a]	36.7[a]	< 0.0001
Indole-3-carbinol (I3C)	8.7[b]	6.5[a]	5.8[a]	< 0.0001	5.0[a]	7.6[b]	8.4[b]	< 0.0001
Total hydrolysis products (HPS)	434.9[b]	216.2[a]	488.5[b]	< 0.0001	398.8[b]	171.5[a]	569.2[c]	< 0.0001

Mean values with different superscripts in the same row for each group (Variety and Treatment) significantly different

Table S5d: Correlation value for drivers of liking in black kale

Variables	Succinic	GLY/VAL/I	GLU.ACID/	GIBN/4-H	ERN/BAN/	Alcohol:1	Aldehyde:A	Aldehyde	Ester:Acet	Ester: (But	Fur/Terp:	Fur/Terp:	Fur/Terp:	Hydrocarb	Hydrocarb	Ketone:3P	Sulfur:Car	Acid_Ni_P	Acid_Ni_P	App: Gree	Odour:swi	MF:Moist	Taste:Stall	Flavour:St	AE:bitter/	Gustin_BT	Gustin_BT	Gustin_TL	Gustin_TL	TAS2R38_I	TAS2R38_I	TAS2R38_T	TAS2R38_	Taste perc	Liking:MF,	Liking:App	Cluster 1	Cluster 2
Glucose/Fructose	-0.113	0.086	0.593	-0.242	-0.410	-0.226	-0.390	-0.620	0.017	0.305	-0.802	0.025	-0.448	0.022	-0.174	-0.166	0.361	-0.354	0.015	-0.006	0.122	-0.194	0.327	-0.168	-0.255	0.468	-0.453	-0.190	0.176	-0.256	-0.213	0.364	-0.550	0.051	-0.358	0.425	-0.165	0.006
Sucrose	-0.078	-0.163	0.048	0.156	-0.253	0.262	0.434	0.256	0.460	0.144	0.239	0.683	-0.129	0.516	0.147	0.228	0.260	0.184	0.287	-0.389	0.373	0.325	0.231	0.381	0.386	0.273	0.256	-0.279	-0.150	0.289	0.224	-0.206	-0.247	-0.280	-0.220	-0.194	-0.272	-0.317
Ctric/Malic	0.000	0.364	0.514	-0.068	-0.144	-0.476	-0.595	-0.203	-0.302	-0.074	-0.405	-0.386	-0.480	-0.453	-0.138	-0.350	0.059	-0.291	-0.408	0.251	-0.264	-0.377	-0.093	-0.389	-0.470	-0.180	-0.191	0.309	-0.058	-0.089	-0.340	0.213	0.102	0.373	0.099	0.286	0.193	0.274
Succinic	**1**	0.778	-0.503	0.885	0.746	0.723	0.677	-0.041	0.514	0.437	-0.258	0.366	0.478	0.412	0.555	0.736	0.667	0.708	0.241	-0.800	0.800	0.781	**0.735**	**0.766**	**0.788**	**0.653**	0.366	**-0.785**	-0.207	0.368	0.095	-0.888	-0.159	**-0.745**	-0.379	**-0.812**	-0.807	-0.749
GLY/VAL/LEU/ISO/THR/SER/MET/PHY/LYS/TYR	0.778	**1**	0.000	0.594	0.639	0.460	0.366	-0.266	0.432	0.235	-0.532	0.239	0.208	0.198	0.461	0.537	0.491	0.724	-0.147	-0.618	0.620	0.549	**0.704**	0.559	0.520	0.444	0.420	**-0.663**	-0.116	0.097	0.300	-0.580	-0.293	-0.537	-0.445	-0.430	-0.602	-0.602
GLU.ACID/TRYP	-0.503	0.000	**1**	-0.550	-0.567	-0.657	-0.709	-0.203	-0.148	-0.420	-0.332	-0.093	-0.801	-0.231	-0.527	-0.470	-0.265	-0.340	-0.670	0.457	-0.412	-0.625	-0.200	-0.546	-0.581	-0.276	-0.272	0.290	0.364	-0.419	-0.073	0.626	-0.058	0.559	0.180	0.565	0.451	0.456
AAA vs ASP.ACID	0.002	0.000	0.000	0.084	-0.311	-0.062	0.090	0.454	-0.130	0.296	0.236	-0.071	-0.306	-0.296	0.624	-0.140	0.053	0.095	0.050	-0.185	0.073	0.086	-0.009	-0.030	0.048	0.153	-0.272	-0.012	0.192	0.206	-0.488	-0.223	0.346	0.155	0.237	-0.139	0.036	0.151
ALA/PRO/ASP/GLU/HIS	0.092	0.000	0.000	0.178	0.177	0.549	0.559	-0.215	0.763	-0.068	-0.030	0.793	0.020	0.786	0.021	0.604	0.257	0.470	0.181	-0.478	0.511	0.468	0.504	0.540	0.592	0.409	0.364	-0.575	0.092	0.230	0.491	-0.245	-0.387	-0.507	-0.303	-0.308	-0.346	-0.482
GRPN/GBSN/Total GLS	0.336	0.443	-0.206	0.000	0.442	0.071	-0.089	-0.401	-0.276	0.250	-0.523	-0.475	0.579	-0.295	0.101	-0.003	0.004	0.105	-0.020	0.032	0.048	0.042	0.151	0.021	-0.064	0.090	-0.028	-0.164	-0.039	-0.444	0.196	0.037	-0.287	-0.167	-0.342	0.159	-0.211	-0.142
GIBN/4-HOH/NEO	0.885	0.594	-0.550	**1**	0.656	0.763	0.780	0.103	0.659	0.365	0.017	0.605	0.338	0.594	0.511	0.783	**0.764**	0.622	0.409	-0.853	0.802	0.842	**0.660**	**0.840**	**0.846**	0.549	0.568	**-0.624**	-0.505	**0.730**	0.104	**-0.965**	-0.090	**-0.766**	-0.343	**-0.891**	-0.779	-0.793
4-MeOH	-0.073	-0.065	-0.056	0.000	0.481	0.290	0.214	-0.263	0.477	-0.679	0.194	0.384	0.162	0.528	-0.488	0.430	-0.227	0.296	-0.183	0.056	-0.043	0.097	0.007	0.197	0.229	-0.322	0.586	-0.018	-0.055	0.124	0.669	0.014	-0.038	-0.200	0.031	-0.143	0.135	-0.168
BITC/ER/IB/SF	-0.278	-0.227	-0.172	-0.040	0.000	-0.264	-0.201	0.302	-0.343	-0.288	0.523	-0.322	-0.029	-0.351	-0.084	-0.294	-0.285	-0.285	-0.007	0.358	-0.481	-0.238	-0.559	-0.279	-0.328	-0.688	0.240	0.722	-0.485	0.302	-0.070	0.097	0.457	0.330	0.319	0.139	0.435	0.307
ERN/BAN/SFN/TOTAL HPS	0.746	0.639	-0.567	**0.656**	**1**	0.798	0.697	-0.263	0.576	0.027	-0.116	0.325	0.626	0.459	0.384	0.823	0.323	0.820	0.142	-0.604	0.581	0.729	0.580	**0.720**	**0.745**	0.284	0.655	-0.597	-0.218	0.342	0.519	-0.696	-0.136	**-0.723**	-0.331	**-0.642**	-0.527	-0.679
IBN	0.398	0.135	-0.545	0.492	0.000	0.359	0.494	0.307	0.000	**0.857**	0.048	0.122	0.405	0.035	0.733	0.126	0.559	0.104	0.718	-0.555	0.518	0.529	0.303	0.455	0.386	0.512	0.052	-0.296	-0.500	0.333	-0.164	-0.512	-0.218	-0.435	-0.433	-0.285	-0.608	-0.444
BPN	-0.143	-0.498	-0.486	-0.229	0.000	-0.052	0.079	0.534	-0.369	-0.156	**0.610**	-0.329	0.449	-0.221	-0.061	-0.171	-0.587	-0.033	-0.014	0.322	-0.322	-0.159	-0.485	-0.191	-0.052	-0.295	-0.241	0.171	0.380	-0.303	-0.096	0.062	0.465	0.202	0.468	-0.111	0.306	0.321
Alchohol:2pent(Z)/1octen-3-ol/1hep/1oct/2-oct(E)/phen	-0.253	-0.511	-0.618	-0.138	0.115	0.000	0.126	0.452	-0.358	-0.176	0.739	-0.327	0.483	-0.240	0.029	-0.161	-0.503	-0.103	0.206	0.310	-0.388	-0.078	-0.568	-0.136	-0.091	-0.538	0.116	0.467	-0.166	0.059	0.069	0.040	0.443	0.154	0.334	0.000	0.355	0.246
Alcohol:1pent3/2pent(E)/1hex/3hex(E)&(Z)/2hex(E)&(Z)	0.723	0.460	-0.657	0.763	0.798	**1**	0.956	-0.266	0.794	0.373	-0.122	0.667	0.598	0.751	0.571	0.948	0.651	0.781	0.576	-0.895	0.881	0.969	0.803	**0.957**	**0.961**	0.649	0.636	-0.800	-0.369	0.569	0.491	-0.808	-0.404	-0.956	-0.589	**-0.699**	-0.803	-0.907
Alcohol:1-pent	0.157	0.026	-0.003	-0.053	0.305	0.000	-0.104	-0.036	0.047	-0.509	0.062	-0.156	-0.070	-0.011	-0.266	0.171	-0.355	0.206	-0.510	0.197	-0.177	-0.160	-0.074	-0.170	0.023	-0.099	-0.242	-0.106	0.716	-0.178	-0.194	-0.033	0.483	0.171	0.557	-0.301	0.280	0.257
Aldehyde: Prop/Hex/2Hex/2Hep/2,4Hex/2Octe/2Non/2E	-0.279	-0.519	-0.501	-0.337	0.205	0.025	0.000	0.000	-0.305	-0.255	0.381	-0.398	0.481	-0.177	-0.159	-0.083	-0.549	-0.121	0.116	0.394	-0.396	-0.163	-0.430	-0.216	-0.147	-0.389	-0.088	0.311	0.162	-0.181	0.109	0.241	0.268	0.138	0.278	0.139	0.381	0.282
Aldehyde: Pent/2Pent/Oct/Non vs 2,4Hep	0.677	0.366	-0.709	0.780	0.697	0.956	**1**	0.000	0.738	0.371	0.103	0.687	0.603	0.717	0.606	0.876	0.585	0.765	0.590	-0.877	0.838	0.957	0.681	**0.947**	**0.965**	0.562	0.658	-0.723	-0.411	0.604	0.454	-0.850	-0.285	-0.901	-0.495	**-0.753**	-0.761	-0.862
Aldehyde: Prop/But2/But3/2,4Dec(E,E)	-0.041	-0.266	-0.203	0.103	-0.263	-0.266	0.000	**1**	-0.330	-0.137	0.811	-0.137	-0.032	-0.277	0.030	-0.304	-0.329	-0.097	-0.158	0.196	-0.286	-0.174	-0.539	-0.183	-0.084	-0.396	-0.078	0.350	0.025	0.080	-0.375	-0.182	0.702	0.344	**0.601**	-0.305	0.270	0.322
Ester:Acetic/3Hex (E)&(Z)/2Hex(Z)	0.514	0.432	-0.148	0.659	0.576	0.794	0.738	-0.330	**1**	0.000	-0.150	0.935	0.083	0.937	0.176	0.910	0.632	0.680	0.257	-0.778	0.770	0.761	0.769	**0.829**	**0.834**	0.509	0.709	-0.694	-0.304	0.625	0.531	-0.633	-0.401	-0.780	-0.464	**-0.619**	-0.625	-0.800
Ester: (But acid/But acid 3hex	0.437	0.235	-0.420	0.365	0.027	0.373	0.371	-0.137	0.000	**1**	-0.418	0.030	0.418	0.032	0.690	0.149	0.669	0.050	0.737	-0.564	0.600	0.499	0.516	0.421	0.325	0.766	-0.160	-0.481	-0.325	0.113	-0.168	-0.356	-0.510	-0.500	**-0.644**	-0.103	-0.715	-0.486
Fur/Terp: Fur/2Fur/Furfural/2Fumet	-0.258	-0.532	-0.332	0.017	-0.116	-0.122	0.103	0.811	-0.150	-0.418	**1**	0.000	0.000	-0.061	-0.127	-0.143	-0.446	-0.096	-0.039	0.266	-0.384	-0.141	-0.618	-0.149	-0.038	-0.590	0.150	0.499	-0.087	0.302	-0.139	-0.099	0.720	0.294	**0.638**	-0.263	0.436	0.320
Fur/Terp: Myr/D-carv	0.366	0.239	-0.093	0.605	0.325	0.667	0.687	-0.137	0.935	0.030	0.000	**1**	0.000	0.951	0.068	0.759	0.625	0.491	0.328	-0.707	0.702	0.669	0.631	**0.763**	0.748	0.447	0.676	-0.566	-0.411	0.637	0.476	-0.554	-0.403	-0.693	-0.440	-0.555	-0.588	-0.738
Fur/Terp: Limon	0.478	0.208	-0.801	0.338	0.626	0.598	0.603	-0.032	0.083	0.418	0.000	0.000	**1**	0.172	0.403	0.399	0.147	0.426	0.489	-0.374	0.408	0.551	0.278	0.529	0.495	0.263	0.335	-0.417	-0.289	-0.043	0.448	-0.405	-0.296	-0.604	-0.457	-0.294	-0.509	-0.524
Hydrocarbon:Hep 2,2 vs 3Ethyl 1,2,3/1Dod/Oct/Nona	0.412	0.198	-0.231	0.594	0.459	0.751	0.717	-0.277	0.937	0.032	-0.061	0.951	0.172	**1**	0.000	0.832	0.613	0.483	0.402	-0.686	0.710	0.693	0.656	**0.787**	0.776	0.484	0.656	-0.611	-0.389	0.587	0.527	-0.537	-0.458	-0.767	-0.482	-0.562	-0.620	-0.776
Hydrocarbon: 1,4Dimet vs cym/Trid	0.104	0.479	0.682	-0.039	-0.192	-0.152	-0.310	-0.559	0.148	0.205	-0.827	0.085	-0.513	0.000	0.000	-0.027	0.405	-0.038	-0.222	-0.157	0.225	-0.066	0.457	-0.041	-0.121	0.425	-0.218	-0.291	0.151	-0.162	-0.115	0.145	-0.451	-0.009	-0.341	0.248	-0.219	-0.099
Hydrocarbon:Hep/Cym	0.555	0.461	-0.527	0.511	0.384	0.571	0.606	0.030	0.176	0.690	-0.127	0.068	0.403	0.000	**1**	0.397	0.465	0.549	0.474	-0.685	0.609	0.684	0.531	0.552	0.554	0.563	0.134	-0.520	-0.170	0.328	-0.035	-0.635	-0.127	-0.523	-0.378	-0.390	-0.564	-0.487
Ketone:3Pent/2,3Oct/ion	0.736	0.537	-0.470	0.783	0.823	0.948	0.876	-0.304	0.910	0.149	-0.143	0.759	0.399	0.832	0.397	**1**	0.631	0.817	0.358	-0.858	0.844	0.901	0.814	**0.920**	**0.948**	0.584	0.682	-0.796	-0.281	0.602	0.491	-0.802	-0.325	-0.896	-0.473	**-0.762**	-0.728	-0.869
Ketone: 1pent	-0.145	-0.447	-0.587	-0.187	0.297	0.087	0.094	0.157	-0.251	-0.293	0.505	-0.350	0.501	-0.144	-0.102	0.000	-0.534	-0.008	0.071	0.323	-0.348	-0.089	-0.422	-0.144	-0.040	-0.394	-0.028	0.267	0.171	-0.085	0.060	0.074	0.414	0.105	0.379	-0.059	0.350	0.247
