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Glucosinolates, myrosinase hydrolysis products and flavonols found in rocket (*Eruca sativa* and *Diplotaxis tenuifolia*)

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Abstract

Rocket species have been shown to have very high concentrations of glucosinolates and flavonols, which have numerous positive health benefits with regular consumption. In this review we highlight how breeders and processors of rocket species can utilize genomic and phytochemical research to improve varieties and enhance the nutritive benefits to consumers. Plant breeders are increasingly looking to new technologies such as HPLC, UPLC, LC-MS and GC-MS to screen populations for their phytochemical content to inform plant selections. Here we collate the research that has been conducted to-date in rocket, and summarise all glucosinolate and flavonol compounds identified in the species. We emphasize the importance of the broad screening of populations for phytochemicals and myrosinase degradation products, as well as unique traits that may be found in underutilized gene bank resources. We also stress that collaboration with industrial partners is becoming essential for long-term plant breeding goals through research.

Key words: *Brassicaceae,* Isothiocyanates, Plant breeding, Indoles, Nitriles

Introduction

In recent years, several species of minor leafy-crops have risen to prominence as potentially important commercial and edible species. One example is rocket, which has quickly gained popularity in the Western diet. Originally found as an obscure crop in Mediterranean and Middle-Eastern countries, rocket has become popular largely due to the pungent aromas and tastes associated with it. Glucosinolates (GSLs)/isothiocyanates (ITCs) and flavonols derived from many species [1–4] have been shown to infer significant protection against cancer and heart disease [4–11]. In Western countries, diets are generally lacking in fruits and vegetables. Despite government initiatives (such as the “5-a-day” campaign in the UK and USA), these diseases are increasingly leading to premature deaths [12]. Plant breeders aim to maximize levels of such beneficial compounds, but with little genomic information about rocket species presently available, this is a formidable task. This review will give an overview of research in rocket, an outbreeding crop, and how breeders and processors can utilize it to enhance beneficial compounds.

Rocket species

Rocket (also known as arugula, rucola and roquette) is a leafy vegetable crop that has gained substantial popularity across the world, particularly over the last fifteen years [13–16]. Two main species are predominantly farmed as salad crops; these are *Eruca sativa* (‘salad’ or ‘cultivated’ rocket; sometimes referred to as *Eruca vesicaria* subsp. *sativa*) and *Diplotaxis tenuifolia* (‘wild’ rocket). Both species share a peppery taste and aroma that is very distinctive [17]. They have been reported to contain high levels of vitamin C, GSLs, flavonols and phenolics [18–25]. These are all known to have both anti-oxidant and anti-cancer properties, and are also implicated in lowering the risk of cardiovascular and cognitive disease. For excellent information on these beneficial effects and their underlying causes, see Drewnowski & Gomez-Carneros [26], Keum et.al [27], D'Antuono et.al [28], Egea-Gilbert et.al [29], Degl'Innoocenti et.al [30], Bjorkman et.al [31] and Jeffery et.al [32].

Taxonomy and domestication

A distinction should be made that both *Eruca* and *Diplotaxis* species have overlapping characteristics, and that one can be easily mistaken for the other by the untrained eye, and/or before a certain level of maturity has been reached [28]. It is also arguable that *D. tenuifolia* is the least 'wild' of the two species even though the common name is 'wild rocket'. It is featured and favored in commercial products and breeding programs, and is likely to be more domesticated than *Eruca* species as a result. *Diplotaxis* varieties are generally uniform phenotypically, with *Eruca* varieties being more diverse in this respect [23]. No direct genomic evidence has been presented in the literature to suggest one species is any more or less genetically variable than the other. Variability in GSL data seems to support the hypothesis that *Diplotaxis* species are more 'wild' [33], though it is not conclusive, as only a relatively small number of cultivars have been tested. This is a point that needs clarification through research and extensive breeding, as neither species can be considered fully domesticated [29]. For example, germination rates are variable, reproductive organs are typically small, seedpods shatter and disperse freely (rather than staying on the plant), and physical defenses such as leaf hairs are still present in many commercial varieties [34].

Phytochemicals in *Eruca sativa* and *Diplotaxis tenuifolia*: types and structures

Glucosinolates

GSLs are β-thioglucoside *N*-hydrosulphates that are responsible for the sharp and bitter-tasting flavors found in cruciferous vegetables [35,36]. In combination with the enzyme myrosinase (thioglucoside glucohydrolase, EC 3.2.1.147), GSLs are hydrolyzed to create isothiocyanates, nitriles, thiocyanates, epithionitriles, indoles, oxazolidine-2-thiones, cyanopithioalkanes, ascorbigens, goitrogens and epithioalkanes [37–49]; see Figure 1. Many of these hydrolysis products have antibacterial, antifungal and insect repellant effects [50–55]. GSLs and ITCs are being increasingly used as 'biofumigants' to suppress soil borne pathogens, nematodes and weeds. Some of the volatile products have the opposite effect of attracting species that can tolerate high GSL concentrations, such as types of ovipositing insect [56,57].

The conditions under which hydrolysis of GSLs occurs will affect the respective proportions of the chemicals produced; pH, iron ions, thiol ions, temperature and hydration play a particularly prominent role in this process *in vivo* [58]. The separation of GSLs in specialist 'S-cells' from myrosinase in myrosin cells means that the two components only come into contact upon tissue disruption; for example when damaged via chewing or digestion [59–69]. It is the biological activity of the ITC hydrolysis products in humans that are of most interest in rocket [50]. GSLs can be hydrolyzed within the intestinal tract by gut microflora that are known to have specific myrosinase activity [70–73], but the efficacy of their action is not yet well determined.

GSL concentrations can vary and change over time depending on environmental conditions and stress [7]. Other factors affecting GSL profiles include the plant age, organ type, developmental stage, ambient air temperature, level of water stress, photoperiod, agronomic practice, degree of wounding, and geographical origin of the variety/species [74–81]. These can often affect the profiles of *all* phytonutrients contained within tissue, not just GSLs [82], and they are all factors that plant breeders aim to mitigate through development of genetically advanced and uniform breeding lines.

GSLs and the ITC derivatives have been an integral part of the human diet for millennia because of the presence of them in the family *Brassicaceae* [64–66,83–89]. GSLs are evolutionarily recent secondary metabolic products having arisen 10-15 million years ago [90,91], acting to prevent pathogen attack and dissuade herbivory. They are known in only a few angiosperm families of the order *Brassicales*, which includes the *Brassicaceae* [92–100], and of which *Eruca* and *Diplotaxis* are members.

