# Taste and health promoting factors of Brassica bioactive compounds

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# Final report (milestone 190)

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# MEDIA SUMMARY


CSIRO scientists (located in Sydney and Adelaide) used sensory and consumer science, phytochemistry and cell biology to determine the trade-off between taste characteristics and health properties of *Brassica* vegetables. The aim was to understand how to increase consumer acceptance.

*Brassica oleracea* vegetables (e.g. broccoli, broccolini, red and green cabbages, cauliflower, Brussels sprouts) contain phytochemicals with health-benefits (e.g. anticancer, antioxidant). However these components are believed to be responsible for the undesirable taste of many *Brassica* vegetables.

A consumer test of 200 adults showed that broccoli and cauliflower were the most popular amongst the 6 *Brassica* vegetables. Information related to the cancer-protective benefits of *Brassica* vegetables had only a small effect on responses to (the least liked) Brussels sprouts only. Overall, taste perception, and to a lesser extent, attitudes, influenced the liking of and intentions to consume *Brassica* vegetables. This means that improving taste (whilst retaining 'healthy' compounds) may be more important than promoting health information in order to increase the popularity of *Brassica* vegetables.

Chemical and sensory characterisation of broccoli, cauliflower, Brussels sprouts, and red cabbage determined the contribution of major phytochemicals (i.e. glucosinolates, free sugars, phenolics) to the taste profiles of these vegetables.

Brussels sprouts and broccoli were found to contain the most glucosinolates. Phenolics were also identified. Red cabbage and cauliflower contained more free sugars than Brussels sprouts and broccoli

Sensory evaluation found sweetness and bitterness were the most important distinguishing attributes, followed by saltiness and astringency (i.e. dryness or mouth puckering). Cauliflower and red cabbage were sweeter than broccoli and Brussels sprouts. Brussels sprouts most bitter and astringent. Broccoli was saltier than all other. There was a good agreement between the trained sensory panels' assessment and consumer perceptions.

Several components, including glucosinolates and phenolics, were found to contribute to the bitterness and astringency of *Brassica vegetable* extracts.

The extracts from the *Brassica* vegetables were found to inhibit one type of cancer cell growth and possessed antioxidant activity. Glucosinolates, the enzyme myrosinase and phenolics probably are responsible for these effects.

This is the first time that a systematic, interdisciplinary attempt has evaluated the contribution of phytochemicals to the taste and health properties of *Brassica* vegetables. The findings indicate that to a large extent, the phytochemicals responsible for the undesirable taste of *Brassica* vegetables are also major contributors to their health properties. Therefore, an improvement in taste properties, by reducing glucosinolates and phenolics would lead to a loss of health properties.

An increase in sweetness, accomplished by increasing sugar content, would reduce perceived bitterness when consuming *Brassica* vegetables. The development of new/improved *Brassica oleracea* varieties with increased sugar content could be beneficial for the industry as it could lead to increased consumer acceptance.

Finally, development of new/improved varieties with increased pigmentation is likely to lead to higher antioxidant activities whilst maintaining their growth-inhibiting action against cancer.

## TECHNICAL SUMMARY

*Brassica oleracea* vegetables (e.g. broccoli, broccolini, red and green cabbages, cauliflower, Brussels sprouts) are known to contain several phytochemicals (e.g. glucosinolates, isothiocyanantes, phenolics) with health-benefiting (e.g. anticancer, antioxidant) properties. These components are believed to be responsible for the undesirable taste associated with many *Brassica* vegetables.

An interdisciplinary approach combining sensory and consumer science, phytochemistry and cell biology was employed to determine the relative contribution, or trade-off, between taste characteristics and health properties of phytochemicals in *Brassica* vegetables with a view of increasing consumer acceptance.

A consumer test was conducted with 200 adults all reporting medium-high physical activity as a marker/control of health conscious behaviour and reporting either low or high vegetable intake. Broccoli and cauliflower were the most popular in terms of hedonics and intention to consume amongst a group of 6 *Brassica* vegetables. The provision of information related to the cancer-protective benefits of *Brassica* vegetables had only a small effect as it appeared to influence responses to (the least liked) Brussels sprouts only. Overall, taste characteristics, and, to a lesser extent, attitudes dominated the liking of and intentions to consume *Brassica* vegetables. This means that improving taste (whilst retaining 'healthy' compounds) may be more important than promoting health information in order to increase the popularity of *Brassica* vegetables.

Chemical and sensory characterisation of whole and fractionated extracts from selected Australian-grown *Brassica* vegetables (broccoli, cauliflower, Brussels sprouts, red cabbage) was carried out in order to determine the contribution of major phytochemicals (i.e. glucosinolates, free sugars, phenolics) to the taste profiles of these vegetables.

The glucosinolate (GS) and phenolic profiles were determined using liquid chromatography coupled to photodiode array detection and mass spectrometry. Ten GS were quantified across the vegetables investigated. Brussels sprouts (186.3 µg/g FW) followed by broccoli (164.1 µg/g FW) were found to contain the most GS. The phenolic profiles of all samples were dominated by signals at 280 and 326 nm likely indicative of phenolic acids. As expected, red cabbage was the only vegetable with a significant anthocyanin signal (574 µg/g FW). Red cabbage (26.7 mg/g FW) and cauliflower (18.7 mg/g FW) were found to contain a higher concentration of free sugars than Brussels sprouts (12.6 mg/g FW) and broccoli (10.2 mg/g FW).

Sensory evaluation of the whole extracts found sweetness (cauliflower and red cabbage sweeter than broccoli and Brussels sprouts) and bitterness (Brussels sprouts more bitter than the rest) were the most important distinguishing attributes followed by saltiness (broccoli saltier than rest) and astringency (Brussels sprouts more astringent than the rest). There was generally a good agreement between the results from the sensory assessment of the whole extracts and the taste intensity ratings generated from the consumer acceptance test using steamed *Brassica* vegetables.

Fractionation of the whole *Brassica* extracts, followed by sensory evaluation of the fractions revealed that fraction 1 was the most taste active fraction across all *Brassica* whole extracts. Sweetness (likely due to free sugars), umami (possibly due to amino acids), astringency and saltiness were the main attributes to the taste profile of this fraction. Sub-fractionation showed that this fraction was also bitter (GS and others) but the sweetness (due to sugars) was counteracting bitterness.

Several components within each extract were found to contribute to the bitterness and astringency of whole *Brassica* extracts. Total and individual GS content alone could not explain the sensory results for either of these attributes. Phenolics and/or other components are also likely to be responsible for these undesirable taste sensations.

The extracts from the *Brassica* vegetables exhibited both *in-vitro* antiproliferative and antioxidant activity. The myrosinase-treated whole extracts from all four *Brassica* vegetables showed comparable growth-inhibiting activity against colon adenocarcinoma cells (HT-29). Only myrosinase-treated fractions possessed antiproliferative activity with fraction 1 being the most active across all vegetables. Isothiocyanates (ITC) were likely to be responsible for the observed activity against the HT-29 cells although the GS composition (GS are ITC precursors) did not correlate with the observed activity suggesting that myrosinase rather than substrate availability was more important during the GS to ITC conversion.

The whole extract from red cabbage exhibited the highest antioxidant activity amongst the four *Brassica* extracts assayed due to the presence of cyanidin-based anthocyanins in that extract. Only a few fractions from each whole extract showed notable antioxidant activity. The contribution of fraction 1 to the antioxidant activity of each extract varied from 20% (red cabbage) to 78% (cauliflower).

This is the first time, to our knowledge, that a systematic, interdisciplinary attempt was made to evaluate the contribution of key phytochemicals to the taste and health properties associated with *Brassica* vegetables. The findings indicate that to a large extent, the phytochemicals responsible for the undesirable taste of *Brassica* vegetables are also major contributors to their health properties. Therefore, an improvement in taste properties, by reducing GS and/or phenolic content, would lead to a loss of health properties. The bitter GS gluconapin was the only component that could potentially be reduced without affecting much the health properties of red cabbage.

An increase in sweetness, accomplished by increasing sugar content, would reduce perceived bitterness when consuming *Brassica* vegetables. The development of new/improved *Brassica* oleracea varieties with increased sugar content could be beneficial for the industry as it could lead to increased consumer acceptance.

As shown by red cabbage, the presence of pigments such as anthocyanins in the chemical profile markedly improves the antioxidant properties of a vegetable without affecting its antiproliferative properties. Therefore, development of *B. oleracea* varieties with increased anthocyanins would be beneficial for public health.

The multi-disciplinary ‘toolbox’ developed and used in this study can now be applied to any other vegetable or fruit for the benefit of the Australian Industry and the public.

# 1. INTRODUCTION

Diets rich in vegetables and fruit are highly encouraged by nutritionists as they have been linked with several beneficial human-health effects (*1, 2*). Several plant-based components that are present in the everyday diet are reported to be physiologically active (*1, 3, 4*) and should form the basis for dietary-based chronic disease prevention. *Brassica* is a genus of plants which belongs to the Brassicaceae family or otherwise known as the mustard family. Many species within this genus are well known and widely used. *B*. oleracea, including vegetables such as broccoli, cabbage, kale, kohlrabi, cauliflower and Brussels sprouts, is the most commonly encountered species.

## 1.1 Major phytochemicals found in *Brassica* crops

### 1.1.1 Glucosinolates (GS) and their derivatives

A group of compounds generating a lot of interest are glucosinolates (GS). GS are β-thioglucoside N-hydroxysulfates [also known as (*Z*)-(or *cis*)-N-hydroximinosulfate esters or S-glucopyranosyl thiohydroximates], with a side chain (R) and a sulfur-linked β-D-glucopyranose moiety (*5*).
All Brassica plants that have been investigated are able to synthesise GS (*6*) although several non-*Brassica* species may contain one or more GS in their chemical profile (*5*). To date more that 120 different GS have been identified and the ones mostly studied are those found in *Brassica* vegetables: the aliphatic, aromatic and heterocyclic (e.g. indole) GS (*5, 7*). Although highly variable, the GS content in *Brassica* vegetables can reach 1% dry weight in some tissues (*5*); in most GS-containing plants this value is attributed to a small number of GS (usually less than 12) (*8*) (Table 2). Despite this, GS distribution can be quite different across various plant organs both in qualitative and quantitative terms (*9*).

GS content can vary quite significantly even between plants cultivated under the same conditions on the same site (*10*). Studies have shown that genetic and environmental/agronomic factors exert considerable control on the levels of GS found in a plant (*8, 11*). Moisture, soil type, fertiliser application, plant spacing, post-harvest handling/storage but also pathogen/pest attacks are all known factors that may affect total and/or individual GS content (*8, 10, 12*). Seasonal effects on GS content have also been reported (*10, 13, 14*) but limited evidence exists on the linkage between GS content and specific climatic factors (*15, 16*).

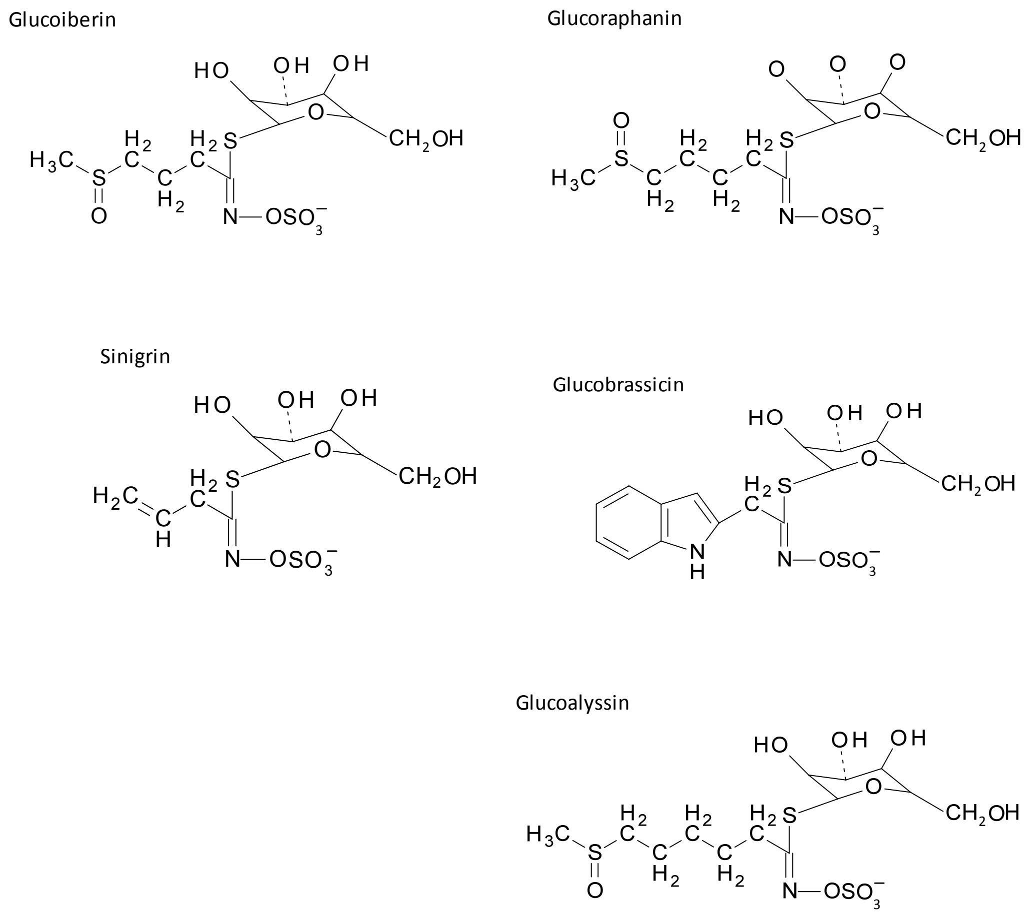


**Figure 1.** Structures of selected GS found in *B. oleracea* plants

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Studies have indicated that synthesis of tryptophan-derived (i.e. indolyl) GS was regulated primarily by environmental factors whilst synthesis of methionine-derived (i.e. aliphatic) GS was controlled to a large extent by genotype (*17*). Therefore, genetic manipulation of *Brassica* vegetables for the production of varieties with high aliphatic GS content may be possible; an up regulation of indolyl GS on the other hand would require a clear understanding of the interaction mechanism between environmental factors and genes (*12*).

*HYDROLYSIS OF GS BY MYROSINASE*

GS are known to be very stable and relatively non-reactive under normal conditions. However following tissue damage, by biotic or abiotic factors, GS are known to produce isothiocyanates (ITC) (and several other products) through the action of the glycoprotein myrosinase (*18*)(Figure 2). The nature of the end products of myrosinase-catalysed GS hydrolysis is determined by the substrate, pH conditions, availability of ferrous ions, and the level and activity of specific protein factors such as the epithiospecifier protein (*19*). The most common products of GS hydrolysis are isothiocyanates (although other products are also formed) (*8*). The nature of the products formed following GS hydrolysis is important. ITC are associated with many of the positive physiological effects attributed to GS however many of the other GS breakdown products are known to be goitrogenic and/or toxic (*8, 20*).

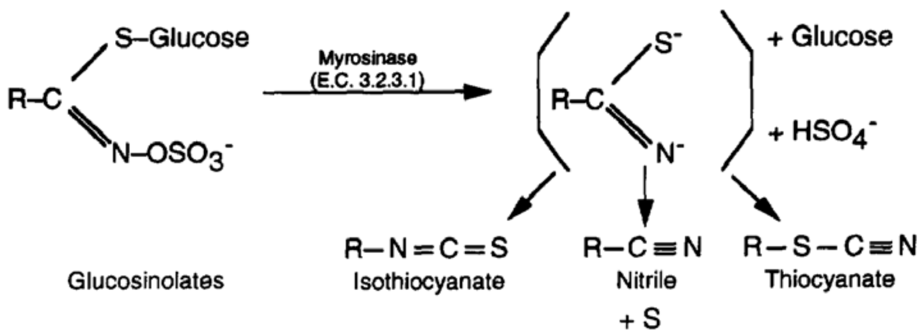


**Figure 2**. Myrosinase-catalysed breakdown of GS [from (*20*)].

*ISOTHIOCYANATES (ITC)*

More than 120 ITC have been identified to date (*21*) (Figure 3). However, most studies deal with only very few ITC as these compounds tend to be very unstable and therefore their extraction and purification or synthesis is not an easy task. In addition, earlier reports about the potential of sulforaphane [4-methylsulfinyl)butyl isothiocyanate], the hydrolysis product from glucoraphanin, as a natural inducer of anticarcinogenic protective enzymes (*3*) have focused the attention of investigators on this molecule. Additionally, indole-3-carbinol (I3C) a derivative of the very unstable brassicin (3-indolylmethylisothiocyanate), has also shown strong potential as an agent against hormone-related cancers (*22, 23*).

The nature of ITC produced upon myrosinase-catalysed hydrolysis will be similar to that of the GS used as substrate (i.e. side-chain of GS remains in the ITC). In terms of ITC concentration, found in vegetables following cellular damage, availability of myrosinase and substrate are the two critical factors. Therefore any process that will have an effect on the levels of either the substrate or the enzyme will indirectly affect the level of ITC produced. However, bioavailability of ITC (as with any bioactive agent) ultimately determines whether physiologically adequate amounts are absorbed by the body (*24*).

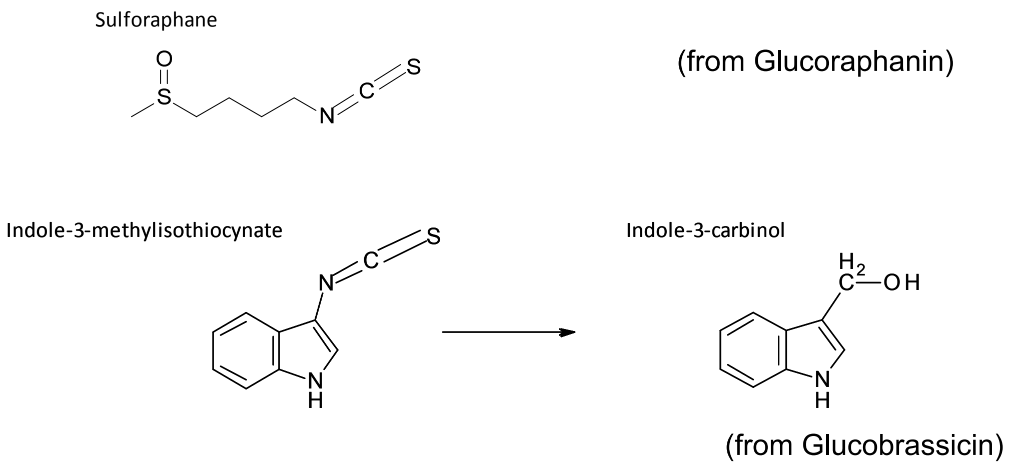


**Figure 3.** Selected ITC found in *Brassica* vegetables and their precursors (in brackets). Indole-3-methylisothiocyanate has never been isolated as it rapidly converts to indole-3-carbinol.

### 1.1.2 Phenolics

In addition to the extensively studied GS and their derivatives, *Brassica* vegetables are known to contain simple and more complex phenolic components. The most widespread and diverse group of phenolics in plants are the flavonoids (termed this way because they are structured according to the $C_6$-$C_3$-$C_6$ flavone skeleton) (*25*) (Figure 4). Simpler phenolic components in plants include benzoic and cinnamic acid derivatives (*25*). Although not extensively studied, phenolic components are believed to be responsible for the high antioxidant capacity observed for some *Brassica* species as a study using whole cruciferous extracts and purified GS has shown that GS possess rather weak antioxidant properties (*26*). The pigmented (e.g. red, purple) *Brassica* species in particular appear to produce high values in total phenolic assays likely as a result of the presence of anthocyanins (natural red/purple flavonoid pigments) in these vegetables (*27*).

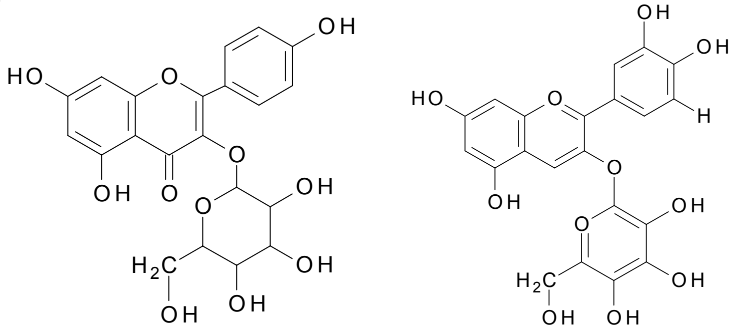


**Figure 4.** Selected flavonoids found in *B.* oleracea  crops.

## 1.2 Health-benefiting properties of *Brassica* phytochemicals

It is widely recognised that oxidative stress is a major contributor to the development of major chronic diseases such as cancer and cardiovascular disease (*28*). Oxidative stress is simply the cellular imbalance between production and elimination of reactive oxygen species (*29*). Regular consumption of vegetables is believed to promote reduction of oxidative stress through the action of antioxidants. Thus the antioxidant capacity of a fruit/vegetable could be very useful in preventing the initiation of chronic diseases whilst additional disease-specific activity may also be exerted (e.g. cancer antiproliferative activity) by plant-derived phytochemicals (*30*).

### 1.2.1 Antioxidant properties

The strong antioxidant properties of phenolic acids and other flavonoids (such as anthocyanins) in general found in *Brassica* vegetables are well established (*31*). More interesting however is the finding that ITC can also have direct and indirect antioxidant activity. Methylthioalkyl ITC for example exhibit potent scavenging ability against radical species or peroxides (*21*); this ability is due to the side chain (rather than of the –N=C=S function), which is conserved from the parent GS (*21*) and therefore is observed only for these ITC (i.e. not generic ITC property). The indirect activity of ITC is generic and it arises through transcriptional activation of multiple antioxidant proteins; this is by far the route by which ITC exert most of their antioxidant activity (*21*).

### 1.2.2 Anticancer properties

A very recent comparative study evaluating the antiproliferative effect of extracts from 34 commonly consumed vegetables found *Brassica* vegetables to have potent inhibitory activity against most cancer cell lines used (including kidney, prostate, lung and breast malignant cells) (*28*). This activity is likely to

be derived from the action of ITC, formed from the myrosinase-catalysed hydrolysis of GS, found in the damaged cells of *B. oleracea* plants (*32*); GS themselves do not possess antiproliferative properties (*33*).
ITC are known to target multiple stages of the cancer process: tumour initiation, promotion and progression phases, and also by suppressing the final steps of carcinogenesis, i.e. angiogenesis and metastasis (*29*). The glucoraphanin-derived sulforaphane, found to be abundant in broccoli and Brussels sprouts, has been the most studied ITC and its cancer-fighting ability is so well established that it is currently subjected to human clinical trials (*34*).

## 1.3 Taste properties of *Brassica* vegetables

There is considerable evidence that one of the main determinants of food choice is liking (hedonics). Taste is often reported to be the main driver of liking. GS, and in some cases their decomposition products, appear to be implicated to the undesirable taste associated with many *Brassica* vegetables. Most literature describes a bitter effect mainly for sinigrin, gluconapin and progoitrin, but also for glucobrassicin and neoglucobrassicin, but in different intensities, while in contrast, alkyl glucosinolates provide no contribution to the attribute "bitter" (*35-38*). There are also studies that find no association between GS and bitterness or taste overall (*39, 40*). Almost all the findings to date are based on correlations of compositional and sensory data; information on the sensory qualities of individual components or even vegetable extracts is very scarce. The only data of this nature has been provided by Fenwick and others who concluded that the bitterness associated with certain cultivars of Brussels sprouts was linked to the presence of the GS sinigrin and progoitrin (*36*). Sinigrin was found to be bitter itself whilst the bitterness associated with progoitrin was due to its decomposition product, the goitrogen 5-vinyloxazolidine-2-thione (68). In the same study, 78% of assessors found gluconapin to be bitter as well (*36*). Taste trials, carried out many years later, showed that both bitterness and taste preference for sprouts are correlated with the content of sinigrin and progoitrin thus supporting the findings by Fenwick et al. (*37*). A couple of recent studies however have questioned the relationship between GS and taste. According to Baik et al. no GS (including progoitrin) were linked to bitterness in 19 cultivars of broccoli (*39*). In addition, the taste of the GS-rich tropical tree *Moringa olefeira*, assessed in a masked protocol, was found not to be related to GS content (*40*). These studies suggest that other factors, additional to GS or ITC content, are contributing to the bitterness or astringency of *Brassica* vegetables. Phenolic compounds are well known to contribute to the bitterness and/or astringency of many foods (*41*) and it is probable that they do make a contribution to the taste quality of *Brassica* species although this was not investigated in any of the studies reported to date. The nature and amount of sugars present can also play a role at the perceptual level as studies suggest that the sweet and bitter tastes are closely related (*42, 43*).