Ketone:Acet	0.058	-0.092	-0.409	0.142	-0.089	0.228	0.456	0.509	-0.046	0.450	0.350	0.144	0.471	0.035	0.461	0.000	0.059	0.184	0.425	-0.279	0.252	0.331	0.011	0.321	0.287	0.128	0.190	-0.155	-0.283	0.009	0.212	-0.249	-0.149	-0.260	-0.264	-0.118	-0.297	-0.261
Sulfur:Car/Thio/ThioEster vs 1But	0.667	0.491	-0.265	**0.764**	0.323	0.651	0.585	-0.329	0.632	0.669	-0.446	0.625	0.147	0.613	0.465	0.631	**1**	0.311	0.636	-0.855	0.871	0.751	0.830	**0.752**	**0.662**	0.832	0.324	-0.668	-0.556	0.605	0.063	-0.655	-0.566	-0.773	**-0.704**	-0.478	-0.890	-0.817
Sulfur: DMTS/DMMM	0.236	-0.336	-0.864	0.374	0.371	0.484	0.583	0.394	0.156	0.049	0.648	0.162	0.538	0.288	0.214	0.375	0.000	0.202	0.476	-0.219	0.160	0.394	-0.070	0.342	0.456	0.030	0.187	-0.078	-0.148	0.441	-0.027	-0.473	0.343	-0.307	0.193	-0.574	-0.130	-0.191
Sulfur: DMDS	0.480	0.626	-0.030	0.297	0.793	0.479	0.350	-0.339	0.549	-0.395	-0.222	0.295	0.255	0.390	-0.004	0.641	0.000	0.774	-0.391	-0.302	0.319	0.368	0.442	0.422	0.485	0.071	0.523	-0.513	0.206	-0.015	0.584	-0.348	-0.082	-0.409	-0.110	-0.413	-0.205	-0.395
Sulfur: DMS	-0.069	0.056	-0.149	-0.130	0.039	0.315	0.400	-0.076	0.109	0.359	-0.098	0.154	0.437	0.097	0.451	0.119	0.000	0.357	0.299	-0.285	0.303	0.339	0.257	0.330	0.272	0.234	0.213	-0.346	-0.045	-0.230	0.548	-0.008	-0.504	-0.345	-0.516	0.178	-0.276	-0.319
Acid_Ni_Py:Oct vs pyr/4(H)py	-0.105	-0.390	-0.544	0.056	0.114	-0.039	0.146	0.742	-0.277	-0.311	0.916	-0.218	0.298	-0.208	0.016	-0.116	-0.484	0.000	0.000	0.256	-0.368	-0.072	-0.593	-0.113	-0.014	-0.584	0.159	0.452	-0.105	0.192	-0.086	-0.157	0.709	0.216	0.565	-0.271	0.372	0.272
Acid_Ni_Py: Hex/Benz	0.708	0.724	-0.340	0.622	0.820	0.781	0.765	-0.097	0.680	0.050	-0.096	0.491	0.426	0.483	0.549	0.817	0.311	**1**	0.000	-0.725	0.687	0.773	0.676	**0.773**	**0.830**	0.385	0.635	-0.756	-0.017	0.279	0.538	-0.738	-0.149	**-0.702**	-0.318	**-0.672**	-0.551	-0.688
Acid_Ni_Py: Benzprop	0.241	-0.147	-0.670	0.409	0.142	0.576	0.590	-0.158	0.257	**0.737**	-0.039	0.328	0.489	0.402	0.474	0.358	0.636	0.000	**1**	-0.552	0.553	0.603	0.399	0.548	0.460	0.565	0.172	-0.303	-0.621	0.503	0.069	-0.376	-0.467	**-0.619**	**-0.603**	-0.193	-0.618	-0.567
App: Green/shiny/moist/cooked	-0.800	-0.618	0.457	-0.853	-0.604	-0.895	-0.877	0.196	-0.778	-0.564	0.266	-0.707	-0.374	-0.686	-0.685	-0.858	-0.855	-0.725	-0.552	**1**	-0.982	**-0.965**	-0.906	**-0.952**	**-0.928**	-0.807	-0.532	0.858	0.408	-0.602	-0.298	0.864	0.457	0.923	**0.653**	**0.700**	0.919	0.930
App:Brown marks/veins/oily	0.135	-0.173	-0.719	0.238	0.362	0.233	0.399	0.643	-0.116	-0.147	0.797	-0.118	0.525	-0.087	0.257	0.122	-0.328	0.272	0.121	0.000	-0.108	0.202	-0.337	0.146	0.243	-0.370	0.295	0.175	-0.141	0.238	0.066	-0.373	0.562	-0.053	0.360	-0.427	0.125	0.019
App:bubbly/veins	0.367	0.683	0.066	0.025	0.530	0.042	-0.105	-0.294	-0.118	-0.104	-0.424	-0.417	0.207	-0.359	0.231	0.100	-0.165	0.446	-0.486	0.000	0.003	0.026	0.183	-0.018	-0.002	-0.041	0.037	-0.206	0.316	-0.358	0.145	-0.081	0.063	-0.016	-0.009	-0.013	0.004	-0.008
Odour:sweet/cooked vs stalky/leafy/metallic/sulfurous	**0.800**	**0.620**	-0.412	**0.802**	0.581	**0.881**	**0.838**	-0.286	**0.770**	**0.600**	-0.384	**0.702**	0.408	**0.710**	**0.609**	**0.844**	**0.871**	**0.687**	0.553	-0.982	**1**	0.937	**0.945**	**0.941**	**0.905**	0.871	0.476	-0.915	-0.365	0.487	0.331	-0.797	-0.571	**-0.942**	**-0.727**	**-0.641**	-0.962	-0.949
MF:Crunchy/Warming/Fibrious/Toughness vs Moist	**0.781**	0.549	**-0.625**	**0.842**	**0.729**	**0.969**	**0.957**	-0.174	**0.761**	0.499	-0.141	**0.669**	0.551	**0.693**	**0.684**	**0.901**	**0.751**	**0.773**	**0.603**	**-0.965**	**0.937**	**1**	**0.835**	**0.981**	**0.964**	0.701	0.631	-0.810	-0.457	0.611	0.416	-0.875	-0.414	**-0.959**	**-0.635**	**-0.718**	-0.876	-0.940
Taste:Stalk bitter/leaf: bitter/metallic vs leaf:sweet/salty	**0.735**	**0.704**	-0.200	**0.660**	0.580	**0.803**	**0.681**	-0.539	**0.769**	0.516	**-0.618**	**0.631**	0.278	**0.656**	0.531	**0.814**	**0.830**	**0.676**	0.399	-0.906	**0.945**	**0.835**	**1**	**0.840**	**0.795**	**0.874**	0.402	**-0.927**	-0.221	0.357	0.361	**-0.643**	**-0.656**	**-0.874**	**-0.755**	-0.482	-0.887	-0.883
Flavour:leafy/sulphury vs sesame/burnt	0.046	0.402	0.626	-0.112	-0.139	-0.073	-0.272	**-0.745**	0.193	0.222	-0.911	0.115	-0.389	0.095	-0.067	0.027	0.410	-0.061	-0.106	-0.151	0.249	-0.035	0.502	0.000	-0.104	0.461	-0.180	-0.325	0.109	-0.207	0.037	0.228	**-0.622**	-0.099	-0.479	0.341	-0.256	-0.165
Flavour:Stalky/peppery/sulphury	**0.766**	0.559	-0.546	**0.840**	**0.720**	**0.957**	**0.947**	-0.183	**0.829**	0.421	-0.149	**0.763**	0.529	**0.787**	0.552	**0.920**	**0.752**	**0.773**	0.548	**-0.952**	**0.941**	**0.981**	**0.840**	**1**	**0.971**	0.672	0.708	-0.821	-0.488	0.585	0.514	-0.848	-0.475	**-0.975**	**-0.665**	**-0.716**	-0.888	-0.974
AE:bitter/throat/residue/lingering/metallic vs salty	**0.788**	0.520	-0.581	**0.846**	**0.745**	**0.961**	**0.965**	-0.084	**0.834**	0.325	-0.038	**0.748**	0.495	**0.776**	0.554	**0.948**	**0.662**	**0.830**	0.460	**-0.928**	**0.905**	**0.964**	**0.795**	**0.971**	**1**	**0.646**	**0.644**	**-0.833**	-0.321	0.592	0.424	**-0.895**	-0.302	**-0.922**	-0.491	**-0.825**	-0.813	-0.900
Taste percep: Bitter vs sweet	**-0.745**	-0.537	0.559	**-0.766**	**-0.723**	**-0.956**	**-0.901**	0.344	**-0.780**	-0.500	0.294	**-0.693**	**-0.604**	-0.767	-0.523	**-0.896**	**-0.773**	**-0.702**	**-0.619**	**0.923**	**-0.942**	**-0.959**	**-0.874**	**-0.975**	**-0.922**	**-0.735**		**0.843**	0.483	-0.491	-0.535	**0.758**	**0.604**	**1**	**0.769**	**0.606**	0.920	0.984
Taste percep: Savoury	-0.152	-0.409	-0.506	-0.304	0.250	0.096	0.051	-0.089	-0.264	-0.117	0.191	-0.351	**0.634**	-0.094	-0.179	-0.027	-0.451	-0.070	0.154	0.308	-0.254	-0.093	-0.283	-0.116	-0.069	-0.211	-0.105	0.111	0.185	-0.342	0.209	0.222	0.051	0.000	0.090	0.130	0.192	0.141
Liking:MF/Taste/OvAll/Consump Int	-0.379	-0.445	0.180	-0.343	-0.331	-0.589	-0.495	**0.601**	-0.464	**-0.644**	**0.638**	-0.440	-0.457	-0.482	-0.378	-0.473	**-0.704**	-0.318	**-0.603**	**0.653**	**-0.727**	**-0.635**	**-0.755**	**-0.665**	-0.491	**-0.665**	-0.416	**0.623**	0.540	-0.126	-0.567	0.267	**0.943**	**0.769**	1	0.000	**0.795**	**0.796**
Liking:App and Consump Int	**-0.812**	-0.430	0.565	**-0.891**	**-0.642**	**-0.699**	**-0.753**	-0.305	**-0.619**	-0.103	-0.263	-0.555	-0.294	-0.562	-0.390	**-0.762**	-0.478	**-0.672**	-0.193	**0.700**	**-0.641**	**-0.718**	-0.482	**-0.716**	**-0.825**	-0.403	-0.449	0.579	0.164	**-0.630**	-0.018	**0.934**	-0.211	**0.606**	0.000	1	0.558	0.592
Cluster 1	-0.807	-0.602	0.451	-0.779	-0.527	-0.803	-0.761	0.270	-0.625	-0.715	0.436	-0.588	-0.509	-0.620	-0.564	-0.728	-0.890	-0.551	-0.618	0.919	-0.962	-0.876	-0.887	-0.888	-0.813	-0.868	-0.410	0.860	0.459	-0.388	-0.294	0.738	0.638	0.920	0.795	0.558	**1**	0.935
Cluster 2	-0.749	-0.602	0.456	-0.793	-0.679	-0.907	-0.862	0.322	-0.800	-0.486	0.320	-0.738	-0.524	-0.776	-0.487	-0.869	-0.817	-0.688	-0.567	0.930	-0.949	-0.940	-0.883	-0.974	-0.900	-0.715	-0.685	0.825	0.550	-0.505	-0.551	0.757	0.633	0.984	0.796	0.592	0.935	**1**
Gustin_BT:AG and AA	**0.653**	0.444	-0.276	0.549	0.284	0.649	0.562	-0.396	0.509	**0.766**	-0.590	0.447	0.263	0.484	0.563	0.584	0.832	0.385	0.565	-0.807	0.871	0.701	**0.874**	0.672	**0.646**	**1**	0.000	**-0.862**	-0.095	0.247	-0.003	-0.545	-0.596	**-0.735**	**-0.665**	-0.403	-0.868	-0.715
Gustin_BT:GG	0.366	0.420	-0.272	0.568	0.655	0.636	0.658	-0.078	0.709	-0.160	0.150	0.676	0.335	0.656	0.134	0.682	0.324	0.635	0.172	-0.532	0.476	0.631	0.402	0.708	**0.644**	0.000	**1**	-0.307	**-0.639**	0.540	**0.783**	-0.532	-0.264	**-0.635**	-0.416	-0.449	-0.410	-0.685
Gustin_TL:AG and AA	**-0.785**	**-0.663**	0.290	**-0.624**	-0.597	-0.800	-0.723	0.350	-0.694	-0.481	0.499	-0.566	-0.417	-0.611	-0.520	-0.796	-0.668	-0.756	-0.303	0.858	-0.915	-0.810	**-0.927**	-0.821	**-0.833**	**-0.862**	-0.307	**1**	0.000	-0.175	-0.342	**0.671**	0.539	**0.843**	**0.623**	0.579	0.860	0.825
Gustin_TL:GG	-0.207	-0.116	0.364	-0.505	-0.218	-0.369	-0.411	0.025	-0.304	-0.325	-0.087	-0.411	-0.289	-0.389	-0.170	-0.281	-0.556	-0.017	**-0.621**	0.408	-0.365	-0.457	-0.221	-0.488	-0.321	-0.095	**-0.639**	0.000	**1**	**-0.610**	-0.333	0.356	0.365	0.483	0.540	0.164	0.459	0.550
TAS2R38_BT:Rare/AVI/AVI AND PAV/AVI	0.368	0.097	-0.419	**0.730**	0.342	0.569	**0.604**	0.080	**0.625**	0.113	0.302	**0.637**	-0.043	0.587	0.328	**0.602**	**0.605**	0.279	0.503	-0.602	0.487	0.611	0.357	0.585	0.592	0.247	0.540	-0.175	**-0.610**	**1**	0.000	**-0.677**	0.073	-0.491	-0.126	**-0.630**	-0.388	-0.505
TAS2R38_BT:PAV/PAV	0.095	0.300	-0.073	0.104	0.519	0.491	0.454	-0.375	0.531	-0.168	-0.139	0.476	0.448	0.527	-0.035	0.491	0.063	0.538	0.069	-0.298	0.331	0.416	0.361	0.514	0.424	-0.003	**0.783**	-0.342	-0.333	0.000	**1**	-0.106	-0.565	-0.535	-0.567	-0.018	-0.294	-0.551
TAS2R38_TL:Rare/AVI/AVI AND PAV/AVI	-0.888	-0.580	0.626	**-0.965**	-0.696	-0.808	-0.850	-0.182	-0.633	-0.356	-0.099	-0.554	-0.405	-0.537	-0.635	-0.802	-0.655	-0.738	-0.376	0.864	-0.797	-0.875	**-0.643**	-0.848	**-0.895**	-0.545	-0.532	**0.671**	0.356	**-0.677**	-0.106	**1**	0.000	**0.758**	0.267	**0.934**	0.738	0.757
TAS2R38_TL:PAV/PAV	-0.159	-0.293	-0.058	-0.090	-0.136	-0.404	-0.285	**0.702**	-0.401	-0.510	**0.720**	-0.403	-0.296	-0.458	-0.127	-0.325	-0.566	-0.149	-0.467	0.457	-0.571	-0.414	**-0.656**	-0.475	-0.302	-0.596	-0.264	0.539	0.365	0.073	-0.565	0.000	**1**	**0.604**	**0.943**	-0.211	0.638	0.633

