University of Reading

Natural Processing for Beverages: From a *Hibiscus sabdariffa* (Roselle) Beverage perspective

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Abstract

The beverage industry is tending towards the production of healthier, low calorie beverages containing only naturally derived ingredients. The use of these ingredients presents an opportunity for a rise in functional beverages but could also present beverage quality challenges. Using *Hibiscus sabdariffa* (Roselle) extracts, known for its functional properties, this research investigates some quality considerations that may result due to relevant changes to the production, storage and consumption of the beverage to suit clean label considerations. These issues relate to processing, calories reduction and stability. Several research questions have been generated under these categories and addressed in subsequent chapters of the thesis. From a Roselle beverage perspective, pasteurisation (in-bottle, 85^{o}C for 20 minutes) and cold storage (below 4^{o}C) were satisfactory for processing of the beverage. Stevia Rebaudioside A (SRA) was explored as a sugar replacer and displayed promising results such as improving the sensory attributes of the unsweetened Roselle extract. In physical and chemical tests, it matched the attributes of unsweetened beverages. However, it did not perform as well as sugar in either consumer studies and when combined with spice flavours. The spices (cinnamon, ginger and cloves) used for their flavouring and preserving properties did not improve consumer liking of the beverages in general but cinnamon demonstrated stabilising effects on the anthocyanins in a sugar sweetened Roselle beverage through a suspected co-pigmentation mechanism. Furthermore, cinnamon and ginger were deemed congruent with the Roselle flavour profile based on their performance in the consumer study. Further investigative work on spice and sweetener synergies and the understanding of their mechanisms were recommended.

Declaration

I confirm that the work presented in this thesis is my own and has been generated as a result of original research work. Materials from other sources have been acknowledged.

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List of Abbreviations

AAE	Ascorbic acid Equivalent
ACE	Angiotensin-converting-enzyme
ACEi	Angiotensin-converting-enzyme inhibition
Ax	Absorbance reading at wavelength x
C3G	Cyanidin 3-glucoside
C3S	Cyandin 3-sambubioside
CAD	Commercial air-dried
Colour (L*, a*, b*)	Colour (Lightness, redness and yellowness)
CVD	Cardiovascular diseases
D3S	Delphinidn 3-sambubioside
Day X	Time-point recorded in number of days from the preparation of the Roselle beverage/extract
FAO	Food and Agriculture Organization
FC	Folin Ciocalteu
FD	Freeze dried (or drying)
FIIRO	Federal Institute of Industrial Research Oshodi
FRAP	Ferric ion reducing antioxidant power
Frz	Frozen
FSA	Foods Standards Agency
GAE	Gallic acid equivalent
GI	Glycemic index

HCl	Hydrochloric acid
HDPE	High Density
HPLC	High-performance liquid chromatography
IC_{50}	Half maximal inhibitory concentration
K	Rate constant
MCA	Multiple correspondence analysis
MW	Molecular Weight
NaOH	Sodium Hydroxide
PCA	Principal component analysis
PCAD	Pasteurised Commercial Air dried
PFD	Pasteurised Freeze dried
Pst	Pasteurised
PTD	Pasteurised Tray dried
QDA	Quantitative Descriptive Analysis
RB	Roselle beverage
Reb A	Rebaudioside A
Ref	Refrigerated
SD	Standard deviation
SRA	Stevia Rebaudioside A
TA	Total anthocyanins/ Titratable acidity (where relevant)
TD	Tray dried (or drying)
TP	Total phenolic

TPTZ	2,4,6-Tri(2-pyridyl)-s-triazine
Unpst	Unpasteurised
Unswt	Unsweetened
WHO	World Health Organisation

Chapter 1 – General Introduction

1.1 Background

Consumer demand for better health has resulted in the beverage industry moving towards healthier clean label products. For rapid progress to be made, there is the need for researchers to provide relevant information to enable developers make informed decisions. Using the Roselle beverage as a template, knowledge gaps have been identified in certain aspects of the production process which may be applicable to a wider range of products. Consequently, the scope for research is quite large. However, this thesis investigates key foundational issues in relation to processing, calorie reduction and beverage stability. This thesis expects to link contemporary beverage trends with relevant research to provide pertinent answers.

1.2 Introduction to the Thesis

The main challenges in the production of clean label functional and healthy drinks include:

a. Safe processing which ensures the preservation of health giving compounds.
b. Processing of the beverages without the use of synthetic or chemical preservatives, and without compromising on taste and quality.
c. The difficulties of sugar replacement with healthier alternatives without affecting the taste and quality of the product.

The Roselle beverage has been identified as an ideal subject to explore the broad concepts of clean labelling, sugar replacement and functional beverage production.

Consequently, the research aims are:

1. To investigate the impact of pasteurisation on the physical and bioactive properties of a Roselle drink.

2. To study the effect of sugar replacement with stevia glycosides on consumer acceptability and the stability of the Roselle beverage.

3. To explore the use of spices as flavouring and preservatives in Roselle beverages

To achieve these aims, the research objectives are:

1. To compare pre and post pasteurised extracts for differences in their physical properties i.e. colour and pH; bioactive compounds and properties i.e. total anthocynanins, FRAP (Antioxidant) capacity and Angiotensin converting enzyme (ACE) inhibitory activity of the Roselle extract; and their keeping quality during typical beverage storage conditions.
2. To sensorially profile unsweetened and sweetened (Sugar and Stevia Rebaudioside A) Roselle beverages and measure the consumer liking of the sweetened beverages in order to identify the effects of sugar replacement.
3. To explore the use of some typical spices i.e. cinnamon, ginger and/or cloves, as flavourings to enhance the taste of the Roselle beverages and mask any off-flavours in the beverage; and to measure the preservative effect on the spices on key physical and bioactive properties of the Roselle beverage.

A multidisciplinary approach is necessary in order to make a meaningful contribution to the development of the functional beverage sector. Thus, this thesis is divided into parts (Figure 1.1) which align with the research aims and objectives.

This current chapter introduces the Roselle beverage; its constituent ingredients with their benefits and properties, typical processing conditions, challenges and the

justification for the research topic and methodologies. In Chapter 2, industry trends and concepts; and related background work in this current research are detailed.

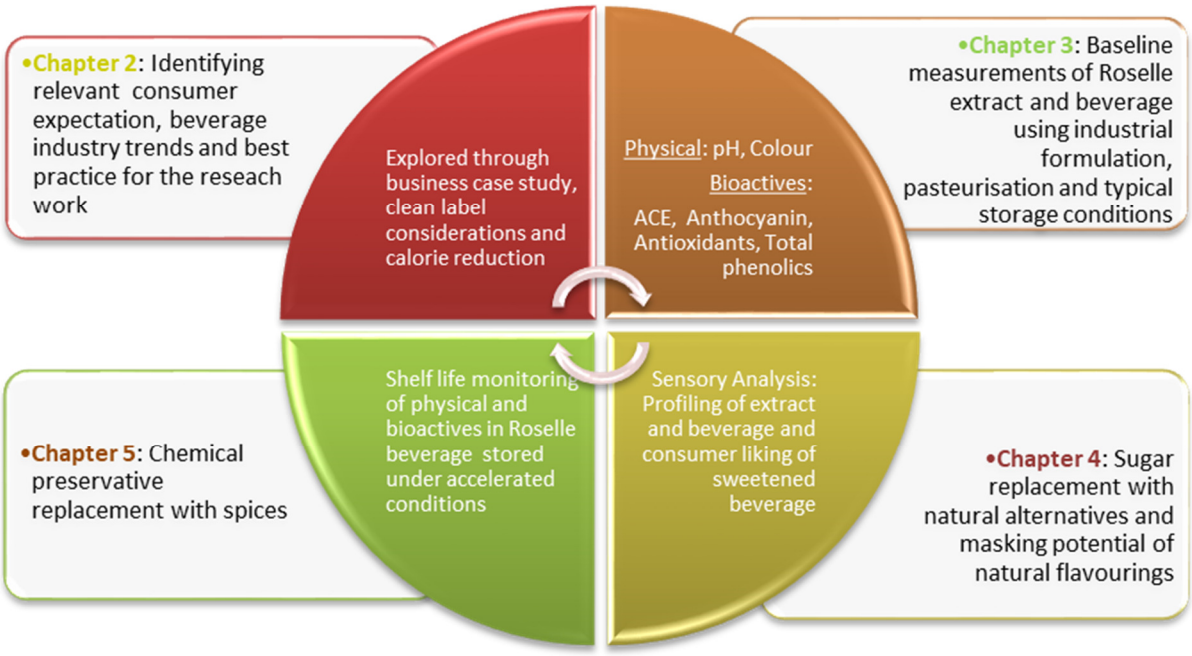


Figure 1.1: Thesis layout of research design

Following the contemporary Roselle beverage procedures and industry trends and concepts, Chapter 3 proceeds to explore the effect of pasteurisation and storage on the physical and bioactive properties in the Roselle extract. The hypotheses of Chapter 3 are: (1) pasteurisation will affect the physical and bioactive properties of the Roselle extract (2) storage at different temperature conditions will influence the rate of change in the measured physical and bioactive properties of the Roselle extract (3) there are correlations between the properties of the Roselle extract.

Subsequently, Chapter 4 provides a descriptive sensory profile of Roselle extracts using typical drying, extraction and pasteurisation before delving into studying the sensory properties of a typical Roselle beverage (RB) with sweeteners and flavours. The hypotheses of this study in Chapter 4 are: (1) variations in processing conditions will affect the sensory

profile of a Roselle infusion (2) Stevia Rebaudioside A (SRA) may enhance aspects of the sensory profile of Roselle infusions (3) consumers can discriminate between sugar and SRA sweetened beverages and prefer sugar-sweetened RB; (4) familiarity with Roselle may affect liking for RB; and (5) spices may mask off-notes in RB sweetened with Reb A.

In Chapter 5, the sweeteners and two spices (cinnamon and ginger) from the sensory work were explored for stabilising potential on key physical and bioactive attributes of the Roselle beverage. The hypotheses for this chapter are (1) there is an effect of Just about right (JAR) levels of sweeteners on the physical and bioactive compounds in the Roselle beverage (2) cinnamon and ginger spices may affect the physical and bioactive compounds in the Roselle beverage (3) the combination of sweeteners with spices may offer stabilisation to Roselle anthocyanins.

The thesis concludes with key findings of the research work and recommendation for future work.

1.3 Literature review

1.3.1 Roselle

The main ingredient in a Roselle beverage is the Roselle calyx. Grown mostly in tropical and sub-tropic regions, it is used as medication, tea, natural colorant and an ingredient in functional beverages (Gruenwald, 2009). Roselle properties such as its red colour, low pH and pectin content are useful properties for a beverage. Moreover, its phenolic content makes it ideal for a functional beverage.

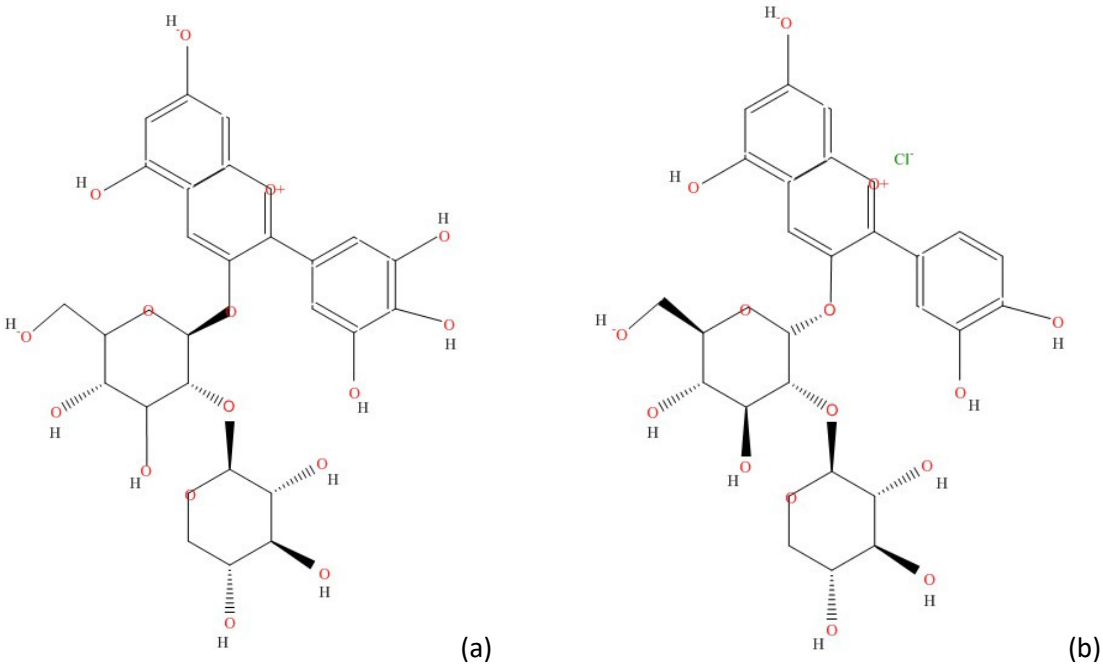
Several studies have revealed that Roselle extracts are rich in polyphenolic compounds. These are bioactive compounds consisting of aromatic rings attached to one or more hydroxyl groups (Vattem & Maitin, 2016). Roselle polyphenols consist of flavonoids e.g.

anthocyanins, protocatechuic acid and quercetin (Carvajal-Zarrabal et al., 2012) gossypetin, hibiscetrin (Ali, Wabel, & Blunden, 2005), hibiscetine and sabdaretine, many of which are antioxidants. Roselle also contains phytosterols i.e. sterols like j3-sitosterol and ergosterol (Ali et al., 2005), eugenol and some suspected compounds, such as gossypin, gossytrin, rutin, isoquercitrin, kaempferol 3-rhamnoglucoside, kaempferol 3-glucoside, cannabiscitrin, myricetin (Makhsudova, Pakudina, & Sadykov, 1967). However, the compounds of most interest in Roselle extracts are its anthocyanins (Figure 1.2); mainly Delphinidin 3-sambubioside and Cyanidin 3-sambubioside (Cisse et al., 2012, Tsai et al., 2002), although some other studies have also reported Delphinidin 3-glucosides and Cyanidin 3- glucosides as minor anthocyanins (Du & Francis, 1973).

In general, anthocyanins are bioactive compounds which provide both the medicinal and sensory properties associated with a plant (Vattem & Maitin, 2016). The content of anthocyanins in Roselle were reported to be in the range of 1.5 to 2.5 g/100 g dry weight (Cisse, Bohuon, et al., 2012; Du & Francis, 1973). Roselle anthocyanins are highly water soluble (Polyphenols Laboratories, 2017a, 2017b). The key properties of Roselle anthocyanins are shown in Table 1.1. Both anthocyanins are positively charged ions which react rapidly, hence, promoting its strong antioxidant capabilities.

Table 1.1: Chemical properties of Roselle anthocyanins (National Center for Biotechnology Information, 2017a, 2017b)

Anthocyanin name	Delphinidin 3-sambubioside	Cyanidin 3-sambubioside
Molecular weight (g/mol)	598 g/mol	617 g/mol
Chemical formula	$C_{26}H_{29}O_{16}^{+}$	$C_{26}H_{29}O_{15}^{+}$



(a) (b)

Figure 1.2: Chemical structures of (a) Delphinidin 3-sambubioside and (b) Cyanidin 3-sambubioside found in *Hibiscus sabdariffa* (Roselle) extracts (ChemDraw).

Roselle also contains vitamins and minerals including aluminium, manganese, magnesium, potassium, sodium, calcium, phosphorus and iron (Janick & Paull, 2008; Mahadevan, Shivali, & Kamboj, 2009).

1.3.2 Properties of Roselle

Of particular importance to this study are the health benefits credited to Roselle, particularly those confirmed in human trials (Table 1.2). To date, these trials have revolved around reduction of cholesterol and high blood pressure (BP) in diabetics. McKay, Chen, Saltzman, and Blumberg (2010) and H. Mozaffari-Khosravi, B. A. Jalali-Khanabadi, M. Afkhami-Ardekani, and F. Fatehi (2009) attribute BP reducing properties to anthocyanins and flavonoid components such as hibiscin, hibiscretin, delphinidin-3-glucoside, delphinidin-3-sambubioside and cyanidin-3-sambubioside and other phytochemicals.

Human studies (Table 1.2) display the functional potential of Roselle beverages. Three 240-mL servings/day of brewed hibiscus tea for 6 weeks was sufficient to show a reduction in Diastolic BP of pre- and mildly hypertensive consumers as seen in the study by McKay et al. (2010).

Of equal importance are the sensory properties of Roselle. A variety of sensory studies have also been carried out on Roselle products including beverages, syrups, jams and sauces. The sensory studies carried out on Roselle beverages are expounded on in Chapter 4 of this thesis. For other foods, D'Heureux-Calix and Badrie (2004) carried out a customer acceptability tests on Roselle sauces and the results suggested that the red colour was the most liked attribute. Other measured attributes included tanginess/acidity, flavour, aroma, mouth-feel and acceptability. The overall product acceptability ranged from slightly to moderately liked.

Until now, the bioactive properties which include the antioxidant capacity and anthocyanin content have been the most studied aspect of Roselle. Further discussions on relevant studies are detailed in Chapter 3.

Table 1.2: Some human studies that support the benefits of Roselle

Title of research	No. of participants, age and duration of study	Conclusions of study	Reference
The effect of sour tea (Hibiscus sabdariffa) on essential hypertension	54 Patients Mean age: 52.6±7.9 Duration: 15 days	This study proves the public belief and the results of in vitro studies concerning the effects of sour tea on **lowering high blood pressure**.	Haji Faraji and Haji Tarkhani (1999)
Hibiscus sabdariffa extract reduces serum cholesterol in men and women	42 patients Aged 18 – 75 Duration: 4 weeks	The observation of **lowered serum cholesterol** in these subjects suggests that HSE may be effective in hypercholesterolemic patients.	Lin et al. (2007)
The effects of sour tea (Hibiscus sabdariffa) on hypertension in patients with type II diabetes	60 diabetic patients with mild hypertension Duration: 30 days	Consuming Sour tea (ST) infusion had **positive effects on BP in type II diabetic patients with mild hypertension**. This study supports the results of similar studies in which antihypertensive effects have been shown for ST.	H. Mozaffari-Khosravi, B. A. Jalali-Khanabadi, M. Afkhami-Ardekani, and F. Fatehi (2009)
The Efficacy of Karkadeh Tea in Controlling Post-Prandial Blood Glucose Levels	8 individuals	Karkadeh tea appears to have an effect in terms of slowing the rate of rise in blood glucose following consumption of a high glycemic index food, but that ultimately it induces a greater degree of glucose absorption *cf.* other types of imbibed fluids.	Harrison, Cooper, Suliman, and AlAlami (2009)
Hibiscus Sabdariffa L. Tea (Tisane) Lowers Blood Pressure in Prehypertensive and Mildly Hypertensive Adults	65 pre- and mildly hypertensive adults, Aged 30–70 years Duration: 6 weeks	Consumption of hibiscus tea, in an amount readily incorporated into the diet, **lowers BP** in pre- and mildly hypertensive adults and may be a component in recommendations for people with these conditions.	McKay et al. (2010)
Effects of Roselle on arterial pulse pressure and left ventricular hypertrophy in hypertensive patients	10 patients Mean age 50±5 Duration: 4 weeks	These findings empirically suggest **favourable cardiovascular effects** of Roselle in patients with established moderate essential hypertension.	Al-Shafei and El-Gendy (2013)

1.3.3 Toxicity and safe levels

Roselle has a long history of use (Du & Francis, 1973). Research carried out by Akindahunsi and Olaleye (2003) suggest that Roselle extract is safe to consume in moderate doses (Hopkins, Lamm, Funk, & Ritenbaugh, 2013). Lim (2014) suggests 150 – 180 mg/kg per day as a safe level but cautions on excessive consumption due to Roselle extracts association with liver damage and toxicological testicular damage in rats. Furthermore, in other rat studies, Roselle extract consumption, up to 5000 mg/kg body weight, did not lead to acute toxicity (Sireeratawong, Itharat, Khonsung, Lertprasertsuke, & Jaijoy, 2013), The recommended safe level of Roselle intake on humans still needs to be established.

1.3.4 Introduction to Roselle beverages

The Roselle beverage called Zobo (or Zoborodo) in Nigeria is a flavoured infusion derived from cooking calyx of hibiscus sabdariffa with other spices and flavours in water and further sweetened to taste. It is also mostly preserved using a combination of heat and chemical treatments, both of which are commonly used to preserve soft drinks (BDSA, 1999). Similar forms of the Nigerian Zobo drink are found in other parts of Africa, and in the Caribbean, South America and Asia under different names including Sorrel, Bissap, Jamaica and Karkade. The Nigerian drink and its counterparts in other countries are typically sweetened with sugar using amounts comparable to the amounts found in other existing non-alcoholic beverages.

In addition to sweetening, Roselle beverages may also be flavoured to improve palatability. A variety of flavours may be used in Roselle beverages and there are no restrictions on the types of natural or synthetic flavouring that may be used industrially as long as they have been approved by the appropriate body and used within the recommended limits. Hence,

there is much room for innovation in this area. The focus of this research will be on natural flavours (e.g. extracts and fruits juices) due to clean label.

Roselle drinks made from combining Roselle extract and juices from apple, orange, pineapple, pear, mango, guava and papaya and lime have been explored by Fasoyiro, Ashaye, Adeola, and Samuel (2005), Ukwubile, Otalu Jr, and Babalola (2013), Mgaya, Remberg, Chove, and Wicklund (2014) and Onuoha and Fatokun (2014). The challenges faced when using natural flavourings include hazing, colour instability and reduced shelf life. Suggestions were made for the use of citric acid and sodium benzoate to combat these occurrences in Roselle beverage (Ukwubile et al., 2013).

Similarly, spices are used in Roselle beverages more for organoleptic than health benefits. However, the added benefits of such additives are widely known and could positively impact the functionality, stability and popularity of Roselle beverages. Rupasinghe and Yu (2012) studied the use of herbs and spices in beverages as antimicrobials but mainly in relation to the bacterial activity. Although some beverages contain spice extracts, there are limited studies on the effect of spices on beverage properties in general.

1.3.5 Production of Roselle Beverage in Industry

A process flow chart for the production of Roselle beverages by FIIRO (Federal Institute of industrial Research Oshodi) which has been adapted for use in this research is shown in Figure 1.3. To minimise moisture loss due to boiling, the extraction in this research was carried out at 90^{o}C for 25 minutes. The choice and alterations in the methodology used in this current research work are details in section 1.9. The highlighted processing stages are the specific areas of interest to this research work. These are where the research aims, objectives and research questions are founded.

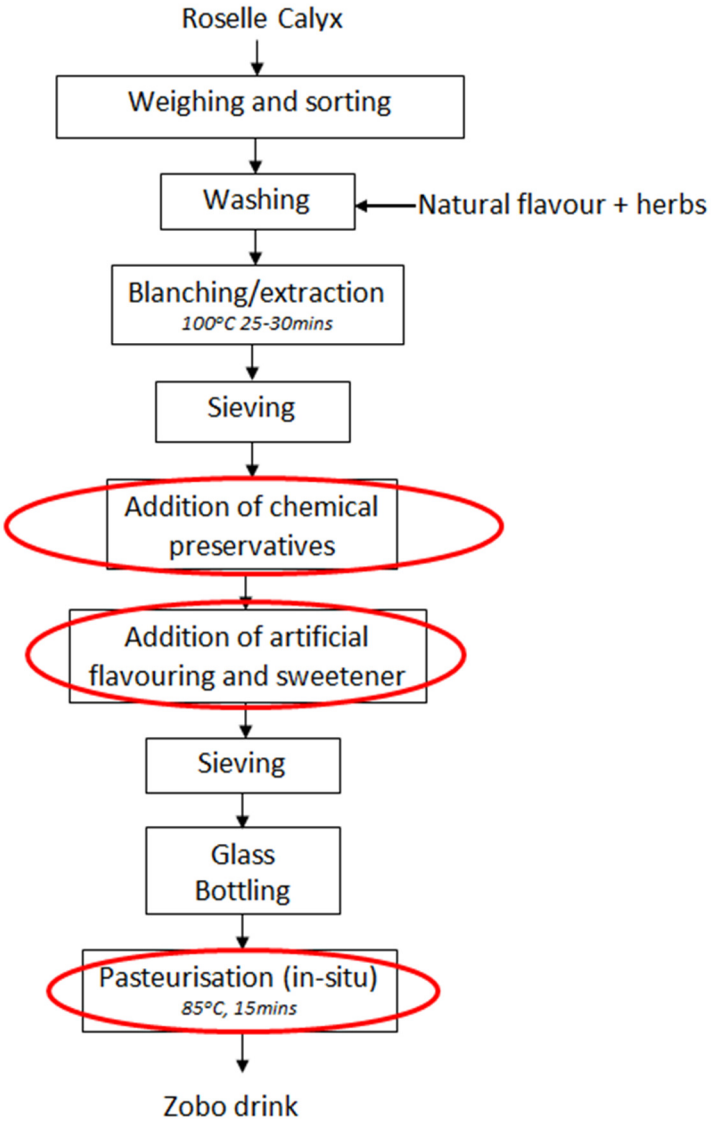


Figure 1.3: Suggested Industrial Zobo production process (FIIRO training) with red highlights of specific areas addressed in this research work.

1.3.6 Sugar replacement in Roselle based drinks

Replacing sugar as the major sweetener in food and beverages has been a major industry objective since the latter part of the twentieth century. Roselle beverages are typically sweetened with industrial sweeteners such as sucrose (white sugar) or natural sweeteners e.g. honey, fruit juice, maple syrup, nectars, simple sugars (fructose or dextrose) and sugar alcohols. Although high in sweetening quality, these sweeteners fail in their ability to satisfy consumer's requirements health vis-à-vis calorie reduction. Indeed, the use of sugar in Roselle beverages seems counterintuitive to some of the documented natural benefits of Roselle extracts.

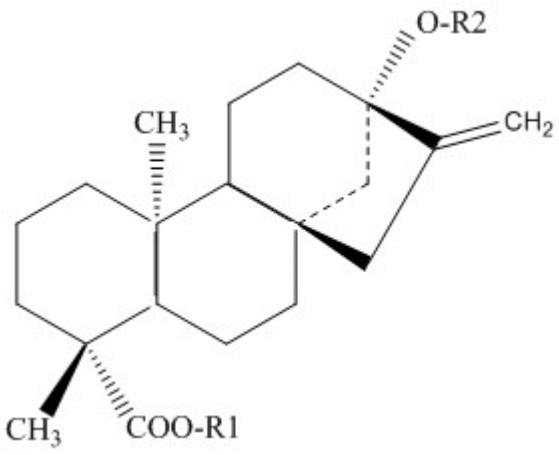
Sugar in aqueous solutions provides elevation of boiling point; lowering of vapour pressure; increase in viscosity; increase in osmotic pressure and heat of solution (Woodroof & Phillips, 1980). In formulations, sugar affects pH and changes irreversibly with time and temperature. These changes could lead to sweeter and less viscous beverages due to the conversion of sucrose to dextrose (glucose) and fructose. Sugar gives a certain mouth feel and aids volatility of some flavour compounds. Consequently, replacement of sugar with other sweeteners does create sensory challenges in addition to other technical challenges. Some sweeteners are preferred to sugar for their glycaemic advantage and suitability for diabetic diets, as sucrose and glucose containing sugars require insulin for their metabolism. Fructose, also called fruit or natural sugar, is reported to be well tolerated by diabetics (50-80 g daily) and is also favourable to formulations as it not only provides sweetness but also acidity, reduced calories and buffering power (Corti, 1999). However, a study by Stanhope et al. (2009) reveals that fructose leads to dyslipidemia and increased volume of visceral adiposity in overweight or obese adults. It is also suspected to participate in increasing the

risk factors for hypertension, diabetics and cardiovascular diseases in general by raising uric acid levels (Johnson et al., 2007).

Whereas polyols such as sorbitol, xylitol and maltitol do not require insulin for metabolism, therefore they are better suited for diabetics (Grabitske & Slavin, 2008). However, they are not presently approved for use in beverages in the EU and do not appear to meet clean label requirements. Of the sugar alcohols, only erythritol (1.6%) has been approved by the EFSA European Food Safety Authority (European Food Safety Authority, 2015) as a flavour enhancer in beverages.

Therefore, intense sweeteners are purported to be the current healthy solution for sweetening beverages, of which only the Stevia glycosides satisfy clean label conditions and are approved for use in the EU. Low-calorific sweeteners from the plant source Stevia are becoming more popular although incorporation into beverages is limited. Extracts from the Stevia rebaudiana plant has been associated with off-flavours (Corti, 1999). Not all of the 8 stevia glycosides in stevia give the required flavour and safety properties. The two most favourable, in terms of safety and flavour, are Stevia Rebaudioside A and stevioside; which constitutes 3 - 8% and 1% of the stevia leaf respectively. Stevia rebaudioside A (Figure 1.4) is preferred for its taste and found to be stable in carbonated beverages, including in its response to heat and acidity (Chang & Cook, 1983). It is also considered to be 200 – 450 times the potency of sugar (International Stevia Council, 2010; I. Prakash, G. DuBois, J. Clos, K. Wilkens, & L. Fosdick, 2008). In addition, Perez-Ramirez, Castano-Tostado, Leon, Rocha-Guzman, and Reynoso-Camacho (2015) demonstrated that stevia can add extra functionality to Roselle beverages. 1.4 – 1.5% stevia used reduced the loss of antioxidants as well as the degradation of anthocyanins and total polyphenols in the beverage (Perez-Ramirez et al., 2015).

In addition to actual sweeteners, acids in a beverage solution may improve sweetness profiles by interacting competitively at the taste receptors rather than interacting directly with sweeteners (Prakash, Bishay, Desai, & Walters, 2001).



	R1	R2
Rebaudioside A	β-Glc	β-Glc-β-Glc(2→1) | β-Glc(3→1)

Figure 1.4: Structure of steviol and stevia Reb A (Lemus-Mondaca, Vega-Gálvez, Zura-Bravo, & Ah-Hen, 2012). Steviol (R1 = R2 = H) is the aglycone of the steviol glycosides. "Glc" representing glucose moieties. Rebaudioside A is chemically known as 13-[(2-O-β-D-glucopyranosyl-3-O-β-D-glucopyranosyl-b-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid, β-D-glycopyranosyl ester with chemical formula $C_{44}H_{70}O_{23}$ and a molecular weight of approximately 967g/mol.

1.3.7 Preservation of Roselle beverages

The low pH of Roselle beverages (typically less than pH 3.0) as with many soft drinks should make it unsuitable for bacterial activity especially food poisoning bacteria. However, it may be subject to yeast and mould spoilage and indeed quality and nutrition deterioration over time, which is not unusual for acidic beverages in general.

According to Woodroof and Phillips (1980), microbiological spoilage in soft drinks is mostly due to yeast (i.e. *Saccharomyces Torulopsis - Candida, Hansenula Pichia*) and is characterised by floating particles, haze and formation of sediments. Other likely spoilage

organisms include lactic acid bacteria, moulds (*Aspergillus Penicillum*), acetic acid bacteria (*Acetomanas Acetobacter*) and ubiquitous flora (aerobic yeast such as *Rhodotorula* and *Bacillus*). Another study by D'Heureux-Calix and Badrie (2004) in which Roselle extract was stored at 20^{o}C for 8 weeks revealed that microbial activity was due to yeast, mould, lactic acid bacteria and total mesophilic aerobes.

The specific type of yeast and mould that may thrive in a Roselle beverage is subject to the local environment. Braide, Oranusi, and Peter-Ikechukwu (2012) suggested that in African climates, natural or ubiquitous microbiological flora found in the growing habitat of Roselle i.e. soil, water and vegetation include Bacillus and Saccharomyces species. There is also the variability of microorganisms that may be introduced during production of the beverage, all of which affect the keeping quality the Roselle beverage and therefore poses a challenge to microbiological based research on Roselle beverages.

In industry, pasteurisation is considered to be a cost effective preservation and safety solution to food. It works by reducing the number of micro-organisms, especially bacteria, in a beverage to minimal levels. Therefore, this basic preservation technique is the very minimum expected. It is also widely used across the beverage industry. Section 1.9.2 looks further at pasteurisation in relation to Roselle beverage research.

However, pasteurisation is usually combined with chemical preservatives to be effective. The preservatives typically used in Roselle beverages include benzoic, sorbic and sulphuric acids, which may be are effective against bacteria, yeast and some other fungi (Houghton, 1984; Woodroof & Phillips, 1980). In the Nigerian Roselle drink, citric acid, sodium benzoate and sodium metabisulphite are usually used in combination within acceptable limits to stabilise colour, extend shelf life and vitamin C content. (Braide et al., 2012; Olawale, 2011; Ukwubile et al., 2013).

However, the clean label movement advocates the use of natural preservation or natural preservatives such as spices which have been demonstrated to have antimicrobial properties (Rupasinghe & Yu, 2012). Essentials oils from a variety of spices, including cinnamon (*Cinnamomum zeylanicum*), were investigated by Simić et al. (2004) which showed strong antifungal properties of cinnamon due to its trans-cinnamaldehyde properties. Similarly, amongst several essential oils, cinnamon, ginger and guinea pepper were explored by Sessou, Farougou, and Sohounhloué (2012) and showed antimicrobial effects on bacteria as well as fungi. The antimicrobial activities of these spices were attributed to its constituent phenolics, such as eugenol.

Natural additives to be used to extend shelf life or act as antioxidants are also advocated to replace synthetics additives. In a study by Odukoya et al. (2005), the use of spices, such as ginger (*Zingiber officinale*), cloves (*Syzygium aromaticum* L.) and guinea pepper (*Xylopia aethiopica*), were researched for their potential use as antioxidants in food. Of all the spices measured in their study ginger and cloves had the highest total phenolic content although this did not automatically translate into the highest antioxidant capacity or reducing power. In a Roselle beverage, Ashaye, Olanipekun, Ige, Farinde, and Akinkunmi (2013) investigated the use of Alligator pepper (*Aframomum danielli*) and concluded that it had a mild preservative potential on the chemical properties of the beverage. Onuoha and Fatokun (2014) on the other hand, suggest the use of lime juice as a natural preservative in a Roselle beverage because of its strong acidity which gives it antibacterial properties.

Roselle beverage deterioration could also be due to a variety of intrinsic factors such as ingredients and formulation, water activity, acidity/pH and chemical oxidation; or extrinsic factors such as microbial activity, processing and storage temperatures, packaging material, light exposure, and enzymatic oxidases occurring during storage (Badrie & Schauss, 2010;

IFST, 1993; Woodroof & Phillips, 1980). Physical and chemical deterioration are perhaps more reproducible than microbiological deterioration and will be the focus of this research work.

Good storage stability increases the chance of good quality products reaching the consumer. Understanding and improving storage stability within the boundaries of clean labelling is important to this research and indeed the beverage industry.

1.3.8 Methodologies used in the measure of Roselle properties

a. Extraction

As discussed in chapter 3, several optimal extraction conditions have been reported by various researchers. Optimal conditions appear to be relative to the compound(s) of interest. In several studies, higher extraction temperatures resulted in higher anthocyanin content (Cisse, Vaillant, Kane, Ndiaye, & Dornier, 2012; Perez-Ramirez et al., 2015) although it contradicts the results from other studies (Chumsri, Sirichote, & Itharat, 2008). However, Cisse, Bohuon, et al. (2012) show the importance of solid to solvent ratios and particle size in the extraction process. Higher anthocyanin extraction yields were obtained with solid-to-solvent ratios of 1:30 (kg Roselle:kg solvent) compared to a solid-to-solvent ratio of 1:10. The study also demonstrated that for extraction temperatures between 60^{o}C and 90^{o}C, anthocyanin content peaked 10 minutes into extraction time and decreased thereafter. For the different temperature conditions, the anthocyanin content at the peaks did not appear to be significantly different. This suggests that temperature may not be the primary concern in anthocyanin extraction.

This research uses a solid to solvent ratio of 1:200, thus expecting a much higher extraction yield compared to the Cisse, Bohuon, et al. (2012) study. Furthermore, extraction

conditions in this current study were closely based on an existing industrial recipe (Figure 1.3) in order to replicate similar challenges that may be faced. Consequently, whole dried Roselle calyx was used in the extraction process rather than finely ground calyx. The extraction time of 25-30 minutes was adopted for the study but was set at 25 minutes. However, the following alterations were made to the industrial process. Rather than extracting at boiling temperature (100^{o}C), extraction temperature was set at 90^{o}C to avoid excessive loss of moisture as a result of boiling.

b. Pasteurisation

As with extraction conditions, pasteurisation conditions vary in studies of Roselle. Cisse, Vaillant, et al. (2012) used 100^{o}C for 5 minutes and observed significant differences in anthocyanins, antioxidants and colour between pasteurised and unpasteurised samples. In all properties, pasteurised extracts had lower values. Whereas, Perez-Ramirez et al. (2015) pasteurised at 95^{o}C for 15 minutes and observed a 6 - 7% loss in anthocyanins but no losses with total polyphenolic content or antioxidant capacity. In a different study by Bechoff et al. (2014) Roselle beverages were pasteurised at 85^{o}C for 20 minutes although the aim of their study was not to investigate the effect of pasteurisation However, these pasteurisation conditions are quite similar to conditions for the industrial process (Figure 1.3) pasteurised at 85^{o}C for 15 minutes. Therefore, pasteurisation conditions for this present study were set at 85^{o}C for 20 minutes, in-situ. However, for sensory work, pasteurisation conditions were altered to 95^{o}C for 3 seconds to match the HTST equipment capabilities.

c. Storage

In keeping with similar storage studies on Roselle extracts, a storage time of six-months was initially adopted for this research work. There is the challenge of maintaining cold chains in the storage of beverages in countries with interrupted power supply, hence it is useful to observe the effect of storage temperature changes on the quality of the beverages. Hence as detailed Chapter 3, extracts were stored at different temperatures (frozen: -21^{o}C, refrigerated: 4^{o}C and room temperature: 21^{o}C).

However, in Chapter 4, for food quality and safety reasons, Roselle extracts were either freshly prepared or refrigerated (4^{o}C), packed in pre-sterilised glass bottles and consumed within 2 days. On the other hand, Roselle beverages (with sweeteners and spices) were frozen in pre-sterilised polyethylene bottles and defrosted on the day of consumption.

In Chapter 5, accelerated storage conditions (40^{o}C for 30 days) were employed. The selection of the conditions was based on the results of a previous trial run of temperatures ranging between of 30 – 60^{o}C. Although all temperature conditions were suitable from an anthocyanin perspective, 40^{o}C was selected based on reduced moisture losses, anticipated minimal effects on antioxidant capacity and equipment availability. Observing cautions on accelerated storage conditions, moisture losses could potentially introduce inaccuracies in measurements and must be avoided (Franks, 1993). The beverages stored under accelerated conditions included an unsweetened Roselle extract which could be directly compared to the results in Chapter 3.

d. Masking of off-notes

Several methods have been employed in improving the taste of functional beverages. In relation to bitterness and astringency, Shahidi and Alasalvar (2016) mention the use of

complex flavouring which could suppress off-notes and compliment overall flavour; subduction which have employed the use of selected flavours to compliment lingering tastes and flavours; encapsulation of bitter or astringent ingredients; and introduction of ingredients that would interfere with bitterness receptors or signalling pathways i.e. bitter blockers. The literature on the use of spices as masking agents for off-flavours in beverages is limited although spices extracts are popularly used in beverages internationally. Therefore, it is explained in Chapter 4 why spice and the particularly selected spices used in this study were considered for masking of SRA off-flavours in the Roselle beverage.

e. Analytical methods

Table 1.3: Laboratory methods used to analyse aqueous Roselle extract in this study

S/N	Measured parameter	Method used	Reference	Apparatus
1	Antioxidant capacity	Ferric reducing antioxidant power (FRAP) assay	Benzie and Strain (1999)	GENios TECAN Plate reader
2	Angiotensin converting enzyme inhibition (ACEi)	FAPGG substrate	Various (Henda et al., 2013; Murray, Walsh, & FitzGerald, 2004; Shalaby, Zakora, & Otte, 2006)	GENios TECAN Plate reader
3	Colour	Hunters lab	L*a*b* colour system	CT-1100 ColorQUEST HunterLab
4	pH	pH meter	n/a	Mettier-Toledo SevenEasy pH meter
5	Titratable acidity	AOAC 1984	AOAC (1984)	Orion star A111 pH meter
6	Total anthocyanin content	pH buffer differential and HPLC	Various (Lee, Durst, & Wrolstad, 2005; Obouayeba et al., 2014)	Amersham pharmacia biotech Ultrospec 1100 pro
7	Total phenolic content (TP)	Folin Ciocalteu	V. L. Singleton and J. A. Rossi (1965)	Perkins Elmer UV/VIS Spectrometer

In Table 1.3, the laboratory methods used to monitor the physical and bioactive properties of the Roselle extracts/beverages are shown. For the real time measurements, samples were analysed at specific time-points which were daily intervals for the first five days; 2-3 days intervals for the next 10 days; 5 days intervals up until day 30, 10 days intervals up to day 60 and monthly intervals thereafter until day 180. Most tests (except colour and ACE were carried out on the exact day of the time-point, therefore, equipment available and method rapidity and reproducibility were the primary criteria used in methods selection.

1. Antioxidant capacity – Several methods for antioxidant capacity have been employed in the measurements in extracts/beverages. With standard Roselle samples, Tsai, McIntosh, Pearce, Camden, and Jordan (2002) compared some methodologies: ferric ion reducing antioxidant power (FRAP), oxygen radical absorbance capacity (ORAC) and total antioxidant status (TAS) antioxidant assays, and demonstrated linear relationship between FRAP and TAS (R^2 =0.9411) and FRAP and ORAC (R^2 =0.9288). In a separate study on vegetable juices, (Wootton-Beard, Moran, & Ryan, 2011), FRAP also correlated well with the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) assay, $ABTS^+$ (R^2 = 0.87) whilst $ABTS^+$ was strongly associated with Folin Ciocalteu method (R^2 = 0.89). However, the 2,2-diphenyl-1-picrylhydrazyl assay, DPPH and FRAP were poorly correlated (R^2 = 0.53) as were DPPH and Folin Ciocalteu method (R^2 = 0.50). Therefore apart from DPPH, any of the methods investigated will provide quite similar results. Moreover, one of the methods could be used to consistently estimate the antioxidant capacity of a Roselle beverage. Consequently, the FRAP assay was selected particularly for its rapidity and large sample capacity. It is based on the ferroin analog reducing ability of phenolics. Tripyridyltriazine (TPTZ) contains FE^{3+} which is reduced to FE^{2+} in acidic conditions (de la Rosa et al., 2009). Using the GENios TECAN plate reader allows

several samples to be analysed quickly and at the same time and therefore reduces the margin for error. The results are expressed in gram Ascorbic acid equivalent per litre (g AAE/L).