## 1.4 Health-information and consumer acceptance

Change in taste acceptance is learnt through a wide range of associative learning that may be pairings with liked stimuli including sensory, post-ingestive effects (e.g. satiation), social acceptance or cognitive (e.g. health information) in nature. Such learning becomes integrated into outcome expectancies (i.e. the expectation that a positive outcome will occur) widely thought to be a driver of behaviours.

One mechanism for increasing the liking of innately disliked food is the provision of information reporting the health benefits of a food. For example, past research has included health information when seeking to increase vegetable (and fruit) consumption in clinical trial settings (*44, 45*). That study succeeded in increasing fruit consumption more than vegetable consumption. Other researchers have used health information to effect positive hedonic changes towards novel foods with health benefits. However, It is unknown whether such information may influence liking of vegetables that are widely reported to be disliked because of their taste properties.

### 1.4.1 Selection of health information used

HAL/Ausveg has considered information pertaining to vegetables and has created guidelines (*46*). A critical review found this document to be unsuitable for the current study for several reasons. For example, the interpretations of market research descriptive data (HAL Vegetracker 2008 – 2010), used as justification for the chosen approach, are questionable. For example, "*95% agree that eating vegetables regularly can help reduce the risks of cancer, heart disease and obesity – this indicates that the chronic disease prevention/control aspects of vegetables are almost universally known*" is a mistaken interpretation. A more realistic interpretation is that there is a tendency for people to agree rather than disagree. Knowledge can only be elicited by open responses to questions (*47*), controlling for people's access to information by personal interview or controlled laboratory settings. The Brand Story chosen format avoids explicit reference to disease risk reduction and suggests that (certain) vegetables are "good for" a range of human conditions; however, there are frequently no or weak evidence to substantiate these claims. Furthermore there is no evidence that such messages are effective in encouraging consumption. Reviews by the authors considered a wide range of health information pertaining to vegetables and *Brassicas* in particular. Weak effects for reduction of cancer risk have recently been reported for vegetables in general (*48*) although there is a lack of measures of specific vegetables. In respect to *Brassica* vegetables, whilst epidemiological evidence is lacking (possibly due to a lack of tools to measure *Brassica* intake) the mechanisms and laboratory evidence are unambiguous. Criteria for selection information were evidence based; lack of ambiguity; a credible and trusted source. As a consequence the Brand Story (2010) strategy was rejected and a recent Cancer Council statement was chosen (Figure 1)(*49*). The hypothesis was that if information on protecting against cancer is known (recalled) then such health knowledge may over-ride any taste aversion to *Brassica* vegetables.

### 1.4.2 Health and taste attitude scale (HTAS)

Attitudes are known to interact with sensory perception and the acceptance of foods (*50*). The Health and Taste Attitude Scale (HTAS) was developed to measure how differing attitudes may influence food choice (*51*) and validated across selected European cultures (*52*). In the current study three sub-scales from the HTAS were hypothesized to be relevant and were included. The general (food for) health attitudes subscale taps into health motivations and those scoring highly on this scale were hypothesized to report liking and intention to consume *Brassica* vegetables. Nevertheless original reports (*51*) found no negative correlation between 'foods for health' and 'foods for pleasure'. In other words, health and pleasure orientation could occur simultaneously. In contrast those reporting high scores on the foods for reward sub-scale were hypothesized to be orientated more towards hedonic aspects of food choice.

Brassica vegetables are not novel and current consumption is likely to be related to liking hence current consumption should be considered when attempting to effect change. In the current study vegetable intake was measured at screening using a simple validated measure of portions (*53*). Specific vegetable consumption was also measured using a validated vegetable frequency questionnaire (*54*). The tool was designed to give an overall estimate of usual vegetable consumption frequency (not quantities) and included some specific *Brassica* vegetables of interest to the current study (cabbage, cauliflower and broccoli)

In the current study perceived tastes (sweet, sour, bitter, umami and salt) and overall flavour impact (strength) were measured and hypothesized to be predictors of liking and intentions to consume.

## 2. AIM & OBJECTIVES

The overall aim of this project was to determine the relative contribution, or trade-off, between taste properties and health-promoting benefits of phytochemicals, in order to improve consumer acceptance of vegetables within the *B. oleracea* species.

An interdisciplinary, integrated approach was used to achieve this aim. Main individual tasks carried out within the project included:

- A **consumer acceptance** test that measured taste perception and acceptance of various *B. oleracea* vegetables and evaluated the influence of health-information on the intention of consumers to consume these vegetables;
- A descriptive **sensory assessment** of taste on whole and fractionated extracts from *B. oleracea* vegetables using trained panellists;
- *in-vitro* **antioxidant** (FRAP) and **anticancer** (HT-29 colon adenocarcinoma cells) assays that evaluated the potential physiological activities of whole and fractionated extracts from *B. oleracea* vegetables;
- **identification and quantitative determination** of phytochemicals in whole and fractionated extracts from *B. oleracea* vegetables using a range of analytical techniques (e.g. liquid chromatography, mass spectrometry and others).

A diagram of the whole process is shown below (Figure 5). Information obtained from all these activities was combined and formed the basis of the conclusions and recommendations presented at the end of this report.

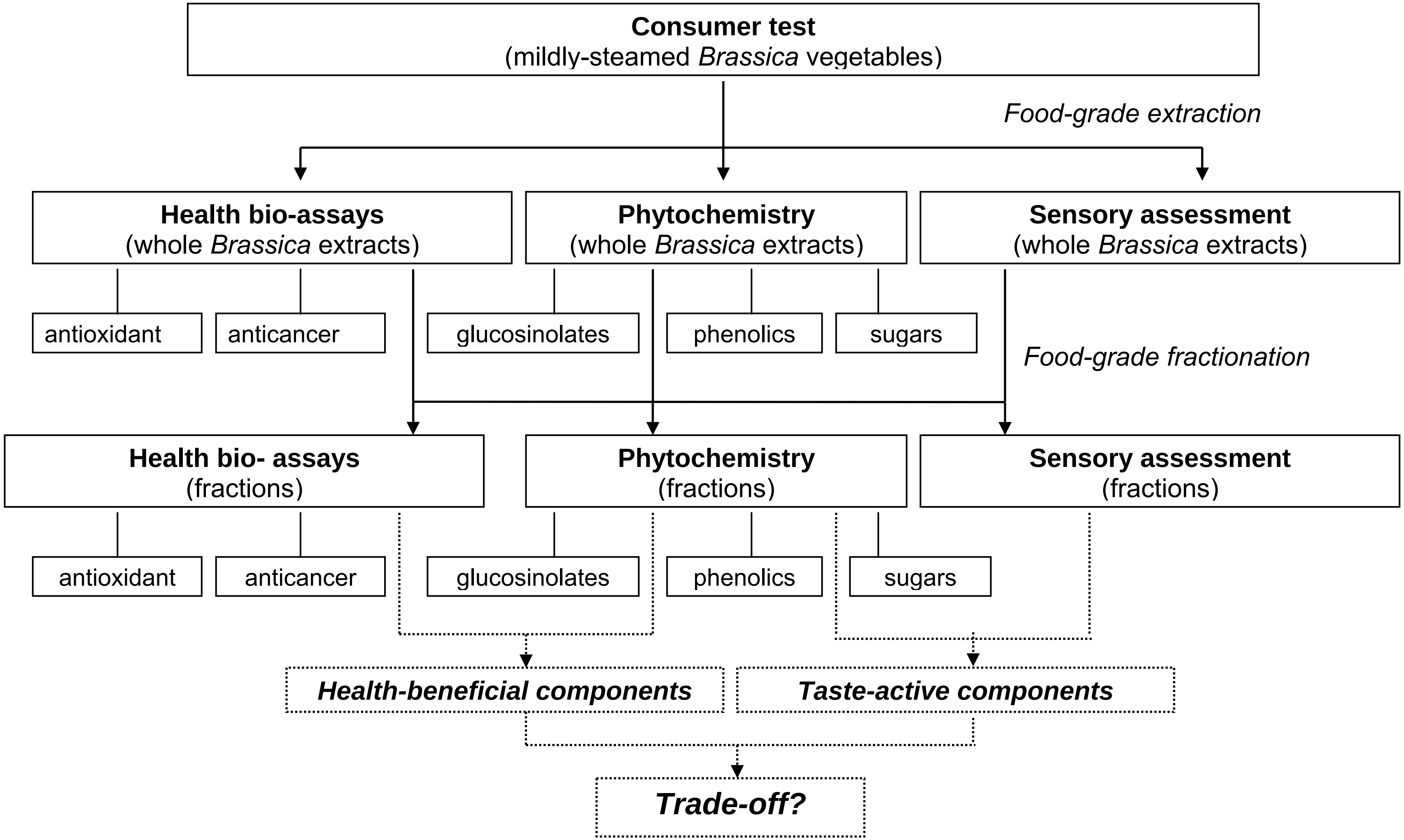


**Figure 5.** Diagram of the various tasks (solid lines) and outcomes (broken lines) associated with this study.

# 3. METHODOLOGY

## 3.1 Consumer acceptance trial

### 3.1.1 Sample sourcing/selection

*Brassica* vegetables were selected based upon:

- Common and less common types
- Popular and unpopular types
- Guaranteed supply from the same growers over the period of testing (see Table 1)
- Reasonable consumer burden (number of samples tasted in one session)

The vegetables were purchased from Adelaide Produce Markets, Pooraka SA on a weekly basis, stored at 4°C for a maximum of 5 days, prepared according to CSIRO food handling procedures (CSIRO FNS Risk Assessment Process for Foods for Human Consumption approved) and served within 30 minutes. Vegetables selected are shown by wholesaler, grower and location of origin in Table 1.

**Table 1.** Vegetables selected by wholesaler, grower and location

| Vegetable | Wholesaler | Grower and location |
|---|---|---|
| Red cabbage | Mercurio Bros | T. Musolino & Co., Virginia, SA |
| Green cabbage | Mercurio Bros | T. Musolino & Co., Virginia, SA |
| Cauliflower | Mercurio Bros | T. Musolino & Co., Virginia, SA |
| Brussel sprouts | Mercurio Bros | A.E. Cranwell & Sons, Nairne, SA |
| Broccoli | WildSalad | Swanport, Murray Bridge, SA |
| Broccolini | Bache Bros | Pitchford Produce, Currency Creek, SA |

Each vegetable was steamed for 6 minutes just prior to serving. Approximately 20g servings of each were presented monadically in a randomised balanced design, minimising first order and carry over effects (*55*).

### 3.1.2 Information

A statement from The Cancer Council Australia was simplified and presented verbally and visually to information treatment groups prior to any responses. All participants were measured for knowledge regardless of their assignment to groups. For ethical reasons the same information was given to non-information groups after the study.

> Vegetables are recommended for their important role as a low-energy-dense source of nutrients (vitamins, minerals, phytochemicals and fibre) and for their contribution to weight management, as well as for their probable cancer-protective effect. Ensuring an adequate intake of vegetables is likely to reduce the risk of some cancers.
>
> A recent scientific review suggests cruciferous vegetables (including, broccoli, broccolini, cabbages, cauliflower, Brussels sprouts) may be important because they contain compounds that inhibit carcinogenesis.
>
> Adapted from The Cancer Council Australia 2007. *National Cancer Prevention Policy 2007−09.* NSW: The Cancer Council Australia.

**Figure 6.** Health information used during the consumer test.

### 3.1.3 Participants

An accredited market research company was commissioned to provide participants according to the specified inclusion and exclusion criteria (below). Competitive quotes were sought from three companies and Macgregor Tan Ltd was chosen. Ethical approval was received from CSIRO Food and Nutritional Sciences Human Research Low Risk Review Panel and informed consent was obtained. Central location tests (Adelaide, South Australia) were undertaken mid-March (late summer) 2011.

*Sample size*

Variation in hedonic responses (the main dependent variable) was unknown; however, past experience in similar domains by the senior researchers suggest that 40-50 participants per group would be sufficient to detect differences at the 5% level. Hence 200 consumers were sought to attend one central location session of approximately one hour. Consumers were compensated for their time with a $30 retail voucher.

*Inclusion and exclusion criteria*

Recruitment was based upon the following inclusion and exclusion criteria:

**Inclusion:** Adults 18-55years, 50/50 female/male, reporting moderate – high physical activity (Booth, 2000; IPAQ)

**Exclusion:** reporting low physical activity, vegetarians, pregnant women, any allergies to vegetables, residency <3years, not able to understand English

All potential participants were screened for physical activity and invited to participate if reporting moderate to high physical activity as defined by the validated International Physical Activity Questionnaire (IPAQ) (*56, 57*). See Appendix 1 for definitions.

*Design*

In a 2 (low vegetable consumption [L] and high vegetable consumption [H]) x 2 (information [i] or no information [n]) design, 50 consumers in each group (N = 200 in total) were sought.

All were screened and first allocated to one of two groups by vegetable consumption, with half the number of participants female and half male, using a validated scale (*53*):

- Less or equal to 2 portions/d (excluding potatoes and potato products) = low
- Greater or equal to 3 portions/d (excluding potatoes and potato products) = high

The two consumption groups were then randomly allocated to an information or no-information condition.

| | |
|---|---|
| Group Li<br>≤ 2/servings vegetables/day<br>Health information<br>N = 50 | Group Hi<br>≥3/servings vegetables/day<br>Health information<br>N = 50 |
| Group Ln<br>≤ 2/servings vegetables/day<br>No information<br>N = 50 | Group Hn<br>≥3/servings vegetables/day<br>No information<br>N = 50 |

H = high vegetable consumption; L = low vegetable consumption
i = information; n = no information

**Figure 7.** Design of the consumer test.

*Participant tasks*

We first sought affective responses (hedonics) to the vegetable samples, then, in a later task, separately sought analytical responses (taste perception). Tasks were separated because analytical thinking tends to diminish hedonic responses and is not typical of real world behaviour.

- Health information (Figure 6) presentation to 50% of sample;
- Hedonics (9 point labelled scale) randomised presentation of 6 *Brassica* vegetable samples (Appendix 2);
- Taste intensity: 6 (separate) vegetable samples x 5 tastes and flavour. (Appendix 3);
- HTAS health, pleasure and reward subscales (*51*) (Appendix 4);
- Validated Vegetable FFQ (*54*) (Appendix 5);
- Intention to consume; socio-demographics and supplement use; two questions on recall of health information (Appendix 6).

### 3.1.4 Analysis

All analysis was undertaken using SPSS v 17 and a $p < 0.05$ value chosen as the level of statistical significance.

**Q1.** *Is the specific cancer protection information supplied recalled by the Information treatment groups more frequently than the no-information groups?*

The open responses to two questions were collapsed and analysed so as to determine if the information groups (Hi and Li) recalled the cancer protection message in either / or responses to a greater extent than those in the no-information group (Hn and Ln). Responses were coded (by two researchers for inter-rater reliability) for ‘cancer protection’; ‘general knowledge’; ‘vague’ and ‘incorrect/blank’ categories. Chi square tests determined if one group recalled the cancer protection message statistically significantly more that the other group.

**Q2**. *Will health benefit information increase reported liking for brassica vegetables when controlling for current vegetable consumption?*

Analysis of variance (ANOVA) was undertaken with hedonic score (for each vegetable) as a within subjects factor and information group and reported vegetable intake as a between subjects factor.

**Q3.** *Will health benefit information increase reported intentions to consume Brassica vegetables when controlling for current vegetable consumption?*

ANOVA was undertaken and intention to consume score (for each vegetable) as a within subjects factor and information group and reported vegetable intake as a between subjects factor.

***Q4.*** *How are the taste/flavour characteristics of the Brassica vegetables perceived by consumers and do they differ by vegetable, consumption and information intervention group?*

Initially ANOVA was undertaken and each taste / flavour score (for each vegetable) as a within subjects factor and information group and reported vegetable intake as a between subjects factor. Subsequently ANOVA was undertaken on the whole sample and post-hoc tests used to determine statistically significant differences in perceptions of tastes / flavours across vegetables.

**Q5.** *What predicts a) liking (hedonics) and b) intention to consume Brassica vegetables?*

Will socio-demographics (age, gender, educational status); current consumption (reported vegetable portions/d) and/or specific Brassica consumption frequency); attitudes towards foods; information; and sensory perception predict Brassica vegetables a) liking and b) intention to consume?

In order to capture the wider influences upon hedonics and intentions, a series of stepwise multivariate regression analysis was undertaken, placing each independent variable in competition in order to determine significant predictors of the dependent variables: a) hedonic score and b) intention to consume each vegetable. Independent variables were entered stepwise:

Step 1 (socio-demographics)

Gender (M = 1, F = 2)
Education status
Age (group)

Step 2 (intake and intervention)

Current vegetable intake (high / low)
Information (group)

Step 3 (attitudes)
Food for Health
Food for Pleasure
Food for Reward

Step 4 (sensory perception specific to each *Brassica* vegetable)

Bitterness
Saltiness
Sweetness
Umami
Sourness
Overall flavour intensity

---

## 3.2 Phytochemistry

### 3.2.1 Sample sourcing (used in all tasks apart from the consumer test)

Fresh *Brassica* vegetables were obtained from Sydney Markets (Sunfresh Ltd) and were immediately subjected to freeze-drying using an industrial unit (Virtis Genesis 35EL). The same freeze-dried, homogenised material was used for all tasks with the exception of the consumer test which was performed on fresh, intact *Brassica* vegetables obtained in Adelaide (details in section 3.1.1).

### 3.2.2 Sample extraction

Freeze-dried material (600 mg) of *Brassica* vegetables was placed in food grade aqueous ethanol (70%, 5 mL). The mixture was then sonicated (70 °C for 30 min.) and centrifuged (9000G for 20 min.). The supernatant was then removed. The extraction process was repeated three times in total for the same material and the supernatants were combined and centrifuged (15000G for 15 min.). The resulting supernatant was finally filtered (0.2 μm), and concentrated (3 mL). This whole extract and its fractions were then subjected to antiproliferative (MTT) assays and chemical analysis (by HPLC and LC/MS).

### 3.2.3 Separation and fractionation of extracts

A food-grade semi-preparative high performance liquid chromatograph (HPLC) equipped with a photodiode array detector and an automated fraction collector (Shimadzu, Rydalmere, NSW) was used. Separation of compounds was achieved using a $C_{18}$ commercial liquid chromatography column (Luna $C_{18(2)}$, 250 mm length x 10 mm i.d. x 5 μm particle size). The mobile phase consisted of pure water (A) and certified food grade ethanol (>95% purity, CSIR, NSW) at a flow rate of 4 mL/min. Desolvation of fractions was then carried out using a dedicated food-grade conventional rotary evaporator (Heidolph Laborota 4011, John Morris Scientific, NSW).

### 3.2.4 High performance liquid-chromatography (HPLC)

The whole extracts as well as their fractions were monitored (190-800 nm) using an analytical scale system similar to that described in section 2.3 above running on a water/acetonitrile mobile phase (1 mL/min.). All analyses were carried out in duplicate.

### 3.2.5 Liquid chromatography-mass spectrometry (LC/MS)

LC/MS-MS analysis was carried out on a Quantum triple stage quadruple (TSQ) mass spectrometer (ThermoFinnigan, NSW, Australia) equipped with a quaternary solvent delivery system, a column oven, a photo-diode array detector and an autosampler. An aliquot (5 μL) of the whole extract (or its fractions) was chromatographed on a Synergi Hydro $C_{18}$ column (150 mm x 2.1 mm, 5 μm particle size, Phenomenex, NSW, Australia) which was maintained at 30 °C. The mobile phase consisted of 0.5% formic acid in water (A) and 0.5% formic acid in acetonitrile (B) at the rate of 200 μL/min (no splitting). A linear gradient was used (0% B to 100% B over 20 min). Ions were generated using an electrospray source in the negative (GLS) or positive (phenolics) mode. MS detection and/or quantification in the selected reaction monitoring (SRM) mode and the common neutral loss (CNL) mode was carried out depending on the nature of the analytes.

### 3.2.6 Quantification of analyte

Glucosinolates were quantified against external calibration curves using glucotropaeolin as an internal standard (spiked at 2.1 mg/mL). Glucoraphanin, glucoallysin and glucoerucin were expressed against the curve of glucoiberin. Gluconapin and progoitrin were quantified against the curve of sinigrin whilst glucobrassicin, neoglucobrassicin and 4-methoxyglucobrassicin were quantified using the curve from glucotropaeolin. The concentration of calibration solutions was determined by measuring the absorbance at 235 nm and using published molar absorptivity values (*58*). Phenolic components were also quantified using external calibration curves (syringic acid at 280 nm, chlorogenic acid at 326 nm, rutin at 370 nm and cyanidin-3-glucoside at 520 nm).

## 3.3 Sensory evaluation

### 3.3.1 Assessors

The panel consisted of 10 assessors (8 female, 2 male; mean age 45.2 years with SD of 5.1 years). Assessors were sensory assessors from CSIRO, who had previously been screened for taste and smell acuity and had extensive experience in descriptive sensory analysis across a range of products.

### 3.3.2 Sensory laboratory

All sensory testing took place in the sensory laboratory at CSIRO's North Ryde facility, which has been designed in accordance with International Standards on Sensory Analysis (ISO 6658:1985).

### 3.3.3 Descriptive analysis

The trained panel carried out descriptive analysis to assess the basic taste properties (Table 1) of whole extracts and fractions from four *Brassica* vegetable extracts (broccoli, cauliflower, Brussels sprouts and red cabbage). Whole extracts were evaluated in duplicate in two sessions over two days. Fractions were evaluated in duplicate in two sessions over four weeks using a standard method of assessment (fractions from one vegetable were assessed each week). The assessors were asked to rate the perceived intensities of the basic taste attributes *sweet, salt, sour, bitter, umami (savoury),* and *astringent* aftertaste. The assessors were asked to note any other taste characteristics they perceived in the text box provided. Prior to evaluation, reference standards for all the basic taste attributes except astringency were provided to assessors to clarify the attributes (Table 1). Water (Noble Pureau, Noble Beverages, NSW) was used as palate cleanser. As taste evaluation was the purpose of this study, a nose peg was used by the panellists during assessment.

All evaluations were carried out in the individual sensory booths under white light. A six minute inter-stimulus interval was imposed between samples and a five minute break was imposed after the seventh sample to reduce panellist fatigue. Between samples panellists were required to cleanse their palate by drinking water.

The products were blind-coded with random 3-digit code and the order of sample assessment was randomised to account for first order and carryover effects. The experimental design was produced using the design generation package – CycDesigN (Whitaker, D. Williams, E.R. and John, J.A. (2002)

CycDesigN Version 2: *A package for the computer generation of Experimental Designs*. CSIRO, Canberra, Australia). Attributes were rated on 100mm unstructured line scales anchored at 5 and 95%, respectively, with extremes for each descriptive term. Data were recorded and stored using the Compusense sensory data acquisition software (version 4.6, 2004; Compusense Inc., Guelph, Ontario, Canada).

