Applied Horticultural Research Pty Ltd

Final Report for Horticulture Australia Ltd

VG08148 (completed February 2012).

Developing a nutrient and/or health claim label for packaged baby leaf spinach and rocket

Gordon Rogers *et al.*

Horticulture Australia Ltd Final Report

Project VG08148 Developing a nutrient and/or health claim label for packaged baby leaf spinach and rocket.

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This project was funded with a voluntary contribution from Harvest Freshcuts and by Horticulture Australia Ltd. This project aimed to firstly clarify the food labelling guidelines which were complex and under review. It then collected data that could be used to support a nutrient claim label on fresh cut spinach and rocket taking into account the change in nutrient content as a result of growing season and postharvest storage. In addition some consumer focus groups were run to determine how best to present this information to consumers.

Horticulture Australia

TABLE OF CONTENTS

1 MEDIA SUMMARY

Babyleaf spinach and rocket are major components of salad leaf mixes, including the popular packaged fresh-cut mixes. They are also high in some of the important vitamins and antioxidants such as Vitamin C, vitamin A, folate (iron) and glucosinalotes.

Current food labelling regulations allow claims to be made on packages, which inform consumers about the amounts of these healthy bioactives foods contain. Many factors can affect the levels of these bioactives including weather, variety and handling. The main aim of this project has been to measure the impact of these factors on the levels of bioactives.

This project first reviewed the current regulatory system as it relates to nutrition labelling of fresh produce. Next, it reviewed the scientific literature to find what has been published about the levels of Vitamin C, vitamin A and folate in spinach and rocket. Finally, it measured the levels of these bioactives in spinach and rocket including the influence of season, location and storage.

It was found while location, season and storage all affected the levels of Vitamin C, vitamin A and folate in rocket and spinach, and that after 12 days in storage levels were high enough that the following claimed could be made:

- Baby Leaf Spinach/Rocket form part of a healthy diet of fruits and vegetables
- Baby Leaf Rocket is a source of vitamin C, folate and Vitamin A (beta-carotene)
- Baby Leaf Spinach is a source of folate and Vitamin A (beta-carotene)

Models were developed to determine the estimated levels of vitamin C and folate in blends of salad mixes using the average values from these trials. Nutrient claim labels were developed for the Macro label for Woolworths.

Consumer studies suggested the use of a nutritional claim as the predominant message in marketing salad products was questioned by consumers, and deemed not as important as flavour or variety. While heavy users of leafy greens are health motivated, the proposed nutritional claim platform does not act as a strong, compelling point of difference for launching a range of products into the market.

Further consumer research is recommended to confirm the value placed on nutrient labelling by consumers. It may be that is more that it is new and unexpected information rather than that is regarded as having limited value by consumers.

2 TECHNICAL SUMMARY

Babyleaf spinach and rocket are major components of salad leaf mixes, including the popular packaged fresh-cut mixes. They are also high in some of the important vitamins and antioxidants such as Vitamin C, vitamin A, folate (iron) and glucosinalotes.

Current food labelling regulations allow claims to be made on packages, which inform consumers about the amounts of these healthy bioactives foods contain. Many factors can affect the levels of these bioactives including weather, variety and handling. The main aim of this project has been to measure the impact of these factors on the levels of bioactives.

This project first reviewed the current regulatory system as it relates to nutrition labelling of fresh produce. Food Standards Australia New Zealand is developing a new food standard that may allow the use of more health claims for foods on packaging and in advertising. At present nutrient content and nutrient function claims for foods are generally permitted, but claims about health enhancement or reduction of risk of diseases are not permitted (with one exception about folate and birth defects).

The following types of claims could be used immediately for baby spinach/rocket:

- *Baby spinach/Rocket form part of a healthy diet of fruits and vegetables.*
- *Baby spinach/Rocket are a natural source of antioxidants*
- *Baby spinach/Rocket are a natural source of antioxidants that help protect the cells from oxidative damage*
- *A natural source of the antioxidants – to help keep your body healthy*
- *Antioxidants in Baby spinach/Rocket help protect the body against free radical damage*
- *Baby spinach/Rocket are a source of vitamin C, folate and beta-carotene*

  The following claim may be able to be used when the new Nutrition and Health Claims standard comes into force:
- *Eating a diet rich in fruits and vegetables as part of an overall healthy diet may reduce risk for stroke and perhaps other cardiovascular diseases*

It is unlikely that other claims about reduced risk of cancer or cardiovascular disease could be made without substantial further research.

Next, the project reviewed the scientific literature to find what has been published about the levels of Vitamin C, vitamin A and folate in spinach and rocket, and the findings are presented in section 5 of the report.

Finally, the levels of these bioactives were measured in spinach and rocket including the influence of season, location and storage. The total folate and vitamin C content of baby spinach and wild rocket leaves were analysed from samples taken from different growing districts around Australia in summer and winter seasons. The samples were stored for 12 days at 0°C, 5°C or under a commercial protocol.