2. Angiotensin converting enzyme (ACE) inhibition – ACE is further described in Chapter 3. This test has been included as an anti-hypertensive test of the Roselle beverage. It measures changes in optical density at 340 nm due to the cleaving of ACE to FAPGG (Fagyas, 2014). Similar polyphenol rich foods, such as tea and such as pomegranate juice, have been observed to be ACE inhibitors (Aviram & Dornfeld, 2001; Dong, Xu, Liang, Head, & Bennett, 2011). Although several studies have alluded to the ACE inhibitory capacity of Roselle extracts, the most prominent study which tries to explain the mechanism was Ojeda et al. (2010). In his study, the FAPGG (N-[3-(2-furyl) acryloyl]L-phenylalanyl glycyl glycine) substrate was used. ACE hydrolysis of FAPGG was quantified (Herrera-Arellano et al., 2007) and the activity measured at 345 nm with isocratic reverse-phase HPLC. Lisinopril was used as the control. To achieve comparable results, the FAPGG substrate was similarly used in this study but adapting the method for use on the GENios TECAN plate reader to enable for a quicker and more robust analysis. Also instead of Lisinopril, Captopril, another popular antihypertensive which also works by inhibiting the ACE enzyme, was used as a control and the IC_{50} compared to literature. The results for ACE inhibition capacity are expressed in %.
3. Colour - The CT-1100 ColorQUEST HunterLab was set to measure transmittance and calibrated using standard plates. L*, a* and b* Readings were obtained for extracts and b to a ratios used to calculate hue angles which were used to make comparisons between extracts with reference to Figure 1.4 below. The results are expressed in absolute values for colour lightness and chroma and in degrees for hue angle.

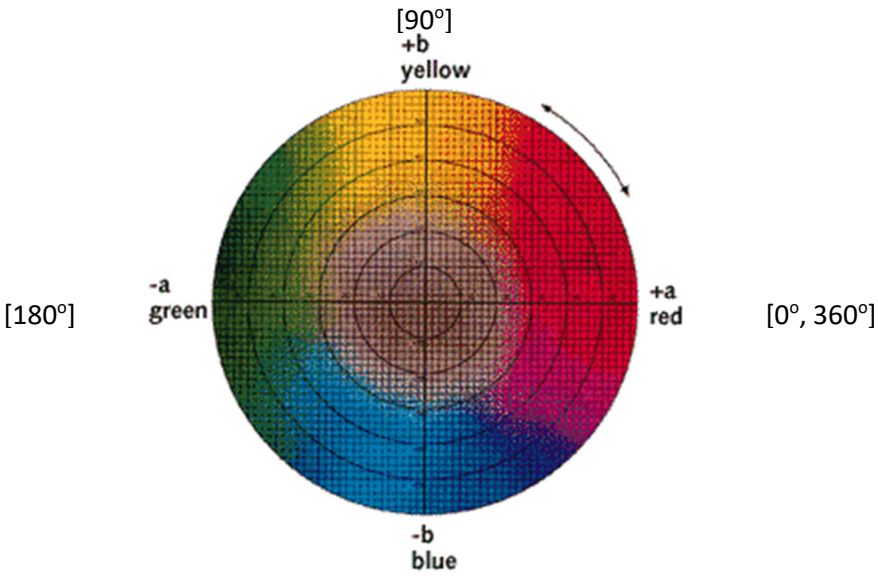


Figure 1.4: CIE LAB colour chart (Oocities, 2009)
[Hue angles in square brackets]

4. pH – this was done routinely using pre-calibrated pH meters. Results are expressed in 2 decimal numbers.
5. Titratable acidity – The standard AOAC method was followed and the results were expressed as % malic acid, similar to the Mgaya - Kilima, Remberg, Chove, and Wicklund (2014) study since Roselle contains malic acid (Da-Costa-Rocha, Bonnlaender, Sievers, Pischel, & Heinrich, 2014).
6. Total anthocyanin content – the pH buffer deferential method is simple, quick and reliable. It measures changes in absorbance of monomeric anthocyanin at two pH (pH 1.0 and pH 4.5) at 520nm. At pH 1.0 anthocyanins are structurally transformed to their coloured oxonium form and at pH 4.5, they are transformed to their hemiketal form. The results are expressed in milligram delphinidin 3-sambubioside equivalent per litre (mg D3S/L). HPLC was used to validate the pH differential result in Chapter 3.

7. Total phenolic content – The Folin Ciocalteu method is quite common in contemporary research. In this method, phenols are oxidised using a molybdotungstate reagent to yield a coloured product measured at 760nm which can be compared to a standard plot of a phenolic compound such as gallic acid. The results in this study are expressed at milligram gallic acid equivalent per millilitre (mg GAE/ml).

f. Sensory methods

The sensory work comprised of analytical methods i.e. quantitative descriptive analysis, used by trained assessors. Consumer studies followed with affective methods i.e. hedonic tests used in consumer liking tests, Just About Right (JAR) scales to measure flavour intensities and Check All That Apply (CATA) questionnaires to measuring consumer emotions.

Firstly, due to the absence of a lexicon for Roselle beverages and the limited offerings of Roselle in the UK, Roselle was assumed to be quite unfamiliar to a UK panel. Therefore, a panel of professional food tasters (MMR Reading - 1 man and 10 women) were trained for 4 days to introduce them to Roselle beverages and enable them individually come up with descriptive terms using unsweetened and sweetened Roselle extracts (9% sugar equivalent) and a selection of the spice flavoured Roselle beverages.

As a group, the descriptive terms were pooled and streamlined in an open discussion under the supervision of a panel leader, until a consensus was reached on the appropriate terms and their definitions. Using Roselle extracts of varying concentrations (5 g/L to about 100 g/L), and various food, non-food and pure chemical standards, the descriptive terms were further trimmed by the assessors Finally, a mock scoring exercise was carried out prior to Quantitative Descriptive Analysis (QDA) to verify the definitions of the selected vocabulary.

Profiling and quantitative descriptive analysis using QDA (Tragon) were carried out in 4 sessions with the 11 trained assessors, to obtain results in duplicates. Further details of the training sections are provided in Chapter 4.

Chapter 2 –Making Healthier beverages

Abstract

This chapter gives a less technical context for the research topic, showing why the study is important, and how certain decisions came about, which influenced the research questions in Chapters 3 – 5. Starting with a brief history of functional beverages, the current industrial outlook to functional beverage is also explored. Information exclusive to this research work is discussed under a business case study, calorie reduction and clean label considerations. Under these subheadings, related activities, initial trials and failed experiments not discussed in subsequent chapters are revealed. Information from this chapter should educate a much wider audience beyond small scale manufacturers and researchers

2.1 Beverages - Then and now

Non-alcoholic beverages are a pleasurable means of hydrating the body. Due to osmolality, these drinks are more readily absorbed into the body than water (Ashurst, 2005). Beverages can be sources of important nutrients required for good health and are also key features for entertainment and relaxation. As far back as in the 1600s, cordials contained herbs e.g. dandelion, nettle; and spices such as ginger, cloves and cinnamon (Emmins, 1991). The herbs and spices were sometimes added as part of the process to produce safer alternatives to contaminated water but were particularly found to be beneficial to invalids (Emmins, 1991). In fact, these types of beverages predate fruit flavoured beverage. Eventually, in the 1700s, other soft drinks such as artificial mineral waters, soda waters, lemonade and ginger beer and sherbets were developed some of which were delivery vehicles for vitamin C to prevent scurvy among sailors and treat other ailments.

The ginger beer, in particular, has survived several centuries and is still a favourite today. These early beverages could be considered to be the progenitors to our modern day functional beverages, which could be as life-changing and enduring. Clearly, beverages were never intended to be carriers of empty calories. However, in recent years, the reputation of non-alcoholic beverages such as fruit juices and soft drinks, has been tarnished especially as it is associated with poor health particularly due to its high sugar content. Beverages are one of the main sources of sugar in daily consumption worldwide. For example, Public Health England revealed the National Data and Nutrition survey for 2012 – 2014 which indicates that soft drinks account for 27% (Figure 2.1) of the total sugar intake for 19-64 age group, of all food groups (Tedstone et al., 2017). As the highest contribution to total sugar intake, sugar reduction strategies in soft drinks and beverages are an important area of research.

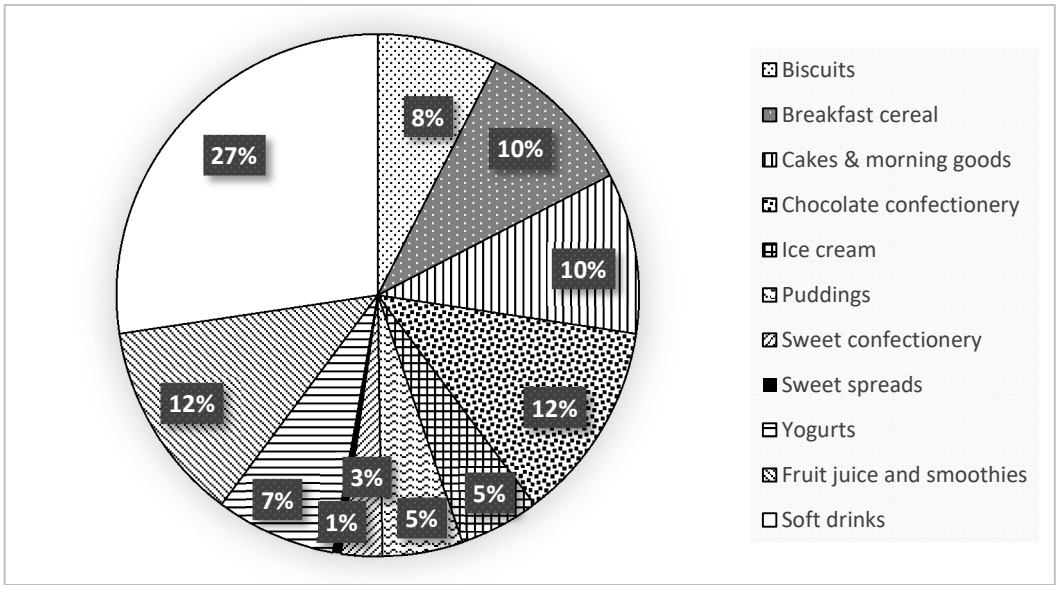


Figure 2.1: Data published by Public Health England on the National Diet and Nutritional Survey food groups relevant to the sugar reduction programme and soft drinks; specifically showing percentage total sugar intakes for 19-64 years old (NDNS 2012-2014) (Tedstone et al., 2017).

In a review of the joint report of the World Health Organisation (WHO)/Food and Agriculture Organisation (FAO) expert consultation on diet, nutrition and their relation to

chronic diseases, Nishida, Uauy, Kumanyika, and Shetty (2004) indicated that the consumption of free sugar rich drinks leads to higher energy consumption than with drinks artificially sweetened with low calorific sweeteners; eventually leading to weight gain. According to WHO (2014), the term "free sugars" refers to all monosaccharides and disaccharides added to foods by the manufacturer, cook or consumer, plus the sugars that are naturally present in honey, syrups and fruit juices.

The health concerns regarding sugar particularly in beverages are not unfounded. A meta-analysis on the health impact of sugar rich drinks shows a correlation between increased sugar consumption and a greater risk of developing diabetes and obesity related chronic diseases such as cardiovascular diseases (Malik, Akram, Shetty, Malik, & Njike, 2014). Globally, the number of obese people has tripled since 1975 (World Health Organisation, 2017b). Furthermore, 415 million people worldwide are reported to be living with diabetes as of 2015 (International Diabetes Federation, 2015) and 31% of global mortality figures in 2015 are due to cardiovascular diseases (CVD) (World Health Organisation, 2017a). Therefore, in addition to several other campaigns, there has been a push for the reduction of sugar in foods and the development of food and beverage products that will combat these alarming trends.

For improved health, the recommendations by WHO/FAO included the reduction of free sugars; natural or added, to less than 10% of total calorie intake (Nishida et al., 2004). The rationale for these recommendations was their negative impact on health leading to obesity, poor dentition and CVD (Nishida et al, 2004). More recently, WHO recommended halving daily sugar intake to a target of 5% total calorie intake which is about 26g for an average adult with a normal body mass index (World Health Organisation, 2014b).

In relation to this, national discussions are being held in various countries including the UK about regulating sugar consumption by implementing a sugar tax on sugar sweetened products. Already the sugar task has been introduced in other countries such as Mexico, France, Denmark and part of the US with mixed results. In Mexico, a 10% tax on soft drinks containing added sugars led to a 6 - 10% reduction in soft drinks purchase and 7% rise in purchase of milk and 100% fruit juices (Colchero, Popkin, Rivera, & Ng, 2016). Similarly, in Philadelphia (USA), the sugar tax which commenced in January 2017 decreased sales of carbonated soft drinks by 55%; ready to drink coffee and tea sales by 37% and refrigerated juice drinks by 47%, although corresponding sales in no tax neighbouring states increased (Menayang, 2017). Needless to say, a sugar tax would be a challenge for beverage companies and may negatively affect the economy. Consequently, the route to survival for beverages is through re-formulation and the emergence of new brands to accommodate WHO/FAO recommendations and providing healthier options for consumers.

Beyond sugar concerns, beverage innovation in recent years has tended towards creating food and beverage products with added benefits to consumers (Corbo, Bevilacqua, Petruzzi, Casanova, & Sinigaglia, 2014; European Food Information Council, 2006) and these products are categorized as functional foods and drinks. Functional drinks/beverages have no universal definition but may be described as containing natural or added ingredients that provide benefits such as weight loss, improved gut health, overall wellbeing etc. (Corbo et al., 2014). Lifestyle changes have encouraged the growth of the functional foods and beverage market. Therefore, there is a need for further research to maximise the potentials of functional foods and provide a wide range of effective alternatives in the market.

Another trend in the food and beverage sector that is worth considering in this thesis is clean labelling. The “clean label” trend is growing in popularity within the soft drink

category in general (Gelski, 2016). The concept of clean labelling encompasses such a broad and somewhat evolving criterion, therefore no formal definition exists. According to Mintel (Price, 2016), consumers perceive clean label to mean "naturalness" or "made from all-natural ingredients" and synonymous with "free-from". Therefore, this thesis seeks to explore related issues under the current perceptions of clean label requirement such as the use of "all-natural" ingredients and "free-from" added sugar and preservatives.

Furthermore, many tested and novel botanicals and functional ingredients suitable for use in clean label and functional beverages have been explored in recent years. These ingredients range from fruits, plants, herbs and spices to dairy products and varying combinations. The sourcing of these ingredients has assumed global reach. Thus, local ingredients from various parts of the world are gaining worldwide interest. As discussed in the previous chapter, one such ingredient is the *Hibiscus Sabdariffa* plant (Roselle), which has health benefits and functionalities and is grown in many countries in several continents including Africa, Asia, and North America. These countries have commercially available Roselle beverages which makes it an ideal case study and the focus of this research.

The market value of functional and fortified beverages in 2015, which included fortified waters, fruit and herbal teas, was reported by Euromonitor to be over 90 billion dollars globally (Arthur, 2016). Furthermore, the functional beverages market, in particular, is forecasted to experience global growth of about 9% at a compound annual growth rate between 2017 and 2022 (Mordor Intelligence, 2017). A huge segment of this market, thus far, has consisted of energy, sport and nutraceutical drinks and yet is reported to be the fastest growing in the beverage category. Essentially this market is driven by the prevailing risk of chronic diseases. However, the term "Naturalness" appears to be a concurrent trend with nutraceutical drinks. This is where herbs and spices may be applicable.

These recent data shows that the market is rife with the re-emergence of beverages flavoured with herb and spices. However, there is surprisingly little research into the effects of incorporating herbs and spices in beverages. Apart from the investigation of functional properties of beverages, such as the probiotic viability of drinking yoghurt or the fortification of beverages with fibre or particular nutrients, sugar reduction has been the primary focus of beverage research in recent year. This, of course, is a very important aspect but should be done in tandem with other product development objectives. Therefore, to encourage wider research into the incorporation of herbs and spices to beverages, this chapter reveals findings, failings and learning outcomes from otherwise undocumented aspects of my research, including the participation in several competitions in which the Roselle beverage flavoured with herbs and spices were the products on offer. The information is discussed under 3 subheading namely:

1) Business case-study
2) Clean label consideration
3) Calorie reduction

2.2 Business case study

In this section, an investigation of the consumer and industry reception to a clean label functional beverage is carried out through my participation in competitions (sub-section 1 – 3) and a beverage symposium (sub-section 4). Hence, a background is laid for the positioning of Roselle beverages in the current functional beverage market, as well as, an introduction to some issues of particular importance to this study.

1 EU TradeIT Entrepreneurial Summer Academy - 2014, Tralee. Ireland

A poster entry for this event highlighted the benefit of Roselle and the potential for producing a functional beverage. On the final day of the summer academy, each participant was interviewed on their potential product and the focus of the interview was (1) the product and its unique selling point (2) the target market and how will they be reached? (3) Intellectual property management (4) sources of funds. This entry won an award for Food Innovation.

2 Henley Business School Ideafest - 2014, Reading, UK

A Roselle beverage called “Hi’FAB” was entered for the competition. Hi’FAB was described as a tasty and refreshing drink for any occasion that is formulated with health promoting natural ingredients and did not contain chemical preservatives. Its main ingredient, the hibiscus extract was highlighted for its usefulness in reducing cardiovascular disease (CVD) risks, such as, regulating blood pressure and lower cholesterol. The presentation supplied a description of the beverage and the unique selling point, information on the target market, product competition, required finance, profit margin and a brief financial forecast. Hi’FAB was the winning product for the Ideafest competition.

3 Biotechnology Yes, Nottingham, UK

Entering the Biotechnology YES competition in a team of 4, it was decided to expand on the foundations from the previous competitions. Therefore, it became necessary to ensure there was sensory backing for the marketing of this beverage. Hence, a series of informal tasting sessions were held in October 2014 in Reading in which 77 people participated. The tasting sessions varied the quantities and types of natural sweeteners and the quantities and types of spices.

The Roselle beverage products on offer for tasting included Original (sweetened Roselle extract), Ginger (medium and extra strength) and Cinnamon (medium and extra strength). The beverages were sweetened with either honey or maple syrup both of which were selected for their preferential use over sugar and cardiovascular or antidiabetic properties. As several studies link the consumption of high glycemic index (GI) foods to the increased risk or diabetes or CVDs and advocate consumption of low GI foods, both sweeteners compare preferably to sugar (GI Group, 2017). GI values below 55 are reported to have a moderate effect on the rise of blood glucose, therefore, with GI of 35 – 58 and 54 (per 25 g serving) for honey and maple syrup respectively, several studies support their use as alternatives to sugar for diabetics and CVD risk suffers (Apostolidis, Li, Lee, & Seeram, 2011; Erejuwa, 2014; St-Pierre et al., 2014; Yaghoobi et al., 2008).

Comments from participants included “natural”, “tasty”, “good aroma”, “never tried anything similar”, “refreshing and natural”, “good non-alcoholic option”, “good non-carbonated option” and “tastes like Christmas”. Negative comments revolved around the strength of the spices and the level of sweetness but none in relation to the Roselle flavour. The Roselle beverage was entered under the name “Bozo” which, unfortunately, and unknown to us, has negative connotations in North America. This was picked upon by the competition panel. Although not the winning entry, the general feedback was positive.

4 Innovation in Non-Alcoholic Beverages 2017

There was a clear emphasis on sugar reduction, the use of natural ingredients and added functionality to beverages for most of the brands presented. One new beverage category was the unsweetened flavoured waters/beverages which were represented by brands like Dash waters and JB Rabbitt. In both waters, vegetable infusions were mixed with sparkling or still water and left unsweetened. However, the majority of the brands represented at

the event contained reduced levels of sucrose, fruit juices or intense sweeteners such as steviol glycosides.

Cost concerns in relation to sugar replacement with intense sweeteners were not apparent at the symposium. Since the price of certain emerging sweeteners such as the steviol glycosides are not currently standardised due to differences in type, quality and purity, there is difficulty in ascertaining the cost implications of replacing sugar with steviol glycosides. However, the highest price of Stevia Rebaudioside A (SRA, up to 99% purity) observed towards the second half of the year 2017 did not exceed £150 per kg (Alibaba) whereas the price of sugar in the year 2017 did not exceed £300 per tonne. Therefore for 1 kg of sweetener, the price ratio of sugar to SRA could be about 1:500. However, considering that the SRA extract may be up to 450 times (International Stevia Council, 2010; I. Prakash, G. E. DuBois, J. F. Clos, K. L. Wilkens, & L. E. Fosdick, 2008) as sweet as sugar, the impact of cost may still be minimal.

Conversations with several beverage producers including Dash waters and JB Rabbitt indicated that pasteurisation is still widely used within the beverage industry for preservation. Hot filling was mentioned by some producers as the alternative. On asking why HPP and other new technologies were not used, the response was that it was not readily available or cost effective for small producers. Consumers do not appear to mind the concept of pasteurisation in relation to natural foods since it is widely used on popular products like milk.

2.3 Clean label considerations

Following earlier findings from the business case study, it was important to look more closely at the “clean label” concept. As earlier mentioned, “Naturalness” is a key factor in the clean label trend. The Food Advisory Committee (FSA, 2008) defines “Natural” to mean

a food "comprised of natural ingredients e.g. ingredients produced by natural, not the work of man or interfered with by man". Where any chemicals, or additives/flavouring which altered compositions are used, the term does not apply. Any processing, including pasteurisation, to ensure food safety, is also precluded from the use of the term natural. However, a natural product can be described by the processing it has been subjected to. Furthermore, beverages in general (except milk and juices) are made from several ingredients, therefore, they cannot be described as natural but can be described as "made from natural ingredients". Hence, if pasteurised and constituting only of natural ingredients, the Roselle beverage used in this study can be described as "Pasteurised Roselle drink made from natural ingredients".

The clean label concept also encompasses "the free from" trend and where certain ingredients have not been used to the advantage of any group of people, it may be declared as well particularly if the precluded additive is typically found in the class of foods. This could be but should not be confused with the term "natural". Therefore, the lack of non-natural additives either as flavouring or preservatives could be declared as "free from non-natural additives". Traditional or physical processing of natural additives is acceptable under the natural ingredient requirements.

Only a few clean label sweeteners exist. To fit the "naturalness" demand, these sweeteners must be from natural ingredients and must be naturally derived. Sugar is considered to be a clean label sweetener (Kerry Foods, 2017) despite its calories and link to poor health. Apart from sugar, other clean label sweeteners are shroud with controversies coupled with a lack of adequate toxic information particularly in relation to human epidemiology, short-term mutagenicity, and acceptable daily intake, to ensure the safety of consumers. Some short time or animal studies have been carried out.

Beyond sweeteners, other additives such as flavouring and preservatives need to align with clean label requirements. One of the drivers for the clean label movement is consumer intolerance to food additives. In 1986, 0.03 – 0.15% of the population were reported to be intolerant to food additives (Millstone, 1986). Artificial additives such as benzoic acid and sodium benzoate have been implicated in hypersensitivity and intolerance. It is difficult to ascertain what the current figures are, as in many cases, intolerances are not reported or documented.

As part of this research work, the preservative (microbiological) effect of cinnamon and ginger was also explored as alternatives to typical preservatives. However, the results were fundamentally inconsistent. This is an important aspect of the clean label campaign and perhaps the biggest challenge to small producers. Further research in this area is recommended.

2.4 Calorie reduction

Early in the research work, calorie reduction was discovered to be just as important as the glycemic index in the choice of sweeteners particularly with the proposal of the sugar tax in the UK to commence in 2018. First, there is a need to understand the sugar content of beverages. For sugar-sweetened beverages, in particular, an average range of 5 - 12% was reported by Hess, Latulippe, Ayoob, and Slavin (2012). Harvard School of Public Health (2014) estimates that a regular can of soda (340 ml) contains approximately 40 g (12%) sugar. In similar portion sizes, 100% natural juices such as Minute Maid Orange Juice; Mott's Plus for Kids' Health Juice Apple Grape; Naked® Juice 100% Juice Pomegranate Blueberry and Welch's® 100% Grape Juice were reported to contain 41 g, 48 g, 54 g and 63 g (12-19%) sugar respectively. However, sugar is not in isolation. The quantities of maple syrup or honey used in beverages and their calorie contributions are only marginally less

than for sucrose. Hence, natural intense sweeteners replaced maple syrup and honey which were the first considerations in this current work on Roselle beverages. Besides benefits to the body, intense sweeteners also favour dental health, thus are considered to be consumer protectors (Millstone, 1986).

In deciding the appropriate sweetener to use for calorie reduction, several sweeteners were explored which are shown in Table 2.1. Based on the business case study, about 8 - 9% sweetness was thought to be ideal for the Roselle beverage. Hence, in the initial stages, different quantities of all the sweeteners (except sweetener G) were dissolved in water and tasted until the sweetness was gauged to be similar to the sweetness of 80 g/L. The quality of sweeteners used relative to the sweetness of 80 g/L are shown in Table 2.1. The exception was the Cargill Stevia Rebaudioside A (Stevia Reb A) which had a clearly specified ratio of 1:250 sugar to Stevia Reb A on the manufacturers' specification sheet.

Table 2.1: Sweeteners considered for use in the initial stages of the Roselle beverage research work

S/N	Sweetener	Source	Code	Quantity used (g/L)
1	Sucrose	Tate and Lyle	A	80
2	Truvia*	The Silver Spoon Company	B	40
3	Stevia Rebaudioside A	Pure Circle	C	0.4
4	Steviol Glycosides	Pure Circle	D	0.1
5	Stevia Leaf**	Just Ingredients	E	11
6	Miraculin***	ChinMau Biotech Co., Ltd	F	80 (8 tablets)
7	Stevia Rebaudioside A, 80% purity	Cargill	G	0.32

*Truvia (sweetener B) consists of Erythritol, steviol glycosides and natural flavourings

**The stevia leaf (sweetener D, 10 g) was extracted in 200 g of vodka (Smirnoff, 40% alcohol) by steeping for 36 hours and bringing to boil and leaving to simmer for 30 minutes to boil off the alcohol (WellnessMama.com, 2011).

***Miraculin (sweetener F) tablets were ground to powder, however, solubility was very low. In addition, an equivalent sweetness with sucrose was not achieved, and when added to Roselle extract, the solution became a light brown. Upon storage overnight, there was rapid microbial growth. Hence no further work was carried out with Miraculin.

Subsequently, when the other sweeteners were dissolved in unpasteurised Roselle extract (as prepared in Chapter 3), an informal panel was carried out with 8 colleagues to:

1. Assess if the right level of sweetness was obtained;
2. Assess if the appropriate quantities of sweeteners had been used to give an equivalent sweetness with sugar;
3. Decide on which sweeteners to proceed with in future work

Only Sweeteners A – E were presented to the informal panel. A summary of results from the panel is shown in Table 2.2

Table 2.2: Summary of feedback from informal tasting session

Code	Sweetener	Mean sweetness rating (mean score out of 5)	Appearance	Odour	Colour/change with sweetener	Flavour	Off-note	Aftertaste	Comments
A	Sucrose	Most sweet (5)	Good, clear, transparent	Acidic, flowery	Red/Brightening or lightening of red colour, crystal	Very good, fresh, nice, sweet, sour, fruity	None	Sweet	Liked the most, sourness is just right, candy-ish, quite sweet
B	Truvia	Less sweet than A but similar sweetness to C and D (3)	Good, clear, like wine	Sweet, flowery	Red/Slight lightening of red colour, crystal	Good, fruity, sour, candy, sweet	Sweet, sour	Sweet	Good, Liked
C	Stevia Rebaudioside A	Less sweet than A but similar sweetness to B and D (3)	Good, clear	Strange, herbal	Red/No visible colour change in red colour, crystal	Sweet, Strong, rough/acid, like saccharine, bitter, fruity	Strange, Strong – sweetness, bitter (very)	Strong - sweet, bitter	Not liked, horrible, bitter, would not drink ever!
D	Steviol Glycosides	Less sweet than A but similar sweetness to C and D (3)	Good, clear	Strange, fruity, tropical	Red/No visible colour change in red colour	Unusual, acidic (tangy), sour, bitter	Sweet, bitter (very), medicinal	Tangy, acidic, bitter, sweet	Not good, tangy, would not drink ever!
E	Stevia Leaf	Least sweet (2)	Good, cloudy, opaque, turbid, translucent	Bitter, nice aroma, like apple, meaty, terrible	Red/Darkening or browning of red colour - magenta	Sweet, like aspartame, bad, sour, fruity	Sweet, bitter, metal, animal	Sweet, bitter, metallic	A favourite, Taste natural – more real, Tangy, tastes like artificial sweetener

To conclude on the results from Table 2.2

1. The quantity of sucrose used was satisfactory.
2. There was clear discrimination between the sweeteners and the level of sweeteners varied from the sucrose sweetened sample. In theory, this would have meant an increase in the quantity of the selected sweetener, until the sugar equivalent was obtained. However, this is not easily done with stevia sweeteners as the sweetness profile is not typically dose respondent.
3. Sweeteners C and D were deemed unsatisfactory for future work based on the strong dislike by participants. Although the results for sweetener E were satisfactory, it may not have passed ethical requirement for use in a consumer panel since only steviol glycosides and not the stevia leaf or plant has not been approved for use in the EU (Food Standards Agency, 2011; Scientific Committee on Food, 1999)

Consequently, sweetener G (Cargill Stevia Rebaudioside A, Table 2.1) was sourced, tasted and found to be an improvement over sweeteners C and D, and therefore selected for use in future work.

2.5 Conclusion

The beverage industry is moving towards a healthier, more natural future as this is what consumers demand. This will require a lot more research on sweeteners and other additives. In the case of sweeteners, the beverage industry is yet to find sweeteners that offer good quality taste and also meet health demands. In the meantime, using sweeteners in combination appears to be the best solution as the synergy between certain sweeteners is able to temporarily navigate taste challenges. However, the Cargill Stevia Reb A was used independently in future chapters since very little data exists on non-sugar sweetened Roselle beverages in general. Finally, clean label preservation remains a key aspect but undeveloped part of this campaign. Experimenting with herbs and spices in beverages to discover synergies and congruent flavours is a recommended area for future research.

Chapter 3 – The Effect of Pasteurisation and Storage temperature on the Bioactives and Physical Compounds in a *Hibiscus Sabdariffa* (Roselle) Beverage

Abstract

The impact of traditional processing and storage temperatures on important physical and bioactive properties of *Hibiscus Sabdariffa* (Roselle) extracts such as total phenolic content, total monomeric anthocyanin, Ferric reducing antioxidant power (FRAP), angiotensin-I-converting-enzyme inhibition (ACEi), colour (hue-angle and chroma) and pH is investigated in this study. Following aqueous heat extraction (90^{o}C, 25 mins) of Roselle (5 g/L), there was no significant effect of pasteurisation (85^{o}C, 20 mins) on the measured parameters except with ACEi, where upon 180 days of storage, ACEi activity was better maintained in pasteurised extracts. In general, refrigeration and freezing storage allowed for significantly improved the stability of all the properties compared to room temperature storage. It was observed that ACEi, FRAP antioxidant and hue angle were only partially dependant on anthocyanin content and therefore anthocyanin degradation products were also considered to also influence these properties. Chroma was strongly correlated to anthocyanin content.

3.1 Introduction

Hibiscus Sabdariffa (Roselle) is mostly grown in tropical and sub-tropic regions of the world and used as local medication, tea and food and beverage ingredient. It has a high pectin content and acidic flavour, therefore, it finds broad application as a food ingredient and provides a unique taste in beverages.

The Roselle plant is made up of 15-30% organic acids such as ascorbic, citric, hibiscus, malic, oxalic, protocatechuic, stearic, tartaric, indolyl-acetic, succinic and gallic (Ali et al., 2005; Mahadevan, Shivali, & Kamboj, 2009; Sinela et al., 2017). Roselle has gained increased popularity in the last decade and several pertinent studies have been carried out on its health benefits. Roselle tea was demonstrated to lower elevated blood pressure (Haji Faraji & Haji Tarkhani, 1999; McKay et al., 2010); lower cholesterol in vivo (Lin et al., 2007) and potentially slowing down the increase of blood glucose levels (Harrison et al., 2009; Peng et al., 2011).

Thus far, the health benefits of Roselle have been ascribed to its polyphenolic content which may have antioxidant properties. The blood pressure lowering effects have been attributed in part to its Angiotensin-I-converting-enzyme (ACE) inhibitory activity (Herrera-Arellano et al., 2007). ACE, secreted in the blood vessels of the lung and kidney, modulates blood pressure by promoting the production of Angiotensin II; a vasocontrictor, and retarding bradykinin, a vascodilator (Winters, 2014). Roselle inhibits ACE production and synergistically promotes diuresis to provide antihypertensive effects. Whilst the latter can only be monitored in urine samples, the former is measurable in the extract.

Various studies reveal that Roselle extracts are rich in polyphenols; including quercetin, gossypetin, hibiscetrin, hibiscetine and sabdaretine. However, identified bioactive compounds in Roselle are broadly classified into flavonoids, of which anthocyanins are a part (Lin, Chen, & Wang, 2011; Perez-Ramirez et al., 2015). Identified anthocyanins in Roselle were delphinidin-3-sambubioside, cyanidin- 3-sambubioside, delphinidin-3-glucoside and cyanidin 3-glucoside (Du & Francis, 1973). The first two were found to be most abundant, thus classed as major anthocyanins. These major anthocyanins, according

to Ojeda et al. (2010), are responsible for ACE inhibition activity; achieving this through competition with the substrate for active sites.

A few shelf life studies on Roselle and numerous shelf life studies on other juices and plant extracts have shown that anthocyanins are prone to degradation. In a study on berry juices, Hellström, Mattila, and Karjalainen (2013) demonstrated this phenomenon further noting the importance of the beverage matrix and encouraging further research. As advised, the simple beverage matrix in this study is viewed holistically to understand how the properties of the beverage occur concurrently during processing and storage.

Since this study is concerned with the properties of a ready-for-consumption Roselle beverages and not in the optimisation of extraction procedures, results of aqueous extraction from other studies are of the most relevance. A few storage studies of different time frames but not exceeding six months have been conducted, showing some similar aspects of chemical analysis to this study, For instance, such studies have showed that anthocyanin degradation followed a first order reaction rate and that extraction temperatures were more important than pasteurisation in the degradation of anthocaynins during storage, with higher extraction temperatures having a more detrimental effect (Aurelio, Edgardo, & Navarro‐Galindo, 2008; Cisse, Vaillant, et al., 2012).

Furthermore, various shelf life results reveal that optimal conditions of extraction are relative to the compound of interest. For instance, a study with extracts obtained under two different conditions; (1) 25^{o}C for 240 minutes and (2) 90^{o}C for 16 minutes, showed better colour retention for the former but higher total phenolic content and antioxidant capacity for latter, with similarities in anthocyanin content for both (Wong, Yusof, Ghazali, & Man, 2003). The study determined optimum extraction condition of 60^{o}C for 3.5 hours based on the relative stability of anthocyanin and ascorbic acid. Whereas, Perez-Ramirez

et al. (2015) determined optimal conditions (95^{o}C for 60 minutes) in favour of ABTS and DPPH antioxidant activity and polyphenolic content in their study. At this temperature, there was no effect of extraction time on the antioxidant levels.

Some other relevant studies include work by Fasoyiro et al. (2005) and Mgaya‐Kilima et al. (2014), which focused on the properties of fruit flavoured Roselle beverages with 14 days and 6 month storage respectively. In the former, only physical, sensory and microbiology properties were monitored leaving out bioactive properties. In the latter, extraction conditions were at 50^{o}C for 30 minutes; beverages contained sodium benzoate (1 g/L), and only room and refrigerated storage conditions were explored. Hence, there remains a gap in information for a simple real life study presenting transferable information for product development purposes.

Therefore, considering the variation in Roselle beverage formulations and optimal conditions from the various studies, this study steers away from achieving optimal conditions under lab conditions but rather adopts a base formulation and pasteurisation and storage conditions similar to those used by small scale producers in developing countries. Thus, this study takes a typical industrial approach i.e. using hot water extraction at a set condition and focuses on the concurrent effects of pasteurisation and storage conditions on the physical and bioactive properties of the beverages which include total phenolic content, total anthocyanins, ACE inhibition, antioxidant capacity (FRAP), colour and pH. The work aims to provide a holistic view on the concurrent changes in these properties and to obtain an improved understanding of the causes and/or mechanisms of these changes.

3.2 Materials and Methods

3.2.1 Materials

Dried Roselle calyces (Country of origin: Nigeria; Variety/species: unknown) was purchased from Just Ingredients (UK). 2,4,6-Tri(2-pyridyl)-s-triazine (TPTZ), Ferric chloride (97% purity), Ascorbic Acid (99%), Gallic acid (98% purity), ACE enzyme (1.0 units/mg protein), Tris base, (N-[3-(2-furyl)acryloyl]-Phe-Gly-Gly (FAPGG), Captopril (98% purity), were obtained from Sigma-Aldrich, UK. Anthocyanins; Delphinidin 3-Sambubioside (90% purity), Cyandin 3-Sambubioside (95% purity) were obtained from Extrasynthese, France.

3.2.2 Preparation of Liquid Roselle Extract

Dried Roselle calyx, was stored at room temperature in its original transparent packaging in a dark cupboard. Preparation was carried out using an industrial recipe from the Federal Institute of industrial Research Oshodi (FIIRO, 2012) Nigeria with slight modifications. Briefly, an aqueous extract of Roselle was obtained by steeping 5 g of dried Roselle calyx in one litre of hot water for 25 minutes in a shaking (86 rev/min) water bath (Grant OLS 200), maintained at 90^{o}C. The extract was filtered using a Buchner filter, Buchner flask and Whatman No. 4 filter paper, then cooled on ice. Half the volume of extract was further pasteurised in-bottle at 85^{o}C for 20 minutes (Bechoff et al., 2014) and referred to as the "pasteurised extract". The pasteurised extract was cooled on ice. The other half was left untreated and referred to as the "unpasteurised extract".

3.2.3 Storage

Extracts in this study were stored under three different conditions: UK room temperature 21^{o}C (SD 1^{o}C) refrigeration temperature 4^{o}C (SD 1^{o}C) and freezing temperature -21^{o}C (SD

1^{o}C) and were regularly monitored using a thermometer (ETI 800-100). Extracts were stored for 180 days in transparent steriline vials in plastic containers in a temperature monitored dark cupboard at room temperature or in the fridge or freezer. There was no further control of light during storage. The 180 day storage period was selected based on storage practicalities and timeframes used in previous studies (Mgaya-Kilima et al., 2014; Mgaya-Kilima, Remberg, Chove, & Wicklund, 2015). During analysis, the vials were kept cool on ice.

3.2.4 Identification and quantification of bioactive compounds

3.2.4.1 HPLC-DAD

Freshly prepared, pasteurised or stored Roselle extract was filtered using 13 mm 0.22 µm PVDF filters (Kinesis, UK). HPLC column specifications were 150 x 4.6 mm ACE Excel 5 C18 from Hichrom Limited (Reading, Berkshire). This was used in an Agilent 1100 series HPLC system with dual UV/VIS detector (Agilent G1315A/B and G1365A/B diode array detectors). The injection volume was 100 µL. The two mobile phases used for elution were: A - 94.9% Milli-Q Water combined with 0.1% Formic acid (v/v; Sigma, Germany) and Acetonitrile, ACN (v/v; Sigma-Aldrich, Germany); and B - 99.9% ACN with 0.1% Formic acid (v/v). The elution gradient proposed by Obouayeba et al. (2014) was shortened to 5-15% B (0-5 min), 15-25% B (5-15 min), 25-100% B (15-20 min) and 100-%% B (20-25 min). The main anthocyanins in Roselle were detected at 520 nm and identified based on retention times, spiking and comparing spectra with an external standard (Extrasynthesis, France: Delphinidin 3-sambubioside MW ~ 597 g/mol and Cyandin 3-sambubioside MW: 617 g/mol).