A study by Pasini et al. [33] of 37 rocket accessions (*Diplotaxis* and *Eruca*) showed that GSL profiles were all very similar, regardless of the species. In total, twelve GSL compounds were found across all accessions; Table 1 illustrates all known GSL compounds identified to-date in rocket. These include 4-mercaptobutyl GSL (glucosativin) [21], 4-methylthiobutyl GSL (glucoerucin) [101], and 4-methylsulfinylbutyl GSL (glucoraphanin) [28], which constitute the three most abundant GSLs in rocket.

Flavonols

Flavonols are diphenylpropanes (C_6–C_3–C_6) [102] and are another important group of chemicals found within rocket species. Flavonols in rocket are found with sugar conjugates, and typically occur in relatively large quantities [103]. The aglycones found (such as quercetin and kaempferol) are glycosylated and acylated, which in turn affects their biological properties [18]. A study by Martínez-Sánchez et al. [18] identified over 50 different flavonol compounds across four different species. Watercress, mizuna and two species of rocket were all found to accumulate very different compounds within their leaves, and in varying quantities. Wild rocket showed high levels of quercetin-3,3',4-triglucosyl (43.5 mg per $100g^{-1}$ fw) and salad rocket had mostly kaempferol-3,4'-diglucosyl (97.8mg per $100g^{-1}$ fw). The group also showed a correlation between quercetin derivatives and high antioxidant activity, despite the significant variations seen between species.

Studies conducted on rocket tissues have identified significant concentrations of polyglycosylated flavonols. The core aglycones of these are kaempferol, quercetin and isorhamnetin [102]; Table 2 provides an up-to-date list of all flavonol compounds identified in rocket to-date. Martinez-Sanchez et al. [18] showed that *Eruca* species accumulate kaempferol derivatives, whereas *D. tenuifolia* accumulates predominantly quercetin instead, meaning that the two chemicals could be used as an identification marker between the two species [104]. Isorhamnetin aglycones are common to both species but typically in much lower concentrations [33]. The specific aglycones also infer varying degrees of anti-oxidant activity. For example, quercetin derivatives have a higher activity than kaempferol and isorhamnetin. The differences in structure (the arrangement of hydroxyl groups and glycosylation) affect anti-oxidant activity by allowing the molecules to act as hydrogen/electron donors, single oxygen scavengers, or as reducing agents [105].

Phytochemicals and the relation with quality: taste and aroma

It is thought that the presence of glucosativin, glucoerucin and their hydrolysis products within rocket leaves is what gives them a characteristic flavor [44]. Many of the health beneficial GSLs and ITCs are thought to be responsible for strong tastes that *some* consumers find repellant [106]. It seems that to many people, these compounds contribute very little to a pleasurable eating experience and are actively avoided [83]. Conversely however, some people *do* prefer these strong tastes and aromas, and will actively seek to consume rocket when it is available. Growers in Italy often prefer the subsequent cuts because of the more intense tastes and aromas that are produced [107] and some will even 'sacrifice' the first cut in favour of the subsequent leaf growth. This highlights a divide between consumers that may be indicative of underlying genotype(s) for taste perception and preference.

The breeding process in rocket varieties to-date has effectively made the species 'milder' in taste when compared to plants that grow naturally in the wild. Whether this has been intentional or as a result of selecting for other unrelated traits (such as leaf morphology) is debatable. Some recent commercial varieties have been bred for a 'hotter' taste, such as '*Wildfire*', by Tozer Seeds (Surrey, UK).

A study by Pasini et al. [17] demonstrated how breeding for sensory traits could be achieved, by highlighting which glucosinolates contributed to specific taste and aroma elements in rocket. It was found that progoitrin/epiprogoitrin is responsible for bitter taste attributes, despite being only a minor component of the overall GSL profile of rocket (4.3-11.4% of total GSL concentration). The perceived pungency of leaves was positively related to the overall GSL content of accessions, and the levels of glucoraphanin negatively contributed to the typical 'rocket' flavour. The study also highlighted an important difference between rocket and other *Brassica* sensory studies [108], in that bitterness was perceived as a favorable characteristic according to panelists. The flavonol compound kaempferol-3-(2-sinapoyl-glucoside)-4'-glucoside also significantly and positively contributed to flavor attributes in *Eruca* accessions. This would indicate that GSL compounds are not totally responsible for flavor in rocket. The study itself stopped short of saying how or if the information obtained would be used in breeding programs, but with study into rocket flavor components, milder (and/or stronger) varieties could be bred more efficiently once the responsible compounds are properly identified [26].

Health promoting properties of glucosinolate-myrosinase products and flavonols of rocket

Isothiocyanates

ITC hydrolysis products have been identified in rocket [45], such as 4-(methylthio)butyl ITC (erucin) [109,110] which is known to show anti-proliferative activity in human lung carcinoma A549 cells, hepatoma (HepG2) cells, colon cancer cells, prostate cancer cell lines (PC3, BPH-1 and LnCap) and leukemia cells [111]. Erucin is a structurally reduced analog of sulforaphane, (which is predominantly found in broccoli) and has shown promising anti-cancer properties *in vitro* (e.g. anti-proliferation of human erytroleukemic K562 cells) [112]. Research into the chemopreventative and anti-genotoxic nature of ITCs has shown promising results [113] (see Figure 2). Other studies involving chemically induced genotoxicity have shown very strong anti-genotoxic effects of *E. sativa* extracts [13] which is in agreement with other *Brassicaceae* studies [114,115]. Identifying specific cultivars of rocket with elevated levels of erucin and glucoraphanin would be an important first-step in developing superior varieties from a human nutrition standpoint.

The results of GSL/ITC research prompted an investment in broccoli breeding in the last decade. A similar concerted effort could be made for rocket which contains similar compounds, and which are potentially just as efficacious in humans [116]. Erucin for example, has been shown to have very similar, and even superior, biological activity to sulforaphane [117]. One paper has specifically demonstrated that the concentrations of rocket ingested in an average daily diet is significant enough to infer a cancer preventative effect [13]. The metabolism of ITCs in humans via the mercapturic acid pathway has been investigated. ITCs are conjugated with glutathione and degraded by N-acetylation, initiating an increase of phase II detoxification enzymes; see Figure 3 for detailed pathway breakdown of erucin [113].

