Importance of high vegetable consumption in controlling weight

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This report outlines the final results and recommendations of a series of studies conducted across a number of institutions examining various aspects of the role of vegetables in weight loss. The aim of the research was to obtain further evidence of the role of vegetable consumption in addressing overweight and obesity in the Australian population. This would provide the industry and consumers with information on how vegetables may be useful in managing appetite and body weight.

This project has been funded by HAL using the vegetable levy and matched funding from the Australian Government. Additional support was provided by the research institutions, including key staff and students from the University of Wollongong, Curtin University, the University of Queensland and the Department of Agriculture, Fisheries and Forestry, Queensland.

31 March 2013

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MEDIA SUMMARY

This project aimed to provide further scientific evidence on the role of vegetable consumption in weight loss. Food and nutrition scientists worked together to show that vegetables contain bio-active components (phytochemicals) that may influence the development of fat cells, they have a large bulk and low energy density that enables a greater feeling of fullness when eating compared to other food types in the same amounts, and they form an integral part of a healthy diet for weight loss. Analyses of Australian vegetables confirmed their phytochemical composition, and the foods analysed were found to be popular with participants in the weight loss trial (especially lettuce, potato, broccoli and onion). The satiety benefits were best when vegetables were prepared in larger pieces, and these effects supported the role of vegetables as good choices for snacks between meals. A bitter taste did not seem to have an effect on how much a person eats, but the volume of vegetables did. Participants in the year-long dietary trial were not hungry on a high vegetable diet and appeared able to continue the healthy eating plan with relative ease. They were able to keep off around 6 kg of body weight, and reduce waistlines at the same time. Future research can be developed from what was learnt here. Weight loss occurs when total energy (calories) consumed is less than energy expended. It is difficult to attribute weight loss to a single food group when this is a total diet effect, but we can combine knowledge to argue why vegetables are so valuable in this scenario. We can target more of the type of vegetables that are known to have greater levels of certain phytochemicals (e.g. chlorogenic acid) and growers can look to those vegetables and new cultivars. Future research can work on better biomarkers of vegetable intakes (for example from urine samples), that enable us to link weight loss effects with higher vegetable intakes, and we can be more targeted in our dietary advice for this purpose. We also need to know more about the effects of cooking and storage. We need to work out more ways to incorporate vegetables into *every* meal of the day, as well as snacks, to assist people in increasing their levels of vegetable consumption overall, and to be confident in preparing a wide variety of vegetables for all occasions.

TECHNICAL SUMMARY

There were a number of problems concerning vegetables addressed in this research. These related to the scientific knowledge base on chemical composition, form and function, and the scientific methods by which we better understand this and create the evidence for effects. The research also addressed overarching translational issues, where scientists from different disciplines built bridges to incorporate the different forms of knowledge generated from these disciplines. This combined effort created a stronger argument for the role of vegetables in weight loss. Early results from the 2011-2013 Australian Health Survey found that only 8.3% adults consumed the recommended $\geq$5 servings of vegetables/day, with 52.9% females and 58.3% males consuming $\leq$ 2 serves/day (1). An opportunity lies in motivating increased consumption as a strategy for weight management, especially given the proposed mechanisms of low energy density and increased satiating effects (2). The scientific evidence supporting the role of vegetables in weight loss remains somewhat qualified, partly because weight loss results from a total diet not a single food group. There are implications for the R&D value chain. It goes to measuring the phytochemical composition of different Australian vegetables (with implications for how this might be modified and/or utilised in a commercial sense). These developments can be taken up into short feeding studies examining the impact of vegetable form and preparation on appetite and weight loss trials incorporating both sets of knowledge. There are also methodological challenges. These include the need to develop laboratory techniques to study Australian vegetables and the biological effects resulting from vegetable consumption. Randomised Controlled Trials (RCTs) that expose the value of consuming single foods in the context of a whole diet represent another challenge. In this project we combined the efforts of food and nutrition scientists with skills in analytical chemistry, food technology, nutritional physiology, biostatistics and dietetics to conduct a number of studies. An RCT was conducted with reference to foods examined in the laboratory and the information provided to participants took note of the potential appetite effects at meal snack occasions. The outcomes are summarised below.

- The inconsistency in published results from RCTs examining the effects of vegetables on weight loss may be explained by vastly different elements in trial designs.
- There are several classes of polyphenols in vegetables that appear to suppress the growth of fat cells by modifying the adipocyte lifecycle. In addition to these compounds having anti-inflammatory effects, this knowledge provides plausible explanations on the impact of vegetables in weight loss.
- Australian varieties of vegetables commonly consumed by participants in the RCT (broccoli, onion, potato) were found to have levels of phenolic acids similar to those found in overseas databases. The methods developed showed good linearity and accuracy, but caution is required in comparing polyphenols contents reported in the literature.
- Vegetable form influences the early stages of appetite: vegetables prepared in larger chunks provide the maximum benefit. The impact is greatest after about 2 hrs, supporting the value of vegetables at all meals, including snacks.
- Bitterness does not appear to influence appetite, but food volume does, so there is good reason to include vegetables such as leafy greens in meals targeting weight loss.
- Diets that contain a high proportion of vegetables lead to substantial weight loss that is well sustained after one year. A secondary effect to the weight loss is reduction in risk factors for cardiovascular disease risk and type 2 diabetes. These results may relate to permanent changes in food intake patterns.

Future research needs to extend these areas of knowledge and build the interdisciplinary effort as proven so valuable here.

INTRODUCTION

Obesity is a major health problem affecting millions of people throughout the globe (3). In Australia, the rates of obesity in adults continues to increase to the extent that around 60% of adults are overweight or obese (4) and weight management remains a significant target for population health. The process of declining health and disease consequences associated with being overweight are being constantly exposed in the scientific literature. Not only is the increased risk of developing type 2 diabetes and cardiovascular disease apparent, but this may be related to the low grade inflammation that is associated with the fat cells present in the obese state (5) . Managing body weight is dependent on the balance between energy (calories/kilojoules) consumed and energy expended. Further research in this area is also exposing more detail on the control mechanisms of weight management (6) enabling a deeper understanding of the energetics of body weight and the conditions that would support healthy weight. This includes an understanding of other influencers as well, such as appetite control and the ability to maintain healthy food choices as part of an established lifestyle. Research that addresses some or all of these issues lies at the forefront of understanding the importance of individual foods in controlling weight.

Food consumption is pivotal in weight management, and some foods are likely to be better than others in supporting weight control. From a nutritional balance perspective, foods that deliver required nutrients for a reasonable energy cost are a primary consideration. As a class of food, vegetables form a highly nutritious group delivering significant amounts of key nutrients in the diet and with a relatively low energy contribution. Thus it is not surprising that dietary guidelines across the globe refer to the inclusion of vegetables in a healthy diet (2, 7).

The purpose of dietary guidelines is to protect the health of the population and in recent iterations the methodologies for developing guidelines have used more defined methods for examining the scientific evidence base for the advice construction (2). Likewise regulatory authorities refer to the scientific evidence base for the management of health claims on foods (8-10). For example, the 2013 Australian Dietary Guidelines (2) referred to suggestive evidence of an association between the consumption of vegetables with a reduced risk of weight gain (11). The highest level of evidence is provided by randomised controlled trials (RCTs) of food consumption in humans. The literature reviewed for the ADG referred to the period after 2002 but some inconsistency in the research was noted. A further review of why this is so would shed light on the research that needs to be done to strengthen the evidence base from RCTs. Additional research provides supportive explanations for these findings and directions for future investigations. For example, having a greater knowledge of the composition of vegetables and of the potential effects on appetite and on body fat may help to explain the mechanisms by which observed effects may occur.

The ability to address these different research questions means that multiple research capacities need to be brought together. Understanding the mechanisms of action of foods requires studies of food composition and the actions of identified chemical compounds thus identified. Mechanisms of action can also be studied through feeding foods and measuring short term effects on physiological properties, such as appetite and satiety. The type of food and the form in which it is consumed is also likely to affect these outcomes. Together this research provides important information that might explain effects seen from dietary trials, bearing in mind that the highest level of evidence is provided from human consumption studies.

In this project, a multidisciplinary team was formed from four institutions across Australia (University of Wollongong, University of Queensland, Curtin University, Queensland Department of Agriculture Fisheries and Forestry), to undertake a set of studies that aimed to make a substantial contribution to knowledge and the evidence base for consuming a high proportion of vegetables for weight management. This included studies of the phytochemical content of selected vegetables, satiety studies comparing the effects of different types and forms of vegetables, and an RCT examining the effect of a high vegetable consumption on weight loss.

MATERIALS AND METHODS

Six separate studies were conducted during the project period comprising systematic literature reviews, analyses of vegetables for their phytochemical properties, satiety studies with different types and forms of vegetables, and a yearlong dietary trial of high vegetable consumption.

1. Literature reviews

A systematic literature review (SLR) was conducted by the team at Wollongong to review the evidence from weight loss trials of the effect of a high vegetable consumption and to inform the design elements of a long term weight loss trial targeting high vegetable consumption. Using the PICO format as defined by the National Health and Medical Research Council (12), the research question was defined as 'Does a higher intake of vegetables (Intervention) result in greater weight loss (Outcome) in overweight adults (Population) than a lower level of consumption (Comparator)'. Details of the search strategies and analytical processes are outlined in the manuscript which has been accepted for publication in the journal, Critical Reviews in Food Science and Nutrition (Appendix 1).

A second literature review was developed by the teams at Queensland's Department of Agriculture, Fisheries and Forestry (DAFF) and Curtin University examining the phytochemical composition and potential anti-obesity properties of vegetables that would provide possible explanations for effects observed in human trials. Key articles from the scientific literature were sourced to describe proposed mechanisms of action of phytochemicals on adiposity. These mechanisms were described in terms of compounds that are prominent in vegetables, such as phenolic acids and flavonoids. The major vegetable sources of phytochemicals with potential anti-obesogenic activity were then described, and finally the effect of cooking methods on phytochemical levels was outlined. A manuscript entitled "Vegetables containing phytochemicals with potential anti-obesity properties: a review" has been accepted for publication in the journal, Food Research International (Appendix 2) (13).

2. Vegetable analyses

Given the theoretical positions above, there is a need to have up to date Australian data on the phytochemical composition of locally sourced vegetables. Analysis of the phytochemical content of significant vegetables was conducted at the laboratories of Queensland's Department of Agriculture, Fisheries and Forestry (DAFF).

To link with other components within this project, the main vegetable groups reportedly consumed by the study sample in the dietary trial conducted at Wollongong were chosen for analysis. Diet History data from baseline (Oct 2010-Feb 2011; n=113) and after 3mo (Feb – May 2011; n= 109) were analyzed. The AUSNUT 2007 database in FoodWorks (Xyris, version 6.0.2562) was used to determine the average daily amount (g) of vegetables consumed. Using these results and information available from the USDA Database for Flavonoid Content of Selected Vegetables (Release 3, 2011) and Phenol Explorer (14) , four vegetables (potato, sweet potato, broccoli, onion) were selected as containing known appreciable levels of polyphenols of interest. Frozen mixed vegetables were added to the list based on trial participant consumption patterns and were purchased from Coles and Woolworths in Brisbane. The Wollongong team purchased sweet potato, broccoli, onion and potato samples from Wollongong and they were transported to Brisbane for processing and analysis. On receipt in the laboratories they were divided into either raw samples which were

lyophilised prior to analysis or cooked samples that underwent standard cooking treatment such as steaming, and lyophilised prior to analysis.

Extraction protocols and HPLC methods for analysis of phytochemicals with potential anti-obesity effects (quercetin, kaempferol, myricetin, apigenin, luteolin and chlorogenic acids, as caffeic acid) were established. Details of the materials and methods are contained in the report contained in Appendix 9.

3. *Satiety studies*

Knowing that the phytochemical composition of vegetables may in themselves influence appetite, we conducted a number of meal based studies. However, as vegetables are consumed in many forms, we examined these effects under various conditions of form and taste. It has also recently been demonstrated that stimulation of bitter receptors in the stomach of mice results in secretion of ghrelin (a hormone associated with satiety), an increase in gastric residence time, and a decrease in subsequent feed intake (15). This is consistent with a role for prolonged gastric residence in giving the body time to assess the potential danger associated with bitter substances (an evolutionary advantage) as well as potentially enhancing satiety. As some vegetables have a characteristic bitterness, we set out to test whether bitterness could affect perceived satiety by giving subjects a drink before the test meal which varied in bitterness up to the maximum acceptable bitterness.