3.2.4.2 Analysis of chemical and bioactive compounds

a Total Monomeric Anthocyanins

Total monomeric anthocyanins were determined using the pH differential method (Lee et al., 2005); the extract was mixed individually with pH 1.0 or 4.5 pH buffer solutions in a ratio 1:4 and left for 20 minutes. The absorbance of test portions at pH 1.0 and 4.5 was determined spectrophotometrically (Amersham Pharmacia Biotech Ultrospec 1100 pro UV spectrophotometer) at 520 nm and 700 nm. Anthocyanin pigment concentrations were expressed in delphinidin 3-sambubioside (D3S) equivalents. Calculations were carried out using the following equation:

$$Anthocyanin\ pigment\,(D3S, mg/L) = \frac{\left(A \times MW \times DF \times 10^3\right)}{\left(\varepsilon \times 1\right)} \quad (1)$$

Where $A = \left(A_{520nm} - A_{700nm}\right)_{pH\,1.0} - \left(A_{520nm} - A_{700nm}\right)_{pH\,4.5}$; MW (molecular weight) = 597 g/mol for delphinidin-3-sambubioside; DF = dilution factor; 1 = pathlength in cm; □ = 26 000 molar extinction coefficient in L x mol^{-1} x cm^{-1} for cyanidin-3-glucoside and 10^3 = factor for conversion from g to mg & cm. The results were expressed in milligram delphinidin 3-sambubioside per Litre (mg D3S/L)

b Antioxidant capacity

Antioxidant capacity was measured using the Ferric Reducing Antioxidant Power (FRAP) Assay which was proposed by Benzie and Strain (1999) with some modification. This method was selected for its rapidity and reproducibility which was essential to this study. The FRAP reagent was prepared by mixing acetate buffer, 2,4,6-Tri(2-pyridyl)-s-triazine (TPTZ) solution and Ferric chloride solutions in a 10:1:1 ratio. Extracts/standard (10 µl) was

measured into microcentrifuge tubes. FRAP reagent (300 µl) was added to the content of the microcentrifuge tubes and vortexed. The content of each tube (100 µl) was transferred to a Nunc 96 well plate. Absorbance was measured immediately in a GENios TECAN plate-reader at 595 nm. Ascorbic acid (Sigma, Germany) standards with concentrations ranging from 10 to 1000 µmol/L were used to generate standard plots ($R^2 \geq 0.99$) and an equation to calculate the antioxidant capacity of extracts as compared to ascorbic acid concentrations. The results were expressed in gram Ascorbic Acid Equivalent per Litre (g AAE/L).

c Total Phenolic content

Folin Ciocalteu (FC) colorimetry (V. Singleton & J. A. Rossi, 1965) was used to determine total phenolic contents of the extracts. Extract/standard (0.2 mL) was added to 6.0 mL of distilled water in 10 mL volumetric flasks after which 0.5 mL Folin - Ciocalteu reagent (Sigma-Aldrich, Germany) was added and mixed. After 1 min and before 8 min, 1.5 mL of 20% Sodium Carbonate (Fisher Scientific, UK) solution was also added and the volume adjusted with water to 10 mL. The colour generated after 2 hours was read at 760 nm using an Amersham Pharmacia Biotech Ultrospec 1100 pro UV spectrophotometer. Gallic acid (Sigma-Aldrich, Germany) standards with concentrations ranging from 0.05 - 1 g/L were used to generate standard plots and an equation for the calculation of total phenolic concentration in each extract. The results were expressed in milligram Gallic Acid Equivalent per Litre (mg GAE/L)

d ACE inhibition

Angiotensin-I-Converting Enzyme Inhibitory activity (ACEi%) was measured using a method based on a combination of previous studies by Murray et al. (2004), Shalaby et al. (2006) and Henda et al. (2013). Tris buffer solution (50 mM; adjusted to pH 7.5) and 0.88 mM

FAPGG (N-[3-(2-furyl)acryloyl]-Phe-Gly-Gly, Sigma, adjusted to pH 7.5) were prepared; and the former was stored at refrigerated temperature while the other was stored at -21°C. A 25 mL solution (adjusted to pH 7.5) of 0.3029 g Tris base (Sigma) and 0.8766g NaCl dissolved in deionised water, was mixed with 25 mL glycerol. The resulting solution (4 mL) was mixed with 1U ACE (Sigma) to obtain a concentration of 250 mU ACE. Captopril, with concentrations ranging between 0.7 to 20 nM, was freshly prepared on the day of analysis for use as the positive control.

FAPGG (150 µl) was pipetted into a 96 well NUNC plate and incubated at 37°C in the Tecan microplate reader for 1 min. Control (10 µl; 50 nM tris buffer solution) or inhibitor (Roselle extract, anthocyanin standard, captopril or 5M HCl) was added to the well and finally 10 µl 250 mU ACE. The absorbance at 340 nm was measured every minute over 30 minutes at a constant temperature of 37°C. The slope of the plot of absorbance against time was used in the equation below to calculate percentage ACE inhibition (ACEi%). ACEi results were presented in percentages (%).

$$ACEi\% = 100 \times \left(1 - \frac{Slope_{inhibitor}}{Slope_{control}}\right) \qquad (2)$$

To calculate IC_{50} the inverse concentration of extracts was plotted against the inverse of ACEi%. IC_{50} for captopril (Positive control standard) was 0.018 µM (SD 0.007). 5M HCl was used as a negative control standard.

3.2.4.3 Physical tests: Colour and pH

Colour was measured by the use of a CT-1100 ColorQUEST HunterLab taking measurements for transmittance. Standard black plates were used for calibration. L*, a* and b* readings were obtained and used to calculate Chroma and hue angles using the following equations.

$$Hue\ angle\left(^{o}\right)=\arctan\left(\frac{b*}{a*}\right) \quad (3)$$

$$Chroma=\left[(a*)^{2}+(b*)^{2}\right]^{\frac{1}{2}} \quad (4)$$

The hue angle and chroma may be used on a CIE 1979 L*a*b* colorimetric system diagram to identify colour and monitor changes. Chroma was presented in absolute values ranging from 0 to 100 while the unit for hue angle was degrees.

pH was measured using a pH meter (Mettier-Toledo SevenEasy) calibrated using buffer 4.0 and 7.0 buffer solutions (Sigma-Aldrich).

3.3 Theory/Calculation

3.3.1 Chemical kinetics

Natural logarithms of the concentration of phenolic and antioxidant capacity were plotted against time in days to confirm that these reactions follow first order kinetics. The plots showed a linear trend for all cases and from the slopes, the first order reaction rate constant (k) was obtained. The half-life ($t^{1/2}$) which is the time taken for the phenolic compound(s) or activity to reach half its initial value, was determined by applying the equation derived from the first order reaction equation as described below:

$$t_{½}=\frac{Ln\,2}{k} \quad (5)$$

3.3.2 Statistical treatment

Three batches of Roselle were prepared and each batch was analysed in triplicate. Statistical analyses were carried out using Linear mixed models for repeated measures

(Brown & Prescott, 2014) on SPSS Statistical Software (IBM, Version 24) to compare groups based on processing (n=2) and storage type (n=3). For simultaneous comparison of parameters, a Bonferroni adjustment was carried out manually on Microsoft Excel. Results with $P<0.05$ (95% confidence level) were considered significantly different.

3.4 Results and Discussion

3.4.1 Initial values and effect of Pasteurisation

Table 3.1: Initial (Day 0) values of the physical properties and bioactivities of Roselle extracts; pre and post pasteurisation

	Unpasteurised	**Pasteurised**
	Mean values ± SD	
Total Phenolic content (mg GAE/L)	131.7 ± 7.5[a]	127.0 ± 13.1[a]
FRAP Antioxidant capacity (g AAE/L)	140.2 ± 21.4[a]	130.1 ± 22.8[a]
Monomeric anthocyanins (mg D3SE/L)	42.3 ± 3.6[a]	39.5 ± 2.2[a]
ACE inhibition (%)	31.8 ± 8.7[a]	34.3 ± 4.4[a]
Colour - hue angles (°)	14.0 ± 0.9[a]	13.8 ± 0.9[a]
Colour – chroma	61.8 ± 1.5[a]	60.8 ± 1.7[a]
pH	2.86 ± 0.02[a]	2.81 ± 0.03[b]

Initial values are based on analysis carried on an aqueous Roselle extract prepared with 5 g Roselle steeped in a litre of water and maintained at 90°C for 25 minutes. Pasteurised extracts where further treated to in-bottle heating at 85°C for 20 minutes. Each point represents mean values + SD. Acronyms used are GAE: Gallic acid equivalent; AAE: Ascorbic acid equivalent; D3SE – cyanidin-3-equivalent; ACE- Angiotensin-I-covering enzyme. Letters (superscripts) a and b indicate significant differences between unpasteurised and pasteurised extracts for each property.

Initial values for total phenolic content, monomeric anthocyanins, antioxidants, ACE inhibition, colour (hue angle and chroma) and pH measured in Roselle extracts before and after pasteurisation are shown in Table 3.1. With the exception of pH, pasteurisation had no significant effect on the studied parameters.

The mean total phenolic (TP) content was 132 mg GAE/L (approximately 26 mg GAE/g dry Roselle). These results compare with documented values of 23 mg/g dry weight (Tsai et al.,

2002) where 1g per 100 ml of dried Roselle cultivar F141 (Taiwan) was extracted for 3 minutes by boiling. The mean initial content of total anthocyanins (TA) was 42 mg D3S/L Roselle extract (approximately 8.61 mg D3S/g Roselle). These values compared with results from the Mgaya - Kilima et al. (2015) study, 32.9 and 48.0 mg C3G/L, which were obtained by extracting Roselle (from Tanzania) in water (1 g in 10 ml) at 50°C for 30 min. For both TP and TA, variations in initial content are expected due to raw material species and sources, extraction time and temperature (Ramirez-Rodrigues, Balaban, Marshall, & Rouseff, 2011). Two major anthocyanins were found in the Roselle extracts (Figure 3.1), which is similar to findings by Ramirez-Rodrigues et al. (2011). Chromatographs before and after pasturisation were similar in profile for the anthocyanins. These anthocyanins were identified as delphinidin 3-sambubioside, D3S (R_T = 5.861 minutes) and cyanidin 3-sambubioside, C3S (R_T = 6.835 minutes) by comparing with standards (Figure 3.1).

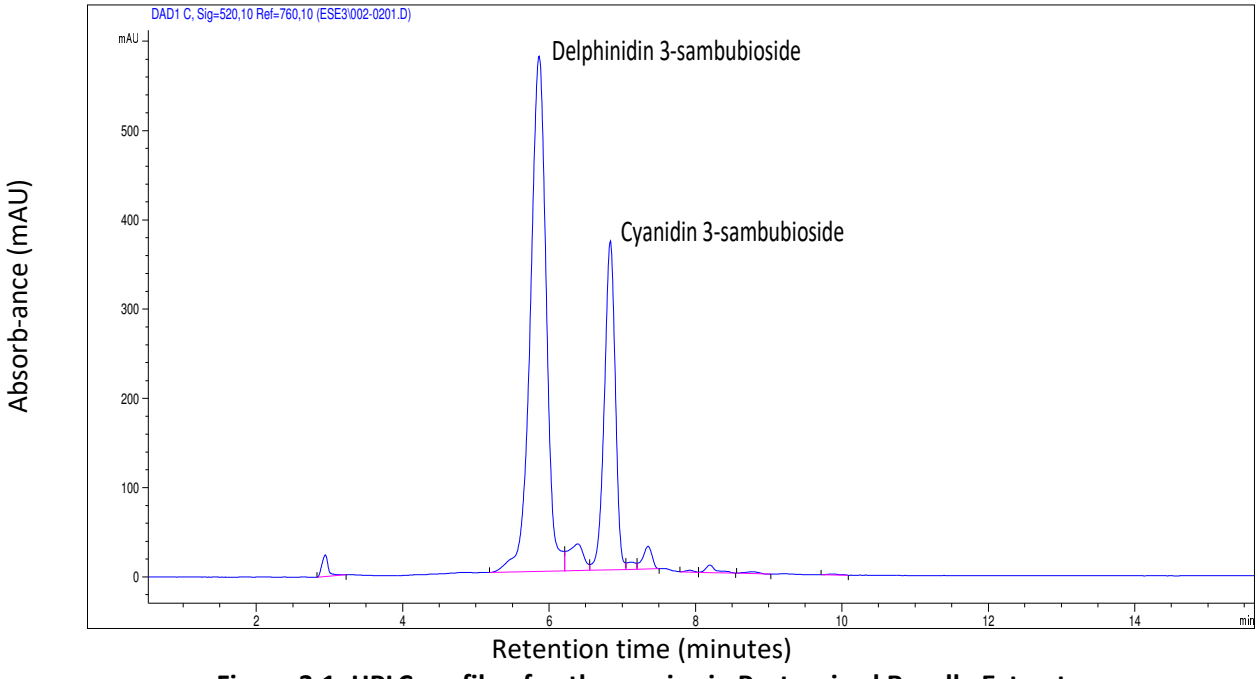


Figure 3.1: HPLC profile of anthocyanins in Pasteurised Roselle Extract

The initial concentration for D3S and C3S were 65 mg/L (*SD 0.002*) and 14 mg/L (*SD 0.000*) respectively. The ratio of D3S to C3S was 82:18, which is similar to the 85:15 ratio obtained

by Tsai et al. (2002) but varies in comparison to Bridle and Timberlake (1997) ratio: 71:29. Differences in Roselle varieties and processing conditions between studies may account for the variations.

Prior to pasteurisation, the Roselle extract showed a mild (32%) ACEi activity (Table 3.1). Thus, ACEi% was measured for the major Roselle anthocyanins. D3S showed a stronger mean ACEi% (26% $\pm$ 1%) compared to C3S (20% $\pm$ 9%) at 1 mg/mL. As the quantified concentration of anthocyanins in the Roselle extract is in much smaller amounts than 1 mg/mL, this raises some interesting questions as to whether the anthocyanins are solely responsible for ACEi in the Roselle extracts or whether these anthocyanins and other phenolic acids act synergistically. These results differ from the strong ACEi effect measured by Ojeda et al. (2010) but the differences in actual values of ACEi values may stem partly from differences in methodologies employed between the studies (Henda et al., 2013).

140 g AAE/L (approximately 28 mg AAE/g Roselle) was measured as the antioxidant capacity (FRAP) in Roselle extract. This value is 14 – 59% as active as the FRAP antioxidant activities of thirteen varieties of green tea studied Benzie and Szeto (1999). Tsai et al. (2002) observed that the antioxidant capacity of Roselle was 16 – 25% as active as the antioxidant capacities of the three varieties of green tea measured in their study.

Overall, under the processing conditions used in this study, pasteurisation did not cause significant degradation of the bioactive content of Roselle extract. Similarly, after pasteurisation, the hue angle and chroma did not vary significantly indicating that the colour of the Roselle extract was still intact. This outcome differs from some other studies. For instance, Cisse, Vaillant, et al. (2012), who used pasteurisation conditions 100^{o}C for 5 minutes, demonstrated detrimental effects on the beneficial physiochemical compounds and phenolic compounds in Roselle extracts (1:15 calyx to water). Upon pasteurisation of

an extract produced under more intense conditions (100°C for 30 minutes), a 7% loss in anthocyanin; 8% loss in colour density and 10% loss in colour strength was observed. However, although Perez-Ramirez et al. (2015) also recorded a 6-7% loss in anthocyanins due to pasteurisation (95°C for 15 minutes), no losses in TA or antioxidant capacity was observed, for a Roselle extract produced at 95°C for 60 minutes. In both studies, extraction and pasteurisation temperatures were more stringent than this present study. In this present study, pasteurisation temperature was less than extraction temperature. This may account for the minimal post-pasteurisation alterations to the properties of the extract.

3.4.2 Effect of storage time and temperature

3.4.2.1 Total phenolic content (TP)

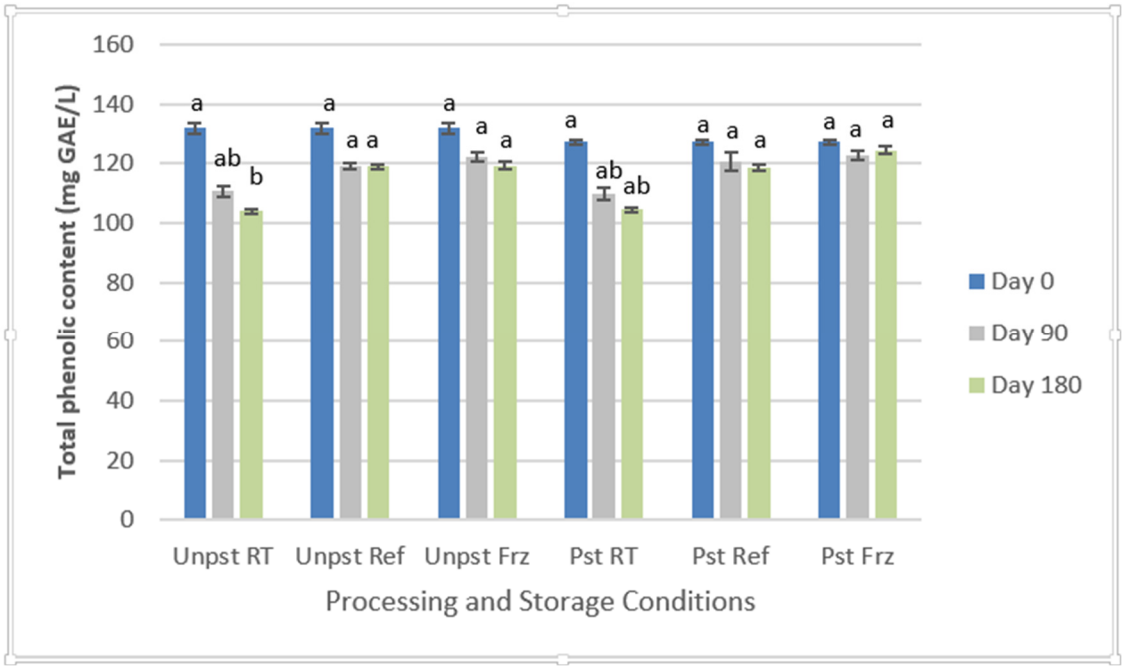


Figure 3.2: Total phenolic (mg Gallic acid equivalent/L) profile of Roselle extract during 180 days storage. Each bar/point represents mean values ± SD. Results shown for 3 time points (Days 0, 90 and 180) for the following samples: Unpasteurised extract stored at room temperature 21°C (Unpst RT), Unpasteurised extract stored refrigerated 4°C (Unpst Ref), Unpasteurised extract stored frozen -21°C (Unpst Frz), Pasteurised extract stored at room temperature 21°C (Pst RT), Pasteurised extract stored refrigerated 4°C (Pst Ref) and Pasteurised extract stored frozen -21°C (Pst Frz). Letters (superscripts) a – b indicate significant difference across individual properties.

Comparing initial values (Day 0) with final results over a six-months period (Day 180), the storage time did not significantly (P = 0.295) affect the total phenolic content (TP). Even under room temperature storage, although there was a significant difference between day 0 and day 180 samples for unpasteurised extracts, the losses in TP were relatively small (21%, Figure 3.2). These losses could be due to the anthocyanin degradation which is later discussed in this chapter. On the other hand, Day 0 and Day 180 extracts were not significantly difference in TP, for room temperature stored pasteurised samples. Room temperature stored extracts displayed an extensive shelf life with a half-life of up to 577 days for both unpasteurised and pasteurised extracts and coefficient of determination (R^2) of 0.8915 and 0.8957 respectively. The rate constant was 0.0012 day^{-1} in both cases. The effect of storage temperature was also significant ($P < 0.05$).

As expected, there was a significant effect (p = 0.022) of storage temperature. Improved shelf life under colder conditions compared with room temperature was observed. Combining pasteurisation and storage temperature conditions, no significant effect was observed. However, maximum mean percentage loss of TP for unpasteurised and pasteurised extracts stored for 180 days were 21 and 16% under room temperature storage; 14 and 11% under refrigerated storage and 11 and 4% under frozen storage, which show slightly improved retention of TP content for pasteurised extracts.

While this is a favourable result for any beverage, it may be deceptive as a measure of overall quality. The hydroxyl group on phenolic compounds make them highly reactive, therefore changes are expected during storage which is further explored in measurements of total anthocyanin content and total antioxidant capacity (FRAP).

3.4.2.2 Total Anthocyanin content (TA)

During the 180 day storage, significant effects due to storage temperature and storage time were observed, but there were no significant differences between pasteurised and non-pasteurised extracts (Figure 3.3). Minimal losses of TA were incurred in extracts stored at 5°C and -21°C. However, extracts stored at room temperature (21°C) lost more than half the initial value in 180 days. During the 180 day storage, significant effects due to storage temperature and storage time were observed, but there were no significant differences between pasteurised and non-pasteurised extracts (Figure 3.3).

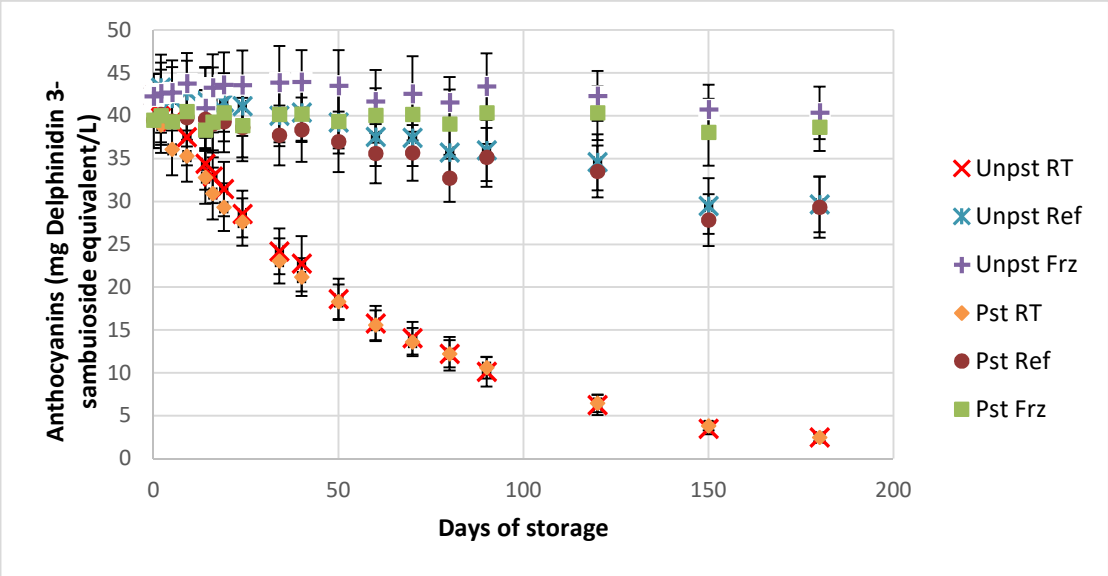


Figure 3.3: Monomeric anthocyanin (mg Delphinidin 3-sambubioside equivalent/L) profile of Roselle extract during 180 days storage. Each point represents mean values ± SD. Extracts analysed at different time points were: Unpasteurised extract stored at room temperature 21°C (Unpst RT), Unpasteurised extract stored refrigerated 4°C (Unpst Ref), Unpasteurised extract stored frozen -21°C (Unpst Frz), Pasteurised extract stored at room temperature 21°C (Pst RT), Pasteurised extract stored refrigerated 4°C (Pst Ref) and Pasteurised extract stored frozen -21°C (Pst Frz).

Minimal losses of TA were incurred in extracts stored at 5°C and -21°C. However, extracts stored at room temperature (21°C) lost more than half the initial value in 180 days. This agrees with previous studies on anthocyanin losses in Roselle extracts and other plant

foods (Andersen, Jordheim, Wallace, & Giusti, 2013; Cisse, Bohuon, et al., 2012). For unpasteurised and pasteurised extracts stored at room temperature, kinetics for the degradation of anthocyanins followed first order reaction model with R^2 of 0.9988 (*SD 0.001*) and 0.999 (*SD 0.001*) respectively, indicating a very good fit. The degradation rate of reactions for anthocyanins unpasteurised (0.016 $\pm$ 0.002 day^{-1}) and pasteurised (0.015 $\pm$ 0.002 day^{-1}) extracts were not significantly different.

Due to differences in extraction, pasteurisation and storage conditions, exact comparisons of reaction rates are difficult. However, the closest set of results could be observed in the study by Cisse, Vaillant, et al. (2012) which revealed an anthocyanin degradation rate of 0.008 day^{-1} (actual value: 9.83 x 10^{-8} s^{-1}) for the product of extraction and storage at of 30^{o}C for 240 minutes and a 20^{o}C for 182 days respectively. Under similar storage conditions, the product of the extraction at 100^{o}C for 30 minutes had an anthocyanin degradation rate of 0.013 day^{-1} (actual value: 13.89 x 10-^{8} s^{-1}). The extraction conditions of this present study, are quite similar to this latter set of conditions, hence the similarities in degradation rates, allowing for differences in raw materials and production methodologies. The half-lifes of anthocyanin were 43 (*SD 5*) and 45 (*SD 4*) days for unpasteurised and pasteurised extracts respectively, stored under room temperature conditions.

Anthocyanin degradation during storage could be due to oxidative degeneration which refers to the most reactive compounds being preferably oxidised than more stable scission phenolic compounds. The products formed from anthocyanin degradation could be phenolic acids (such as hydrobenzoic acid) and aldehydes (Patras, Brunton, O'Donnell, & Tiwari, 2010). Furthermore, Sinela et al. (2017) specifically identified gallic acid and protocatechuic acid as degradation products. The mechanism of degradation is supported by the results obtained in this study, where a minimal decrease of total phenolic content

(Figure 3.2) were observed simultaneously with important losses of anthocyanins (Figure 3.3).

Reduction in anthocyanin content separately measured by HPLC and pH differential methods were in agreement (Table 3.2). This supports the review findings by Lee, Rennaker, and Wrolstad (2008) on the good correlation (R = 0.931) between anthocyanin measurements (with HPLC and pH differential method), for 4 different studies.

Table 3.2: Mean anthocyanins losses (%) at Day 180 from day 0 measured with pH differential method and HPLC

Processing & storage conditions	Mean average % losses of monomeric anthocyanins - pH differential method ± SD	Mean overall losses (%) of anthocyanin measured by HPLC ± SD	Mean average % losses of D3S mg/ml at Day 180 of storage ± SD	Mean average % losses of C3S mg/ml at Day 180 of storage ± SD
Unpst RT (21°C)	95 ± 2	94 ± 2	95 ± 0	91 ± 0
Unpst Ref (4°C)	30 ± 9	30 ± 12	30 ± 1	22 ± 0
Unpst Frz (-21°C)	5 ± 7	3 ± 3	0 ± 1	0 ± 0
Pst RT (21°C)	94 ± 1	94 ± 1	96 ± 0	90 ± 0
Pst Ref (4°C)	26 ± 8	31 ± 7	31 ± 1	24 ± 0
Pst Frz (-21°C)	2 ± 3	6 ± 5	3 ± 1	3 ± 0

Table 3.2 - Each point represents mean values + SD. Extracts analysed at day 0 and day 180 and the percentage losses expressed: Unpasteurised extract stored at room temperature 21°C (Unpst RT 21°C), Unpasteurised extract stored refrigerated 4°C (Unpst Ref 4°C), Unpasteurised extract stored frozen -21°C (Unpst Frz -21°C), Pasteurised extract stored at room temperature 21°C (Pst RT 21°C), Pasteurised extract stored refrigerated 4°C (Pst Ref 4°C) and Pasteurised extract stored frozen -21°C (Pst Frz -21°C)

With pH differential method, up to 95% loss of anthocyanin (D3S equivalent) was observed over the 180 days of storage. D3S and C3S measured and quantified by HPLC at the end of the storage period also showed significant losses of similar proportions to losses of total anthocyanin (Table 3.2). Moreover, with both methods, minimal losses were observed with freezing (less than 10%) and medium losses (about 30%) with refrigeration over the 180 days of storage. Percentage losses appeared slightly higher for D3S than for C3S.

3.4.2.3 Antioxidant Capacity

There were significant losses in antioxidant capacity (FRAP) during the 180 day storage as shown in Figure 3.4 which was influenced by storage temperature and time. Antioxidants as free radical scavengers are highly reactive with other compounds including phenolic acids (Howard, Prior, Liyanage, & Lay, 2012; Skrede, Wrolstad, & Durst, 2000; Tiwari, Brunton, & Brennan, 2013). In all storage conditions, antioxidant losses were observed, although the antioxidant capacity decreased more rapidly at higher temperatures. This is as expected from previous studies on plant juices/extract (Andersen et al., 2013). In addition, the observed losses also appear to be particularly substantial before day 60 and stabilise thereafter, which indicates a non-linear degradation path during storage.

Whilst the antioxidant capacity of Roselle extracts may be largely connected to Roselle anthocyanins, other phenolic compounds contained in the extract also provide antioxidant effects (Tsai et al., 2002). Comparing antioxidant values with anthocyanin values which are also non-linear with respect to time, there appears to be some correlation. A plot of FRAP against anthocyanin capacity gives a coefficient of determination (R^2) values of 0.8668 and 0.8659 for unpasteurised and pasteurised extracts respectively, which are quite similar to Tsai et al. (2002) $R^2 = 0.8375$ for a plot of FRAP against Anthocyanin content measured at 520 nm. These values are indicative of only a partial dependence of FRAP antioxidant capacity on anthocyanin concentration.

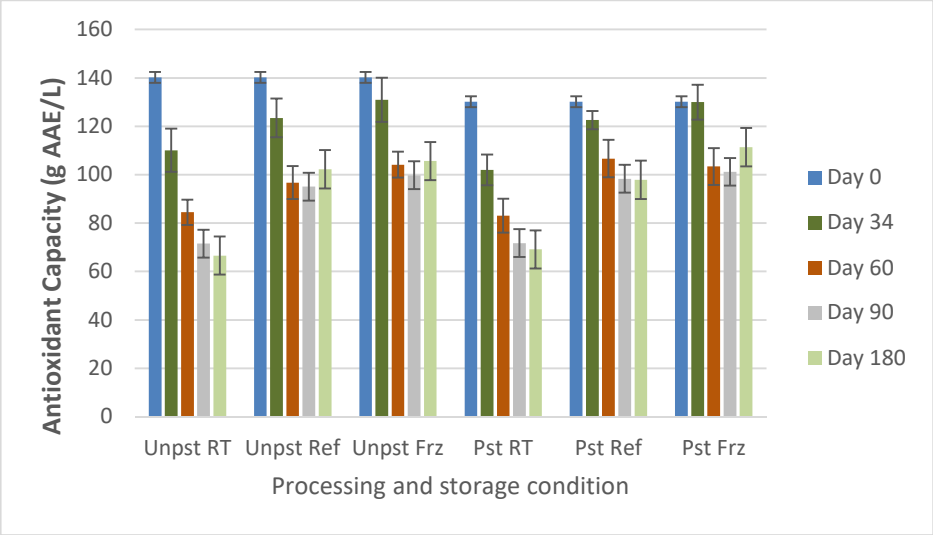


Figure 3.4: Antioxidant capacity, FRAP (g Ascorbic acid equivalent/L) profile of Roselle extract during 180 days storage. Each bar/point represents mean values ± SD. Extracts analysed at various time points but only 5 time points shown (Days 0, 34, 60, 90 and 180) for the following samples: Unpasteurised extract stored at room temperature 21°C (Unpst RT), Unpasteurised extract stored refrigerated 4°C (Unpst Ref), Unpasteurised extract stored frozen -21°C (Unpst Frz), Pasteurised extract stored at room temperature 21°C (Pst RT), Pasteurised extract stored refrigerated 4°C (Pst Ref) and Pasteurised extract stored frozen -21°C (Pst Frz).

This correlates with Tsai et al. (2002) findings on 51% influence of anthocyanin on FRAP activity, with 24% of remaining activity attributed to other phenolic compounds. The formation of gallic acid and protocatechuic acid as anthocyanin degradation product in Roselle extracts may be largely responsible for antioxidant stability observed after 60 days. Whilst gallic acid in low concentrations may be pro-oxidant, in higher concentration, it is a strong antioxidant due to its scavenging effect on the hydroxyl radical (Badhani, Sharma, & Kakkar, 2015; Yen, Duh, & Tsai, 2002). Although less efficient than gallic acid, protocatechuic acid has also been reported to have strong antioxidant properties (Brand-Williams, Cuvelier, & Berset, 1995; Tseng et al., 1998). In the hierarchy of antioxidant activity (trolox) of polyphenols, delphinidin and cyanidin compounds were rated higher than gallic acid (Rice-evans, Miller, Bolwell, Bramley, & Pridham, 1995). The above reported observations may explain the initial (up to 60 days) rapid decrease in antioxidant capacity observed in Figure 3.4. As anthocyanin levels decreased, degradation products which are

antioxidant and more storage stable were formed, thus counteracting reductions in FRAP (antioxidant) capacity due to the anthocyanin decrease. This explains the smaller changes in antioxidant capacity observed after 60 days (Figure 3.4). Furthermore, after 180 days at room temperature storage, pasteurised extract had a 94% reduction in anthocyanins (Table 3.2), whereas FRAP (antioxidant) capacity decreased only by about 50% (Figure 3.4).

In summary, given the data observed here on changes in anthocyanins, polyphenols and antioxidant capacity over time, which are in agreement with previous studies, it can be concluded that initially anthocyanins were partly responsible for the FRAP antioxidant property of the extract but subsequently, anthocyanin degradation products contributed to the antioxidant properties of the extracts, which explains the improved stability after day 60 for all storage conditions. For extracts stored at room temperature, the rate of reaction of antioxidant degradation was 0.0038 and 0.0037 day^{-1}; half-life was 182 and 187 days; R^2 was 0.753 and 0.757 for unpasteurised and pasteurised extracts respectively.

Over the 180 day storage period of unpasteurised and pasteurised extracts respectively, mean antioxidant losses (in percent) were 52 and 38% for room temperature storage; 36 and 31% for refrigerated and 27 and 17% for frozen.

Roselle extracts stored in refrigerated and frozen conditions for 180 days were colour stable as chroma and hue angle were constant throughout storage (Figure 3.5). However, storage at room temperature showed a consistent change in the colour of Roselle extracts as there was a decrease in chroma and increase in hue angle over the 180 day storage. Over time, these extracts became lighter (increasing L), less red (decreasing a) and more yellow (increasing b) concurrently. Hue angles appeared slightly more stable for pasteurised extracts which is an indication that pasteurisation favoured colour retention in stored extracts.

3.4.2.4 Colour

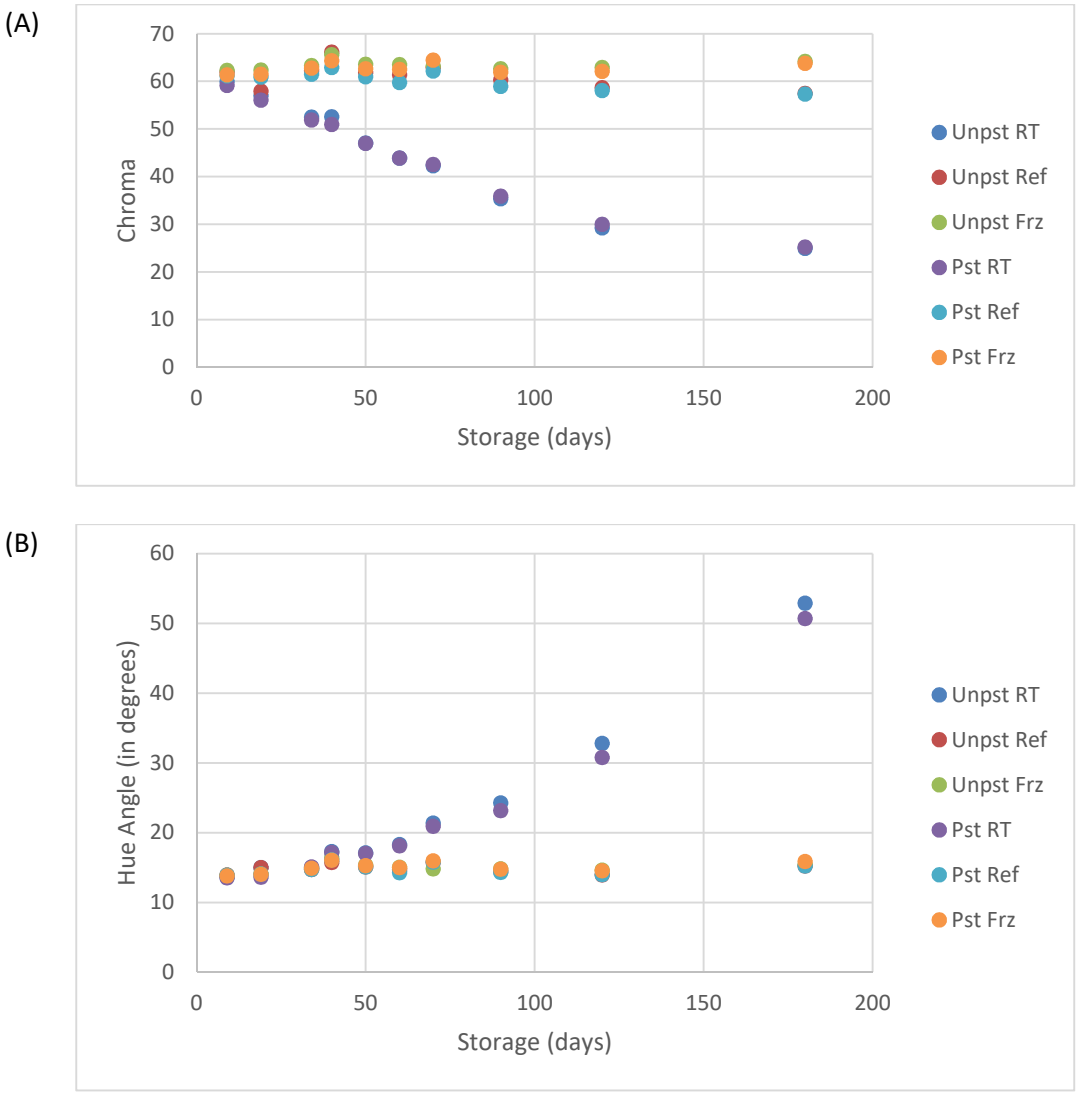


Figure 3.5: Colour profile in Roselle extract stored for 180 days (A) Chroma and (B) hue angle. Each point represents mean values. Extracts analysed at different time points were: Unpasteurised extract stored at room temperature 21°C (Unpst RT), Unpasteurised extract stored refrigerated 4°C (Unpst Ref), Unpasteurised extract stored frozen -21°C (Unpst Frz), Pasteurised extract stored at room temperature 21°C (Pst RT), Pasteurised extract stored refrigerated 4°C (Pst Ref) and Pasteurised extract stored frozen -21°C (Pst Frz)

As Roselle colour has been ascribed to its anthocyanins (Bridle & Timberlake, 1997), it is expected that anthocyanin measurements are positively correlated with hue angle and chroma attributes. For extracts stored at room temperature, chroma showed a strong correlation with anthocyanins (R^2 = 0.9328, 0.9376 for unpasteurised and pasteurised

respectively) but hue angle a weak correlation with anthocyanins (R^2 = 0.5885, 0.6112 for unpasteurised and pasteurised respectively).

3.4.2.5 pH

There was no significant difference in pH after 180 days storage. On day 180, pasteurised extracts had a mean pH of 2.81 + 0.01 across the different strage temperatures which were slightly lower than the pH values of unpasteurised extracts (2.85 + 0.01). This trend is similar to what was observed with the initial pH values on Day 0 of the study.

3.4.2.6 ACE inhibition

ACEi% was initially calculated at 31.8% and 34.3% for unpasteurised and pasteurised extracts from this study, respectively. Further storage of up to 180 days was conducted for both pasteurised and unpasteurised extracts under room temperature, refrigeration and freezing conditions and ACE inhibition measured on day 180. The reduction of ACEi (in percent) in relation to the initial ACEi is shown in Table 3.3.

Table 3.3: Losses in ACE inhition of extracts stored for 180 days

Storage condition	**Loss in ACEi from Day 0 (%) ± SD**	
	Unpasteurised	**Pasteurised**
Room Temperature (21°C)	99.3 ± 23.6	58.5 ± 12.3
Refrigeration (4°C)	60.7 ± 36.6	25.2 ± 2.6
Freezing (-21°C)	38.4 ± 7.4	18.5 ± 11.3

Mean values of Day 0 and Day 180 used to calculate percentage loss of ACEi. Table 3 - ACEi refers to Angiotensin-I-Converting Enzyme. Results are calculated in percentage losses of ACEi based on mean values (± SD) for Day 0 and Day 180 storage of Roselle extract made from steeping Roselle 5g Roselle in a litre of water at 90°C for 25 minutes. Pasteurised extracts further heated in-bottle 85°C for 20 minutes

After 180 days storage, ACEi% reduced under all conditions. The reduction pattern was dissimilar from reductions in anthocyanin content (Table 3.2) as higher percentage reductions in ACEi activity were observed compared to percent reductions in anthocyanin

content, especially with unpastuerised extracts. Interestingly, pasteurised and unpasteurised extracts showed significant difference where anthocyanin contents were similar. Pasteurised extracts demonstrated higher ACE inhibition than unpasteurised extracts even at RT. These demonstrate that degradation of anthocyanins was only partially responsible for the reduction in ACEi% as small changes in anthocyanin content at freezing temperature led to important changes in ACEi%, particularly for unpastuerised extracts.

3.5 Conclusion

In this study, the effects of pasteurisation and storage temperature on the bioactive properties; polyphenolic content, antioxidant capacity and ACE inhibition, and physical properties; colour and pH, of a Roselle extract were investigated. For the first 2 months of storage at room temperature, anthocyanin degradation led to decreases in FRAP antioxidant capacity, ACEi and colour hue and chroma. However, the formation of degradation products helped stabilise FRAP (antioxidant) activity and maintain total phenolic content throughout the 180 days. Furthermore, while pasteurisation had no significant effect on the initial physical properties and bioactivities measured in this study, it led to an enhanced retention of ACEi activity over a 180 day storage period.

During the storage period, effects of storage time and temperature were observed on the properties of the extract. Room temperature storage expedited decreases in anthocyanins, FRAP antioxidant capacity, ACEi% and colour. Whereas, at refrigerated and freezing temperatures, changes were minimal. Interestingly, total phenolics did not decrease significantly throughout the 180 days of storage and under all storage conditions possibly due to the phenolic compounds formed as degradation products of anthocyanins.

Total phenolic content and pH were relatively stable throughout the duration of storage. On the other hand, the properties most prone to degradation, such as anthocyanins where strongly correlated with only colour chroma. FRAP antioxidants, colour hue angle and ACEi were only partially related with anthocyanins. These properties were hypothesised to be also partially influenced by other polyphenols in the Roselle extract especially the anthocyanin degradation products. These relationships may give insights into the mechanisms of degradation of polyphenolic compounds in Roselle extracts and need to be further investigated.

The information presented in this study is important to beverage producers as it demonstrates that simple processing methods as used in this study may still yield good quality products. Thus with respect to the physical and bioactive properties of Roselle products, pasteurisation treatment followed by refrigeration or freezing is ideal. For refrigerated Roselle products, a shelf life of up to 60 days without a significant loss in quality is achievable, but 45 days is advised. Changes expected during this period are with the antioxidant (FRAP) and ACE inhibitory activity. A better understanding of the mechanisms of degradation of both activities in Roselle extracts is recommended for future studies.