**Table 2.** Definition of taste attributes and reference standards used during the sensory evaluation of the *Brassica* fractions.

| Category | Attribute | Definition | Anchor | Reference Standards |
|---|---|---|---|---|
| Taste | Sweet | The perceived intensity of the sweet taste - defined by basic taste solution | Low to high | Sucrose (2% and 16% ) |
| | Salty | The perceived intensity of the salty taste - defined by basic taste solution | Low to high | Salt (0.2% and 0.7%) |
| | Sour | The perceived intensity of the sour taste - defined by basic taste solution | Low to high | Citric acid (0.05% and 0.2%) |
| | Bitter | The perceived intensity of the bitter taste - defined by basic taste solution | Low to high | Caffeine (0.05% and 0.2%) |
| | Umami | The perceived intensity of the umami taste - defined by basic taste solution | Low to high | MSG (0.18% and 0.45%) |
| Aftertaste | Astringency | The perceived intensity of dry puckering sensation one minute after swallowing | Low to high | |

## 3.4 Physiological activity of *Brassica* extracts

### 3.4.1 Antioxidant activity

The antioxidant activity of the extracts was determined by the ferric reducing activity (FRAP) assay. The FRAP reagent was initially prepared consisting of 10 mL acetate buffer (300 mmol/L), 10 mL of $FeCl_3$ (20 mmol/L) and 1 mL TPTZ solution (10 mmol/L). The acetate buffer (pH 3.6) consisted of 3.1 g of sodium acetate and 16 mL acetic acid per litre of water. The TPTZ solution consisted of 31.2 mg of TPTZ in 10 mL HCl. Initially, the extracts in water were added to FRAP reagent in a 96-well microplate (Sarstedt Australia) followed by shaking (15 sec.). After incubation (8 min.), the absorbance was read at 595 nm using a spectrophotometer (Labsystems Multiskan MS; Thermo Fisher Scientific). The reducing capacity of the extracts was expressed as μmol of Iron (II) per gram of dry weight (μmol $Fe^{2+}$/ gDW) based on a $FeSO_4$ standard curve.

### 3.4.2 Enzyme treatment

Myrosinase (thioglucoside glycohydrolase, E.C. 3.2.3.1, Sigma-Aldrich) was dissolved (2.5 mg/mL) in 0.1M $Na_2HPO_4$/$NaH_2PO_4$ buffer at pH 6.56 (*59*). Aliquots (1 mL) from whole extracts or fractions were treated with the enzyme preparation (1 mL) at 30 °C for 60 min. Analysis of the treated extracts/fractions showed that no GLS substrates were detectable following treatment.

### 3.4.3 Antiproliferative activity

Colon adenocarcinoma (HT-29) cells, obtained from the American Type Culture Collection, were cultured in McCoy's 5A modified medium containing 10% FBS and 1% P-S at 37°C under a humidified atmosphere containing 5% $CO_2$. A colorimetric assay based on the WST-1 reagent was used to assess the antiproliferative activity of the extracts. Cells ($1x10^4$ cells in 100 μL medium) were plated into each well of the 96-well microplates. After they were incubated for 24 h, the cells were treated with various concentrations of the extracts for another 24 h. Then WST-1 reagent was added to each well and the microplates were incubated for another 1.5 hours to allow formation of the formazan dye that subsequently was measured (450 nm, Victor 2 1420 Spectrophotometer, Perkin Elmer, US) to determine cell proliferation rate.

# 4. RESULTS-DISCUSSION

## 4.1 Consumer acceptance test of *Brassica* vegetables

### 4.1.1 Results

*Participants*

Two hundred participants completed all tasks. Table 3 below shows participant characteristics.

**Table 3.** Characteristics of participants in the consumer test.

| N = 200 | Percentage |
|---|---|
| **Gender** | |
| Male | 49.5 |
| Female | 50.5 |
| **Age distribution (years)** | |
| 18-30 | 31 |
| 31-40 | 19.5 |
| 41-50 | 31 |
| 51-55 | 16.5 |
| **Perceived culture** | |
| Australian | 89.5 |
| **Education status (highest)** | |
| Some high school | 6 |
| High school | 20 |
| Technical | 23 |
| University | 51 |
| Dietary supplement use[1] | 48 |

[1] no relationship between dietary supplement use and vegetable intake. i.e. there were equal numbers in both groups (Chi square p > 0.05).

*Overall hedonic and intention ratings across the Brassica vegetables*

**Table 4.** Overall hedonic scores (9 point scales) and differences by vegetable (n = 200).

| Vegetable | Mean | SEM | |
|---|---|---|---|
| Cauliflower | 7.43 [a] | 0.099 | |
| Broccoli | 7.36 [a] | 0.094 | |
| Broccolini | 7.01 [b] | 0.106 | |
| Green cabbage | 6.82 [bc] | 0.109 | |
| Red cabbage | 6.62 [c] | 0.107 | |
| Brussels sprouts | 5.52 [d] | 0.154 | |

Values not sharing a common subscript letter [a] are statistically significantly different (p< 0.05) from one another.

Table 4 shows that cauliflower and broccoli were the most liked and Brussels sprouts the least liked overall.

**Table 5.** Overall intention to consume (7 point scales) and differences by vegetable (n = 200).

| Vegetable | Mean | SEM |
|---|---|---|
| Broccoli | 6.03 [a] | 0.088 |
| Cauliflower | 5.63 [b] | 0.111 |
| Green cabbage | 5.04 [c] | 0.124 |
| Broccolini | 4.67 [d] | 0.129 |
| Red cabbage | 4.36 [e] | 0.124 |
| Brussels sprouts | 3.77 [f] | 0.149 |
| Values not sharing the same subscript letter [a] are statistically significantly ($p < 0.05$) different from one another | | |

Table 5 shows statistically significant differences between each *Brassica* vegetable for intention to consume in the near future.

*Q1. Is the specific health information supplied recalled by the Information treatment groups more frequently than the no-information groups?*

Analyses of the distributions of the four response categories revealed that 45% participants in the information groups recalled (had knowledge of) 'cancer protection' more frequently ($p < 0.000$) than participants in the no-information groups (only 4%). This suggests that cancer protection is not uppermost in consumers' minds when thinking about the health benefits of vegetables or *Brassica* vegetables. Proportions recalling / knowing 'general knowledge' were 47% for the information groups and 72% for the no information groups. Additionally 22% in the no information groups were vague or incorrect in contrast to only 5% of the information groups. This suggests that a large proportion of participants in the information groups recalled the health messages and that cancer protection is somewhat unknown.

*Q2. Will health benefit information increase reported liking for brassica vegetables (when controlling for current vegetable consumption)?*

Figure 8 shows mean scores (standard error of the mean) for hedonics of each *Brassica* vegetable by consumer group.

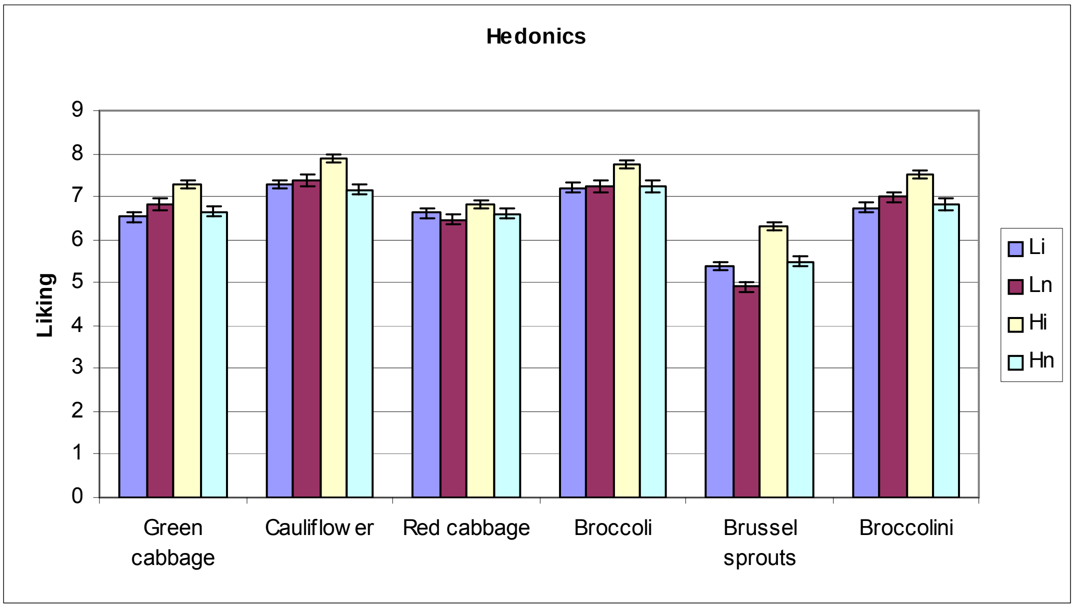


**Figure 8**. Hedonic scores (9-point scale) for vegetables by reported vegetable intake and information group (n=200, 50 in each group).

**Table 6.** ANOVA on *Brassica* hedonics, significant effects of current consumption (reported portions of vegetables daily) and information (group).

| **Vegetable** | **Effects of information and vegetable consumption upon** | **Significance (p)** |
|---|---|---|

| | **hedonics** | |
|---|---|---|
| Cauliflower | Vegetable Consumption x Information | 0.037 |
| Broccoli | - | - |
| Broccolini | Vegetable Consumption x Information | 0.026 |
| Green cabbage | Vegetable Consumption x Information | 0.028 |
| Red cabbage | - | - |
| Brussels sprouts | Vegetable Consumption<br><br>Information | 0.014<br><br>0.032 |

In ANOVA, information independently influenced liking of (the least liked) Brussels sprouts (Table 6). Refer to the Figure 8 (above) for descriptive statistics. For three vegetables, information influenced liking when interacting with current vegetable intake (see Figures 9-11 below). In all three examples (green cabbage, cauliflower and broccolini) information and high consumption interacted to increase liking. In other words, participants who report high consumption (predisposed to eating vegetables) were influenced by the health information to report an increase in their liking of three *Brassica* vegetables.

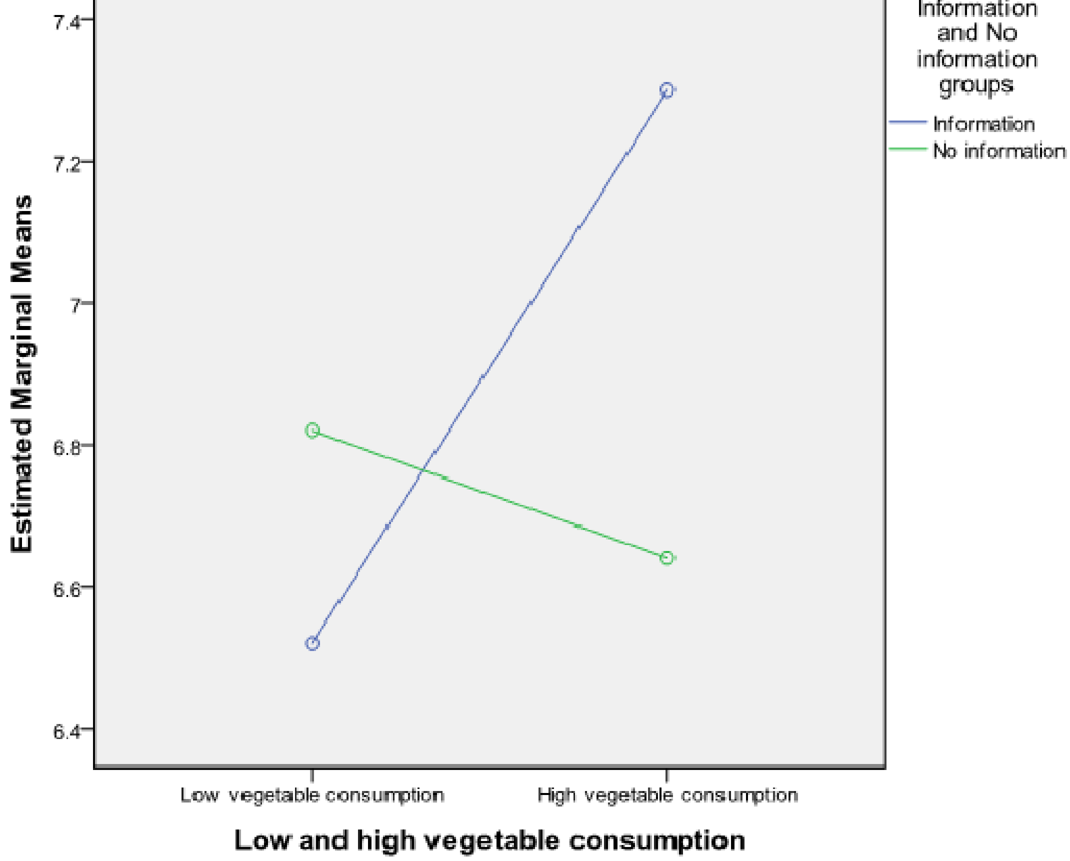


**Figure 9.** Interaction between general vegetable consumption and information for green cabbage.

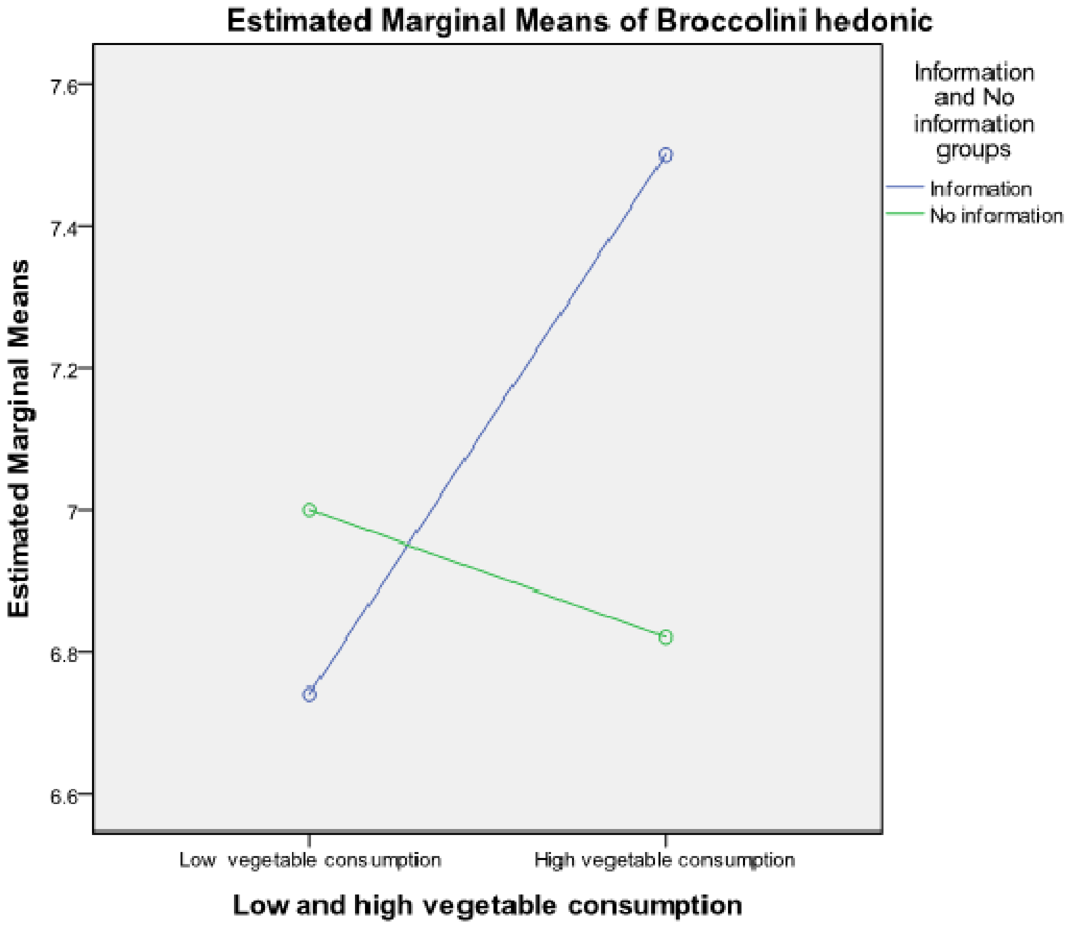


**Figure 10.** Interaction between general vegetable consumption and information for broccolini

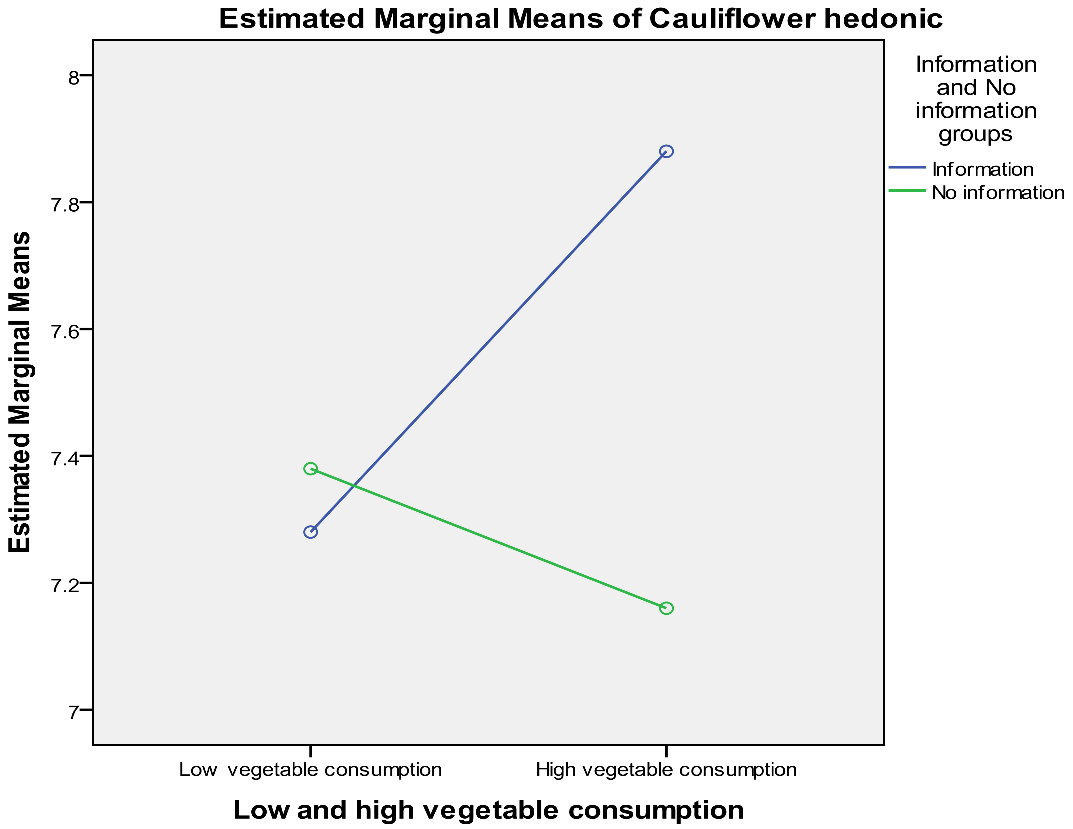


**Figure 11**. Interaction between general vegetable consumption and information for cauliflower.

*Q3. Will health benefit information increase reported intentions to consume Brassica vegetables (when controlling for current vegetable consumption)?*

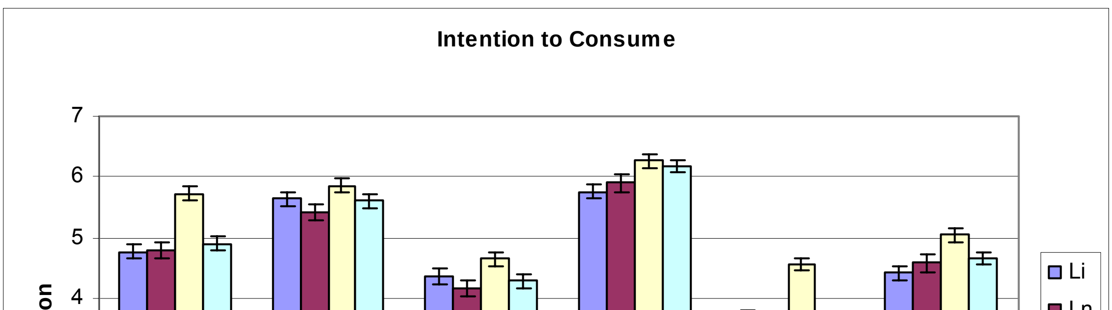

**Figure 12**. Intention scores (7-point scale) for vegetables by reported vegetable intake and information group (n=200, 50 in each group).

**Table 7**. ANOVA on intentions to consume *Brassica* vegetables, significant effects of current consumption (reported portions of vegetables daily) and information (group).

| Vegetable | Effects of information and vegetable consumption upon intention to consume | Significance (p) |
|---|---|---|
| Cauliflower | - | - |
| Broccoli | Vegetable Consumption | 0.027 |
| Broccolini | - | - |
| Green cabbage | Vegetable Consumption | 0.028 |
| Red cabbage | - | - |
| Brussels sprouts | Information<br><br>Vegetable Consumption<br>x<br>Information | 0.018<br><br>0.035 |

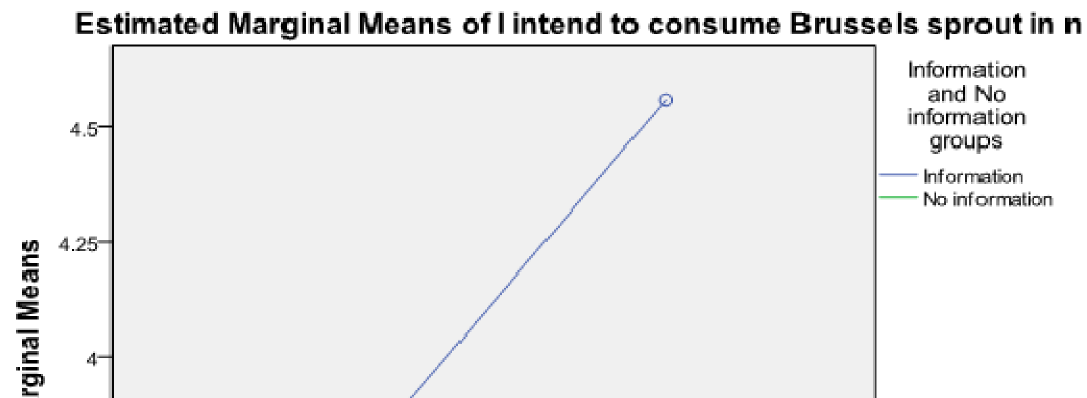

**Figure 13**. Interaction between general vegetable consumption and information for Brussels sprouts.

In ANOVA (Table 7), information independently influenced the intentions to consume (the least liked) Brussels sprouts. Also, an interaction with current consumption was found. In other words, when vegetable intake was high, information had a greater effect on responses to Brussels sprouts. For two other vegetables (broccoli, green cabbage), current vegetable consumption influenced intentions to consume positively.

*Q4. How are the taste/flavour characteristics of the Brassica vegetables perceived by consumers and do they differ by vegetable, consumption and information intervention group?*

Note that the perceived tastes (e.g. bitterness) are scores reported by participants based upon their tasting of the vegetable samples. Analysis investigated whether within a vegetable and within a taste there were differences across groups.

Out of 36 tests (6 taste/flavour x 6 vegetables by consumption/information group) all ratings were found to be not statistically significantly different ($p > 0.05$). Hence current general vegetable consumption and health information knowledge would appear to be not directly associated with the perceptions of taste and flavour of selected *Brassica* vegetables.

Sensory perceptions are illustrated in the spiderplot (Figure 14) below and differences across vegetables were tested across the whole sample (Table 8). Green cabbage was perceived to be the sweetest with Brussels sprouts the least sweet. Mean saltiness perception did not differ greatly across the selected *Brassica* vegetables (mean scores) however some differences were found. Brussels sprouts were statistically significantly the most sour and most bitter and green cabbage the least bitter. Umami did not differ greatly however Broccoli was perceived to have most umami taste. Similarly flavour impact did not differ greatly however red cabbage was perceived to have the least flavour impact.