Nitriles

Along with ITCs, nitriles are the most abundant bioactive compounds produced by GSL hydrolysis [116]. The hydrolysis of glucoraphanin for example, yields predominantly sulforaphane and sulforaphane nitrile. The ratio in which the two are formed depends greatly upon the environmental conditions and the plant cultivar that is used [117]. A low pH medium tends towards the formation of nitriles, whereas high pH forms ITCs [118,119]. The presence of thiol and iron ions favors nitriles, and high temperature and hydration produce more ITCs [120,121]. This can have substantial consequences for any potential health benefits that might be inferred from eating rocket [119]. The nitrile form is approximately three orders of magnitude less efficacious than the ITC in inducing quinone reductase (phase II enzyme), and thus infers a reduced enzymatic and anticarcinogenic response. Nitriles also compete with ITCs in this induction, and reduce potential positive effects further [38]. As the ratio of these compounds may depend on plant variety, care must be taken in rocket breeding when selecting plants for GSL content, as this may not be reflective of the bioactives produced in subsequent hydrolysis reactions [122]. Other underlying genetic factors may influence which degradation pathway is taken.

Indoles

Indoles are the predominant autolysis product of indole glucosinolates such as glucobrassicin, as their ITC counterparts are unstable [85]. Glucobrassicin has been detected as a minor GSL in rocket species [33], and the predominant indole species produced is indole-3-carbinol. This compound is known to be cancer-preventative [125,126], particularly in reproductive organs *in vitro* and *in vivo*. A condensation product of indole-3-carbinol, 3,3'-diindolymethane, is also responsible for beneficial physiological effects. Both compounds have been shown to reduce cell proliferation in breast, prostate, cervical and colon cancer cell lines. They also show distinct differences from ITCs such as sulforaphane [127], and inhibition of tumor development in the stomach, breast, uterus, tongue and liver of rodents [128–135]. Experiments in rodents have shown an increase in drug-metabolizing enzymes in the stomach, liver and small intestines of individuals consuming both ITCs and indoles. This is suggestive of enhanced detoxification phase II enzymes (such as quinone reductase, glutathione reductase and glutathione transferase) [134], and a mechanism by which these phytochemicals infer chemopreventative effects [135,136].

Typically indoles inhibit cell proliferation through cytostatic mechanisms, whereas ITCs induce cytotoxicity within cell lines (at above 12.5μM concentrations), which ultimately leads to increased apoptosis [137,138]. This indicates that both types of compound could act and be effective at different stages of cancer development [11]. Indoles have been shown to induce programmed cell death in prostate, breast and osteocarcinoma cell lines [139] and G_1 cell cycle arrest in breast and prostate cancer cell lines [142,143]. It is these cytostatic effects on cell proliferation that has been suggested as the mechanism responsible for the lack of apoptosis effects in indoles [141].

Using information on GSL content in rocket, the ITC and indole effects can be potentially maximized in new varieties, and be of a greater benefit to human health when considered in tandem, rather than separately [127].

Oxazolidine-2-thiones & goitrogens

The hydrolysis of β-hydroxy-alkyl GSL compounds (e.g. progoitrin; a minor GSL in rocket) can produce oxazolidine-2-thiones such as goitrin (5-vinyloxazolidine-2-thione) [142–148]. It is these compounds that are largely attributed to the thyroid condition of goiter in mammals [149], but the action of microflora in the gut is thought to mediate the problems associated with high oxazolidine-2-thione intake [150,151]. That being said, oxazolidine-2-thiones interfere with thyroxine synthesis [154] and are therefore likely to have an adverse biological effect regardless of gut microflora action or bodily iodine status [3]. A study by Nishie and Daxenbilcher [155] showed that these compounds are not teratogenic or embryotoxic however.

These molecules contribute significantly to the bitter taste of rocket that some people perceive quite strongly [154]. The detection of these compounds may be mediated in a similar genetic fashion as PROP (propylthiouracil), for example [155,156]. By using phytochemical data in rocket breeding programs these oxazolidine-2-thione components could be reduced, potentially improving consumer acceptance (depending on the target consumer) and avoiding any possible adverse health effects associated with over-consumption.

Ascorbigens

Ascorbigens are formed via the reaction of indole-3-carbinol and 3,3’-diindolymethane with ascorbic acid in the stomach during myrosinase-catalyzed degradation of indoly-3methyl glucosinolates [157,158]. In this manner it is thought that ascorbigens have a role in cancer-modulation [159] via quinone reductase induction [114]. As has been highlighted previously, this has important implications for breeding for plant varieties with enhanced chemopreventative effects.

Epithioalkanes

Epithioalkanes are formed as part of the myrosinase reaction with GSLs at low pH with epithiospecifier protein and ferrous ions. These GSLs typically have a side-chain with a double bond, such as sinigrin [160,161]. It is uncertain whether these compounds produce any significant bioactive effect in humans, but the ratio in which they are produced alongside ITCs, nitriles and indoles may impact on these compounds' efficacy as anti-carcinogens.

Flavonols

The antioxidant and anti-inflammatory function of flavonols in the human diet are well known and include protecting the colonic epithelium from free radical damage [164–167]. They can induce the up-regulation of enzymes (such as cytochrome P450), that may lead to a decreased risk of cancer, cardiovascular disease, immune dysfunction, atherosclerosis and chronic inflammation [168,169].

Factors affecting phytochemical content

Breeding and cultivation

Rocket has been consistently shown to be a good dietary source for flavonols, GSLs and anti-oxidants. However, there can be large differences between plants of the same germplasm accession due to a combination of genetic and environmental variability. This is probably due to the outbreeding nature of the species [104] and a lack of overall uniformity in varieties. Commercial varieties cannot be considered truly domesticated because of this tendency for outcrossing, and the susceptibility of plants to inbreeding depression (a loss of genetic variability due to repeated self-pollination or crossing with a closely related individual). Development of advanced open-pollinating breeding lines (lines that are allowed to cross-pollinate freely in a population of selected individuals), or even F_1 hybrids (superior varieties produced by crossing distinctly different, elite inbred lines), could potentially minimize such variation.

Throughout the food chain there are many aspects that can have an adverse effect on GSL levels within leaves (Figure 4). These include the cultivar choice, cultivation practice, climatic conditions, photoperiod, sulphur and nitrogen availability, harvest date, time spent in storage, the temperature of wash water, levels of physical damage to leaves, packaging atmosphere and food preparation methods [30–32,170–173].

Harvesting

Rocket species have the ability to re-grow their leaves repeatedly after cutting, which allows for several harvests to take place under optimal conditions [107]. In parts of southern Italy, it is not unheard of for up to seven harvests to occur from a single planting. This has obvious cost-saving benefits for growers, but multiple harvests also induce stress responses in rocket that may be detrimental to the flavor and aesthetics of the crop. Stress drives up the production of secondary metabolites such as GSLs and anthocyanins, which will produce very strong, bitter tastes. There are other detrimental effects of multiple harvests; leaves become progressively smaller and more 'skeletal' in appearance with each cutting, for example. High anthocyanin levels also affect the color of leaves, turning them an undesirable pink, purple or red. Color has been found to be one of the most important characteristics consumers look for in rocket [174], and so the loss of fresh appearance can ultimately lead to rejection of crops by supermarkets and processors.