Appendix XI: Supplementary tables for Chapter 6

Table S6a: Effect of domestic cooking and red cabbage variety on myrosinase activity (U/g-1 DW), protein content (mg/g-1 DW) and specific activity (U/mg-1 protein DW)

	Variety			Significance	Treatment			Significance
	RM	RD	RL	*P-value*	Raw	ST	SF	*P-value*
Myrosinase activity (*U.g-1 DW*)	18.3^{b}	29.3^{c}	12.2^{a}	< 0.0001	37.6^{c}	6.0^{a}	16.1^{b}	< 0.0001
Protein content (*mg.g-1 DW*)	14.8^{b}	14.0^{a}	14.6^{b}	< 0.0001	21.5^{c}	10.7^{a}	11.3^{b}	< 0.0001
Specific activity (*U.mg-1 protein DW*)	1.1^{b}	1.9^{c}	0.8^{a}	< 0.0001	1.8^{c}	0.6^{a}	1.4^{b}	< 0.0001

Mean values with different superscripts in the same row for each group (Variety and Treatment) significantly different at p<0.0001

Table S6b: Effect of red cabbage variety and cooking method on amino acid (µg/g DW), sugars (mg/g DW) and organic acid (mg/g DW) concentration

		Variety			Significance	Treatment			Significance
Code	Compound	RM	RD	RL	*P-value*	Raw	ST	SF	*P-value*
	Amino acids (µg/g DW)								
Ala	Alanine	4.4^{ab}	4.0^{a}	4.8^{b}	0.003	4.8^{b}	4.4^{ab}	4.0^{a}	0.005
Gly	Glycine	0.2^{b}	0.1^{a}	0.2^{ab}	0.003	0.2^{c}	0.2^{b}	0.2^{a}	< 0.0001
AAA	α-Aminobutyric acid	$<0.1^{a}$	0.1^{b}	0.1^{c}	<0.0001	0.1^{b}	0.1^{b}	$<0.1^{a}$	< 0.0001
Val	Valine	1.2^{b}	1.1^{ab}	1.0^{a}	0.018	1.3^{c}	1.1^{b}	0.9^{a}	< 0.0001
Leu	Leucine	0.3^{b}	0.3^{b}	0.2^{a}	< 0.0001	0.3^{c}	0.25^{b}	0.19^{a}	< 0.0001
Iso	Isoleucine	0.8^{b}	0.7^{a}	0.6^{a}	<0.001	0.8^{c}	0.7^{b}	0.5^{a}	< 0.0001
Thr	Threonine	0.8^{b}	0.8^{b}	0.6^{a}	< 0.0001	0.9^{c}	0.8^{b}	0.6^{a}	< 0.0001
Ser	Serine	4.4^{b}	3.4^{a}	3.6^{a}	< 0.001	4.8^{c}	2.9^{a}	3.6^{b}	< 0.0001
Pro	Proline	1.3	1.6	1.4	0.201	1.6^{b}	1.5^{ab}	1.2^{a}	0.037
Asp	Asparagine	2.9^{b}	2.5^{ab}	2.4^{a}	0.041	3.0^{b}	2.6^{ab}	2.2^{a}	0.003
Asp.A	Aspartic acid	5.3	5.1	4.7	0.101	5.4^{b}	5.6^{b}	4.0^{a}	< 0.0001
Met	Methionine	$<0.1^{ab}$	$<0.1^{a}$	0.1^{b}	0.028	0.1^{b}	$<0.1^{ab}$	$<0.1^{a}$	0.005
Glu.A	Glutamic acid	4.1^{b}	3.1^{a}	3.1^{a}	0.007	2.8^{b}	5.8^{c}	1.6^{a}	< 0.0001
Phy	Phenylalanine	0.1^{b}	0.1^{a}	0.1^{a}	0.002	0.1^{b}	0.1^{b}	0.1^{a}	< 0.0001
Glu	Glutamine	54.1^{b}	40.2^{a}	37.9^{a}	< 0.0001	52.2^{c}	44.4^{b}	35.7^{a}	< 0.0001
Lys	Lysine	0.2^{c}	0.1^{b}	0.1^{a}	< 0.0001	0.2^{b}	0.1^{a}	0.1^{a}	0.001
His	Histidine	1.0^{b}	0.8^{b}	0.5^{a}	< 0.0001	0.8^{ab}	0.9^{b}	0.7^{a}	0.021
Tyr	Tyrosine	0.2^{c}	0.1^{b}	0.1^{a}	< 0.0001	0.1	0.1	0.1	0.040

Tryp	Tryptophan	0.13^{b}	0.11^{b}	0.06^{a}	< 0.0001	0.1^{b}	0.1^{a}	0.1^{a}	< 0.001
TAA	Total amino acids	81.6^{b}	64.3^{a}	61.5^{a}	< 0.0001	79.7^{b}	71.7^{b}	56.0^{a}	< 0.0001
	Sugars(mg/g DW)								
	Sucrose	40.1^{c}	33.9^{b}	23.7^{a}	< 0.0001	33.7	33.1	31.0	0.282
	Glucose	63.0^{b}	55.3^{a}	62.1^{b}	< 0.001	67.8^{b}	64.5^{b}	48.1^{a}	< 0.0001
	Fructose	52.4	49.0	50.7	0.117	57.3^{b}	55.3^{b}	39.6^{a}	< 0.0001
	Total sugars	155.6^{b}	138.2^{a}	136.5^{a}	< 0.0001	158.8^{b}	152.9^{b}	118.6^{a}	< 0.0001
	Organic acids(mg/g DW)								
	Citric	39.1^{a}	64.6^{b}	64.8^{b}	< 0.0001	58.6^{b}	62.2^{b}	47.8^{a}	0.003
	Malic	49.8^{a}	54.5^{ab}	58.5^{b}	0.009	58.6^{b}	58.8^{b}	45.4^{a}	< 0.0001
	Succinic	35.1^{a}	40.6^{a}	47.6^{b}	< 0.0001	53.1^{c}	24.7^{a}	45.5^{b}	< 0.0001
	Total organic acids	124.0^{a}	159.7^{b}	170.9^{b}	<0.0001	170.3^{b}	145.7^{a}	138.7^{a}	< 0.001

Mean values with different superscripts in the same row for each group (Variety and Treatment) significantly different at $p<0.05$

Table S6c: Glucosinolate (mg/g-1 DW) and glucosinolate hydrolysis products (µg/g-1 DW: sulforaphane equivalent) of red cabbage

	RM			RD			RL			Significance
Compound	Raw	ST	SF	Raw	ST	SF	Raw	ST	SF	*P-value*
Glucosinolates (mg/g-1 DW)										
Sinigrin (SIN)	3.4^{ab}	3.0^{ab}	2.7^{a}	4.0^{abc}	3.1^{ab}	2.7^{a}	4.6^{bc}	5.3^{c}	2.6^{a}	< 0.0001
Gluconapin (GPN)	1.4^{ab}	1.1^{a}	1.1^{a}	1.2^{ab}	1.2^{ab}	1.1^{a}	2.6^{c}	1.7^{b}	1.2^{ab}	< 0.0001
EPI/progoitrin (PROG)	3.5^{ab}	3.4^{ab}	2.8^{ab}	3.3^{ab}	4.2^{b}	2.6^{a}	3.8^{ab}	3.6^{ab}	3.1^{ab}	0.024
Glucoerucin (GER)	3.1^{ab}	2.3^{a}	2.5^{a}	4.3^{bc}	3.4^{abc}	3.9^{abc}	5.0^{c}	3.5^{abc}	3.3^{ab}	< 0.0001
Glucoiberin (GIBN)	2.0^{ab}	2.0^{a}	1.8^{a}	2.7^{abc}	2.5^{abc}	2.1^{ab}	3.7^{c}	3.4^{bc}	2.3^{ab}	< 0.001
Gluconasturtiin (GNAS)	4.0^{ab}	3.0^{ab}	2.8^{ab}	14.6^{d}	12.6^{cd}	10.7^{c}	6.1^{b}	2.5^{a}	2.0^{a}	< 0.0001
Glucoraphanin (GRPN)	2.9	2.8	2.6	4.4	4.6	3.3	4.9	4.2	3.4	0.708
Glucobrassicin (GBSN)	8.0^{cd}	6.8^{bcd}	6.5^{bcd}	8.8^{d}	5.2^{abc}	4.7^{ab}	4.4^{ab}	3.3^{a}	2.6^{a}	< 0.0001
4-hydroxyglucobrassicin (4-HOH)	0.5^{ab}	0.3^{a}	0.3^{a}	0.9^{cd}	0.5^{abc}	0.3^{ab}	1.2^{d}	0.7^{bc}	0.6^{abc}	< 0.0001
4-methoxyglucobrassicin (4-MeOH)	2.9^{ab}	2.3^{a}	2.5^{a}	5.8^{bc}	5.1^{abc}	6.0^{c}	9.5^{d}	5.1^{abc}	4.0^{abc}	< 0.0001
Neoglucobrassicin (NEO)	0.6^{a}	0.6^{a}	0.5^{a}	1.6^{c}	0.8^{ab}	1.0^{ab}	1.6^{c}	1.3^{bc}	1.1^{abc}	< 0.0001
Total glucosinolates (GLS)	32.3^{ab}	27.6^{a}	26.2^{a}	51.7^{e}	43.5^{cde}	38.3^{bcd}	47.5^{de}	34.6^{abc}	26.2^{a}	< 0.0001
GLS hydrolysis products (µg/g^{-1} DW; sulforaphane equivalent)										
Allyl thiocyanate (ATC)	0.5^{ab}	0.3^{ab}	0.3^{ab}	0.3^{ab}	0.6^{bc}	1.2^{d}	0.1^{a}	0.3^{ab}	1.0^{cd}	< 0.0001
Allyl-ITC (AITC)	0.4^{ab}	1.7^{b}	1.8^{b}	0.3^{a}	3.1^{cd}	2.6^{bcd}	0.3^{a}	2.3^{bc}	3.5^{d}	< 0.0001
1-cyano-2,3-epithiopropane (CETP)	24.5^{b}	0.6^{a}	1.0^{a}	22.1^{b}	0.2^{a}	1.6^{a}	30.5^{b}	0.7^{a}	0.5^{a}	< 0.0001
3-Butenyl-ITC (3BITC)	2.3^{ab}	5.7^{c}	5.0^{bc}	1.5^{a}	13.2^{e}	4.9^{bc}	2.4^{ab}	9.5^{d}	2.5^{ab}	< 0.0001