In addition to this, we noted that different vegetables may also play different roles, particularly in relation to glycaemic (blood glucose) effects that can influence satiety. This is particularly the case with starchy vegetables. For this reason we compared effects of potato and sweet potato variants on satiety, this time including glycaemic response. Potato (*Solanum tuberosum*) is a widely grown and important part of the Australian diet being a good source of carbohydrates and some vitamins and minerals. Dietary guidelines however recommend the consumption of a wide variety of vegetables. In light of this, greater understanding of the nutritional properties and health related effects such as satiety and glycaemia (blood glucose) of other starchy vegetables such as sweet potato (*Ipomoea batatas*) cooked and consumed in the different ways commonly used for potato is required.

i) Effect of vegetable form

At the University of Queensland, a randomised crossover trial was conducted with 16 participants consuming 4 test breakfasts containing carrots of different forms in a dish of pasta and tomato sauce. Training meals comprising mixed vegetables (carrot, potato, peas, beans) were provided at the beginning (for familiarisation) and end (for consistency) of each study, resulting in a total of 6 visits to the laboratory. The test dates were scheduled on similar days of activity when participants were feeling generally well. Participants were instructed to consume the entire meal and monitor their sensation of fullness for the next 4 hours. The order of presentation of the meals was randomised using a Latin square design.

ii) Effect of bitter flavour on satiety

Again at the University of Queensland, 16 panellists attended 6 sessions to assess acceptance levels for broccoli and pasta breakfast meals. Each meal consisted of 200g broccoli with 55g pasta and 100g Dolmio extra garlic pasta sauce and a Latin square design was applied for randomisation. There were 4 test meals with additional familiarisation and consistency test meals at the beginning and end of the test meal visits respectively. These isocaloric latter meals contained 200g mixed vegetables, 50g pasta and 50g sauce and comprised a similar volume. Bitterness was introduced through a lemon flavoured drink concurrently consumed with variable quantities of quinine hydrochloride (0, 80, 0, 80 ul/l) and gentian

(0,0,240,240ul/l respectively). Commercial tonic water contains 80ul/l QHCL, and the threshold level was determined as 7ul/l from a panel of n=20. The same panel produced a threshold for gentian of 240ul/l, after which the taste was considered unpleasant. Panellists made their own lunch from wholemeal bread, butter, chicken or grilled vegetables and feta (vegetarian) sandwich fillers, steamed vegetables and low fat vanilla yoghurt. The choice was based on the USDA "Choose my plate' sample menu plan with excess amounts supplied. The amount of food consumed was measured by weighing remaining foods and calculating the kilojoule values. Further details of the study design have been outlined in a full paper prepared for submission for publication (Appendix 10) (16).

iii) Comparative effects of sweet potato vs. potato on satiety

The aim of this study performed by Curtin University was to compare the post-prandial (post-meal) effects in human participants of consuming equal energy and equal available carbohydrate portions of the most widely grown sweet potato (var. Beuaregard) and potato (var. Nadine) either microwave-cooked and eaten hot or microwaved-cooked, cooled overnight and eaten cool.

A preliminary in vitro glucose release study was conducted to inform the post prandial human study. Using glucometry, a rapid in vitro starch digestibility assay was conducted examining glucose release from free glucose, maltodextrins and starch to compare effects from freshly steamed warm sweet potato, and cooled orange skinned sweet potato, purple skinned sweet potato and conventional white potato. Values for free glucose (released at time 0 of pancreatin/amyloglucosidase digestion), rapidly released glucose (released at 0-20min), slowly released glucose (released between 20-120min) and unreleased glucose (still as starch at 120min) were expressed as g/100g dry glucose equivalents (from glucose, maltodextrins and starch).

Another preliminary study compared three methods of measuring the oxidative stress biomarker F2-isoprostane that may be elevated in the obese using: (a) isotope dilution gas chromatography mass spectrometry (GC-MS); (b) enzyme linked immuosorbent (ELISA) assay and (c) liquid chromatography electro spray tandem mass spectrometry (LC-ESI-MS/MS) (performed by Dr Shaofang Wang of ChemCentre, Bentley, WA). Plasma samples from the Wollongong RCT group were provided from 10 participants from both study arms at 2 time points for this method comparison.

The sweet potato (~80 kg) and potato (~80 kg) were obtained from a single harvest and single location in WA. They were pre-processed by cubing (no peel), steam blanching, light vacuum packing into plastic pouches and flat-pack blast freezing into ~120 representative single serves of ~ 50 g available carbohydrate (based on FSANZ nutritional composition database). Microwave cooking time of single-serves from frozen was determined by cooking to the same peak force (g) by instrumental hardness using a texture analyser. The accurate weight of cooked "served hot" and cooked, chilled and "serve cold" sweet potato and potato to give 50 g available carbohydrate (as required for the human study) was determined by direct analysis; the proximate and dietary fibre content of the samples was determine by standard AOAC methods and calculation of available carbohydrate by difference. The food safety of the samples prepared as to be eaten was confirmed by microbiological testing. A more in depth analysis of the carbohydrate composition of the sweet potato and potato samples as eaten was developed by measuring their glucose, sucrose, total starch and fructose and digestible starch + maltodextrins resistant starch content (Megazyme International kit methods)and in vitro starch digestibility (17). The level of chlorogenic acid (phytochemical with potential blood

glucose lowering properties) and carotenoids (antioxidants and vitamin A precursors) were measured by Queensland Department of Agriculture, Fisheries and Forestry using HPLC methods.

The test meals were designed using the direct analytical data and comprised primarily the sweet potato/potato served with salad dressing, spring onions and salt. The weight of each test meal was controlled with water. The available energy content was controlled at 1500kJ, available carbohydrate at 50g and the energy density of each meal was equal. The fat and protein content of the meals were balanced using vegetable oil and whey protein powder. The volume of the test meals were balanced using drinking water. The clinical study experimental design was a single blind randomised cross-over post-meal study of four treatments consisting primarily of the sweet potato/potato described above. Approval for the study was gained from Curtin University Human Ethics Committee and the study was registered with the Australian New Zealand Clinical Trials Register. Selection criteria for participants were: healthy; age range 18-65 years; body mass index range 18.5- 29.5 kg/m². Exclusion criteria were: smokers; pregnant women; food allergy; excessive alcohol consumption; history of diabetes and cardiovascular diseases. Recruitment was performed via flyers, posters, radio, email and internet announcements. Screening was done by questionnaire.

Each participant assessed each of the four test meals in a randomised order, one meal each two weeks. On the evening before the test, a standard dinner was eaten and then the participant fasted overnight. The following morning the participant attended Curtin University and fasting finger prick and venous blood samples were collected. The fasting satiety (appetite) perceptions were rated by questionnaire. The test meal was consumed within10 minutes after which the palatability of meal was rated by questionnaire. Following this, participants re-rated their satiety immediately, 25, 40, 55, 85, 115 and 175 mins after start of the meal. Finger prick samples were collected at 15, 30, 45, 60, 90 and 120 mins after the start of meal and venous blood sample at 30, 60, 120 and 180 mins. After 180 mins a buffet was provided and the amount consumed recorded. Then food intake for the rest of the day was recorded by participants. Glucose was measured on finger-prick blood using a HemoCue analyser. Sixteen participants took part in the satiety and finger-prick blood glucose aspect of this study with 12 completing all treatments as reported here. The post-prandial response was calculated from the area under the curve (AUC) for the incremental value versus time from start of meal. An extension of this research funded by Curtin University involves a sub-cohort of 9 participants who provided venepuncture samples which will be used to measure other appetite and health related biomarkers.

4. *Randomised Controlled Trial: effect of high vegetable consumption on weight loss*

A single blind parallel randomised controlled trial was conducted through the University of Wollongong between 2010 and 2012. The study was approved by the University of Wollongong Human Research Ethics Committee and registered with ANZCTN (ACTRN12610000784011). Participants were recruited from the Wollongong area by advertising in the local media and randomized to either the control or intervention diet group. All participants were given a personal diet prescription based on core food groups (vegetables, fruit, grain foods, meat/fish/eggs, milk/cheese/yoghurt) and monthly sessions with the dietitians was provided for the first 3 mo. Energy intake was restricted to 80% of estimated energy requirements and diets were modelled to provide 45 - 50% carbohydrate, 20 - 25% protein and 25 – 30 % fat. In keeping with national Dietary Guidelines, all participants

were encouraged to eat 5 serves of vegetables each day, but the serve size in the control group material was 0.5 cup cooked vegetables, or 1 cup raw vegetables and for the intervention group was double that (1 cup of cooked vegetables and 2 cups of raw vegetables). The intervention group was given further specific advice on ways of using vegetables. Anthropometric measurements, diet history interviews and health questionnaires were completed at baseline, 3, 6 9 and 12 months and email/health messages contact was maintained. Data was entered into OpenClinica Version 3.1.2 software for clinical research, using the double-entry method for completeness. Dietary data was analysed using FoodWorks 2009, version 6.0.2562. Vegetables were categorised in order to analyse reported vegetable intake. Data were analysed using SPSS version 19.0. Primary analyses were conducted using a linear mixed model which uses all available data regardless of whether the subjects complete the study. The analysis was conducted on an intention to treat basis. Secondary analyses were conducted using Spearman's rho bivariate correlations between change in weight and change in percentage of dietary kilojoules consumed from vegetables. Further details are contained in a manuscript prepared for publication (Appendix 6) (18).

RESULTS AND DISCUSSION

1. Literature reviews

A full review entitled 'Effects of vegetable consumption on weight loss: a review of the evidence and implications for design of randomised controlled trials' was accepted for publication in the journal Critical Reviews in Food Science and Nutrition (19) (Appendix 1). Briefly, the review found 16 trials which fitted the research question and criteria for appraisal, but of those 5 reported a greater weight loss from a high vegetable consumption, 9 showed no difference and one showed weight gain. A closer look at the study designs showed that those comparing a high vegetable diet with 'usual intake' and/or included behavioural counselling in the treatment showed better effects. Study design elements relating the test diets appeared to be an important factor in demonstrating the effect of vegetable consumption, as did the manner in which data analysis was conducted.

From the perspectives of mechanisms of action of vegetables, the literature shows that several classes of polyphenols contained in vegetables appear to suppress the growth of adipocytes by modifying the adipocyte lifecycle, and they may also have anti-inflammatory effects, which is now a recognised feature of the excess adiposity. Many vegetables contain significant levels of these compounds but they may be affected by food processing. Full details of this review can be found in the published manuscript (Appendix 2) (13). A further review in relation to inflammatory processes is being prepared for submission for publication (Appendix 3)

2. Vegetable analyses

Analysis of the eating patterns of trial participants in Wollongong exposed 32 vegetable categories. The top 10 : tomato (69.9g), potato (58.2g), cucumber (27.5g), carrot (25.7g), lettuce (19.4g), broccoli(17.0g), mixed vegetables (15.3g), leafy greens(12.2g), onion(12.0g), avocado (12.0g) contributed to 74% of the total (365g/day). After 3mo (n=109 subjects), 7 remained in the top 10 (contributing 72% of average consumption, 505g/day), with legumes (35.0g), capsicum (22.8g), pumpkin (15.4g) replacing leafy greens, onion, avocado. Tomato remained top ranked but potato dropped from 2nd to 5th rank (58.2g vs36.5g), and mixed vegetables shifted from 7th to 4th (15.3 g vs. 39.1g). The final group for analysis included sweet potato, potato (to also relate to the satiety study) broccoli, onion (given the high quercetin content), and mixed frozen vegetables

The degree of recovery of phytochemicals from the selected vegetables and other method validation procedures indicated that changes in extraction procedures were necessary. The changes in extraction protocols and HPLC methods enabled the analysis of quercetin, kaempferol, myricetin, apigenin, luteolin, and 5 phenolic acids. Flavone and flavonol levels were measured in broccoli and raw onion samples. The frozen mixed vegetables produced indecipherable results leading to the decision to abandon that analysis. Detailed results of the phytochemical analyses are contained in Appendix 9.

3. Satiety studies

i) Effect of vegetable form

The effect of the physical form of vegetables on their perceived satiety was tested by incorporating 200g of carrots into a pasta-based breakfast meal. The 4 treatment types were 1) Raw shredded, 2) Steamed shredded, 3) Steamed cubed and 4) Raw pureed carrots. Following the meal, subjects recorded their satiety state using a Labelled Magnitude Scale

hunger score (20) every 15 mins for the first hour after the meal and every 30 mins during the subsequent 3 hours. After the 4 hour test period, subjects consumed as much as they wanted of a pizza meal and the amounts of food consumed was calculated.

During the first 45 minutes (cognitive phase), subjects recorded assessments of at least 'moderately full', but significantly lower scores were reported for the pureed carrot meal ($p<0.05$) (Figure 1). No additional chewing was required while consuming the meal with pureed carrots, in contrast to the other three meals, which may have contributed to this perceived difference.

At the post-ingestive stage (60 – 120 minutes) the effect of the pureed carrot meal was the most profound. The average hunger score of the pureed carrot meal was significantly lower than either raw shredded, steamed cubed or steamed shredded meals ($p<0.05$) (Figure 1). The pureed particles may have required less gastric processing because they were smaller than the average size after chewing, and hence may have shortened gastric residence times.

At the post-absorptive stage (150 – 240 minutes), the panellists became hungry and at this stage there was no significant difference in hunger scores between meals.

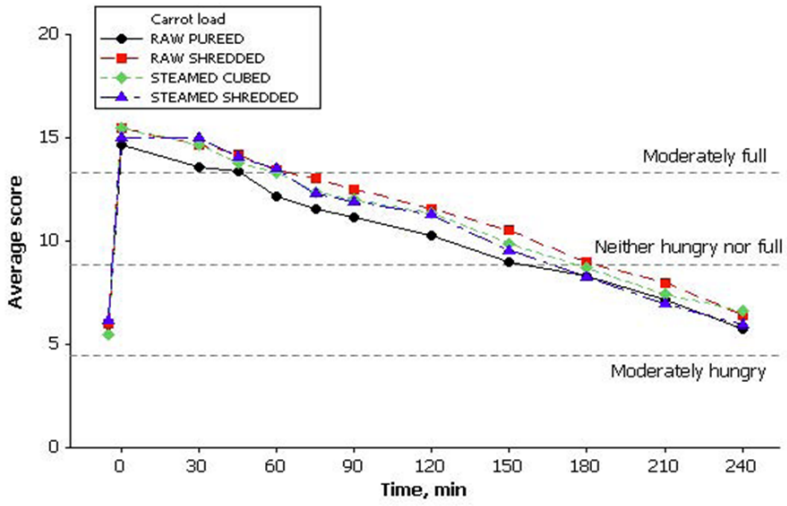


FIGURE 1 Average hunger scores for the 4 treatment type meals.

At the conclusion of the satiety trial after 4 hours, there was no significant difference in hunger scores and no statistical difference in pizza lunch intake for the four breakfast meals was observed. The reason for this could be related to the time between breakfast and lunch being too large, leading to excessive hunger levels (similar to pre-breakfast).