Chapter 4 – Sugar and Stevia Rebaudioside A Sweetened Hibiscus Sabdariffa (Roselle) Beverages – Sensory Profiles, Consumer Preference and Potential Masking with Spice Flavours

Abstract

The increasing popularity of *Hibiscus sabdariffa* (Roselle) beverages has led to numerous studies on its sensory and functional properties. The focus has been mostly on the latter while several gaps remain with the former. In response to these gaps, this study presents a lexicon for Roselle extracts and investigates the effect of sweetening with sugar (sucrose) and a sugar alternative, Stevia Rebaudioside A (SRA, 80% purity), on consumer liking of the beverage. Furthermore, the masking potential of natural spice flavours (cinnamon, cloves and ginger) on the off-flavours of SRA within the beverage were investigated. A commercial air-dried Roselle was compared to fresh Roselle dried by two different methods (tray or freeze-drying), and all samples were aqueously extracted and pasteurised. The lexicon was developed by a trained panel, and the extracts were profiled using quantitative descriptive analysis. The lexicon was modified for rating the sweetened beverages, succeeded by a consumer study with 141 healthy participants. Consumer liking for the sugar-sweetened (80 g/L) beverages was significantly ($p < 0.05$) higher than for SRA-sweetened (0.32 g/L) beverages and the addition of spices did not improve consumer liking of the beverages. Consumer liking was associated with emotive language whereby words indicative of negative emotions were associated with a dislike of the beverage and the inclusion of clove flavour. The inclusion of cinnamon and ginger displayed a congruency with the flavour profile of Roselle extracts leading to positive emotion associations and higher liking scores.

4.1 Introduction

Hibiscus Sabdariffa (also known as Roselle, Karkade, Bissap, Zobo, Jamaica, Sorrel) is a versatile food ingredient grown mostly in tropical regions. Health benefits attributed to Roselle, in vitro and in vivo, include antidiabetic, antioxidant, antihypertensive and hypocholesterolemic effects. Gruenwald (2009) classified it as a green botanical ingredient, which may be used in functional beverages and may require masking of its taste. Sometimes called "sour tea", Roselle has been described in relevant literature as cranberry-like (D' Heureux‐Calix & Badrie, 2004), acidic in flavour and distinctively floral, berry-like in aroma (Monteiro et al., 2016). In the last decade, numerous studies have been carried out on the health benefits (in vitro and in vivo) of Roselle extracts; physical, chemical and microbiological properties of Roselle infusions/extracts; optimum processing, storage and packaging condition for stability of phenolic content and the sweetening of Roselle beverages.

However, only a few studies exist on the sensory properties of Roselle beverages. A recent study on the consumer liking for Roselle beverages was carried out by Monteiro et al. (2016) which showed higher consumer preference for the unsweetened beverage subjected to a novel under-vacuum pre-treatment (62^{o} Brix) compared to sweetened ready to drink syrup (65^{o} Brix) and ready-to-drink infusions (15^{o} and 17^{o} Brix). For consumers, the unique attributes of the preferred beverage included a more intense red colour, sweet taste; fruity, honey, hibiscus odour and hibiscus taste, which set it apart from other samples that were described as more astringent, acidic and bitter. Other attributes unveiled by the study

include cranberry-like and aronia-like odours. Some additional attributes such as acidity, irritant and fermented taste were revealed in a previous study (Bechoff et al., 2014).

A much earlier study by Chen, Huang, Ho, and Tsai (1998) centred on the volatile compounds of unprocessed and processed Roselle calyx (frozen and dried at 50^{o}C and 75^{o}C), which highlighted differences in volatile content upon processing. There has not been a follow up study to determine whether these differences in volatiles might affect the sensory perception of Roselle extract. Several other studies have identified phenolic compounds and anthocyanins of Roselle extracts, which include Hibiscus acid, gallic acid, protocatechuic acid, quercetin, sambubiosides and glucosides (Obouayeba et al., 2014; Ramirez‐Rodrigues, Plaza, Azeredo, Balaban, & Marshall, 2011; Sinela et al., 2017), and which have been shown to clearly influence sensory properties of Roselle beverage. Various studies have proceeded to demonstrate an effect of processing conditions such as drying, extraction and preservation, on the phenolic compositions, bioactive and physical properties of Roselle extract. However, only a very recent study has explored the effects of simple processing conditions on the sensory quality of the Roselle extracts by profiling hibiscus infusions and teas along with other Roselle beverages (Monteiro et al., 2017).

As observed in studies by Aurelio et al. (2008) and Monteiro et al. (2016), sugar (sucrose) is the foremost sweetener for Roselle beverages in the current market. Nonetheless, reducing sugar in beverages has become a worldwide priority due to its calorific load and link to poor health such as obesity, type 2 diabetes and cardiovascular diseases (Malik et al., 2014). Currently, Steviol glycosides are the only EU approved clean label alternative to sugar. Stevia Rebaudioside A (SRA), the most popular of the steviol glycosides, particularly in beverage products, provides better solubility and consumer liking compared to

stevioside but still lags in consumer acceptance compared to sugar (Carakostas, Prakash, Kinghorn, Wu, & Soejarto, 2012; Lemus-Mondaca, Vega-Gálvez, Zura-Bravo, & Ah-Hen, 2012; Lindley, 2008).

Off-notes in sweeteners and indeed stevia products may be masked using other sweeteners, bulking agents, flavouring or flavour enhancers (Ley, 2008). Thus, select herbs and spices, many of which are generally recognised as safe (GRAS), may offer flavour-enhancing solutions for SRA although there are limited studies in this area. Cinnamon (Cinnamomum zeylanicum nees), cloves (Syzygium aromaticum L) and ginger (Zingiber officinale Roscoe), selected for their frequency of use in 39 online Roselle beverages recipes (Table A1.1) used the current study. Cinnamon, ginger and cloves were suggested as spicy notes used individually or as mixtures in combination with fruity notes and vanillin, to mask astringency in pharmaceutical products (Abraham & Mathew, 2014). Ginger oil was used in combination with thaumatin and magnesium gluconate to mask a bitter pharmaceutical acetaminophen (Ley, 2008). Moreover, spices are rich in polyphenols, some of which have been demonstrated to modify taste properties. For instance, hydroxycinnamic acid (a metabolite of cinnamic acid found in cinnamon) displays bitter taste inhibition by competing with bitter compounds at taste receptor sites (Ley, 2008; Riemer, 1994).

In designing this study, several considerations have been made. Firstly, the potential of processing to alter the sensory profile of Roselle extract/beverage. Some form of drying of fresh Roselle is required to enable repeatable research work. Therefore, freeze-drying was employed to obtain samples closest to fresh (Krokida & Philippopoulos, 2006; Michalczyk, Macura, & Matuszak, 2009) and these are compared with tray-drying. During extraction, it is believed that the anthocyanins, responsible for the colour of Roselle extracts, are

affected by extraction conditions (Cisse, Vaillant, et al., 2012), therefore several extraction conditions are used. Furthermore, under refrigerated or frozen storage conditions, Roselle extract demonstrates good bioactivity and ideal physical and chemical properties for a beverage i.e. low pH, colour stability and high anthocyanin and antioxidant content (Chapter 3), therefore, the beverages are stored using both conditions.

Secondly, to create a realistic and more complex profile on which the impact on the liking of the beverages between familiar and unfamiliar consumers was assessed, juice flavours (lemon and pineapple) were also added to the formulations. These were selected for their particular use in Roselle beverages and ready availability in the UK. Whilst pineapple juice was merely used as a typical congruent juice flavour, lemon juice may augment mouthfeel in beverages sweetened with intense sweeteners (Kappes, Schmidt, & Lee, 2006).

Finally, consumer choices are thought to be driven by the emotional context of products. Indeed authors have argued that the emotional context may drive liking, and that sensory characteristics can drive the emotional context (Gutjar et al., 2015; King & Meiselman, 2010; Ng, Chaya, & Hort, 2013). Some studies have linked familiarity with a food to higher liking (Prescott, Young, Zhang, & Cummings, 2004). Consequently, as Roselle beverages are quite uncommon in the UK and may, therefore, be unfamiliar to the majority of UK consumers, this study (conducted in the UK) also considers the emotional profile as well as the effect on the liking of the Roselle beverage by both familiar and unfamiliar consumers.

No sensory lexicon for Roselle extracts was available until quite recently (Monteiro et al., 2017), therefore a lexicon had to be developed earlier on at the start of this current study. This demonstrates the novelty of this area of research. This study is also one of earlier studies to explore the effects of simple heat treatment on the sensory profile of Roselle

extracts. Furthermore, few studies have explored the use of Stevia Rebaudioside A (SRA) in clean label beverages and more so in Roselle beverages. Thus, starting with a basic profiling of Roselle extracts (unsweetened), the study progresses to profiling sweetened beverages (sugar and SRA) and concludes with a consumer liking study. The consumer liking study will transcend describing the effect of sweeteners and flavours on consumer liking, to investigate potential drivers of consumer liking.

4.2 Methodology

4.2.1 Sample Preparation

4.2.1.1 Roselle Extract

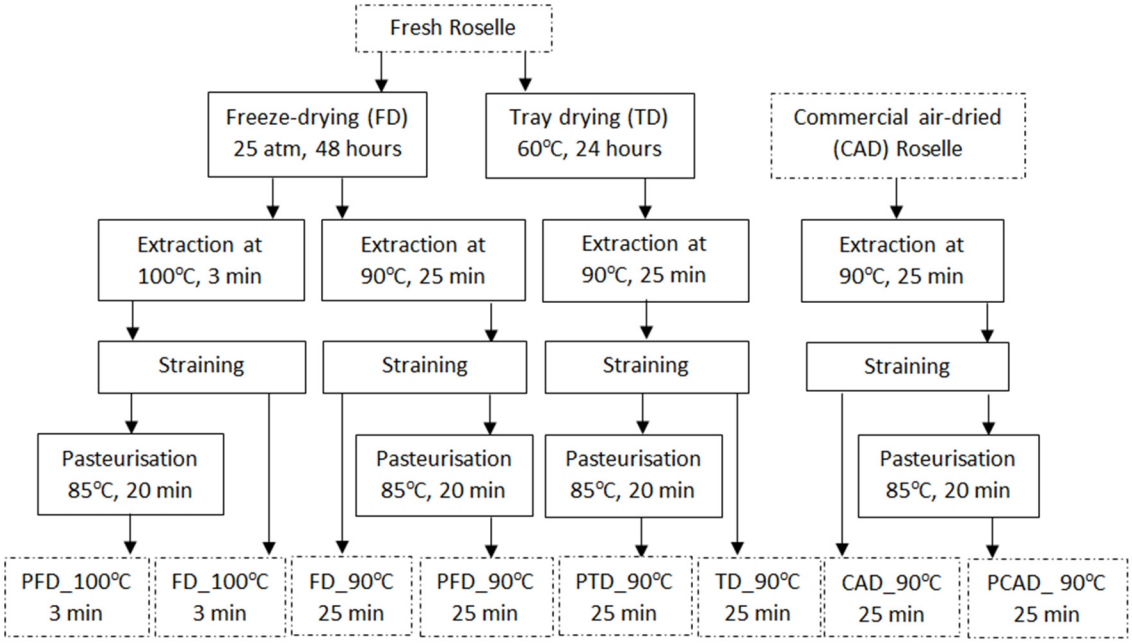


Figure 4.1: Roselle extracts production process

Commercial air-dried Roselle (Just Ingredients, UK; Country of Origin: Nigeria) and Fresh Roselle procured from the town centre market in Reading (Country of origin: Jamaica) were used for this study. The latter was washed and air-dried for 5 minutes before further drying

using either a tray drier (60^{o}C for 24 hours) or freeze drier (48 hours, vacuum pressure 25 Atm). On completion of drying, tray dried samples (TD) achieved an average moisture content of 1.76 $\pm$ 0.07%, while freeze-dried (FD) samples had a moisture content of 5.62 $\pm$ 0.40%. The commercial air-dried (CAD) Roselle had an average moisture content of 7.82 $\pm$ 0.23%.

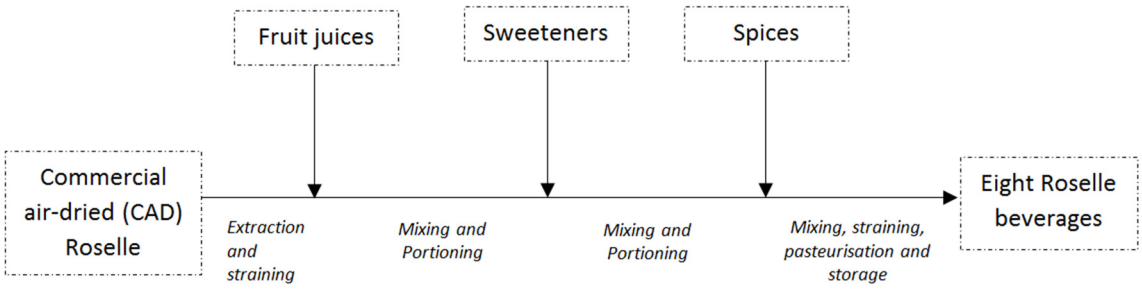
FD Roselle (5 g) was stirred into 1L boiling water (100^{o}C) for 3 minutes. Another batch of FD Roselle was mixed in hot water (5 g Roselle per Litre water) for 25 minutes and the temperature maintained at 90^{o}C. Similarly, TD and CAD Roselle (5 g/L each) were extracted separately in hot water (maintained at 90^{o}C) for 25 minutes. All Roselle-water mixtures were strained (stainless steel strainer) to obtain the extract, which was hot filled into swing top glass bottles (Wilkinson, UK) and immediately cooled on ice. Half the volume of each sample was further pasteurised at 85^{o}C for 20 minutes and immediately refrigerated (4^{o}C). A diagrammatic representation of the extract preparation process and the resulting 8 products are shown in Figure 4.1.

It should be noted that the commercially air-dried Roselle used in this study originated from a different country and apart from the method of drying, the specific details of drying conditions could not be established. Therefore it has been included in the profiling study as a reference.

4.2.1.2 Roselle Beverage

An aqueous extract of commercially air-dried Roselle (Just Ingredients, UK) was obtained by steeping Roselle calyx in water contained in a jacketed vessel set to 90^{o}C for 25 minutes (Chapter 3). After sieving, pineapple and lemon juices (40 mL/L and 10 mL/L respectively; Cobell, UK) were added to the extract and divided into two portions. The juices were

included to model a more complex beverage profile as may be found in the market. Each portion was independently sweetened with sugar (80 g/L) or Stevia Reb A (0.32 g/L; 80% purity - Cargill, UK) and further split into 4 portions which were flavoured with spices; 1 g/L cinnamon, 1 g/L ginger or 0.5 g/L cloves (ground, Just Ingredients, UK), with one portion left unflavoured (no spice). These were left refrigerated overnight (18 hours) and the spices were strained with a muslin cloth before being pasteurised in a high temperature short time pasteuriser (APV International Limited, UK) for 95°C, 3s then packed and stored frozen (-18°C) in sterile high-density polyethylene bottles (HDPE - Medfor, UK). The spices were included as flavouring and potential off flavour maskers. Figure 4.2 shows the production process resulting in eight beverages.



Extraction conditions	Sweetener quantities	Flavouring quantities	Pasteurisation & storage
90°C for 25 min	8% sugar equivalent	1 g/L cinnamon and ginger	95°C for 3s
	1:250 sugar to SRA	0.5 g/L cloves	-18°C storage

Figure 4.2: The Roselle beverage production process

4.2.2 Microbiological, Physical and Chemical tests

Bottled samples of the Roselle beverages were sent to Geneius Laboratories Ltd (Cramlington, UK) for food safety analysis. Consumer work commenced with satisfactory results (negative for pathogens and low counts for hygiene indicators). Colour was

measured using a CT-1100 ColorQUEST HunterLab as described in Chapter 3. An Orion star A111 pH meter (Thermo scientific, UK) was used for pH measurements which was calibrated using Orion application buffer solutions pH 4.01, pH 7.0 and pH 10.01 (Thermo Scientific, UK). The official AOAC 1984 method was used for titratable acidity (TA) with some modifications. 50 mL of extract was titrated undiluted with 0.1M NaOH (Fisher Scientific) until pH 8.2. TA was expressed as % malic acid.

4.2.3 Sensory methods

4.2.3.1 Sensory Profile of Roselle Extract

Quantitative Descriptive Analysis (QDA^{TM}) was used. With the aid of a facilitator and the use of necessary standards, a trained panel of 11 people developed a consensus of sensory descriptors for appearance, odour, taste, flavour, mouth-feel of Roselle beverage samples. These descriptors (Table A1.3, Appendix) were entered into Compusense cloud (Guelph, Ontario, Canada) for scoring which was carried out in air conditioned (23 $\pm$ 1^{o}C) sensory booths, under artificial daylight, in the Sensory Science Centre (Department of Food and Nutritional Sciences, University of Reading). Each assessor was provided with plain crackers and water (served at room temperature) to clean their palate between samples.

Eight extracts (Section 4.2.1.1) were randomly assigned 3-digit numbers. Extracts were freshly prepared on the day of the profiling session, refrigerated and served at room temperature. In three sessions and within one week, 4-6 samples per assessor were assessed to obtain results in duplicates. Extracts (50 mL) were presented to each assessor in a balanced order in clear 170 mL sniffer glasses. Assessors scored intensities of 4 appearance terms, 8 odour terms and, 5 taste terms, 9 flavour terms, 3 mouth-feel terms

and 6 after-effect terms on anchored unstructured line scale (Scale 1-100). Attributes, terms and anchor points are shown in Table A1.3.

4.2.3.2 Sensory Profile of Roselle Beverage

Separate profiling sessions were held for Roselle beverage, which followed similar protocols to Section 4.2.3.1. Eight Roselle sweetened beverages (henceforth referred to as RB) were produced as detailed in Section 4.2.1.2. They were prepared in advance, frozen, defrosted and refrigerated prior to the panel sessions. The 8 RBS were randomly assigned 3-digit numbers. Each panellist assessed four different samples per session. Four sessions were held on consecutive days to obtain duplicate results. Each assessor was presented with 100 mL of RBs in clear 350 mL glass cups, in a balanced order. Assessors scored intensities for 4 appearance terms, 12 odour terms and, 6 taste terms and 14 flavour terms, 6 mouth-feel terms and 10 after-effect terms on anchored unstructured line scale (Scale 1-100). Attributes, terms and their respective anchor points are shown in Table A1.4.

4.2.3.3 Consumer liking Testing

This study was given favourable option for conduct by the School of Chemistry, Food and Pharmacy Research Ethics Committee (Reference: 42/15) before participants were recruited. Participants (114) were untrained, 18 to 65, and resident in the Reading area. To encourage a balance between familiar and unfamiliar consumers, recruitment was targeted to include groups from countries of origin with familiarity to Roselle. Consent was obtained from all participants prior to participation in the study.

In the sensory booths, 50 mL RBs were presented to consumers monadically in transparent 200 mL plastic cups. A balanced design with random allocation of samples was used. Consumers were asked to score liking (overall, appearance, aroma and flavour) using a 9

point hedonic scale, anchored from dislike extremely to like extremely. To assess whether sweetness, flavour and astringency intensities were appropriate for each consumer "Just About Right scales" (JAR) scales were used; 5 point scales anchored from 1: Much too little to 5: Much too Much. To assess consumption and purchase intents, 5 point scales an anchored from 1: Definitely would not drink/purchase to 5: Definitely would drink/purchase. To evaluate the emotional contextualisation of the products "Check All That Apply" (CATA) questions were provided using the esSense Profile terms (King and Meiselman, 2010). The questionnaires ended with demographic questions and familiarity with Roselle. Questions, anchor point or answer choices are shown in Table A1.5.

4.2.4 Analysis

For profiling data, Analysis of Variance (ANOVA) and Principal Component Analysis (PCA) were carried out using SENPAQ (Version 3.2); where in the two-way ANOVA assessors were fitted as a random effect, samples as fixed effects and the main effects were tested against the sample by assessor interaction. For the consumer study, hedonic liking data was analysed using three-way ANOVA (XLSTAT version 2017.1) to determine the effect of sweetener (n = 2), spice type (n = 3), familiarity (n = 2), demographics (such as ethnicity or gender), consumption intent (n = 5) or purchase intent data (n = 5) on liking. Multiple pairwise comparisons were performed using Tukey's HSD at a significance level of $p \leq 0.05$. Principal Component Analysis (PCA) and Agglomerative Hierarchical Clustering (AHC; Ward's Agglomerative method; Euclidean distance dissimilarity) were also carried out using XLSTAT. AHC was carried out on consumer overall liking data and ANOVA was used to identify differences within clusters. Just About Right (JAR) data were analysed by penalty analysis and ANOVA using XLSTAT. ANOVA was also used for physical and chemical data, to

compare the properties of beverages (n=8). Tukeys (HSD) test was performed for simultaneous paired comparisons. Results with P<0.05 (95% confidence level) were considered significantly different.

4.3 Results and Discussion

4.3.1 Descriptive analysis

4.3.1.1 Roselle Extracts

Physical and chemical properties

As shown in Table 4.1, the low pH results demonstrate that all samples were acidic.

Table 4.1: Physical and chemical properties of the Roselle beverages used for sensory profiling

Sample	**pH**	**Colour hue (Hunter Lab)**			**Titratable acidity (% malic acid)**
		Lightness, L	Hue angle	Chroma	
FD_100°C,3min	3.01 ± 0.07^{d}	59.9±0.8^{c}	9.1±2.6^{a}	52.3±5.2^{ab}	0.09±0.00^{bc}
FD_90°C,25min	2.80 ± 0.03^{a}	51.2±1.1^{a}	14.5±1.2^{bcd}	62.5±0.6^{c}	0.10±0.01^{c}
TD_90°C,25min	2.88 ± 0.02^{bc}	57.6±1.5^{abc}	13.0±0.2^{abc}	56.0±1.8^{abc}	0.09±0.00^{bc}
CAD_90°C,25min	3.11 ± 0.06^{e}	56.6±2.4^{abc}	16.3±0.2^{cd}	48.8±2.8^{a}	0.07±0.00^{a}
PFD_100°C,3min	2.94 ± 0.02^{cd}	57.9±2.5^{abc}	11.3±0.3^{ab}	55.1±0.2^{abc}	0.09±0.00^{b}
PFD_90°C,25min	2.82 ± 0.01^{ab}	52.4±2.0^{a}	14.5±0.9^{bcd}	60.3±2.9^{bc}	0.09±0.00^{bc}
PTD_90°C,25min	2.88 ± 0.02^{bc}	58.4±0.6^{bc}	13.4±0.2^{bcd}	54.0±0.1^{abc}	0.09±0.00^{b}
PCAD_90°C,25min	3.09 ± 0.04^{e}	58.0±1.9^{abc}	17.3±0.4^{d}	46.8±1.0^{a}	0.07±0.00^{a}

This table shows eight extracts half of which have been pasteurised and the other half left unpasteurised. The extracts are as follows: Freeze dried Roselle extract processed for 3 min at 100°C (FD_100°C,3min); freeze-dried Roselle extract processed for 25 min at 90°C (FD_90°C,25min); Tray dried Roselle extract processed for 25 min at 90°C (TD_100°C,25min); Commercial air dried Roselle extract processed for 25 min at 90°C (CAD_90°C,25min); freeze-dried Roselle extract processed for 3 min at 100°C then pasteurised (PFD_100°C,3min); freeze dried Roselle extract processed for 25 min at 90°C then pasteurised (PFD_90°C,25min); tray dried Roselle extract processed for 25 min at 90°C then pasteurised (PTD_90oC,25min); and commercial air dried Roselle extract processed for 25 min at 90°C then pasteurised (CAD_90°C,25min). Letters (superscripts) a – e indicate significant difference across individual properties.

The acidity is due to the phenolic content consisting of up to organic acids including unique acids such as hibiscus acid and generic acids such as ascorbic, citric, maleic, gallic acid to

mention a few (Ali et al., 2005; Mahadevan et al., 2009; Sinela et al., 2017). The acidity of Roselle extract may be one of the reasons for the use of sweeteners.

There were significant differences in the acidity of Roselle extracts in this study depending on the processing conditions. The most acidic sample was FD_90^{o}C,25min and the least acidic samples were CAD_90^{o}C,25min and PCAD_90^{o}C,25min. This result suggests that the method of drying and extraction may have a minor effect the acidity of the Roselle extract. However, it is interesting to note that differences in acidity did not stem from the moisture content of the dried samples as TD had a lower moisture content (1.76 $\pm$ 0.07%) compared to FD (5.62 $\pm$ 0.40%) and CAD Roselle (7.82 $\pm$ 0.23%). With titratable acidity, these differences between the extracts were not as magnified. Indeed, only the CAD samples were found to significantly differ from the others.

Lightness (L), hue angle and chroma are shown in Table 4.1. In general, there were no significant differences between samples pre and post pasteurisation confirming findings in Chapter 3. However, between drying methods, FD_100^{o}C,3min significantly differs from FD_90^{o}C,25min and PFD_90^{o}C,25min. As expected FD_100^{o}C,3min is lightest compared to other samples because it is the least processed. However, when processed at 90^{o}C for 25 min, FD, TD and CAD Roselle extracts were similar in lightness before and after pasteurisation. Similarly, with this more intense processing, the redness intensity as indicated by the hue angle was more pronounced than in the less processed sample. The hue angles also indicate that CAD extracts possessed the deepest shade of red relative to other extracts studied. However, the low chroma for the CAD samples suggests a greyish tone or tendency towards discolouration, which is significantly less in the FD samples.

Roselle Extract sensory attributes

Assessors identified 35 descriptive terms (Table 4.2) where 20 of these descriptive terms were significantly different between samples ($P<0.05$; Table A1.6). The lexicon was very much similar to the lexicon developed by Monteiro et al. (2017) as the terminologies used were synonyms. Only a few terminologies differed vastly such as the spicy notes. In general, there were no significant differences observed between pasteurised and unpasteurised extracts. However, as shown in Figure 4.3, differences were observed between extracts from Roselle dried and processed under different conditions

Appearance: Descriptors used to describe the appearance of Roselle extracts are shown in Table 4.2. Roselle extracts were red in colour as indicated by the hue angle values when viewed on a Lab colour chart. In agreement with colorimeter results, the panel could not distinguish between pre and post-pasteurised extracts (Table A1.6) and there was no significant discrimination between the various drying methods. However, there were differences between the lightly processed (3min, 100^{o}C) and heavily processed (25min, 90^{o}C) FD extracts. FD_100^{o}C,3min were significantly lighter in colour than the FD_90^{o}C,25min. The PCA bi-plots also indicate that CAD was identified as the reddest in colour. Other differences in appearance that were observed between the extracts, such as, bubbles and sediments (Table A1.6), may be due to pouring and filtering respectively. However, a key appearance quality was cloudiness, which was particularly observed in extracts of Roselle which had been freeze-dried.

Odour: Odour terms used to describe the extracts are shown in Table 4.2. Significant differences between the extract in this study were only observed for sweet, red berry fruit, veggie note, allspice and dusty odours (Table A1.6). The differences in odour terms did not

appear to be due to pasteurisation or processing time with the exception of dusty odour in FD_90^{o}C,25min and PFD_90^{o}C,25minextracts. The PCA bi-plots (Figure 4.3) indicate that the odour attributes, in general, were stronger with FD and TD samples than in CAD.

Table 4.2: Sensory lexicon (descriptors and definitions) for Roselle extracts

Attribute and descriptors	**Definitions**
Appearance	
Red Colour	Shade of red associated with Roselle extract/beverages
Bubbles	Vesicle of beverage filled with air
Sediments	Particles at the bottom of the extract/beverage
Cloudy	Perceived translucence of the extract/beverage
Odour	
Sweet	An odour associated with sugary foods
Red berry fruit	Aroma(s) associated with red berry fruits
Orchard fruit	Aroma(s) associated with orchard fruit
Floral	A subtle aroma note associated with dry rose petals
Veggie note	Earthy aroma associated with root vegetables
Allspice	Spicy odour associated with the ground allspice
Cooked	A jammy odour associated with processed fruits
Dusty	Odour associated with dried storage
Taste	
Sweet	Taste associated with sugary food
Acid	Sensation associated with lemons
Bitter	Taste associated with quinine
Salty	Taste associated with NaCl
Metallic	Unusual off note taste sensation in the mouth
Flavour	
Red berry fruit	Flavour(s) associated with red berry fruits
Orchard fruit	Flavour(s) associated with orchard fruits
Brown Fruits	Flavour(s) associated with brown fruits
Veggie note	Earthy flavour associated with root vegetables
Floral	A subtle flavour note associated with dry rose petals
Allspice	Spicy flavour associated with the ground allspice
Cooked	A jammy flavour associated with processed fruits
Dry wood	Flavour associated with dried wood
Dusty	Odour associated with dried storage
Mouthfeel	
Mouth coating	Degree to which the beverage coats the mouth
Salivating	Degree to which saliva is induced upon drinking of the beverage
Drying (Astringent)	Degree of Astringency or mouth puckering
After-effect	
Sweet	A lingering taste associated with sugary foods
Acid	A lingering lemony taste
Salty	A lingering taste associated with NaCl
Bitter	A lingering distinct taste associated with quinine
Allspice	A lingering spicy taste
Drying	Astringent, mouth puckering sensation

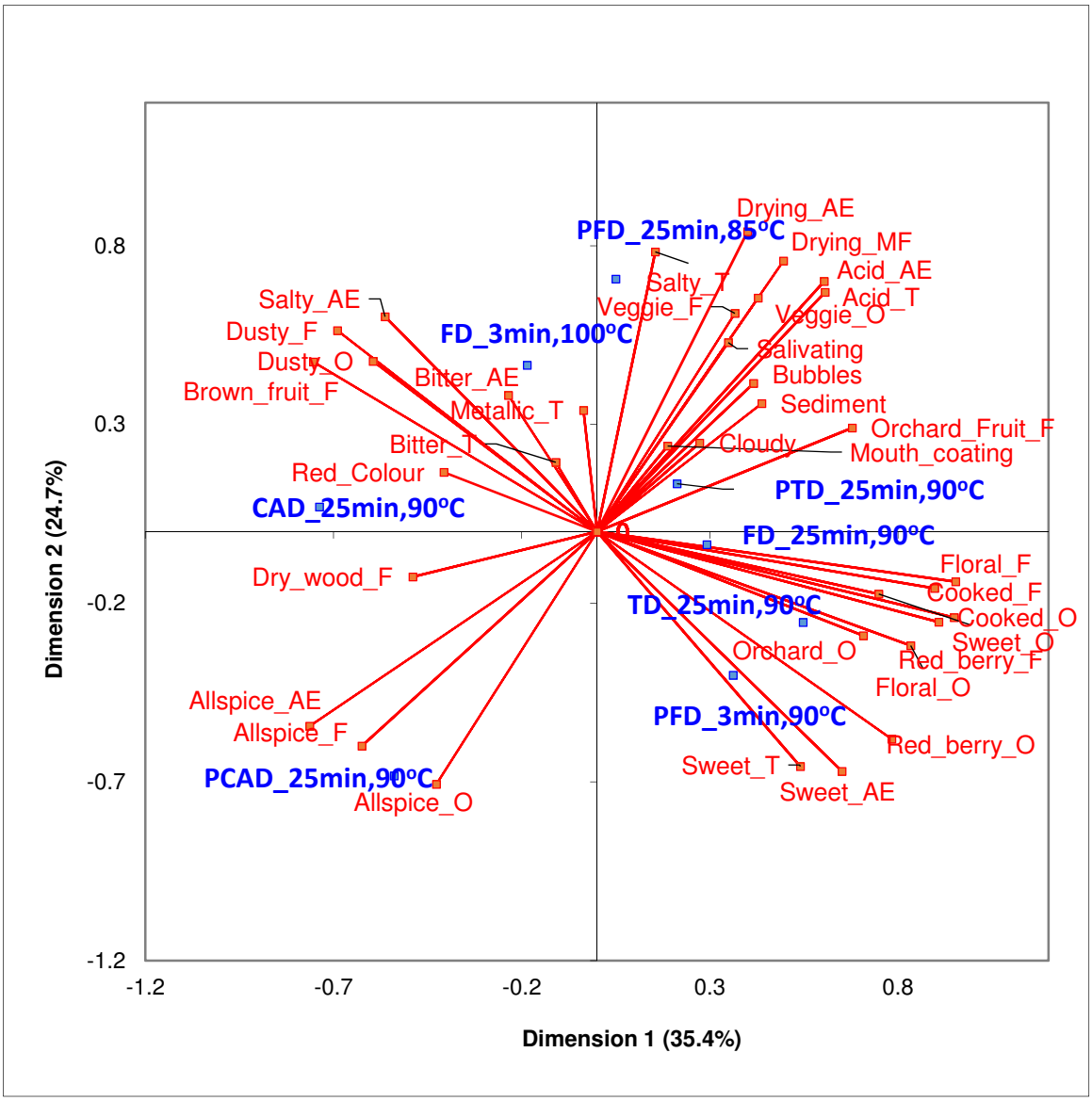


Figure 4.3: PCA biplot of Roselle extract attributes. The extracts are as follows: FD_100°C,3min - Freeze dried Roselle extract processed for 3 min at 100°C, FD_90°C,25min - freeze-dried Roselle extract processed for 25 min at 90°C, TD_100°C,25min - Tray dried Roselle extract processed for 25 min at 90°C; CAD_90°C,25min - Commercial air dried Roselle extract processed for 25 min at 90°C; PFD_100°C,3min - freeze-dried Roselle extract processed for 3 min at 100°C then pasteurised; PFD_90°C,25min - freeze dried Roselle extract processed for 25 min at 90°C then pasteurised; PTD_90oC,25min - tray dried Roselle extract processed for 25 min at 90°C then pasteurised; CAD_90°C,25min - and commercial air dried Roselle extract processed for 25 min at 90°C then pasteurised. Descriptors are followed by atrributes: _O - Odour attribute, _T - Taste attribute, _F - Flavour attribute, _MF - mouthfeel attribute, and _AE - After-effect attribute.

Taste and Flavour: Of all the descriptors of the taste attribute (Table 4.2), the dominant taste was acidity. There were significant differences between the acidity of extracts. FD_90°C,25min and PFD_90°C,25min were significantly more acidic than CAD_90°C,25min

and PCAD_90^{o}C,25min which was broadly in-line with pH results. Bitter and sweetness were rated at similar levels to one another in the extracts and although there were significant differences between extracts in sweetness, there were no significant differences in sample pairs (Tukey HSD test).

Of the flavour descriptors (Table 4.2), only the terms: floral, allspice, cooked and dusty were significantly different between the extracts. The PCA biplot (Figure 4.3) flavour attributes indicate that CAD extracts possess a dusty, brown fruit flavour and all spice flavour that makes it different from FD and TD extracts. The latter appears to have more of the floral, red berry fruit and orchard fruit flavour.

Mouthfeel: In general, the mouthfeel attribute of the Roselle extracts was minimal. There were no significant differences between the mouthfeel of the extracts as the differences were very small.

***Aftertaste*:** Drying/astringent and acid aftertaste were different between the extracts. Although not statistically significant, TD and FD extracts were rated more acidic aftertaste than CAD. Similarly, the PCA biplot indicates a higher drying aftertaste in FD and TD extracts compared to CAD extracts. The latter was significantly higher in an allspice aftertaste.

It is surmised that the observed differences between extracts produced from CAD and the other Roselle may have been due to a number of unknown factors including air drying conditions, pre and post drying storage conditions, and the age of the dried Roselle. Overall, the appearance of the extract seemed the most affected by changes to processing conditions. The effect of drying method in this study on the sensory attributes of Roselle extracts was minimal. However, freeze dried extracts were observed to be more cloudy than extracts from other drying methods. On the other hand, a change in extraction

conditions i.e. temperature and time, resulted in a significant change in the colour of the extract. This correlates with previous studies on the effect of extraction conditions anthocyanin content and indeed colour (Cisse, Vaillant, et al., 2012). Finally, there was no significant effect of pasteurisation on the sensory attributes of the extracts.

4.3.1.2 Roselle Beverages (RB)

Table 4.3 shows the pH, colour measurements and titratable acidity of the beverages used in the consumer study. All beverages were in the acidic range.

Table 4.3: Physical and chemical properties of Roselle beverages

Sample	pH	Colour hue (LAB)			Titratable acidity (% malic acid)
		Lightness, L	Hue angle	Chroma	
RB_sugar_no spice	3.22±0.00^{c}	65.1±0.9^{b}	16.5±0.7^{a}	35.9±0.4^{c}	0.11±0.00^{c}
RB_sugar_cinnamon	3.12±0.01^{ab}	65.2±0.2^{b}	18.5±0.7^{ab}	36.6±0.3^{cd}	0.10±0.00^{ab}
RB sugar_cloves	3.13±0.04^{ab}	61.4±0.3^{a}	16.2±0.6^{a}	39.4±0.1^{e}	0.12±0.00^{d}
RB_sugar_ginger	3.08±0.00^{a}	69.4±1.0^{c}	17.0±0.7^{ab}	32.0±0.7^{a}	0.09±0.00^{a}
RB_SRA_no spice	3.14±0.01^{b}	69.6±1.1^{c}	16.9±1.2^{ab}	31.5±0.3^{a}	0.10±0.00^{b}
RB_SRA_cinnamon	3.08±0.01^{a}	60.3±1.5^{a}	19.3±0.7^{b}	37.3±0.2^{d}	0.13±0.00^{e}
RB SRA_cloves	3.10±0.01^{ab}	67.3±1.1^{bc}	18.8±1.2^{ab}	31.2±0.3^{a}	0.10±0.00^{ab}
RB_SRA_ ginger	3.12±0.01^{ab}	65.5±2.3^{b}	17.9±1.3^{ab}	33.4±0.5^{b}	0.12±0.00^{de}

The table shows eight Roselle beverages for use in sensory profiling and consumer liking study. The beverages are as follows. Roselle beverage with no spice sweetened with sugar (RB_sugar_only); Roselle beverage with no spice sweetened with stevia Reb A (RB_stevia_only); Roselle beverage with cinnamon sweetened with sugar (RB_sugar_cinnamon); Roselle beverage with cloves sweetened with sugar (RB_sugar_cloves); Roselle beverage with ginger sweetened with sugar (RB_sugar_ginger); Roselle beverage with cinnamon sweetened with stevia Reb A (RB_stevia cinnamon); Roselle beverage with cloves sweetened with sugar (RB_stevia cloves); and Roselle beverage with ginger sweetened with stevia Reb A (RB_stevia ginger). Letters (superscripts) a – d indicate significant differences across individual properties.

The addition of lemon and pineapple juices to the Roselle extract did not alter the pH significantly but doubled the titratable acidity (from 0.06 + 0.00 to 0.13 + 0.00 % malic acid). Both juices are rich in malic acid which may account for this change. Similarly, sugar did not alter the pH of the pre-sweetened extract (3.23 $\pm$ 0.05) but SRA made the extract slightly more acidic. Furthermore, regardless of the sweetener used, the addition of spices increased acidity, which is expected since the selected spices are rich in organic acids. However, within beverages containing the spices, there were no significant differences in pH. There were significant differences in titratable acidity between beverages, although the reason for the difference is not clear. The richness and range of phenolic acids in spices may be responsible.

The spices created differences in the colour of the beverages. With sugar-sweetened beverages, the cinnamon flavoured beverage was closest to the unflavoured (no spice) beverage, however, cloves made the beverage darker, and ginger made the beverage lighter. All samples were of similar red colour as indicated by their hue angles, however, chroma values revealed a greyish or discoloured tone with the ginger flavoured beverage. For SRA sweetened beverages, some colour leaching (colour separation observed on the lid of the storage container) was observed which may account for the lightness in colour of almost all the beverages except the cinnamon flavoured beverage. Amongst the SRA - sweetened beverages, the reddest, darkest and less grey beverage was the cinnamon flavoured beverage.

Profiling result on the attributes of Roselle beverages

Fifty-two (52) descriptors were agreed upon by assessors but only 24 of the terms (Table A1.5 - Appendix) were significantly different between samples ($P < 0.05$). The addition of

other ingredients i.e. sweeteners, spices, lemon and pineapple juices to the extracts introduced new terms. Odour terms such as soapy and off note i.e. fishy/putty; Taste terms such as savoury; flavour terms such as pineapple juice, liquorice, soapy and off note; mouthfeel terms such as warming, tingling, residue, and throat catching; and after-effect terms such as soapy and liquorice were added. With the use of the commercially dried Roselle, the characteristic allspice odour, flavour and after-effects; and the drying mouth observed in the extracts appeared to be lost. This may be as a result of any of the ingredients used i.e. the juices or sweeteners.

As a result of added sweetness, in particular, aspects of the sensory profile improved in the beverage compared to the extracts. In addition to the desired sweetness (odour, taste and after effect), dusty odours and flavours; acidity, salty, bitterness and metallic tastes; and salty and bitter aftertaste were also reduced. The sweetened Roselle beverages were also salivating and less mouth coating. In addition, the veggie odour and flavours were lost. There was no significant loss of floral flavour. However, some desirable properties such as the fruity odours and flavours were reduced whilst undesirable properties such as cooked flavour became more pronounced and savoury taste and liquorice flavour and after-effect were introduced. The latter was due to the addition of SRA.

Appearance: As indicated by the colour LAB hue angle and chroma, Roselle samples were a red colour. The colorimetry differences (differences in lightness and chroma) were not observed by the trained panel, probably due to similarities in hue angle values. This is a favourable observation as colour differences may affect liking and alert consumers to differences in samples from the onset, thus biasing the data.

Odour: Table A1.7 (Appendix) shows significant differences in the odour of the beverages which were expected due to the distinct odours of the spices. Although sweetness levels using both sweeteners were well-matched, scores for cinnamon and clove odour were higher in the sugar-sweetened whereas ginger odour scored higher in SRA sweetened beverages. This may be a testament to flavour interactions. The red berry fruit, pineapple juice, orchard fruit and floral odour notes were also affected by the sweetener type. SRA beverages gave a more intense red berry fruit, pineapple juice, and orchard fruit odours compared to their sugar counterparts. This trend was almost mirrored for the floral odour but for the SRA_ ginger beverage which scored lower than its sugar counterpart. The higher sweet odour scores for SRA sweetened beverages (Table A1.7) coupled with the more acidic nature of SRA Roselle beverages (Table 4.2) supports findings by Bonnans and Noble (1993), which links the perception of fruitiness intensity with sweetness and acidity.

Taste and Flavour: There were no significant differences between the sweetness of the control samples i.e. sugar and SRA sweetened beverages with no spice, suggesting that sweetness levels were comparable between sugar and SRA. Unflavoured (no spice) SRA - sweetened beverages were more liquorice, acidic, bitter, metallic, savoury and salty compared with their sugar counterparts. Furthermore, Unflavoured (no spice) sugar beverages possessed stronger cooked (jammy) and pineapple juice flavour compared with unflavoured SRA beverages. The latter were characterised by a strong liquorice flavour. This cooked flavour was probably due to HTST pasteurisation of the beverage after sweetening.