The samples were analysed after 0, 4 and 12 days in storage. There was a significant variation in the levels of total folate and vitamin C content due to production region. The levels of these nutrients were higher in winter crops compared to summer crops. The levels of both folate and vitamin C diminished in storage; however, the reduction was less for leaves stored at 0°C compared to 5°C, or the commercial protocol of 5°C for 4 days and then 7°C for 8 days. Wild rocket leaves were higher in both vitamin C and total folate content compared to baby spinach but had a faster rate of nutrient loss during storage. The nutrient levels were higher in winter-grown crops and the nutrient levels were maintained at higher levels when the leaves were stored at 0°C.

It was found while location, season and storage all affected the levels of Vitamin C, vitamin A and folate in rocket and spinach, that after 12 days, the levels were high enough that the following claimed could be made:

- Baby Leaf Spinach/Rocket form part of a healthy diet of fruits and vegetables
- Baby Leaf Rocket is a source of vitamin C, folate and Vitamin A (beta-carotene)
- Baby Leaf Spinach is a source of folate and Vitamin A (beta-carotene)

Models were developed to determine the estimated levels of vitamin C and folate in blends of salad mixes using the average values from these trials. Nutrient claim labels were developed for the Macro label for Woolworths.

Consumer studies suggested the use of a nutritional claim as the predominant message in marketing salad products was questioned by consumers, and deemed not as important as flavour or variety. While heavy users of leafy greens are health motivated, the proposed nutritional claim platform does not act as a strong, compelling POD for launching a range of products into the market.

Further consumer research is recommended to confirm the value placed on nutrient labelling by consumers. It may be that is more that it is new and unexpected information rather than that is regarded as having limited value by consumers.

3 INTRODUCTION

Over the past decade, there has been a boom in the baby leaf salad industry worldwide, resulting in it being the most rapidly expanding of all the fruit and vegetable retail categories. The recent growth in the demand for baby leaf salads has been largely attributed to demand for convenience (Wagstaff et al. (2007). However, consumers are becoming increasingly interested in the health benefits of the foods they eat (Williams, 2006) and sales of packaged baby leaf spinach and rocket may be able to take advantage of this consumer desire to make healthy eating choices.

Research has shown that spinach and rocket are excellent sources of active phytonutrients compounds which could have specific health benefits, these include glucosinolates in rocket (Pappa, Strathmann, Lowinger et al.,2007) and antioxidants particularly flavenoids and phenols (Chu, Sun, Wu et al. 2002 as well as folate in spinach (Hart, Wright, Wolfe, et al 2006; FSANZ Standard 1.1 A.2 Transitional Standard – Health Claims).

This project aimed to clarify the revised food labelling laws, FSANZ (2008) as they relate to fresh fruit and vegetables, using packaged baby leaf spinach and rocket as case studies.

Williams (2006) states that "Consumers are becoming increasingly health conscious and most agree that eating healthily is a better way to manage illness than medication….there is an observed "push" from food companies seeking new profit opportunities and a "pull" from an educated health-conscious consumer". The question is whether or not packaged baby leaf spinach and rocket can position itself to be part of that market.

This project aimed to address the following three issues:

1. How does the new FSANZ code (Proposal P293) apply to packaged baby leaf spinach and rocket? Are there opportunities to use the new labelling laws for either a nutrient claim, a general health claim or a high level health claim?

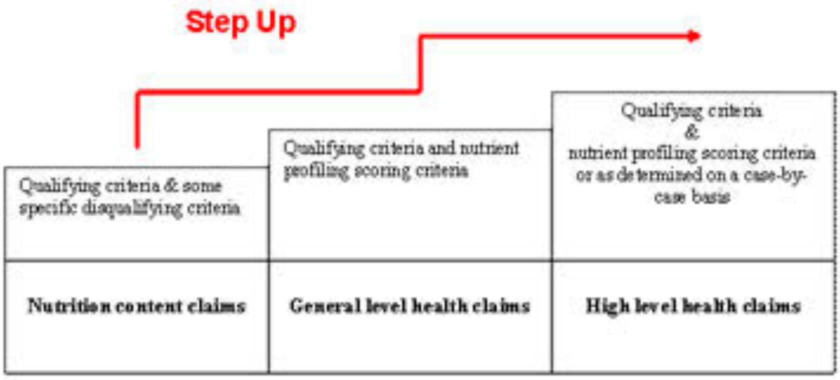
2. Do consumers of packaged baby leaf spinach and rocket see an advantage in having additional nutrient or health claim data on the package?

3. How are "average" values of nutrients or bioactives (antioxidants or folate for example) determined for packaged baby leaf spinach and rocket when research has shown that seasonal growing conditions, variety and storage conditions significantly affect shelf life and visual quality (VG05068: Optimising crop management and postharvest handling for baby leaf salad vegetables).