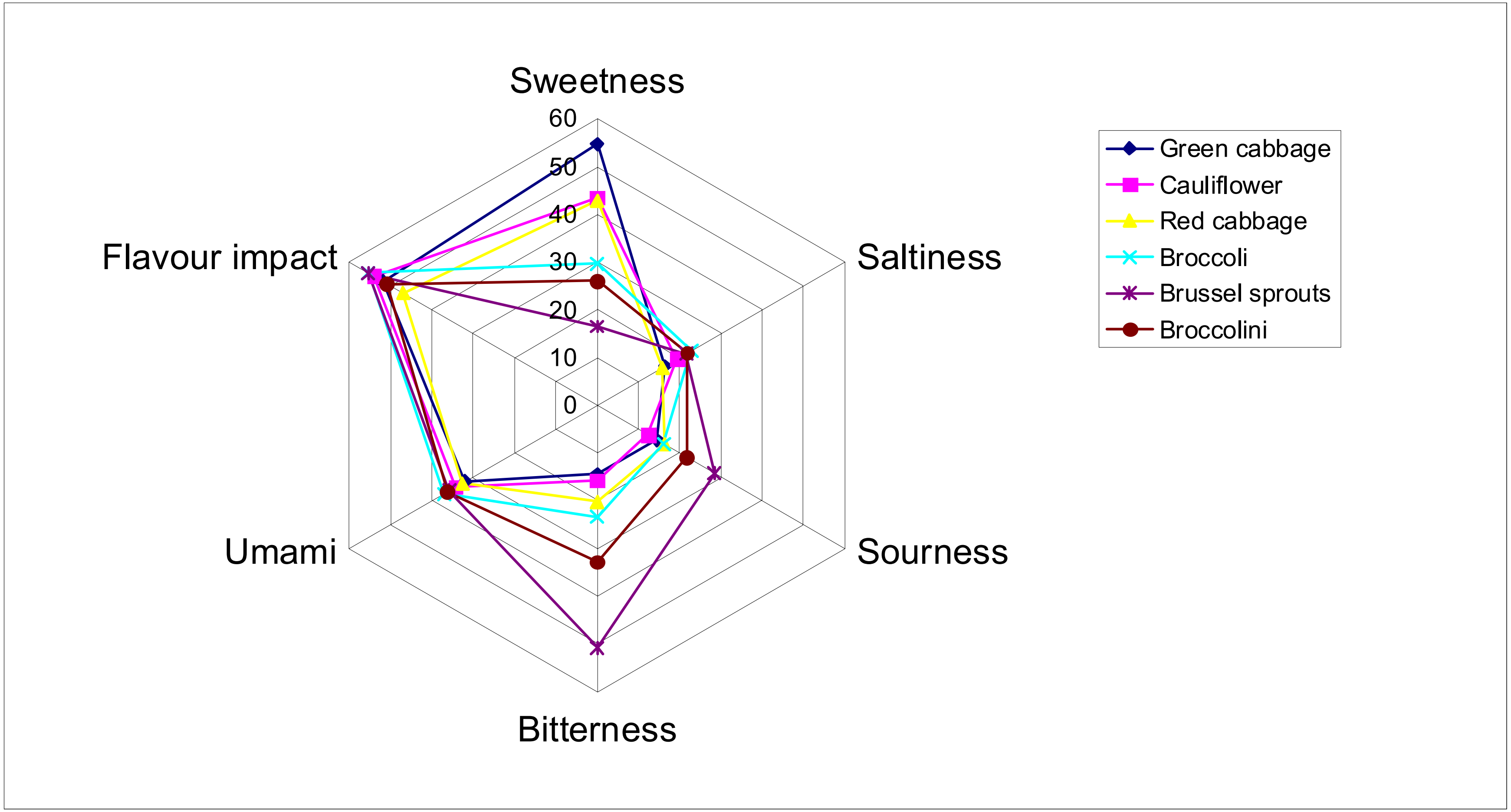


**Figure 14.** Taste/flavour ratings of the various *Brassica* vegetables as assessed by the participants in the consumer test.

**Table 8.** Vegetables by perceived taste and flavour (mean, sem) and tests across vegetable scores (100mm scale) n = 200. Values not sharing a common subscript letter $^{a}$ are statistically significantly different ($p$< 0.05) from one another.

| | | | | | | |
|---|---|---|---|---|---|---|
| **Sweetness** | **Green cabbage** | **Cauliflower** | **Red cabbage** | **Broccoli** | **Broccolini** | **Brussels sprouts** |
| F=120.234; p=.000 | $54.75^{a}$ (1.81) | $43.52^{b}$ (1.77) | $42.93^{b}$ (1.70) | $29.71^{c}$ (1.62) | $26.07^{d}$ (1.51) | $16.55^{e}$ (1.21) |
| **Saltiness** | **Broccoli** | **Broccolini** | **Brussels sprouts** | **Cauliflower** | **Green cabbage** | **Red cabbage** |
| F=12.043; p=.000 | $22.91^{a}$ (1.51) | $21.86^{a}$ (1.42) | $21.57^{ab}$ (1.46) | $19.40^{b}$ (1.27) | $16.50^{c}$ (1.23) | $15.81^{c}$ (1.15) |
| **Sourness** | **Brussels sprouts** | **Broccolini** | **Red cabbage** | **Broccoli** | **Green cabbage** | **Cauliflower** |
| F=28.630; p=.000 | $28.34^{a}$ (1.83) | $21.37^{b}$ (1.43) | $16.34^{c}$ (1.28) | $16.08^{c}$ (1.29) | $14.49^{cd}$ (1.29) | $12.30^{d}$ (1.05) |
| **Bitterness** | **Brussels sprouts** | **Broccolini** | **Broccoli** | **Red cabbage** | **Cauliflower** | **Green cabbage** |
| F=118.786; p=.000 | $51.21^{a}$ (2.01) | $32.52^{b}$ (1.78) | $22.83^{c}$ (1.45) | $19.97^{c}$ (1.38) | $15.64^{d}$ (1.24) | $14.44^{d}$ (1.13) |
| **Umami** | **Broccoli** | **Broccolini** | **Brussels sprouts** | **Cauliflower** | **Red cabbage** | **Green cabbage** |
| F=3.832; p=.002 | $37.49^{a}$ (1.87) | $36.43^{ab}$ (1.83) | $35.59^{abc}$ (1.83) | $34.24^{bcd}$ (1.79) | $33.09^{cd}$ (1.71) | $32.17^{d}$ (1.69) |
| **Flavour impact** | **Broccoli** | **Brussels sprouts** | **Cauliflower** | **Green cabbage** | **Broccolini** | **Red cabbage** |
| F=5.819; p=.000 | $55.48^{a}$ (1.62) | $55.24^{a}$ (1.94) | $53.96^{ab}$ (1.52) | $52.17^{ab}$ (1.59) | $51.17^{b}$ (1.59) | $46.92^{c}$ (1.51) |

Q5. *What predicts a) liking (hedonics) and b) intention to consume Brassica vegetables?*

Will socio-demographics (age, gender, educational status); current consumption (reported vegetable portions/d) and/or specific Brassica consumption frequency); attitudes towards foods; information; and sensory perception predict Brassica vegetables a) liking and b) intention to consume?

Multivariate models

More comprehensive testing of data was undertaken placing a wide range of measures (see methods above) as independent variables (predictors) in competition with one another to determine significant predictors of the dependent variables, hedonics and intentions to consume. Note that the 'perceived' tastes (e.g. bitterness) are scores reported by participants based upon their tasting of the vegetable samples.

Reliability of selected independent variables

Attitudes

Each of the HTAS sub-scales used were found to have acceptable - good internal consistency, specifically, General (Food for) Health interest (Cronbach's alpha 0.85); Food for Pleasure (Cronbach's alpha 0.77) and Food for Reward (Cronbach's alpha 0.57). The lower alpha for Food for Reward is generally considered acceptable for exploratory research and may be related to the small number of items.

Univariate tests suggested that for Food for Health, differences ($p < 0.000$) may occur between the 4 consumption/information groups; however, pair wise post hoc Tukey tests found no significant differences ($p > 0.05$). Similarly for Food for Pleasure a trend was observed ($p = 0.051$) but no pair wise differences were found ($p > 0.05$). Nevertheless differences may occur between individuals (as modelled in the multivariate analysis, below).

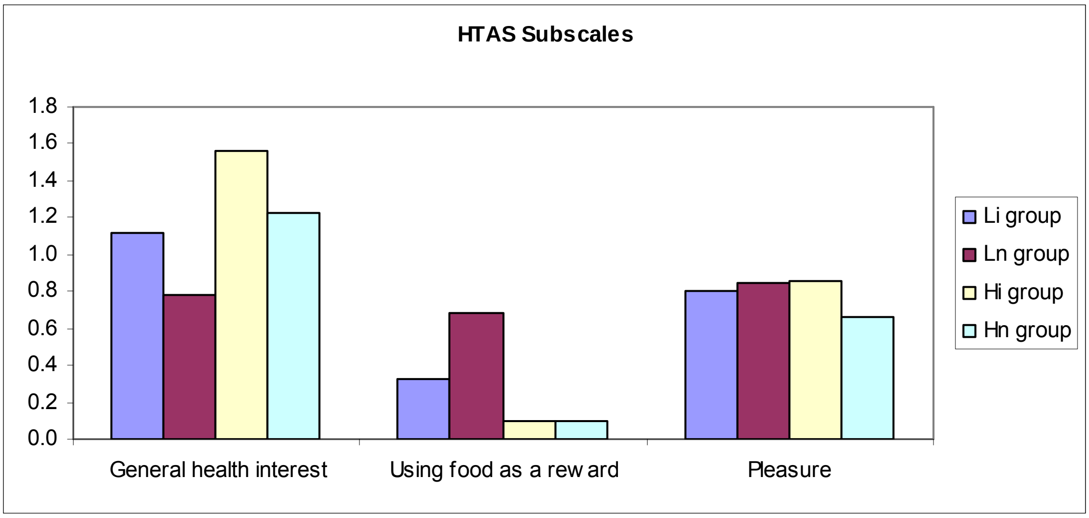


**Figure 15**. Health and Taste Attitude Scale (HTAS) sub-scales scores across groups ($p$ >0.05).

Multivariate regression analysis included a wide range of measured variables (see methods above), including socio-demographics, information and consumption, sensory perception and attitudes as independent variables, entered into models in competition with one another, with individual's scores for hedonics (9 point scale) and intentions to consume (7 point scale) selected *Brassica* vegetables as the dependent variables (Tables 9-14). Statistically significant values (standardised betas) reported indicate that for one (standardized, or standard deviation) unit change in the independent variable (left hand columns) a corresponding change occurs in the dependent variables (hedonics and intention) according to that value. For example a one unit change in perceived bitterness is associated with a 0.38 decrease in both liking (hedonics) of and intentions to consume broccoli (Table 9). Summation of the beta values represents the total amount of change associated with all the variables. For example, the significant variables in one of the models for broccoli account for more than 1 standard deviation in hedonic score.

**Table 9**. Multivariate predictors of hedonics and intentions to consume broccoli (n=200).

| **Broccoli** | **Hedonics (adj $R^2$ 0.37)** Standardised beta | **Intention (adj $R^2$ 0.20)** Standardised beta |
|---|---|---|
| Bitterness | -0.38*** | -0.38*** |
| Flavour impact | 0.31*** | 0.24*** |
| Gender (female) | 0.19** | 0.20** |
| Sweetness | 0.17* | - |
| Vegetable intake | - | 0.14* |

***p<0.000; **p<0.01; *p< 0.05

**Table 10.** Multivariate predictors of hedonics and intentions to consume green cabbage (n=200).

| **Green cabbage** | **Hedonics (adj $R^2$ 0.27)** Standardised beta | **Intention (adj $R^2$ 0.19)** Standardised beta |
|---|---|---|
| Sourness | -0.35*** | -0.17* |
| Flavour impact | 0.31*** | 0.24*** |
| Food for pleasure | 0.15* | 0.20** |
| Age | 0.14* | 0.14* |
| Food for reward | - | -0.19* |

***p<0.000; **p<0.01; *p< 0.05

**Table 11**. Multivariate predictors of hedonics and intentions to consume cauliflower (n=200).

| **Cauliflower** | **Hedonics (adj $R^2$ 0.24)** Standardised beta | **Intention (adj $R^2$ 0.13)** Standardised beta |
|---|---|---|
| Bitterness | -0.32*** | - |
| Flavour impact | 0.40*** | 0.24** |
| Gender (female) | - | 0.21** |
| Food for reward | - | -0.16* |

***p<0.000; **p<0.01; *p< 0.05

**Table 12**. Multivariate predictors of hedonics and intentions to consume red cabbage(n=200).

| **Red cabbage** | **Hedonics (adj $R^2$ 0.27)** | **Intention (adj $R^2$ 0.11)** |
|---|---|---|
| | Standardised beta | Standardised beta |
| Flavour impact | 0.42*** | 0.27*** |
| Gender (female) | 0.14* | - |
| Sourness | -0.30*** | - |
| Food for health | - | 0.16* |

***p<0.000; **p<0.01; *p< 0.05

**Table 13**. Multivariate predictors of hedonics and intentions to consume Brussels sprouts (n=200).

| Brussels sprouts | Hedonics (adj $R^2$ 0.17) | Intention (adj $R^2$ 0.19) |
|---|---|---|
| | Standardised beta | Standardised beta |
| Age | 0.31*** | 0.21** |
| Sourness | -0.24** | -0.17* |
| Umami | 0.16* | - |
| Foods for health | 0.16* | 0.19** |
| Flavour impact | - | 0.23** |
| Information | - | 0.14* |

***p<0.000; **p<0.01; *p< 0.05

**Table 14**. Multivariate predictors of hedonics and intentions to consume broccolini (n=200).

| **Broccolini** | **Hedonics (adj $R^2$ 0.30)** | **Intention (adj $R^2$ 0.28)** |
|---|---|---|
| | Standardised beta | Standardised beta |
| Sweetness | 0.27*** | 0.30*** |
| Sourness | -0.25*** | 0.21** |
| Foods for health | 0.18** | 0.24*** |
| Bitterness | 0.17* | - |
| Foods for pleasure | 0.13* | - |
| Saltiness | - | -0.15* |

***p<0.000; **p<0.01; *p< 0.05

Tables 9 – 14 show that sensory perception dominates, with attitudes contributing to a lesser degree, to the acceptance of *Brassica* vegetables. Not surprisingly being female and of older age were positive predictors of acceptance of some *Brassica* vegetables. Notably 'foods for pleasure' was only a minor positive predictor. In contrast 'food for reward' was a negative predictor in only one case.

Information has only a small effect upon intentions to consume one vegetable, Brussels sprouts (consistent with univariate analysis) but may be important for this particular vegetable. Additionally this suggests that when a food is unpopular information has the potential to influence responses positively.

In summary the multivariate models identify where variation in consumer characteristics and perceptions is associated with the liking and intentions to consume *Brassica* vegetables. These analyses suggest where interventions may effect successful change.

### 4.1.2 Discussion

Successful recruitment of two hundred physically active adults found approximately half to be educated to tertiary level and half to be dietary supplement users, characteristics often found amongst health conscious consumers. There was no evidence that these otherwise health conscious consumers differ in their use of dietary supplements. In other words, those reporting consuming fewer vegetable portions do not 'compensate' by consuming dietary supplements more than those who report consuming higher intakes of vegetables. The sample was also younger than the general population, possibly related to their active lifestyles.

Both the hedonic and intentions data reflect the current market popularity of broccoli relative to other *Brassica* vegetables.

Both the information and the non-information groups were asked what they thought were the health benefits of vegetables and *Brassicas* in particular. Information pertaining to the cancer protection afforded by *Brassica* vegetable consumption is not generally recalled ('known') by those not immediately informed (no-information groups) during the study. However in general, the majority of the intervention group (95%) could recall or state either specific or general health benefits of vegetables in contrast to participants in the no information groups, of whom almost 1 in 4 could not state any 'correct' knowledge. Using this more rigorous methodology, this finding conflicts with the recent Brand Story (2010) interpretations (and the basis of Ausveg Health promotion strategies) and could be a valuable novel piece of information for consumers; however, caution is warranted in the light of other findings (below).
With a focus just upon consumption and information there would appear to be some influence upon liking and intentions to consume (the least liked) Brussels sprouts. The multivariate models, taking into account a greater number of possible influences, confirm that it is possible that information may have some small effects upon the popularity of Brussels sprouts (intentions to consume). Those predisposed (reporting higher vegetable consumption) may be more influenced.

Whilst it is notable that the most popular *Brassica*, broccoli, was not perceived to be particularly relatively sweet on average, multivariate analysis found sweet perception as predictor of broccoli and broccolini liking and intentions to consume. Identifying, changing or highlighting the 'sweetness of *Brassicas* (for example, changing free sugars content) may be useful in changing acceptance.

Tables 9-14 show that the independent variables measured explain moderate variance in liking and intentions to consume *Brassica* vegetables with a tendency for liking to be better explained. In this respect the liking models support the original rationale of this project (i.e. that sensory perception is associated with liking). Given the wide range of behaviours and situations (not measured) that could impact upon intention to consume widely reported in the literature (e.g. (*45, 60*) it is not surprising that the intention to consume models only explain a low-moderate variance. For a-theoretical models with a focus on specific variables, these are nevertheless reasonable and expected. The better models, generally predictors of liking, can explain around 1 standard deviation variation in the dependent variables. Importantly many of the predictors are potentially malleable and reflect the focus of the larger project in which the current task is located.

Sensory perception seems to dominate liking as their importance as predictors reflects the variance across the participants. What influences that variance is unknown but could include experience (exposure) or genetic variation in taste perception (*61*).

Attitudes towards foods for health were relevant in some cases. Age and female gender, characteristics that are known to be associated with 'healthy' eating, were sometimes positive predictors. Notably age was the strongest positive predictor for the least liked and most bitter vegetable, Brussels sprouts. The age range was not wide (18-55y), due to deliberately excluding older participants who may suffer declines in sensory ability, so this age effect is unlikely to be due to age related physiological changes. Rather it is likely to be a function of exposure and/or conditioning i.e. a traditional British vegetable strongly associated with festive occasions. However the models did not explain more than a moderate amount of variance in liking or intentions.

### 4.1.3 Conclusion

In general, the data obtained in this consumer test suggested that taste characteristics are the main driving force behind vegetable preference and need to be addressed in order to increase popularity of *Brassica* vegetables. Attitudes are potentially malleable and also have potential for changing acceptance of *Brassica* vegetables

These consumer results laid the foundation for further investigating the components responsible for the sensory and health-beneficial properties associated with *Brassica* vegetables.

## 4.2 Chemical and sensory characterisation of *Brassica* extracts

### 4.2.1 Background

The consumer test highlighted the need to identify the phytochemicals responsible for the undesirable (e.g. bitterness) and desirable (e.g. sweetness) taste characteristics of *Brassica* vegetables. Four *B.* oleracea members (broccoli, cauliflower and red cabbage, Brussels sprouts) were selected to be investigated further based on availability, market popularity but also on hedonic and intention to consume data (i.e. high and low,Tables 4 and 5).

### 4.2.2 Results-Discussion

*Chemical composition of whole Brassica extracts*

Due to (interstate) quarantine constraints the material used for the remainder of the study was sourced from different suppliers (located in Sydney, NSW) and therefore was of the same type but not identical to the one used for the consumer test (suppliers located in Adelaide, SA). Nevertheless, the component groups in all samples believed to be potentially responsible, based on the available literature, for the taste characteristics of *Brassica* vegetables (i.e. GS, phenolics, sugars) were quantitatively determined. This was performed in order to get a measure of the differences between the different samples (Tables 15 and 16). Although some differences between the samples were observed (e.g. progoitrin in broccoli, sinigrin in cauliflower, Table 15), overall the *Brassica* vegetables obtained from the Sydney and Adelaide wholesalers were similar in terms of GS and phenolic composition. GS content can vary quite significantly even between plants cultivated under the same conditions on the same site (*10*). For example a 27-fold difference in GS levels between individual accessions was observed for broccoli grown under controlled conditions (*10*).

Table 17 compares the individual and total GS content in the *Brassica* vegetables used in this study with those reported by two previous studies. The comparison shows very clearly the large quantitative and qualitative GS variation that exists in *Brassica* vegetables. The vegetables used in this study have clearly less total GS content than those in the comparison studies. Genetic and environmental/agronomic factors are known to have an impact on the levels of GS found in a plant (*8, 11*).

As can be seen from Table 17, gluconapin (for red cabbage), glucobrassicin and neoglucobrassicin are the major contributors to the GS profiles of the vegetables used in this study. Brussels sprouts exhibited the highest total GS concentration followed by broccoli and red cabbage; cauliflower produced the least total concentration of GS.

Identification of every component in each extract and at each wavelength can be a very difficult and very time-consuming exercise when dealing with very complex extracts. Different phytochemicals however are known to absorb at distinct wavelengths based on their structure. Table 16 below shows the total concentration of components absorbing at the various wavelengths across the whole extracts from the *Brassica* vegetables investigated. It is likely that the peaks at 280 nm and 326 nm are due to phenolic acids, glycosides are likely to dominate the signal at 370 nm and anthocyanins responsible for the signal at 520 nm. As can be seen phenolic acids appear to dominate the profiles (ie. signals at 280 and 326 nm) for all extracts. As expected red cabbage was the only vegetable that exhibited a significant concentration of anthocyanins (responsible for its red pigmentation). LC/MS analysis identified 19 anthocyanins in the whole extract from the red cabbage used in this study. Most of these were based on a core of cyaniding-3-*O*-diglucoside-5-*O*-glucoside that was either non-acylated or mono-acylated or di-acylated with *p*-coumaric*,* caffeic, ferulic and sinapic acids as reported previously (*62*).

**Table 15.** Concentration of individual and total glucosinolates in the various *Brassica* vegetables used in this study. Values represent the mean from two determinations per vegetable type.

| | Concentration (µg/g DW[a]) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Glucosinolate** | **Broccoli** | | **Cauliflower** | | **Red cabbage** | | **Brussels sprouts** | | **Broccolini** | **Green cabbage** |
| | **Syd[b]** | **Adel[c]** | **Syd** | **Adel** | **Syd** | **Adel** | **Syd** | **Adel** | **Adel** | **Adel** |
| Glucoiberin | 38.2 | 9.9 | 3.4 | 21.5 | 9.6 | 15.1 | 3.9 | 33.5 | 0.3 | 29.1 |
| Progoitrin | 126.9 | n/d | 9.1 | 5.7 | 120.5 | 168.2 | 22.9 | 60.4 | 61.7 | 23.9 |
| Sinigrin | 15.5 | 0.6 | 17.0 | n/d | 47.8 | 40.6 | 97.9 | 5.3 | n/d | 122.7 |
| Glucoraphanin | 86.7 | 65.6 | 1.1 | 2.1 | 164.4 | 102.7 | 17.4 | 9.5 | 67.3 | 4.5 |
| Gluconapin | 137.3 | n/d | 5.7 | 25.8 | 609.0 | 488.1 | 127.1 | 340.2 | 112.0 | 67.1 |
| Glucoerucin | 3.1 | 21.7 | 0.0 | 125.9 | 0.0 | 10.1 | 0.0 | 19.5 | 16.9 | 14.5 |
| Glucobrassicin | 384.9 | 583.6 | 417.7 | 280.7 | 187.4 | 348.2 | 565.1 | 1322.9 | 572.3 | 275.2 |
| Glucoalyssin | 4.1 | 6.8 | 4.5 | 3.1 | 1.9 | 3.8 | 6.3 | 14.1 | 6.5 | 3.1 |
| 4-Methoxyglucobrassicin | 146.5 | 279.4 | 94.2 | 90.7 | 118.5 | 89.4 | 136.5 | 158.4 | 178.2 | 220.0 |
| Neoglucobrassicin | 751.3 | 2315.1 | 86.3 | 167.9 | 26.8 | 106.1 | 491.7 | 154.9 | 664.2 | 127.3 |
| **Total** | **1694.5** | **3282.7** | **639** | **723.4** | **1285.9** | **1372.3** | **1468.0** | **2118.7** | **1679.4** | **887.4** |

[a] dry weight
[b] Syd: vegetables obtained from Sydney-based wholesalers
[c] Adel: vegetables obtained from Adelaide-based wholesalers

**Table 16**. Concentration of phenolics[a] in the various *Brassica* vegetables used in this study. Values represent the mean from two determinations per vegetable type.

| | Concentration (µg/g DW) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Wavelength (nm)** | **Broccoli** | | **Cauliflower** | | **Red cabbage** | | **Brussels sprouts** | | **Broccolini** | **Green cabbage** |
| | **Syd**[b] | **Adel**[c] | **Syd** | **Adel** | **Syd** | **Adel** | **Syd** | **Adel** | **Adel** | **Adel** |
| **280** | 5997 | 6664 | 5080 | 4269 | 4158 | 8690 | 5205 | 7550 | 6095 | 2670 |
| **326** | 9822 | 12400 | 4603 | 4178 | 15342 | 22003 | 14362 | 14611 | 18538 | 3358 |
| **370** | 1375 | 1551 | 738 | 569 | 2613 | 5483 | 2207 | 2701 | 6413 | 636 |
| **520** | 35 | 38 | 41 | 38 | 4915 | 10063 | 12 | 25 | 39 | 15 |

[a] It is likely that the signals at 280 nm and 326 nm are due to phenolic acids, glycosides are likely to dominate the signal at 370 nm and anthocyanins are responsible for the signal at 520 nm.
[b] Syd: vegetables obtained from Sydney-based wholesalers
[c] Adel: vegetables obtained from Adelaide-based wholesalers

**Table 17.** Comparison of individual and total glucosinolate content between the *Brassica* vegetables used in this and previous studies (*58, 63*).

| | Broccoli | | | Cauliflower | | | Brussels Sprouts | | | Red cabbage |
|---|---|---|---|---|---|---|---|---|---|---|
| **Glucosinolate** | **Concentration (μmol/100 g FW)** | | | | | | | | | |
| | **Aus[a]** | **UK[b]** | **USA[c]** | **Aus[a]** | **UK[b]** | **USA[c]** | **Aus[a]** | **UK[b]** | **USA[c]** | **Aus[a]** |
| Glucoiberin | 0.9 | 17.1 | 12.9 | 0.1 | 1.3 | 41.6 | 0.1 | 1.5 | 58.7 | 0.2 |
| Progoitrin | 3.1 | 3.3 | 0.1 | 0.2 | 0.4 | 8.6 | 0.8 | 2.4 | 133.0 | 3.2 |
| Sinigrin | 0.4 | 1.4 | 0.2 | 0.4 | 5.3 | 42 | 3.5 | 8.6 | 155.0 | 1.4 |
| Glucoraphanin | 1.9 | 29.4 | 105.0 | n/d[e] | 0.3 | 4.5 | 0.5 | 0.5 | 15.1 | 3.9 |
| Gluconapin | 3.6 | 2.9 | 0.1 | 0.1 | 3.4 | 1.3 | 4.3 | 2.8 | 108.0 | 17.1 |
| Glucoerucin | 0.1 | n/a[d] | 1.0 | n/d | n/a | 2.6 | n/d | n/a | n/d | 0.0 |
| Glucobrassicin | 8.3 | n/a | 37.7 | 8.2 | n/a | 153.0 | 16.0 | n/a | 374.0 | 4.4 |
| Glucoalyssin | 0.1 | 3.9 | 1.1 | 0.1 | n/d | 2.6 | 0.2 | 0.3 | 11.0 | 0.0 |
| 4-methoxyglucobrassicin | 3.0 | n/a | 38.5 | 1.7 | n/a | 40.4 | 3.6 | n/a | 84.4 | 2.6 |
| Neoglucobrassicin | 15.2 | n/a | 11.4 | 1.6 | n/a | 23.4 | 13.0 | n/a | 0.0 | 0.6 |
| **Total** | **36.6** | **62.4** | **208.0** | **12.5** | **13.5** | **320.0** | **42.0** | **17.2** | **939.0** | **33.5** |

[a] Aus: this study (Syd values taken from Table 15).
[b] UK-based study (*63*)
[c] USA-based study (*58*)
[d] n/d = not detected
[e] n/a= not available/not reported

The whole extracts contained only trace amounts of isothiocyanates (ITC). Experiments (data not shown) confirmed that this was due to myrosinase unavailability rather than lack of GS substrate. This indicated that myrosinase was inactivated before it could convert GS to ITC. The absence of ITC in the whole extracts was desirable as it allowed the GS to be accurately determined. Also, the taste properties of whole extracts with no or very little ITC represented more accurately the profiles of the *Brassica* vegetables evaluated during the consumer test. It is well known that thermal treatment inactivates myrosinase (e.g. 90% activity lost following steaming for 7 minutes) (*64*). This suggests that the steamed *Brassica* vegetables evaluated during the consumer test would have had little myrosinase activity remaining during evaluation as vegetables were steamed for 6 min. before serving to consumers.