The sweetened beverage lost their distinguishing characteristics with the addition of cinnamon. Cinnamon introduced a more florally, fruity (cranberry and raspberry), cooked

and juicy (pineapple) flavour to the SRA - sweetened beverage. These descriptors, more pronounced in SRA - sweetened than sugar-sweetened beverages, did not overshadow the characteristic liquorice flavour of SRA. However; the strong flavour of cloves most highlighted the liquorice flavour note in SRA-sweetened beverages. Although ginger did not seem to enhance the flavour attribute of SRA - sweetened beverages, it did enhance the fruity and floral flavours of sugar-sweetened beverages.

In relevant samples, cinnamon and clove flavour scored higher in sugar-sweetened beverages than the SRA sweetened beverages. Conversely, ginger flavour scored higher in SRA sweetened beverages than in sugar-sweetened beverages. Moreover, the addition of the spices; ginger and cloves, were particularly observed to introduce further bitterness, which was more pronounced in SRA - sweetened than sugar - sweetened beverages; whereas cinnamon flavoured samples were significantly less bitter. With the exception of clove flavoured beverages, the addition of spices reduced the liquorice flavour of SRA sweetened Roselle beverages. Furthermore, the SRA_cinnamon scored the highest for red berry fruit taste although in other cases the sugar-sweetened beverages generally scored higher then SRA -sweetened beverages with or without spices.

Mouthfeel: There were three mouthfeel attributes that were significantly different between the Roselle beverages, which were warming, tingling and throat catching. These effects were particularly prominent in ginger flavoured beverages and not in the other beverages.

Aftertaste: Bitterness, astringency, liquorice and salty were the distinguishing aftertastes of unflavoured (no spice) SRA sweetened beverages compared to unsweetened sugar - sweetened beverages. This largely remained unchanged with the addition of spices,

although the addition of cinnamon reduced saltiness in SRA-sweetened beverages; cloves heightened bitterness in sugar-sweetened beverages and ginger added some acidity and to SRA sweetened beverages. The liquorice aftertaste appears to be due to SRA and/or ginger or cloves in the formulation. Higher astringent after-effect was observed in all beverages with SRA irrespective of the spice used.

4.3.2 Consumer study

4.3.2.1 Overview of study

Table 4.3 shows the demographics of consumers in the study. There were more female (66%) than male (34%) participants. The most represented age group were the 19-29 year olds and participants represented at least 12 ethnic groups.

Comparisons between profiling data and Just About Right data

Profiling data for Roselle beverages showed higher sweet odour scores for SRA sweetened beverages compared to sugar-sweetened beverages, although the difference was statistically insignificant. However, no difference was observed for sweet taste. Despite this similarity in the sweetness of the Roselle beverages, the consumer study shows higher JAR scores for sweetness in all sugar-sweetened samples compared to their SRA sweetened counterparts.

The sweetness of sugar-sweetened beverages was assessed to be “just about right” by more participants (68 - 74%) compared to SRA sweetened beverages (40 - 53%) which were predominantly rated as not sweet enough. The response to this cannot be to increase the amount of SRA used as this may increase the off-notes associated with SRA. In this case, the use of another sweetener with the right profile in combination with SRA might be more appropriate.

Furthermore, flavour JARs were also consistently higher in sugar-sweetened beverages compared to the SRA sweetened counterparts. The flavour intensity of RB_stevia_no spice, in particular, was considered "too little" by the majority of participants (45%) but for RB_sugar_no spice, it was "just about right" by 72% of participants. The flavour intensities of the SRA sweetened beverages were expected to improve with the inclusion of spices. However, the increase in flavour JAR ratings when cinnamon was added was small (increasing from 43% to 50%) and where cloves and ginger were used the flavour JAR ratings did not increase.

The sensory profiling ratings for flavour intensities were much higher for the clove and ginger flavours in the SRA sweetened beverages (57.5 and 46.2 respectively) in comparison to the cinnamon variant (28.2), supporting the conclusion from the consumer study that the clove and ginger variants were too strong in flavour. Reducing the concentration of both spices may improve liking scores. For indeed, the highest penalties in overall liking data were observed with the cloves flavoured variants. With SRA as the sweetener, 57% of participants considered the flavour intensity as "too much" with a mean drop of 2.5. Similarly, 55% of participants assessed the flavour intensity of the RB_sugar_clove as "too much" with a mean drop of 2.8. Similarly, with ginger, 22% and 40% of participants considered the flavour intensity "too much" with mean drops in liking from Just about right" of 2.8 and 2.2 for sugar and SRA sweetened beverages respectively.

Concerning astringency, more participants assessed the astringency of SRA sweetened beverages as "too much" (20 - 39%), compared to sugar sweetened beverages (11 – 18%), except where cloves were used (astringency in both variants were assessed to be "too much" by 38% of participants). This concurs with the descriptive analysis for the after-effect

attribute "Astringency"; the Roselle beverage profile had indicated higher astringency after-taste for SRA sweetened beverages in general. Although spices increased beverage astrigency, this was consistent regardless of the sweetener used further.

Therefore, the JAR scores correlated with the profiling data for sweetness, flavour and astringency and therefore clearly showed consumer discrimination between sweeteners and between spice variants.

4.3.2.2 Effect of Sweetener and spices on consumer liking and intent

Grouping together all consumers that scored above or below the middle "neither like nor dislike" category, the results conclude that 82% of consumers liked the sugar-sweetened control sample whilst only 58% liked the SRA sweetened control sample (Figure A1.2 - Appendix). This indicates general liking for the beverage whilst highlighting the disparity between the flavour profiles of sugar and SRA sweetened beverages. Similar trends were observed for consumption intent (Figure A1.3 - Appendix) and purchase intent (Figure A1.4). The mean liking data (Table 4.3) demonstrates that the sugar-sweetened beverages with or without spice were significantly better liked than the SRA sweetened samples. Mean scores for appearance, aroma and flavour liking data were consistent with the mean scores for overall liking, implying a "halo-effect". When spices were added, similar trends were observed for beverages sweetened with either sweetener. Regardless of the sweetener, mean overall liking for cinnamon and ginger flavoured beverages were not significantly different. Overall, liking of the beverages did not improve with the addition of spices. However, liking of beverages flavoured with spices displayed a hierarchal order where, regardless of the sweetener, cloves were the least liked. The hierarchy was also replicated in the consumer and purchase intents (Figure A1.5 – Appendix).

Table 4.3: Mean liking scores of Roselle beverages with or without spice flavour

Sample	Overall Liking	Appearance Liking	Aroma Liking	Flavour Liking
RB_sugar_no spice	6.9±1.6^{a}	7.1±1.3^{a}	6.6±1.5^{a}	6.8±1.7^{a}
RB_sugar_cinnamon	6.3±1.9^{ab}	6.9±1.3^{a}	6.2±1.7^{ab}	6.3±1.8^{a}
RB_sugar_cloves	5.3±2.4^{c}	6.8±1.4^{a}	5.4±2.2^{d}	5.1±2.4^{b}
RB_sugar_ginger	6.2±2.0^{b}	6.9±1.2^{a}	6.2±1.7^{ab}	6.2±2.1^{a}
RB_SRA_ no spice	5.5±1.9^{c}	6.3±1.7^{b}	5.8±1.5^{bc}	5.3±1.9^{b}
RB_SRA_cinnamon	5.4±1.9^{c}	6.3±1.6^{b}	5.8±1.7^{bc}	5.3±2.0^{b}
RB_SRA_cloves	4.5±2.2^{d}	6.1±1.7^{b}	5.1±2.0^{d}	4.3±2.1^{c}
RB_SRA_ginger	5.3±1.9^{c}	6.1±1.7^{b}	5.7±1.6^{bcd}	5.2±1.9^{b}

The table shows eight Roselle beverages for use in sensory profiling and consumer liking study. The beverages are as follows. Roselle beverage with no spice sweetened with sugar (RB_sugar_only); Roselle beverage with no spice sweetened with stevia Reb A (RB_stevia_only); Roselle beverage with cinnamon sweetened with sugar (RB_sugar_cinnamon); Roselle beverage with cloves sweetened with sugar (RB_sugar_cloves); Roselle beverage with ginger sweetened with sugar (RB_sugar_ginger); Roselle beverage with cinnamon sweetened with stevia Reb A (RB_stevia cinnamon); Roselle beverage with cloves sweetened with sugar (RB_stevia cloves); and Roselle beverage with ginger sweetened with stevia Reb A (RB_stevia ginger). Letters (superscripts) a – d indicate significant differences across individual properties.

In order to investigate how the consumers conceptualised the sensory profile of the RBs, "Emotion scoring", as suggested by King and Meiselman (2010), was used. This can help provide further insight into understanding the overall liking scores in Table 4.3. In a subsequent study, King, Meiselman, and Carr (2010) revealed that for "herbs/spices" and "still and carbonated beverages", 33% and 42% (respectively) of the suggested 39 emotion terms were unrelated to overall acceptability. Consumers in this study deemed quite a number of emotion terms relevant to the Roselle beverages. Principal component analysis, PCA, and multiple correspondence analysis, MCA (both not shown) did not show particular discrimination in the use of the emotion terms with the liking of the beverages. Thus, the number of emotion terms used for all beverages were summed up and terms that were most frequently used (up to or more than 5% of the grand total) were selected from Table

A1.8. Five emotion terms were identified as particularly relevant to the liking of the Roselle beverages as shown in Figure 4.3. These 5 terms represent only 13% of the original list of emotion terms.

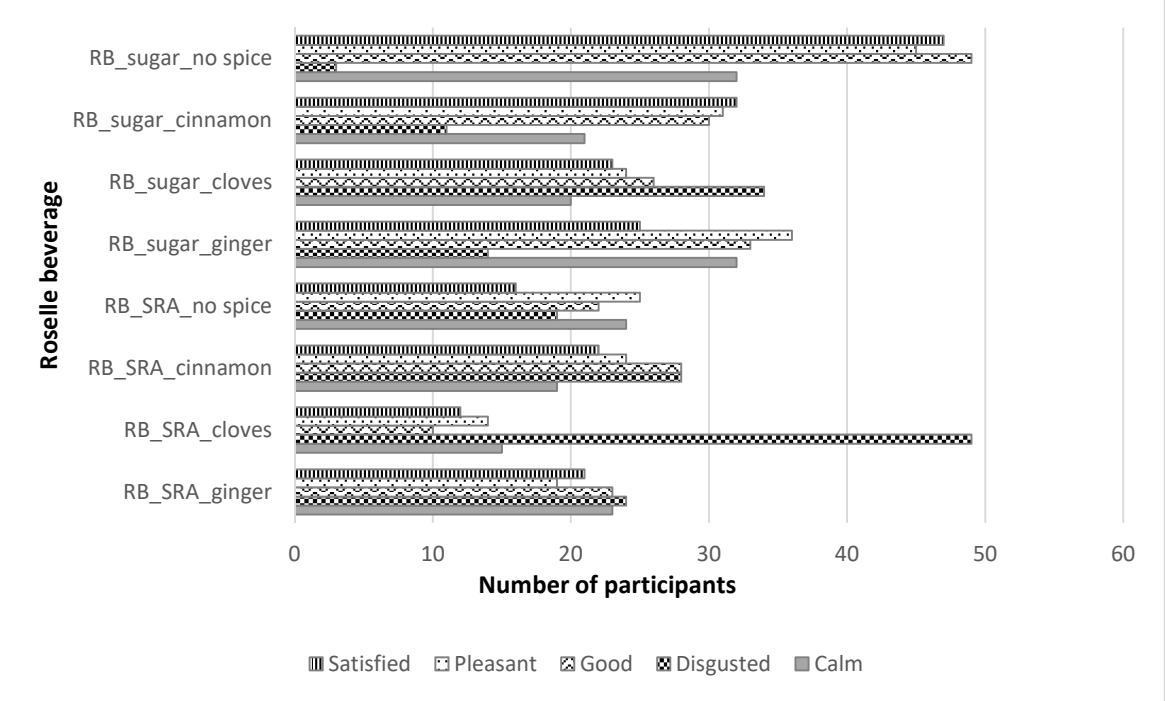


Figure 4.4: The emotion terms most used by consumers in the study of Roselle beverages. The figure shows eight Roselle beverages assessed by liking study. The beverages are as follows: Roselle beverage with no spice sweetened with sugar (RB_sugar_only); Roselle beverage with no spice sweetened with stevia Reb A (RB_stevia_only); Roselle beverage with cinnamon sweetened with sugar (RB_sugar_cinnamon); Roselle beverage with cloves sweetened with sugar (RB_sugar_cloves); Roselle beverage with ginger sweetened with sugar (RB_sugar_ginger); Roselle beverage with cinnamon sweetened with stevia Reb A (RB_stevia cinnamon); Roselle beverage with cloves sweetened with sugar (RB_stevia cloves); and Roselle beverage with ginger sweetened with stevia Reb A (RB_stevia ginger).

Beverages with the highest overall liking scores were associated with the terms: “Calm”, “Good”, “Pleasant” and “Satisfied”. Whereas the use of the emotion term “Disgusting” was associated with the lowest overall liking scores; the clove-flavoured samples in particular. Although these terms may connote different meanings to individual consumers, the selection of emotion terms associated with the liking of the beverage are positive, whereas the words associated with the dislike of the beverage is a strongly negative term. The

positive term may be useful perhaps in quality control and marketing activities for the Roselle beverages.

Finally, there was no significant effect of familiarity with Roselle on the use of the emotion terms (p = 0.332) although the emotion terms "Free", "Interested", "Joyful", "Mild", "Nostalgic" "Understanding" were more significantly used by consumers who were familiar with Roselle (results not shown). Whereas, "Wild" more significantly used by consumers unfamiliar with Roselle. Again, the emotion terms significantly used by consumers familiar with Roselle are positive. Although the term significantly used by consumers unfamiliar with Roselle is not strictly negative, it does indicate caution in the consumption of Roselle beverages.

4.3.2.3 Effect of Familiarity, Gender, Age or Ethnicity on consumer liking of RB

Fifty-nine per cent (59%) of consumers in this study were familiar with Roselle (Table 4.4.). However, familiarity had no significant effect on consumer liking (p=0.148). Further questions asked on purchase intent (where available) revealed that 47% of those familiar with Roselle would not purchase it if available (Figure A1.6). This might explain the lack of correlation between familiarity and liking.

There was no effect of gender on overall liking of the Roselle beverages. There was also no effect of age on overall liking of the Roselle beverages except with RB_SRA_ginger where 19-29 year old participants (43%) demonstrated a significantly (p=0.013) lower overall liking of the beverage compared to 30 – 39 year old participants (26%).

Table 4.4: Consumer demographics and subject characterisation

		Male	Female	Total	
Number of consumers	**n**	48	93	141	
	%	34	66		
Median/Mean Age(Stdev)		30/36 (12)			
Age Groups				**n**	**%**
		19-29		60	43
		30-39		36	26
		40-49		21	15
		50-59		16	11
		60-65		8	6
Ethnicities		Group	Abbrev.	n	%
		Asian/Asian British- Chinese	AC	13	9
		Asian/Asian British- Pakistani	AP	3	2
		Black/African/Caribbean/Black British- African	BA	20	14
		Black/African/Caribbean/Black British- Caribbean	BC	8	6
		Mixed/Multiple - White and Black Asian	MA	4	3
		Mixed/Multiple - White and Black Caribbean	MC	1	1
		Other - Any other	OE	24	17
		Other – Arab	OA	2	1
		Prefer not to declare	N/A	4	3
		White - English/Welsh/Scottish/Northern Irish/British	WB	48	34
		White – Irish	WI	1	1
		White – Other	WO	13	9
Familiarity 41% unfamiliar and 59% familiar with RB					

As can be seen in Table 4.3 the largest ethnic groups in the study were White (English/Welsh/Scottish/Northern Irish/British; WB - 34%) followed by the Black (African/Carribbean/Black British- African; BA - 14%). Seventeen per cent (17%) of participants were categorised as "Other – Any other" (OE), a group largely consisting of Asians ethnicity (Malaysians). Ethnic groups less than 10% representation in this study were considered as not truly representative of ethnic preference and therefore omitted from the analysis. These three ethnicities preferred RB_sugar_no spice the best (Figure A1.7). There was no significant difference between liking of RB_sugar_no spice, perhaps because Roselle is indigenous to Malaysia and many countries in the African continent. Regardless of the

sweetener used, BA demonstrated significantly stronger overall liking for the beverages with ginger and clove spices compared to WB and OE, which suggests familiarity with these spices. However, their liking of SRA sweetened beverages increased with the addition of ginger and cinnamon. An increase in the liking of SRA sweetened beverage with the addition of cinnamon was also observed with WB. For the other beverages except RB_sugar_no spice, liking patterns were similar for WB and OE.

4.3.2.4 Consumer groups with similar liking patterns

Table 4.5: Agglomerative Hierarchical cluster classes (n = 3) and their mean overall liking scores for Roselle beverages (n = 8)

Clusters No	**1**	**2**	**3**
Sample size (n)	*53*	*68*	*20*
%	38	48	14
Overall liking of individual sample	Means (9 point scale)		
RB_sugar_no spice	5.9^{ab}	7.5^{a}	7.4^{a}
RB_sugar_cinnamon	4.7^{c}	7.3^{ab}	6.8^{a}
RB_sugar_cloves	4.3^{c}	6.5^{bc}	3.6^{cd}
RB_sugar_ginger	6.5^{a}	7.1^{ab}	2.6^{d}
RB_SRA_ no spice	4.5^{c}	6.1^{c}	6.1^{ab}
RB_SRA_ cinnamon	4.8^{bc}	6.2^{c}	4.4^{bc}
RB_SRA_ cloves	4.1^{c}	5.0^{d}	4.0^{cd}
RB_SRA_ ginger	4.9^{bc}	6.1^{c}	3.5^{cd}

Letters (superscripts) a – d indicate significant differences in overall liking scores within individual clusters.

Cluster analysis (AHC) demonstrates that consumers could be divided into 3 different clusters based on their overall liking scoring patterns (Table 4.5).

Cluster 1 (38% of participants) disliked all of the RB except with RB_sugar_no spice and RB_sugar_ginger. The largest cluster; Cluster 2 (48% of participants), clearly discriminated their liking between sugar-sweetened and SRA sweetened beverages preferring the

former. Finally, cluster 3 (14% of participants) liked the samples without any spice the most, although they also liked cinnamon flavoured beverage when it was sweetened with sugar. A breakdown of the demographics of participants in each cluster is supplied in Table A1.9 (Appendix) which shows that there were no particularly distinguishing characteristics of the participants within the clusters.

Figure 4.5 provides an internal preference map; the PCA is based on the liking data with the sensory profiling data regressed onto the plot as supplementary variables. This shows that participants in cluster 1 and 2 liked the mouthfeel associated with spices and disliked the bitterness, drying, liquorice and metallic attributes associated with SRA sweetened beverages. Furthermore, cluster 3 favoured the fruity floral notes of the beverage, which were not able to mask the SRA off notes. Thus, based on the clusters analysis for overall liking, the choices of the majority (86%) of consumers suggests a congruency of the ginger and cinnamon flavours with the Roselle beverage. However, these flavours did not significantly mask the off-notes associated with SRA sweetened beverages.

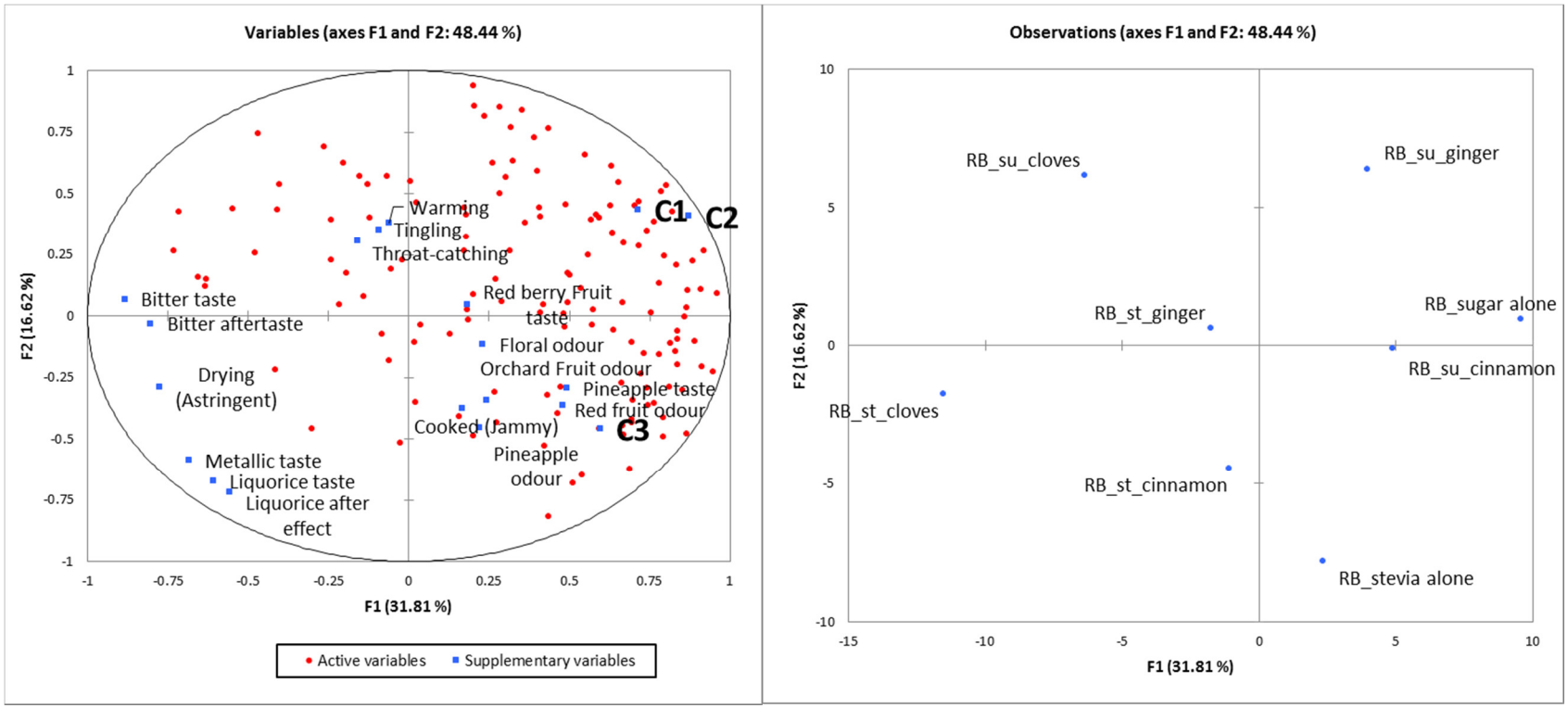


Figure 4.5: PCA biplot of consumer overall liking with Roselle beverages attributes and AHC analysis (in bold italics)

4.4 Conclusion

This study developed a lexicon of Roselle extract by assessing the effect of processing on the sensory attributes of the Roselle extract. Variations in processing conditions only minimally affected the sensory profile. In particular, freeze-drying resulted in a more cloudy extract and the extraction procedure significantly influenced the colour of the extract. However, the perceived differences in sensory attributes due to raw material and drying method were not statistically significant. Pasteurisation had no detrimental effect on the sensory attributes of the extracts.

Sweetening did enhance aspects of the sensory attributes of the Roselle extract particularly reducing dusty odours and flavours; acidity, bitterness, saltiness, and metallic taste; and mouth-coating and salivating mouth-feel. However, fruity and floral odours and flavours did not benefit from the addition of sweeteners.

Although the Roselle beverages were generally liked, overall liking of the Roselle beverage was not synonymous with familiarity with Roselle. Furthermore, consumers clearly discriminated their liking between sugar and SRA sweetened beverages, clearing preferring the former. With the addition of spices, overall liking of the SRA sweetened beverage did not improve for consumers.

In investigating consumer conceptualisation of the Roselle beverages, five emotion terms particularly stood out which were *Satisfied, Pleasant, Good, Disgusted* and *Calm*. Four of the five terms were strongly associated with the beverages with best overall liking, whilst *Disgusted* best conceptualised beverages with the worst overall liking.

There was evidence of a selective preference for spices based on ethnicity. Furthermore, for 86% of consumers, cinnamon and ginger were assessed to be congruent with SRA

sweetened Roselle beverage, although masking of SRA off notes with these spices were not significant. Finally, for the majority of participants (48%), overall liking scores for spice flavoured beverages were rated above 5. This suggests that the use of these spices with SRA may be well received in contemporary beverages.

Chapter 5: Effect of the Spices (Cinnamon and Ginger) on the stability of Anthocyanin in a Roselle beverage.

Abstract

The use of spice flavours in beverages is a niche but growing trend. Having confirmed the congruency between cinnamon, ginger and Roselle sweetened with Stevia Rebaudioside A in Chapter 4, this current study investigates the effect of these spices on the physical and beneficial properties on a Roselle beverages, i.e. anthocyanins, FRAP antioxidant properties and colour during storage under accelerated conditions (40^{o}C for 30 days). Roselle was sweetened with sugar and Stevia Rebaudioside A. The type of sweetener had no effect on the initial properties of the beverage. Furthermore, of both spices, only cinnamon (1 g/L) led to an initial increase in the total phenolic and FRAP (antioxidant) capacity of the Roselle beverage. More interestingly, the inclusion of cinnamon particularly when combined with sugar reduced the degradation of anthocyanins and improved colour stability during storage. The mechanism of the stabilising effect of the sugar - cinnamon combination on anthocyanins is not certain at this stage but it is postulated to be due to a co-pigmentation reaction or the acylation of anthocyanins with a complex formed from the reaction of glucose with phenolic compounds contained in cinnamon.

5.1 Introduction

The polyphenolic compounds in Roselle are largely responsible for its aesthetic and health properties. As shown in Chapter 3, these compounds, which include anthocyanins, are known to be unstable and degrade during storage, hence impacting on the colour, antioxidant capacity and ACE inhibitory activity of the beverage. In general, anthocyanins are sensitive to pH, temperature, oxygen and light (Cortez, Luna-Vital, Margulis, & Mejia, 2017). Oxygen causes anthocyanins to degrade either by oxidation or through enzyme action. Anthocyanins degradation in the presence of enzyme polyphenol oxidase leads to a destruction of the flavylium structure (Patras et al., 2010). Furthermore, increased solid content and condensation reaction with other phenolic compounds, have also been associated with the degradation of anthocyanins. Although sugar is primarily added to beverages to improve their palatability, it may function as a stabilising agent for Roselle anthocyanins. The aglycone form; anthocyanidin, consisting of a hydroxyl flavylium base structure, becomes more stable when linked to sugars; usually glucose (Vattem & Maitin, 2016). A high concentration of sugar is expected to control water activity and consequently prevent the hydration of the flavylium ring which would have led to the deterioration of the anthocyanin (Dougall, Baker, Gakh, Redus, & Whittemore, 1998). A study by Kopjar and Piližota (2011) also show that sugars such as glucose and trehalose provide stabilising effects on anthocyanins. However, low levels of sugar may accelerate degradation of anthocyanins (Dyrby, Westergaard, & Stapelfeldt, 2001).

The potential health benefits of the Roselle beverage, for instance its antidiabetic properties (H. Mozaffari-Khosravi, B.-A. Jalali-Khanabadi, M. Afkhami-Ardekani, & F. Fatehi, 2009; Mozaffari-Khosravi, Jalali-Khanabadi, Afkhami-Ardekani, Fatehi, & Noori-Shadkam, 2009), could be hindered with the inclusion of sucrose (henceforth referred to as sugar) to the

beverage. Although there are several low calorie intensity sweeteners available to replace sugar, only Stevia glycosides currently satisfy consumer clean label demands and have been approved for use in the EU. One particularly pertinent study was carried out by Woźniak, Marszałek, and Skąpska (2014) in which it was observed that 0.05 – 0.2 mg L^{-1} steviol glycosides (stevioside and rebaudioside A) had no effect on the degradation of anthocyanins upon storage, whereas 50 – 200 g L^{-1} sugars (glucose, fructose and sucrose) increased the stability of anthocyanins (cyanidin-3-glucoside and pelargonidin-3-glucoside). Solution temperature, sugar type and concentration where identified as influencial to the level of stability offered by the sugar to the anthocyanin. A synergistic effect was observed when sucrose and steviol glycosides were mixed. Notwithstanding, further investigations on stabilising potential of both sugar and stevia Reb A on anthocyanins in more complex beverage systems is necessary.

A more complex beverage system would contain natural flavours, such as, juices or fruit, vegetable and spice extracts, which due to their phenolic compositions, may further affect anthocyanin stability. Of these natural flavouring, spices are the least studied in beverages. Spices are nutritional additives (Millstone, 1986) and may be useful as natural antioxidants in a beverage matrix, as many spices are considered to be good antioxidant sources. Therefore, this is a significant gap as spices are already in use in many contemporary ready to drink sweetened beverages such ginger ale, ginger beer, root beer, lassi, spiced elderberry infusions, mulled wine and a range of herbal or spicy infusions available internationally. Besides Roselle beverages, spiced elderberry infusions and mulled wine which also contain anthocyanins and may also benefit from information on anthocyanin stability with spices. Consequently, it is also important to understand the type of chemical interactions that could be formed when spices are used in beverages, as these interactions could be either beneficial or undesirable.

Two spices have been selected for this study due to their popularity in the beverage industry and their congruency with Roselle as revealed in Chapter 3. The first, *Cinnamomomum zeylanicum* (cinnamon) is a strong antioxidant containing sugar, resin, phenolic compounds such as catechin, protocatechuic acid, proanthocyanidins, tannins, eugenol, linalool and essential oils (cinnamaldehyde or cinnamic aldehyde) (Chericoni, Prieto, Iacopini, Cioni, & Morelli, 2005; Dudonné, Vitrac, Coutière, Woillez, & Mérillon, 2009; Hamidpour, Hamidpour, Hamidpour, & Shahlari, 2015; Shan, Cai, Sun, & Corke, 2005). Cinnamaldehyde is oxidised to form cinnamic acid. Some studies have shown that anthocyanins acylated with cinnamic acid, increased colour retention with a corresponding decrease in degradation rates (Dougall et al., 1998; Giusti & Wrolstad, 2003). Secondly, *Zingiber officinale* (ginger) is a popular spice containing essential oils, phenolic compounds including oxalic, tartaric acids, gingerols, shogaol, curcumin (Yeh et al., 2014). Both spices have strong antioxidant capacity although cinnamon has about 4 times the activity of ginger (Dragland, Senoo, Wake, Holte, & Blomhoff, 2003). Nevertheless, no information seems to exist on the effects of either of these spices on anthocyanins.

There are of course limitations to the use of spices in beverage formulation. For instance, the sensory characteristics of the spices are not broadly acceptable to all groups of consumers as identified in Chapter 4. Furthermore, although most spices have a good historical record of safe, there is the risk of exceeding recommended limits. In addition, there may be formulation and quality issues, such as, the formation of a gel-like substance when some powdered spices (including cinnamon) are used in solution (perhaps in the presence of sugars, phenolics and/or heat), which does not seem to have been explained scientifically although it is widely discussed by coffee drinkers. This shows the knowledge gap in this area of research.

In this present study, the objectives are to investigate (1) the stabilising potential of sugar and stevia Rebaudioside A on the chemical and physical properties of a Roselle during storage (2) the effect of cinnamon and ginger on the chemical and physical properties of a Roselle beverage during storage (3) possible interactions between the spices, sweeteners and anthocyanins during storage of the Roselle beverage.

5.2 Materials and methods

5.2.1 Beverage preparations

Five grams (5 g) of commercial air dried Roselle (Just Ingredients, UK) was steeped in 1 Litre of water at 90^{o}C for 25 minutes in a Duran flask shaken (86 rev/min) in a water bath (Grant OLS 200). The extract was filtered under vacuum with Whatman filter paper (No. 4) then cooled on ice. The extract was divided into three portions and one portion was left unsweetened while the other portions were sweetened with either white granulated sugar (80 g/L) or Stevia Rebaudioside A (0.32 g/L; 80% purity - Cargill, UK). Each of the three extracts was further split into 3 portions and flavoured with ground dried spice powder; 1 g/L cinnamon or 1 g/L ginger (Just Ingredients, UK), leaving one portion without spice. These were refrigerated overnight (18 hours) and filtered as above. The resulting nine products were stored at 40^{o}C for 30 days.

5.2.2 Storage

Several conditions for the accelerated study were carried out prior to the commencement of this study. The storage conditions tested were 30^{o}C, 40^{o}C and 60^{o}C for 30 days. The anthocyanin and total phenolic results for all conditions were consistent with real time data from Chapter 3. Therefore, as mentioned in Chapter 1 (section 1.3.8), 40^{o}C for 30 days was selected for the study primarily due to reduced moisture losses, anticipated minimal effects on antioxidant capacity and equipment availability. The kinetic results of the accelerated tests

are results are displayed in Figure A2.1. Aliquots of Roselle beverages from section 5.2.1 were stored under accelerated conditions at 40^{o}C for 30 days in a stability cabinet (Sanyo Gallenkamp). Comparing results from the accelerated study to the real time study in Chapter 3, 1 day under accelerated storage was found to be equivalent to 6 days of real time with respect to total anthocyanin measurement only. This timing does not follow for the other measured properties, such as the total phenolic content or FRAP capacity.

5.2.3 Analysis of samples

Chemical tests

Total phenolic content, monomeric anthocyanins, FRAP (antioxidant) capacity were analysed as detailed in section 3.2.4 of Chapter 3.

Physical tests

pH and colour were measured as detailed in section 3.2.4.3 of Chapter 3.

5.2.4 Chemical kinetics

The natural logarithms of the concentration of total monomeric anthocyanins were plotted against time (in days) to confirm adherence to first order kinetics. A linear trend was obtained for almost all samples (R^2 range was 0.81 – 0.98, Table 5.1) and the linear equation was used to obtain the reaction rate constant (k). The half-life ($t_{1/2}$) which is the time taken for the phenolic compound(s) or activity to reach half its initial value, was determined by applying the equation derived from the first order reaction equation (Equation 4).

$$Ln\frac{A_o}{A} = kt \qquad (4)$$

Then the half-life was determined as:

$$t_{½} = \frac{\ln 2}{k} \qquad (5)$$

Where A_o is initial concentration. The equivalent days of real time storage for the accelerate storage were obtained by comparing the rate of reactions between the room temperature storage (Chapter 3) and the accelerated storage in this current study, for each parameter (total phenolic content, anthocyanins and FRAP antioxidant). Assuming initial concentration (A_o) and final concentration (A_e) remain unchanged for real time and accelerated conditions, then the first order equation could be re-written as,

$$Ln\left(\frac{A_o}{A_e}\right) = kt_s = k_a t_a \qquad (6)$$

Where t_s is the real time shelf life; k is the rate constant in real time; t_a is the accelerated shelf life; and k_a is the accelerated rate constant. Therefore for 1 day of accelerated study, the real storage time equivalent for relevant parameters, can be calculated as:

$$t_s = \frac{k_a}{k} \qquad (7)$$

5.2.5 Statistics

Three batches of Roselle were prepared and each batch was analysed in triplicate. Statistical analyses were carried out using Linear mixed models for repeated measures (Brown & Prescott, 2014) on SPSS Statistical Software (IBM, Version 24) to compare groups based on sweeteners (n = 2) and spice flavour (n = 2). Results with $P < 0.05$ (95% confidence level) were considered significantly different. ANOVA (XLSTAT 2016 Addinsoft) was used to compare the properties of Roselle beverages at two time points (Days 0 and 30). Tukeys (HSD) test was performed for simultaneous paired comparisons. Results with $P<0.05$ (95% confidence level) were considered significantly different.

5.3 Results and discussion

To relate conditions for the accelerated study to the real time study in Chapter 3; 1 accelerated storage day at 40°C was found to be equivalent to approximately 6 real time days for anthocyanin alone. It is inaccurate to estimate equivalent real time days for antioxidant and total phenolic content since they do not fit the zero or first order kinetics perfectly. However, for reference, zero order kinetics and first order kinetics were assumed for total phenolic content and antioxidant content as shown in Tables A2.2 and A2.3.

5.3.1 Total Phenolic content

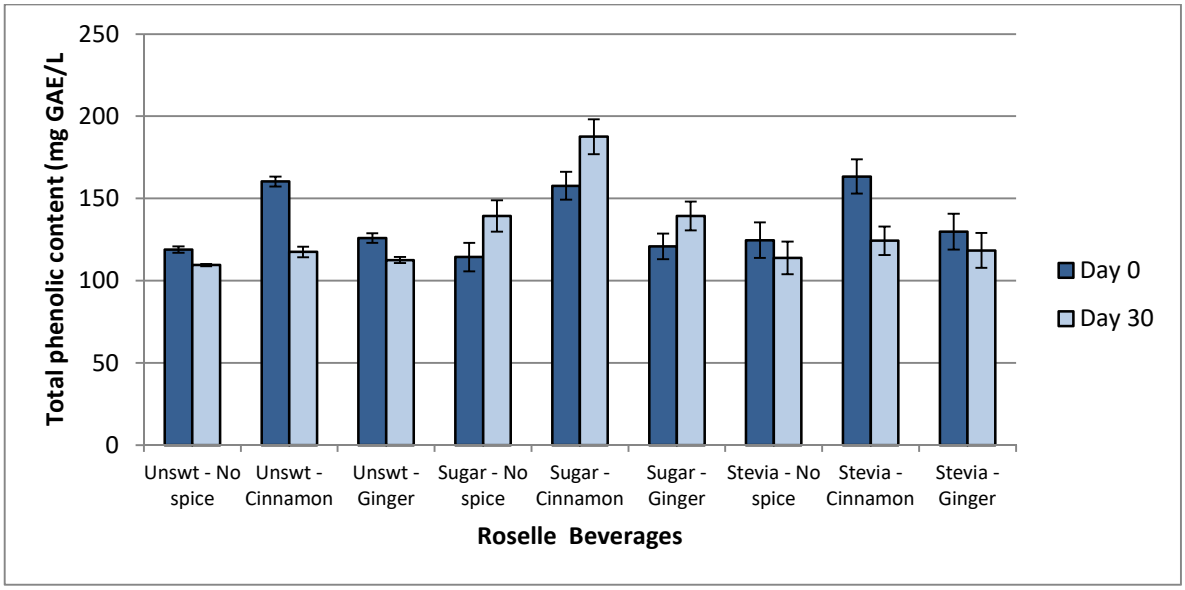


Figure 5.1: Total Phenolic content (mg GAE/L) profile of Roselle beverages unsweetened or sweetened (with sugar or stevia Reb A) and unflavoured or flavoured (with cinnamon or ginger) on Days 0 and 30 of accelerated storage at 40°C. Each bar/point represents mean values ± SD. "Unswt – no spice" refers to Roselle beverage without sweetener and spice; "Unswt – cinnamon" – refers to Roselle beverage with cinnamon flavour but no sweetener; "Unswt – ginger" – refers to Roselle beverage with ginger flavour but no sweetener; "sugar – no spice" refers to Roselle beverage without spice but sweetened with sugar; "sugar – cinnamon" refers to Roselle beverage with cinnamon and sweetened with sugar; "sugar – ginger " refers to Roselle beverage with ginger and sweetened with sugar; "SRA – no spice" refers to Roselle beverage without spice but sweetened with Stevia Reb A; "SRA – Cinnamon" refers to Roselle beverage with cinnamon and sweetened with Stevia Reb A; "SRA – Ginger " refers to Roselle beverage with ginger and sweetened with Stevia Reb A.

As seen in Chapter 3, the total phenolic content in the Roselle extract was quite stable for over 180 days of storage. Storage at room temperature for 180 days only resulted in 21 ± 6% loss in phenolic content in the unpasteurised extract. Therefore, the concern here is how the

sweeteners and herb would affect the already relatively stable system. The addition of sweeteners resulted in no significant change ($p > 0.05$) to the initial total phenolic content of the Roselle beverage (Figure 5.1). Furthermore, the addition of sweeteners did not significantly affect the degradation of phenolic compounds during storage. The addition of spices, on the other hand, contributed to an initial rise in the total phenolic content of the beverage, although statistically, only the rise due to the addition of cinnamon was significant ($p < 0.05$). This rise is accounted for by the phenolic content of the spices. Cinnamon increased the initial total phenolic content more than ginger because it contains over 10 times the quantity of phenolic found in of ginger in aqueous or methanol extracts (Dudonné et al., 2009; Shan et al., 2005).

The total phenolic content of sugar sweetened Roselle beverages significantly improve during storage. However, this result was most likely due to sucrose hydrolysing in the presence of acids to produce reducing sugars which are known to interfere with the Folin-ciocalteu reagent. There was a significant effect of cinnamon on the total phenolic content of the stored sugar sweetened beverage (Table A2.7) although other sugar sweetened beverages did not significantly differ from stevia and unsweetened beverages. Similarly, SRA did not alter the stability of the phenolic content during the storage period although the inclusion of cinnamon to the SRA sweetened beverage also led to better total phenolic retention after 30 days of accelerated storage (40^{o}C). The results from unflavoured SRA beverage (without spices) differ from results in the study by Perez-Ramirez et al. (2015) which observed improved stability on certain phenolic compounds, such as gallic acid, quercetin and rosmarinic acid on the addition of stevia (97% purity; 14 – 15 g/L) although the stevia quantities used were much higher than the present study (0.32 g/L Stevia Reb A).

5.3.2 Anthocyanins

Chapter 3 revealed a 94 ± 2% loss in anthocyanin for unpasteurised extract stored at room temperature for 180 days. Initially, the inclusion of sugars and spices did not significantly ($p=0.353$) alter the anthocyanins (Figure 5.2). As the sweeteners and spices are devoid of anthocyanins, this was expected. Moreover, the quantities of sweeteners (8 % Sugar Equivalent; 80 g/L sugar and 0.32 g/L SRA of 80 % purity) and spices (1 g/L cinnamon or ginger) were relatively small.