In summary, the effect of carrot preparation on satiety was significant in the cognitive and post-ingestive stages. It is recommended to consume larger pieces of vegetables to achieve maximal benefit. The effectiveness of more intact vegetable preparations is particularly marked around 2 hours after consumption. This suggests that intact vegetables in a meal may decrease the tendency to snack before the subsequent meal.

ii) Effect of bitter flavour on satiety

The results of the bitterness studies showed that there was no significant difference in reported satiety scores after meals that were accompanied by either bitter or non-bitter drinks. There was however less satiety afforded by the trial meals based on mixed vegetables, despite

the fact that total meal energy and volume were kept constant. The mixed vegetables had a higher energy than the broccoli so the amount of sauce used was reduced to compensate. This result provides evidence that low energy-dense vegetables are particularly useful for promoting satiety even in meals of the same total energy and volume. Details of this study can be found in the manuscript prepared for submission in Appendix 10.

A split-plot analysis of repeated measures in the Latin Square design with panellist and visits as block factors was conducted on the hunger/fullness trends, for the first 45 minutes, 60 – 90 minutes and 120 – 180 minutes to follow the cognitive, post-ingestive and post-digestive stages of satiety. The trial meals were analysed separately from the test meals. There was no significant difference between the hunger/fullness profiles for the test meals. However, the hunger level before lunch in the test meals was significantly higher than the hunger level before lunch in the trial meals. Despite this, there was no significant difference in the energy consumed in the lunch between the test meals, between the trial meals, or between the test and trial meals.

iii) Comparative effects of sweet potato vs. potato on satiety

The preliminary in vitro study on glucose release compared two different varieties of sweet potato (var. Beauregard) and potato (var. Nadine) after microwaved-cooking and the analysed hot or cooled overnight and tested cold. Levels of rapidly released glucose were found to be: warm potato > warm orange-skinned sweet potato > warm purple-skinned sweet potato ($P < 0.001$ for all comparisons). Rapidly released glucose levels were lower in cooled than warm potato ($P < 0.001$) but similar in cooled and warm orange-skinned sweet potato ($P > 0.01$).

The second preliminary study compared the levels of isoprostanes (a biomarker of oxidative stress that may be elevated in obese people) in a sub-group of plasma samples from Study 4 (*Randomised controlled trial: effect of high vegetable consumption on weight loss*) using three different analytical methods. In comparison to the "gold standard" method of gas chromatography-mass spectrometry (GC-MS), the ELISA was considered to be rapid and easy to perform without specialist equipment. However the ELISA method gave consistently different values than the GC-MS method bringing into question its accuracy. A liquid chromatography mass spectrometry method (sample preparation and analysis) was established and its linearity of response to standard solutions validated. However at this stage its limit of detection (1000 pg/ml) is too high for the quantification of isoprostanes in the serum (range by GC-MS ~50-300 pg/mL). Further method development work to reduce the limit of detection would be required for this method to be of value. (Appendix 4)

The main satiety study compared four different sweet potato/potato forms for their effect on appetite and blood glucose levels (glycaemia). From the compositional analysis of the four different sweet potato/potato forms it was found that the sweet potato had significantly higher ($P < 0.05$) levels of glucose, fructose and sucrose than the potato regardless of the preparation method (**Table 1**). The starch content were however similar. This difference in the sugar content of the samples may have an influence on the glycaemic response to the meals and hence their satiating power.(Appendix 5)

The sweet potato sample had dramatically higher ($P < 0.05$) levels of chlorogenic acid and related polyphenolic phytochemicals than the potato samples (**Table 2**). Since chlorogenic acid from coffee has been shown to affect glucose absorption (21) it may also affect the glycaemic and hence the satiety response of the sweet potato/potato samples in the present study.

TABLE 1

Available carbohydrate composition of the potato and sweet potato samples used in the formulation of test breakfasts[1]

	Content (g/100g dry basis)			
	Glucose[2]	Fructose[2]	Sucrose[2]	Digestible starch plus matodextrins[2]
Potato (hot)	4.4 ± 0.0[3]	1.5 ± 0.1[3]	1.5 ± 0.7[3]	58.0 ± 6.3[3]
Potato (cold)	5.4 ± 0.9[3]	1.6 ± 0.2[3]	1.2 ± 0.1[3]	57.2 ± 0.9[3]
Sweet potato (hot)	12.7 ± 2.4[4]	8.6 ± 1.1[4]	18.0 ± 2.2[4]	50.4 ± 5.0[3]
Sweet potato (cold)	11.7 ± 1.1[4]	8.6 ± 0.8[4]	18.2 ± 1.2[4]	43.0 ± 0.7[3]

[1] All values are means (n=2) ± SD.
[2] Samples were compared using one-way ANOVA with Tukey post-hoc test, $P < 0.05$ was considered significant. A different superscript number in the same data column indicates a significant difference.

TABLE 2

Content of chlorogenic acid and related polyphenolics in the potato and sweetpotatoes[1]

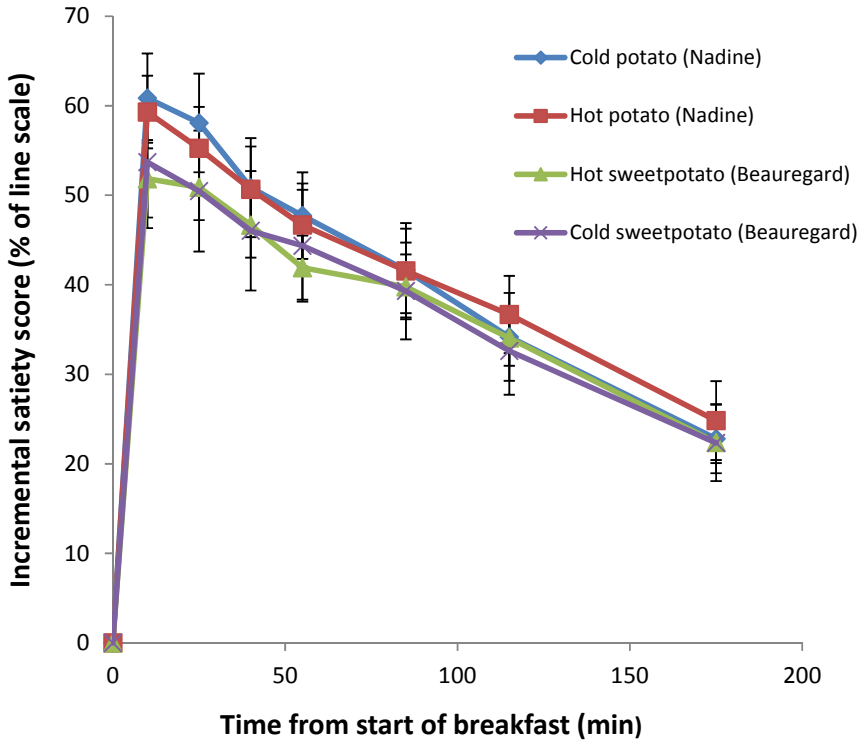
	Content (µg / g dry basis)			
	Chlorogenic acid[2]	3, 4 diCQA[2]	3, 5 diCQA[2]	4, 5 diCQA[2]
Potato (hot)	26.2 ± 0.9[3]	Not detected	Not detected	Not detected
Potato (cold)	39.1 ± 2.1[3]	Not detected	Not detected	Not detected
Sweet potato (hot)	286.9 ± 81.6[4]	144.0 ± 54.5[3]	221.0 ± 113.1[3]	72.1 ± 27.1[3]
Sweet potato (cold)	219.6 ± 5.5[4]	126.2 ± 39.3[3]	175.2 ± 4.8[3]	76.7 ± 26.2[3]

[1] All values are means (n=2) ± SD.
[2] Samples were compared using one-way ANOVA with Tukey post-hoc test, $P < 0.05$ was considered significant. A different superscript number in the same data column indicates a significant difference.

The post-meal satiety response to the four potato/sweet potato test meals is given in **Figure 2**. No significant differences between the treatments (P>0.05) were seen at any of the post-meal time points (**A**) nor the incremental areas under the curve for incremental satiety score (AUC) (**B**).

The glycaemic response to the four potato/sweet potato test meals is given in **Figure 3**. From **Figure 3 (A)** it can be seen that the mean incremental blood glucose value for the cold potato and cold sweet potato were both significantly lower than that of the hot potato and hot sweet potato at 15 minutes after the start of the meal (P <0.05). By 30 minutes after the start of the meal values for the cold potato and cold sweet potato were only significantly lower than that of the hot potato (P < 0.05). No significant differences were observed in the incremental AUC for incremental glucose of the four treatments (p >0.05 for main effect of treatment). These results suggest that cooling of the potato and sweet potato samples may reduce early post-meal glycaemia which may be of value in overall healthy blood glucose control.

A.



B.

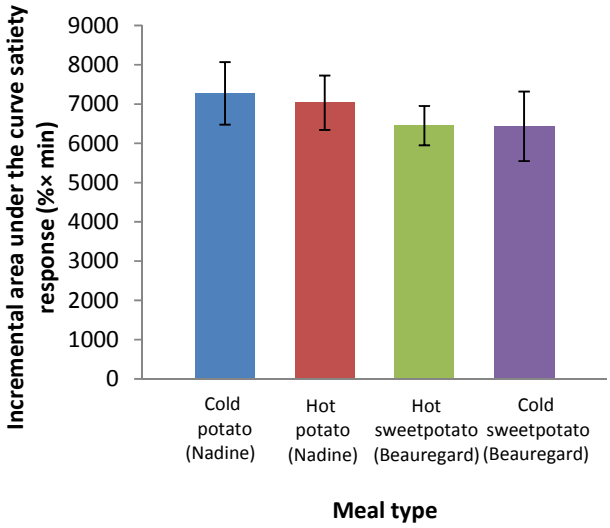
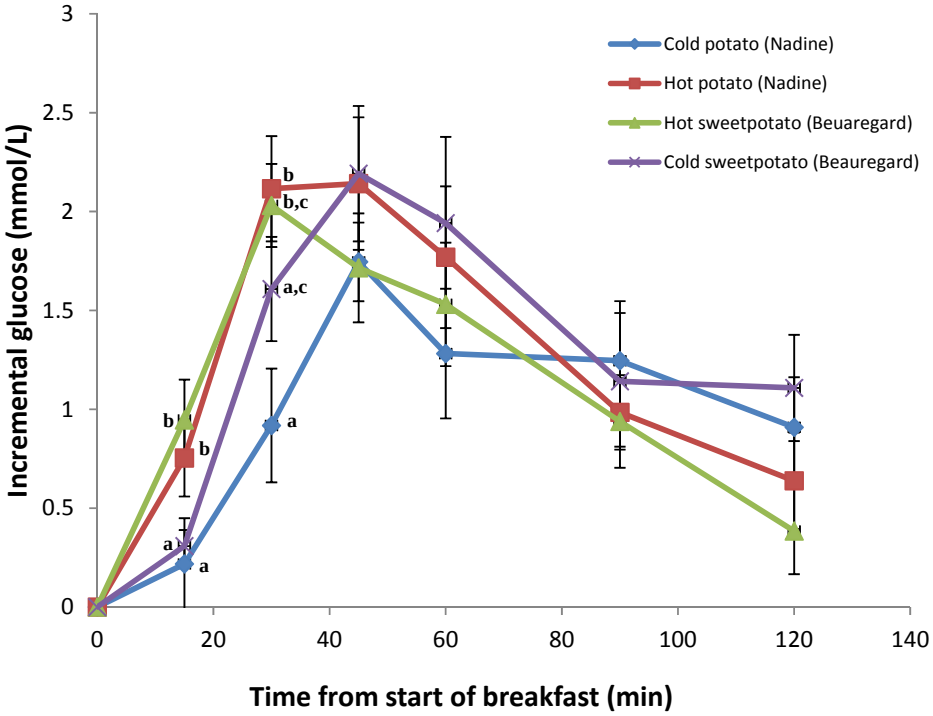


FIGURE 2. Post-prandial satiety after consumption of the potato/sweet potato test breakfasts. A: Profile of incremental satiety score vs. versus time. B: Incremental area under curve. Data are means ± SEMs, *n*=12.

A



B

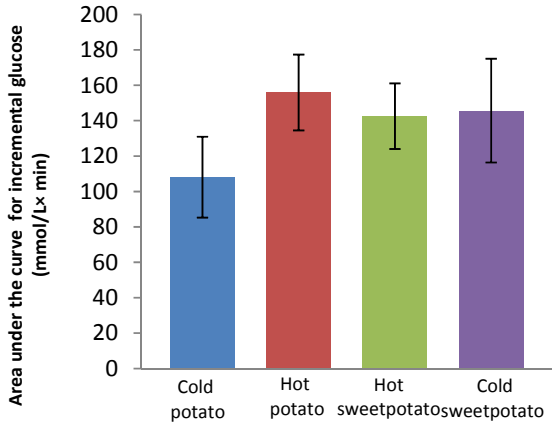


FIGURE 3. Post-prandial glycaemia after consumption of the potato/sweet potato test breakfasts. A: Profile of incremental blood glucose concentration vs. versus time. B: Incremental area under curve for incremental blood glucose concentration above baseline. Data are means ± SEMs, n=16. A different superscript letter at the same time point (A) represents a significant difference (repeated measure ANOVA, $P < 0.05$).