Industrial and culinary processing

There are five main influences that have been identified in affecting GSL levels during processing [94]. These are the action of myrosinase hydrolysis, myrosinase inactivation, the lysis and leaching of GSLs into wash-water, thermal degradation of GSLs, and the loss of ascorbic acid, iron and other enzyme co-factors. Myrosinase inactivation and thermal degradation of GSLs is probably less of an issue in rocket species, as the leaves are not typically cooked. The leaves are not ordinarily frozen, and so freeze-thaw hydrolysis is not likely to be a major factor either. Other factors almost certainly play a significant role in GSL and phytochemical loss in rocket. Verkerk et al. [94] highlighted four key areas that affect GSL levels before reaching the end consumer. These are:

1. The variety / cultivar used
2. Storage and packaging (post-harvest, post-processing & in shops/supermarkets)
3. Industrial processing
4. Consumer preparation methods

If each of these areas can be mitigated through breeding superior varieties, consumers will receive an end product that is of higher nutritive quality and thus provide increased health benefits.

Post harvest storage

Studies on both *Diplotaxis* and *Eruca* species have been conducted to determine the effects of post harvest storage conditions on chlorophyll content and respiration rates [15]. Both species of rocket have been found to have high respiration rates [107] leading to rapidly impaired visual quality, such as stem browning, tissue yellowing and general decay [175]. Provided initial GSL loss can be mitigated through breeding, ITC formation has been shown to increase over nitrile formation during the storage period [176].

Time, temperature, humidity and atmospheric conditions are all optimized for specific crops within the logistics chain, but these factors are often only designed to prevent visual degradation and not phytochemical breakdown [100]. Getting producers, packagers and transporters to change their current practices in order to better preserve the health-promoting compounds in rocket would be a difficult task. Treatments and storage conditions are often integrated parts of protocols and procedures, and changing these would require significant testing on a commercial scale.

New selection tools for breeders

Phytochemical selection

It should not be forgotten that some GSLs and their breakdown products are thought to be toxic, and even carcinogenic, at high concentrations [128]. Breeders and researchers should be mindful that more of a certain compound does not necessarily mean 'better' [177]. Humans seem to be able to tolerate GSLs much better than pigs, rats and rabbits for example; but overconsumption of these compounds may have serious health consequences [64] as high dose-effect relationships are as yet unknown in humans [94]. Few papers in GSL research (regardless of species) have acknowledged the potential for plant breeders to utilize HPLC/UPLC/LC-MS/GC-MS methods within breeding programs to 'monitor' and select plants for their phytochemical content in this manner. These techniques would provide valuable information on breeding lines relatively rapidly, especially for GSL and flavonol breeding [178]. It is not common practice to select rocket plants based on their phytochemical profile at present, but as interest in these compounds increases it will be necessary for breeders to modify their selection criteria and information sources in order to remain competitive in the salad vegetable market [94]. This has been achieved with '*Beneforte*' broccoli (Seminis Vegetable Seeds; subsidiary of Monsanto Company, St. Louis, Missouri, USA; www.beneforte.com) for example. It has also been indicated in hybrid varieties of *Brassica* that ITC/nitrile ratios can be selected for [179].

Genetic resources and Marker Assisted Breeding

European initiatives (such as the EU GENRES project 'Leafy vegetables germplasm, stimulating use'; http://documents.plant.wur.nl/cgn/pgr/leafyveg/) have included rocket species within their remit, indicating the rising prominence of the species, and the desire for more work to be conducted on them. The germplasm accessions stored in gene banks are a valuable genetic resource for breeders to take advantage of [180]. The accessions contained within these collections are highly variable and have unique visual and sensory characteristics that could be introgressed into breeding lines relatively easily [181].

Genetic information about rocket within the published literature is very scarce. Some molecular marker techniques such as Random Amplification of Polymorphic DNA (RAPD), Inter-Simple Sequence Repeats (ISSR) and Amplified Fragment Length Polymorphisms (AFLP) have been used to analyze morphological characteristics of *Eruca vesicaria* [29]. ISSR and AFLP are relatively robust for screening variable populations and discriminating between cultivars [180] but RAPDs are notoriously unreliable and suffer from a lack of reproducibility and resolution. Perhaps one of the most underutilized marker types is SRAP (Sequence Related Amplified Polymorphism). The forward and reverse primers are designed to target arbitrary GC and AT rich sequences of the genome respectively, and are therefore more likely to anneal to active genomic regions [182]. This could be of use in understudied crops such as rocket, as it provides a simple, repeatable and reliable way of screening large populations.

These techniques are now for the most part however, obsolete in advanced molecular plant breeding, as NGS (Next Generation Sequencing) and SNP (Single Nucleotide Polymorphism)/QTL (Quantitative Trait Loci) analyses are far more specific, reliable and cost-effective. SNPs are the most abundant marker type within genomes, and their high density is ideal for studying specific regions in detail [183]. NGS techniques are now relatively affordable, even for relatively small companies. They are widely available in academic institutions, but many companies are bypassing these in favor of dedicated private commercial services [184] or are developing their own in-house facilities. The inability of some research institutions to provide adequate customer service, cost-effectiveness, data storage, and results on time is jeopardizing how much knowledge is in the public domain. Increasingly, both large and small breeding companies are collaborating privately and advancing techniques far beyond those found in academic institutions. Future work by institutes in advanced genomics, sequencing and genotyping is likely to be obsolete in some cases because private research is already finding new innovations, e.g. for data storage and bioinformatics. Because private companies have no obligation to share their knowledge, many of these advances may be unobserved by the mainstream scientific community. Institutes and Universities need to do more to attract business from industry in order to keep up with the pace of private advances in this area.

Transcriptome sequences are now (generally) adequate for breeders to use and make huge advances in only a few years. Linkage mapping and QTL analyses can be conducted on desktop computers, making integration into breeding companies relatively straightforward from an IT point of view, even if the actual sequencing and genotyping are outsourced. Again, this may typically be to private companies providing a dedicated service. The availability of software licenses and advanced training courses from private companies also means plant breeders do not necessarily need the expertise found in Universities and research institutes in order to attain their goals.