The concentration of free sugars is also known to have an impact on the taste profile of vegetables particularly in relation to sweetness. The results from the quantitative determination of the free sugars detected in the *Brassica* vegetables used to obtain whole extracts for sensory assessment are shown below (Table 18).

**Table 18.** Concentration of free sugars in whole *Brassica* extracts.

| | **Fructose** | **Glucose** | **Sucrose** | **Total** |
|---|---|---|---|---|
| | Concentration (mg/g FW) | | | |
| Red Cabbage | 9.5 | 13.4 | 3.8 | 26.7 |
| Cauliflower | 9.3 | 8.0 | 1.4 | 18.7 |
| Brussels sprouts | 4.7 | 4.1 | 1.4 | 12.6 |
| Broccoli | 4.5 | 5.6 | 2.5 | 10.2 |

*Sensory evaluation of Brassica whole extracts using trained panellists*

An aliquot (1 mL) of the whole extract (re-constituted in mineral water) from each *Brassica* vegetable was presented to each panellist per session. Assuming complete analyte recovery during extraction, each 1 mL aliquot contained equivalent amount of phytochemicals found in 4.7-6.2 g of *Brassica* vegetables fresh weight (varied according to vegetable moisture content, Appendix 7). The panellists evaluated the taste-intensity of each sample in terms of *sweet, salt, sour, bitter, umami (savoury),* and *astringent* aftertaste. using 100mm unstructured line scales anchored at 5 and 95%, respectively, with extremes for each descriptive term.

The results obtained are summarised in Table 19 and visualised in Figure 16 below. As can be seen, the extract from Brussels sprouts was rated as significantly more bitter than those from the other vegetables; the extract from cauliflower was significantly less bitter than the extracts from broccoli and red cabbage. The extracts from cauliflower and red cabbage were found to be significantly sweeter than the extracts from Brussels sprouts and broccoli; these were rated similar in sweetness. This result is in complete agreement with the concentration of free sugars found in these extracts (Table 18). The broccoli whole extract was significantly saltier than the other extracts (no significant differences in the saltiness of the rest) whilst the Brussels sprouts extract was found more astringent than the rest. Finally, no significant differences in sourness and umami were found between the *Brassica* whole extracts.

**Table 19.** Summary of descriptive sensory assessment scores for the whole extracts in terms of the various taste attributes. Sweetness, bitterness and saltiness appear to be the main differences between the *Brassica* whole extracts.

| Whole extract | Sweet | Salty | Sour | Bitter | Umami | Astringency (aftertaste) |
|---|---|---|---|---|---|---|
| Brussels sprouts | 24.3[a] | 33.0[a] | 25.5 | 43.4[c] | 31.9 | 29.6[b] |
| Cauliflower | 40.5[b] | 33.1[a] | 20.3 | 18.5[a] | 36.6 | 22.5[a] |
| Broccoli | 21.9[a] | 42.1[b] | 25.5 | 28.6[b] | 41.0 | 25.8[ab] |
| Red cabbage | 39.6[b] | 34.0[a] | 23.0 | 30.5[b] | 36.2 | 27.6[ab] |
| **F-value** | 23.3 | 6.4 | 1.4 | 21.3 | 3.9 | 2.7 |
| ***p*-value** | **<0.0001** | **<0.001** | NS | **<0.0001** | NS | **0.05** |
| **SED** | 2.9 | 2.6 | 2.9 | 3.2 | 2.7 | 2.6 |

Values not sharing the same subscript letter are statistically significantly different from one another.

Overall there was generally good agreement, particularly for sweetness and bitterness, between the results from the descriptive assessment of the taste attributes of the whole extracts using trained panellists and those obtained from the participants in the consumer test following assessment of mildly-steamed vegetables. This was useful, as it suggested that information regarding taste-impact components that was generated from *Brassica* whole extracts (and their fractions) could be used to understand and interpret taste differences observed during consumption of actual *Brassica* vegetables.

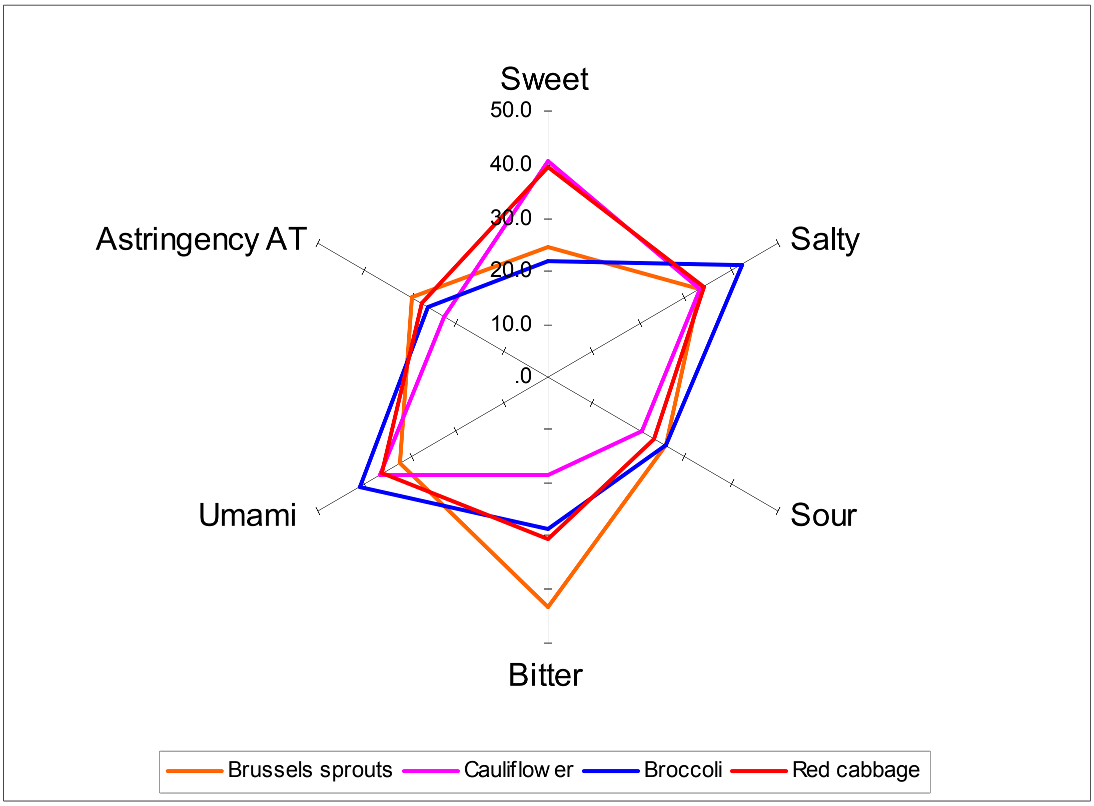


**Figure 16**. Spider plot illustrating the sensory assessment scores of the whole extracts from the four *Brassica* vegetables against each taste attribute (trained panel n=10).

*Fractionation of the whole extracts from Brassica vegetables*

Fractionation of the whole extracts, followed by sensory evaluation and chemical analysis of the resulting fractions, was performed in order to understand the contribution of phytochemicals (e.g. GS, phenolics, sugars) to the taste characteristics associated with each type of *Brassica* vegetable. This direct approach allowed sound assessments relating to the taste contribution of key phytochemicals found in *Brassica* vegetables to be made.

The fractionation of the whole extracts was undertaken using a preparative liquid chromatograph equipped with an automated fraction collector. Several fractions for each whole extract resulted (Figure 17). Although the chromatographic conditions were identical during the fractionation for all vegetable extracts, due to the different profiles observed for each vegetable, the fractions obtained were (sometimes) different in terms of their boundaries. This was to ensure that distinct areas of activity (based on signal between 190-800 nm) were collected within the same fraction. Table 20 below presents the fraction boundaries for each *Brassica* vegetable type.

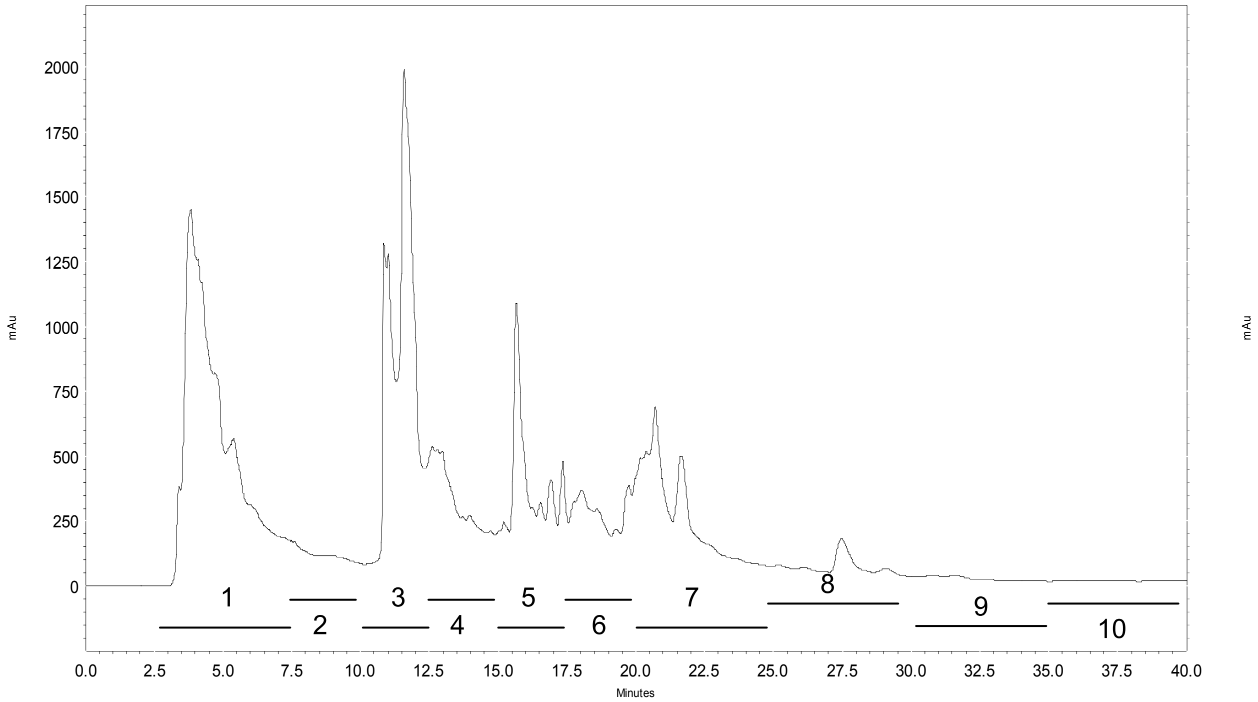


**Figure 17.** Reverse-phase preparative LC/PDA chromatogram of an extract from Brussels sprouts (190-800 nm). Numbered lines indicate the fractions obtained for sensory analysis.

**Table 20.** Number and size (i.e. length in minutes) of fractions collected for each *Brassica* vegetable extract.

| **Fraction** | **Broccoli** | **Brussels sprouts** | **Cauliflower** | **Red cabbage** |
|---|---|---|---|---|
| | **Retention time (min.)** | | | |
| 1 | 2.5-7.5 | 2.5-7.5 | 2.5-7.5 | 2.5-7.5 |
| 2 | 7.5-10 | 7.5-10 | 7.5-10 | 7.5-10 |
| 3 | 10-12.5 | 10-12.5 | 10-15 | 10-15 |
| 4 | 12.5-15 | 12.5-15 | 15-20 | 15-17.5 |
| 5 | 15-17.5 | 15-17.5 | 20-25 | 17.5-20 |
| 6 | 17.5-20 | 17.5-20 | 25-30 | 20-25 |
| 7 | 20-22.5 | 20-25 | 30-35 | 25-30 |
| 8 | 22.5-25 | 25-30 | 35-40 | 30-35 |
| 9 | 25-30 | 30-35 | 40-45 | 35-40 |
| 10 | 30-35 | 35-40 | 45-50 | 40-45 |
| 11 | 35-40 | NA | NA | 45-50 |

*Sensory evaluation of fractions*

Following fractionation, each fraction was concentrated, de-solvated and re-constituted in mineral water before being presented to the trained panellists. The assessment was identical to that carried out for the whole *Brassica* extracts. The amount of phytochemicals assessed was equivalent to 3.8-5.0 g FW per vegetable type per panellist across all fractions per replicate assessment (varied according to vegetable moisture content, Appendix 7).

1) Total taste activity of fractions

Figures 18-21 below illustrate the total taste activity of each fraction in each vegetable. The total taste values represent the sum of the averaged values for all evaluated attributes in each vegetable. Mineral water was used as a ‘no-taste’ control and its scores were subtracted from the scores of all fractions for each attribute. As can be seen fraction 1 exhibited by far the greatest taste activity 1 across all vegetables (Figures 18-21). The contribution of the remaining fractions in the taste activity of each vegetable extract was lower than that of fraction 1 but most individual fractions were deemed by the assessors to have some taste activity.

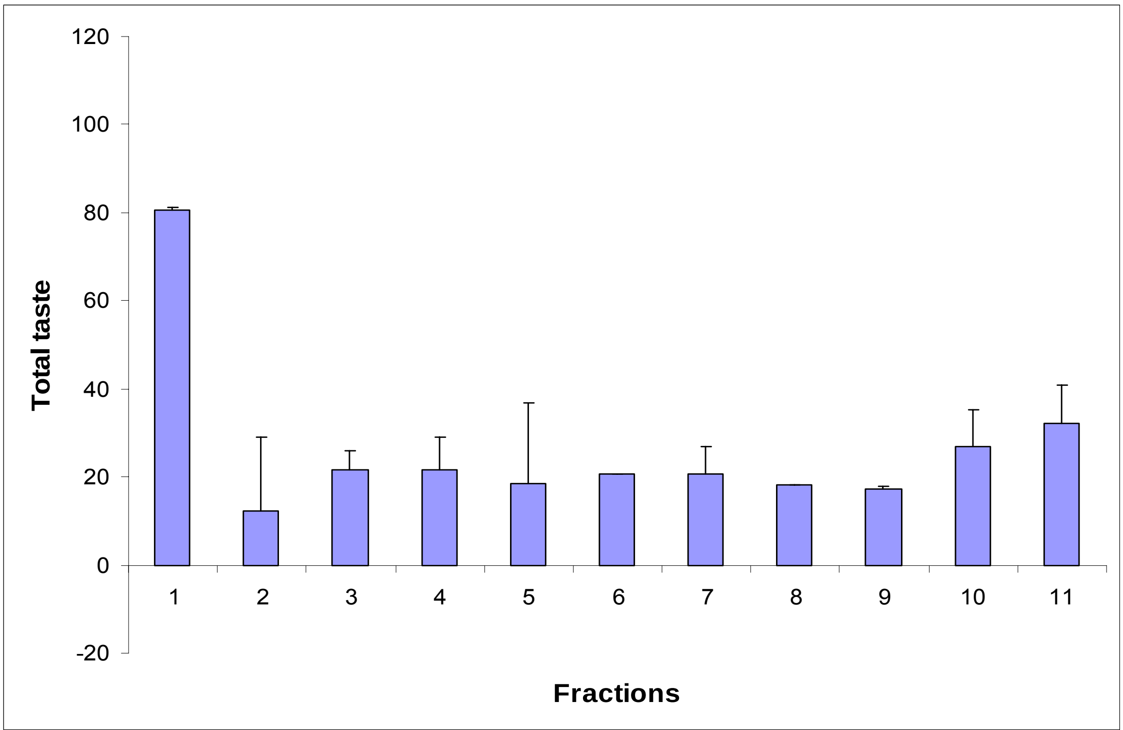


**Figure 18.** Total taste of each fraction from a broccoli extract as determined by sensory evaluation using trained assessors. Values were obtained from the averaged scores for all evaluated attributes. Bars represent the error across the duplicate panel assessments (i.e. total of 20 individual evaluations per fraction).

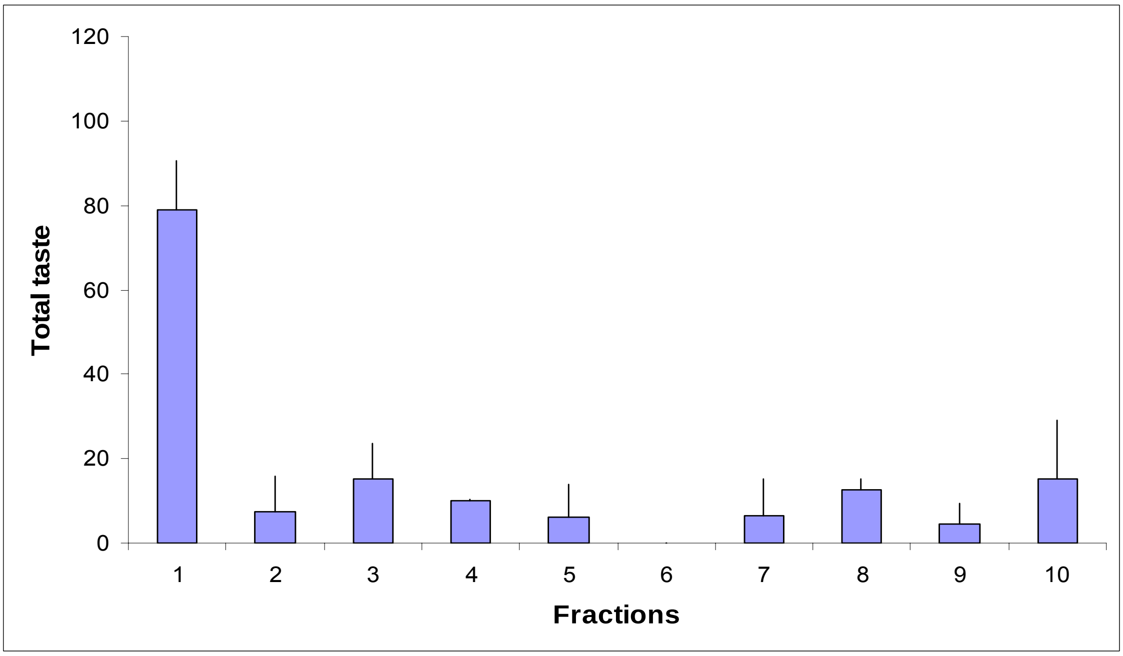


**Figure 19**. Total taste of each fraction from a cauliflower extract as determined by sensory evaluation using trained assessors. Values were obtained from the averaged scores for all evaluated attributes. Bars represent the error across the duplicate panel assessments (i.e. total of 20 individual evaluations per fraction).

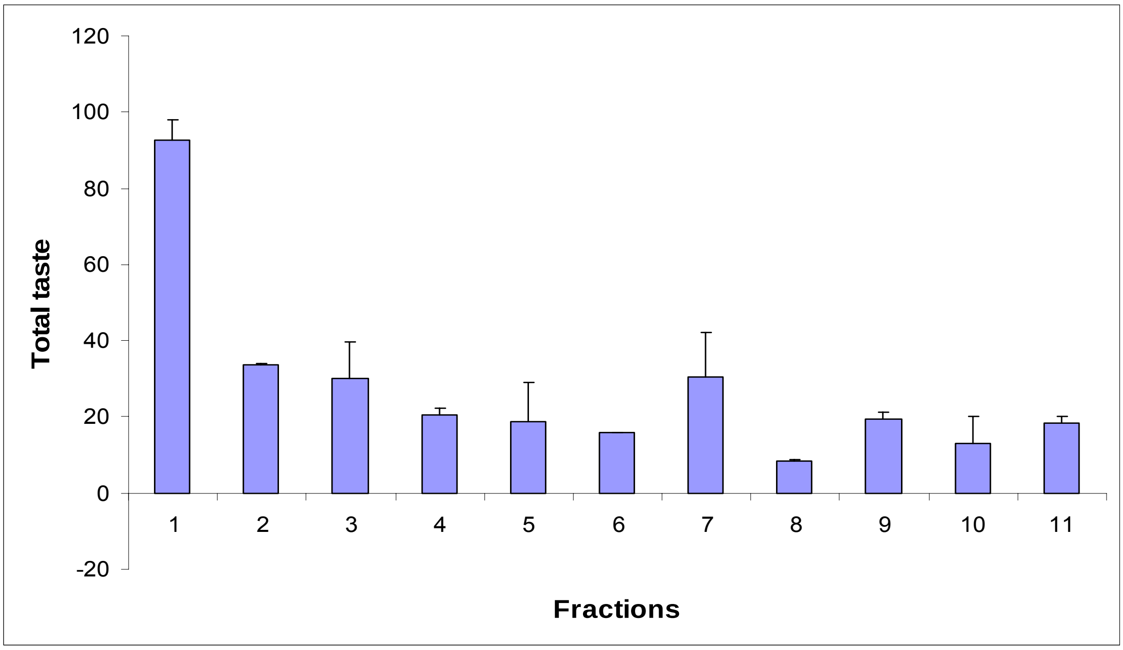


**Figure 20.** Total taste of each fraction from a Brussels sprouts extract as determined by sensory evaluation using trained assessors. Values were obtained from the averaged scores for all evaluated attributes. Bars represent the error across the duplicate panel assessments (.i.e. total of 20 individual evaluations per fraction).