4. Randomised controlled trial: effect of high vegetable consumption on weight loss

Over three quarters of the people enrolled in the study stayed on for the whole 12 months. Both diet advice groups reduced energy intake (P<0.001 time effect) and lost weight (P<0.001 time effect) but there was no difference between groups in amount of weight lost (P=0.776 interaction effect). Mean weight loss for the sample at 12 mo. was -6.5±5.2kg (range -27.8 to + 5kg). The high vegetable advice group consumed more vegetables but the lack of difference in reduced energy intake between dietary advice groups could have masked any changes that could be due to vegetable intake specifically. However, within the total study sample, a higher proportion of energy consumed as vegetables was associated with increased weight loss at the end of the intensive intervention phase of 3 months. Thus people who consumed relatively more calories (kilojoules) from vegetables as opposed to other foods, lost more weight. As weight loss reflects reduced energy intake, this also means that people who ate more vegetables consumed fewer calories. This was not the case at 12mo but this could be due to greater variation in food patterns over time. As would be expected from weight loss, cardiovascular risk factors (fasting blood glucose, insulin, triglycerides) reduced. The level of isoprostanes (a marker of oxidative stress) also decreased over the first 3 months (whole group P<0.001). (Appendix 6)

DISCUSSION

Controlling weight is a major challenge for today's consumer. While body weight reflects a wide range of influencers, dietary intake appears to be the greatest influence (22). This means we need to understand the dietary impact of individual foods much better, especially in an environment where there are multitudes of foods to choose from.

In this research we considered cutting edge knowledge in the composition of vegetables, not just from a content perspective but also from new perspectives on the metabolic consequences of overweight and its association with cardiovascular disease. Body fat itself is now understood to lead to negative inflammatory conditions (23), and we have considered the possible impact of polyphenols in vegetables as being protective in this regard. The review of the literature in this area provided plausible argument for why vegetables would be valuable in the diet, not just to control for energy intake and provide known nutrients such as Vitamin C and fibre, but also because they would deliver important phytochemicals that may prove protective. This is an important new area of research with significant translational implications. Continued research on the polyphenol composition of vegetables will be necessary to keep up in that regard, and this research project has made a substantial contribution both in producing data and in developing the technology for analysis in Australia.

Moving to the level of actual consumption of vegetables, we started with the position that vegetables should be an ideal component in diets for increasing satiety and satiation due to their low energy density and high water and fibre contents. Despite this, there have been relatively few studies of the specific effects of vegetables on perceived satiety, and not in consideration of the important aspects of form and preparation. From those studies that have been reported, vegetables have generally been shown to induce significant increases in satiety and reduction in energy consumption at a subsequent meal, provided at least 200g of vegetables are used (24). A smaller number of studies have suggested that replacing energy-dense foods in a meal with vegetables is a promising tool to decrease energy intake without compromising satiety (25). In general, more intact and less processed forms of vegetables (and fruits) are found to have the greatest response (26). There are not enough studies to conclude whether there are significant differences between the effects of different types of vegetables on satiety and satiation.

The studies carried out as part of the current project have demonstrated:

1) That puree forms of vegetables are unlikely to be as satiating as even relatively small (<1 cm) pieces of vegetables that require some chewing, and
2) That a bitter drink taken with a meal may not induce greater satiety.

The implications from these results are:

1) That consumption of vegetables in more intact rather than puree or juice forms should be emphasized to maximize satiety,
2) That the bitterness associated with some vegetables is unlikely to be a contributory factor to post-meal satiety and that therefore all low energy density vegetables are likely to enhance satiety and
3) No effect of potato/sweet potato from on satiety was observed, however there was some evidence that early post-meal blood glucose levels were lower for cold potato and cold sweet potato than the hot forms and hence may be more beneficial for long-term blood glucose control.

This supports previous findings for potato forms (27) and provides new knowledge on glycaemic response of sweet potato forms. However the study may be underpowered to detect important differences in the mean values of the AUC for both satiety and glycaemia, therefore we propose to continue the study by recruiting more participants prior to publication of the data in journal articles.

This level of detail is informative for moving to longer term studies under free living conditions. Our yearlong clinical trial in which people were advised on eating plans based on the Australian Guide to Healthy Eating (28) (but with particular emphasis on vegetable consumption), showed that substantially increasing the proportion of vegetables in a calorie restricted diet can result in more weight loss in the short term. In interpreting the results it is important to distinguish between the treatment groups (where the treatment was dietary advice) and the whole study sample or cohort (where 'behaviour = vegetable intake' was measured). Under free living conditions, dietary advice is taken up and translated to an individual's circumstances at variable rates, so with both groups following healthy diets, there was no statistical difference in amount of weight lost, but there was an association between weight loss and actual vegetable intake across the whole sample in the intensive phase. Weight loss trials comparing different advice strategies show that so long as energy is restricted weight loss will occur (29, 30) and indeed that was the case in both our diet advice groups. In many ways this observation reflects a limitation of the RCT methodology for examining effects of food consumption patterns. However, conducting the analysis on total group data can expose the relationship between actual foods consumed and weight loss achieved (31), an issue we noted in our recently published critical review of vegetable consumption trials(19). We found one study showing greater weight loss with higher vegetable consumption, but the control group was advised to consume usual diet, which can be highly variable (31). This means you cannot necessarily attribute effects to a single food because there are many concurrent differences in the dietary intake. Our study confirmed the relationship between higher vegetable consumption and weight loss in a trial context where our advice controlled for all other dietary variables, including total energy intake (Appendix 6,7,8). Taken with the understanding that vegetables increase satiety and that compounds within vegetables may also be influencing metabolic effects, this study added confidence in the position that vegetables have an important place in the weight management diet.

All of the studies conducted in this project had limitations. In some cases, laboratory techniques needed to be developed and tested first. It was also not possible to study all vegetables available to the consumer in the satiety studies. Our trial participants were volunteers, so they were more likely to want to follow instructions. Indeed they all consumed recommended amounts of vegetables which is why it was harder to show a difference between groups. However, in a secondary analysis of this trial and other data, we found that those who entered the trials with higher consumptions of energy dense foods (including takeaway meals, sweets and soft drinks) had to make more changes to their diets and lost more weight than those already eating a balanced diet (but not controlling for energy) (32). Thus the amount of food consumed always remains important in weight loss, but there is a message about vegetables *replacing* energy dense foods and snacks that should not be overlooked.

In summary this research provided an excellent example of interdisciplinary research focused on a common problem (and opportunity). The capture of disciplinary expertise was evident in the conduct and reporting of the research, but the cross referencing of information between teams enabled a much stronger and relevant building of the case for vegetables in the context of weight management.

TECHNOLOGY TRANSFER

First and foremost, this research project demonstrated the extent to which research from a range of disciplines can be conducted and integrated to address the evidence base for the role of vegetables in weight management. The conduct of the research reflects many of the steps in technology transfer, but an overview of how this might happen requires consideration. The following key points are made:

1. Systematic literature reviews and clinical trials

Systematic literature reviews (SLR) form the basis for evidence based statements on the effects of foods on health. This methodology is reflected in the processes undertaken to form dietary guidelines (2, 33) and in requirements for dossiers for health claims (8, 10). The recently released 2013 Australian Dietary Guidelines statement of suggestive evidence that the consumption of vegetables is associated with a reduced risk of weight gain (2) is based on this process. In this methodology a hierarchy of evidence is utilized in which multiple human clinical trials (RCTs) are seen to provide the best form of evidence. The SLR conducted in this HAL project critically evaluated published clinical trials involving vegetables and identified a number of study design issues which can be problematic in attempts to expose the value of vegetables in RCTs. The RCT conducted in the HAL project took these issues into account in both the design and discussion of results. Thus the trial added to the evidence base but importantly it contributed to the scientific debate on how we are constructing evidence on food effects in a very complex and interdependent nutrient-food-diet scenario.

Implications:

(a) As a registered trial, the study adds to the evidence base for the effects of vegetable on weight loss (subject to publication in the scientific literature). The message from the trial is that, in line with the Australian Guide to Healthy Eating (AGHE), people who consume recommended serves of vegetables are able to lose weight well and keep it off. The AGHE lists recommended amounts of all core foods to make up a balanced diet, but this puts the focus back on vegetables. It is necessary that they keep this focus because surveys show vegetables are not being eaten at these levels.

(b) The RCT did not occur in isolation. It was conducted in a broader research context in which information from important studies of vegetable polyphenolic content (and a review of their potential actions) and short term appetite effects of vegetable form and preparation were carried out. Knowledge form this research put the RCT in a stronger scientific context, providing direction for some of the details, and plausible explanations for the research outcomes.

2. Vegetable Composition Studies

To reinforce the message that the rich polyphenol content of vegetables is another critical reason to include them in any weight loss strategy, accurate and reproducible methods for isolating and measuring these compounds are essential. However, the diverse chemical nature of these phytochemicals complicates the extraction and hydrolysis steps required for their determination. Numerous extraction methods have been described in the literature but limitations inherent in each has given rise to noticeable discrepancies in the levels reported.

A necessary initial requirement was to adopt a simple, rapid and robust methodology that could isolate and quantify individual polyphenols while allowing comparison with values reported for overseas grown vegetables. To achieve this, previously published methods were

evaluated and two analysis protocols that measured individual phenolic acids and flavonoids respectively were identified. Further optimization of extraction, hydrolysis and analysis conditions were performed before applying to the measurement of phytochemicals in the raw and cooked vegetable samples.

Implications:

(a) The results of this testing will greatly assist in the promotion of vegetables as primary foods for weight-loss efforts by identifying the richest sources of anti-obesity phytochemicals.

(b) The data collected will provide the consumer with information needed in formulating decisions on cooking protocols that preserve these bioactives.

(c) The results of the analysis provided much needed baseline data on polyphenol levels in Australian-grown vegetables.

3. Appetite studies

Short term satiety studies provide a means of testing in a controlled environment which food factors are likely to be important in affecting the re-development of post-meal hunger. The design of the trials was such that each individual was their own control as all participants consumed all test meals. This meant that several statistically-significant effects were obtained. Of particular note was that:

i) pureed carrot was less satiating than more intact forms of carrot, particularly around the two hours post-meal time when snacking may be contemplated,
ii) more energy dense mixed vegetables were less satiating than less energy dense broccoli even for the same total meal energy,
iii) there was no evidence that bitterness is a determining factor of vegetable satiety , and
iv) Cold potato and sweet potato demonstrated lower blood glucose response at early post-meal time points than their hot equivalents.

Implications:

(a) These studies provide further support for the concept that food form in addition to analyzable composition is important in satiety and potentially important in weight management. For maximal satiety benefit, vegetables should not be consumed in pureed or juiced form.

(b) The relatively long lasting satiety provided by low energy-dense vegetables suggests a possible route to demonstrating to consumers the potential of vegetables for weight management. It is a reasonable supposition that consumers would associate enhanced satiety with the potential for weight management, so demonstrations or challenges could be devised that compared the satiating effect of a vegetable-rich meal with a comparator energy-dense meal of equal total energy. For example a range of isoenergy meals with different rice to vegetable ratios could be devised and tested for the potential to demonstrate the satiety of vegetables.

(c) Both cold potato and cold sweet potato may be more beneficial than their hot counterparts for healthy blood glucose control.

RECOMMENDATIONS

General

- Strategic use of this model of cross disciplinary research is recommended. It contextualizes the problem more effectively and is likely to produce synergistic outcomes and have a greater impact under the right conditions. This form of research requires a longer term investment, and is more complex to manage than single projects, but it can be achieved by engaging senior scientists with experience in the area.
- The contractual conditions and milestones reporting is an important component of project management that should be maintained.
- It is recommended that HAL and the vegetable industry work with the institutions in promoting the research when it is published. This can take advantage of the full capacities of the industry and research environments, demonstrating the value of these partnerships as well as the outcomes for consumers and other stakeholders. This may be long term as multiple outcomes can be produced form re-analyzing data and writing reviews on this substantial piece of work.

Specific

- Consider the support of secondary analyses of data from the dietary trial which could draw out more detail specific shifts in dietary behavior (including differ types of vegetables) and the reasons behind these changes
- Consider further investment in translational research which address the barriers to shifting dietary intakes, and considers the position of vegetables in a cuisine context
- Support further research in requisite knowledge for further food based trials and surveys, notably food compositional studies and biomarkers of vegetable intake and of biological effects.
- Continue to support the links between studies of food form and preparation with trials and surveys.

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APPENDICES

Appendices 1 – 8: Summaries of manuscripts associated with the research presented in the report

Appendices 9 & 10: Full reports of studies associated with the research presented in the report

Appendix 1: Literature review 1: Dietary trials on effects of vegetable consumption on weight loss

Tapsell LC, Dunning A, Warensjo E, Lyons Wall P, Dehlsen K. Effects of vegetable consumption on weight loss: a review of the evidence with implications for design of randomized controlled trials. Critical Rev in Food Sci and Nutr [In press. Accepted for publication November 2011.].

This systematic literature review found 16 randomized controlled trials (RCTs) that addressed the question 'does a higher intake of vegetables produce a greater weight loss in overweight adults'? Of these 5 trials found vegetable intake was effective in weight loss, 9 reported no difference, one showed weight gain, and one showed an association between vegetable consumption and weight loss. The differences in outcomes could be explained in terms of elements of trial design. Effects were seen when the comparator group was asked to consume their usual diet, but this would not necessarily enable effects to be attributed to differences in vegetable consumption alone. No effects were seen when energy intakes were similar in both groups. The weight gain effect was found when vegetables were simply added to the usual diet. The critique of these studies exposed the problems for trial design examining the effects of single food groups when total energy intake from all foods is the main factor influencing body weight. Alternative analytical strategies were also identified, where associations between vegetable intakes and weight loss were considered.

Appendix 2: Literature review 2: Phytochemicals in vegetables and potential effects on obesity

Williams D, Edwards D, Hamernig I, Jian L, James AP, Johnson S, Tapsell LC. Vegetables containing phytochemicals with potential anti-obesity properties: a review. Food Research International [In press].

This literature review examined the proposed mechanisms of action of plant phytochemicals in obesity management, outlined the vegetable sources known to contain these compounds, and discussed issues in relation to different cultivars, the distribution of phytochemicals within the vegetable, and the impact of cooking on phytochemical composition. The studies reviewed showed that polyphenol compounds in vegetables can induce the breakdown of fat, and decrease the accumulation of fat, possibly by interfering with the lifecycle of the adipocyte or fat cell. Research to date puts red onions, lettuce, capsicum, curly kale and orange sweet potato as the highest sources of polyphenols. Boiling in water can reduce levels substantially, and gentle stir frying has better retention.