Summary

Of all the research papers concerning rocket species and their phytochemistry, none have directly addressed how information could be used within a working breeding population. Often it is explained or postulated purely as theory rather than actual practice, or only given a cursory mention. Only very rarely is a plant breeding program reflective of theory, due to the large number of environmental factors affecting plant growth, development and reproduction. The progressive selection of rocket plants through conventional/molecular breeding would be a valuable tool for the research community as well as providing an excellent incentive for breeding companies to fund research. The actual monitoring and quantification of GSL/flavonol levels through successive generations (i.e. not just one as has been the case with most studies) would not only validate the heritability of such traits in rocket, but would also provide a 'roadmap' for how other minor crops might be developed for commercial use.

Attention must be paid to the phytochemical content of varieties within breeding populations of rocket. By focusing solely on morphological traits, important phytochemical genotypes may be inadvertently lost from populations; this could be said of all *Brassicaceae* species, not just rocket. The balance of glucosinolate-myrosinase degradation products does seem to have a genetic component to it and so could be selected for also. Utilising genetic resources, the falling costs of sequencing and bioinformatics can produce nutritively superior varieties of rocket in the near future. Plant breeding typically takes longer than the average research project allows for, even with the use of advanced genomic selection methods. This is a situation that could be remedied by long-term industrial collaboration and sponsorship by plant breeding firms.

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Figure captions

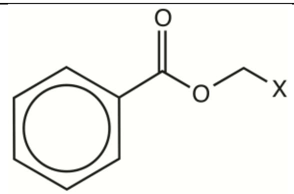
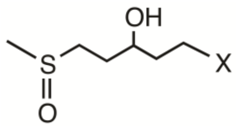
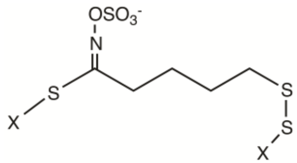
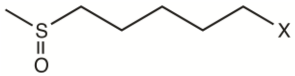
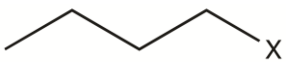
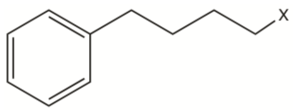
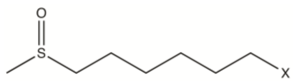
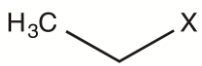
Figure 1: – The glucosinolate-myrosinase reaction and some of the subsequent compounds produced under different conditions, such as pH and the influence of epithiospecifier proteins (ESP) (Adapted from Zhang [9] and Hall et al. [185]).

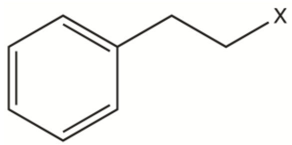
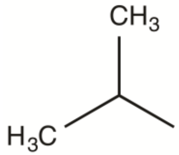
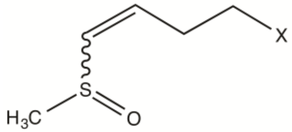
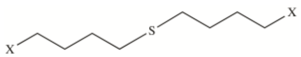
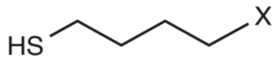
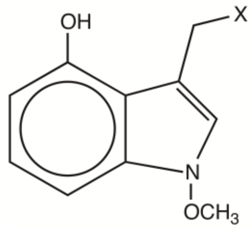
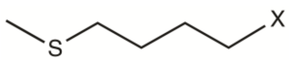
Figure 2: – Pathways of documented ITC action in tumorigenic cells. See Wu et al. [113] for a detailed review of the roles ITCs play in cancer prevention.

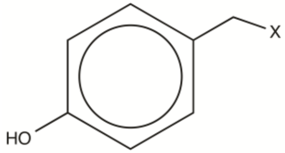
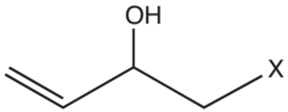
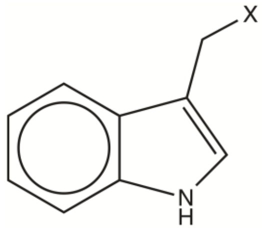
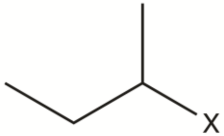
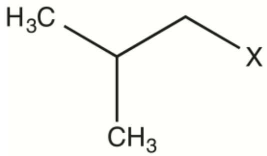
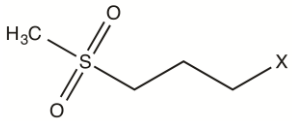
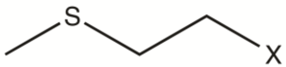
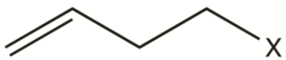
Figure 3: – The mercapturic acid pathway of ITC metabolism in the human body. After ingestion of rocket leaves glucoerucin is hydrolyzed by myrosinase to form erucin. This is released and absorbed in the ileum, where it is transported in the blood to cells around the body. ITCs initiate Phase II detoxification enzymes in this pathway, and are known to aid in cancer prevention. (Adapted from Wu et al. [113]).

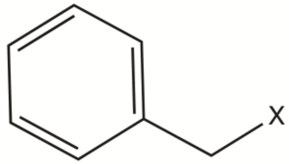
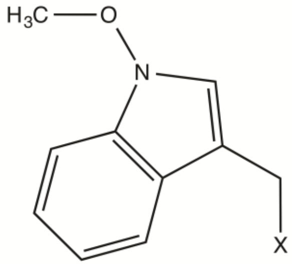
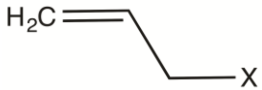
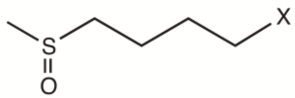
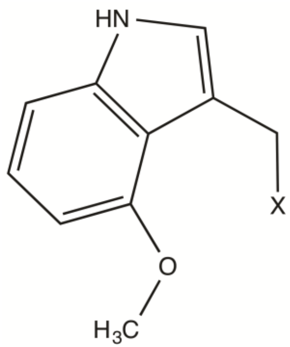
Figure 4: – Factors and conditions within the commercial supply chain that affect GSL and flavonol levels within rocket leaves.