4,5-epithiovaleronitrile (EVN)	21.5^{b}	0.2^{a}	1.1^{a}	21.1^{b}	0.4^{a}	1.5^{a}	25.6^{b}	0.2^{a}	0.5^{a}	< 0.0001
Goitrin (GN)	2.1^{a}	110.0^{de}	71.3^{bc}	2.8^{a}	220.0^{f}	48.4^{b}	2.8^{a}	128.6^{e}	88.7^{cd}	< 0.0001
1-cyano-2-hydroxy-3,4-epithiobutane isomer 1 (CHETB-1)	42.4^{b}	ND	3.5^{a}	36.7^{b}	ND	5.4^{a}	41.6^{b}	ND	4.4^{a}	< 0.0001
1-cyano-2-hydroxy-3,4-epithiobutane isomer 2 (CHETB-2)	53.3^{b}	ND	5.6^{a}	47.3^{b}	ND	7.2^{a}	55.5^{b}	ND	4.3^{a}	< 0.0001
Iberverin (IBVN)	ND	4.1^{d}	3.1^{bcd}	ND	2.7^{bc}	2.7^{b}	ND	3.9^{cd}	3.5^{bcd}	< 0.0001
4-methylthiobutyl nitrile (4MBN)	1.8^{b}	ND	1.4^{b}	1.6^{b}	ND	<0.1^{a}	1.8^{b}	ND	0.6^{a}	< 0.0001
Erucin (ER)	0.2^{ab}	12.5^{f}	3.1^{de}	0.1^{ab}	3.5^{e}	1.0^{abc}	0.1^{a}	1.7^{bcd}	2.2^{cde}	< 0.0001
Erucin nitrile (ERN)	7.7^{d}	0.8^{a}	0.8^{a}	4.8^{c}	0.7^{a}	0.7^{a}	2.6^{b}	0.4^{a}	0.6^{a}	< 0.0001
Iberin (IB)	6.9^{a}	116.1^{b}	106.3^{b}	7.5^{a}	170.2^{c}	121.7^{b}	10.5^{a}	130.6^{b}	140.5^{bc}	< 0.0001
Iberin nitrile (IBN)	47.5^{b}	ND	0.5^{a}	90.8^{c}	ND	6.6^{a}	71.3^{bc}	ND	4.3^{a}	< 0.0001
2-phenylethyl-ITC (PEITC)	0.7^{ab}	2.2^{c}	0.1^{a}	1.0^{b}	2.2^{c}	0.9^{b}	1.1^{b}	1.7^{c}	0.5^{ab}	< 0.0001
Benzenepropanenitrile (BPN)	2.4^{c}	ND	2.4^{c}	2.3^{c}	ND	<0.1^{a}	2.0^{c}	ND	0.6^{b}	< 0.0001
Sulforaphane (SFP)	36.3^{a}	347.5^{c}	315.6^{bc}	32.7^{a}	706.3^{e}	276.0^{bc}	32.4^{a}	566.8^{d}	184.2^{b}	< 0.0001
Sulforaphane nitrile (SFN)	189.1^{b}	3.0^{a}	8.7^{a}	225.5^{b}	3.7^{a}	20.2^{a}	346.1^{c}	4.1^{a}	15.2^{a}	< 0.0001
Indole-3-carbinol (I3C)	3.0^{b}	0.5^{a}	2.2^{ab}	5.6^{c}	0.5^{a}	2.0^{ab}	2.3^{ab}	0.4^{a}	1.0^{a}	< 0.0001
Indoleacetonitrile (1IAN)	55.7^{c}	1.6^{a}	1.4^{a}	83.8^{d}	2.1^{a}	3.0^{a}	25.3^{b}	1.5^{a}	1.7^{a}	< 0.0001
Pentyl-ITC (PITC)	ND	2.6^{c}	1.0^{b}	ND	3.2^{c}	0.9^{b}	ND	1.8^{b}	1.8^{b}	< 0.0001
1H-Indole (1H-I)	1.5^{a}	ND	ND	1.5^{a}	ND	ND	1.1^{a}	ND	ND	< 0.0001
Benzeneacetonitrile (BAN)	1.1^{a}	ND	ND	1.4^{b}	ND	ND	1.0^{a}	ND	ND	< 0.0001
Total hydrolysis products (HPS)	501.0^{ab}	609.5^{ab}	536.5^{ab}	590.7^{ab}	1132.6^{d}	508.7^{ab}	656.5^{b}	854.5^{c}	462.3^{a}	< 0.0001

Mean values with different superscripts in the same row significantly different at p<0.05

Table S6d: Effect of red cabbage variety and cooking method on glucosinolate (mg/g-1 DW) and glucosinolate hydrolysis products (µg/g-1 DW; sulforaphane equivalent) concentrations

	Variety			Significance	Treatment			Significance
Compound	RM	RD	RL	*P-value*	Raw	ST	SF	*P-value*
Glucosinolates (mg.g-1 DW)								
Sinigrin (SIN)	3.0^{a}	3.3^{a}	4.2^{b}	0.003	4.0^{b}	3.8^{b}	2.7^{a}	0.000
Gluconapin (GPN)	1.2^{a}	1.2^{a}	1.9^{b}	< 0.0001	1.8^{c}	1.4^{b}	1.1^{a}	< 0.0001
EPI/progoitrin (PROG)	3.2	3.4	3.5	0.584	3.6^{b}	3.7^{b}	2.8^{a}	0.003
Glucoerucin (GER)	2.6^{a}	3.9^{b}	3.9^{b}	< 0.0001	4.1^{b}	3.1^{a}	3.2^{a}	0.001
Glucoiberin (GIBN)	2.0^{a}	2.4^{a}	3.2^{b}	< 0.0001	2.8^{b}	2.6^{ab}	2.1^{a}	0.015
Gluconasturtiin (GNAS)	3.3^{a}	12.6^{b}	3.5^{a}	< 0.0001	8.2^{b}	6.0^{a}	5.2^{a}	< 0.0001
Glucoraphanin (GRPN)	2.8^{a}	4.1^{b}	4.1^{b}	0.004	4.0	3.9	3.1	0.076
Glucobrassicin (GBSN)	7.1^{b}	6.2^{b}	3.4^{a}	< 0.0001	7.1^{b}	5.1^{a}	4.6^{a}	< 0.0001
4-hydroxyglucobrassicin (4-HOH)	0.3^{a}	0.6^{b}	0.8^{c}	< 0.0001	0.8^{b}	0.5^{a}	0.4^{a}	< 0.0001
4-methoxyglucobrassicin (4-MeOH)	2.6^{a}	5.7^{b}	6.2^{b}	< 0.0001	6.1^{b}	4.2^{a}	4.2^{a}	0.001
Neoglucobrassicin (NEO)	0.6^{a}	1.1^{b}	1.3^{b}	< 0.0001	1.3^{b}	0.9^{a}	0.8^{a}	0.001
Total glucosinolates (GLS)	28.7^{a}	44.5^{c}	36.1^{b}	< 0.0001	43.8^{c}	35.2^{b}	30.2^{a}	< 0.0001
GLS hydrolysis products (µg.g^{-1} DW: sulforaphane equivalent)								
Allyl thiocyanate (ATC)	0.4^{a}	0.7^{b}	0.5^{a}	< 0.0001	0.3^{a}	0.4^{a}	0.8^{b}	< 0.0001
Allyl-ITC (AITC)	1.3^{a}	2.1^{b}	2.1^{b}	0.001	0.3^{a}	2.4^{b}	2.6^{b}	< 0.0001
1-cyano-2,3-epithiopropane (CETP)	8.7	8.0	10.6	0.440	25.7^{b}	0.5^{a}	1.0^{a}	< 0.0001
3-Butenyl-ITC (3BITC)	4.3^{a}	6.5^{b}	4.8^{b}	0.001	2.0^{a}	9.5^{c}	4.1^{b}	< 0.0001

4,5-epithiovaleronitrile (EVN)	7.6	7.7	8.8	0.479	22.7^{b}	0.3^{a}	1.1^{a}	< 0.0001
Goitrin (GN)	61.1^{a}	90.4^{b}	73.4^{a}	< 0.001	2.6^{a}	152.8^{c}	69.5^{b}	< 0.0001
1-cyano-2-hydroxy-3,4-epithiobutane isomer 1 (CHETB-1)	15.3	14.0	15.3	0.714	40.3^{b}	ND	4.4^{a}	< 0.0001
1-cyano-2-hydroxy-3,4-epithiobutane isomer 2 (CHETB-2)	19.6	18.2	19.9	0.715	52.0^{b}	ND	5.7^{a}	< 0.0001
Iberverin (IBVN)	2.4^{b}	1.8^{a}	2.5^{b}	0.005	ND	3.6^{a}	3.1^{b}	< 0.0001
4-methylthiobutyl nitrile (4MBN)	1.1^{b}	0.5^{a}	0.8^{ab}	0.002	1.7^{c}	ND	0.7^{b}	< 0.0001
Erucin (ER)	5.3^{b}	1.5^{a}	1.3^{a}	< 0.0001	0.2^{a}	5.9^{c}	2.1^{b}	< 0.0001
Erucin nitrile (ERN)	3.1^{c}	2.0^{b}	1.2^{a}	< 0.0001	5.0^{b}	0.6^{a}	0.7^{a}	< 0.0001
Iberin (IB)	76.5^{a}	99.8^{b}	93.9^{b}	0.002	8.3^{a}	139.0^{c}	122.8^{b}	< 0.0001
Iberin nitrile (IBN)	16.0^{a}	32.5^{b}	25.2^{ab}	0.001	69.7^{b}	ND	3.8^{a}	< 0.0001
2-phenylethyl-ITC (PEITC)	1.0^{a}	1.4^{b}	1.1^{a}	0.002	0.9^{b}	2.1^{c}	0.5^{a}	< 0.0001
Benzenepropanenitrile (BPN)	1.6^{b}	0.8^{a}	1.1^{a}	< 0.0001	2.2^{c}	ND	1.0^{b}	< 0.0001
Sulforaphane (SFP)	233.1^{a}	338.4^{b}	261.2^{a}	< 0.001	33.8^{a}	540.2^{c}	258.6^{b}	< 0.0001
Sulforaphane nitrile (SFN)	66.9^{a}	83.1^{b}	121.8^{c}	< 0.0001	253.6^{b}	3.6^{a}	14.7^{a}	< 0.0001
Indole-3-carbinol (I3C)	1.9^{ab}	2.7^{b}	1.2^{a}	0.001	3.6^{c}	0.5^{a}	1.8^{b}	< 0.0001
Indoleacetonitrile (1IAN)	19.6^{b}	29.6^{c}	9.5^{a}	< 0.0001	54.9^{b}	1.7^{a}	2.0^{a}	< 0.0001
Pentyl-ITC (PITC)	1.2	1.4	1.2	0.369	ND	2.5^{b}	1.2^{a}	< 0.0001
1H-Indole (1H-I)	0.5	0.5	0.4	0.121	1.4^{a}	ND	ND	< 0.0001
Benzeneacetonitrile (BAN)	0.4^{a}	0.5^{b}	0.3^{a}	0.001	1.2^{a}	ND	ND	< 0.0001
Total hydrolysis products (HPS)	549.0^{a}	744.0^{c}	657.8^{b}	< 0.0001	582.7^{b}	865.5^{c}	502.5^{a}	< 0.0001

Mean values with different superscripts in the same row for each group (Variety and Treatment) significantly different at $p<0.05$