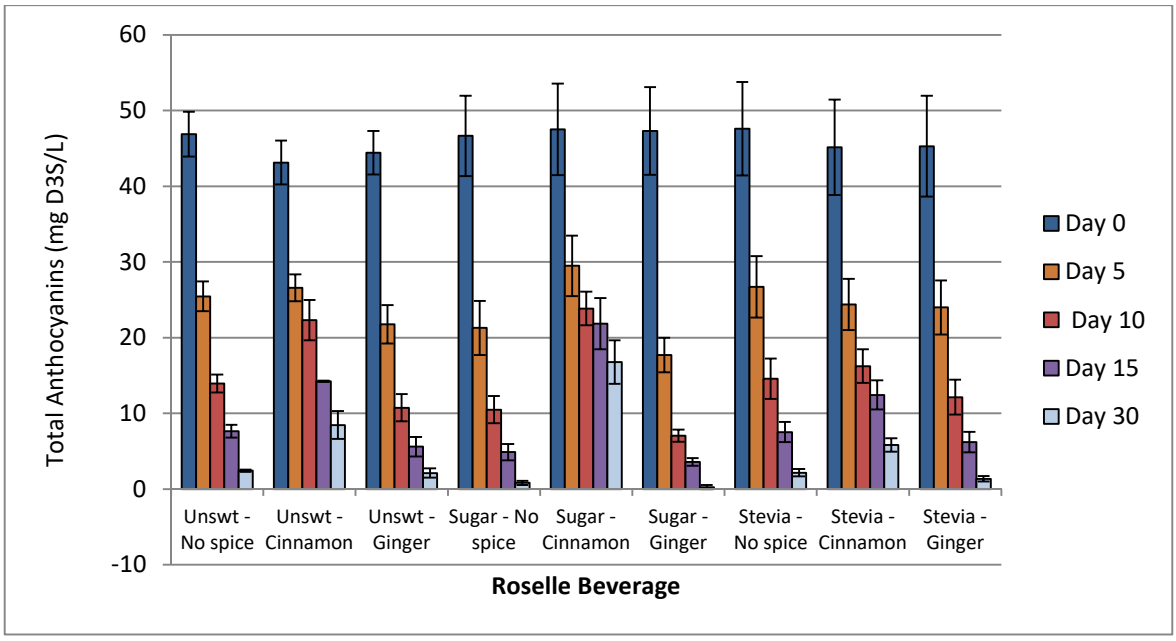


Figure 5.2: Total monomeric anthocyanins (mg Cyanidin-3-glucosided/L) profile of Roselle beverages unsweetened or sweetened (with sugar or stevia Rebaudioside A) and unflavoured or flavoured (with cinnamon or ginger) on Days 0, 5, 10, 15 and 30 of accelerated storage at 40°C. Each bar/point represents mean values + SD. "Unswt – no spice" refers to Roselle beverage without sweetener and spice; "Unswt – cinnamon" – refers to Roselle beverage with cinnamon flavour but no sweetener; "Unswt – ginger" – refers to Roselle beverage with ginger flavour but no sweetener; "sugar – no spice" refers to Roselle beverage without spice but sweetened with sugar; "sugar – cinnamon" refers to Roselle beverage with cinnamon and sweetened with sugar; "sugar – ginger " refers to Roselle beverage with ginger and sweetened with sugar; "SRA – no spice" refers to Roselle beverage without spice but sweetened with Stevia Rebaudioside A; "SRA – Cinnamon" refers to Roselle beverage with cinnamon and sweetened with Stevia Rebaudioside A; "SRA – Ginger " refers to Roselle beverage with ginger and sweetened with Stevia Rebaudoside A.

A study by Tsai, Hsieh, and Huang (2004) using 20 - 60% sugar showed a decrease in the degradation of anthocyanins with increasing sugar content. This effect may have been due

to dilution or the reduction in water activity rather than any particular reactions with the anthocyanins.

During the accelerated storage period, the most change in the beverage was expected to be with the anthocyanins. The sweeteners did not impact on the stability of the anthocyanins during storage (Figure 5.2). Consequently, there were no significant differences in the reaction rates or half-life of the unflavoured beverages (Table 5.1). This varies from the results obtained in the study by Tsai et al. (2004) where the increase in sugar concentrations led to increases in the half-life of the anthocyanins under accelerated storage conditions, although the sugar concentration was much higher in the study.

Table 5.1: Total anthocyanin kinetics data for sweetened and spice-flavoured Roselle beverages (n=8)

Sample	Initial total anthocyanin (mg D3S/L)	Rate constant (day^{-1})	Coefficient of determination (R^2)	Half-life $t_{1/2}$ (days)
unswt_no spice	34 ± 4	0.1022	0.9811	7
unswt_cinnamon	31 ± 4	0.0532	0.9652	13
unswt_ginger	32 ± 4	0.1095	0.9622	6
sugar_no spice	34 ± 8	0.1295	0.9775	5
sugar_cinnamon	35 ± 9	0.0334	0.8132	21
sugar_ginger	34 ± 8	0.1479	0.9303	5
SRA_no spice	35 ± 9	0.106	0.9773	7
SRA_cinnamon	33 ± 9	0.0575	0.9153	12
SRA_ginger	33 ± 10	0.1073	0.9373	6

Total anthocyanin measured in Delphinidin 3-sambubioside equivalent. "Unswt – no spice" refers to Roselle beverage without sweetener and spice; "Unswt – cinnamon" – refers to Roselle beverage with cinnamon flavour but no sweetener; "Unswt – ginger" – refers to Roselle beverage with ginger flavour but no sweetener; "sugar – no spice" refers to Roselle beverage without spice but sweetened with sugar; "sugar – cinnamon" refers to Roselle beverage with cinnamon and sweetened with sugar; "sugar – ginger " refers to Roselle beverage with ginger and sweetened with sugar; "SRA – no spice" refers to Roselle beverage without spice but sweetened with Stevia Rebaudioside A; "SRA – cinnamon" refers to Roselle beverage with cinnamon and sweetened with Stevia Rebaudioside A; "SRA – ginger " refers to Roselle beverage with ginger and sweetened with Stevia Rebaudioside A.

The inclusion of cinnamon to the beverages displayed a stabilising effect on the anthocyanins (Figure 5.2). This effect was significant ($p < 0.05$) even in the absence of sweeteners. However, in combination with sugar, the effect was quite pronounced. Moreover, the rate of anthocyanin degradation was reduced (Table 5.1) and the half-life increased in the

cinnamon - sugar combination. Several compounds contained in the cinnamon extract may be responsible for this effect on the stability of anthocyanins over the 30 days of accelerated storage. They include catechin, protocatechuic acid, proanthocyanidins and cinnamaldehyde. Several studies have reported the ability of these compounds and their reaction products to stabilise anthocyanins through co-pigmentation reactions (Cortez et al., 2017). Cinnamaldehyde is slowly oxidised to form cinnamic acid and studies have shown that anthocyanins are more stable when acylated with cinnamic acid (Dougall et al., 1998; Giusti & Wrolstad, 2003). It is not clear what the mechanism for the stability of the total anthocyanins is and particularly what is the role of the sugar as it seems to enhance the stabilisation. However, considering the increase in total phenolic content in sugar sweetened beverages, phenolics contained in cinnamon may be reacting with the reducing sugars. Several studies revealed better stabilising effects of glucose on anthocyanin compared to sucrose or fructose and demonstrated a co-pigmentation reaction between glucose and chlorogenic acid (Kopjar & Piližota, 2011; Kopjar, Tiban, Pilizota, & Babic, 2009). Perhaps in this current study, the reaction is between glucose and any of the above listed phenolic compounds introduced with the inclusion of cinnamon.

5.3.3 FRAP activity

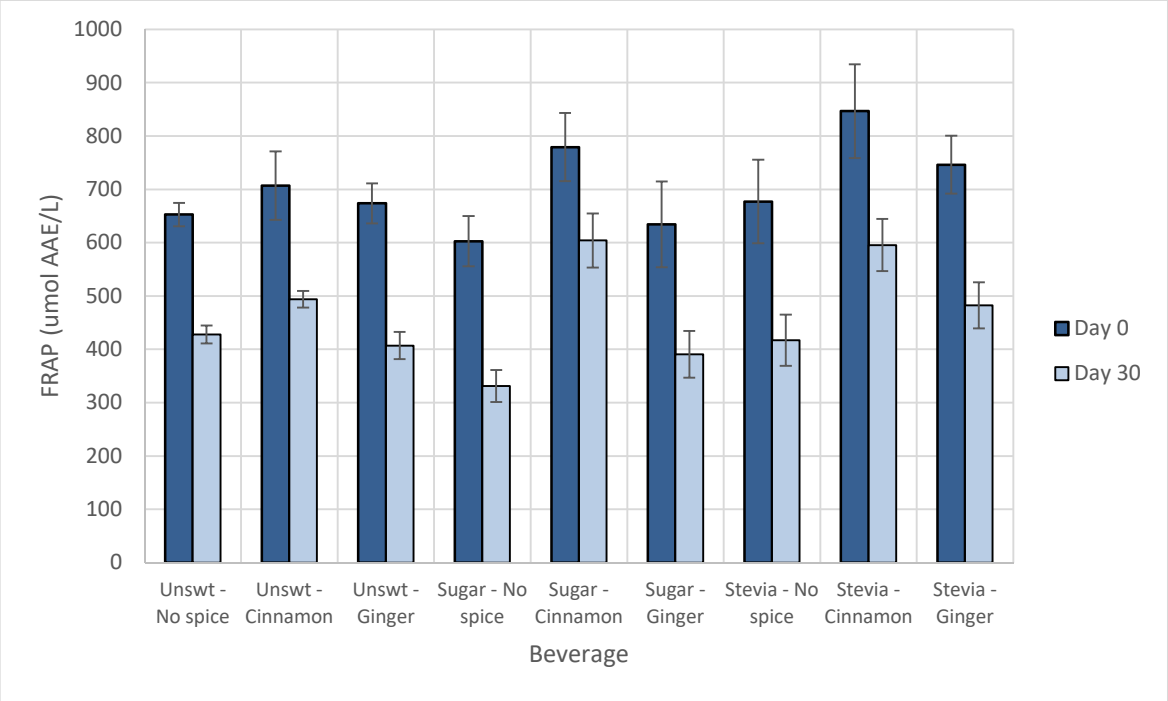


Figure 5.3: FRAP (µmol Ascorbic acid equivalent/L) profile of Roselle beverages unsweetened or sweetened (with sugar or stevia Reb A) and unflavoured or flavoured (with cinnamon or ginger) on Days 0 and 30 of accelerated storage at 40°C. Each bar/point represents mean values + SD. "Unswt – no spice" refers to Roselle beverage without sweetener and spice; "Unswt – cinnamon" – refers to Roselle beverage with cinnamon flavour but no sweetener; "Unswt – ginger" – refers to Roselle beverage with ginger flavour but no sweetener; "sugar – no spice" refers to Roselle beverage without spice but sweetened with sugar; "sugar – cinnamon" refers to Roselle beverage with cinnamon and sweetened with sugar; "sugar – ginger " refers to Roselle beverage with ginger and sweetened with sugar; "SRA – no spice" refers to Roselle beverage without spice but sweetened with Stevia Reb A; "SRA – cinnamon" refers to Roselle beverage with cinnamon and sweetened with Stevia Reb A; "SRA – ginger " refers to Roselle beverage with ginger and sweetened with Stevia Reb A.

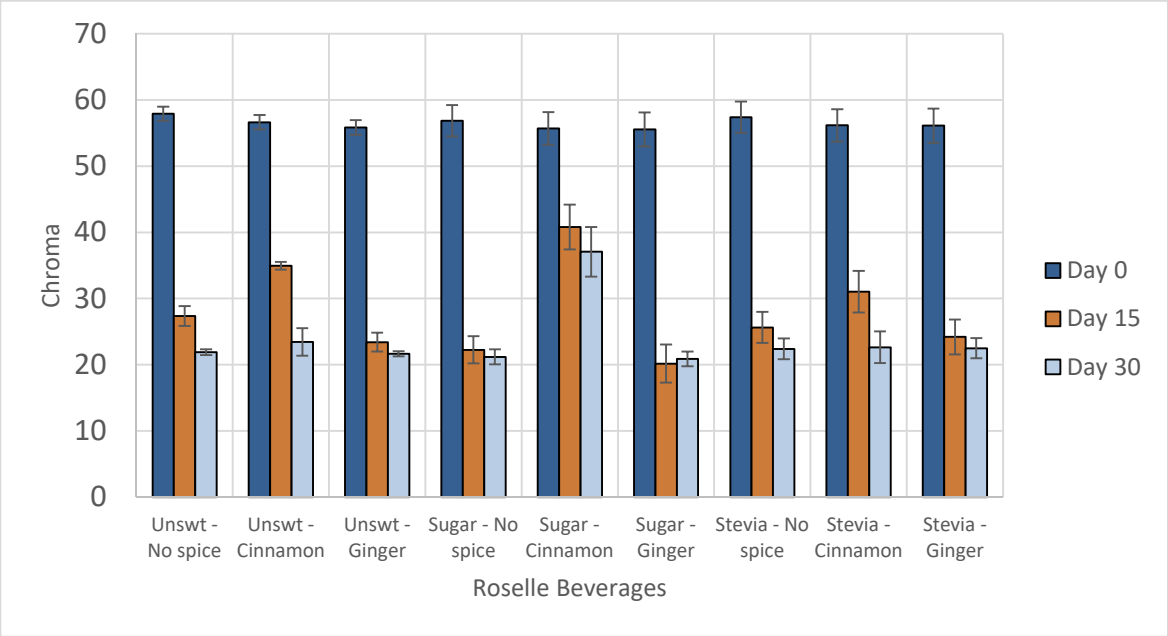
The initial FRAP activity did not significantly ($p > 0.05$) change with the addition of sweeteners (Figure 5.3). The findings in the SRA sweetened beverage are similar to findings by Korir, Wachira, Wanyoko, Ngure, and Khalid (2014) where 3 g/L of stevia did not improve the antioxidant capacity of black tea. However, for sugar, the results in literature are contradictory. When 30 g/L of added sugar was added to black tea the antioxidant capacity reduced (Korir et al., 2014). Conversely, the FRAP antioxidant capacity of mulberry extract increased with the addition of sugar (20 – 60%) although with heating (Tsai, Delva, Yu, Huang, & Dufosse, 2005). For the study by Korir et al. (2014), the mechanism of the reduction in

antioxidant capacity was postulated to be as a result of glucose-gallic complexes. In the Tsai et al. (2005), the increase in FRAP (antioxidant) capacity was attributed to maillard reaction products which have verified in other studies to be effective antioxidants (Kim & Lee, 2009). There was also a significant ($p < 0.05$) increase in initial antioxidant content when cinnamon was added to unsweetened or sweetened Roselle beverages. This corroborates the partial relationship between anthocyanins and antioxidants as discussed in Chapter 3. This effect was not replicated with ginger. Only in the SRA sweetened beverage did ginger effect a significant ($p = 0.194$) initial increase in FRAP (antioxidant) capacity followed by a better anthocyanin stability compared to the unsweetened beverage. Firstly, both spices are considered to be strong antioxidants with cinnamon having about 4 times or more activity than ginger (Dragland et al., 2003), hence, increased antioxidant capacity is expected.

5.3.4 Colour

For all beverages, the correlation between the total anthocyanin content and chroma was high ($R = 0.99 \pm 0.01$). The sweetener type did not affect the initial colour of the Roselle beverage and there was no subsequent effect on the stability of the colour during storage. Although there was no initial effect of the spices on the chroma of the beverage, the inclusion of cinnamon in unsweetened and sweetened beverages led to a reduction in the rate of anthocyanin degradation, particularly within 15 days. Eventually, the anthocyanin degradation on day 30 of the accelerated study was the same as the beverages without cinnamon in the unsweetened and SRA sweetened beverages. However, this was not the case where cinnamon was used in combination with sugar. In this case, there was a significant effect of colour chroma (Figure 5.4a).

(a)



(b)

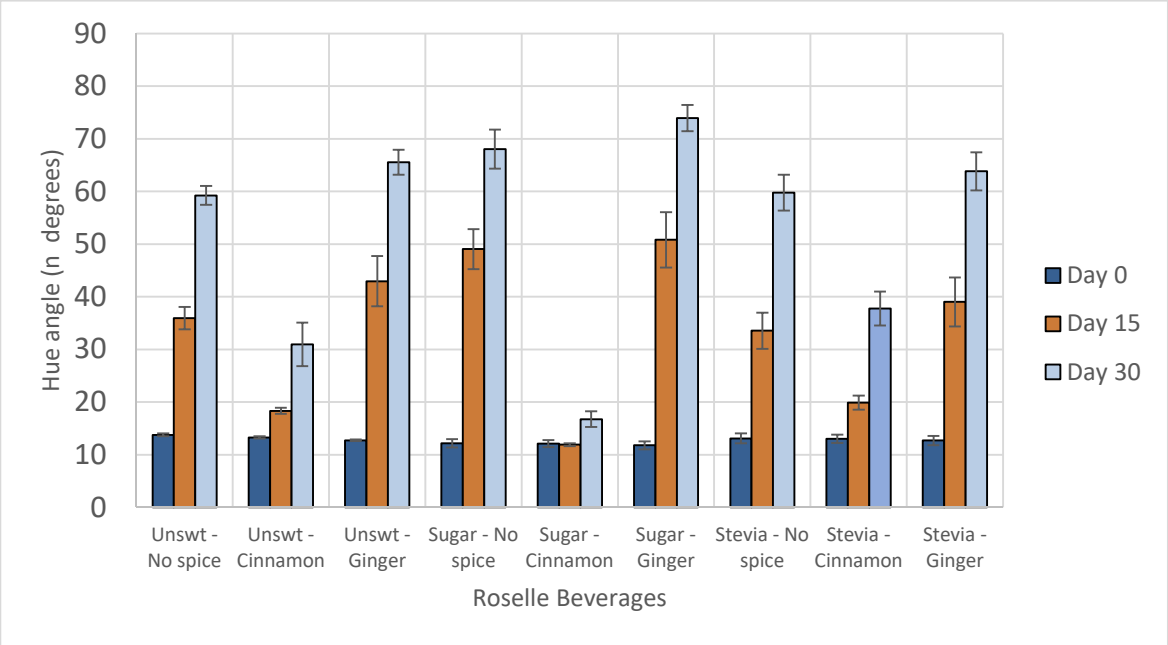


Figure 5.4: (a) Chroma profile of Roselle beverages unsweetened or sweetened (with sugar or stevia Reb A) and unflavoured or flavoured (with cinnamon or ginger) on Days 0, 15 and 30 of accelerated storage at 40°C. (b) Hue-angle profile of Roselle beverages unsweetened or sweetened (with sugar or stevia Reb A) and unflavoured or flavoured (with cinnamon or ginger) on Days 0, 15 and 30 of accelerated storage at 40°C. Each bar/point represents mean values + SD. "Unswt – no spice" refers to Roselle beverage without sweetener and spice; "Unswt – cinnamon" – refers to Roselle beverage with cinnamon flavour but no sweetener; "Unswt – ginger" – refers to Roselle beverage with ginger flavour but no sweetener; "sugar – no spice" refers to Roselle beverage without spice but sweetened with sugar; "sugar – cinnamon" refers to Roselle beverage with cinnamon and sweetened with sugar; "sugar – ginger " refers to Roselle beverage with ginger and sweetened with sugar; "SRA – no spice" refers to Roselle beverage without spice but sweetened with Stevia Reb A; "SRA – cinnamon" refers to Roselle beverage with cinnamon and sweetened with Stevia Reb A; "SRA – ginger " refers to Roselle beverage with ginger and sweetened with Stevia Reb A.

Conversely, colour hue angle (Figure 5.4b) was weakly correlated to total anthocyanins. However, there was no effect of the sweetener on hue-angle. All beverages containing cinnamon had a lower hue angle throughout storage. The sugar – cinnamon beverage maintained the red colour, thus, linking well with the anthocyanin data. The colour stability offered with the sugar - cinnamon combination is perhaps one of the most important findings of this storage as it is would the most obvious indication of the quality of the beverage to consumers. Without this contribution to the beverage, the hue angle of the beverages would increase, as observed with the other beverages in this study, tending towards a more yellow colour during storage.

5.3.5 pH

For most of the Roselle beverages, the pH values did not change significantly throughout the storage period (Figure 5.5). The addition of sweeteners or spices did not significantly alter initial pH values. For all beverages. The pH reduced by day 15 but increased by day 30. This suggests changes occurring in the constituents of phenolic compounds during storage. The pH did not correlate with any of the other measured parameters.

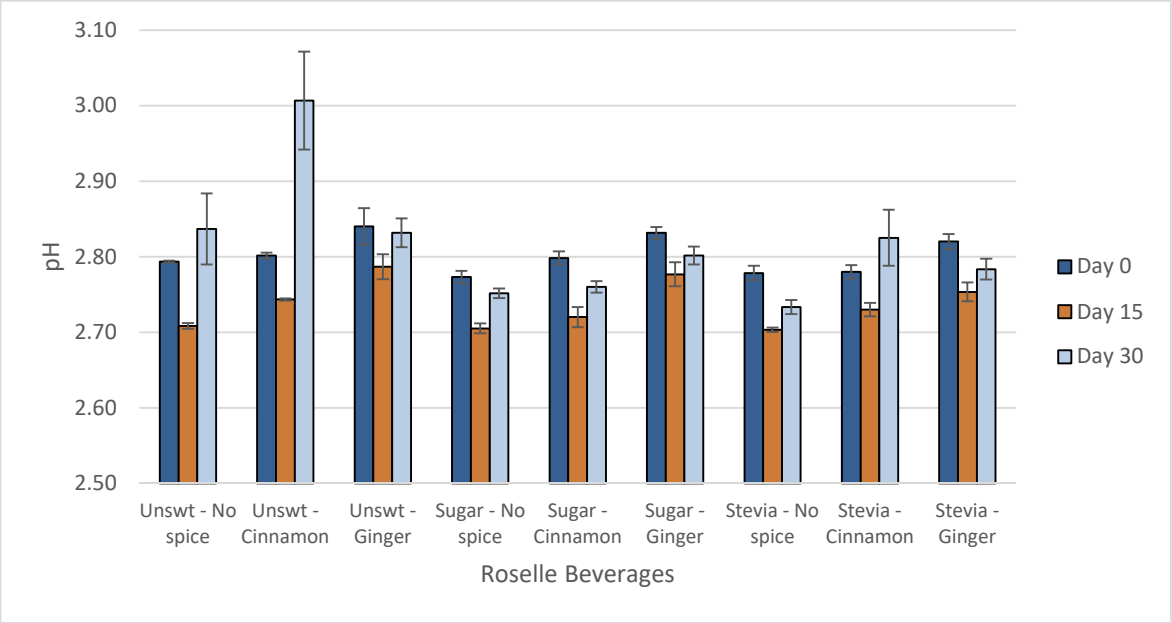


Figure 5.5: pH profile of Roselle beverages unsweetened or sweetened (with sugar or stevia Reb A) and unflavoured or flavoured (with cinnamon or ginger) on Days 0, 15 and 30 of accelerated storage at 40°C. Each bar/point represents mean values + SD. "Unswt – no spice" refers to Roselle beverage without sweetener and spice; "Unswt – cinnamon" – refers to Roselle beverage with cinnamon flavour but no sweetener; "Unswt – ginger" – refers to Roselle beverage with ginger flavour but no sweetener; "sugar – no spice" refers to Roselle beverage without spice but sweetened with sugar; "sugar – cinnamon" refers to Roselle beverage with cinnamon and sweetened with sugar; "sugar – ginger " refers to Roselle beverage with ginger and sweetened with sugar; "SRA – no spice" refers to Roselle beverage without spice but sweetened with Stevia Reb A; "SRA – cinnamon" refers to Roselle beverage with cinnamon and sweetened with Stevia Reb A; "SRA – ginger " refers to Roselle beverage with ginger and sweetened with Stevia Reb A.

5.4 Conclusion

The addition of sweeteners (8% sugar equivalent) to Roselle beverage did not prevent degradation of chemical compounds at the initial stage. In this respect, Stevia Rebaudioside A (SRA) was found to be a good replacement for sugar in the Roselle beverage. However, with the addition of spices, cinnamon particularly improved the stability of anthocyanins and peharps the total phenolic content, consequently, the colour of sugar sweetened beverage was also better retained. This was not observed with SRA or when ginger was used with either sweetener.

The increase in total phenolics over the storage period for the sugar sweetened samples suggests the presence of reducing sugars e.g. glucose, which may assist in the stability of the

beverage in combination with cinnamon. Secondly, there may be co-pigmentation reactions or acylation of the anthocyanins with other phenolic provided from cinnamon or a complex product formed from the reaction of phenolic compounds with sugar. Although the mechanism is not certain yet, the findings in this study show some particular interactions between sugar and cinnamon with the Roselle anthocyanin which leads to improved stability over a 6-months room temperature storage periods (40^{o}C for 30 days accelerated conditions). This should be further investigated to gain improved insight into possible mechanisms. The findings here can also be translated into beverages with anthocyanins.

Chapter 6: General Conclusions and Future work

This concluding chapter of the thesis will summarise key findings in the research work and main contributions to knowledge in the subject field. Also, the potential applications of these findings to an industrial (beverage) process will be evaluated. In the previous chapters, it was made clear that the provision of healthier beverage products is a global concept, however, this must be achieved without compromise on product quality. Furthermore, due to consumer demands for clean label products, there are knowledge gaps in the beverage production process that need to be researched. Consequently, the overall aim of this research work was to use Roselle beverages as a template to investigate relevant concepts within key aspects of a healthy beverage production process. These process steps were in relation to the preservation, sweetening and flavouring of the beverage.

These key aspects of the production process led to the research questions explored in this thesis. First, with preservation, the research questions were whether pasteurisation and storage at different conditions affected the physical and biochemical properties of the Roselle beverage; and to what extent (Chapter 3). The results were considered to be the foundation of the research work which could be linked to existing literature and on which subsequent research work could be built upon.

The key findings of this chapter were that there was no significant effect due to pasteurisation on the initial values of the extract, under the conditions used, but there were significant differences between extracts stored at warm (room temperature) and cold (refrigerated and frozen) storage temperatures. This latter part was as expected but the former contradicted some previous studies.

The differences in pasteurisation temperature and/or time and solids-to-solvent ratio could influence degradation results, hence the differences between these and some of the reported results.

The most interesting effect of pasteurisation on stored extracts was the better retention of ACE inhibitory (ACEi) activity over the 6 months of storage. Although a link was observed between ACEi and anthocyanins, the changes observed in anthocyanin content did not fully account for the changes observed ACEi activity. Therefore, it was concluded that other compounds (most probably polyphenols) also contributed to ACEi activity. The ACE FAPGG assay used was quite sensitive and expensive, therefore, retrospectively, an alternative method for establishing the ACEi property of Roselle would be considered for future work in this area.

It was hypothesised that the anthocyanin degradation products, gallic acid and protocatechuic acid, participated in the stabilising of FRAP (antioxidant) capacity after 60 days of storage at room temperature. A confirmation of this hypothesis would be good for overall knowledge so that perhaps the early incorporating of these degradation products into the formulation may retard the expected losses in antioxidant capacity.

Another key result in relation to pasteurisation was that the sensory profiling of pasteurised and pre-pasteurised Roselle extracts (Chapter 4) were not significantly different which was in conformity to findings in Chapter 3. Even when Roselle was dried differently from the commercially air-dried sample, the results were the same. This was a negative outcome to the first hypothesis in Chapter 4 i.e. variations in processing conditions will affect the sensory profile of a Roselle infusion. This outcome was also the case for tray dried, freeze dried and commercially air- dried Roselle infusions.

Overall, these results support the continued use of pasteurisation in the beverage industry. Due to its relatively low cost and simplicity, it is ideal for small scale producers. Moreover, the knowledge that it is not further detrimental to the physical, chemical and sensory properties of the pre-pasteurised extract, under similar processing conditions, is useful. Also, the strong stability of Roselle beverages displayed at lower temperatures, such as refrigeration and freezing, offer some flexibility with the handling of Roselle beverages in general. The second key aspect under consideration in this thesis was sweetening of the beverage. This is the current hot topic in the beverage industry. It was included in a series of research questions in Chapter 4. The three relevant research hypotheses were: Stevia Rebaudiana A (SRA) may enhance aspects of the sensory profile of Roselle infusions; consumers can discriminate between sugar and SRA sweetened beverages and prefer sugar-sweetened RB and spices may mask off-notes in RB sweetened with SRA. Already the addition of sugar is known to enhance the sensory attributes of many beverages. However, it is unknown whether SRA would do the same. Like sugar, SRA also did improve certain attributes in the Roselle beverage, such as reduced acid, salty and bitter tastes; reduced dusty and veggie odours and flavours. However, it reduced key attributes like fruity odours and flavours and accented cooked flavours. It also increased the characteristic liquorice flavour and after-effect. Hence, it was not surprising that consumers discriminated between sugar and SRA.

Consequently, and in consideration of the last relevant aspect of the healthy beverage processing i.e. flavouring, an attempt was made to mask the off-flavours of SRA with natural flavours i.e. spices (cinnamon, cloves and ginger). This formed the basis of the last research statement in Chapter 4, which was, spices may mask off-notes in RB sweetened with SRA. A masking effect was not observed on SRA sweetened beverages but a

congruency between cinnamon and ginger spices and the Roselle sensory attributes was observed, as consumer liking of cinnamon and ginger flavoured beverages sweetened with SRA was not significantly different from the unflavoured SRA sweetened beverage.

On the issue of sweetening, in response to the research hypothesis: there is an effect of Just about right (JAR) levels of sweeteners on the physical and bioactive compounds in the Roselle beverage, the results in Chapter 5 show that at 8% sugar equivalent, the sweeteners had no significant effect on any of the properties measured. Neither sugar nor SRA altered the degradation path of any of the bioactive compounds during storage. Therefore if consumers could accept SRA or masking of its off-flavours could be achieved with spices SRA could be a satisfactory replacement for sugar in this regard.

Following on from this study, similar quantities of sweeteners (8% sugar equivalent) and spices (1 g/L) were incorporated in Roselle extract to investigate their influence on physical and bioactive properties in Chapter 5. This was in response to the research hypotheses: cinnamon and ginger spices may affect the physical and bioactive compounds in the Roselle beverage, and combinations of sweeteners with spices may stabilise key compounds prone to deterioration in the Roselle beverage. The results showed that cinnamon surpassed ginger in anthocyanin and antioxidant stabilising properties. Moreover, the observed beneficial effect of the sugar - cinnamon mixture, showed the possibilities of the right ingredient combinations in enhancing the stability of Roselle compounds. There was also a significant effect of the SRA - cinnamon mixture on the reduction of losses in FRAP (antioxidant) capacity, although it was significantly less than the sugar – cinnamon effect.

Ultimately, it is believed that all the research questions presented in this thesis were adequately but investigated. There were several limitations to this research work. Firstly, the difficulty in obtaining fresh Roselle restricted the research to the use of commercially

air dried samples with minimal processing information, in most cases. Secondly, there was focused placed on the physical, chemical and sensory properties of the beverage but a microbiological work is also important. Thirdly, with research methodology, there were equipment restrictions and challenges which sometimes impacted on the data that could be obtained, for instance, with the availability of temperature controlled storage facilities, and the breakdown of equipment at certain points during the research work.

However, most importantly, the research designs selected only a small range of Roselle, sweetener and spice. Investigating a wider source/range of these ingredients to provide some more positive outcomes is necessary. There may indeed be advantages in combining sweeteners and combining spices rather than using them individually as carried out in this research which particularly in the consumer study made it easy for consumers to identify specific spices which may have introduced some bias. Furthermore, other sweetener and spice combinations may provide similar synergy as was observed with sugar and cinnamon. There were interesting outcomes to some challenges. For instance, the selection of natural sweeteners led to wider opportunities for learning; and the absence of a Roselle beverage lexicon in existing literature led to the development of a lexicon with a trained panel which was adapted for use with the Roselle beverages as well. Notwithstanding of limitations, this research adds to the body of scientific knowledge by exploring and providing evidence for consumer preference/acceptability of sugar and SRA. In general, there is a gap in stevia related sensory information in beverage research. It also initiates research into spice flavourings which is an under-researched aspect of beverage research, particularly in relation to anthocyanin and antioxidant stability. Furthermore, it offers areas for future research in trying to identify the compounds responsible for the attribute enhancing and diminishing effects.

In conclusion, good quality clean label functional beverages appear to be an achievable concept but still requires further research. Therefore, future work may include investigating the mechanism for ACE inhibition in Roselle beverages. Next, investigating the reasons behind the stability of FRAP (antioxidant) capacity after 60 day storage at room temperature in real time study may provide information on useful compounds with stabilising abilities which may be incorporated during beverage formulation to reduce the loss of FRAP (antioxidant) capacity from the onset. Further storage tests with sweeteners and spices should involve microbiological shelf life studies for holistic results. Now fully aware of the differences in sugar and SRA based on profiling results with the hibiscus beverages, masking the associated off-notes of SRA is another important area for further research. The combination of sweeteners and/or spices could be further investigated for synergistic effects especially in relation to masking of off-notes and anthocyanin and antioxidant stability. This current study may be expanded to anthocyanin rich extracts, natural sweeteners and spices which may further inform the body of knowledge for the beverage industry.

Appendices

Appendix I

Table A1.1: Online recipes for Roselle beverages containing spice/herbs

Country	**Spices used**	**Sources (last accessed 20th March 2017)**
Burkina faso	mint, ginger	http://www.nearof.com/?p=890
Burkina faso	vanilla extract	http://www.internationalcuisine.com/hibiscus-flower-drink/
Canada	mint, ginger, vanilla extract	http://www.food.com/user/593927
Congo	mint, vanilla extract, ginger	http://www.congocookbook.com/beverages/jus_de_bissap.html
Cote d'ivoire	mint, vanilla	https://globalgrazers.wordpress.com/2012/11/10/our-bissap-recipe-a-refreshing-treat-on-a-hot-summers-day/
Dubai	cinnamon	http://www.abudhabiconfidential.ae/wellbeing/karkade-recipe/
Egypt	ginger, allspice berries, cinnamon	http://www.whats4eats.com/beverages/sorrel-punch-recipe
Egypt	vanilla, mint	http://www.gloriagoodtaste.com/wp-content/uploads/2015/08/Karkade-Egypt-21.pdf
Ghana	cloves, cinnamon, vanilla	http://recipes.ghanaculturepolitics.com/2016/08/30/how-to-make-sobolo-sorrel-drink/
Ghana	ginger, hwentia (*Xylopia aethiopica*), pepree (*Aframomum melegueta*), wisaa, ginger	http://www.xocara.com/2015/11/how-to-make-bissap-sobolo-drink-with-michael-ghananie-com/
Ghana	ginger, cinnamon, grains of paradise (*Aframomum melegueta*), vanilla, mint, cloves	http://mywekutastes.com/making-africas-sobolo-drink-bissap-drink/#.WNAT9Pnyi01
Ghana	lemongrass	http://www.naaoyooquartey.com/ganyobinaa/xyqd1zmdlfss76d7x6gflvb3omf958/11/30/2015
Ghana	cloves, ginger	http://bibinigh.com/recipe-for-sorrel-drink-also-known-as-sobolo-in-ghana/
Jamaica	ginger	http://jamaicans.com/hibiscus-tea/
Jamaica	cloves, allspice, cinnamon, mace (Myristica fragrans)	http://www.jamaicatravelandculture.com/food_and_drink/sorrel_drink.htm
Jamaica	ginger, cinnamon	http://globaltableadventure.com/recipe/jamaican-sorrel-drink/
Jamaica	ginger, cloves	http://www.gracefoods.com/recipe-a-z/recipe/4663-sorrel-drink
Malaysia	cinnamon, cloves, star anise	http://honestcooking.com/karkadeh-a-sweet-hibiscus-tea/

Mali	vanilla, ginger	http://globaltableadventure.com/recipe/recipe-vanilla-ginger-bissap-with-poll/
Mali	vanilla, mint	https://cookpad.com/uk/recipes/425528-jus-de-bissap-malian-hibiscus-flower-juice-mali
Mauritania	cinnamon, vanilla extracts	http://nomadunderthebluesky.com/bissap-recipe-a-cold-drink-from-senegal/
Mexico	cinnamon, cloves, allspice, nutmeg	http://allrecipes.com/recipe/214366/agua-de-jamaica-hibiscus-water/
Nigeria	cinnamon	http://www.foodsfromafrica.com/201622tradtional-zobo-drink-hibiscus-punch-recipe-egyptian-karkade/
Nigeria	garlic, ginger	http://www.allnigerianrecipes.com/drinks/zobo-drink.html
Nigeria	cloves, chilli flakes/cayenne pepper, ginger	http://dooneyskitchen.com/my-7-ingredient-zobo-drink/
Nigeria	ginger, cloves	http://www.kaunakitchen.com/zobo-drink/
Nigeria	ginger	http://withdrmalik.org/2016/06/zobo-drink-recipe.html
Nigeria	ginger, garlic, pepper soup spice (cloves, *Xylopia aethiopica, Monodora myristica*), scotch bonnet chilli	http://www.wivestownhallconnection.com/2013/08/how-to-make-nutritious-nigerian-zobo.html
Nigeria	cloves, cinnamon, ginger	https://www.naij.com/1086789-zobo-drink-weight-loss.html
Nigeria	garlic, ginger, cloves, mint	http://zeeliciousfoods.com/zobo-drink-recipe/
Senegal	cloves, allspice seeds, cinnamon, ginger	http://www.seasoningbottle.com/senegalese-bissap/
USA	lemon grass, vanilla	http://www.betumi.com/2009/08/recipe-8-bissap-hibiscus-iced-tea.html
USA	cinnamon, ginger, allspice berries (*Pimenta dioica*)	http://www.simplyrecipes.com/recipes/agua_de_jamaica_hibiscus_tea/
USA	ginger, allspice berries	http://healthiersteps.com/jamaican-sorrel-drink/
USA	cinnamon, mint	http://www.thelittleepicurean.com/2014/08/hibiscus-tea-jamaica.html
USA	Mint	https://manfuelblog.com/2015/08/02/egyptian-karkade-hibiscus-iced-tea-recipe/
USA	cinnamon, ginger, star anise, allspice, lemongrass	http://www.thekitchn.com/recipe-cold-brew-jamaica-hibiscus-iced-tea-recipes-from-the-kitchn-192433
USA	cloves, allspice (pimento), cinnamon, ginger	http://www.osochic.com/2013/12/holiday-christmas-cocktail-sorrel-drink-recipe-bissap-zobo.html
USA	canela, cinnamon, allspice berries (*Pimenta dioica*)	http://www.saveur.com/article/Recipes/Mexico-Sweet-Hibiscus-Drink

The table contains links to online recipes of Roselle beverages, which were selected based on containing at least one herb in the ingredient list and not a repeat of another recipe found

online. In the summary label below, spices were edited for consistency; colloquial names replaced with scientific names and unidentifiable spices/herbs excluded.