Table 1: – Intact Glucosinolates Identified Within Leaves Of Rocket, *Eruca* and *Diplotaxis* Species, By LC-MS (Negative Ion Mode)

R-group	Common name	R-group structure [x]	Mass parent ion	MS^2 spectrum ions (signature ions in bold)	Reference
2-(benzoyloxy) ethyl	-		466	386	33
3-hydroxy-5-(methylsulfinyl) pentyl	-		482	403	
4-(β-D-glucopyranosyldisulfanyl) butyl	Diglucothiobeinin		600	521	33,186
5-(methylsulfinyl) pentyl	Glucoalyssin		450	371	
N-butyl	Dihydrogluconapin		374	294	28
4-phenylbutyl	Glucoamoracin		450	371	
7-(methylsulfinyl) heptyl	Glucoibarin		494	414	
Ethyl	Glucolepiidin		346	266	

2-phenylethyl	Gluconasturtiin		422	343	28
1-methylethyl	Glucoputranjivin		360	280	
4-(methylsulfinyl)-3-butenyl	Glucoraphenin		434	354	
Dimeric 4-mercaptobutyl	DMB		811	731, 569, **405**	21,33,186
4-mercaptobutyl	Glucosativin		406	**259**, 209, 194, 138 97, 96	
4-hydroxy-3-indolymethyl	4- Hydroxyglucobrassicin		463	383, **285**, 275, 267, **259**, 240	33,178
4-(methylthio) butyl	Glucoerucin		420	340, 291, 275, **259**, 242, 227, 195, 178, 163	

4-hydroxybenzyl	Glucosinalbin		424	344, 291, 275, 261, **259**, 246, 231, 228, 182	33,178
(R,S)-2-hydroxy-3-butenyl	Progoitrin/epiprogoitrin		388	332, 308, 301, 275, 259, 210, 195, 136	
3-indolymethyl	Glucobrassicin		447	275, **259**, 251, 205	33,178,187
1-methylpropyl	Glucocochlearin		374	294	85,186
2-methylbutyl	Glucojiaputin		388	308	
5-(methylsulfonyl) pentyl	Glucoerysihienin		466	386	28,33
3-(methylthio) propyl	Glucoiberverin		406	326, 275, **259**, 228, 145	85,178
3-butenyl	Gluconapin		372	292, 275, **259**, 227, 195, 194, 176	28,178

Benzyl	Glucotrapaeolin		408	328, 275, **259**, 241, 230, 212, 195, 166	
1-methoxyindol-3-ylmethyl	Neoglucobrassicin		477	447, **466**, 284, **259**	28,178
2-propenyl	Sinigrin		358	278, 275, **259**, 227, 195, 180, 162	
4-(methylsulfinyl) butyl	Glucoraphanin		436	**372**, 291, **259**, 97, 96	21,33,178,187
4-methoxy-3-indolymethyl	4-Methoxyglucobrassicin		477	291, 275, **259**, 235, 227, 195	178, 188

^{x} = standard GSL molecule according to IUPAC nomenclature

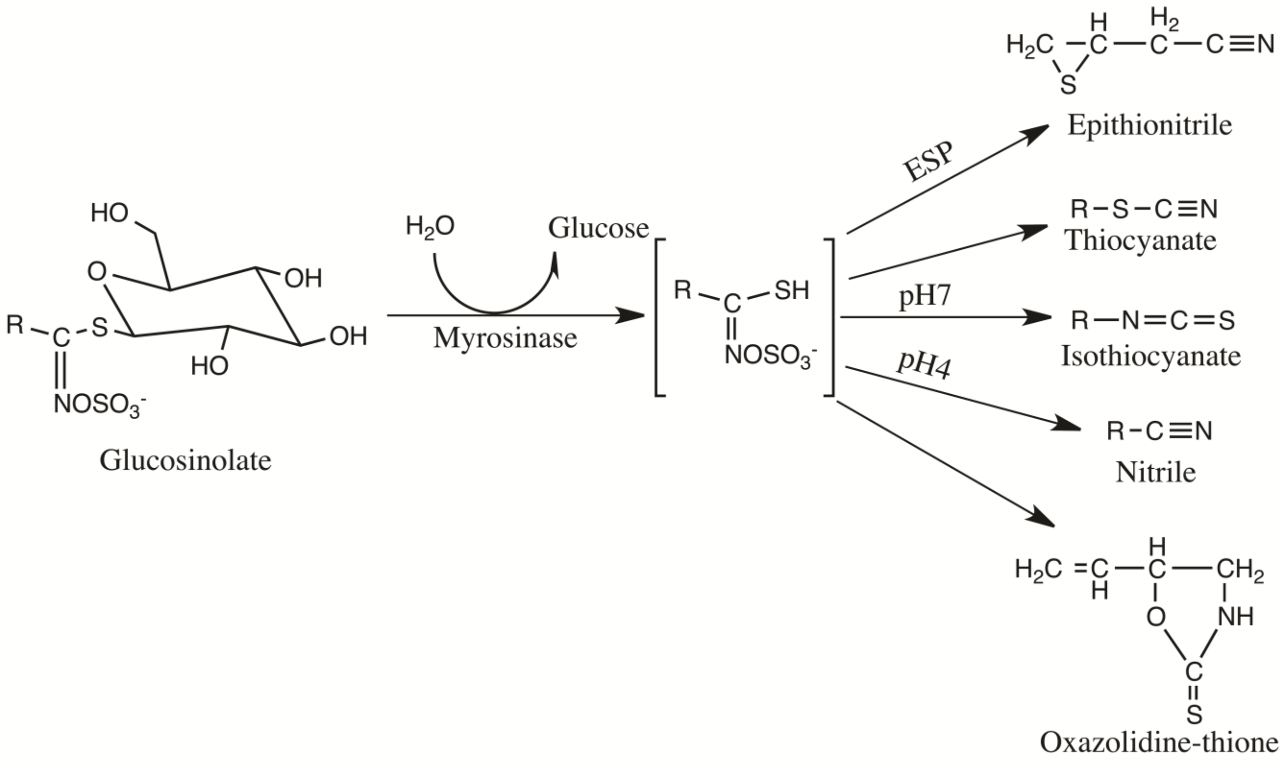
Table 2: – List Of Flavonol Compounds Identified In Leaves Of *Eruca* And *Diplotaxis* Species, By LC-MS (Negative Ion Mode).