Table S6e: Correlation values for drivers of liking in red cabbage

Variables	Sugars: glu/fru/total sugars	OAs: citric/malic/Total OAs	OAs: succinic	AAs: Glu.A	AAs: AAA/Pro	GSLs: SIN/GPN/GER/GIBN/4-HOH/4-MeOH/NEO	GSLs: GER/GNAS/GRPN/T_GSL	GSL: PROG	GSLs: GBSN	GHPs: CETP/EVN/CHETB-1&2/ERN/IBN/SFN/I3C/1IAN/IH-I/BAN VS AITC/IBVN/IB	GHPs: 3BITC/GN/PEITC/SFP/T_GHP VS 4MBN	Alcohol: 1pent3/2pent(E)/3hex(Z)/2hex1ethyl	Alcohol: 2hex(E)/2hep(E) vs 1pent	Alcohol: 1hex	Aldehyde: hep/oct/non vs 2hep(E,E)	Aldehyde: benz	Sulfur: met/CDS/DMDS/thio/DMTS/DMMMS/DMTT vs DMS	Sulfur: ITCP 1&2/ATC/AITC	Nitrile: but/3but/CETP/4MBN/EVN/BSN/BPN	Nitrile: 4MBN/5MPN	Nitrile: 3MBN/BAN	Other: 2fur/fur	Other: hexace	App: marks/shiny/oily/cooked	Odour: metallic/stalky/sulfurous vs sweet/cooked	Odour: sesame/burnt vs stalky/swede	MF: crunchy/warming/fibrous/toughness vs moist	MF: oily	Taste: stalk-bitter/leaf-bitter/leaf-metallic	Taste: leaf-sweet/savoury/salty	Taste: stalk-sweet	Flavour: stalky/earthy/sulfury/peppery	Flavour: sesame/burnt vs stalky/earthy	AE: oily mouthcoating/burnt/salty/nutty vs bitter/earthy	AE: bitter/residue/lingering	Gustin_BT: GG/AG	Gustin_BT: AG/AA	Gustin_TL: AG/AA	Gustin_TL: GG	TAS2R38_BT:PAV/AVI/AVI/AVI/Rare	TAS2R38_BT: PAV/PAV/PAV/AVI/AVI/AVI	TAS2R38_TL: PAV/PAV/PAV/AVI	TAS2R38_TL: AVI/AVI	Taste: sweet vs bitter	Taste: savoury	Liking: taste/mouthfeel/overall/consump.Intent	Liking: taste/consump.Intent vs App	Cluster 1	Cluster 2	Cluster 3
Sugars: glu/fru/total sugars	1	0.546	-0.179	0.460	0.386	0.508	-0.150	0.638	0.466	0.637	0.336	0.264	-0.784	0.435	-0.064	-0.783	0.336	-0.864	0.203	0.224	0.527	-0.568	0.394	-0.937	0.329	-0.918	0.205	-0.922	0.257	-0.849	-0.018	0.361	-0.884	-0.936	-0.025	0.074	0.736	-0.720	0.326	0.197	0.623	-0.497	-0.079	-0.214	-0.912	-0.014	-0.732	0.917	-0.699	-0.821
OAs: citric/malic/Total OAs	0.546	1	0.000	0.285	0.786	0.518	0.384	0.632	-0.169	0.471	0.697	0.231	-0.370	0.166	0.206	-0.495	0.350	-0.383	0.073	-0.152	0.517	-0.805	-0.073	-0.663	0.144	-0.621	0.025	-0.731	0.129	-0.513	-0.706	0.209	-0.609	-0.650	-0.090	-0.066	0.768	-0.677	0.367	-0.067	0.683	-0.417	0.048	-0.388	-0.558	-0.244	-0.507	0.642	-0.490	-0.784
OAs: succinic	-0.179	0.000	1	-0.881	0.074	0.505	0.334	-0.520	-0.090	0.432	-0.550	0.377	0.521	0.585	-0.763	0.291	0.780	0.453	0.670	0.197	0.239	0.224	0.532	0.308	0.777	0.473	0.832	0.353	0.868	0.272	-0.027	0.804	0.527	0.175	0.932	0.928	-0.089	-0.341	-0.477	0.713	0.143	-0.556	-0.121	-0.774	0.368	-0.474	-0.279	0.075	-0.409	0.014
AAs: Glu.A	0.460	0.285	-0.881	1	0.000	-0.208	-0.363	0.645	-0.015	-0.218	0.567	-0.350	-0.696	-0.293	0.685	-0.326	-0.576	-0.575	-0.456	-0.231	-0.101	-0.535	-0.462	-0.530	-0.599	-0.667	-0.757	-0.582	-0.628	-0.363	-0.096	-0.619	-0.691	-0.385	-0.856	-0.804	0.245	0.086	0.662	-0.620	0.040	0.432	0.014	0.648	-0.576	0.438	0.046	0.259	0.172	-0.218
AAs: AAA/Pro	0.386	0.786	0.074	0.000	1	0.461	0.681	0.437	0.096	0.510	0.652	0.469	-0.347	-0.128	0.071	-0.535	0.450	-0.397	0.020	-0.250	0.764	-0.508	0.052	-0.585	0.208	-0.497	0.211	-0.619	0.154	-0.514	-0.792	0.287	-0.526	-0.637	0.069	0.052	0.806	-0.681	-0.190	0.225	0.552	-0.579	-0.184	-0.566	-0.428	-0.655	-0.352	0.383	-0.594	-0.688
GSLs: SIN/GPN/GER/GIBN/4-HOH/4-Me	0.508	0.518	0.505	-0.208	0.461	1	0.000	0.000	0.000	0.501	-0.041	0.300	-0.141	0.541	-0.207	-0.242	0.843	-0.095	0.613	-0.076	0.506	-0.191	0.274	-0.366	0.551	-0.284	0.507	-0.392	0.618	-0.409	-0.217	0.630	-0.226	-0.441	0.432	0.564	0.389	-0.558	-0.196	0.626	0.317	-0.779	-0.043	-0.745	-0.180	-0.397	-0.563	0.535	-0.626	-0.650
GSLs: GER/GNAS/GRPN/T_GSL	-0.150	0.384	0.334	-0.363	0.681	0.000	1	0.000	0.000	0.452	0.276	0.468	-0.001	-0.211	-0.324	-0.072	0.286	-0.035	-0.044	-0.110	0.563	-0.338	0.139	-0.110	0.330	0.076	0.330	-0.066	0.237	0.043	-0.727	0.331	0.015	-0.170	0.348	0.205	0.444	-0.487	-0.408	0.168	0.401	-0.306	-0.433	-0.487	-0.024	-0.719	-0.092	0.007	-0.425	-0.093
GSL: PROG	0.638	0.632	-0.520	0.645	0.437	0.000	0.000	1	0.000	0.158	0.851	-0.148	-0.609	0.062	0.432	-0.588	-0.239	-0.715	-0.061	-0.098	0.150	-0.550	-0.153	-0.730	-0.254	-0.842	-0.298	-0.795	-0.193	-0.547	-0.286	-0.197	-0.817	-0.678	-0.505	-0.414	0.565	-0.378	0.598	-0.336	0.454	-0.008	0.265	0.250	-0.824	0.196	-0.275	0.552	-0.164	-0.618
GSLs: GBSN	0.466	-0.169	-0.090	-0.015	0.096	0.000	0.000	0.000	1	0.541	-0.194	0.665	-0.354	0.001	-0.412	-0.694	0.258	-0.577	-0.246	0.596	0.526	-0.030	0.735	-0.457	0.389	-0.372	0.410	-0.356	0.100	-0.624	0.343	0.365	-0.411	-0.514	0.254	0.148	0.484	-0.425	-0.169	0.317	0.375	-0.368	-0.292	-0.239	-0.440	-0.158	-0.443	0.384	-0.541	-0.289
GHPs: CETP/EVN/CHETB-1&2/ERN/IBN/S	0.637	0.471	0.432	-0.218	0.510	0.501	0.452	0.158	0.541	1	0.000	0.675	-0.382	0.525	-0.680	-0.621	0.712	-0.527	0.312	0.518	0.744	-0.508	0.762	-0.651	0.862	-0.434	0.758	-0.553	0.643	-0.609	-0.151	0.856	-0.430	-0.730	0.625	0.574	0.757	-0.950	-0.113	0.465	0.852	-0.795	-0.306	-0.732	-0.560	-0.417	-0.872	0.801	-0.956	-0.649
GHPs: 3BITC/GN/PEITC/SFP/T_GHP VS 4M	0.336	0.697	-0.550	0.567	0.652	-0.041	0.276	0.851	-0.194	0.000	1	-0.175	-0.482	-0.295	0.628	-0.459	-0.292	-0.489	-0.239	-0.346	0.163	-0.532	-0.465	-0.559	-0.418	-0.644	-0.443	-0.644	-0.416	-0.423	-0.594	-0.363	-0.665	-0.500	-0.607	-0.563	0.543	-0.234	0.372	-0.461	0.352	0.012	0.238	0.186	-0.549	0.015	-0.068	0.246	-0.019	-0.529
Alcohol: 1pent3/2pent(E)/3hex(Z)/2hex	0.264	0.231	0.377	-0.350	0.469	0.300	0.468	-0.148	0.665	0.675	-0.175	1	0.000	0.000	-0.520	-0.479	0.670	-0.221	-0.167	0.330	0.848	-0.254	0.738	-0.307	0.565	-0.152	0.588	-0.259	0.402	-0.374	-0.236	0.592	-0.186	-0.407	0.549	0.377	0.623	-0.656	-0.262	0.546	0.479	-0.510	-0.496	-0.683	-0.219	-0.651	-0.401	0.355	-0.708	-0.321
Alcohol: 2hex(E)/2hep(E) vs 1pent	-0.784	-0.370	0.521	-0.696	-0.347	-0.141	-0.001	-0.609	-0.354	-0.382	-0.482	0.000	1	0.000	-0.148	0.589	0.091	0.911	0.048	0.072	-0.395	0.577	-0.026	0.860	-0.003	0.840	0.159	0.821	0.103	0.655	0.170	0.016	0.866	0.802	0.355	0.277	-0.590	0.450	-0.175	0.080	-0.369	0.167	0.324	-0.141	0.830	0.034	0.377	-0.590	0.467	0.509
Alcohol: 1hex	0.435	0.166	0.585	-0.293	-0.128	0.541	-0.211	0.062	0.001	0.525	-0.295	0.000	0.000	1	-0.570	-0.095	0.536	-0.086	0.810	0.468	0.001	-0.050	0.524	-0.197	0.706	-0.124	0.643	-0.153	0.765	-0.183	0.353	0.718	-0.018	-0.258	0.590	0.722	0.061	-0.462	0.142	0.400	0.415	-0.534	0.250	-0.419	-0.232	0.156	-0.679	0.625	-0.452	-0.379
Aldehyde: 3hex/hex/2hexe(Z)/2hep(E,E	-0.374	-0.577	0.052	-0.449	-0.210	-0.197	-0.234	-0.319	0.305	-0.320	-0.270	0.068	0.499	-0.201	0.000	0.000	-0.032	0.303	-0.088	0.196	-0.217	0.824	0.140	0.438	-0.116	0.353	0.153	0.428	-0.137	0.058	0.487	-0.076	0.354	0.356	0.120	0.103	-0.333	0.401	-0.393	0.191	-0.363	-0.037	0.388	-0.012	0.413	0.070	0.262	-0.477	0.322	0.210
Aldehyde: hep/oct/non vs 2hep(E,E)	-0.064	0.206	-0.763	0.685	0.071	-0.207	-0.324	0.432	-0.412	-0.680	0.628	-0.520	-0.148	-0.570	1	0.000	-0.611	-0.014	-0.469	-0.565	-0.343	-0.101	-0.848	-0.033	-0.900	-0.251	-0.892	-0.172	-0.804	-0.036	-0.240	-0.859	-0.271	0.081	-0.924	-0.854	-0.077	0.490	0.427	-0.641	-0.335	0.509	0.363	0.602	-0.030	0.365	0.461	-0.278	0.604	-0.034
Aldehyde: benz	-0.783	-0.495	0.291	-0.326	-0.535	-0.242	-0.072	-0.588	-0.694	-0.621	-0.459	-0.479	0.589	-0.095	0.000	1	-0.243	0.798	0.194	-0.445	-0.568	0.485	-0.411	0.853	-0.244	0.818	-0.202	0.832	0.037	0.965	0.034	-0.268	0.835	0.869	0.059	0.091	-0.865	0.671	-0.243	-0.007	-0.753	0.522	-0.085	0.270	0.793	0.021	0.706	-0.733	0.603	0.830
Sulfur: met/CDS/DMDS/thio/DMTS/DMI	0.336	0.350	0.780	-0.576	0.450	0.843	0.286	-0.239	0.258	0.712	-0.292	0.670	0.091	0.536	-0.611	-0.243	1	0.000	0.539	0.204	0.675	-0.097	0.649	-0.224	0.826	-0.056	0.833	-0.195	0.812	-0.304	-0.170	0.885	-0.016	-0.354	0.799	0.809	0.423	-0.689	-0.396	0.812	0.431	-0.857	-0.226	-0.954	-0.066	-0.611	-0.601	0.474	-0.776	-0.495
Sulfur: ITCP 1&2/ATC/AITC	-0.864	-0.383	0.453	-0.575	-0.397	-0.095	-0.035	-0.715	-0.577	-0.527	-0.489	-0.221	0.911	-0.086	-0.014	0.798	0.000	1	0.080	-0.166	-0.500	0.540	-0.294	0.930	-0.118	0.917	-0.021	0.887	0.009	0.787	0.075	-0.121	0.931	0.909	0.222	0.182	-0.729	0.601	-0.241	-0.005	-0.543	0.265	0.217	-0.045	0.954	0.040	0.507	-0.712	0.573	0.625
Nitrile: but/3but/CETP/4MBN/EVN/BSN	0.203	0.073	0.670	-0.456	0.020	0.613	-0.044	-0.061	-0.246	0.312	-0.239	-0.167	0.048	0.810	-0.469	0.194	0.539	0.080	1	0.000	0.000	0.223	0.274	-0.003	0.584	0.058	0.606	0.021	0.803	0.061	0.125	0.628	0.158	-0.092	0.580	0.784	-0.131	-0.290	-0.294	0.597	0.070	-0.542	0.129	-0.450	0.007	-0.153	-0.352	0.286	-0.346	-0.213
Nitrile: 4MBN/5MPN	0.224	-0.152	0.197	-0.231	-0.250	-0.076	-0.110	-0.098	0.596	0.518	-0.346	0.330	0.072	0.468	-0.565	-0.445	0.204	-0.166	0.000	1	0.000	-0.031	0.674	-0.148	0.557	-0.037	0.516	-0.039	0.204	-0.382	0.608	0.489	-0.028	-0.182	0.440	0.330	0.195	-0.317	0.185	-0.012	0.570	-0.337	0.254	-0.239	-0.177	0.355	-0.654	0.428	-0.304	-0.213
Nitrile: 3MBN/BAN	0.527	0.517	0.239	-0.101	0.764	0.506	0.563	0.150	0.526	0.744	0.163	0.848	-0.395	0.001	-0.343	-0.568	0.675	-0.500	0.000	0.000	1	-0.473	0.541	-0.617	0.506	-0.467	0.478	-0.587	0.422	-0.532	-0.530	0.555	-0.499	-0.698	0.371	0.298	0.816	-0.817	-0.294	0.572	0.532	-0.617	-0.606	-0.676	-0.487	-0.796	-0.454	0.519	-0.863	-0.555
Other: 2fur/fur	-0.568	-0.805	0.224	-0.535	-0.508	-0.191	-0.338	-0.550	-0.030	-0.508	-0.532	-0.254	0.577	-0.050	-0.101	0.485	-0.097	0.540	0.223	-0.031	-0.473	1	0.000	0.716	-0.112	0.633	0.126	0.720	0.038	0.472	0.558	-0.094	0.660	0.645	0.197	0.260	-0.742	0.626	-0.523	0.286	-0.698	0.136	0.256	0.118	0.656	0.089	0.484	-0.670	0.478	0.551
Other: hexace	0.394	-0.073	0.532	-0.462	0.052	0.274	0.139	-0.153	0.735	0.762	-0.465	0.738	-0.026	0.524	-0.848	-0.411	0.649	-0.294	0.274	0.674	0.541	0.000	1	-0.274	0.819	-0.123	0.842	-0.167	0.697	-0.344	0.306	0.817	-0.098	-0.390	0.770	0.698	0.367	-0.638	-0.213	0.658	0.480	-0.572	-0.285	-0.584	-0.297	-0.312	-0.603	0.518	-0.725	-0.262