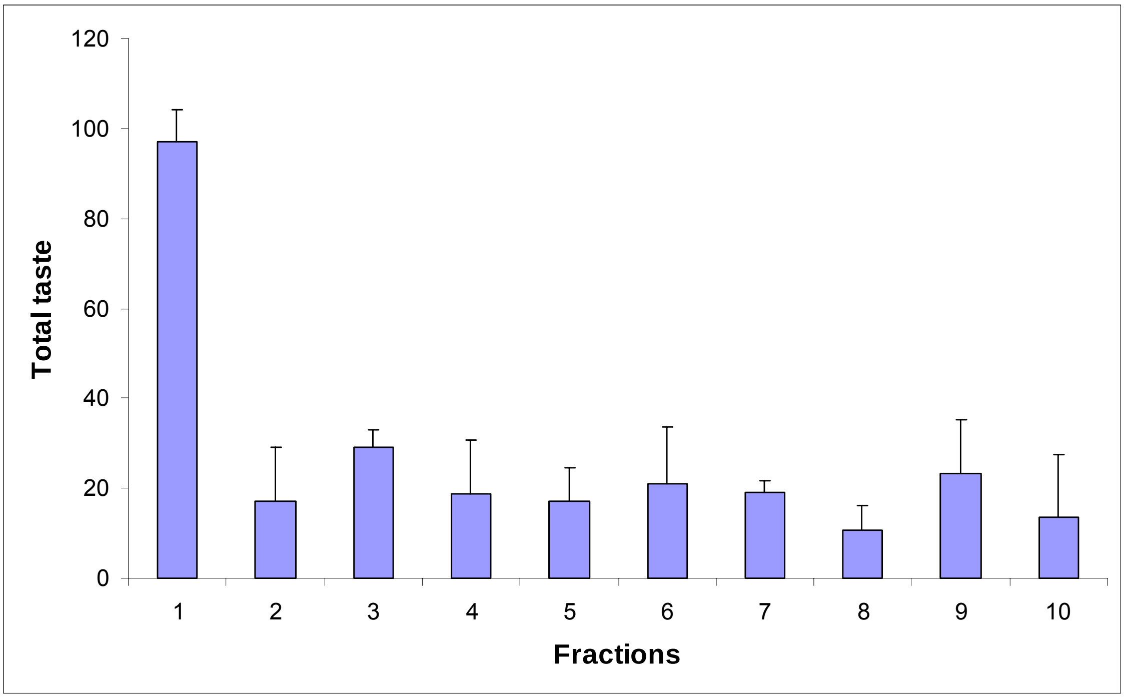


**Figure 21**. Total taste of each fraction from a red cabbage extract as determined by sensory evaluation using trained assessors. Values were obtained from the averaged scores for all evaluated attributes. Bars represent the error across the duplicate panel assessments (i.e. total of 20 individual evaluations per fraction).

2) Taste activity of fraction 1

A closer look at the contribution of each attribute on the total taste activity of fraction 1 (Figure 22) indicates that sweetness, umami, astringency and saltiness were the main contributing attributes to the total taste activity of this fraction across all vegetables. Sourness and bitterness were found to have a minor contribution to the taste activity of this fraction in all vegetable extracts.

Chemical analysis detected several free sugars in this fraction including glucose, fructose and sucrose. In fact, this fraction contained the vast majority of these components (87%-100% of the total sugars present in the whole *Brassica* extracts) (Table 21). These sugars are likely to be primarily responsible for the sweetness of this fraction and are also likely to suppress the bitterness. Most of the glucose detected is likely to have originated from the plants' stored reserves rather than GS hydrolysis. The detection of ITC in trace amounts in these extracts (data not shown) indicates that GS hydrolysis was negligible. This was expected since myrosinase, the enzyme required for GS hydrolysis, is inactivated during the extraction process.

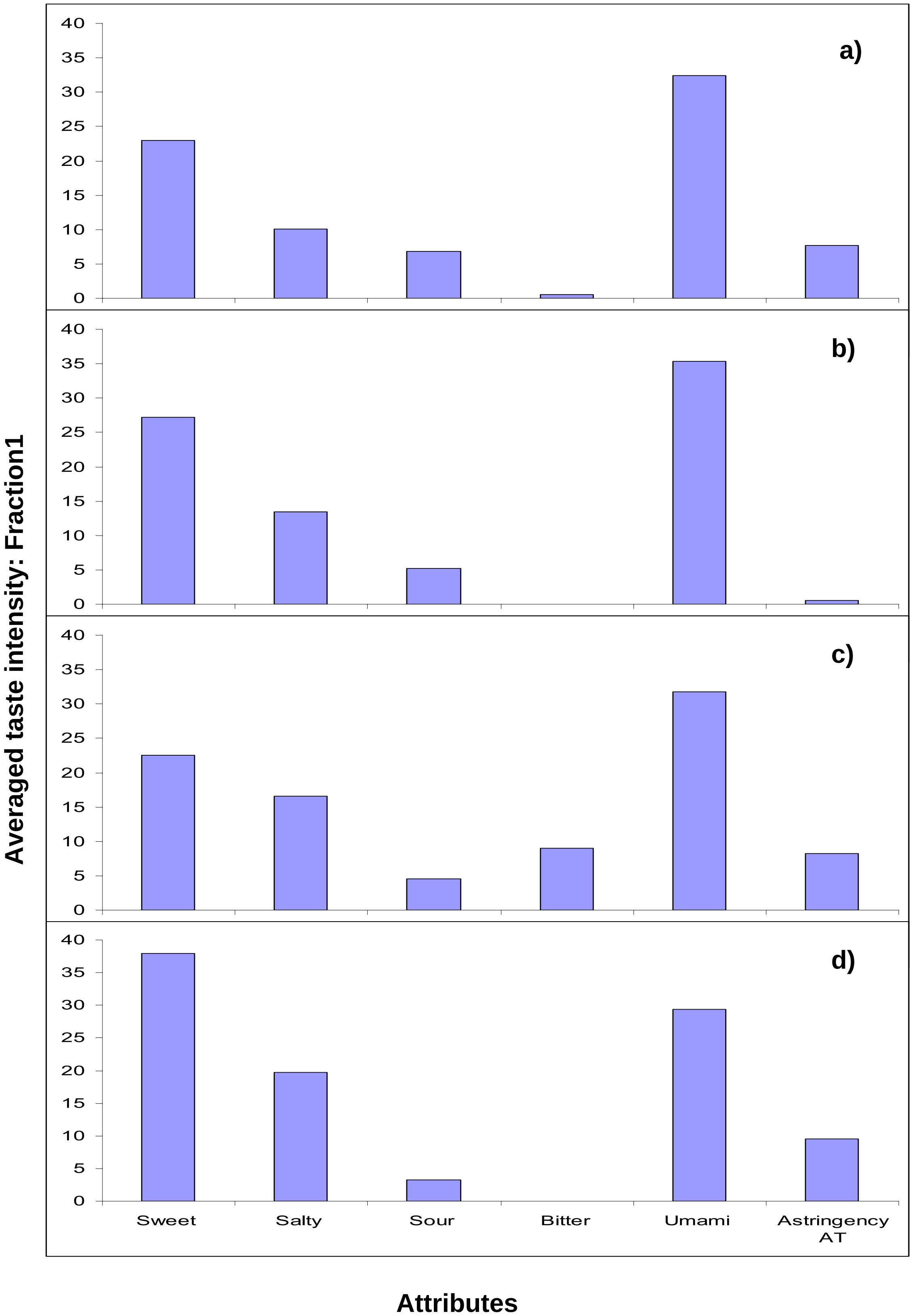


**Figure 22.** Contribution of various attributes to the total taste activity of fraction 1 from broccoli (a), cauliflower (b), Brussels sprouts (c) and red cabbage (d) extracts.

Other hydrophilic components found to be present in this fraction included phenolics and glucosinolates. These components are generally associated with astringency and/or bitterness but are not usually associated with saltiness or umami sensations. It is likely that acidic amino acids (e.g. aspartic, glutamic acid) are responsible for these attributes (*65*). For example, broccoli is known to contain more than 0.5 g of glutamic acid per 100 g of edible material (*66*). In solution, this acid is likely to be present in its anionic form, glutamate, a well known umami-taste inducer (*65*).

**Table 21**. Concentration of free sugars in fraction 1. Numbers in brackets indicate relative percentage against total concentration of sugars detected across all fractions for each vegetable extract.

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| | **Fructose** | **Glucose** | **Sucrose** |
|---|---|---|---|
| | Concentration (mg/mL) | | |
| Broccoli | 10.4 (98%) | 8.8 (95%) | 1.4 (45%) |
| Cauliflower | 20.0 (91%) | 15.2 (91%) | 2.1 (50%) |
| Red Cabbage | 19.3 (87%) | 26.9 (90%) | 5.3 (59%) |
| Brussels sprouts | 11.8 (94%) | 11.0 (94%) | 3.0 (63%) |

To gain more information about the taste profile of fraction 1 further fractionation of this faction was performed for red cabbage and Brussels sprouts. Fraction 1 for these two vegetables was fractionated into sub-fraction 1.1 and subfraction 1.2. Sensory assessment and chemical characterisation of these sub-fractions followed. The results are summarised in Table 22 below.

**Table 22**. Summary of descriptive sensory assessment scores for sub-fractions 1.1 and 1.2 in terms of the various taste attributes.

| **Sub-fraction** | **Sweet** | **Salty** | **Sour** | **Bitter** | **Umami** | **Astringency (aftertaste)** |
|---|---|---|---|---|---|---|
| B.S. Fr. 1.1 | $28.3^{b}$ | $26.2^{b}$ | 12.3 | $11.6^{a}$ | $35.1^{c}$ | 20.2 |
| B.S. Fr. 1.2 | $15.2^{a}$ | $7.9^{a}$ | 13.2 | $22.6^{b}$ | $10.6^{a}$ | 16.0 |
| R.C. Fr.1.1 | $41.2^{c}$ | $23.8^{b}$ | 13.7 | $7.2^{a}$ | $27.7^{b}$ | 17.7 |
| R.C. Fr.1.2 | $30.8^{b}$ | $10.5^{a}$ | 11.2 | $11.0^{a}$ | $10.4^{a}$ | 15.1 |
| **F-value** | 18.4 | 25.6 | 0.3 | 7.9 | 22.1 | 1.9 |
| ***p*-value** | **<0.0001** | **<0.0001** | **NS** | **<0.001** | **<0.001** | **NS** |
| **SED** | 3.5 | 2.6 | 2.8 | 3.3 | 3.7 | 2.3 |

Values not sharing the same subscript letter are statistically significantly different from one another.

The assessment found sub-fraction 1.1 to be significantly sweeter, saltier and more umami than sub-fraction 1.2 for both Brussels sprouts and red cabbage. Sub-fraction 1.1 was significantly more bitter than sub-fraction 1.2 for Brussels sprouts (but not for red cabbage). Finally, no significant differences were found between the two sub-fractions in terms of sourness and astringency.

A very good inverse correlation between the values for bitterness and sweetness for the four sub-fractions was obtained when plotted ($R^2$: 0.937). This is likely to represent differences in the chemical composition of the sub-fractions, but also interaction (e.g. suppression, masking) between the two tastes at the perceptual level. For example, the less sweet/more bitter sub-fraction 1.2 was found to contain the vast majority of GS found in fraction 1 for both red cabbage (82%) and Brussels sprouts (88%). In contrast, the more sweet/less bitter sub-fraction 1.1 contained the vast majority of the free sugars found in fraction 1 (85% for red cabbage and 89% for Brussels sprouts).

For both, red cabbage and Brussels sprouts, the bitterness scores for fraction 1.2 were higher than those for fraction 1 (i.e. 1.1 and 1.2 combined). This confirms the effect of increased sweetness (i.e. from the sugars present in fraction 1.1) on reducing the bitterness of the extracts.

Bitterness and astringency are usually cited as the two most undesirable taste attributes of *Brassica* vegetables (*36, 39, 67*). The following sections will describe the results for these two attributes across fractions from all *Brassica* whole extracts.

*Bitterness across all fractions*

The results of the sensory assessment for bitterness of all the fractions across the *Brassica* extracts are shown below (Figures 23 and 24). Several fractions across all extracts were perceived as being bitter. Fraction 1 in all vegetables appears to be relatively less bitter than other fractions; this is likely to be due to a suppression of bitterness from the high sugar-related sweetness found in this fraction. There were some fractions that were notably more bitter than others (e.g. 3, 7), however overall, the bitterness was spread over several fractions in all vegetable extracts.

In all four extracts, the averaged intensity of bitterness did not correlate with the concentration of any individual GS, total GS, and total phenolics (or combinations of these values e.g. total GS+total phenolics). This is not surprising as it is well known that the taste activity of a component is dependant on both its concentration in the food of interest and its taste threshold.

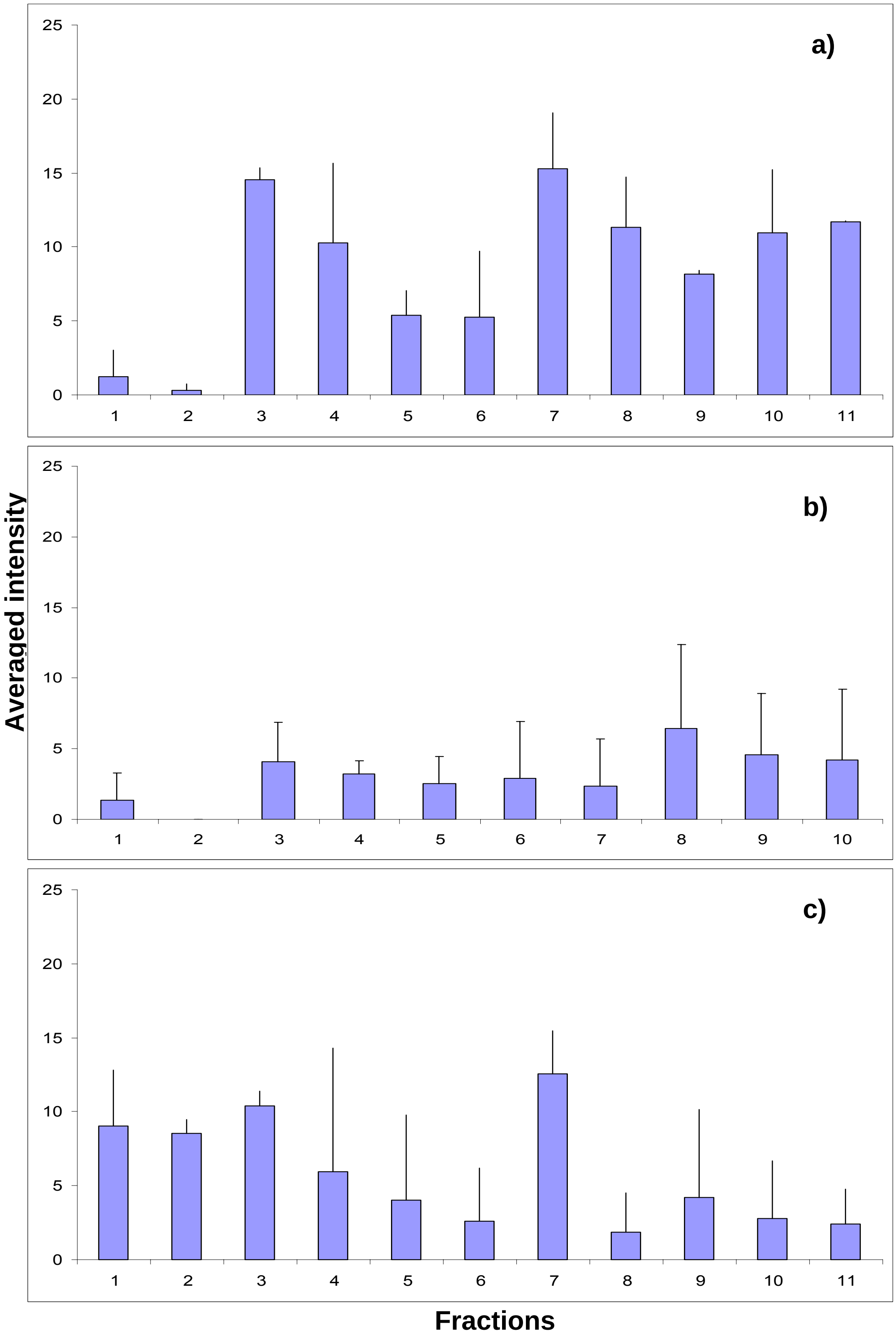


**Figure 23**. Averaged intensity of bitterness in fractions from broccoli (a), cauliflower (b) and Brussels sprouts (c) extracts.

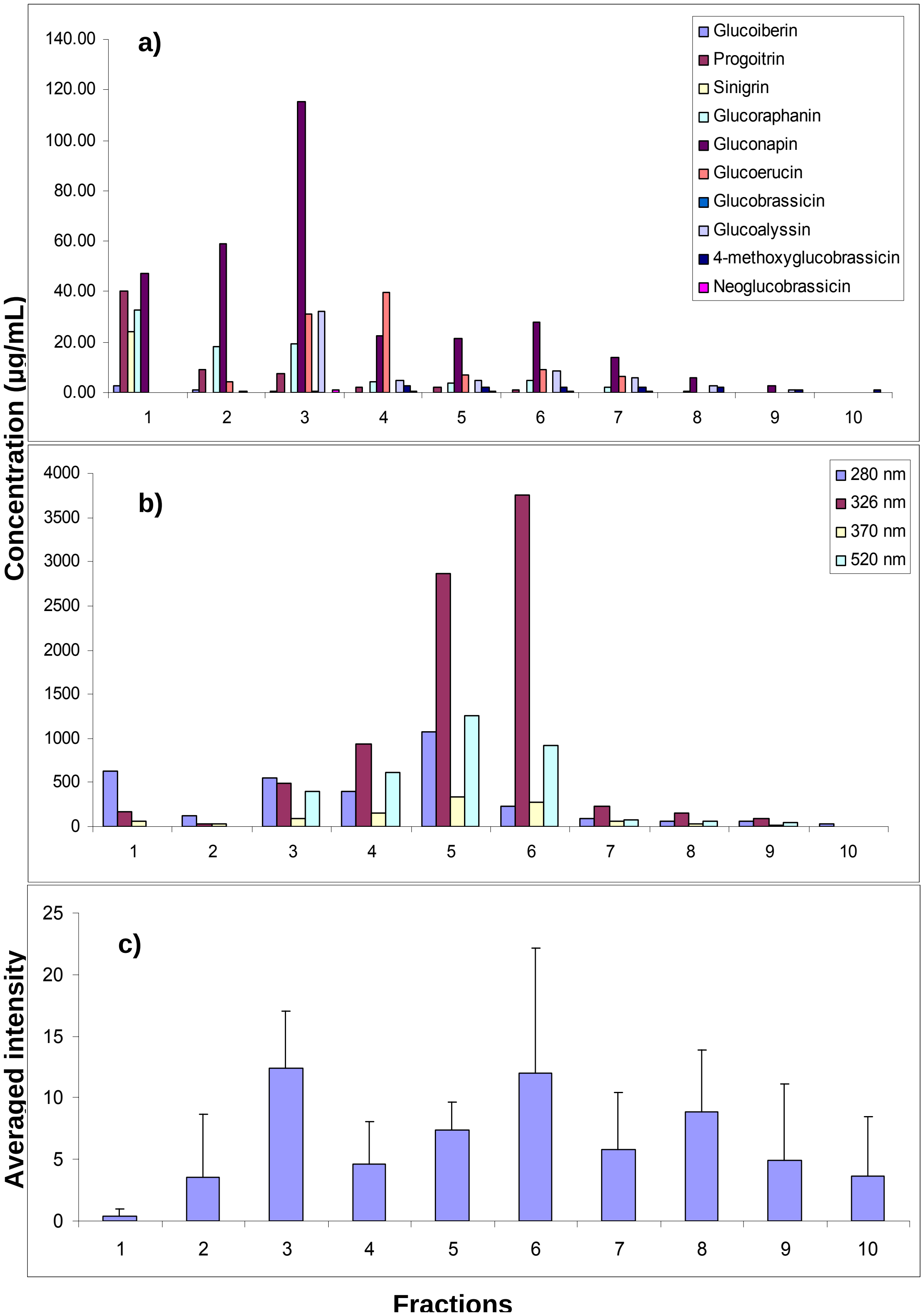


**Figure 24.** Concentration of glucosinolates (a), other photodiode array-detected components (including phenolics) (b), and intensity of bitterness in fractions from a red cabbage extract.

The concentration of phytochemicals (expressed as µg/mL) shown in Figure 24 was the same as the amount evaluated by the assessors for bitterness because the volume of each fraction subjected to sensory assessment was 1 mL. Figure 24 shows that fractions 3 and 7 contained GS such as gluconapin, glucobrassicin and neoglucobrassicin although it is still not known whether these components can be perceived at the amounts found in the fractions subjected to sensory assessment (e.g. gluconapin in fraction 3 ranged from 24-115 µg across the four vegetables). However it is likely that other components (e.g. phenolics) are contributing to the bitterness of *Brassica* extracts. The total GS content of fraction 8 in red cabbage for example is 11 µg and the fraction was still perceived as bitter (Figure 24).

The detection threshold for sinigrin in water has been previously reported as 106 ppm (*68*). In this study, sinigrin was perceived in water at 30 ppm and was rated as bitter from 60 ppm upwards in taste testing by two assessors. Given that the maximum amount of sinigrin present in a fraction was less than 40 ppm (fraction 2 from Brussels sprouts) it is unlikely that it would play a big role in the bitterness of these *Brassica* extracts. Gluconapin however could be well responsible for some of the bitterness found in *Brassica* extracts particularly when present at concentrations close or above 100 ppm (e.g. in red cabbage). The synthesis of gluconapin, an aliphatic GS (i.e. methionine-derived), is primarily controlled by genotype (*17*) and could be the target of breeding programs. In a previous study, 78% of assessors rated gluconapin as bitter (*36*).

*Astringency across all fractions*

Astringency is described sensorially as a puckering, rough, drying mouth-feel resulting from the precipitation of salivary proteins by a chemical thus referred to as astringent. Astringency is not classified as one of the five basic tastes; it is considered an aftertaste due to the time interval required for the sensation to be perceived. To date phenolics such as flavan-3-ols (monomers, dimmers and trimers) as well as phenolic acids such as hydroxybenzoic acids are known to elicit astringency (*69*).

The results from the sensory evaluation of the *Brassica* extracts for astringency are shown in Figures 25 and 26 below. The intensity of astringency for most fractions was low and only very few fractions were definitively rated as astringent (e.g. fractions 6-10 from broccoli and fractions 3, 6, 7 from red cabbage). The astringency of the fractions in each vegetable did not correlate with the levels of a specific component or group of phytochemicals. Figure 26 shows the concentration of GS and other components detected at various wavelengths (each wavelength is indicative of certain components; flavan-3-ols absorb at 280 nm, phenolic acids at 280 and 326 nm, glycosides at 370 nm and anthocyanins at 520 nm) together with averaged astringency for fractions from a broccoli whole extract (concentration of these phytochemicals (expressed as µg/mL) was the same as the amount evaluated by the assessors).