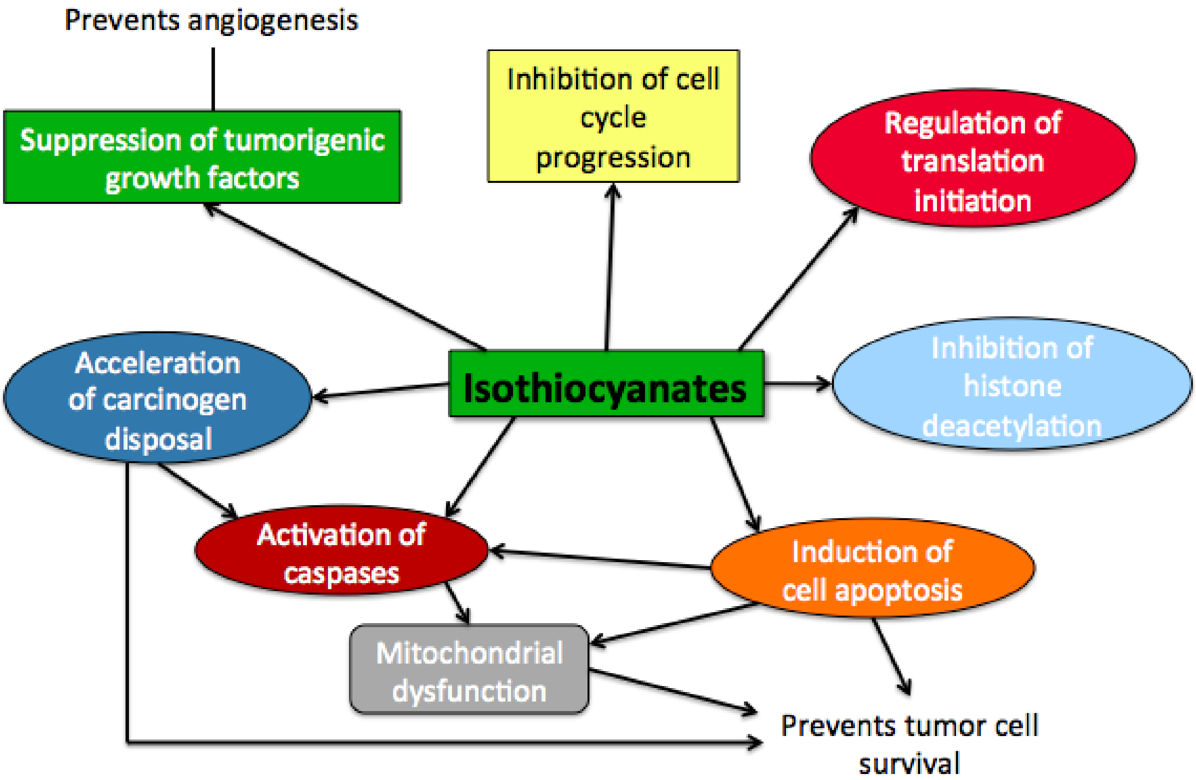
Flavonol compound [a]	*Eruca* [p]	*Diplotaxis* [p]	Mass parent ion	MS^2 spectrum ions (signature ion in bold)	Reference
I 3,4'-diGlc	✓	✓	639	**477**	18,33
I 3-Glc	✓		477	-	
K 3-(2-Sinp-Glc)-4'-Glc	✓		817	-	
K 3,4'-diGlc	✓	✓	609	-	
K 3-Glc	✓		447	**285**	
Q 3-Glc	✓		463	301	
K 3-diGlc-7-Glc	✓		771	609	33
K 3-Sinp-triGlc-7-Glc	✓		1139	977, 771, 609, 429	
Q 3,4'-diGlc-3'-(6-Caf-Glc)		✓	949	787, **625**, 463, 301	
M	✓		317	151	189
Q	✓		301	151	
R	✓		609	300	
Q 3-(2-Caf-Glc)-3'-(6-Sinp-Glc)-4'-Glc		✓	1155	993, 831, 787, 669, **625**, 463, 301	18,25
Q 3-(2-Mcaf-Glc)-3'-(6-Sinp-Glc)-4'-Glc		✓	1185	1023, 817, 669, 655	
Q 3-(2-Fer-Glc)-3'-(6-Fer-Glc)-4'-Glc		✓	1139	977, 639, 463	18,25,33
Q 3-(2-Fer-Glc)-3'-(6-Sinp-Glc)-4'-Glc		✓	1169	1007, 831, 669, 639, 463, 301	

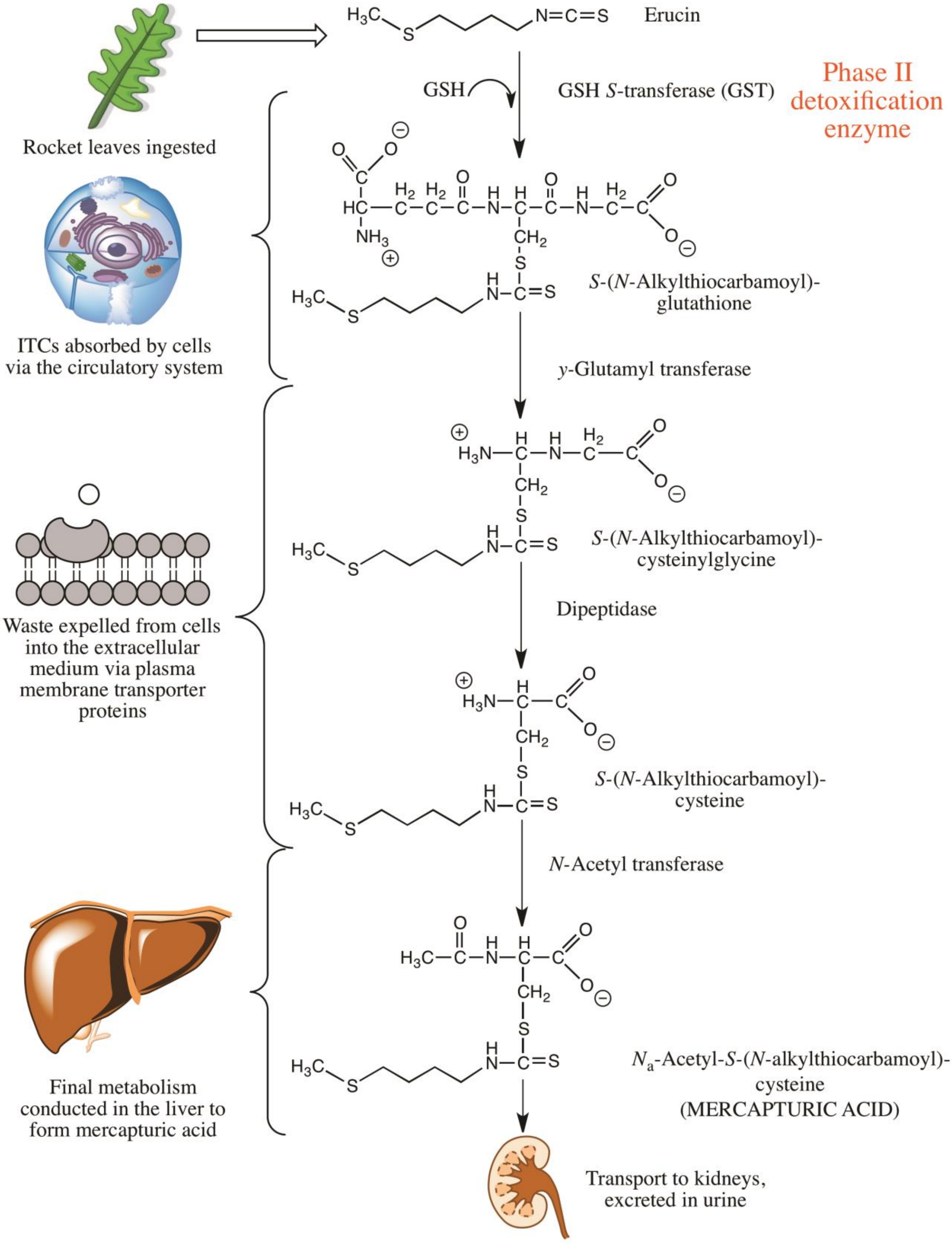
Q 3-(2-Sinp-Glc)-3’-(6-Sinp-Glc)-4’-Glc	✓	1199	1037, 831, 669, 463, 301	
Q 3,3’,4-triGlc	✓	787	625, 463, 301	
Q 3,4’-diGlc-3’-(6-Fer-Glc)	✓	963	801, 639, 463, 301	18,25,33
Q 3,4’-diGlc-3’-(6-Mcaf-Glc)	✓	979	817, 655, 463, 301	
Q 3,4’-diGlc-3’-(6-*p*.Coum-Glc)	✓	933	771, 609, 463, 301	
Q 3,4’-diGlc-3’-(6-Sinap-Glc)	✓	993	831, 669, 463, 301	