App: dark colour/purple/moist/rubbery	-0.144	0.180	-0.784	0.743	0.023	-0.353	-0.188	0.386	-0.478	-0.680	0.600	-0.535	-0.205	-0.652	0.943	0.136	-0.716	-0.012	-0.547	-0.633	-0.338	-0.227	-0.891	0.000	-0.919	-0.184	-0.967	-0.117	-0.822	0.120	-0.349	-0.911	-0.223	0.138	-0.945	-0.913	-0.114	0.503	0.436	-0.716	-0.363	0.666	0.140	0.698	-0.017	0.308	0.558	-0.331	0.614	0.136
App: marks/shiny/oily/cooked	-0.937	-0.663	0.308	-0.530	-0.585	-0.366	-0.110	-0.730	-0.457	-0.651	-0.559	-0.307	0.860	-0.197	-0.033	0.853	-0.224	0.930	-0.003	-0.148	-0.617	0.716	-0.274	1	-0.231	0.958	-0.098	0.983	-0.089	0.876	0.252	-0.254	0.962	0.985	0.142	0.098	-0.877	0.760	-0.301	-0.040	-0.713	0.454	0.153	0.188	0.951	0.115	0.677	-0.856	0.697	0.835
Odour: metallic/stalky/sulfurous vs swe	0.329	0.144	0.777	-0.599	0.208	0.551	0.330	-0.254	0.389	0.862	-0.418	0.565	-0.003	0.706	-0.900	-0.244	0.826	-0.118	0.584	0.557	0.506	-0.112	0.819	-0.231	1	0.000	0.951	-0.118	0.858	-0.270	0.094	0.987	0.032	-0.347	0.911	0.888	0.350	-0.722	-0.351	0.668	0.582	-0.803	-0.232	-0.822	-0.145	-0.395	-0.745	0.549	-0.805	-0.359
Odour: sesame/burnt vs stalky/swede	-0.918	-0.621	0.473	-0.667	-0.497	-0.284	0.076	-0.842	-0.372	-0.434	-0.644	-0.152	0.840	-0.124	-0.251	0.818	-0.056	0.917	0.058	-0.037	-0.467	0.633	-0.123	0.958	0.000	1	0.100	0.980	0.061	0.840	0.195	-0.043	0.991	0.924	0.347	0.267	-0.774	0.591	-0.434	0.067	-0.561	0.282	0.013	-0.013	0.955	-0.031	0.526	-0.774	0.502	0.794
MF: crunchy/warming/fibrous/toughnes	0.205	0.025	0.832	-0.757	0.211	0.507	0.330	-0.298	0.410	0.758	-0.443	0.588	0.159	0.643	-0.892	-0.202	0.833	-0.021	0.606	0.516	0.478	0.126	0.842	-0.098	0.951	0.100	1	0.000	0.870	-0.199	0.139	0.967	0.139	-0.246	0.955	0.939	0.258	-0.630	-0.485	0.772	0.454	-0.804	-0.147	-0.838	-0.035	-0.457	-0.616	0.395	-0.719	-0.293
MF: oily	-0.922	-0.731	0.353	-0.582	-0.619	-0.392	-0.066	-0.795	-0.356	-0.553	-0.644	-0.259	0.821	-0.153	-0.172	0.832	-0.195	0.887	0.021	-0.039	-0.587	0.720	-0.167	0.983	-0.118	0.980	0.000	1	-0.031	0.858	0.318	-0.162	0.977	0.961	0.237	0.176	-0.865	0.708	-0.371	0.000	-0.660	0.401	0.080	0.149	0.938	0.097	0.608	-0.824	0.618	0.858
Taste: stalk-bitter/leaf-bitter/leaf-meta	0.257	0.129	0.868	-0.628	0.154	0.618	0.237	-0.193	0.100	0.643	-0.416	0.402	0.103	0.765	-0.804	0.037	0.812	0.009	0.803	0.204	0.422	0.038	0.697	-0.089	0.858	0.061	0.870	-0.031	1	0.000	0.000	0.897	0.135	-0.218	0.875	0.941	0.141	-0.598	-0.354	0.827	0.293	-0.647	-0.249	-0.721	-0.078	-0.483	-0.479	0.426	-0.671	-0.225
Taste: leaf-sweet/savoury/salty	-0.849	-0.513	0.272	-0.363	-0.514	-0.409	0.043	-0.547	-0.624	-0.609	-0.423	-0.374	0.655	-0.183	-0.036	0.965	-0.304	0.787	0.061	-0.382	-0.532	0.472	-0.344	0.876	-0.270	0.840	-0.199	0.858	0.000	1	0.000	-0.294	0.847	0.885	0.067	0.046	-0.823	0.650	-0.206	-0.052	-0.708	0.599	-0.108	0.303	0.772	-0.010	0.756	-0.770	0.620	0.887
Taste: stalk-sweet	-0.018	-0.706	-0.027	-0.096	-0.792	-0.217	-0.727	-0.286	0.343	-0.151	-0.594	-0.236	0.170	0.353	-0.240	0.034	-0.170	0.075	0.125	0.608	-0.530	0.558	0.306	0.252	0.094	0.195	0.139	0.318	0.000	0.000	1	0.038	0.237	0.249	0.122	0.165	-0.477	0.358	0.086	-0.007	-0.212	0.100	0.373	0.271	0.143	0.642	-0.093	-0.045	0.249	0.245
Flavour: stalky/earthy/sulfury/peppery	0.361	0.209	0.804	-0.619	0.287	0.630	0.331	-0.197	0.365	0.856	-0.363	0.592	0.016	0.718	-0.859	-0.268	0.885	-0.121	0.628	0.489	0.555	-0.094	0.817	-0.254	0.987	-0.043	0.967	-0.162	0.897	-0.294	0.038	1	0.000	-0.383	0.913	0.910	0.385	-0.754	-0.358	0.735	0.575	-0.847	-0.198	-0.869	-0.168	-0.452	-0.738	0.566	-0.825	-0.425
Flavour: sesame/burnt vs stalky/earthy	-0.884	-0.609	0.527	-0.691	-0.526	-0.226	0.015	-0.817	-0.411	-0.430	-0.665	-0.186	0.866	-0.018	-0.271	0.835	-0.016	0.931	0.158	-0.028	-0.499	0.660	-0.098	0.962	0.032	0.991	0.139	0.977	0.135	0.847	0.237	0.000	1	0.921	0.383	0.330	-0.802	0.578	-0.400	0.111	-0.562	0.256	0.073	-0.029	0.943	0.002	0.495	-0.731	0.496	0.765
AE: oily mouthcoating/burnt/salty/nutty	-0.936	-0.650	0.175	-0.385	-0.637	-0.441	-0.170	-0.678	-0.514	-0.730	-0.500	-0.407	0.802	-0.258	0.081	0.869	-0.354	0.909	-0.092	-0.182	-0.698	0.645	-0.390	0.985	-0.347	0.924	-0.246	0.961	-0.218	0.885	0.249	-0.383	0.921	1	0.000	-0.044	-0.900	0.831	-0.186	-0.189	-0.737	0.570	0.171	0.320	0.929	0.222	0.721	-0.869	0.784	0.863
AE: bitter/residue/lingering	-0.025	-0.090	0.932	-0.856	0.069	0.432	0.348	-0.505	0.254	0.625	-0.607	0.549	0.355	0.590	-0.924	0.059	0.799	0.222	0.580	0.440	0.371	0.197	0.770	0.142	0.911	0.347	0.955	0.237	0.875	0.067	0.122	0.913	0.383	0.000	1	0.956	0.060	-0.476	-0.522	0.758	0.290	-0.652	-0.223	-0.800	0.198	-0.476	-0.441	0.207	-0.580	-0.055
Gustin_BT: GG/AG	0.074	-0.066	0.928	-0.804	0.052	0.564	0.205	-0.414	0.148	0.574	-0.563	0.377	0.277	0.722	-0.854	0.091	0.809	0.182	0.784	0.330	0.298	0.260	0.698	0.098	0.888	0.267	0.939	0.176	0.941	0.046	0.165	0.910	0.330	-0.044	0.956	1	0.000	-0.453	-0.516	0.818	0.219	-0.695	-0.128	-0.767	0.147	-0.420	-0.453	0.257	-0.566	-0.126
Gustin_BT: AG/AA	0.736	0.768	-0.089	0.245	0.806	0.389	0.444	0.565	0.484	0.757	0.543	0.623	-0.590	0.061	-0.077	-0.865	0.423	-0.729	-0.131	0.195	0.816	-0.742	0.367	-0.877	0.350	-0.774	0.258	-0.865	0.141	-0.823	-0.477	0.385	-0.802	-0.900	0.060	0.000	1	-0.866	0.151	0.119	0.834	-0.579	-0.210	-0.473	-0.772	-0.378	-0.680	0.759	-0.776	-0.837
Gustin_TL: AG/AA	-0.720	-0.677	-0.341	0.086	-0.681	-0.558	-0.487	-0.378	-0.425	-0.950	-0.234	-0.656	0.450	-0.462	0.490	0.671	-0.689	0.601	-0.290	-0.317	-0.817	0.626	-0.638	0.760	-0.722	0.591	-0.630	0.708	-0.598	0.650	0.358	-0.754	0.578	0.831	-0.476	-0.453	-0.866	1	0.000	-0.433	-0.862	0.756	0.282	0.695	0.682	0.478	0.816	-0.849	0.950	0.765
Gustin_TL: GG	0.326	0.367	-0.477	0.662	-0.190	-0.196	-0.408	0.598	-0.169	-0.113	0.372	-0.262	-0.175	0.142	0.427	-0.243	-0.396	-0.241	-0.294	0.185	-0.294	-0.523	-0.213	-0.301	-0.351	-0.434	-0.485	-0.371	-0.354	-0.206	0.086	-0.358	-0.400	-0.186	-0.522	-0.516	0.151	0.000	1	-0.650	0.282	0.389	0.410	0.472	-0.434	0.678	-0.164	0.394	0.220	-0.234
TAS2R38_BT:PAV/AVI/AVI/AVI/Rare	0.197	-0.067	0.713	-0.620	0.225	0.626	0.168	-0.336	0.317	0.465	-0.461	0.546	0.080	0.400	-0.641	-0.007	0.812	-0.005	0.597	-0.012	0.572	0.286	0.658	-0.040	0.668	0.067	0.772	0.000	0.827	-0.052	-0.007	0.735	0.111	-0.189	0.758	0.818	0.119	-0.433	-0.650	1	0.000	-0.621	-0.417	-0.702	0.022	-0.697	-0.228	0.183	-0.614	-0.147
TAS2R38_BT: PAV/PAV/PAV/AVI/AVI/AV	0.623	0.683	0.143	0.040	0.552	0.317	0.401	0.454	0.375	0.852	0.352	0.479	-0.369	0.415	-0.335	-0.753	0.431	-0.543	0.070	0.570	0.532	-0.698	0.480	-0.713	0.582	-0.561	0.454	-0.660	0.293	-0.708	-0.212	0.575	-0.562	-0.737	0.290	0.219	0.834	-0.862	0.282	0.000	1	-0.628	0.062	-0.517	-0.644	-0.086	-0.885	0.828	-0.724	-0.775
TAS2R38_TL: PAV/PAV/PAV/AVI	-0.497	-0.417	-0.556	0.432	-0.579	-0.779	-0.306	-0.008	-0.368	-0.795	0.012	-0.510	0.167	-0.534	0.509	0.522	-0.857	0.265	-0.542	-0.337	-0.617	0.136	-0.572	0.454	-0.803	0.282	-0.804	0.401	-0.647	0.599	0.100	-0.847	0.256	0.570	-0.652	-0.695	-0.579	0.756	0.389	-0.621	-0.628	1	0.000	0.892	0.272	0.444	0.790	-0.591	0.810	0.723
TAS2R38_TL: Rare	-0.266	-0.435	0.072	-0.107	-0.402	0.267	-0.488	-0.681	-0.091	-0.356	-0.546	-0.210	0.215	-0.076	0.106	0.369	0.095	0.459	0.066	-0.125	-0.279	0.437	-0.222	0.417	-0.071	0.414	-0.089	0.419	-0.119	0.180	0.352	-0.103	0.406	0.432	0.012	0.068	-0.489	0.496	-0.337	0.129	-0.530	0.000	0.000	-0.025	0.579	0.121	0.199	-0.373	0.273	0.278
TAS2R38_TL: AVI/AVI	-0.079	0.048	-0.121	0.014	-0.184	-0.043	-0.433	0.265	-0.292	-0.306	0.238	-0.496	0.324	0.250	0.363	-0.085	-0.226	0.217	0.129	0.254	-0.606	0.256	-0.285	0.153	-0.232	0.013	-0.147	0.080	-0.249	-0.108	0.373	-0.198	0.073	0.171	-0.223	-0.128	-0.210	0.282	0.410	-0.417	0.062	0.000	1	0.177	0.105	0.689	-0.115	-0.016	0.444	-0.213
Taste: sweet vs bitter	-0.214	-0.388	-0.774	0.648	-0.566	-0.745	-0.487	0.250	-0.239	-0.732	0.186	-0.683	-0.141	-0.419	0.602	0.270	-0.954	-0.045	-0.450	-0.239	-0.676	0.118	-0.584	0.188	-0.822	-0.013	-0.838	0.149	-0.721	0.303	0.271	-0.869	-0.029	0.320	-0.800	-0.767	-0.473	0.695	0.472	-0.702	-0.517	0.892	0.177	1	0.000	0.648	0.602	-0.393	0.752	0.495
Taste: savoury	-0.912	-0.558	0.368	-0.576	-0.428	-0.180	-0.024	-0.824	-0.440	-0.560	-0.549	-0.219	0.830	-0.232	-0.030	0.793	-0.066	0.954	0.007	-0.177	-0.487	0.656	-0.297	0.951	-0.145	0.955	-0.035	0.938	-0.078	0.772	0.143	-0.168	0.943	0.929	0.198	0.147	-0.772	0.682	-0.434	0.022	-0.644	0.272	0.105	0.000	1	-0.009	0.581	-0.829	0.586	0.717
Liking: taste/mouthfeel/overal	-0.014	-0.244	-0.474	0.438	-0.655	-0.397	-0.719	0.196	-0.158	-0.417	0.015	-0.651	0.034	0.156	0.365	0.021	-0.611	0.040	-0.153	0.355	-0.796	0.089	-0.312	0.115	-0.395	-0.031	-0.457	0.097	-0.483	-0.010	0.642	-0.452	0.002	0.222	-0.476	-0.420	-0.378	0.478	0.678	-0.697	-0.086	0.444	0.689	0.648	-0.009	1	0.000	-0.004	0.575	0.109
Liking: taste/consump.Intent v	-0.732	-0.507	-0.279	0.046	-0.352	-0.563	-0.092	-0.275	-0.443	-0.872	-0.068	-0.401	0.377	-0.679	0.461	0.706	-0.601	0.507	-0.352	-0.654	-0.454	0.484	-0.603	0.677	-0.745	0.526	-0.616	0.608	-0.479	0.756	-0.093	-0.738	0.495	0.721	-0.441	-0.453	-0.680	0.816	-0.164	-0.228	-0.885	0.790	-0.115	0.602	0.581	0.000	1	-0.893	0.780	0.800
Cluster 1	0.917	0.642	0.075	0.259	0.383	0.535	0.007	0.552	0.384	0.801	0.246	0.355	-0.590	0.625	-0.278	-0.733	0.474	-0.712	0.286	0.428	0.519	-0.670	0.518	-0.856	0.549	-0.774	0.395	-0.824	0.426	-0.770	-0.045	0.566	-0.731	-0.869	0.207	0.257	0.759	-0.849	0.394	0.183	0.828	-0.591	-0.016	-0.393	-0.829	-0.004	-0.893	1	-0.776	-0.832
Cluster 2	-0.699	-0.490	-0.409	0.172	-0.594	-0.626	-0.425	-0.164	-0.541	-0.956	-0.019	-0.708	0.467	-0.452	0.604	0.603	-0.776	0.573	-0.346	-0.304	-0.863	0.478	-0.725	0.697	-0.805	0.502	-0.719	0.618	-0.671	0.620	0.249	-0.825	0.496	0.784	-0.580	-0.566	-0.776	0.950	0.220	-0.614	-0.724	0.810	0.444	0.752	0.586	0.575	0.780	-0.776	1	0.668
Cluster 3	-0.821	-0.784	0.014	-0.218	-0.688	-0.650	-0.093	-0.618	-0.289	-0.649	-0.529	-0.321	0.509	-0.379	-0.034	0.830	-0.495	0.625	-0.213	-0.213	-0.555	0.551	-0.262	0.835	-0.359	0.794	-0.293	0.858	-0.225	0.887	0.245	-0.425	0.765	0.863	-0.055	-0.126	-0.837	0.765	-0.234	-0.147	-0.775	0.723	-0.213	0.495	0.717	0.109	0.800	-0.832	0.668	1