Appendix 3: Literature Review 3: Potential mechanisms which may be affected by vegetable consumption in obesity and chronic inflammation

Johnson S, James A, Jian L, Vogel D, Williams D, Tapsell, L. Protective mechanisms of vegetable phytochemicals for reducing risk of obesity and associated inflammation and oxidative stress. [Manuscript prepared for submission]

This literature review summarised the evidence from cell culture animal model and human studies of the following bio-activities of each major class of vegetable phytochemicals: (a) modification of fat cell lifecycle, (b) anti-inflammatory effects, and (c) metabolic stimulation. It was concluded that evidence primarily in cell culture studies indicated that phytochemicals such as those found in vegetables have potential in the prevention and treatment of obesity. Complex interacting cell signalling pathways are associated with the adipocyte lifecycle therefore combinations of vegetable phytochemicals that target different biochemical pathways in the lifecycle have potential to provide additive or synergistic effects on adipose tissue mass reduction and hence obesity development. Experimental evidence from cellular and animal model studies indicates modulation of inflammation by some classes of vegetable phytochemicals. Human studies to support these cellular and animal model studies are however rare in the literature, in particular, large scale, long term double blind, placebo-controlled intervention trials. Of the vegetable derived phytochemicals capsaicin appears to be the most likely to have effects on weight loss via control of energy metabolism. The evidence for or against a role for quercetin or chlorogenic acid in weight loss is not as strong with only limited evidence in an animal model supporting a role for quercetin in energy expenditure modulation.

Appendix 4: Satiety studies 1: Sweet potato and in vitro glucose responses

Hezam M, Tulsidas K, Johnson S. The effect of sweet potato and potato variety and preparation method on in vitro glucose release. [Submitted]

Slow glucose release from starch is desirable for blood glucose control therefore we determined glucose release from in-vitro digestion of (a) freshly steamed warm and (b) steamed and cooled potato, orange-skinned sweet potato and purple-skinned sweet potato. Levels of rapidly released glucose were: warm potato > warm orange-skinned sweet potato > warm purple-skinned sweet potato. Warm and cooled sweet potato had similar glucose release profiles whereas cooled potato had slower glucose release than warm. Therefore, steamed sweet potato (either hot or cooled) or steamed cooled potato may be more beneficial for blood glucose control than steamed hot potato. This however needs to be confirmed by post-prandial human studies.

Appendix 5: Satiety studies 2: Sweet potato and hormonal responses

Tulsidas K, Solah V, James A, Le Y-P, Williams D, Edwards D, Johnson S. Post-prandial satiety, glycaemic, insulinemic and ghrelin responses of potato and sweet potato meals. [Manuscript in preparation]

Potato (*Solanum tuberosum*) is a widely consumed part of the Australian diet. Dietary guidelines however recommend the consumption of a wide variety of vegetables hence greater understanding of the health related of consuming other starchy vegetables such as sweet potato (*Ipomoea batatas*) is required. The aim of this study was to compare the post-prandial effects on satiety and glycaemia in human participants of consuming equal energy and equal available carbohydrate portions of potato and sweet potato prepared in two ways. Sixteen healthy participants were fed meals of potato or sweet potato containing 50 g available carbohydrate eaten; (a) cooked and eaten hot or; (b) cooked, cooled overnight and served cool. Postprandial perception of satiety, glycaemia and subsequent energy intake was measured. Post-prandial insulinemia and ghrelin responses were measure on a sub-cohort of 9 participants.
Please note: A further cohort of participants and finalisation of analyses will be completed and the manuscript submitted for publication outside the scope of this final project report.

Appendix 6: Randomised Controlled Trial: Vegetable consumption and weight loss

Tapsell LC, O'Shea J, Thorne B, Grafenauer S, Probst Y, Batterham MJ. A higher vegetable intake may lead to greater weight loss effects: a 12mo randomised controlled trial. [Manuscript prepared for submission]

This 12 mo. single blind randomized controlled trial involved intensive dietary counseling with 120 overweight adults for 3mo, with follow up appointments at 6, 9,12mo. Both diet advice groups were advised on diets based on the Australian Guide to Healthy Eating with a 2MJ energy deficit. The information given to the comparator group used serve sizes of vegetables double that of the control group, so the amount of other foods were reduced accordingly to maintain the same energy deficit. Both groups achieved and maintained a substantial weight loss (mean -6.5 $\pm$ 5.2kg) but there was no difference between groups in the amount lost. Secondary analysis showed that the higher proportion of energy consumed as vegetables was associated with increased weight loss at the end of the intensive intervention phase of 3 months.

Appendix 7: Patterns of vegetable consumption in trial participants

O'Shea J, Tapsell LC, Thorne B, Grafenauer S, Probst Y. How targeted advice to eat more vegetables translates to practice: results from a 12 mo. dietary intervention trial [Manuscript prepared for submission]

This analysis described the patterns of vegetable intake over the 12 month trial examining the effect of advice to increase vegetable consumption for weight loss (ACTRN 12610000784011). Vegetable intake increased throughout the trial, and the number of categories also increased as the year progressed. This included a significant increase in the amount of legumes consumed. Tomatoes were the most popular vegetable throughout the trial, despite intakes reducing somewhat over the winter months. The consumption of fried potatoes decreased throughout the trial. Participants in the trial were already consuming a relatively high intake of vegetables but this still was able to be increased in the context of a weight loss trial.

Appendix 8: Baseline dietary patterns and weight loss at 3 months of trial participants

Grafenauer S, Tapsell LC, Beck EJ, Batterham MJ. Baseline dietary patterns are a significant consideration in correcting dietary exposure for weight loss. Eur J Clin Nutr 2013;doi:10.1038/ejcn.2013.26

This study used a cluster analysis statistical technique to examine the dietary patterns of participants in a fish based trial (ACTRN 12608000425392) combined with those of this vegetable trial (ACTRN 12610000784011). The trials were similar in design and enabled an analysis of 231 records of dietary intake at baseline and after 3 mo. Two clusters emerged, one more closely aligned to the Australian Guide to Healthy eating (C1) and the other with relatively high intakes of non-core dietary items, such as takeaway foods, sweets and soft drinks (C2). Those needing the greatest change in diet quality (C1) lost around 5kg more weight ($P<0.05$) and significantly reduced their intakes of the characterizing dietary items. This study showed that cutting back on non-core items can create substantial energy deficits, leaving room to be replaced by more vegetables with a low energy cost.

Appendix 9: Phytochemical analyses of selected vegetables

Williams D, Edwards D. Phytochemical analysis of vegetables. Queensland Department of Agriculture, Fisheries and Forestry.

Introduction

In 1998 the World Health Organisation defined obesity as a "phenotypic manifestation of abnormal or excessive fat accumulation that alters health and increases mortality" (34). Since the publication of this report obesity has risen at an alarming rate in both developed and developing countries and is becoming a major public health concern with incalculable social costs (35, 36). There is a strong association between obesity and chronic diseases such as diabetes, cardiovascular diseases, hypertension, osteoarthritis, some cancers and inflammation-based pathologies (37-39).

While reducing dietary fat content combined with increased physical activity has been shown to be effective in preventing obesity (40, 41), numerous studies show that this simple message is being ignored and alternative strategies are being sought (42-44). Obesity is characterised at the cellular level by an increase in the number and size of adipocytes (fat storage cells) that have differentiated from pre-adipocytes in the adipose tissue (45). This transition from undifferentiated pre-adipocytes into mature adipocytes constitutes the adipocyte life cycle and treatments that regulate both the size and number of adipocytes may provide a valuable adjunct to reduced dietary energy in combating obesity.

With this in mind considerable interest has been aroused world-wide in the potential of dietary phytochemicals to help counteract obesity (46-48). Cell culture and animal model studies have indicated the anti-obesity effects occur through modification of the adipocyte life cycle. A range of mechanisms have been suggested including inhibition of adipogenesis, induction of apoptosis (cell death) and stimulation of lipolysis (47, 49).

Polyphenols are a class of phytochemicals that are likely candidates for anti-obesity agents through studies that suggest they modulate the adipocyte life cycle. The strongest evidence is for phenolic acid derivatives such as chlorogenic acid and related compounds (caffeoylquinic acids) (50, 51), flavonols – quercetin and related compounds (kaempferol, myricetin, isorhamnetin) (49), and the flavones – luteolin and apigenin (49). These classes of polyphenols are widely distributed in plants and therefore are consumed regularly as part of the human diet.

Vegetables provide a major dietary source for phytochemicals with potential anti-obesity properties, with the types and levels varying markedly between species and even cultivars (52, 53). In addition climatic, agronomic and harvest conditions also significantly influence the levels of these phytochemicals in vegetables (54, 55) .

Post-harvest operations, including food-processing have a major influence on the levels of phytochemicals in vegetables and vegetable products. Heat treatment is the most common method for processing vegetables because of its ability to inactivate pathogenic and spoilage microorganisms and endogenous enzymes leading to improved quality and shelf-life (56). Conventional cooking (both domestic and industrial) is known to lower the levels of phytochemicals in processed food products compared to the raw materials (57, 58). In order to retain phytochemicals during the processing of vegetables, the food processor must optimise cooking steps to restrict their degradation.

To advance this field of research, accurate and reproducible methods for isolating and determining the amounts of these polyphenols are required. The diverse chemical natures of these compounds complicate the extraction and hydrolysis steps required for their determination. Although numerous extraction methods for polyphenols have been described in the literature, a common feature is their validation using only one plant material type and for only one specific class (reviewed in (59)). Some of the discrepancies in the literature between levels of individual polyphenols from the same vegetable source could in part be attributed to the differing extraction and hydrolysis protocols used (see Tables 2-4 in the current study). In view of the limitations of many of these previous studies, an initial requirement of our study was to obtain a simple, rapid and robust methodology for the separation and quantification of these polyphenols.

The objectives of the current study were threefold: 1) to optimise extraction and HPLC conditions for the analysis of polyphenols in vegetables, 2) to evaluate the polyphenolic constituents in supermarket - purchased produce, and 3) to determine the impact of cooking on these phytochemical levels. Vegetables were selected for this detailed investigation based on three criteria: 1) they were popular vegetables consumed in the concurrent dietary intervention trial, 2) they are high in polyphenols, and 3) they needed to retain quality without chilling for several days as they were sampled in Wollongong and transported to the laboratory in Brisbane.

Materials

Chemicals

The phenolic acids (chlorogenic and caffeic acids) as well as the flavonols (isorhamnetin, kaempferol, morin, myricetin and quercetin) and the flavones (apigenin and luteolin) were purchased from Sigma-Aldrich. The chlorogenic acid isomers, (3,5-dicaffeoylquinic, 3,4-dicaffeoylquinic and 4,5-dicaffeoylquinic acids) were obtained from Cfm Oskar Tropitzsch (Marktredwitz, Germany). The HPLC-grade methanol, formic acid, 2-propanol and acetonitrile were purchased from Thermo Fisher Scientific. All other chemicals were of analytical grade.

Selection of vegetables

The dietary load of the previously identified polyphenols (i.e. content x intake) for each of the twelve most popular vegetables identified at the 3 month point of the dietary intervention trial was calculated using the following data sources:

- USDA Database for Flavonoid Content of Selected Vegetables, Release 3 (2011)
- Phenol Explorer 2.0 (14)
- Literature data on phenolic acid contents (60-64)

The ranking of the vegetables based on phytochemical load is given in Table 1.

Table 1. Ranking based on anti-obesity phytochemical intake at 3 months of the dietary intervention trial

Ranking	Vegetable
1	**Sweet potato**
2	Lettuce
3	**Broccoli**
4	**Onion**
5	**Potato**
6	Frozen vegetables
7	Tomato
8	Leafy greens
9	Capsicum
10	Carrot
11	Green beans
12	Cucumber

Four vegetables (highlighted accordingly) were selected for detailed polyphenol analysis based on the ranking in Table 1. Note that lettuce was ranked second but was excluded because it is easily perishable and loss of polyphenols, particularly the phenolic acids, during non-refrigerated transportation was a real possibility.

Sampling Program

Vegetables (approximately 500 g) were selected from the fresh fruit and vegetable section at Coles and Woolworths supermarkets in the Wollongong area on 12 November 2011, 12 January, 12 February and 12 July 2012. On the day of purchase the broccoli, onion (brown variety), potato (Sebago var. from Coles and Golden Delight from Woolworths) and sweet potato (orange-fleshed var.) samples were packed in plastic bags, placed in cardboard boxes and transported overnight by Express Post to Brisbane.

Samples of the edible portion of each vegetable were prepared and divided into two sub-samples for analysis on arrival at the laboratory. The vegetables were prepared in the following manner:

- Broccoli - stem was removed and the remainder washed and dried.
- Onion - skin and first layer of the onions removed.
- Potatoes and sweet potatoes - peeled, washed and dried.

Known weights of one sub-sample of the prepared vegetables were then finely chopped in duplicate for lyophilisation over 5 days using a Dynavac freeze-drying unit. The lyophilised samples were weighed and stored at -80°C.

Cooking Treatments

The other vegetable sub-samples were cooked in duplicate as follows:

- Broccoli – 5cm pieces added to boiling water for 5 min.
- Onion – 10g of 5mm slices fried in 10 ml of sunflower oil for 5 min.
- Potatoes and sweet potatoes – 2cm³ diced pieces added to boiling water for 10 min.

After drying with paper towels to remove sunflower oil/water, the cooked material was frozen at -80°C and freeze-dried as outlined above. All freeze-dried samples were ground using a mortar & pestle prior to extraction of phytochemicals. Fresh and dry weight measurements were obtained for all plant material.