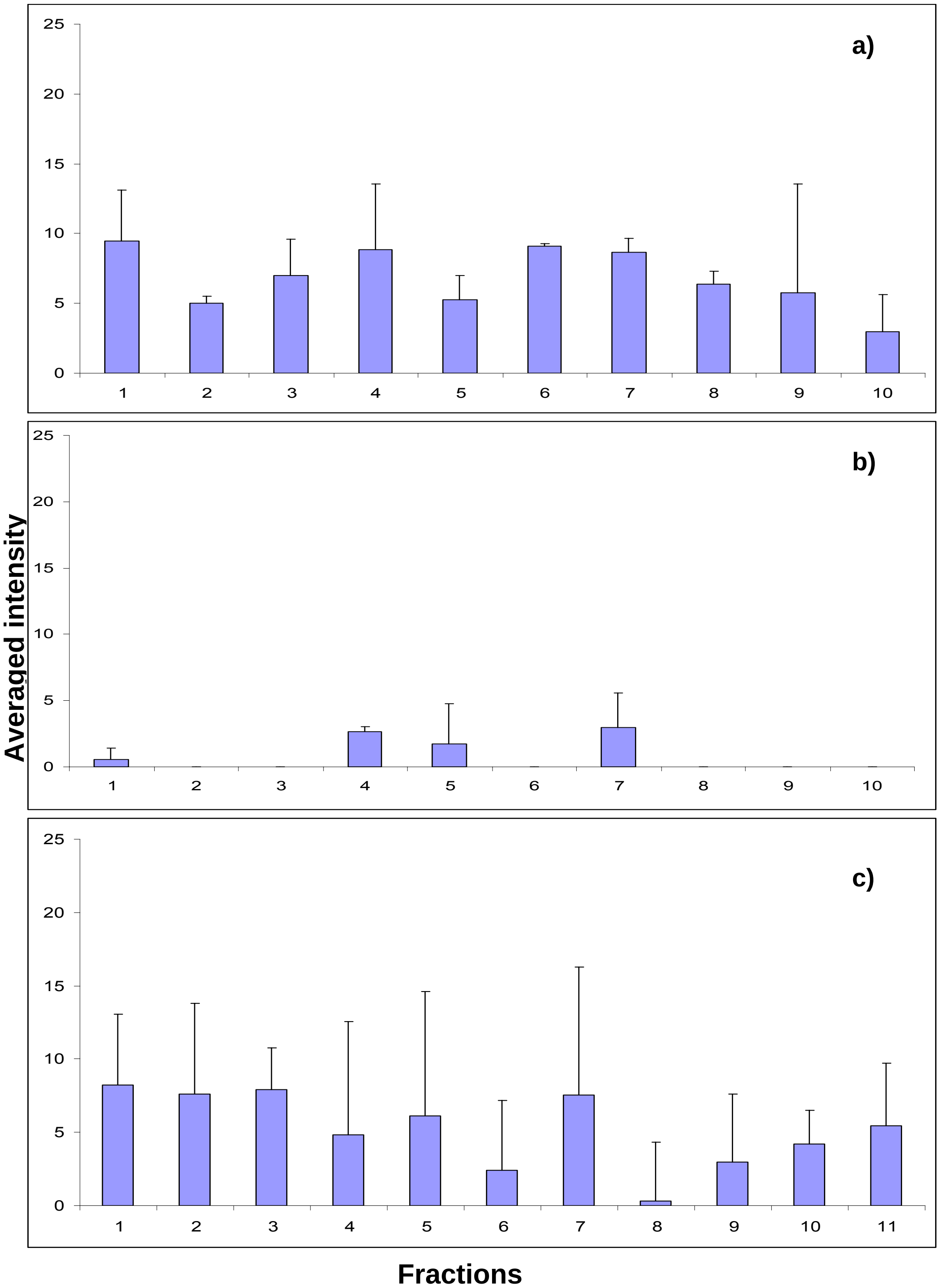


**Figure 25**. Averaged intensity of astringency in fractions from red cabbage (a), cauliflower (b) and Brussels sprouts (c) extracts.

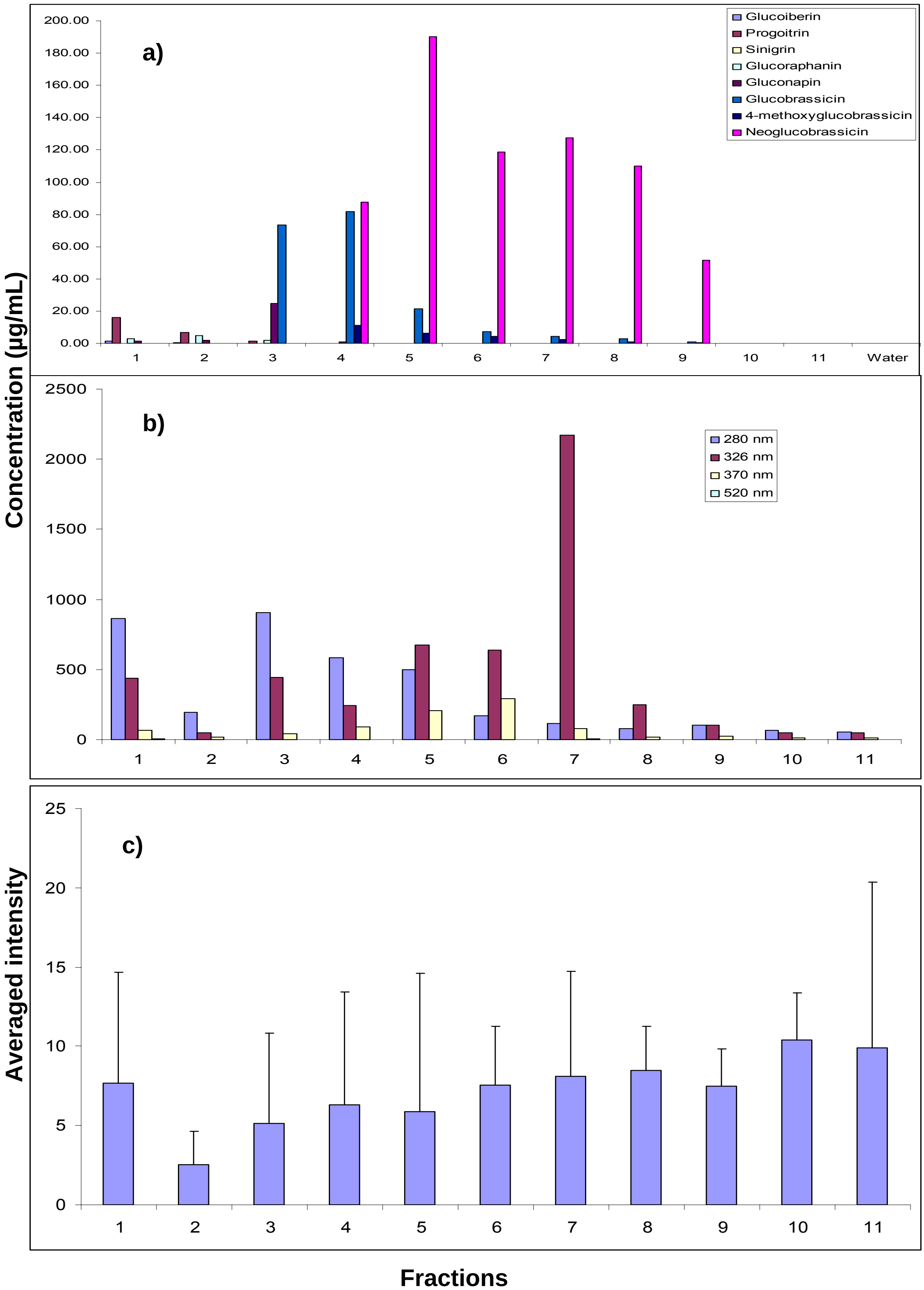


**Figure 26**. Concentration of glucosinolates (a), other photodiode array-detected components (including phenolics) (b), and intensity of astringency in fractions (c) from a broccoli extract.

As can be seen from Figure 26, the available compositional data does not explain the perceived astringency. This suggests that the low astringency found in the *Brassica* whole extracts could originate from a number of components additional to those identified/quantified in this study. *Brassica* vegetables are known to contain a relatively small amount of tannins (0.5 mg/g DW)(*70*) and these could be responsible for the observed (low) astringency. The treatment of tannins before chromatographic separation and quantification requires the use of non food-grade solvents and thus could not be performed in this study.

### 4.2.3 Conclusions from the chemical and sensory assessment of *Brassica* extracts

GS, phenolics and free sugars were the main phytochemicals quantitatively determined in the whole extracts from *Brassica* vegetables used in this study. Although the variation in phytochemical content found in *Brassica* vegetables can be quite large, the chemical profiles of extracts from vegetables sourced in Adelaide (used in the consumer test) and those sourced in Sydney (used throughout the study) were similar.

Gluconapin (particularly for red cabbage), glucobrassicin and neoglucobrassicin were the major contributors to the GS profiles of the vegetables used in this study. Brussels sprouts exhibited the highest total GS concentration followed by broccoli, red cabbage and cauliflower. Phenolic acids were found to dominate the chemical profiles of all *Brassica* extracts. As a consequence of its intense pigmentation, red cabbage was the only vegetable that exhibited a significant concentration of anthocyanins. Red cabbage also contained the highest concentration of sugars followed by cauliflower; Brussels sprouts and broccoli contained significantly less.

Sensory evaluation of the whole extracts found sweetness (cauliflower and red cabbage sweeter than broccoli and Brussels sprouts) and bitterness (Brussels sprouts more bitter than the rest) were the most important distinguishing attributes followed by saltiness (broccoli saltier than rest) and astringency (Brussels sprouts more astringent than the rest). There was generally a good agreement between the results from the sensory assessment of the whole extracts and the perception scores generated from the consumer acceptance test using steamed *Brassica* vegetables.

Fractionation followed by sensory evaluation of the fractions revealed that fraction 1 was the most taste active fraction across all *Brassica* whole extracts. Sweetness (likely due to free sugars), umami (possibly due to amino acids), astringency and saltiness were the main attributes to the taste profile of this fraction. Sub-fractionation showed that this fraction was also bitter (GS and others) but the sweetness (due to sugars) was able to reduce bitterness.

Several fractions within each extract were found to contribute to the bitterness and astringency of whole *Brassica* extracts. Gluconapin in red cabbage could play a role in the bitterness associated with that extract. However, GS alone could not explain the sensory results for either of these attributes. Phenolics and/or other components are likely to be involved to some extent in eliciting these taste sensations.

The finding that components additional to GS are responsible for the undesirable taste associated with *Brassica* vegetables is useful. GS, and in some cases their ITC, have been historically implicated in causing the bitterness and astringency of several *B. oleracea* crops particularly Brussels sprouts (*8, 18, 36*). The results obtained in this study however, have confirmed that components other than GS are contributing to the bitterness and astringency of *Brassica* vegetables. This is in agreement with results from a couple of recent studies carried out on broccoli (*39*) and a non *B. oleracea* plant (*40*).

## 4.3 Potential physiological activities of extracts from *Brassica* vegetables

### 4.3.1 Background

This section describes the *in-vitro* antioxidant and antiproliferative activity of whole and fractionated *Brassica* extracts. This information was required in order to gain a better understanding of the value of phytochemicals in *Brassica* (and any other) vegetables and therefore assist in determining whether there was a relationship between taste and health properties of known components.

### 4.3.2 Antiproliferative activity of various *Brassica* vegetables

The antiproliferative activity of whole extracts from the four *Brassica* vegetables against the colon adenocarcinoma (HT-29) cells, as determined by the WST-1 assay, is presented in Table 23 below. The extracts from Brussels sprouts, broccoli, red cabbage and cauliflower were identical, in terms of composition and concentration, to those used for the sensory and chemical characterisation (reported in previous sections).

The activity of the extracts was evaluated with and without the application of myrosinase. It is accepted that ITC, formed from the myrosinase-catalysed hydrolysis of GS, are likely the major contributors towards the anticancer properties exhibited by several *Brassica* vegetables (*32*); GS themselves do not possess antiproliferative properties (*33*).

The results obtained confirm the higher antiproliferative activity of the myrosinase-treated extracts compared to the myrosinase-free extracts. This highlights the importance of ITC as the main antiproliferative principles found in *Brassica* extracts. Experiments carried out using three commercially available GS (glucoraphanin, sinigrin, glucoiberin) confirmed their relative inactivity against the HT-29 cells (Table 24). These components are part of the GS-profiles of commercially-important *Brassica* crops such as broccoli, cabbage and cauliflower including the vegetables used in this study (Table 15).

**Table 23**. Effect of whole extracts of *Brassica* vegetables on the proliferation of colon adenocarcinoma (HT-29) cells. (Higher % inhibition indicates greater antiproliferative activity).

| **Vegetable** | **Inhibition by extract (%, mean ± std)** | |
|---|---|---|
| | **With myrosinase** | **Without myrosinase** |
| Brussels sprouts | 73 ± 3 | 52 ± 4 |
| Broccoli | 81 ± 2 | No activity |
| Red Cabbage | 78 ± 4 | No activity |
| Cauliflower | 76 ± 4 | No activity |

**Table 24.** Effect of selected GS on the proliferation of colon adenocarcinoma (HT-29) cells. The compounds were applied at a 1.0 mg/mL concentration and were not treated with myrosinase.

| **Glucosinolates** | **Inhibition (%)** |
|---|---|
| Glucoiberin | 48 ± 8 |
| Glucoraphanin | 22 ± 5 |
| Sinigrin | 6 ± 2 |

Myrosinase-treated whole extracts from broccoli, red cabbgage and Brussels sprouts exhibited the same level of inhibition against the HT-29 cells. These extracts were found to have similar GS profiles (Table 15); the whole extract from cauliflower however contained a much smaller concentration of GS without a decrease in antiproliferative activity. This suggests that even a small amount of GS can produce enough ITC to exert antiproliferative activity provided that myrosinase is readily available (i.e not the limiting factor). The antiproliferative effect was found to be dose dependant as expected (Figure 27).

Not much myrosinase remains following cooking or any other thermal treatment of *Brassica* vegetables (*64*) and none was present in the whole extracts generated in this study. However, scientists have confirmed the presence of bacterial myrosinase activity in the human colon that hydrolyses GS to ITC albeit at a smaller rate than plant-derived myrosinase (*71*).

The observed activity from the myrosinase-free Brussels sprouts extract (Table 23) is likely to be primarily due to components other than GS and their products, ITC. Phenolic components, particularly phenolic acids, that were seen as the major contributors of the phenolic make up of most *Brassica* whole extracts in this study (Table 16), could play a role and this has been demonstrated in several studies involving non-*Brassica* extracts (*72, 73*).

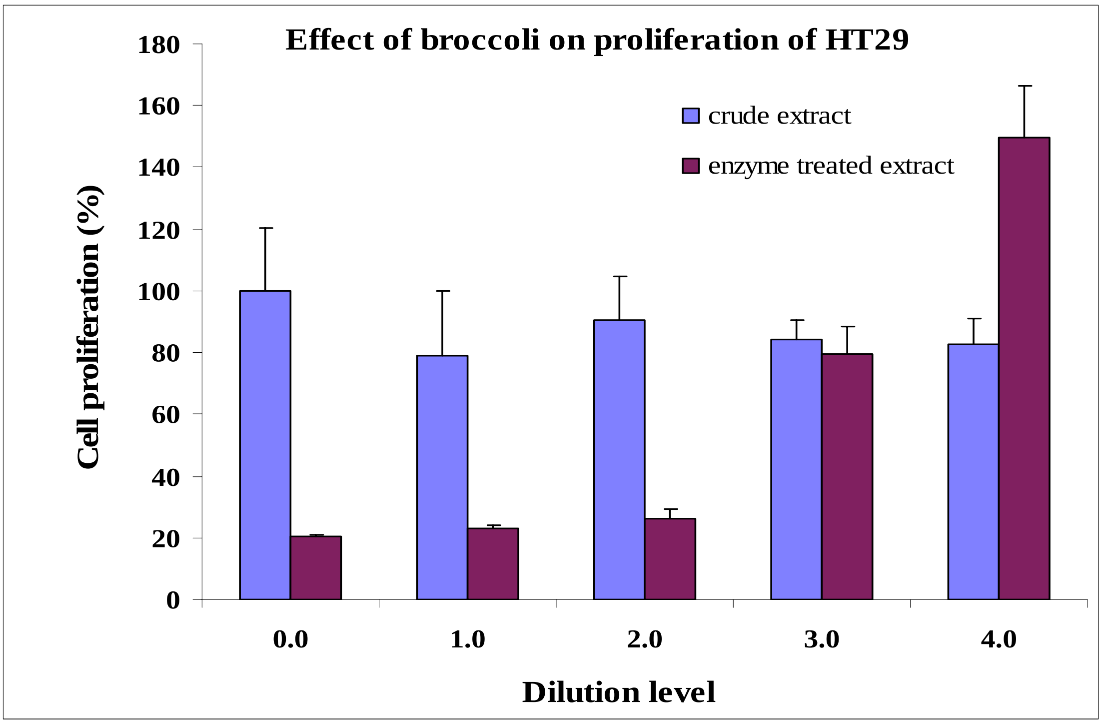


**Figure 27.** Effect of myrosinase treated and non-treated whole extract from broccoli on proliferation of HT-29 colon adenocarcinoma cells (at various dilutions). Similar trends were observed for all extracts.

Fractionation of the myrosinase-treated and non-treated extracts was then performed and the fractions were subjected to the same antiproliferative assay. The fractions were qualitatively and quantitatively identical to those subjected to sensory assessment. Several myrosinase-treated fractions were found to be active against the HT-29 cancer cells (Figure 28) (Table 25). Fraction 1 was the most active fraction across all four vegetables followed by fraction 3 (Table 25). None of the non-treated fractions inhibited cell proliferation.

Fraction 3 from red cabbage did not exhibit significant antiproliferative activity against colon adenocarcinoma cells (37% inhibition, Table 25). Gluconapin, the primary GS in this fraction, is likely to be responsible for some of the bitterness associated with red cabbage (Figure 24). The lack of significant antiproliferative activity suggests gluconapin could be targeted in future breeding programs depending on its contribution to the antioxidant activity of red cabbage. The antioxidant activity of *Brassica* extracts is investigated in section 4.3.3 below.

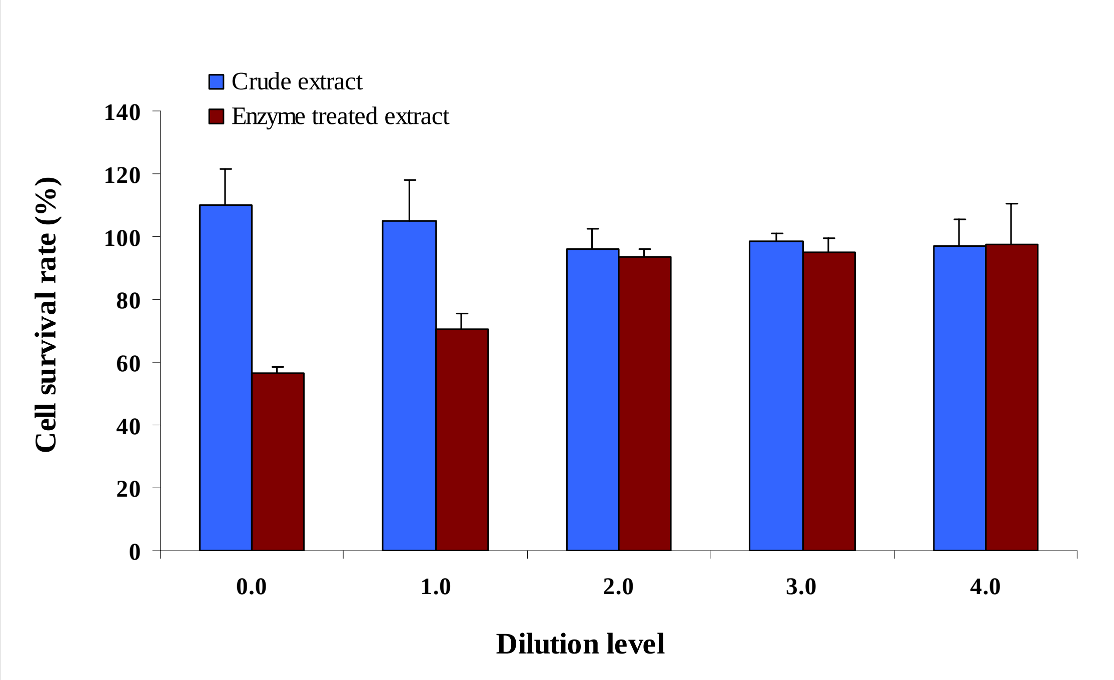


**Figure 28**. Effect of myrosinase treated and non-treated fraction 1 from Brussels sprouts on inhibition of proliferation of HT-29 colon adenocarcinoma cells.

**Table 25.** Antiproliferative activity of myrosinase-treated fractions from *Brassica* whole extracts against HT-29 cells (smaller value indicates higher activity).

| Vegetable | Fraction | Antiproliferative activity (% cell proliferation, mean ± std) |
|---|---|---|
| Brussels sprouts | 1 | 56.3±2.3 |
| | 2 | 84.0±4.7 |
| | 3 | 74.5±2.6 |
| | 4 | 72.2±7.7 |
| broccoli | 1 | 40.3±6.5 |
| | 2 | 73.1±13.8 |
| red cabbage | 1 | 55.7±3.1 |
| | 2 | 66.5±0.5 |
| | 3 | 63.2±1.5 |
| cauliflower | 1 | 56.1±8.1 |
| | 2 | 84.1±6.0 |
| | 3 | 74.9±4.7 |

As with the whole extracts, the results from the fractions (shown above) suggested that GS products (i.e ITC) and not phenolics were likely to be primarily responsible for the observed activity of fraction 1 as myrosinase acts only on GS. However, it was difficult to attribute the activity to a particular ITC as myrosinase was applied to the extracts before fractionation.

Again, the individual and total GS content of fraction 1 from each extract (Table 26) did not correlate with the observed antiproliferative activity (particularly in the case of cauliflower). This possibly highlights the importance of enzyme *versus* substrate availability during the GS to ITC conversion.

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**Table 26**. Individual and total GS content of fraction 1 across the four *Brassica* whole extracts.

| **Glucosinolates** | **Brussels sprouts** | **broccoli** | **red cabbage** | **cauliflower** |
|---|---|---|---|---|
| | Concentration (µg/mL) | | | |
| Glucoiberin | 2.23 | 1.67 | 2.54 | 0.00 |
| Progoitrin | 10.74 | 16.00 | 40.43 | 0.01 |
| Sinigrin | 34.94 | 0.22 | 24.34 | 0.02 |
| Glucoraphanin | 1.84 | 2.93 | 32.70 | 0.00 |
| Gluconapin | 0.00 | 1.66 | 47.27 | 0.00 |
| **Total** | **49.74** | **22.47** | **147.28** | **0.03** |

Finally, it must be noted that inactivity of fractions against HT-29 colon adenocarcinoma cells cannot be used to predict their antiproliferative efficacy against other cell types. The reverse of course also applies for fraction 1.

### 4.3.3 Antioxidant activity of extracts from *Brassica* vegetables

The antioxidant activity of the whole extracts from four *Brassica* vegetables is given in Table 27 below.

**Table 27**. Antioxidant activity, as determined by the FRAP assay, of whole extracts from four *Brassica* vegetables.

| **Vegetable** | **Activity (µmol $Fe^{2+}$/g DW)** |
|---|---|
| Red cabbage | 139.93 ± 1.01 |
| Cauliflower | 77.66 ± 0.41 |
| Broccoli | 61.14 ± 0.81 |
| Brussels sprouts | 60.81 ± 0.94 |

The whole extract from red cabbage exhibited by far the highest antioxidant activity among the extracts assayed (Table 27). It is likely that the substantial amount of cyaniding-based anthocyanins present in red cabbage extract (~ 5 mg/g DW, Table 16) contributed to its higher antioxidant activity compared to that of the other three *Brassica* whole extracts assayed. This is confirmed by the high antioxidant activity of fraction 6 for red cabbage, which was the highest across all fractions across all four vegetables (Table 28-31).

A comparison of the antioxidant (Table 27) and the antiproliferative (Table 23) values indicates that there could be quite a difference in ranking between these two activities. This is not surprising as it simply highlights the different composition of these extracts. A phytochemical can be very good as an antioxidant but not as an antiproliferative agent, the reverse also holds.

Most of the antioxidant activity of each *Brassica* whole extract was found within a few fractions. Fraction 1 was contained the components with the highest antioxidant activity in all vegetables apart from red cabbage where it was second to the anthocyanin-containing fraction. The contribution of fraction 1 to the total antioxidant activity of each vegetable whole extract ranged from 20% in red cabbage to 78% in cauliflower (Tables 28-31).

Fraction 3 from red cabbage showed only moderate antioxidant activity. Gluconapin is a GS component of this fraction. GS are known to exert their *in-vivo* antioxidant activities indirectly via the action of their products ITC; these however are also known to be pro-oxidants at high concentrations (*21*). Therefore, this genetically controlled GS could be targeted for reduction in future breeding programs, as it was implicated to contribute to the bitterness of red cabbage, without major loss of health properties.