Appendix XII: University of Reading Research Ethics Committee Study Evaluation

University of Reading

School of Chemistry, Food and Nutritional Sciences and Pharmacy
Research Ethics Committee

Application Form

SECTION 1: APPLICATION DETAILS

1.1

Project Title: Effect of domestic cooking on the sensory properties and consumer acceptance of different cabbage varieites.

Date of Submission: 21th Sept 2015 Proposed start date: 6th Oct 2015 Proposed End Date: 30th Nov 2015

1.2

Principal Investigator: Dr Lisa Methven

Office room number: 2.65b Internal telephone: 8714

Email address: l.methven@reading.ac.uk Alternative contact telephone:
(Please note that an undergraduate or postgraduate student cannot be a named principal investigator for research ethics purposes. The supervisor must be declared as Principal Investigator)
Other applicants

Name:Omobolanle O. Oloyede Student (delete) Institution/Department: Food and Nutritional Sciences
Email:o.o.oloyede@pgr.reading.ac.uk

Name:Dr Carol Wagstaff Staff (delete) Institution/Department: Food and Nutritional Sciences
Email:c.wagstaff@reading.a.c.uk

....

1.3

Project Submission Declaration

I confirm that to the best of my knowledge I have made known all information relevant to the Research Ethics Committee and I undertake to inform the Committee of any such information which subsequently becomes available whether before or after the research has begun.

I understand that it is a legal requirement that both staff and students undergo Criminal Records Checks when in a position of trust (i.e. when working with children or vulnerable adults).

I confirm that a list of the names and addresses of the subjects in this project will be compiled and that this, together with a copy of the Consent Form, will be retained within the School for a minimum of five years after the date that the project is completed.

Signed..... (Principal Investigator) Date:.....

..... (Student) Date:.....

..... (Other named investigators) Date:.....

..... (Other named investigators) Date:.....

1.4

University Research Ethics Committee Applications

Projects expected to require review by the University Research Ethics Committee must be reviewed by a member of the School research ethics committee and the Head of School before submission.

Signed..... (Chair/Deputy Chair of School Committee) Date:.....

Signed..... (Head of Department) Date:.....

Signed..... (SCFP Ethics Administrator) Date:.....

SECTION 2: PROJECT DETAILS

2.1

Lay summary

Epidemiological studies have shown that the consumption of Brassica vegetables (BV) such as cabbage reduces the risks of cardiovascular diseases, cancer and is reported to have a cytoprotective effect against tissue damage associated with oxidative stress[1-3]. However, the consumption of BV is low, probably due to the bitter taste or pungent flavour. BV contains glucosinolates (GLSs), a class of thioglycosides that uniquely accumulate in these vegetables; these compounds are hydrolysed by an endogenous enzyme (myrosinase) to form isothiocyanates (ITCs) which are mostly responsible for the health properties of BV[4]. However, GLSs and ITCs are also principally responsible for the bitter taste and sulfurous aromas alongside other sulphur volatiles derived from amino acid sulfoxides[5]. Epithiospecifier protein (ESP) is responsible for the formation of simple nitriles and epithionitriles (which possess no anticarcinogenic properties) from GLSs instead of isothiocyanates[6].

The taste receptor of particular relevance to bitterness is TAS2R38. This is a bitter receptor for which there are 3 distinct genotypes; the sensitive PAV/PAV group (25% population), the insensitive AVI/AVI group (25% population) and the medium taste group (PAV/AVI; 50% population). In this study we propose to genotype volunteers for these haplotypes in order to determine whether the effects of bitterness acceptance/rejection in cabbage corresponds to specific taste receptor groups, i.e. the PAV/PAV grouping.

This study will evaluate methods of processing cabbage, that minimise ESP activity and but avoid complete denaturation of the myrosinase. We predict that we will reduce bitter taste, whilst maintaining the availability of the bioactive ITCs. Non-volatile taste and volatile flavour compounds will be studied, as well consumer acceptability, consumer perception of taste and aroma and any correlation between these and bitter taste receptor genotype.

We hypothesise that through plant genotype selection and processing ESP and bitter taste can be minimised and bioavailability of bioactive flavour compounds maximised. It is hypothesised that consumer acceptability will be improved and will not relate to human bitter taste receptor genotype as the volatile profile will be more directly linked to liking than the bitter taste.

References:

1. Latté, K. P., Appel, K.-E. & Lampen, A. (2011). Health benefits and possible risks of broccoli – An overview. *Food and Chemical Toxicology,* **49**, 3287-3309.
2. Herr, I. & Buchler, M. W. (2010). Dietary constituents of broccoli and other cruciferous vegetables: implications for prevention and therapy of cancer. *Cancer Treat Rev,* **36**, 377-83.
3. Guerrero-Beltran, C. E., Calderon-Oliver, M., Pedraza-Chaverri, J. & Chirino, Y. I. (2012). Protective effect of sulforaphane against oxidative stress: recent advances. *Exp Toxicol Pathol,* **64**, 503-8.
4. Mithen, R. F., Dekker, M., Verkerk, R., Rabot, S. & Johnson, I. T. (2000). The nutritional significance, biosynthesis and bioavailability of glucosinolates in human foods. *Journal of the Science of Food and Agriculture,* **80**, 967±984.
5. Baik, H.-Y., Juvik, J. A., Jeffery, E. H., Wallig, M. A., Kushad, M. & Klein, B. P. (2003). Relating Glucosinolate Content and Flavor of Broccoli Cultivars. *Journal of Food Science,* **68**, 1043-1050.
6. Lambrix, V., Reichelt, M., Mitchell-Olds, T., Kliebenstein, D. J. & Gershenzon, J. (2001). The Arabidopsis Epithiospecifier Protein Promotes the Hydrolysis of Glucosinolates to Nitriles and Influences Trichoplusia ni Herbivory. *The Plant Cell,* **13**, 2793–2807.

(This box may be expanded as required)

2.2

Procedure

Please briefly describe what the study will involve for your participants and the procedures and methodology to be undertaken (*you may expand this box as required*).

Screening

Information Sheet (Appendix C) will be distributed.
Consent form to be signed (Appendix B) by participants

Sensory Testing:

6 cabbage varieties will be presented to consumers over two sessions on two separate days. Upon arrival at the Food and Nutritional Sciences Department in the University of Reading, informed consent (Appendix C) will be taken from participants by a trained researcher before any measurements are taken. All sensory testing will be performed in individual sensory booths within an environmentally-controlled sensory laboratory. Consumers will be given cabbages and asked to taste them. They will be asked to rate their overall liking as well as liking of flavour, aroma and texture of the product using a balanced nine-point hedonic scale. The scale has a centred neutral category, and the scale point labels have adverbs that represent psychologically equal steps or changes in hedonic sensitivity (See below for an example):

Overall liking of the product:
Like extremely
Like very much
Like moderately
Like slightly
Just about right/ neither like nor dislike
Dislike slightly
Dislike moderately
Dislike very much
Dislike extremely

The classification of participants as bitter tasters, non-bitter tasters and medium bitter tasters is based on the results of PCR genotyping. A buccal swab will be taken of each volunteer before testing begins, from which DNA will be extracted for genotyping of the taste receptor TAS2R38. No other genetic information will be tested for or retained.

Sample Preparation:

Cabbages will be harvested from the farm the weekend before each consumer panel and placed in paper bags for transport. Cabbages will be washed and sliced under food-safe conditions in the Food and Nutritional Sciences department. The washed cabbages will then be cooked (stir-fry and steaming).

The cabbage varieties will be tested in two separate sessions, each session containing 9 samples (3 varieties each). Cabbages will be presented with 3 digit random codes on a small plate. Sample size will be two pieces of cabbage. Yoghurt

and water will be given as palate cleansers between samples.

(Note: All questionnaires or interviews should be appended to this application)

2.3

Where will the project take place?

Sensory Science Centre, Food and Nutritional Sciences Department, Whiteknights Campus, University of Reading.

2.4

Funding

Is the research supported by funding from a research council or other *external* sources (e.g. charities, business)? No

If Yes, please give details:

Please note that *all* projects (except those considered as low risk, which would be the decision of the School's internal review committee and require Head of Department approval) require approval from the University Research Ethics Committee.

2.5

Ethical Issues

Could this research lead to any risk of harm or distress to the researcher, participant or immediate others? Please explain why this is necessary and how any risk will be managed.

Although some of the cabbages may be unpleasant to taste for some participants, none of them will cause harm or distress during tasting. All participants will be asked before the experiment begins if they have any mustard allergies. Cabbage is not known to cause any allergic reactions in humans, but it is closely related to mustard species; this action is taken as a precaution. All samples used in the study that have been grown at Tozer seed LTD will be washed of any soil or contaminants and will have a documented HACCP (hazard analysis of critical control points) procedure. No fertilizers, pesticides or herbicides have been used during the growing of the plant material. If the participant no longer wishes to taste the leaves they can stop the test at any point during taste testing.

(*this box may be expanded as required*)

2.7

Payment

Will you be paying your participants for their involvement in the study? Yes

If yes, please specify and justify the amount paid

Yes, volunteers will be paid £10 for both visits. Each volunteer will be required to attend the Department of Food and Nutritional Sciences for two visits that will last between 30-45 minutes.

Note: excessive payment may be considered coercive and therefore unethical. Travel expenses need not to be declared.