Methods

Phenolic acids

Extraction and Hydrolysis

Under most acidic conditions commonly used for polyphenol extraction, caffeic acid and its related phenolic acids are severely degraded thereby excluding the simultaneous analysis with other polyphenol classes (59). Phenolic acids have greater stability under neutral or alkaline conditions (65). As phenolic acids have particularly pronounced anti-obesity effects, it is vital to select analytical procedures in which losses of these compounds are minimised. With this in mind the analysis of individual phenolic acids (chlorogenic acid and caffeoylquinic acids (CQA), 5-CQA; dicaffeoylquinic acid isomers: 3,4-diCQA; 3,5-diCQA; 4,5-diCQA) were based on the procedure of Padda and Picha (66) but with several important modifications. The lyophilised vegetables (1 g) were placed in a centrifuge tube and 7ml of 80 % aqueous methanol were added. The tubes were capped and immersed in a water bath at 80°C for 10 mins. The mixture was vigorously shaken, cooled and centrifuged (≈ 3000 x g, 15 mins). The clear supernatant was diluted to 10 ml with extracting solution and 1.5 ml of the diluted supernatant pushed through a 0.45 µm syringe filter into a HPLC vial to be stored at -80°C prior to analysis.

Identification & quantification

Five standard mixtures containing caffeic acid, chlorogenic acid (5-caffeoylquinic acid, CQA) and dicaffeoylquinic acid isomers (3,4-diCQA; 3,5-diCQA; 4,5-diCQA) were prepared in methanol at a concentration range of 2-200 µg/ml (60).

Samples were analysed using a Shimadzu HPLC system consisting of a system controller (SCL-10Avp), degasser (GastorGT-104), low pressure gradient forming switching valve (FCV-10ALvp), pump (LC-10ADvp), auto-sampler (SIL-10ADvp), column oven (CTO-10AC), UV diode array detector (SPD-10AV) linked to Class VP software (Version 6.14 SP1). Optimal separation of the individual phenolic acids was achieved on a reversed-phase C18 Gemini, 5 µm, 4.6 x 150 mm column (Phenomenex) with matching guard column. Both columns were maintained at 30°C. After testing several mobile phases the best suited was a mobile phase of 1 % (v/v) formic acid in H_2O: acetonitrile : 2-propanol (70:22:8, v/v/v), pH 2.5 under isocratic conditions with a flow rate of 0.75 ml/min as it provided optimal

separation with a reasonably low back pressure. An aliquot of 20 µL of sample was injected and peaks were detected at 320 nm and identified and quantified by comparison to commercial standards. The elution order was: chlorogenic acid; caffeic acid; 3,4-diCQA; 3,5-diCQA; 4,5-diCQA. Total phenolic acids were expressed as caffeoylquinic acid content as outlined by (60, 65).

Method validation

This optimised HPLC method was evaluated with regard to linearity and accuracy (67). Linearity was tested according to the relationship between different concentrations of phenolic acids and peak areas. The accuracy was assessed from analytical recovery studies that were performed by spiking selected vegetable samples with known concentrations of each phenolic acid.

Flavonoids

Extraction and Hydrolysis

Flavonols and flavones are the most widely occurring and structurally diverse of all the polyphenols (68). Most occur in plants mainly as *O*-glycosides (69) and due to this wide range, complex HPLC profiles can result. As most of these *O*-glycoside compounds are not generally commercially available, most peaks are not readily identified or quantified. Hydrolysis (acid, alkaline or enzymatic) to their aglycone forms simplifies the HPLC profile and allows quantification, as many aglycones can be obtained from commercial suppliers.

There are numerous reports (59, 70, 71) that testify to the effectiveness of acid hydrolysis in releasing the free flavonoids. Therefore this mode of extraction was adopted in the current study. However, Nuutila et al.,(59) suggested that the high concentration of mineral acids under refluxing conditions may be too harsh and may degrade certain flavonoids, e.g. myricetin. We therefore decided to examine a range of hydrolysis conditions and adopt one that minimised any degradation of the flavonoids of interest. Therefore the flavonoids were extracted as follows: Ground lyophilised vegetables (0.5 g) were vortexed for 30 sec with 20ml of 60% aqueous methanol containing the flavonol morin (30µg/ml) as an internal standard to monitor any losses during sample preparation. Ascorbic acid (20 mg) was added immediately followed with 5 ml 6M HCl and vortexed for 30 seconds before mixing at 80°C for 1hr. After cooling the samples were centrifuged (≈ 3000 x g, 2min) and 1.5 ml of the supernatant was filtered (0.45 µm) into a HPLC vial to be stored at -80°C prior to analysis.

Identification & quantification

Six standard mixtures were prepared in methanol containing apigenin, isorhamnetin, kaempferol, luteolin, morin, myricetin and quercetin at concentrations ranging from 5-100 µg/ml (60).

Samples were analysed using the same Shimadzu HPLC system as above with a reversed-phase C18 Acclaim Polar Advantage 2, 3 µm, 4.6 x 150 mm column (Dionex) with matching guard column at 30°C.
The mobile phases consisted of 0.1% (v/v) formic acid in water (Phase A) and 0.1% (v/v) formic acid in acetonitrile (Phase B). Separation of the polyphenols was achieved by gradient elution (Phase B diluted in Phase A) at a flow rate of 1.5 ml/min.

- 20 – 40 % B in 12 mins
- 40 – 60 % B in 9 mins

- 60 – 100 % B in 2 mins
- 100% B for 2 mins
- Re-equilibration: 100 – 20 % B in 1 min; 20% B for 4 mins.

The injection volume was 10 µL and simultaneous monitoring was performed at 280 nm, 325 nm & 365nm. The order of elution was: myricetin; morin (internal standard); quercetin; apigenin; kaempferol.
Preliminary HPLC analyses were conducted on all extracts to establish that morin was not present in detectable quantities.

Method validation

A method validation protocol was performed for the determination of flavonoid content similar to the one outlined above for the phenolic acids.

Results and Discussion

Stability of standards in extraction/hydrolysis solution

In an effort to simplify the HPLC separation Hertog et al. (69) suggested preparing standards in the extraction or acid hydrolysis solutions to ensure the same environment as the samples. As the stability of standards is critical for quantification the stability of the chlorogenic acid, caffeic acid, kaempferol and quercetin standards under acid hydrolysis conditions was investigated by HPLC analysis over time (0 – 20 hrs).

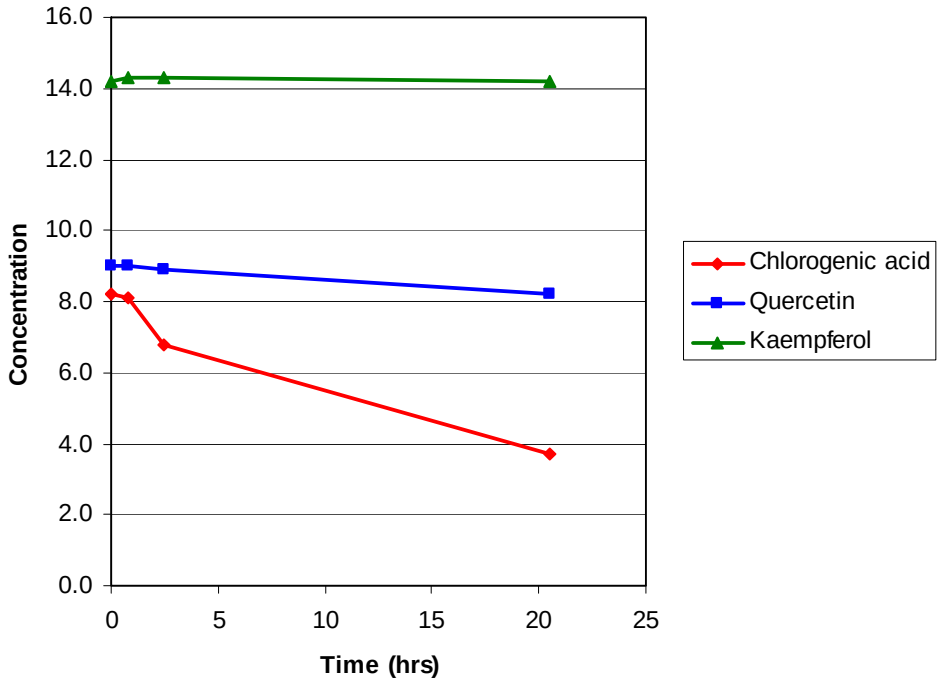


Figure 1. Stability of standards in extraction / hydrolysis solution

The concentration of chlorogenic acid was reduced by 55% (see Figure 1) over the duration of the experiment. As chlorogenic acid decreased, the caffeic acid peak increased, indicating hydrolysis of chlorogenic acid in this environment to caffeic acid. Also quercetin decreased by approximately 10 % while kaempferol levels remained constant. Similar testing was performed on standards prepared in methanol as recommended by Padda and Picha (72). No breakdown was observed for any of the polyphenol standards when this solvent was used.

Phenolic acids

Method validation for phenolic acids analysis

The optimised method adopted gave good linearity and accuracy. A linear relationship between peak areas and concentration tested (2-200 µg/ml) was found for all the phenolic acids tested. Recoveries, which determine the accuracy, for individual phenolic acids ranged from 87-108%.

Phenolic acids in raw vegetables

The total phenolic acid content expressed as caffeoylquinic acid content of potato and sweet potato is presented in Table 2. Also included in the table are previously reported values in the same units for ease of comparison.

Table 2. Phenolic acid content (mg/kg FW) of potato and sweet potato

Vegetable	Caffeoylquinic acids	Reference
Potato-Nov11-Coles	**195**	**(73)**
Potato-Nov11-Woolworths	**216**	**(73)**
Potato-Jan12-Coles	**138**	**(73)**
Potato-Jan12-Woolworths	**127**	**(73)**
Potato-Feb12-Coles	**124**	**(73)**
Potato-Feb12-Woolworths	**225**	**(73)**
Potato-Jul12-Coles	**138**	**(73)**
Potato-Jul12-Woolworths	**97**	**(73)**
Potato-small russet	133	(61)
Potato-large russet	142	(61)
Potato tubers	170	(61)
Sweetpotato-Nov11-Coles	**788**	**(73)**
Sweetpotato-Nov11-Woolworths	**1305**	**(73)**
Sweetpotato-Jan12-Coles	**600**	**(73)**

Sweetpotato-Jan12-Woolworths	**489**	**(73)**
Sweetpotato-Feb12-Coles	**938**	**(73)**
Sweetpotato-Feb12-Woolworths	**536**	**(73)**
Sweetpotato-Jul12-Coles	**626**	**(73)**
Sweetpotato-Jul12-Woolworths	**864**	**(73)**
Sweet potato-cream fleshed	31 – 46	(63)
Sweet potato-orange fleshed	190 – 580	(62)
Sweet potato-purple fleshed	1150	(62)
Sweet potato-white fleshed	93 – 910	(62)
Sweet potato stele	505 – 12205	(74)

Results from our study and those previously reported for potato (61) agree closely as well as confirming that this vegetable is a rich source of phenolic acids. Previous researchers have observed that tuber size and variety have little effect on phenolic acid content of potatoes, with smaller varieties only showing insignificantly lower values than larger ones (61).

There was a marked difference in phenolic acid content noted in our analyses between sweet potato and potato. The range of phenolic acid content for the orange-fleshed variety of sweet potato tested in our study (489-1305 mg/kg FW) was considerably higher than that observed for potato (97-225 mg/kg FW). The broad range of values noted in our study agrees with those reported by Harrison et al.(74). These authors examined sixteen sweet potato cultivars and noted wide variations in phenolic acid content between the periderm (skin layer), cortex (layer under periderm) and stele (the remaining edible portion) (Table 2). (72) also found high levels of phenolic acids in young immature leaves and in small sweet potato roots particularly in the orange-fleshed and purple-fleshed varieties (Table 2).

It has been reported that different sweet potato cultivars grown under the same conditions contain as many as five chlorogenic acid isomers (75). Our study supported this observation as significant amounts of three chlorogenic acid isomers were present in all the sweet potato extracts tested: 3,4-diCQA (11-112 mg/kg FW); 3,5-diCQA (125-449 mg/kg FW) and 4,5-diCQA (4-14 mg/kg FW).

Phenolic acids in cooked vegetables

The impact of cooking on the phenolic acid content of potato and sweet potato are presented in Figure 2. The values presented are the average of all potato and sweet potato samples received after sampling on the 12th of July.

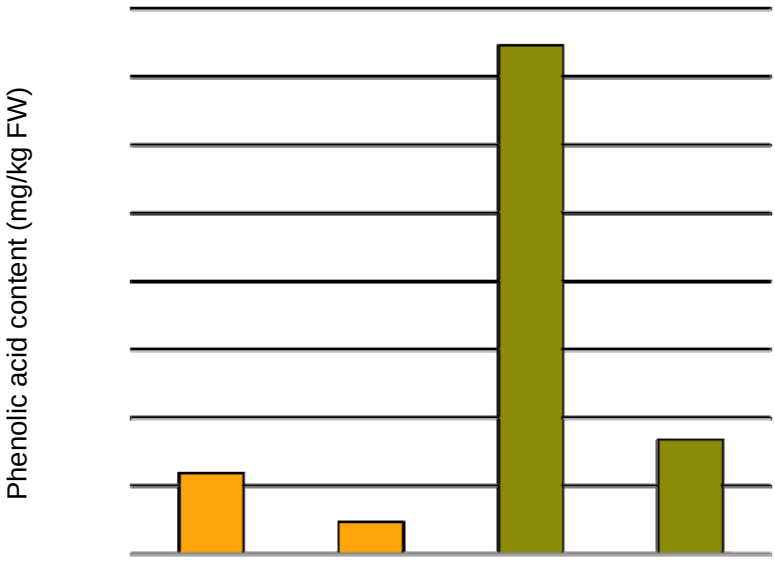


Figure 2. Effect of boiling for 10 min on the total phenolic acid content of potato and sweet potato.