No correlation between the antioxidant values and the taste ratings (for any of the attributes) could be established across all four *Brassica* fractionated extracts. No definite conclusions should be drawn from this as each fraction contained multiple components that could have had a synergistic or antagonistic role towards a specific activity. In addition, taste and antioxidant activity thresholds differ for each phytochemical.

**Table 28**. Antioxidant activity of fractions from the whole extract of broccoli.

| Fraction | Activity (μmol $Fe^{+2}$/fraction ± std) |
|---|---|
| 1 | 20.14 ± 0.50 |
| 2 | 2.81 ± 0.05 |
| 3 | 4.82 ± 0.14 |
| 4 | 2.27 ± 0.02 |
| 5 | 3.36 ± 0.02 |
| 6 | 2.86 ± 0.08 |
| 7 | 7.26 ± 0.09 |
| 8 | 1.19 ± 0.01 |
| 9 | 0.87 ± 0.02 |
| 10 | 0.43 ± 0.01 |
| 11 | 0.40 ± 0.01 |

**Table 29.** Antioxidant activity of fractions from the whole extract of Brussels sprouts.

| Fractions | Activity (μmol Fe+2/fraction ± std) |
|---|---|
| 1 | 18.28 ± 0.35 |
| 2 | 3.27 ± 0.20 |
| 3 | 7.64 ± 0.10 |
| 4 | 3.13 ± 0.08 |
| 5 | 5.04 ± 0.12 |
| 6 | 5.44 ± 0.34 |
| 7 | 9.39 ± 0.03 |
| 8 | 1.51 ± 0.06 |
| 9 | 0.56 ± 0.03 |
| 10 | 0.41 ± 0.00 |

**Table 30.** Antioxidant activity of fractions from the whole extract of cauliflower.

| Fractions | Activity (μmol Fe+2/fraction ± std) |
|---|---|
| 1 | 48.32 ± 1.76 |
| 2 | 3.08 ± 0.32 |
| 3 | 3.44 ± 0.24 |
| 4 | 2.85 ± 0.18 |
| 5 | 2.85 ± 0.03 |
| 6 | 0.45 ± 0.01 |
| 7 | 0.28 ± 0.01 |
| 8 | 0.13 ± 0.01 |
| 9 | 0.21 ± 0.03 |
| 10 | 0.19 ± 0.01 |

**Table 31**. Antioxidant activity of fractions from the whole extract of red cabbage.

| Fractions | Activity (μmol Fe+2/fraction ± std) |
|---|---|
| 1 | 22.97 ± 0.32 |
| 2 | 2.60 ± 0.07 |
| 3 | 13.18 ± 0.54 |
| 4 | 14.88 ± 0.15 |
| 5 | 8.98 ± 0.39 |
| 6 | 45.68 ± 2.84 |
| 7 | 2.64 ± 0.05 |
| 8 | 2.04 ± 0.05 |
| 9 | 1.09 ± 0.02 |
| 10 | 0.23 ± 0.01 |

### 4.3.4 Conclusion

The extracts from the *Brassica* vegetables exhibited both antiproliferative and antioxidant activity. The myrosinase-treated whole extracts from all four *Brassica* vegetables inhibited growth of colon adenocarcinoma cells. Only (a few) myrosinase-treated fractions possessed antiproliferative activity with fraction 1 being the most active across all vegetables.

ITC were likely to be responsible for the observed activity against the HT-29 cells although the GS composition of the whole extracts/fractions did not correlate with the observed activity suggesting that myrosinase rather than substrate availability was more important during the GS to ITC conversion.

The whole extract from red cabbage exhibited the highest antioxidant activity amongst the four *Brassica* extracts assayed likely due to the presence of cyanidin-based anthocyanins in that extract.

A comparison between the antioxidant and the antiproliferative activities of *Brassica* vegetables showed that these do not correlate due to the different chemical composition of the extracts. Red cabbage exhibited the best antioxidant activity, primarily due to its pigments, whilst all four vegetable extracts were equally good at inhibiting growth of colon adenocarcinoma cells.

Fraction 3 from red cabbage exhibited moderate antioxidant activity and antiproliferative activity. This suggests that gluconapin, a likely contributor of the bitterness associated with red cabbage, could be targeted for reduction in future breeding programs as it is primarily genetically controlled.

Only a few fractions from each whole extract showed notable antioxidant activity. The contribution of Fraction 1 to the antioxidant activity of each extract varied from 20% (red cabbage) to 78% (cauliflower). No correlation between the antioxidant values and the taste ratings (for any of the attributes) could be established across all four *Brassica* fractionated extracts.

## 4.4 Overall comparison of the *Brassica* vegetables used in this study

A comparison of the four *Brassica* vegetables investigated in this study is shown below (Table 32). It must be noted that these findings represent the chemical composition of *these* vegetables; the ranking in some of these factors can quite easily change if a different set of vegetables is utilised due to the high natural variation associated with *Brassica* vegetables (*10, 14*).

**Table 32.** Comparison of the health, chemical, sensory and consumer properties of the four *Brassica* vegetables and their extracts.

| | Consumer | Sensory | Phytochemistry | Health |
|---|---|---|---|---|
| **Broccoli** | =Most liked<br>High intention to consume | Highest in saltiness | High total glucosinolate concentration<br>Lowest sugar content | High in antiproliferative activity<br>Low antioxidant |
| **Cauliflower** | =Most liked<br>Highest intention to consume | High in sweetness<br>Lowest in bitterness | Lowest total glucosinolate content<br>Lowest total phenolic content<br>High sugar content | High in antiproliferative activity<br>Low antioxidant (when compared to red cabbage) |
| **Red cabbage** | Low intention to consume | Highest in sweetness | High total glucosinolate concentration<br>Highest total phenolic content<br>Highest sugar content | High in antiproliferative activity<br>Highest in antioxidant activity |
| **Brussels sprouts** | Least liked<br>Lowest intention to consume<br>Health information may increase intention to consume | Highest in bitterness<br>Highest in astringency | Highest total glucosinolate content<br>Low sugar content | High in antiproliferative activity<br>Low antioxidant (when compared to red cabbage) |

# 5. CONCLUSION

## 5.1 Trade-off between health and taste

This is the first time, to our knowledge, that a systematic, interdisciplinary attempt was made to evaluate the contribution of key phytochemicals to the taste and health properties associated with *Brassica* vegetables.

The consumer test results indicated that health information has only a small effect in influencing consumer liking and intention to consume the least desirable Brussels sprouts. Where there was an effect, it was found amongst consumers with high vegetable intake.

Overall, sensory perception was found to be the main predictor for liking and intention to consume *Brassica* vegetables. This suggested that improving the taste associated with *Brassica* vegetables is more important than just ensuring consumers are aware of their health-benefit.

Both the participants in the consumer test and the trained panellists used during the sensory evaluation of the extracts agreed that bitterness and sweetness were the two main taste attributes of the *Brassica* vegetables/extracts. Not surprisingly, the ranking in sweetness correlated well with the content of free sugars found in the extracts. More importantly, however, sweetness was able to suppress perceived bitterness.

This can be very useful considering that to a large extent, the phytochemicals responsible for the undesirable taste of *Brassica* vegetables were also found to be major contributors to their health properties (GS via ITC as main anticancer principles, phenolics as main antioxidants). Therefore, an improvement in taste properties, by reducing GS and/or phenolic content, would lead to a loss of health properties. However, an increase in sweetness, accomplished for example by increasing sugar content, would reduce perceived bitterness when consuming *Brassica* vegetables. Evaluation of new/improved varieties could then readily detect an increase in consumer acceptance.

The aliphatic GS gluconapin was the only component that was implicated in the bitterness associated with red cabbage but not found to contribute much to the health properties of that vegetable (based only on the results from the bioassays obtained in this study). Given that aliphatic GS are primarily genetically controlled reduction of gluconapin through breeding may be possible.

## 5.2 CSIRO multi-disciplinary toolbox

The area of "sensometabolomics" has made significant advances in recent years driven by the need to identify the principles imparting, desirable and undesirable, tastes in foods. In the past the focus was always placed on the quantitatively dominant components in each fresh or processed food (*74*). This often resulted in incorrect conclusions being drawn about the cause of the problem and any potential solutions to overcome it.

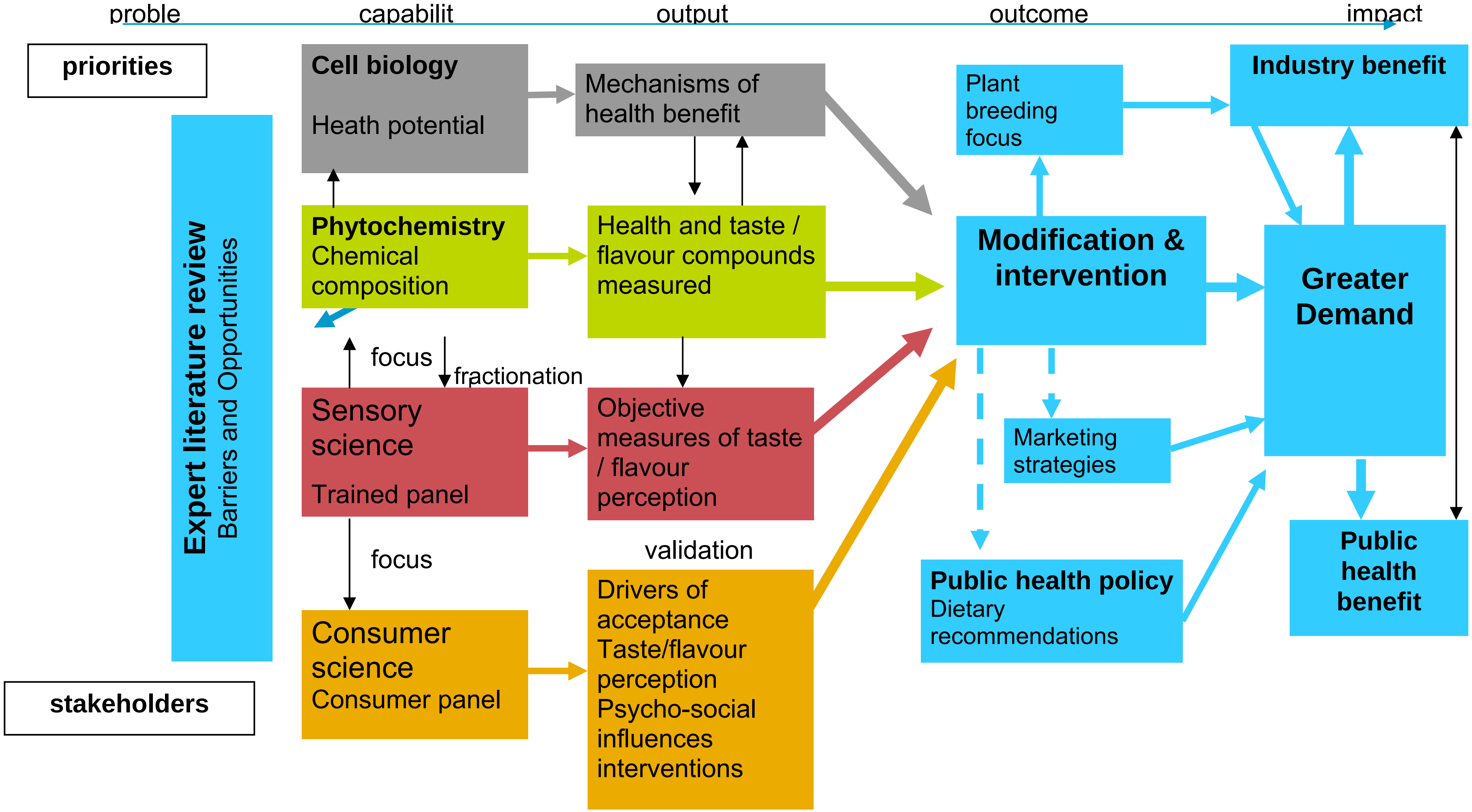


**Figure 29**. Diagram of the CSIRO multidisciplinary toolbox.

In recent years, the integration of key disciplines such as chemistry, cell biology, and sensory and consumer science enabled the discovery of many taste active principles in fresh and processed foods that often also exhibited health-benefiting properties (bitter biacetylenes and oxylipins in carrots (*75, 76*); kokumi (i.e. eliciting mouthfulness) peptides in cheese (*77*); bitter oxylipins in thermally-processed avocado (*78*)).

At CSIRO Food and Nutritional Sciences, we have integrated all these disciplines and created a multi-disciplinary 'toolbox' (Figure 29) that can now be applied to any fresh or processed food product. Information from the application of the toolbox can be used to guide plant breeding programs, guide public health policy and dietary recommendations, used in marketing strategies and others. The ability to integrate 'under one roof', the chemical composition, sensory characteristics, health properties and consumer acceptance of any food product is unique and can yield great benefits to Australian Industry and the public.

## 6. TECHNOLOGY TRANSFER

- This project was previewed in *Vegetables Australia* (vol. 5.2, p. 45, September/October 2009).
- Dissemination of these findings to the Australian Vegetable Industry through industry journals and conferences will be conducted where appropriate.
- Submission of two manuscripts to scientific journals and presentation of results to an international scientific conference is anticipated.
- The authors request assistance from HAL to find a suitable forum / media to communicate the findings to industry stakeholders.

# 7. RECOMMENDATIONS

## 7.1 Scientific

- Age was a predictor of the acceptance of Brussels sprouts however further investigation is needed to determine whether this was an age or age cohort effect.

- Further work is required in identifying the unknown major phenolic components found in most *Brassica* whole extracts (particularly those responsible for the signal at 280 and 326 nm).

- The determination of taste thresholds for all GS and major phenolics identified in this study will allow for a better assessment of their taste activity. Isolation and purification under food grade conditions, however, will be required.

- The focus of this study was primarily on hydrophilic (i.e. water-soluble) components. *Brassica* vegetables also contain many lipophilic (i.e. oil-soluble) components such as carotenoids. A study focusing on the taste activity and health-properties of lipophilic components in *Brassica oleracea* would complement the findings from this study.

## 7.2 Industry

- The development of new/improved *Brassica* oleracea varieties with increased sugar content could be beneficial for the industry as it could lead to increased consumer acceptance.

- Marketing could highlight the sweetness, anticancer and antioxidant characteristics of cabbages, particularly red cabbage.

- Introduction of pigments such as anthocyanins to crops such as broccoli would enhance antioxidant activity whilst maintaining (if not enhancing) anticancer properties.

- Specific health information may assist with the acceptance of Brussels sprouts.

- Future breeding programs could target the reduction of the bitter-contributor gluconapin in *Brassica* oleracea vegetables without a major loss to health properties.

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# APPENDICES

**Appendix 1**

**1. Low (category 1)**
This is the lowest level of physical activity. Those individuals who not meet criteria for categories 2 or 3 ar considered inactive.

**2. Moderate (category 2)**
Any one of the following 3 criteria
• 3 or more days of vigorous activity of at least 20 minutes per day OR
• 5 or more days of moderate-intensity activity or walking of at least 30 minutes per day OR
• 5 or more days of any combination of walking, moderate-intensity or vigorous intensity activities achieving a minimum of at least 600 MET-min/week.

**3. High (category 3)**
Any one of the following 2 criteria
• Vigorous-intensity activity on at least 3 days and accumulating at least 1500 MET-minutes/
week OR
• 7 or more days of any combination of walking, moderate-intensity or vigorous intensity activities achieving a minimum of at least 3000 MET-minutes/week

**Appendix 2**

Hedonics - randomised presentation of 6 *Brassica* vegetables

**ID:___________**

**VEGETABLE RATINGS**

**After tasting each of the vegetables, please indicate your liking or disliking of this vegetable *by circling the option that best represents your opinion*.**

**Green cabbage**

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Dislike extremely | Dislike very much | Dislike moderately | Dislike slightly | Neither like/dislike | Like slightly | Like moderately | Like very much | Like extremely |

Please, press the buzzer on your right side to indicate you have finished this sample.

**Cauliflower**

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Dislike extremely | Dislike very much | Dislike moderately | Dislike slightly | Neither like/dislike | Like slightly | Like moderately | Like very much | Like extremely |

Please, press the buzzer on your right side to indicate you have finished this sample.

**Red cabbage**

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Dislike extremely | Dislike very much | Dislike moderately | Dislike slightly | Neither like/dislike | Like slightly | Like moderately | Like very much | Like extremely |

Please, press the buzzer on your right side to indicate you have finished this sample.

**Broccoli**

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Dislike extremely | Dislike very much | Dislike moderately | Dislike slightly | Neither like/dislike | Like slightly | Like moderately | Like very much | Like extremely |

Please, press the buzzer on your right side to indicate you have finished this sample.

**Brussel sprout**

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Dislike extremely | Dislike very much | Dislike moderately | Dislike slightly | Neither like/dislike | Like slightly | Like moderately | Like very much | Like extremely |

Please, press the buzzer on your right side to indicate you have finished this sample.

**Broccolini**

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Dislike extremely | Dislike very much | Dislike moderately | Dislike slightly | Neither like/dislike | Like slightly | Like moderately | Like very much | Like extremely |

Please, press the buzzer on your right side to indicate you have finished this sample.

**Appendix 3**

Taste intensity (randomised presentation of 6 *Brassica* vegetables)

PLEASE ASSESS THE VEGETABLE TASTE FOR EACH OF THE TERMS LISTED BELOW. PLEASE NOTE WHAT WE MEAN BY EACH OF THE TERMS

- Sweet - as in sugar
- Bitter- as in coffee
- Sour - as in lemons
- salty - as in chips
- umami / savoury / brothy - as in soy sauce or meat
- Flavour impact - overall flavour

ID: _______

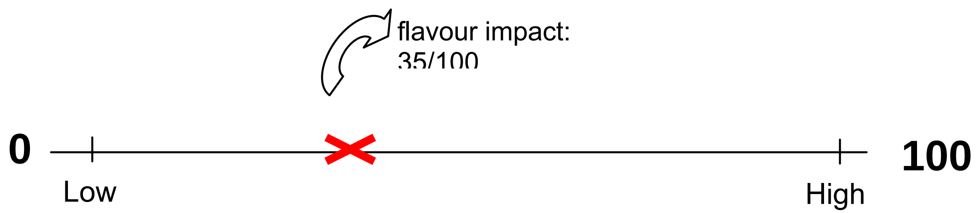
**VEGETABLE ATTRIBUTE INTENSITIES**

PLEASE ASSESS THE VEGETABLE TASTE FOR EACH OF THE TERMS LISTED BELOW.

RATE THE PERCEIVED INTENSITY OF EACH DESCRIPTOR. PLACE A CROSS ON THE LINE SCALE AT THE POINT CORRESPONDING TO THE INTENSITY.

EXAMPLE:

**Flavour Impact**




Vegetable: ____________

FLAVOUR/TASTE:

**Sweet**

Low High

**Salty**

Low High

**Sour**

Low High

**Bitter**

Low High

**Umami / savoury/ brothy**

Low High

**Flavour Impact**

Low High

Please, press the buzzer on your right side to indicate you have finished this sample.

**Appendix 4**

HTAS sub scales (item order was randomised in 10 versions)

ID: _______

**Please indicate your agreement with the following statements by circling the appropriate number on the scale.**

| | **Strongly disagree** | | | | | | **Strongly agree** |
|---|---|---|---|---|---|---|---|
| 1. I am very particular about the healthiness of food | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| 2. I always follow a healthy and balanced diet | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| 3. It is important for me that my diet is low in fat | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| 4. It is important for me that my daily diet contains a lot of vitamins and minerals | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| 5. I eat what I like and do not care about the healthiness of food | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| 6. I do not avoid any foods, even if they may raise my cholesterol | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| 7. The healthiness of my food has little impact upon my food choices | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| 8. The healthiness of snacks makes no difference to me | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| 9. I do not believe that food should always be a source of pleasure | 1 | 2 | 3 | 4 | 5 | 6 | 7 |

| | Strongly disagree | | | | | | Strongly agree |
|---|---|---|---|---|---|---|---|
| | | | | | | | |
| 10. The appearance of a food makes no difference to me | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| 11. It is important for me to eat delicious foods on weekdays as well as weekends | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| 12. When I eat, I concentrate on enjoying the taste of food | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| 13. I finish my meal even when I do not like the taste of food | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| 14. An essential part of the weekend is eating delicious food | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| 15. I reward myself by buying something really tasty. | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| 16. I indulge myself by buying something really delicious. | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| 18. When I am feeling down I want to treat myself with something really delicious. | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| 19. I avoid rewarding myself with food. | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| 20. In my opinion, comforting oneself by eating is self-deception. | 1 | 2 | 3 | 4 | 5 | 6 | 7 |

*Please press the buzzer when you have finished*

**Appendix 5**

ID: _______

Vegetable FFQ (*54*)

Not shown

§ Louise A.Mainvil, Caroline C.Horwath, Joanne E.McKenzie, Rob Lawson (2010) Validation of brief instruments to measure adult fruit and vegetable consumption *Appetite* published online 2010.

*Please press the buzzer when you have finished*

**Appendix 6**

Socio-demographics

1. Please indicate your gender (*tick one box*):

    1) Male ☐

    2) Female ☐

2. Please indicate your present age (*tick one box*):

   1) 18-30 ☐

   2) 31-40 ☐

   3) 41-50 ☐

   4) 51-55 ☐

There are many different ways in which people think of themselves. Which of the following describe the culture(s) you view yourself as being part of? ***(tick every option that applies)***

1. ☐ Aboriginal / Torres Strait Islander
2. ☐ Australian
3. ☐ British/ English/ Scottish/ Welsh
4. ☐ Chinese
5. ☐ Dutch
6. ☐ German
7. ☐ Greek
8. ☐ Indian
9. ☐ Irish
10. ☐ Italian
11. ☐ Vietnamese
12. ☐ Other(s) *Please state* ........................
...........................................................
...........................................................
...........................................................

3. What is your highest level of education achieved? (*tick one box*)

1) Never attended school ☐

2) Primary school ☐

3) Some high school ☐

4) Completed high school ☐

5) Technical or trade certificate (not university) ☐

6) University qualifications or equivalent qualification ☐

ID: _______

4. Do you supplement your diet with vitamins/minerals &/or herbal supplements?

**NO** ☐ **YES** ☐

**If yes**, please list below

| Brand | Type | Dose (eg 5mg) | How often |
|---|---|---|---|
| Eg. Blackmores | Vitamin E | 200 mg | Daily |
| | | | |
| | | | |
| | | | |

5. I intend to consume broccoli in the near future?

| Strongly disagree 1 | 2 | 3 | 4 | 5 | 6 | Strongly agree 7 |
|---|---|---|---|---|---|---|

6. I intend to consume broccolini in the near future?

| Strongly disagree 1 | 2 | 3 | 4 | 5 | 6 | Strongly agree 7 |
|---|---|---|---|---|---|---|

7. I intend to consume red cabbage in the near future?

| Strongly disagree 1 | 2 | 3 | 4 | 5 | 6 | Strongly agree 7 |
|---|---|---|---|---|---|---|

8. I intend to consume green cabbage in the near future?

| Strongly disagree 1 | 2 | 3 | 4 | 5 | 6 | Strongly agree 7 |
|---|---|---|---|---|---|---|

ID: ________

9. I intend to consume Brussels sprouts in the near future?

| Strongly disagree 1 | 2 | 3 | 4 | 5 | 6 | Strongly agree 7 |
|---|---|---|---|---|---|---|

10. I intend to consume cauliflower in the near future?

| Strongly disagree 1 | 2 | 3 | 4 | 5 | 6 | Strongly agree 7 |
|---|---|---|---|---|---|---|

11. Please state what you think are the health benefits of eating vegetables? (*write in*)

______________________________________________________________________________

____________________________________________________

12. Please state what you think are the health benefits of the vegetables you have just tasted. (*write in*)

______________________________________________________________________________

____________________________________________________

*Please press the buzzer when you have finished*

**Appendix 7**

The moisture content of the four (Sydney-sourced) *Brassica* vegetables used in this study is given below.

| Vegetable | Moisture (%) |
|---|---|
| Broccoli | 90.3 |
| Cauliflower | 91.2 |
| Brussels sprouts | 87.3 |
| Red cabbage | 89.5 |

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