2.8

Data protection and confidentiality

What steps will be taken to ensure participant confidentiality? How will the data be stored?

Volunteers will be given a participant number, and throughout data collecting they will be referred to by this number. Data linking the name of the participant to their participant number will be kept in a locked filing cabinet in a locked office of Food and Nutritional Sciences

2.9

Consent

Please describe the process by which participants will be informed about the nature of the study and the process by which you will obtain consent

The information sheet and a cover letter will be given to the potential participants, and those willing to participant can contact the researchers directly. A consent form will be posted (or given as appropriate) to them, and a second will be signed and witnessed when they arrive at Food and Nutritional Sciences to take part.

Please note that a copy of consent forms and information letters for all participants must be appended to this application.

2.10

Genotyping

Are you intending to genotype the participants? Which genotypes will be determined?

Yes. Genotypic categorization will be done in bitter taste receptor TAS2R38

Please note that a copy of all information sheets on the implications of determining the specific genotype(s) to be undertaken must be appended to this application.

SECTION 3: PARTICIPANT DETAILS

3.1

Sample Size

How many participants do you plan to recruit? Please provide a suitable power calculation demonstrating

	how the sample size has been arrived at or a suitable justification explaining why this is not possible/appropriate for the study. We aim to recruit between 100 and 120 participants.
3.2	Will the research involve children or vulnerable adults (e.g. adults with mental health problems or neurological conditions)?No If yes, how will you ensure these participants fully understand the study and the nature of their involvement in it and freely consent to participate? (Please append letters and, if relevant, consent forms, for parents, guardians or carers). Please note: information letters must be supplied for all participants wherever possible, including children. Written consent should be obtained from children wherever possible in addition to that required from parents.
3.3	Will your research involve children under the age of 18 years? No Will your research involve children under the age of 5 years? No
3.4	Will your research involve NHS patients, Clients of Social Services or will GP or NHS databases be used for recruitment purposes? No Please note that if your research involves NHS patients or Clients of Social Services your application will have to be reviewed by the University Research Ethics Committee and by an NHS research ethics committee.
3.5	**Recruitment** Please describe the recruitment process and append all advertising and letters of recruitment. Volunteers aged between 18- 65 years will be recruited via posters across campus and in local shops, job centres etc. Email will be sent to general circulation lists across the University. Web sites such as Gum tree will be used. Some volunteers will be contacted directly though the Nutrition Unit database (people that have agreed to be contacted about further studies). We will also take flyers to halls of residence that are used by Reading University.

Important Notes

1. The Principal Investigator must complete the Checklist in Appendix A to ensure that all the relevant steps and have been taken and all the appropriate documentation has been appended.

2. If you expect that your application will need to be reviewed by the University Research Ethics Committee you must also complete the Form in Appendix B.

3. For template consent forms, please see Appendices C.

Appendix A: Application checklist

This must be completed by an academic staff member (e.g. supervisor)

Please tick to confirm that the following information has been included and is correct.
Indicate (N/A) if not applicable:

Information Sheet

Is on headed notepaper	☐	
Includes Investigator's name and email / telephone number	☐	
Includes Supervisor's name and email / telephone number	☐	
Statement that participation is voluntary	☐	
Statement that participants are free to withdraw their co-operation	☐	
Reference to the ethical process	☐	
Reference to Disclosure	☐	N/A ☐
Reference to confidentiality, storage and disposal of personal information collected	☐	
Consent form(s)	☐	

Other relevant material

Questionnaires	☐	N/A ☐
Advertisement/leaflets	☐	N/A ☐
Letters	☐	N/A ☐
Other (please specify)	☐	N/A ☐

Expected duration of the project (months) ☐

Name (print) …… **Signature ……**

University of Reading

School of Chemistry, Food & Nutritional Sciences and Pharmacy

Whiteknights
PO Box 266, Reading RG6 6AP, UK
phone +44 (0)118 378
fax +44 (0)118 931 0080

Appendix B

Consent Form

Study name: Cabbage Study

Investigators:

Dr Lisa Methven: l.methven@reading.ac.uk
Omobolanle O Oloyede: o.o.oloyede@pgr.reading.ac.uk
Dr. Carol Wagstaff: c.wagstaff@reading.ac.uk

Department Addresses:
Department of Food & Nutritional Sciences, University of Reading, PO Box 226, Whiteknights, Reading, Berkshire, RG6 6AP

Please INITIAL the box

1.	I have had explained to me the purposes of the project and what will be required of me, and any questions I have had have been answered to my satisfaction. I agree to the arrangements described in the Information Sheet in so far as they relate to my participation.	
2.	I understand that participation is entirely voluntary and that I have the right to withdraw from the project any time.	
3.	I have received a copy of this Consent Form and of the accompanying Information Sheet.	
4.	I have read the Information Sheet and been told the reasons why a buccal cell sample is required. I consent to a buccal cell sample being taken for taste receptor genotyping	yes ☐ no ☐

I have had explained to me that consent for my contact details and personal information to be added to the Hugh Sinclair Unit of Human Nutrition Volunteer Database is entirely voluntary. Accordingly I consent as indicated below:

5.	I consent to my contact details being stored on the Nutrition Unit Volunteer Database.	yes ☐ no ☐
6.	I consent to my screening information (including date of birth, height, weight, smoking status, long-term use of medication...) being stored on the Nutrition Unit Volunteer Database.	yes ☐ no ☐

I understand that this application has been reviewed by the School of Chemistry, Food and Pharmacy School Research Ethics Committee and has been given a favourable ethical opinion for conduct.

Name .....

Signed .....

Date .....

Witnessed by:

Name.....Signature.....

Date.....

University of Reading

School of Chemistry, Food & Nutritional Sciences and Pharmacy

Whiteknights
PO Box 266, Reading RG6 6AP, UK
phone +44 (0)118 378
fax +44 (0)118 931 0080

Appendix C

Participant Information sheet: Cabbage Study

We would be very grateful for your participation in this study as we believe it may help us to understand how human perceptions of flavours in cabbage are influenced by the chemicals they contain.

Who would we like to participate in the study?

- We are looking to recruit healthy volunteers from University of Reading.

What does the research involve for me personally?

Activity
• Taste a range of cabbages and indicate whether I like their appearance, overall taste and mouthfeel. • Participation will involve two separate visits, taking approximately 45 minutes on each visit. Visits will be at the sensory science centre within the Department of Food and Nutritional Sciences. • An important part of this study is to assess how individuals differ in their perception of taste. It is known that specific genes are associated with individual variations in taste perception. Our genes carry the genetic information, or 'instructions', about which proteins the body makes including receptors in taste buds. The receptor that we are interested in is called TAS2R38, and genetic differences in this receptor have been linked with so-called 'super-tasters' (people who can detect flavours more strongly than other people). In order to determine your genotype for this taste receptor (only according to your consent) you will be asked to provide a buccal cell sample using a proprietary sterile swab of cells from the soft tissues lining the inside of the mouth. Using this sample we will be able to collect the DNA from the cells in order to characterise the taste receptor genes. The samples will be destroyed as soon as they are no longer needed. Details of your genotype will be stored for future use on the Hugh Sinclair Unit of Human Nutrition Volunteer Database (according to your consent), so that you could be contacted for future studies involving your particular genotype. Also, we will tell you the result of your genotype if you wish.

Consent

The study is entirely voluntary, and only those who give informed consent will be allowed to take part in the study. You can decide not to participate at any point and all participants are free to withdraw at any time.

Confidentiality

- We require very little information that is personal to you.
- Your name will be recorded in order to allocate you a participant number.

- The list of names and participant numbers will be kept in a locked cabinet
- Once the study is completed your identifiable data will be destroyed.
- Consent forms will be kept for five years

Are there any adverse consequences to your health as a result of being a volunteer on this study?
Cabbage is a member of the same family of plants as mustards. If you have a mustard allergy, you may wish to consult your GP before participating in this study. There are no known health risks associated with consuming cabbage, and this is just a precautionary measure. (All products and ingredients to be tested are not known to carry any risks at the levels to be used in this study.)

What are the potential benefits of the study?
This study will help gain an understanding of people's preferences of cabbage flavour and taste, as well as indicating the strength of certain flavours present within the cabbage. The information will then be used to help understand links between perception and the chemical compounds present in cabbage tissue. There is little direct benefit to you.

Will any expenses be incurred during the study?
You will be paid £10 on completion of both visits as a thank you for taking part in our research.

Who has reviewed the study?
The School of Chemistry, Food and Pharmacy School Research Ethics Committee have reviewed the study and given a favourable ethical opinion for conduct.

How do I arrange to take part?
If you wish to take part in the study please contact the research team who sent you this information leaflet.

If you have any concerns or complaints about the research, we will do our best to resolve them. Please contact: ***(as appropriate)***

Omobolanle o oloyede: o.o.oloyede@pgr.reading.ac.uk

Dr Lisa Methven: l.methven@reading.ac.uk

Dr Carol Wagstaff: c.wagstaff@reading.ac.uk

Study Number: 37/15

Volunteers Needed for Cabbage tasting

- **Are you aged 18-65 years of age?**
- **Do you eat cabbage at all?**
- **Are you happy to taste a range of cabbages for us?**

£10 - Payment

- Are you happy to donate cells to determine your tasting sensitivity using a cheek cell swab (which is painless and non-invasive)?

If YES to all those questions, this study needs you!!

- You will attend the Sensory Science Centre at the University of Reading for **TWO 45 minutes visit** (Once in **October** and another in **November).**

-You will be reimbursed for your time on the second visit

If you are interested you can just book yourself in via the doodle poll link: http://doodle.com/poll/q8uy38g2z44n7tur and sign the consent form when you arrive. If however you would prefer more information in advance then please contact:

Bola Oloyede; email:o.o.oloyede@pgr.reading.ac.uk.

Contact Bola Oloyede
o.o.oloyede@pgr.reading.ac.uk

Consumer Study
Contact Bola Oloyede
o.o.oloyede@pgr.reading.ac.uk

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o.o.oloyede@pgr.reading.ac.uk

Recruitment e-mails for University Community

E-mail 1:
Hello Everyone,

- Are you aged 18-65 years of age?
- Do you like cabbages?
- Are you happy to taste a range of cabbages for us?
- Are you happy to donate cells to determine your tasting sensitivity using a cheek cell swab which is painless and non-invasive?

If YES to all those questions, this study needs you!!

We will be asking you to taste rocket leaves and tell us how much you like them, and how strong you think the flavours are. If you are interested in taking part in the study, please contact Omobolanle (Bola) Oloyede (o.o.oloyede@pgr.reading.ac.uk) who will provide you with an information sheet.

Many thanks,

Bola

E-mail 2:
Hi Everyone,

Just a quick reminder that cabbage tasting is coming up. Anyone who hasn't yet volunteered, please contact me as soon as possible, through phone and e-mail.

Many thanks,

Bola

15

Taste Receptor Gene Factsheet

Genes contain the information to make all the proteins our body needs. Humans have around 25,000 genes, 99.9% of which are exactly the same in all people. There is much interest in the genes that differ between people and how they impact our health. At the University of Reading, we are interested in how these variations affect people's response to foods. For this reason, in some of our studies we ask you to provide a blood or saliva sample that we use to determine if you have variations of a particular gene. In this particular study we will ask you for a buccal (mouth) cell sample only.

Your buccal cell sample will be tested for variations in a *'super-taster' receptor* gene (TAS2R38) and this factsheet is designed to explain what these receptors do in the body and what impact variations in this gene may have, if any, on your health.

What is TAS2R38, and what does it do?

There are numerous different protein receptors on the tongue that detect tastes. The TAS2R38 gene codes for a protein receptor that detects the bitterness of certain chemicals, such as a group known as 'glucosinolates' that are found in Brassica vegetables. It has been linked with people who are so-called 'super-tasters', for their ability to detect flavours that other people can't.

Does everyone have the same TAS2R38 gene?

The genetic code for these receptors can differ slightly from person to person.

For TAS2R38, several forms of this gene have been identified worldwide, but there are two common types (taster and non-taster) found outside of Africa. These types differ slightly resulting in changes in the protein receptors on your tongue. The type known as PAV is identified as the bitter-taster, and the type called AVI is the non-taster type. Individuals can have two bitter sensitive PAV types, two insensitive AVI types, or one of each. About 25% of people have the bitter tasting types, 50% have both a bitter tasting and non-tasting (medium-tasting) type, and another 25% have all non-tasting types.

How does this affect me as an individual, and what can I do about it?

Individual differences in the ability to taste certain chemicals (such as the synthetic bitter chemical n-6-propylthiouracil (PROP), and glucosinolates in brassica vegetables) are related to variability in coding for the TAS2R38 gene. Whether this difference in taste perception has any effect on dietary choice and on health has yet to be fully researched, and there are no firm conclusions to date. This is partly because there are many other factors, such as environmental and socioeconomic conditions, as well as experience, that play a much larger part in how we choose and consume food.

Implications for health insurance

The genotyping we do is what is called 'predictive testing' and as such there is no need to disclose the results of these tests, at present or any time in the future, to your insurance company.

Why are researchers interested in this gene?

We are interested to further determine if individuals of different taste receptors respond differently to food and dietary choice. In the future, rather than providing everyone with general dietary advice, it may be that a more personalised approach is taken, providing advice to suit an individual's genetic make-up.

Sources of further information

It must be emphasised that genotyping is a relatively new area which is still in the research stage, with information in this area far from complete. If you would like to read more on this topic, you may find the following web site of the Human Genetics Commission useful, www.hgc.gov.uk

If you have any questions or would like further information please contact the study investigators, or Dr Lisa Methven.

Dr Lisa Methven
Associate Professor in Food and Sensory Science,
Department of Food and Nutritional Sciences, University of Reading.
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0118 378 8714