The percentage loss of phenolic content of potatoes after boiling was 61%. This agrees closely with the 66% reduction of phenolic acid content reported by Tudela, (76) after boiling fresh-cut potato strips. Further agreement was provided by Miglio et al. (77) who observed a general decrease in all phenolic acids for vegetables that underwent their cooking treatments. Other studies involving potato found that phenolic acids were absent in baked potatoes, while frying resulted in losses of over 50% (61, 77).

The losses after boiling sweet potato were even more pronounced, with a reduction of 78% found in our study (Figure 2). This large reduction in phenolic acid content agrees closely with two other research groups (61, 77) with both stating that prolonged boiling of sweet potato resulted in losses of phenolic acids of 60 – 100%. An exception to this trend was provided by Takenaka et al. (75) when they reported a slight increase in the chlorogenic acid isomers (3-caffeoylquinic, 4-caffeoylquinic,3,4-decaffeoylquinic and 4,5-dicaffeoylquinic acids) after boiling sweet potatoes. A later investigation (64) supported this finding when they stated that steam cooking resulted in statistically insignificant increases in the concentration of individual phenolic acids identified in sweet potato root tissues. It was suggested by Truong et al., (64) that the slight increase in chlorogenic acid could be attributed to release of bound phenolics and the inactivation of polyphenol oxidase during boiling. Polyphenol oxidase (PPO) is an enzyme present in many plants and after tissue damage, e.g. cutting, the enzyme catalyses the formation of a brown pigment from phenolic compounds. It was suggested that cooking treatments resulted in PPO inactivation that

reduced the enzymatic degradation compared to the raw vegetable with a subsequent increase of phenolic acids.

Flavonols

Method validation for flavonol analysis

Again the modified method gave good linearity and accuracy. A linear relationship between peak areas and concentration tested (5-100 µg/ml) was found for all the flavonoids tested. Recoveries for individual flavonols ranged from 89-98%.

Flavonols in raw vegetables

Quercetin is the major dietary flavonoid of the flavonol class found in vegetables. Other phytochemicals that are structurally close to quercetin include kaempferol, myricetin and isorhamnetin.

The content of quercetin and its related compounds are presented in Table 3.

Table 3 Flavonol content (mg/kg FW) of onions

Vegetable	Quercetin	Kaempferol*	Isorhamnetin*	Reference
Onion-Nov11-Coles	**854**	**25**	**52**	**(73)**
Onion-Nov11-Woolworts	**883**	**36**	**25**	**(73)**
Onion-Jan12-Coles	**596**	**22**	**40**	**(73)**
Onion-Jan12-Woolworths	**968**	**24**	**77**	**(73)**
Onion-Feb12-Coles	**1201**	**26**	**30**	**(73)**
Onion-Feb12-Woolworths	**920**	**9**	**74**	**(73)**
Onion-Jul12-Coles	**1044**	**38**	**140**	**(73)**
Onion-Jul12-Woolworths	**1088**	**23**	**92**	**(73)**
Onion-red-dry skin	1900			(78)
Onion-red-outer fleshy layer	660			(78)
Onion-red-edible portion	600			(78)
Onion-yellow	270 - 1187			(79)
Onion-yellow	214	6	0.2	(80)
Onion-white	185 – 634			(70)

* blank entries indicate component levels not reported in study

Our values for the quercetin content of the brown onions were consistently high and very similar to the values reported by most other researchers (Table 3). However, our values were much higher than those given by the USDA in 2007 for US grown onions. In contrast to many other researchers (70, 78) significant levels of kaempferol (9-38 mg/kg FW) were detected in our testing. Another point of departure in our results was the presence of considerable amounts of the flavonol isorhamnetin.

Most researchers have noted that levels vary between cultivars as well as between the different layers of the onion bulb (Table 3). Studies of red onions showed the dry skin fraction contained 3 times the level of quercetin than the outer fleshy layer and the inner edible portion (78). The outer layers of onions were shown by Chu et al.(81), to contain up to 10 times the levels of quercetin and kaempferol than the inner layers (onion variety not specified), with low levels of myricetin present (81)(81)(80)(81)(81)(80)(80)(80)(80)(80)(80)(80)(80)(80)(80)(80)(80)(80)(80)(80)(80)(80)(80)(80)(80)(80)(79)(78)(78)(78)(78)(78)(77)(77)(77)(77)(76)(76)(76)(76)(75)(74)(73)(74)(73)(72)(71)(70)(69)(68)(68)(67)(66)(65)(64)(63)(62)(61)(60)(59)(58)(57)(56)(55)(55)(54)(53)(53)(52)(51)(51)(51)(50)(50)(50)(50)(50)(50)(50)(49)(49)(48)(48)(47)(46)(45)(44)(43)(42)(41)(40)(39)(39)(38)(37)(36)(37)(36)(35)(34)(33)(32)(31)(30)(29)(28)(27)(26)(25).
Differing hydrolysis conditions as well as the inconsistent removal of onion skin and outer layers may account in part for the variation in values for quercetin content given by many researchers.

Table 4 Flavonol content (mg/kg FW) of broccoli

Vegetable	Quercetin	Kaempferol	Isorhamnetin*	Reference
Broccoli-Nov11-Coles	**35**	**57**	**BDL**	**(73)**
Broccoli-Nov11-Woolworths	**38**	**67**	**7**	**(73)**
Broccoli-Jan12-Coles	**40**	**69**	**BDL**	**(73)**
Broccoli-Jan12-Woolworths	**40**	**81**	**25**	**(73)**
Broccoli-Feb12-Coles	**49**	**81**	**9**	**(73)**
Broccoli-Feb12-Woolworths	**40**	**71**	**21**	**(73)**
Broccoli-Jul12-Coles	**38**	**77**	**20**	**(73)**
Broccoli-Jul12-Woolworths	**31**	**34**	**51**	**(73)**
Broccoli	40	40		(82)
Broccoli	30 – 37	60 – 72		(83)

* blank entries indicate component levels not reported in study

BDL – below detection limit

Broccoli has been reported by several sources as possessing high quercetin content (82, 83).. Our values presented in Table 4 agree with their published values but it should be noted that the levels are much lower than those given for onions. The detection of significant amounts of kaempferol in our analyses also agrees with these other investigations.

Flavonols in cooked vegetables

Generally, the level of phytochemicals in vegetables decreases exponentially with increases in cooking duration and magnitude (55). However, there have been reported cases where heating aids the extractability of these phytochemicals leading to an apparent concentration increase (84). Therefore, the content of phytochemicals reported in vegetables after cooking is a net result of the combined effects of degradation and leaching during cooking and changes in phytochemical extractability during analysis.

The effect of cooking on the quercetin content of broccoli and onions are presented in Figure 2. The values presented are the average of all samples received after sampling on the 12th of July.

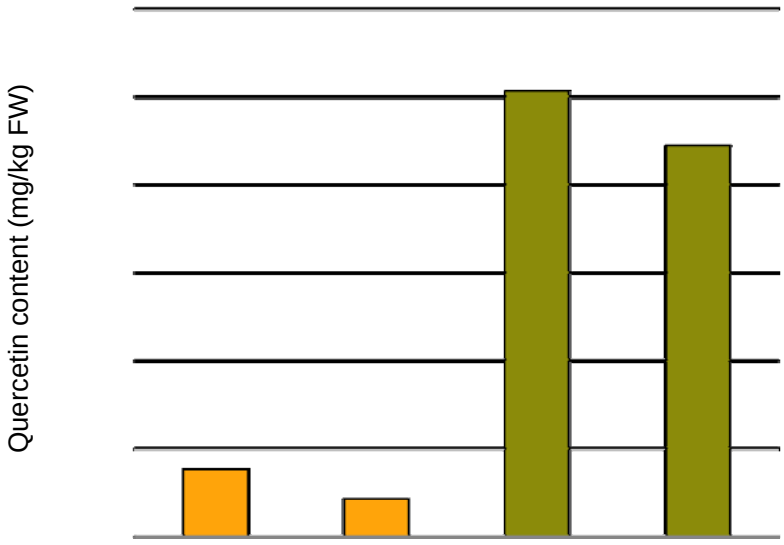


Figure 3. Effect of cooking on the quercetin content (mg/kg FW) of broccoli and onion.

Boiling broccoli for 5 min gave a reduction of 45% in the quercetin level (Figure 3). This was certainly less than the levels reported by Miglio et al.(77), of 90% for quercetin and 85% for kaempferol. These authors noted lower reductions on steaming (losses of 40% and 1%, respectively) and frying (70% and 45%, respectively). They stated that the greater diffusion of phytochemicals into an aqueous, boiling cooking medium compared to steaming and

frying is to be expected for these water-soluble compounds and accounted for the different levels.

Frying of onions in sunflower oil gave a reduction in quercetin levels of only 12% (Figure 3). It should be noted that the sunflower oil used in our study for frying the onions did not contain any flavonol either before or after frying. This value agrees closely with that presented by Crozier et al. (70). These authors reported that frying onions in sunflower oil resulted in a reduction of only 21%, possibly due to the less effective extraction of the hydrophilic quercetin by the hot oil compared to hot water. These researchers also reported a similar reduction of quercetin in tomatoes after undergoing the same cooking treatments(70).

Reductions of between 44-53% in the levels of the quercetin glycosides were reported during 60 min boiling of onions (85). An earlier study had demonstrated that 15% of quercetin was lost on boiling onions for only 5 min (86). Both groups of authors suggested that this reduction was due to thermal degradation of the quercetin but they could not discount leaching of the water soluble quercetin.

Flavones

Flavones are much less common than flavonols in vegetables (87). Natural flavones consist of glycosides of apigenin and luteolin which are corresponding flavones to the flavonols, kaempferol and quercetin respectively. The only significant vegetable sources of flavones identified to date are parsley and celery (70, 82, 88). A finding corraborated by our study as none of the vegetables examined contained detectable amounts of these flavones.

CONCLUSIONS

In recent years reports suggesting beneficial anti-obesity properties of the polyphenols, phenolic acids and flavonoids have increased interest in vegetables as an important source of these plant bioactives. A vast amount of literature, although little on Australian-grown produce has emerged comparing different vegetables as sources of these compounds. However, limitations in the analysis methods used previously meant we have had to optimise extraction and HPLC conditions in order to develop a simplified and robust methodology that quantifies individual phenolic acids and flavonoids. Both methods developed illustrated good linearity and accuracy. In saying that, the method developed for the analysis of flavonoids is still a compromise between efficient production of the aglycones and their degradation.

Using these analysis protocols the estimates of individual phenolic acids and flavonoids obtained on selected Australian-grown vegetables agreed closely with most other published findings from overseas. However caution should still be used in comparing the polyphenol content of different plant materials reported in scientific literature.

Previous studies suggest that the cooking method has a pronounced influence on the levels of these phytochemicals in vegetables and our data certainly confirmed this statement. Boiling vegetables in water resulted in the greatest losses of these water-soluble phytochemicals perhaps by leaching or thermal degradation. In contrast, frying appeared to result in the least loss.

Recommendations:

For any future dietary trials that assess the intake of anti-obesity phytochemicals, a closer fit of analysis performed and the clinical study should be adopted. This could take the form of:

- The cultivars/varieties of selected vegetables should be specified. The levels of anti-obesity phytochemicals vary markedly between cultivars.
- The selected vegetables should be sourced over at least one year as climatic and harvest conditions also significantly influence the levels.
- The history of storage should be available as this also is a major cause of variation in polyphenol content.
- Increase the number of selected vegetables to include potentially the most potent anti-obesity vegetables e.g. red capsicum and red curly kale as well as potent vegetables not commonly cooked e.g. red-leafed lettuce.
- Procedures that indicate the commonly used cooking methods by the participants of the dietary trial for employment in the laboratory before detailed analysis.
- Collection of participants' urine at regular intervals to provide a more accurate assessment of anti-obesity phytochemical intake.

We believe that an expanded clinical trial with a closer fitting analysis regime would provide much useful information to promote the cause of fighting obesity with vegetables. The extra data would go some way to determining the levels of phytochemical intake that would be expected to be beneficial in combating obesity. Recommendations that detail amounts to be consumed and cooking protocols for vegetables high in these phytochemicals could be a desired outcome of this approach. There is an ever-increasing amount of scientific literature detailing the content of these phytochemicals in vegetables (raw and cooked) but surprisingly little, if any pertaining to the levels of Australian-grown vegetables.

Acknowledgements

I wish to thank Emeritus Professor Christa Critchley for her contribution to proof-reading the manuscript as well as providing informed comments and suggestions. Also thanks to Avis Houlihan formerly of DAFF who undertook much of the work in obtaining references, collating and developing the earlier versions of this manuscript. The technical assistance by Ingrid Hamernig formerly of DAFF was invaluable.

Appendix 10: Satiety studies 3: effect of bitterness

Thavaraj P, Kravchuk O, Roura E, Gidley M. Effect of bitterness on satiety. University of Queensland.

Introduction

Bitter flavour is one of the 5 basic tastes that humans can perceive. Animals use the sense of taste to select foods to eat and evaluate their quality (89). Bitter taste is associated with unripe, spoiled and toxic food stuff and therefore is used as a cautionary signal (90). Since many plant toxins and rancid oils are bitter, the bitter taste receptors were thought to detect and protect the organism from consuming poisonous substances (91). However, there has not been a steady correlation between the level of bitterness and toxicity (92). It is for their own protection that plants secrete toxins and natural pesticides, thereby avoiding being ingested by animals (93). Most plant based substances, phenols, isoflavones, terpenes, glucosinolates and flavonoids have protective antioxidant, anticarcinogenic, and tumour blocking properties and are beneficial for human beings however, due to their bitter flavour, make the plant unpalatable (94) thus leading to rejection of bitter fruits and vegetables. Human beings are sensitized to bitter flavour in some vegetables and other foods so methods have been developed by which the level of bitterness in food is decreased by selective breeding and/or debittering processes (95).