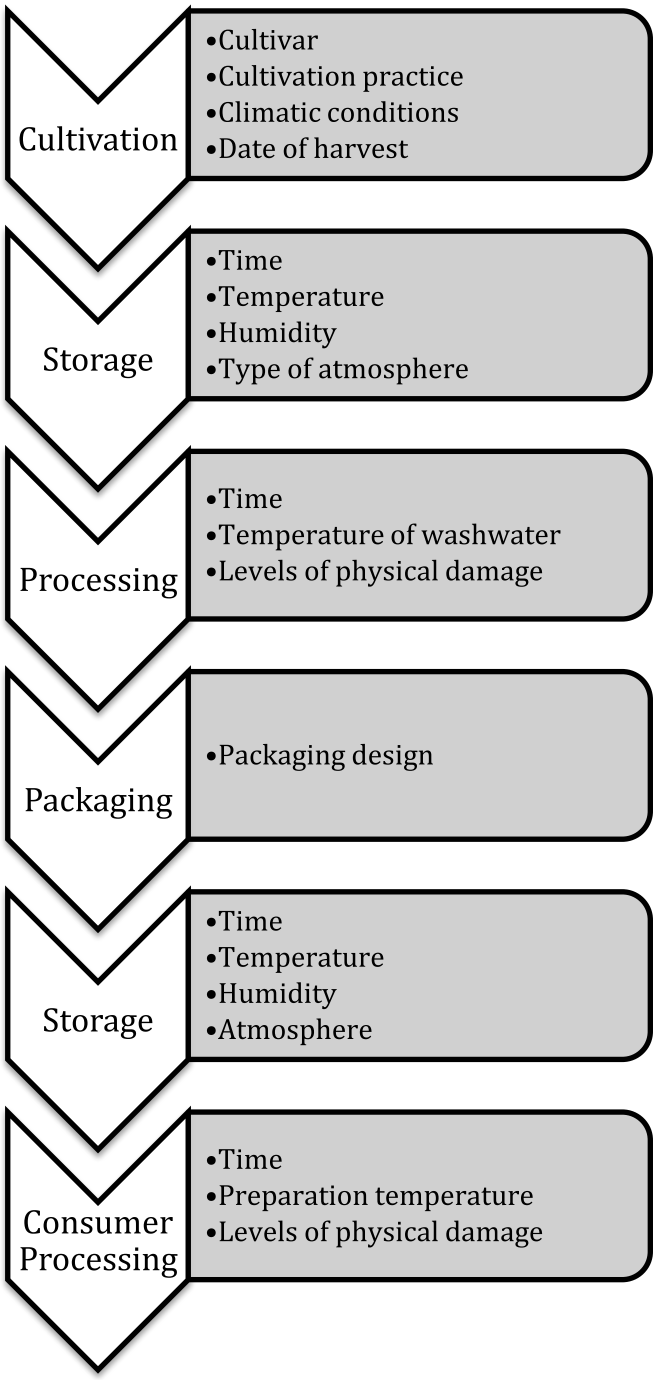
[a] = Abbreviations: Caf, caffeyol; Mcaf, methoxycaffeyol; *p*.Coum, *p*-coumaroyl; Fer, feruloyl; Sinp, sinapoyl; Glc, glucoside; Q, quercetin; K, kaempferol; I, isorhamnetin; M, myricetin; R, rutin

[p] = ✓ compound positively identified in species


Glucosinolate
H2O
Glucose
Myrosinase
ESP
Epithionitrile
Thiocyanate
pH7
Isothiocyanate
pH4
Nitrile
Oxazolidine-thione


Prevents angiogenesis
Suppression of tumorigenic growth factors
Inhibition of cell cycle progression
Regulation of translation initiation
Acceleration of carcinogen disposal
Isothiocyanates
Inhibition of histone deacetylation
Activation of caspases
Induction of cell apoptosis
Mitochondrial dysfunction
Prevents tumor cell survival


Erucin
GSH
GSH S-transferase (GST)
Phase II
detoxification
enzyme
Rocket leaves ingested
S-(N-Alkylthiocarbamoyl)-
glutathione
ITCs absorbed by cells
via the circulatory system
γ-Glutamyl transferase
S-(N-Alkylthiocarbamoyl)-
cysteinylglycine
Waste expelled from cells
into the extracellular
medium via plasma
membrane transporter
proteins
Dipeptidase
S-(N-Alkylthiocarbamoyl)-
cysteine
N-Acetyl transferase
Final metabolism
conducted in the liver to
form mercapturic acid
Nα-Acetyl-S-(N-alkylthiocarbamoyl)-
cysteine
(MERCAPTURIC ACID)
Transport to kidneys,
excreted in urine


Cultivation
•Cultivar
•Cultivation practice
•Climatic conditions
•Date of harvest
Storage
•Time
•Temperature
•Humidity
•Type of atmosphere
Processing
•Time
•Temperature of washwater
•Levels of physical damage
Packaging
•Packaging design
Storage
•Time
•Temperature
•Humidity
•Atmosphere
Consumer Processing
•Time
•Preparation temperature
•Levels of physical damage

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