Table A1.2: Frequency of spice usage in Roselle beverage online recipes from Table A1.1

Spice name (Scientific name)	Occurrence in recipes
African pepper (*Xylopia aethiopica*)	2
Alligator pepper (*Aframomum melegueta*)	2
Allspice (*Pimenta dioica*)	9
Calabash nutmeg (*Monodora myristica*)	1
Cayenne pepper (*Capsicum annuum*)	1
Cinnamon (*Cinnamomum cassia*)	**18**
Cloves (*Syzygium aromaticum*)	**14**
Garlic (*Allium chinense*)	3
Ginger (Zingiber officinale)	**24**
Lemongrass (*Cymbopogon citratus*)	3
Mace (*Myristica fragrans*)	1
Mint (*Mentha spicata*)	10
Nutmeg (*Myristica fragrans*)	1
Scotch bonnet chilli (*Capsicum chinense*)	1
Star anise (*Illicium verum*)	2
Vanilla (*Vanilla planifolia*)	11
Total count	103

Table A1.3: Profiling vocabulary list for Roselle Extract

Vocabulary classification	Term	Description	Reference	Anchors
Appearance	Red Colour	Shade of red associated with Roselle extract/beverages	Roselle extract (5g/L) at 90^{o}C for 25 minutes	Light to Dark
	Bubbles	Vesicle of beverage filled with air	N/A	None to Lots
	Sediments	Particles at the bottom of the extract/beverage	N/A	None to Lots
	Cloudy	Perceived translucence of the extract/beverage	N/A	Not to Very
Odour	Sweet	Odour associated with sugary foods	N/A	Not to Very
	Red berry fruit	Aroma(s) associated with red berry fruits	a combination of Cranberry juice and raspberry fruit	Not to Very
	Orchard fruit	Aroma(s) associated with orchard fruit	a combination of pear and ripe apple fruits	Not to Very
	Floral	A subtle aroma note associated with dry rose petals	dry rose petals	Not to Very
	Veggie note	Earthy aroma associated with root vegetables	Raw turnip	Not to Very
	Allspice	Spicy odour associated with the ground allspice	Ground dried allspice	Not to Very
	Cooked	Jammy odour associated with processed fruits	Strawberry jam	Not to Very
	Dusty	Odour associated with dried storage	Hessian string/sack/bag	Not to Very
Taste	Sweet	Taste associated with sugary food	N/A	Not to Very
	Acid	Sensation associated with lemons	N/A	Not to Very
	Bitter	Distinct taste of quinine	N/A	Not to Very
	Salty	Taste associated with NaCl	N/A	Not to Very
	Metallic	Unusual off note taste sensation in the mouth	N/A	Not to Very
Flavour	Red berry fruit	Flavour(s) associated with red berry fruits	a combination of cranberry juice and raspberry fruit	Not to Very
	Orchard fruit	Flavour(s) associated with orchard fruits	a combination of pear and ripe apple fruits	Not to Very
	Brown Fruits	Flavour(s) associated with brown fruits	Dates and Raisins	Not to Very
	Veggie note	Earthy flavour associated with root vegetables	Raw Turnip	Not to Very
	Floral	A subtle flavour note associated with dry rose petals	Dry rose petals	Not to Very

	Allspice	Spicy flavour associated with the ground allspice	Ground dried llspice	Not to Very
	Cooked	Jammy flavour associated with processed fruits	Strawberry jam	Not to Very
	Dry wood	Flavour associated with dried wood	Sharpened pencil shavings	Not to Very
	Dusty	Odour associated with dried storage	Hessian string/sack/bag	Not to Very
Mouthfeel	Mouth coating	Degree to which the beverage coats the mouth	N/A	Not to Very
	Salivating	Degree to which saliva is induced upon drinking of the beverage	Cranberry juice	Not to Very
	Drying	Degree of Astringency or mouth puckering	N/A	Not to Very
Aftereffect	Sweet	A lingering taste associated with sugary foods	N/A	Not to Very
	Acid	A lingering lemony taste	Lemon	Not to Very
	Salty	A lingering taste associated with NaCl	N/A	Not to Very
	Bitter	A lingering distinct taste associated with quinine	N/A	Not to Very
	Allspice	A lingering spicy taste	Ground allspice	Not to Very
	Drying	Astringent, mouth puckering sensation	N/A	Not to Very

Table A1.4: Profiling vocabulary list for Roselle beverage

Vocabulary classification	**Term**	**Definition**	**Reference**	**Anchors**
Appearance	Red Colour	Shade of red associated with Roselle extract/beverages	Roselle extract (5g/L) at 90^{o}C for 25 minutes	Light to Dark
	Bubbles	Vesicle of beverage filled with air	N/A	None to Lots
	Sediments	Particles at the bottom of the extract/beverage	N/A	None to Lots
	Cloudy	Perceived translucence of the extract/beverage	N/A	Not to Very
Odour	Sweet	Odour associated with sugary foods	N/A	Not to Very
	Red berry fruit	Aroma(s) associated with red berry fruits	a combination of cranberry juice and raspberry fruit	Not to Very
	Pineapple juice	Aroma associated with pineapple juice	UHT pineapple juice	Not to Very
	Orchard fruit	Aroma(s) associated with orchard fruit	Standards – a combination of pear and ripe apple fruits	Not to Very

	Floral	A subtle aroma note associated with dry rose petals	Dry rose petals	Not to Very
	Ginger	Aroma associated with ginger	Ground dried ginger	Not to Very
	Cloves	Aroma associated with cloves	Ground dried cloves	Not to Very
	Cinnamon	Aroma associated with cinnamon	Ground dried cinnamon	Not to Very
	Cooked	Jammy odour associated with processed fruits	Strawberry jam	Not to Very
	Dusty	Odour associated with dried storage	Hessian string/sack/bag	Not to Very
	Soapy	Aroma associated with bar soap	Unperfumed hypoallergic soap	Not to Very
	Off note	Fishy and putty aroma associated with adhesives or plastics	N/A	Not to Very
Taste	Sweet	Pleasant taste associated with sugary food	N/A	Not to Very
	Acid	Sensation associated with lemons	N/A	Not to Very
	Bitter	Distinct taste associated with quinine	N/A	Not to Very
	Salty	Taste associated with NaCl	N/A	Not to Very
	Metallic	Unusual off note taste sensation in the mouth	N/A	Not to Very
	Savoury	Taste associated with spicy foods	N/A	Not to Very
Flavour	Red berry fruit	Flavour(s) associated with red berry fruits	a combination of UHT cranberry and raspberry juice	Not to Very
	Pineapple juice	Flavour(s) associated with pineapple juice	UHT pineapple juice	Not to Very
	Orchard fruit	Flavour(s) associated with orchard fruits	a combination of pear and ripe apple fruits	Not to Very
	Floral	A subtle flavour associated with dry rose petals	Dry rose petals	Not to Very
	Liquorice	Flavour associated with liquorice	N/A	Not to Very
	Ginger	Flavour associated with ginger	Ground dried ginger	Not to Very
	Cloves	Flavour associated with cloves	Ground dried cloves	Not to Very
	Cinnamon	Flavour associated with cinnamon	Ground dried cinnamon	Not to Very
	Cooked	Jammy taste associated with processed fruits	Strawberry jam	Not to Very
	Dusty	Odour associated with dried storage	N/A	Not to Very

	Soapy	Aroma associated with bar soap	N/A	Not to Very
	Off note	fishy and putty i.e. adhesive, plastic odour	N/A	Not to Very
Mouthfeel	Warming	Sensation of increased temperature created by spicy foods after swallowing	N/A	Not to Very
	Mouth coating	Degree to which the beverage coats the mouth	N/A	Not to Very
	Tingling	Stinging sensation on the tongue	N/A	Not to Very
	Residue	Feeling of the presence of sediments in the mouth	N/A	Not to Very
	Salivating	Degree to which saliva is induced upon drinking of the beverage	N/A	Not to Very
	Throat catching	Sensation created in the throat as a result of spiciness	N/A	Not to Very
Aftereffect	Sweet	A lingering taste associated with sugary foods	N/A	Not to Very
	Acid	A lingering lemony taste	N/A	Not to Very
	Salty	A lingering taste associated with NaCl	N/A	Not to Very
	Bitter	A lingering distinct taste associated with quinine	N/A	Not to Very
	Soapy	A lingering taste of soap	N/A	Not to Very
	Ginger	A lingering taste of ginger	N/A	Not to Very
	Cloves	A lingering taste of cloves	N/A	Not to Very
	Cinnamon	A lingering taste of cinnamon	N/A	Not to Very
	Drying	A lingering astringent or mouth puckering sensation	N/A	Not to Very
	Liquorice	A lingering taste associated with liquorice	N/A	Not to Very

Table A1.5: Consumer liking test questions

Sample liking questions		
Attribute/ Question	**Scoring method**	**Anchor points**
Overall liking	9 point hedonic scale	1 Dislike Extremely 2 Dislike Very Much 3 Dislike Moderately 4 Dislike Slightly 5 Neither Like nor Dislike 6 Like Slightly 7 Like Moderately 8 Like Very Much 9 Like Extremely
Appearance liking	9 point hedonic scale	1 Dislike Extremely 2 Dislike Very Much 3 Dislike Moderately 4 Dislike Slightly 5 Neither Like nor Dislike 6 Like Slightly 7 Like Moderately 8 Like Very Much 9 Like Extremely
Aroma liking	9 point hedonic scale	1 Dislike Extremely 2 Dislike Very Much 3 Dislike Moderately 4 Dislike Slightly 5 Neither Like nor Dislike 6 Like Slightly 7 Like Moderately 8 Like Very Much 9 Like Extremely
Flavour liking	9 point hedonic scale	1 Dislike Extremely 2 Dislike Very Much 3 Dislike Moderately 4 Dislike Slightly 5 Neither Like nor Dislike 6 Like Slightly 7 Like Moderately 8 Like Very Much 9 Like Extremely
Sweetness	JAR scale	1 Much too Little 2 Too Little 3 Just-about-right 4 Too Much 5 Much Too Much
Flavour Intensity	JAR scale	1 Much too Little 2 Too Little 3 Just-about-right

		4 Too Much 5 Much Too Much
Astringency	JAR scale	1 Much too Little 2 Too Little 3 Just-about-right 4 Too Much 5 Much Too Much
Consumption and Purchasing Intent		
Question	**Scoring method**	**Anchor points**
Consumption intent	5 point scale	1 Definitely would not drink 2 Probably would not drink 3 May or may not drink 4 Probably would drink 5 Definitely would drink
Purchasing intent	5 point scale	1 Definitely would not purchase 2 Probably would not purchase 3 May or may not purchase 4 Probably would purchase 5 Definitely would purchase
CATA	Multiple choice (tick)	Active, Adventurous, Affectionate, Aggressive, Bored, Calm, Daring, Disgusted, Eager, Energetic, Enthusiastic, Free, Friendly, Glad, Good, Good-natured, Guilty, Happy, Interested, Joyful, Loving, Merry, Mild, Nostalgic, Peaceful, Pleasant, Pleased, Polite, Quiet, Satisfied, Secure, Steady, Tame, Tender, Understanding, Warm, Whole, Wild, Worried
Demographics		
Question		**Anchor points**
Gender	1 2	MALE FEMALE
Ethnicity	1 2 3 4 5 6 7 8 9 10 11 12 13 14 15	WHITE- English/Welsh/Scottish/Northern Irish/British WHITE- Irish WHITE- Other MIXED/MULTIPLE ETHNIC GROUP- White and Black Carribean MIXED/MULTIPLE ETHNIC GROUP- White and Black African MIXED/MULTIPLE ETHNIC GROUP- White and Black Asian ASIAN/ASIAN BRITISH- Indian ASIAN/ASIAN BRITISH- Pakistani ASIAN/ASIAN BRITISH- Bangladeshi ASIAN/ASIAN BRITISH- Chinese BLACK/AFRICAN/CARRIBBEAN/BLACK BRITISH- African BLACK/AFRICAN/CARRIBBEAN/BLACK BRITISH- Carribbean OTHER ETHNIC GROUP- Arab OTHER ETHNIC GROUP- any other Prefer not to declare
Working Status	1 2	Working Unemployed

	3	Student
	4	Other
Food Sector	1	YES
	2	NO
Wage	1	Less than £15,000 per annum
	2	Approx £15,000 per annum
	3	More than £15,000 per annum
	4	Does not want to say
Completed qualification	1	Did not graduate from secondary school
	2	Secondary school graduate
	3	Apprenticeship/ trade
	4	Diploma
	5	Bachelor's degree
	6	Master's degree
	7	Doctorate (PhD)
Familiarity with Roselle		
Question	**Responses**	
Familiarity	1	YES
	2	NO
Normally Buy	1	Yes Frequently (Approximately once a week)
	2	Yes Sometimes (Approximately once a month)
	3	Rarely (Less than once per month)
	4	Never

Table A1.6: Profiling results on Attributes of Roselle Extracts

	CAD_90°C,25min		FD_90°C,25min		FD_100°C,3min		PCAD_90°C,25min		PFD_90°C,25min		PFD_90°C,25min		PTD_90°C,25min		TD_90°C,25min		Significance of sample (p=value)
Red Colour	53.4	a	52.8	a	43.8	bc	52.0	ab	53.3	a	40.6	c	49.5	ab	47.7	abc	<.0001
Bubbles	1.4	c	10.3	a	6.2	abc	2.0	bc	7.1	ab	2.3	bc	1.8	bc	5.3	abc	<.0001
Sediment	1.4	ab	7.0	ab	4.3	ab	1.0	b	7.7	a	7.3	ab	1.3	ab	2.0	ab	0.002
Cloudy	1.3	ab	5.3	ab	5.7	ab	0.9	b	4.8	ab	8.0	a	1.3	ab	0.1	b	0.003
Sweet Odour	13.3	b	21.2	ab	17.4	ab	17.1	ab	17.8	ab	20.7	ab	20.2	ab	23.1	a	0.02
Red berry Fruit Odour	22.4	a	29.4	a	25.0	a	27.8	a	24.8	a	30.8	a	29.1	a	30.4	a	0.04
Orchard Fruit Odour	9.1	a	10.4	a	10.9	a	10.0	a	9.6	a	11.3	a	11.0	a	14.8	a	0.83
Veggie Odour	9.3	bc	10.9	bc	13.2	abc	4.2	c	16.8	ab	9.0	bc	22.7	a	12.8	abc	0.0005
Floral Odour	6.9	a	13.1	a	9.6	a	8.3	a	8.1	a	12.9	a	9.8	a	11.9	a	0.43
Allspice Odour	3.8	ab	1.9	ab	0.7	b	6.1	a	2.5	ab	3.9	ab	3.2	ab	2.3	ab	0.03
Cooked Odour	6.0	a	10.4	a	8.0	a	8.5	a	9.0	a	10.4	a	11.0	a	13.5	a	0.24
Dusty Odour	24.7	a	10.2	c	24.0	ab	18.5	abc	25.4	a	13.4	bc	10.8	c	17.6	abc	<.0001
Sweet Taste	10.9	a	13.2	a	10.3	a	14.6	a	12.8	a	15.4	a	11.6	a	16.8	a	0.04
Acid Taste	25.6	bc	32.1	ab	29.4	abc	23.6	c	33.2	a	29.2	abc	29.0	abc	28.1	abc	0.001
Bitter Taste	10.4	a	10.0	a	10.8	a	12.5	a	13.6	a	11.0	a	11.1	a	11.0	a	0.67
Salty Taste	5.2	a	6.2	a	7.0	a	5.1	a	7.9	a	5.9	a	5.4	a	5.3	a	0.24
Metallic Taste	3.8	a	5.8	a	3.9	a	3.9	a	4.7	a	3.4	a	4.1	a	2.8	a	0.66
Red berry Fruit Flavour	27.7	a	32.0	a	27.6	a	29.3	a	32.3	a	32.8	a	28.6	a	32.8	a	0.38
Orchard Fruit Flavour	9.9	a	9.7	a	11.9	a	9.6	a	12.9	a	12.9	a	12.4	a	13.5	a	0.60
Brown fruit Flavour	5.5	a	2.5	a	3.4	a	3.3	a	4.7	a	2.8	a	3.0	a	2.0	a	0.48
Veggie Flavour	6.1	ab	8.2	ab	6.9	ab	4.4	b	11.9	ab	3.9	b	14.0	a	8.8	ab	0.01
Floral Flavour	6.6	a	11.8	a	8.5	a	7.7	a	10.1	a	13.7	a	12.8	a	13.0	a	0.04
Allspice Flavour	4.6	ab	2.7	b	1.0	b	7.2	a	2.7	b	2.2	b	2.1	b	2.8	b	0.001
Cooked Flavour	3.4	a	6.5	a	4.0	a	4.0	a	7.4	a	9.6	a	6.9	a	11.0	a	0.04
Dry wood Flavour	9.9	a	7.6	a	10.6	a	12.1	a	10.0	a	7.5	a	6.5	a	10.9	a	0.51
Dusty Flavour	26.2	a	11.1	b	23.7	a	19.1	ab	25.0	a	11.1	b	16.5	ab	16.7	ab	0.0001
Mouth coating	13.3	a	14.4	a	15.8	a	17.1	a	18.4	a	15.0	a	17.1	a	16.0	a	0.12
Salivating	24.5	a	22.9	a	23.3	a	22.2	a	27.0	a	23.4	a	25.2	a	26.5	a	0.45
Drying Mouthfeel	25.0	a	25.8	a	25.9	a	21.4	a	29.8	a	24.1	a	28.2	a	27.9	a	0.17
Sweet After-effect	7.7	a	8.9	a	7.7	a	9.7	a	8.4	a	10.6	a	9.3	a	11.4	a	0.47

Acid After-effect	18.4	a	20.6	a	21.2	a	16.6	a	22.0	a	18.4	a	21.9	a	21.9	a	0.03
Salty After-effect	5.9	a	4.1	a	6.6	a	5.0	a	5.5	a	4.5	a	5.8	a	4.4	a	0.48
Bitter After-effect	7.4	a	7.1	a	8.1	a	6.8	a	7.0	a	7.3	a	6.7	a	6.9	a	0.99
Allspice After-effect	2.5	ab	0.7	ab	0.1	b	3.0	a	0.6	ab	0.9	ab	0.9	ab	0.4	ab	0.02
Drying After-effect	25.1	ab	25.4	ab	25.3	ab	18.6	b	29.2	a	23.3	ab	25.9	ab	26.1	ab	0.01

Table A1.7: Profiling results on Attributes of Roselle beverages

	RB_SRA_cinnamon		RB_SRA_cloves		RB_SRA_ginger		RB_sugar_cinnamon		RB_sugar_cloves		RB_sugar_ginger		RB__SRA__no spice		RB__sugar_no spice		Significance of sample (p=value)
Red Colour	46.9	ab	51.6	a	50.1	ab	42.4	ab	46.7	ab	39.1	b	45.3	ab	42.1	ab	0.02
Bubbles	9.9	a	0.3	b	0.2	b	7.9	ab	4.6	b	3.5	b	1.6	b	3.8	b	<.0001
Sediment	19.7	a	31.9	a	33.2	a	31.7	a	29.8	a	21.8	a	21.1	a	25.0	a	0.05
Cloudy	16.6	a	18.8	a	15.0	a	24.5	a	14.5	a	9.4	a	10.7	a	12.1	a	0.26
Sweet Odour	34.0	a	29.4	a	34.6	a	22.8	a	25.7	a	30.7	a	32.8	a	29.7	a	0.09
Red berry Fruit Odour	15.0	a	10.1	a	17.0	a	9.5	a	9.1	a	13.9	a	16.9	a	14.8	a	0.03
Pineapple Juice Odour	4.5	a	4.9	a	14.7	a	0.0	a	2.2	a	2.8	a	14.2	a	9.5	a	0.01
Orchard Fruit Odour	6.6	a	3.4	a	8.9	a	0.0	a	2.5	a	6.0	a	9.0	a	6.9	a	0.00
Floral Odour	7.0	a	2.2	a	3.7	a	0.0	a	2.1	a	7.6	a	6.2	a	3.6	a	0.02
Ginger Odour	3.1	a	2.5	a	20.1	a	0.0	a	0.0	a	18.3	a	0.3	a	0.1	a	<.0001
Cloves Odour	1.5	b	58.7	a	0.1	b	0.0	b	63.6	a	0.0	b	0.1	b	0.0	b	<.0001
Cinnamon Odour	21.9	a	1.4	b	1.1	b	35.9	a	0.1	b	1.1	b	0.5	b	0.3	b	<.0001
Cooked Odour	9.1	a	6.6	a	9.2	a	0.0	a	4.5	a	7.5	a	11.2	a	10.8	a	0.16
Dusty Odour	9.3	a	7.3	a	3.4	a	4.2	a	6.4	a	2.7	a	2.7	a	1.2	a	0.17
Soapy Odour	1.5	a	4.5	a	1.0	a	0.0	a	3.7	a	4.6	a	1.0	a	0.3	a	0.41
Off-note Odour	0.2	a	0.1	a	0.2	a	0.0	a	1.9	a	0.1	a	1.4	a	1.0	a	0.74
Sweet Taste	43.1	a	39.7	a	39.8	a	38.7	a	42.2	a	40.2	a	39.6	a	45.2	a	0.88
Acid Taste	7.6	abc	8.1	abc	9.8	ab	11.6	a	6.6	abc	8.0	abc	8.8	abc	4.8	ac	0.08
Bitter Taste	5.6	ab	10.3	a	10.2	a	3.8	ab	9.9	a	5.6	ab	6.9	ab	2.0	b	0.01
Salty Taste	1.6	a	2.7	a	1.9	a	4.6	a	1.4	a	1.2	a	3.8	a	2.1	a	0.17
Metallic Taste	1.5	a	3.8	a	2.4	a	0.0	a	0.5	a	0.1	a	2.6	a	0.1	a	0.04
Savoury Taste	1.5	a	3.0	a	3.5	a	11.2	a	2.7	a	3.0	a	5.3	a	0.1	a	0.63
Red berry Fruit Flavour	20.9	a	12.8	a	16.6	a	6.2	a	13.5	a	21.5	a	16.2	a	17.0	a	0.01

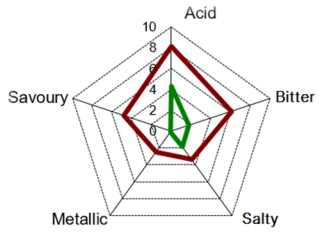
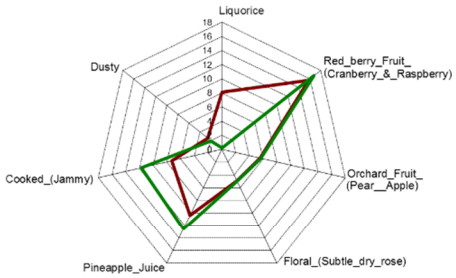
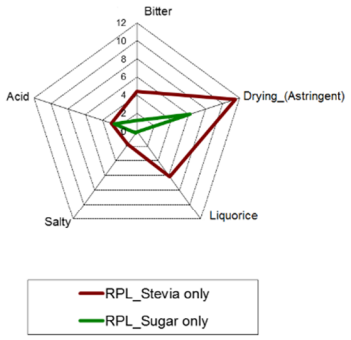
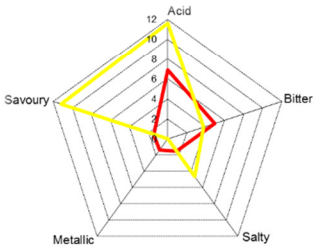
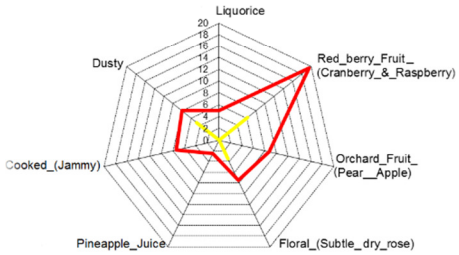
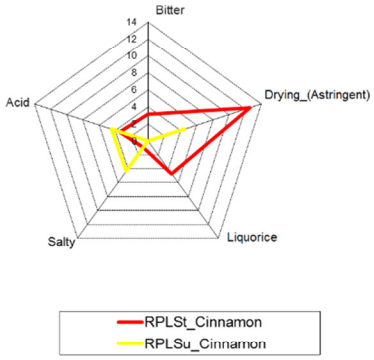
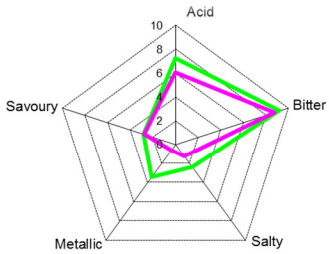
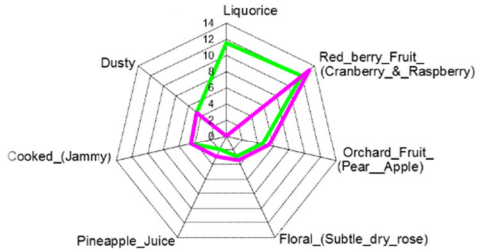
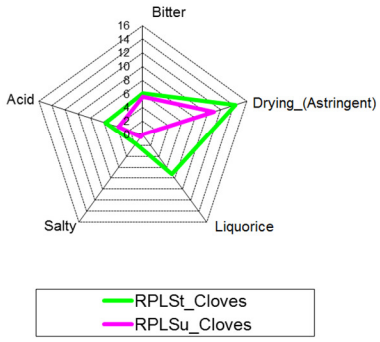
Pineapple Juice Flavour	2.7	a	2.0	a	6.7	a	0.0	a	2.9	a	1.8	a	10.2	a	13.5	a	0.03
Orchard Fruit Flavour	9.7	a	5.1	a	6.0	a	0.0	a	5.9	a	7.0	a	6.0	a	6.0	a	0.25
Floral Flavour	8.2	a	3.0	a	3.1	a	3.5	a	3.4	a	8.2	a	5.3	a	5.4	a	0.07
Liquorice Flavour	5.7	ab	13.0	a	6.5	ab	0.0	ab	0.0	b	0.1	b	9.2	ab	0.3	b	0.0004
Ginger Flavour	3.5		2.8		46.2	a	0.0	b	0.0		32.2	b	1.0		0.1		<.0001
Cloves Flavour	0.3	b	57.5	a	0.1	b	0.0	b	67.3	a	0.4	b	0.0	b	0.1	b	<.0001
Cinnamon Flavour	28.2	ab	1.3	ac	0.5	ac	40.1	a	0.1	ac	0.4	ac	0.1	ac	0.3	ac	<.0001
Cooked Flavour	8.2	a	5.1	a	8.7	a	0.0	a	5.0	a	10.4	a	8.0	a	13.1	a	0.27
Dusty Flavour	8.9	a	5.2	a	4.9	a	4.8	a	5.1	a	2.3	a	2.8	a	2.2	a	0.27
Soapy Flavour	0.9	a	3.8	a	0.8	a	0.0	a	4.5	a	4.5	a	0.7	a	0.3	a	0.24
Off-note Flavour	1.0	a	0.1	a	0.3	a	0.0	a	1.0	a	0.1	a	0.6	a	0.2	a	0.65
Warming	1.9		3.9		26.5	a	0.0	a	1.4		20.0	a	0.3		0.0		<.0001
Mouth coating	7.9	ab	7.8	ab	10.5	a	9.7	ab	7.1	ab	6.2	ab	6.2	ab	5.0	b	0.06
Tingling	1.1		2.4		11.8	a	0.0	a	0.8		8.3	a	0.4		0.1		<.0001
Residue	3.0	a	2.8	a	4.9	a	8.2	a	4.5	a	1.6	a	1.5	a	0.4	a	0.11
Salivating	8.5	a	9.2	a	12.6	a	4.0	a	9.1	a	8.8	a	6.9	a	8.2	a	0.29
Throat-catching	1.1	b	2.2	b	9.2	a	0.0	ab	1.1	b	5.9	ab	0.9	b	0.0	b	0.001
Sweet After-effect	25.8	a	22.5	a	26.2	a	29.7	a	27.1	a	26.0	a	23.6	a	30.0	a	0.49
Acid After-effect	3.9	a	6.4	a	6.4	a	4.5	a	4.2	a	4.2	a	3.3	a	3.0	a	0.07
Salty After-effect	1.1	a	1.9	a	1.1	a	4.3	a	0.4	a	0.6	a	2.0	a	0.2	a	0.04
Bitter After-effect	3.5	a	6.7	a	7.1	a	0.0	a	6.2	a	3.0	a	4.8	a	1.4	a	0.03
Soapy After-effect	0.2	a	3.6	a	0.1	a	0.0	a	3.9	a	2.7	a	0.3	a	0.2	a	0.30
Ginger After-effect	1.2		1.6		27.2	a	0.0	a	0.0		22.0	a	1.2		0.1		<.0001
Cloves After-effect	0.1		36.0	ab	0.1		0.0	b	43.1	a	0.1		0.0		0.1		<.0001
Cinnamon After-effect	14.2	ab	0.6	ac	0.4	c	29.0	a	0.7	ac	0.0	c	0.1	c	0.0	c	<.0001
Drying After-effect	13.6	a	15.4	a	15.4	a	4.5	a	12.0	a	9.2	a	12.6	a	6.8		0.01
Liquorice After-effect	5.3	ab	8.2	a	5.9	ab	0.0	ab	0.0	b	0.1	b	6.9	ab	0.1	b	0.003

Table A1.8: Emotional terms for consumer study of Roselle beverages – check all that apply (CATA)

Emotional terms (n = 39) frequency of use in relation to the Roselle beverage									
Emotion term	**SRA**				**Sugar**				**Usage Total**
	Cinnamon	**Cloves**	**Ginger**	**No Spice**	**Cinnamon**	**Cloves**	**Ginger**	**No spice**	
Active	8	4	6	4	15	8	11	9	65
Adventurous	12	11	8	5	9	15	10	5	75
Affectionate	4	3	1	4	10	7	6	6	41
Aggressive	7	11	9	6	3	7	8	2	53
Bored	15	21	21	33	13	11	12	10	136
Calm	19	15	23	24	21	20	32	32	186
Daring	7	11	6	2	6	4	6	2	44
Disgusted	28	49	24	19	11	34	14	3	182
Eager	7	3	5	2	7	5	7	4	40
Energetic	6	7	9	8	12	12	17	12	83
Enthusiastic	9	8	6	3	7	11	8	11	63
Free	6	4	3	10	12	7	7	11	60
Friendly	17	9	7	9	15	8	13	20	98
Glad	5	4	1	5	13	10	8	16	62
Good	28	10	23	22	30	26	33	49	221
Good-natured	11	11	10	11	19	15	14	16	107
Guilty	4	4	3	3	3	1	2	2	22
Happy	15	8	8	12	31	15	13	31	133
Interested	11	8	11	11	16	19	27	28	131
Joyful	11	10	2	8	24	8	15	19	97
Loving	5	4	2	6	11	5	13	12	58
Merry	8	6	4	5	15	11	15	10	74
Mild	25	12	25	25	20	13	18	15	153
Nostalgic	8	7	6	6	5	12	7	6	57
Peaceful	10	7	8	13	19	11	17	22	107
Pleasant	24	14	19	25	31	24	36	45	218
Pleased	14	13	14	15	27	12	31	35	161
Polite	5	8	4	3	5	3	3	4	35
Quiet	9	8	12	19	17	6	8	7	86
Satisfied	22	12	21	16	32	23	25	47	198
Secure	5	2	3	3	9	6	3	6	37
Steady	11	12	12	12	7	8	4	13	79
Tame	5	2	5	6	3	5	4	4	34
Tender	6	3	5	6	5	5	7	12	49
Understanding	4	3	4	7	3	4	4	3	32
Warm	18	11	11	12	18	17	26	20	133
Whole	6	5	4	3	5	7	8	9	47
Wild	7	11	4	2	1	5	4	4	38
Worried	12	16	16	14	6	13	12	5	94
								Grand total	3589

Table A1.9: Demographics of participants (n=141) within clusters based on overall liking

Classifications	**Cluster, n = 141**			
Gender	**1**	**2**	**3**	Total
Female	33	48	12	93
Male	20	20	8	48
Age bracket				
19-29	26	22	12	60
30-39	12	20	4	36
40-49	7	11	3	21
50-59	4	11	1	16
60-65	4	4		8
Ethnicity				
BLACK/AFRICAN/CARRIBBEAN/BLACK BRITISH- African	13	6	1	20
OTHER ETHNIC GROUP- any other	9	13	2	24
WHITE- English/Welsh/Scottish/Northern Irish/British	12	27	9	48
Familiarity				
NO	24	26	8	58
YES	29	42	12	83

	Taste	Flavour	After-effects
No herb			
Cinnamon			
Cloves			

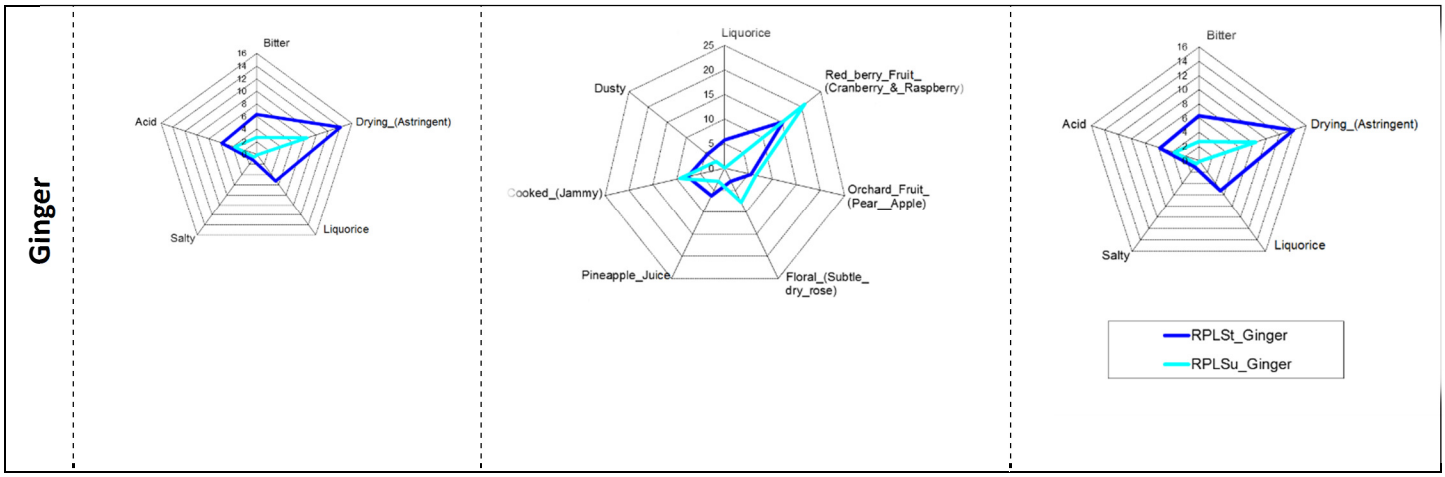
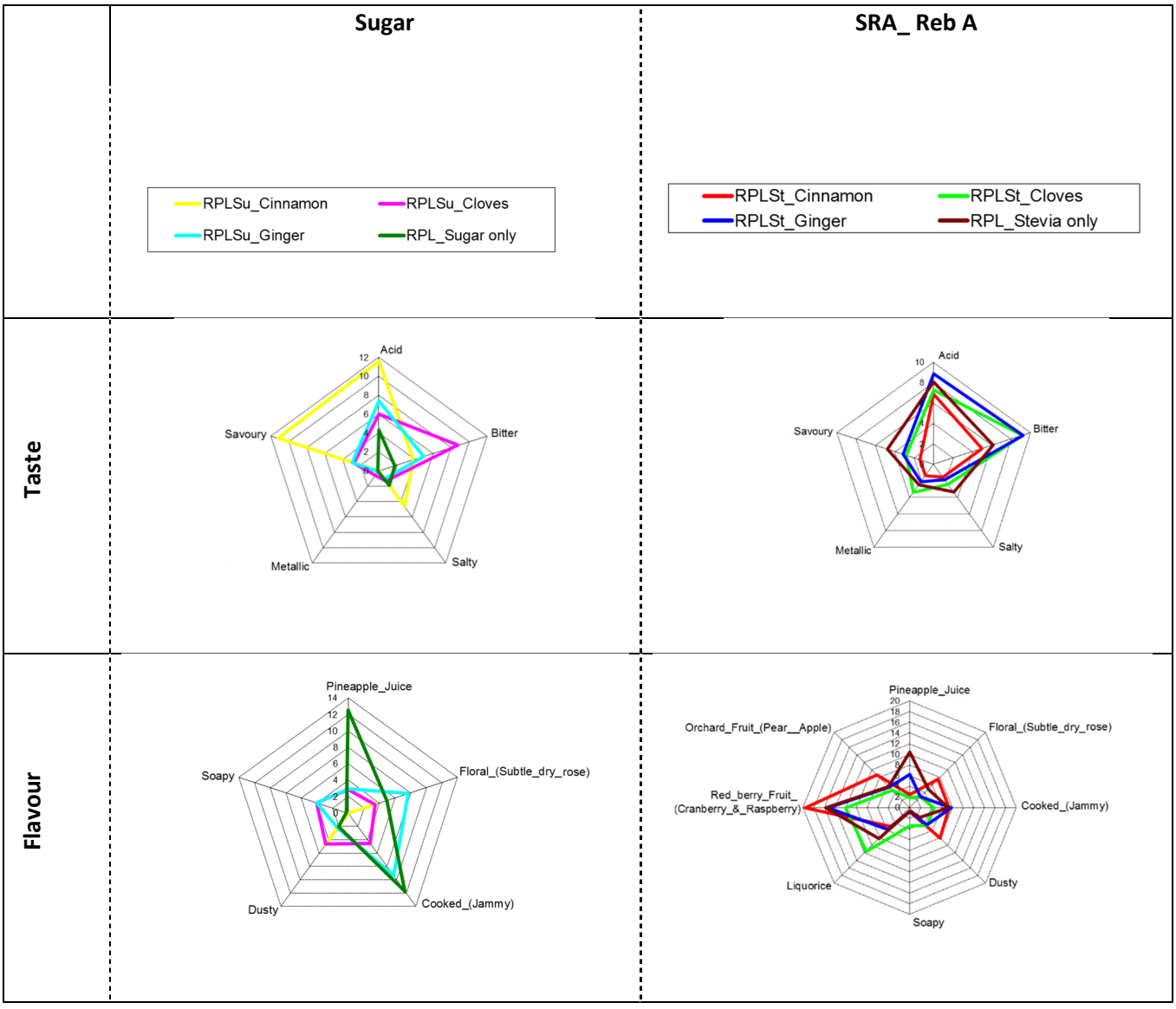


Figure A1.1: Taste, flavour and After-effects attributes of Roselle beverages with spices
Where RB_ refers to Roselle extract + Pineapple juice + lemon juice mixture; St refer to SRA and Su refers to sugar



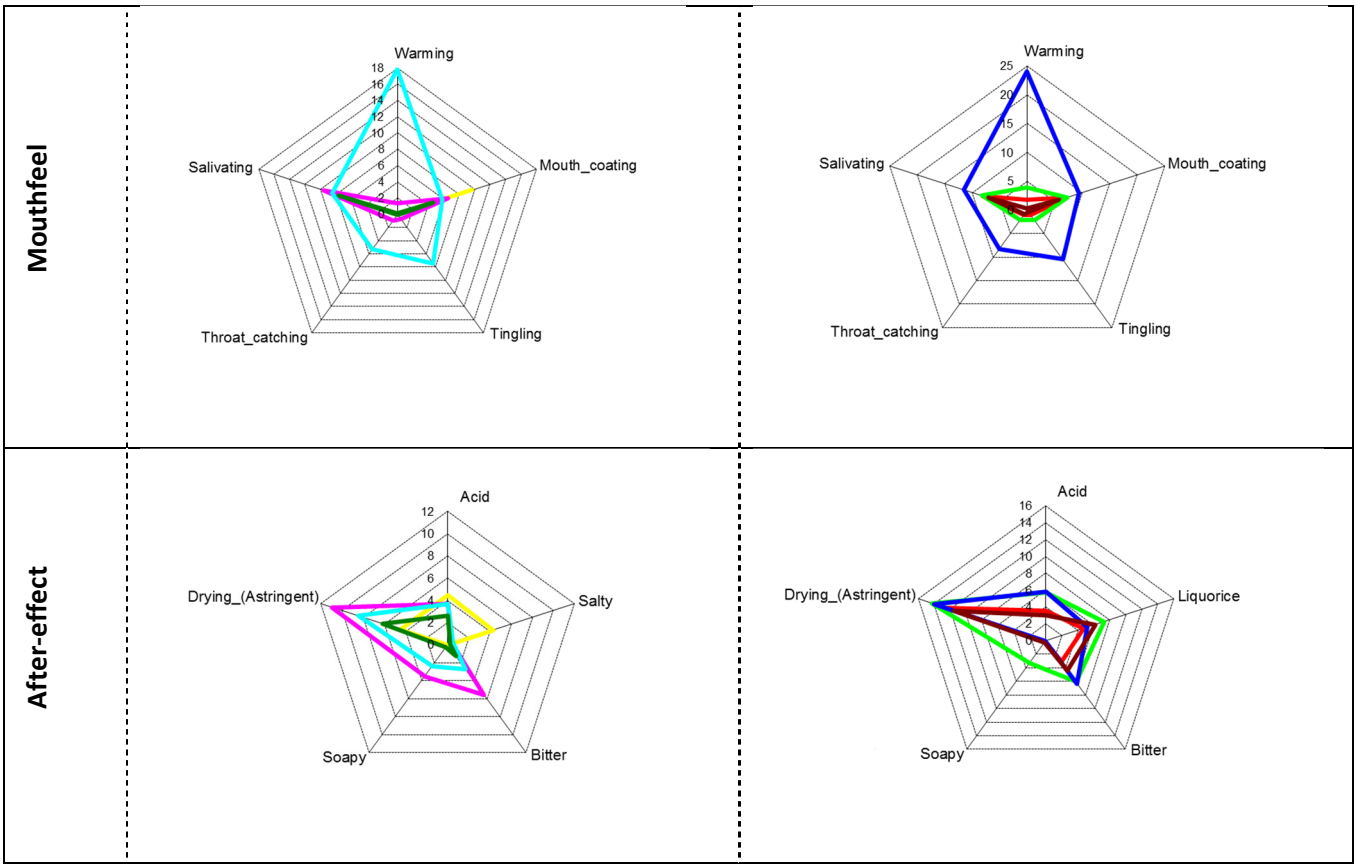
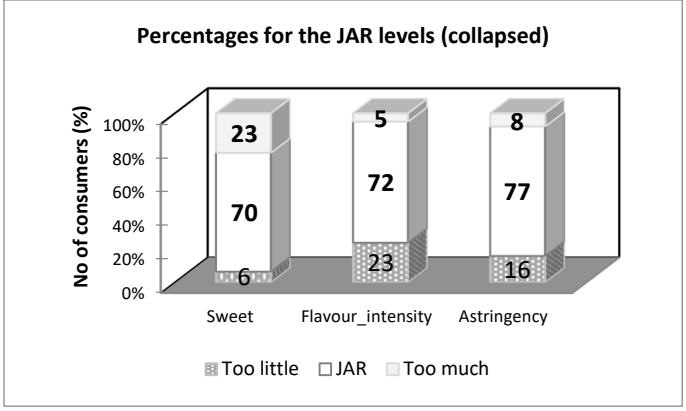
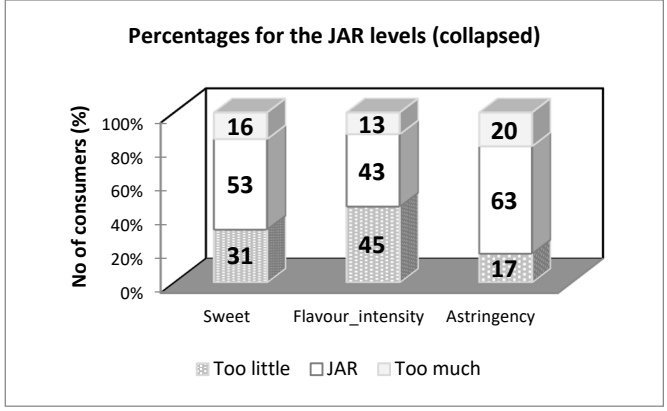


Figure A2: Attributes of Roselle beverages based on sweetener

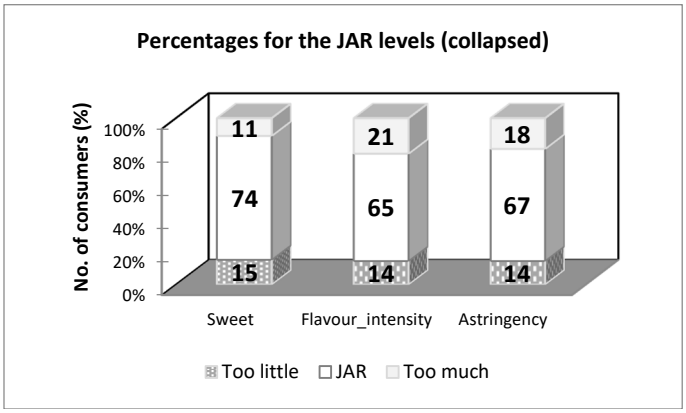
Where RB_ refers to Roselle extract + Pineapple juice + lemon juice mixture; St refer to SRA_ Reb A and Su refers to sugar (scale 0-100)