It has been shown that there are about 25 human taste receptor (hTAS2R) genes that belong to a super family of G protein coupled receptors (GPCR) (91). Though it is a small number of bitter taste receptors, humans perceive bitter flavours from thousands of structurally different compounds (90). Certain human taste receptors are broadly tuned to recognize numerous compounds while some remain very specific to a few bitter agonists; e.g. hTAS2R7, hTAS2R14 and hTAS2R46 have a broad agonist spectrum whereas hTAS2R4 is narrowly tuned to detect Propylthiouracil and denatonium benzoate(96, 97). Bitter taste receptors are not only found in the tongue but also in the human stomach and intestines (98-100). This finding has triggered interest to study their function in the gut and has led to the proposal of their role as part of a nutrient sensing mechanism (100, 101).

The molecular mechanisms through which taste cells detect tastants and activate intracellular signalling eventually leads to depolarization (102). Through studying the mechanism associated with bitter taste detection, the role of alpha-gustducin (a signal transducer) as a type 2 taste cell specific G protein that provided a molecular tool for taste cell transduction was elucidated (99, 103). Mice that lacked a-gustducin were unable to detect sweet or bitter tastes, which lead to the conclusion that it played an essential role in bitter and sweet taste perception (103).
Bitter tasting vegetables due to the antioxidants, flavonoids, terpenes, isoflavonoids they contain, play a key role in promoting good health (104). Apart from this, bitter flavour promotes satiety; which is defined as 'process that leads to inhibition of further eating, decline in hunger, increase in fullness after a meal has finished', also known as post-ingestive satiety or inter-meal satiety (105). Bitter foods are known to increase the production of satiety hormones such as CCK, PYY (hormones that inhibit gastric emptying) due to the activation of complex machinery that mediates different taste modalities with specific chemosensory receptors in the stomach, duodenum and the small and large intestine (100) (106). Low acceptance of bitter flavoured vegetables could be a barrier to the adoption of satiety inducing diets (104).
The aim of this study was to investigate the effects of bitter flavour on satiety using bitter ingredients in a meal.

Materials and methods

1. Threshold test:

The bitter compounds used were gentian extract and quinine hydrochloride (QHCl). Threshold tests were conducted for both compounds separately in order to estimate the concentration of them to be added to a lemon drink consumed with test meals. There were 15 and 20 panellists respectively who participated in the threshold taste testing of Gentian and QHCl. Bench top screening was done prior to the tests to select a suitable range of concentrations of the compounds. 6, 7, 8, 9 and 10 µl of QHCl and 120, 160, 200, 240 and 280 µl of Gentian were used for threshold testing. 7 µl and 240 µl were found to be the threshold concentrations.

2. Preference test:

A preference ranking test was conducted for the meal containing broccoli and pasta varying only the sauce. 3 types of sauces, namely Woolworths select tomato and mushroom sauce, Dolmio extra garlic pasta sauce and Dolmio carbonara pasta sauce were tested to find out the most preferred sauce in a broccoli based pasta meal. 8, 5 and 2 out of 15 panellists preferred the Dolmio extra garlic pasta sauce, Dolmio carbonara pasta sauce and Woolworths select tomato and mushroom sauce respectively.

3. Satiety test:

The satiety test was conducted in the sensory laboratory of the University of Queensland with 16 panellists.

Ethics approval and Subject recruitment- Information about the study was posted around the St. Lucia campus of the University of Queensland in the form of flyers as well as being electronically distributed. Potential participants were interviewed face to face or by telephone. Questionnaires were filled out to identify any allergies, food preferences/ dislikes, medication the panellists were under and any past or current medical conditions that could have affected the panellists themselves or the study and were eliminated. A three factor questionnaire was required to be filled in by all prior to commencement of the experiments. This questionnaire assessed eating behaviour based on three categories namely, 1) cognitive restraint of eating, 2) hunger and 3) disinhibition. Any panellist scoring above 27, i.e. more than 10 in factor 1 and or factor 2 and more than 7 in factor 3 were eliminated. Following this and prior to the start of experiments, signed consent was obtained from all the panellists.

Design- The experiment had a randomised balanced crossover design with four test meals served during four visits to 16 panellists. The order of presentation was randomised using a Latin square design. There were a total of 6 visits that each panellist had to attend during which the 1st visit was a training session to familiarise the participants with test procedures following this there were 4 test sessions where the panellists were served test meals in a randomised order and during the 6th visit, they were served the practice meal again.

Test food- The broccoli test meals were prepared in the Food Sensory laboratory in Hartley Teakle building, University of Queensland. The broccoli was chopped into bite sized florets

(rinsed and cleaned), 200g portions were wrapped in aluminium foil and steamed for 12 minutes at 120 °C in a UNOX spa XVC 054 steam oven. Foil wrapping was done to ensure no loss of water took place. 55g of Zaferelli fusilli pasta was boiled for 20 minutes in 300 ml water and strained. The pasta and 100 ml of Dolmio extra garlic sauce were heated in a pan for 2 minutes and the broccoli was added, stirred for a minute and served hot in a bone china bowl. 250 ml of lemon cordial with no or different levels of the bitter agents were served alongside the meal and consumption of the entire meal and drink was mandatory. Practice breakfast meals contained 200g of Birds Eye Thai style stir fry mixed vegetables instead of 200g of broccoli. This mix had beans, carrots, baby corn, broccoli and bamboo shoot. 50g of Zaferelli fusilli pasta as well as 50g of Dolmios extra garlic sauce were used for the practice meals which were served with 250ml of blank lemon cordial. The meals' energy content was approximately 1160- 1170 kJ.

Ingredients	**Amount**	**Energy content (kJ)**
Zaferelli fusilli Pasta	55 g	836
Broccoli	200 g	68
Dolmios extra garlic sauce	100 g	259 1163

Table 1: Amounts and energy content of ingredients in the broccoli based meal

Ingredients	Amount	Energy content (kJ)
Zaferelli Fusilli Pasta	50 g	760
Mixed vegetables	200 g	282
Dolmios extra garlic sauce	50 g	129.5 1171.5

Table 2: Amounts and energy content of ingredients in the mixed vegetable practice meal

Concentrations of bitter ingredients in meals	Quinine Hydrochloride QHCl (µl)	Gentian (µl)
A	0	0
B	80	0
C	0	240
D	80	240

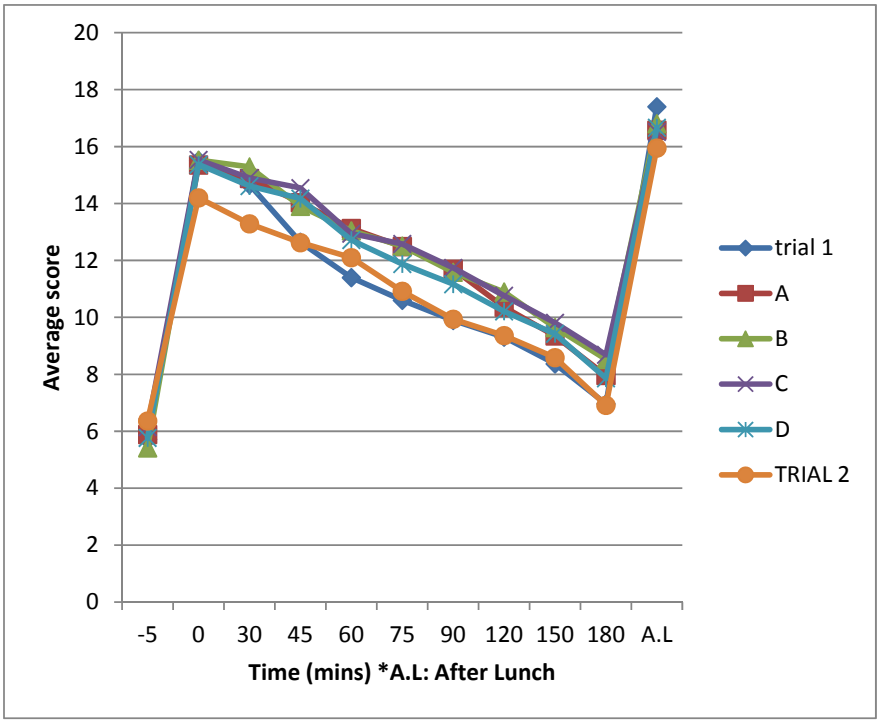
Table 3: Concentrations of bitter ingredients in the lemon drink

Daily procedures: All panellists were asked to fast for 10-12 hours prior to experimental sessions. They were also requested to maintain a similar level of activity prior to the sessions since physical activity plays a role in appetite levels. Breakfast was served warm along with the drink and hunger scores were obtained for before breakfast as well as just after breakfast. The panellists were then allowed to leave and continue rating their hunger levels on a labelled magnitude scale every 15 minutes during the 1st hour and every 30 minutes after that (107). They were asked to return for a buffet lunch after 3 hours of consuming breakfast.

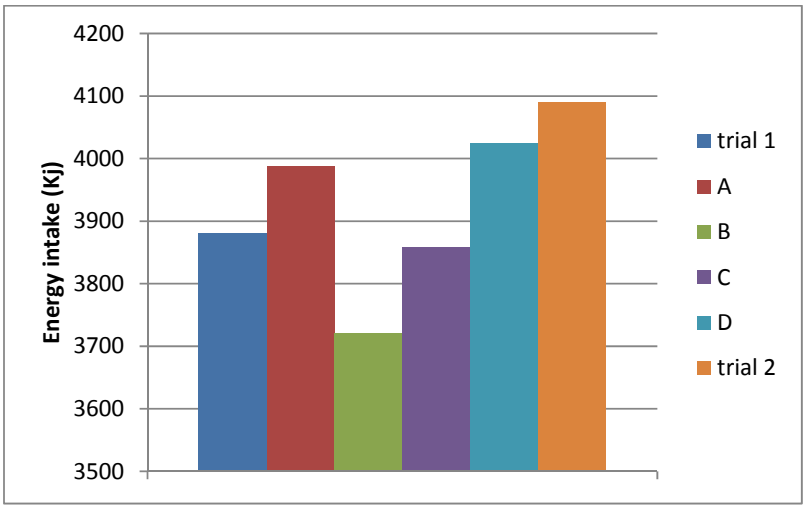
Participants were requested to drink only water, diet coke and tea or coffee without sugar. A drinks diary was maintained. The lunch meal was based on the USDA choose my plate diet planner and consisted of 6 slices of bread, 200g of bids eye Thai style stir fry, 18g of butter, 190g of tandoori chicken sandwich filler, 400g of ski delight vanilla yoghurt and 250 ml of water. Participants were allowed to consume as much or as little as they wanted. More food was provided upon request. A rest of the day food diary was maintained after every experimental session.

Results

The results showed that there was no significant difference in reported satiety scores after meals that were accompanied by either bitter or non-bitter drinks (Figure 2, A to D). There was however less satiety afforded by the trial meals based on mixed vegetables, despite the fact that total meal energy and volume were kept constant. The mixed vegetables had a higher energy than the broccoli so the amount of sauce used was reduced to compensate. This result provides evidence that low energy-dense vegetables are particularly useful for promoting satiety even in meals of the same total energy and volume.



Graph 1: Average scores of hunger vs. time for the different treatments A, B, C and D



Graph 2: Graph showing total energy intake during the 6 visits

Conclusion

All meals promote satiety however, the test meals containing broccoli were more efficient in delaying the return of hunger than the trial meals based on mixed vegetables. Of the four broccoli meals, the meal containing 80 and 240 µls of QHCL and gentian was least effective in promoting satiety despite being most bitter to taste. After an hour of consuming breakfast, the hunger score for meal D was 12.72 and ranged between 12.9- 13.1 in meals A, B and C. Just 15 minutes later, hunger ratings dropped for meal D to 11.8 and 12.5 for meals A, B and C. 120 minutes after breakfast, hunger scores of meal A were similar to that of meal D (10.3 and 10.2 respectively). Meals B and C were most efficient in delaying the return of hunger with meal C being slightly more effective (hunger score of 9.8 and 9.6 for meals C and B respectively at 150 minutes).

The hunger score for just after breakfast, indicated as time 0 on graph.1, was lower in trial 2 (14.2) than all the other meals (15.4-15.5). The difference was very clear after the 75th minute reading with the trial meals 1 and 2 having hunger scores of 10.6 and 10.9 and the other meals ranging between 11.8 and 12.6.

The Lunch intake results show that most amount of energy intake, 4090 kJ was after the trial 2 meal. Though trial meals 1 and 2 were identical and also had the same just before lunch hunger (180th minute) score of 6.9, the energy intake after the former was 3881 kJ, lower than trial B. Energy intake was lower after all the treatment meals in comparison to trial meal 2, with treatment D seeing a larger energy intake than after treatments A, B and C. energy intake after breakfast meal treatment B was lowest and was 3720 kJ. Treatment D, containing 80 and 240 µl of QHCL and gentian respectively was most bitter to taste and seemed to have stimulated the appetite. Interestingly, meals B and C contained 80 and 0 µl of QHCl, gentian and 0 and 240 µl of QHCl, gentian. When the two compounds were incorporated together, they increased the energy intake by 304 kJ than if only QHCl was present and by 166 kJ than if only gentian was present. The energy intake after treatment D was quite closer to that of treatment A, where no bitter compound was present. The difference in energy intake between having both bitter compounds (meal D) and no bitter compound at all (meal A) was 101 kJ.

In conclusion, there was not much difference seen between the treatment meals and their ability to increase satiety however the difference is clear between the mixed vegetable trial

meals and broccoli based meals which were of the same energy intake and volume. This may be related to the higher energy content of the mixed vegetables compared with broccoli which was compensated by lower amounts of sauce in order to keep total energy the same. Overall, this trial has shown that there is greater potential for enhancing satiety through choice of vegetables than modified bitterness and that vegetables with lower energy density may be more satiating even with constant total meal energy.