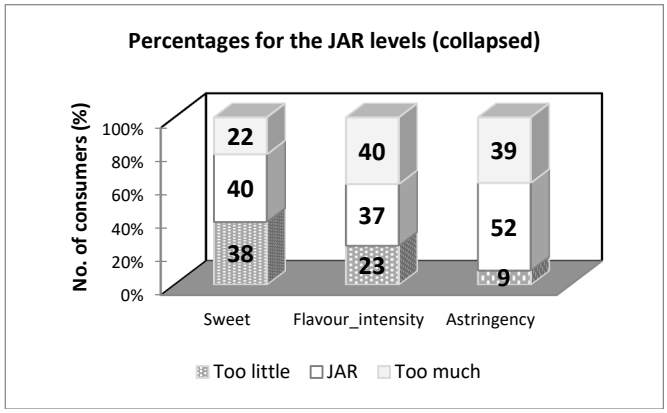
RB_sugar_no spice



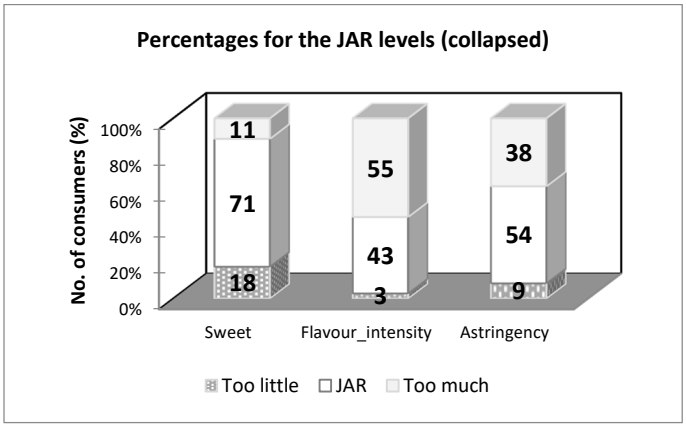
RB_SRA__no spice



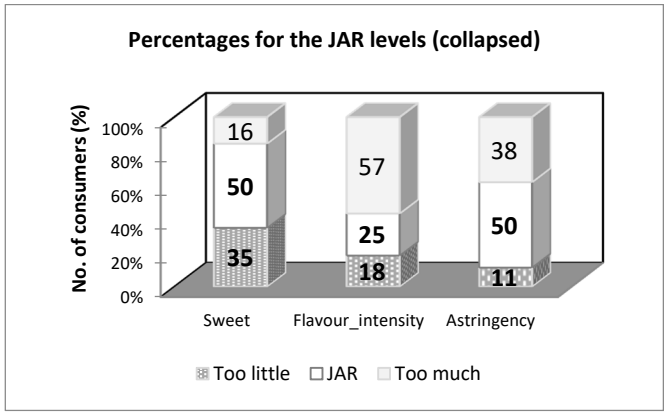
RB_sugar_ginger



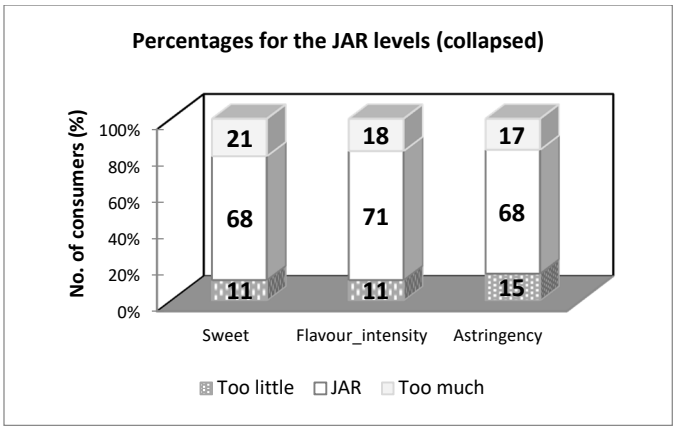
RB_SRA_ginger



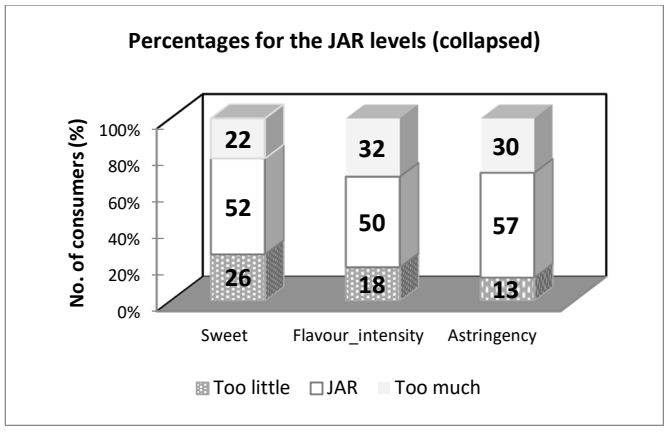
RB_sugar_cloves



RB_SRA_cloves



RB_sugar_cinnamon



RB_SRA_cinnamon

Figure A1.3: Just About Right (JAR) for taste intensities of Roselle Beverages

The 5 points JAR scales were collapsed into 3 points categories ("too little", "Just about right" and "too much")

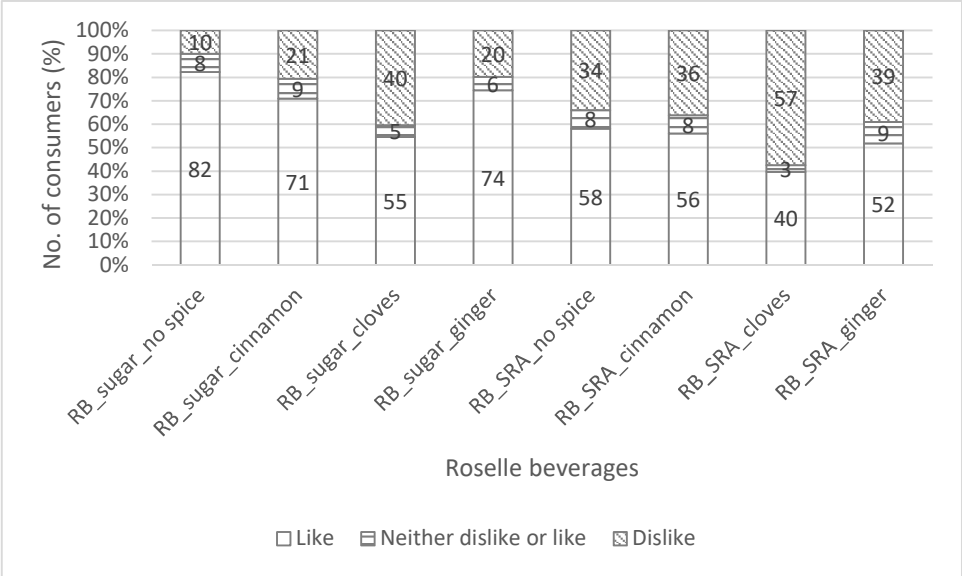


Figure A1.4: Consumer overall liking of Roselle beverages explain line scales (y-axis)

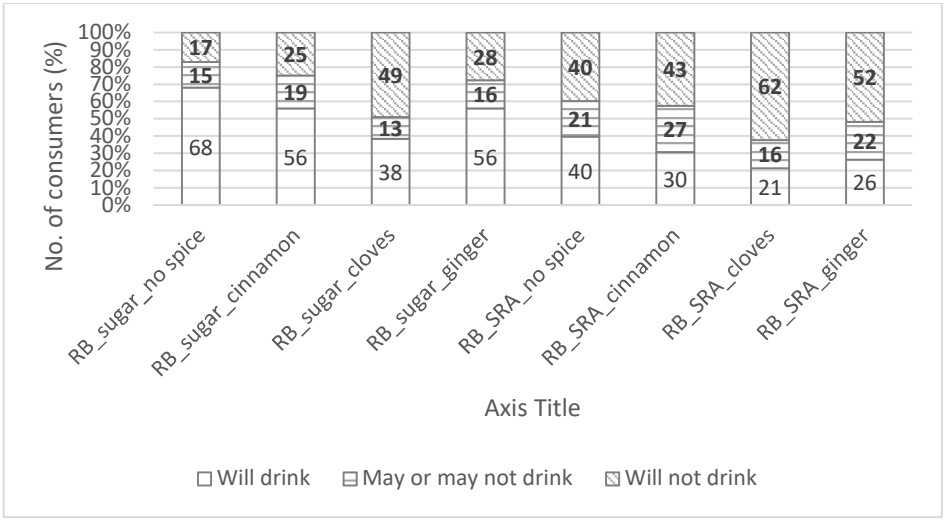


Figure A1.5: Consumer consumption Intent for Roselle Beverages

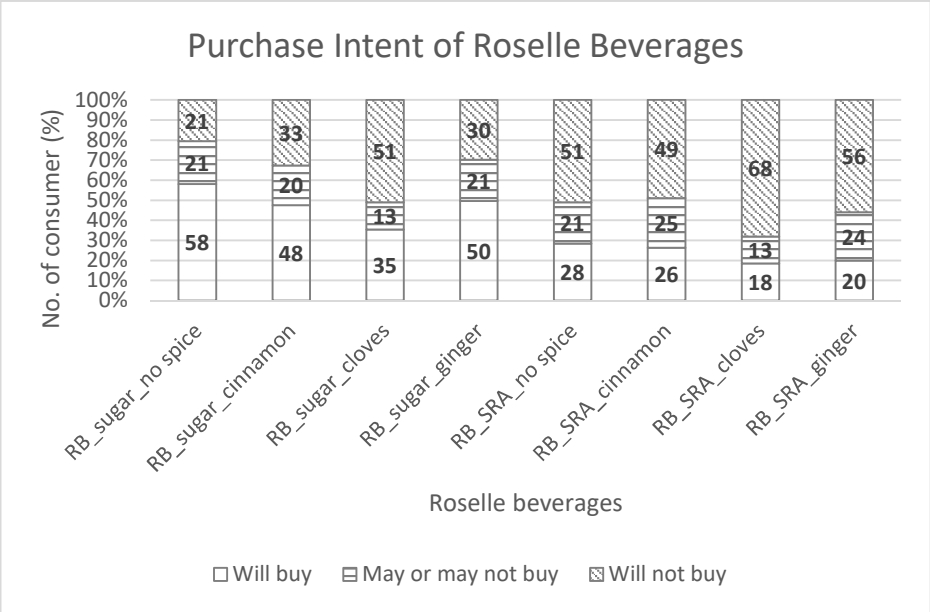


Figure A1.6: Consumer purchase Intent for Roselle Beverages

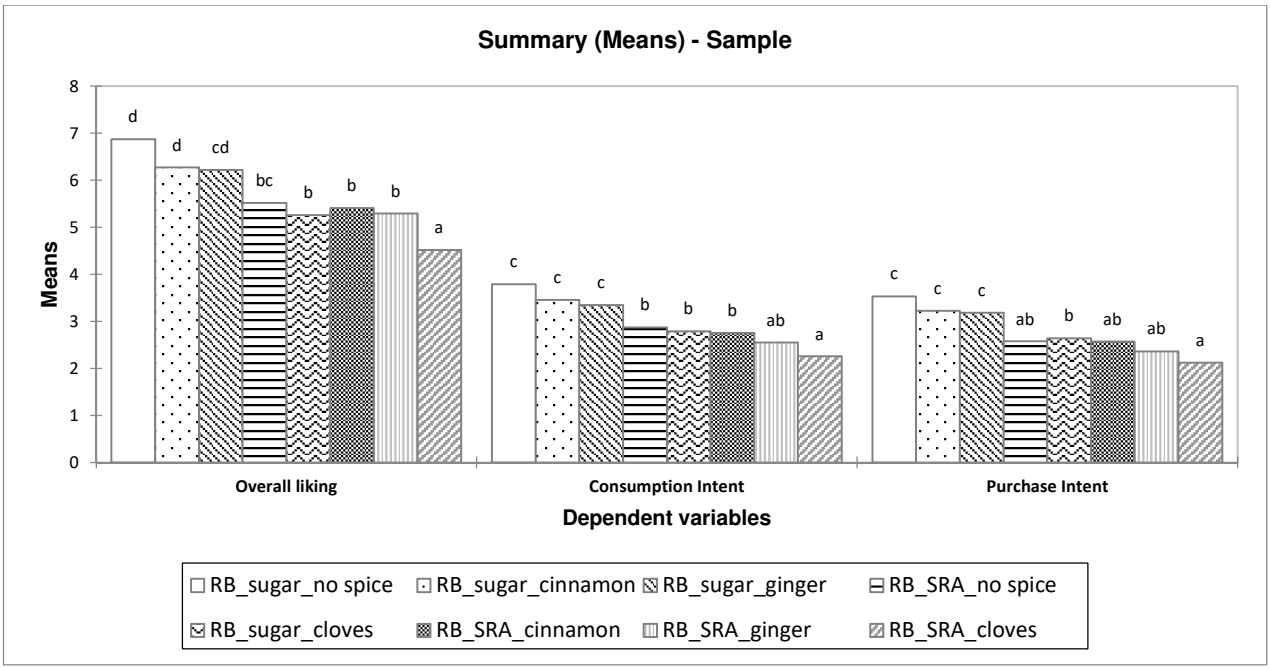


Figure A1.7: Relationship between Overall Liking, Consumption Intent and Purchase Intent

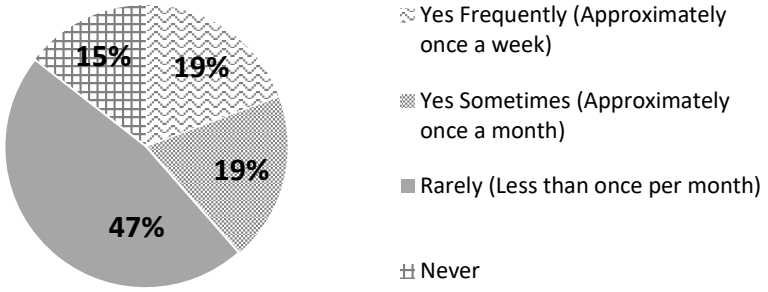


Figure A1.8: Frequency of purchase of Roselle beverages by consumers familiar with Roselle

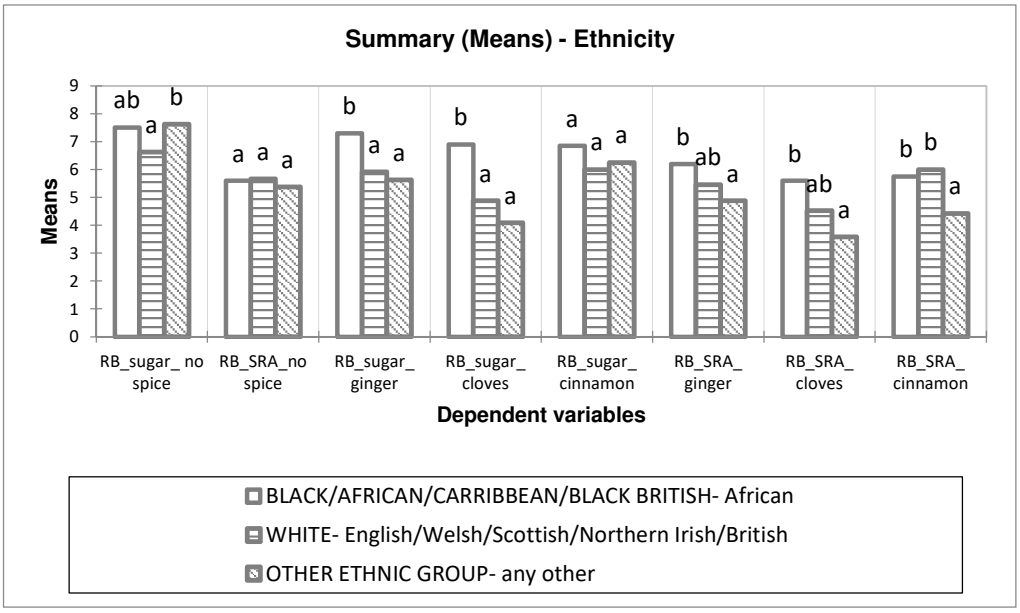


Figure A1.9: Effect of Ethnicity on overall liking of the beverage

Appendix II

Table A2.1: Summary of kinetic data from a previous study (Chapter 3)

Parameter	Rate constant (day^{-1})	Coefficient of determination (R^2)	Half-life $t_{1/2}$ (days)
Total phenolic content	0.0012	0.8915	577
Anthocyanin	0.0161	0.9988	43
Antioxidant	0.0037	0.753	182

Table A2.2: Total phenolics content kinetics data for sweetened and spice-flavoured Roselle beverages (n=8) stored under accelerated conditions 40°C for 30 days

Sample	Total phenolic content (mg GAE/L)	Rate constant (mg GAE L^{-1} day^{-1})	Coefficient of determination (R^2)	Half-life $t_{1/2}$ (days)
Unsweetened RB no spice	119 ± 4	0.3726	0.7907	181
Unsweetened RB cinnamon	160 ± 6	0.441	0.7226	169
Unsweetened RB ginger	126 ± 6	1.331	0.9437	136
Sugar RB no spice	114 ± 17	0.4192	0.3564	203
Sugar RB cinnamon	158 ± 17	0.3889	0.2216	267
Sugar RB ginger	121 ± 16	0.2265	0.2221	160
SRA RB no spice	125 ± 22	0.3905	0.7127	184
SRA RB cinnamon	163 ± 21	0.4434	0.7433	45
SRA RB ginger	130 ± 22	1.4311	0.8831	181

RB – Roselle beverage, SRA – Stevia Rebaudioside A

Table A2.3: FRAP capacity kinetics data for sweetened and spice-flavoured Roselle beverages (n = 8) Stored under accelerated conditions 40°C for 30 days

Sample	Initial total antioxidants (mg AAE/L)	Rate constant (day^{-1})	Coefficient of determination (R^2)	Half-life $t_{1/2}$ (days)
Unsweetened RB no spice	653 ± 44	0.015	0.8097	46
Unsweetened RB cinnamon	707 ± 128	0.0119	0.6821	58
Unsweetened RB ginger	674 ± 75	0.0152	0.7933	46
Sugar RB no spice	603 ± 94	0.0179	0.794	39
Sugar RB cinnamon	779 ± 128	0.0063	0.3442	110
Sugar RB ginger	634 ± 161	0.0179	0.794	39
SRA RB no spice	677 ± 157	0.0158	0.8913	44
SRA RB cinnamon	847 ± 176	0.0078	0.3854	89
SRA RB ginger	746 ± 109	0.0123	0.7611	56

RB – Roselle beverage, SRA – Stevia Rebaudioside A

Table A2.4: Type III Tests of Fixed Effects for total phenolic content (from SPSS)

Source	Numerator df	Denominator df	F	Sig.
Intercept	1	278.822	25178.241	.000
sweetener	2	278.822	39.277	.000
spice	2	278.822	164.626	.000
sweetener * spice	4	277.706	5.134	.001
Day	12	39.903	2.296	.025
sweetener * Day	24	39.903	1.739	.060
Day * spice	24	39.903	.529	.950

Dependent Variable: TP.

Table A2.5: Type III Tests of Fixed Effects for total anthocyanins (from SPSS)

Source	Numerator df	Denominator df	F	Sig.
Intercept	1	173.482	4535.259	.000
sweetener	2	173.482	1.048	.353
spice	2	173.482	37.617	.000
sweetener * spice	4	166.017	13.917	.000
Day	12	55.728	149.595	.000
sweetener * Day	24	55.728	.765	.761
Day * spice	24	55.728	2.316	.005

Dependent Variable: AnCy.

Table A2.6: Type III Tests of Fixed Effects for FRAP (antioxidants) capacity (from SPSS)

Source	Numerator df	Denominator df	F	Sig.
Intercept	1	226.260	10547.282	.000
sweetener	2	226.260	15.606	.000
spice	2	226.260	61.105	.000
sweetener * spice	4	235.015	2.564	.039
Day	12	39.798	13.519	.000
sweetener * Day	24	39.798	.374	.994
Day * spice	24	39.798	.540	.944

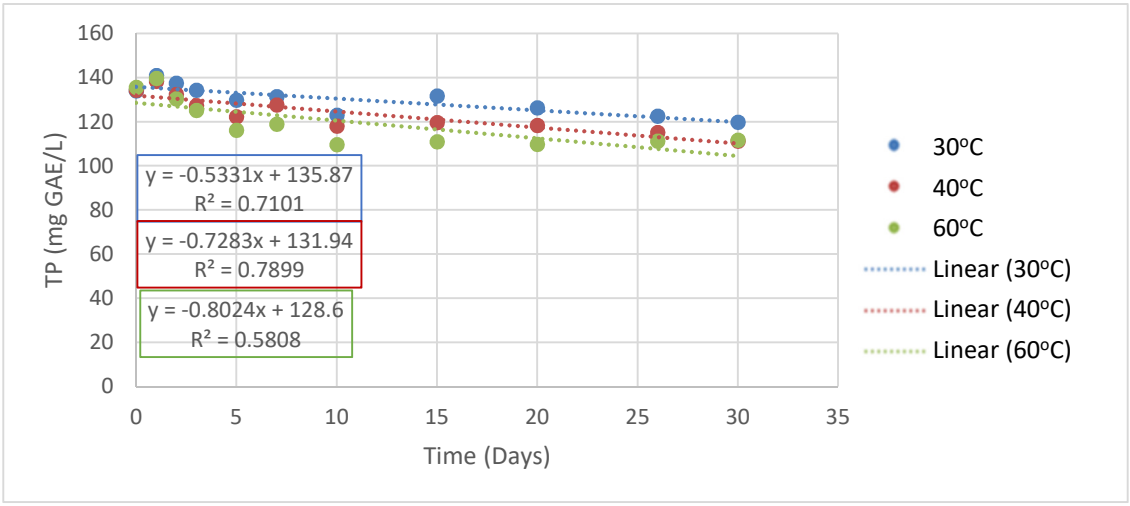
Dependent Variable: AnOX.

Table A2.7: Least square means in descending order for total phenolics content, anthocyanins and FRAP (Antioxidant) for spice flavoured sweetened Roselle beverages on Days 0 and Day 30 (from XLSTAT)

TP	Mean TP	**AnCy**	Mean AnCy	**AnOX**	Mean AnOX
Sugar - Cinnamon*30	188 ^{d}	SRA - No spice*0	48 ^{b}	SRA - Cinnamon*0	847 ^{e}
SRA - Cinnamon*0	163 ^{cd}	Sugar - Cinnamon*0	48 ^{b}	Sugar - Cinnamon*0	779 ^{de}
Unswt - Cinnamon*0	160 ^{bcd}	Sugar - Ginger*0	47 ^{b}	SRA - Ginger*0	746 ^{cde}
Sugar - Cinnamon*0	158 ^{abcd}	Unswt - No spice*0	47 ^{b}	Unswt - Cinnamon*0	707 ^{bcde}
Sugar - No spice*30	139 ^{abcd}	Sugar - No spice*0	47 ^{b}	SRA - No spice*0	677 ^{bcde}
Sugar - Ginger*30	139 ^{abcd}	SRA - Ginger*0	45 ^{b}	Unswt - Ginger*0	674 ^{bcde}
SRA - Ginger*0	130 ^{abc}	SRA - Cinnamon*0	45 ^{b}	Unswt - No spice*0	653 ^{abcde}
Unswt - Ginger*0	126 ^{abc}	Unswt - Ginger*0	44 ^{b}	Sugar - Ginger*0	634 ^{abcde}
SRA - No spice*0	125 ^{abc}	Unswt - Cinnamon*0	43 ^{b}	Sugar - Cinnamon*30	604 ^{abcde}
SRA - Cinnamon*30	124 ^{abc}	Sugar - Cinnamon*30	17 ^{a}	Sugar - No spice*0	603 ^{abcde}
Sugar - Ginger*0	121 ^{abc}	Unswt - Cinnamon*30	8 ^{a}	SRA - Cinnamon*30	596 ^{abcde}
Unswt - No spice*0	119 ^{abc}	SRA - Cinnamon*30	6 ^{a}	Unswt - No spice*30	522 ^{abcde}
SRA - Ginger*30	118 ^{abc}	Unswt - No spice*30	2 ^{a}	Unswt - Cinnamon*30	494 ^{abcd}
Unswt - Cinnamon*30	118 ^{abc}	SRA - No spice*30	2 ^{a}	SRA - Ginger*30	483 ^{abcd}
Sugar - No spice*0	114 ^{abc}	Unswt - Ginger*30	2 ^{a}	SRA - No spice*30	417 ^{abc}
SRA - No spice*30	114 ^{ab}	SRA - Ginger*30	1 ^{a}	Unswt - Ginger*30	407 ^{ab}
Unswt - Ginger*30	113 ^{ab}	Sugar - No spice*30	1 ^{a}	Sugar - Ginger*30	391 ^{ab}
Unswt - No spice*30	110 ^{a}	Sugar - Ginger*30	0 ^{a}	Sugar - No spice*30	331 ^{a}

Where a-e indicate significant differences and SRA refers to Stevia Rebaudioside A

(a)



(b)

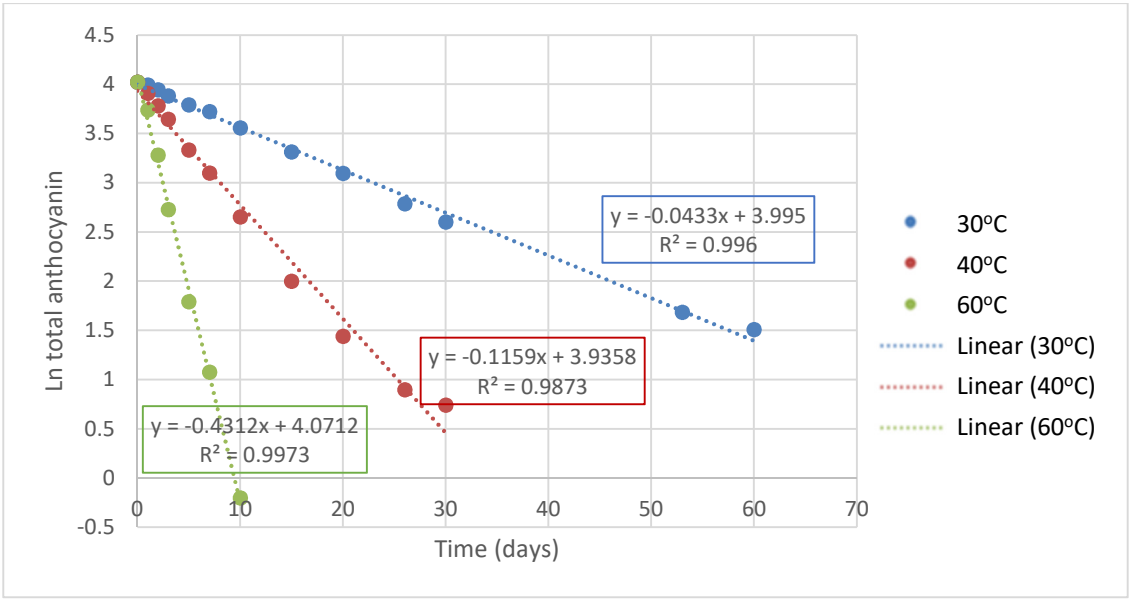


Figure A2.1: Results from accelerated study tests at 30°C, 40°C and 60°C (a) results from the total phenolic content of Roselle extracts (b) results from total anthocyanin content of Roselle extracts.

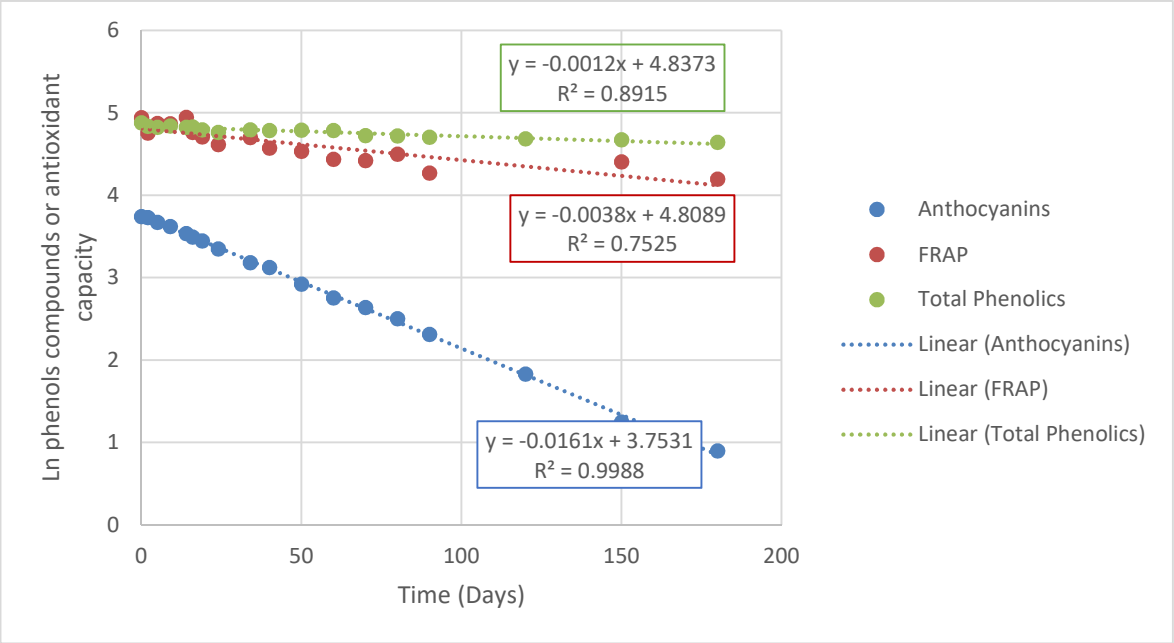


Figure A2.2: First order plot for degradation of phenolics in unpasteurised Roselle extract from Chapter 3

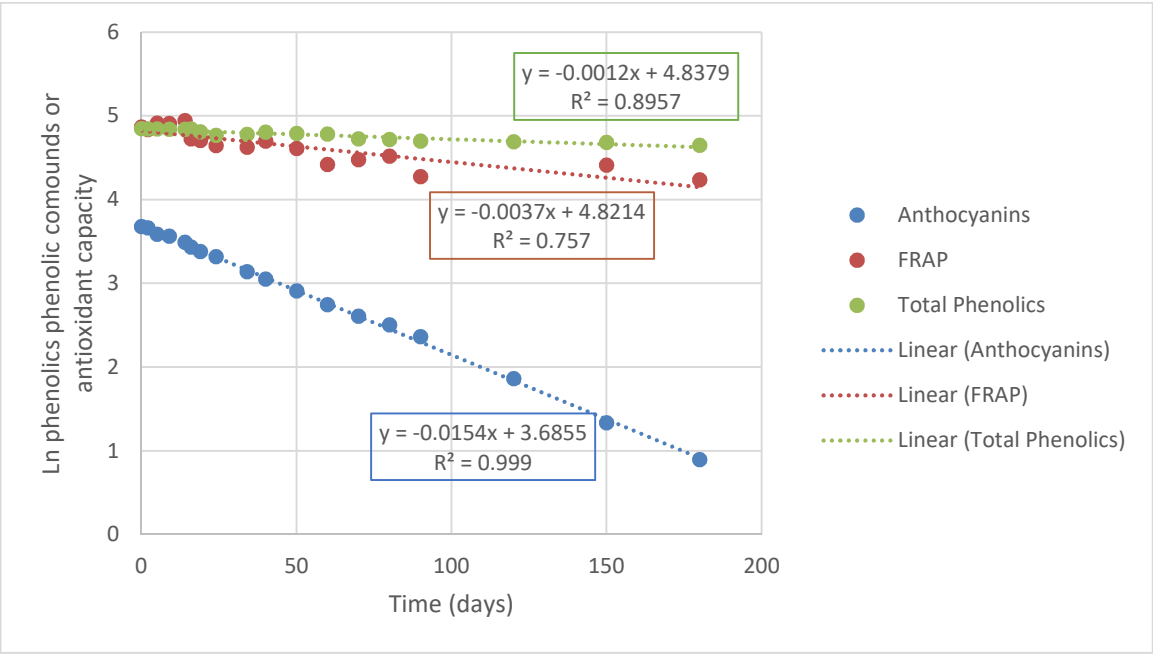


Figure A2.3: First order plots for the degradation of phenolics in pasteurised Roselle extract from Chapter 3

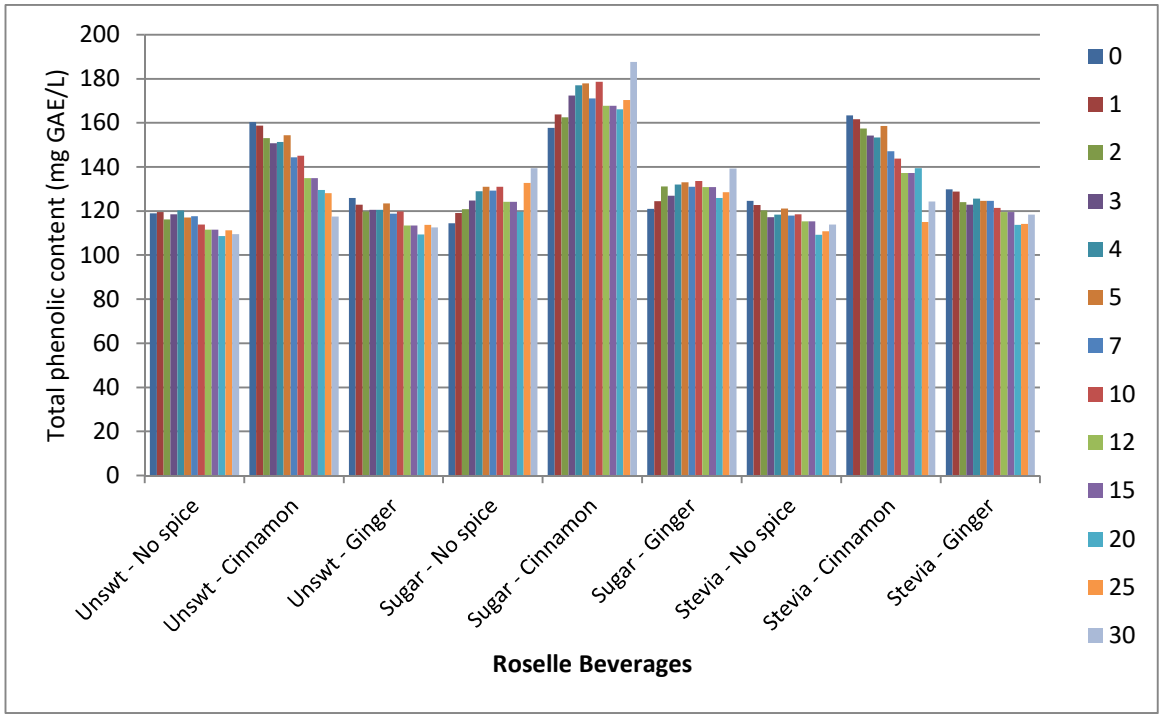


Figure A2.4: Plot of full results of the total phenolic content (mg GAE/L) of Roselle beverages stored from day 0 to 30 under accelerated conditions of 40°C. "Unswt – cinnamon" – refers to Roselle beverage with cinnamon flavour but no sweetener; "Unswt – ginger" – refers to Roselle beverage with ginger flavour but no sweetener; "sugar – no spice" refers to Roselle beverage without spice but sweetened with sugar; "sugar – cinnamon" refers to Roselle beverage with cinnamon and sweetened with sugar; "sugar – ginger " refers to Roselle beverage with ginger and sweetened with sugar; "SRA – no spice" refers to Roselle beverage without spice but sweetened with Stevia Reb A; "SRA – cinnamon" refers to Roselle beverage with cinnamon and sweetened with Stevia Reb A; "SRA – ginger " refers to Roselle beverage with ginger and sweetened with Stevia Reb A.

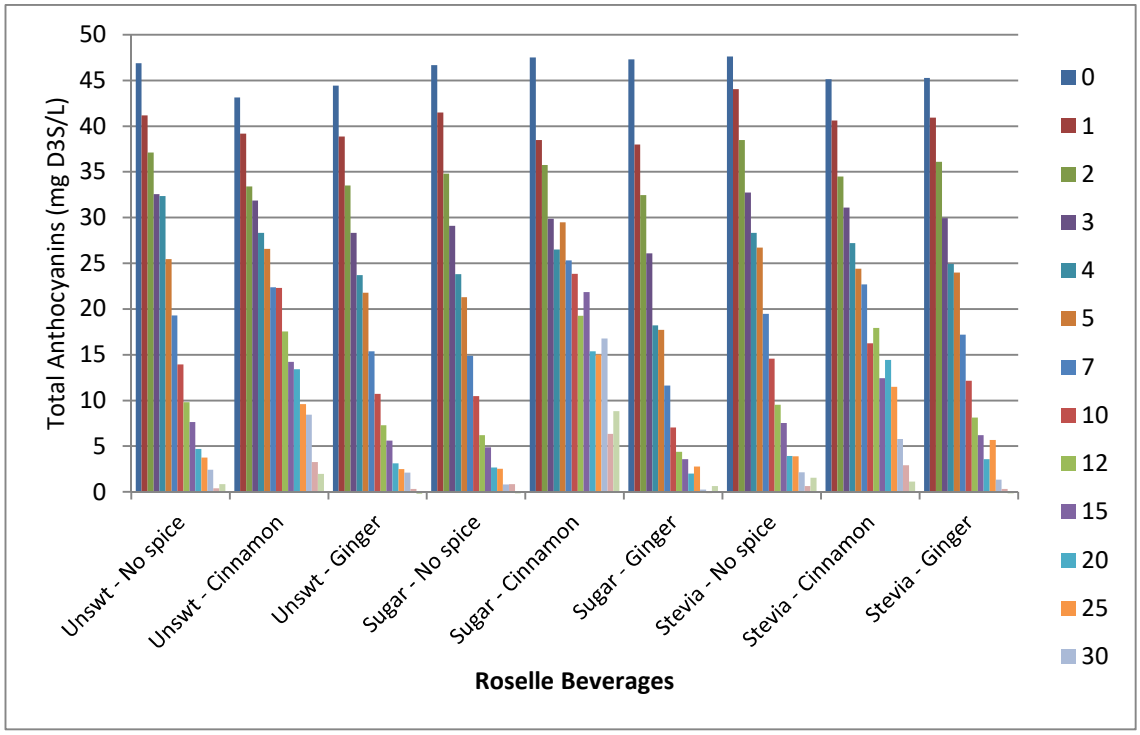


Figure A2.4: Plot of full results of the total monomeric anthocyanins (mg D3S/L) of Roselle beverages stored from day 0 to 30 under accelerated conditions of 40°C. "Unswt – cinnamon" – refers to Roselle beverage with cinnamon flavour but no sweetener; "Unswt – ginger" – refers to Roselle beverage with ginger flavour but no sweetener; "sugar – no spice" refers to Roselle beverage without spice but sweetened with sugar; "sugar – cinnamon" refers to Roselle beverage with cinnamon and sweetened with sugar; "sugar – ginger " refers to Roselle beverage with ginger and sweetened with sugar; "SRA – no spice" refers to Roselle beverage without spice but sweetened with Stevia Reb A; "SRA – cinnamon" refers to Roselle beverage with cinnamon and sweetened with Stevia Reb A; "SRA – ginger " refers to Roselle beverage with ginger and sweetened with Stevia Reb A.

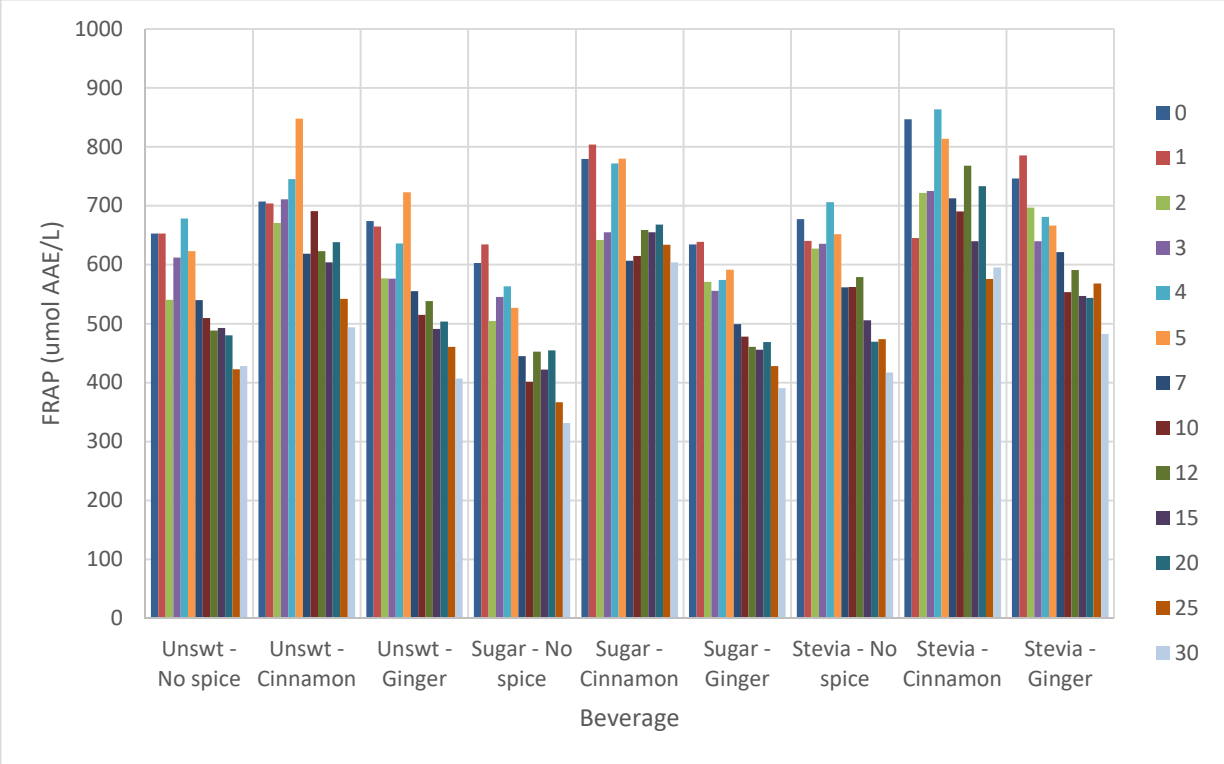


Figure A2.5: Plot of the full results of FRAP activity (µmol AAE/L) of Roselle beverages stored for 30 day storage under accelerated conditions of 40°C for 30 days "Unswt – cinnamon" – refers to Roselle beverage with cinnamon flavour but no sweetener; "Unswt – ginger" – refers to Roselle beverage with ginger flavour but no sweetener; "sugar – no spice" refers to Roselle beverage without spice but sweetened with sugar; "sugar – cinnamon" refers to Roselle beverage with cinnamon and sweetened with sugar; "sugar – ginger " refers to Roselle beverage with ginger and sweetened with sugar; "SRA – no spice" refers to Roselle beverage without spice but sweetened with Stevia Reb A; "SRA – cinnamon" refers to Roselle beverage with cinnamon and sweetened with Stevia Reb A; "SRA – ginger " refers to Roselle beverage with ginger and sweetened with Stevia Reb A.

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