The uncertainty over the application of the new FSANZ code (Proposal P293) is due in part to the fact that the code is still evolving. For example in "September 2008, the three month statutory period for completing the First Review of Proposal P293 was extended to ten months by the Ministerial Council to April 2009" .

A summary of the revised Regulation of claims is available from the Food Standards Australia and New Zealand website (www.foodstandards.gov.au) by searching on *Proposal P293*. At the time of writing, the new standard was still not finalised, with the most recent discussion paper dated 17th February 2012 and calling for further submissions.

In the draft standard, the level of a particular claim determines how that claim is regulated, including the evidence required for substantiation. This principle is outlined in the graphic shown below:



Nutrition content claims and health claims on food labels or in associated advertising for food sold in New Zealand and Australia will be required to be substantiated by scientific evidence. This is to ensure claims are soundly based and do not mislead consumers. Verification of the health benefit is required for all general level health claims.

Nutrition content claims

Nutrition content claims will need to be substantiated, either by analysis or by calculating the content of the claimed component in the food.

General level health claims

To substantiate general level health claims, manufacturers must use either the FSANZ list of nutrient function statements (example Standard 1.1A.2 where a Folate claim is listed to be appropriate for some vegetables and fruits notably, English spinach and Harvest FreshCuts Vegetable Medley), derive claims from the food-disease relationships from high level health claims, use authoritative sources or complete a systematic review as specified in the Scientific Substantiation Framework. Suppliers must have records to substantiate such claims.

High level health claims

Food-disease relationships must be pre-approved by FSANZ and will form the basis for high level health claims. Separate provisions for substantiation of high level health claims are therefore not required.

The current Food Standards Code summarises the nutrient claim in Standard 1.2.8 FSANZ, 2008, as follows:

> **Nutrition claim** means a representation that states, suggests or implies that a food has a nutritional property whether general or specific and whether expressed affirmatively or negatively, and includes a reference to -
>
> (a) energy; or
> (b) salt, sodium or potassium; or
> (c) amino acids, carbohydrate, cholesterol, fat, fatty acids, fibre, protein, starch or sugars; or
> (d) vitamins or minerals; or
> (e) any other nutrient; or
> (f) a biologically active substance (eg. an antioxidant);

The nutrient claim is based on the “average” level of the component in that product but what is an average level? The code uses the following description;

> **Average quantity** in relation to substances such as sodium, potassium, fatty acids, amino acids and vitamins and minerals in a food is the quantity determined from one or more of the following
>
> (a) the manufacturer’s analysis of the food; or
> (b) calculation from the actual or average quantity of nutrients in the ingredients used; or
> (c) calculation from generally accepted data;

which best represents the quantity of the substance that the food contains, allowing for seasonal variability and other known factors that could cause actual values to vary.

The requirements concerning *Nutrition Information Panels* are also contained in Standard 1.2.8. Although FSANZ is responsible for defining food standards, enforcement of those standards is managed by other government departments such as State and Territory Health Departments, the New Zealand Food Safety Authority, and AQIS. There are important provisions in State and Territory Food Acts, the New Zealand *Food Act 1981*, and the *Imported Food Control Act 1992* which provide various defences for failure to comply with food standards. The ACCC also helps to regulate food standards and labelling through the Trade Practices Act (1974).

The application of these standards is only useful if consumers are interested in having more information on the packaged label. Williams (2006) sates that “there is a high level of scepticism about all aspects of information on food labels, including health claims, and concern is often expressed over manufacturers using claims as a sales tool”. Therefore an important part of this project was the use of consumer focus groups to establish if they saw additional nutrient or health information as useful and how they liked that information presented on a package of baby leaf spinach and rocket.

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4 REGULATORY REVIEW

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4.1 Summary of Regulatory Review

Food Standards Australia New Zealand is developing a new food standard that may allow the use of more health claims for foods on packaging and in advertising. At present nutrient content and nutrient function claims for foods are generally permitted, but claims about health enhancement or reduction of risk of diseases are not permitted (with one exception about folate and birth defects).

The following types of claims could be used immediately for baby spinach/rocket:

- *Baby spinach/Rocket form part of a healthy diet of fruits and vegetables.*
- *Baby spinach/Rocket are a natural source of antioxidants*
- *Baby spinach/Rocket are a natural source of antioxidants that help protect the cells from oxidative damage*
- *A natural source of the antioxidants – to help keep your body healthy*
- *Antioxidants in Baby spinach/Rocket help protect the body against free radical damage*
- *Baby spinach/Rocket are a source of vitamin C, folate and beta-carotene*

The following claim may be able to be used when the new Nutrition and Health Claims standard comes into force:

- *Eating a diet rich in fruits and vegetables as part of an overall healthy diet may reduce risk for stroke and perhaps other cardiovascular diseases*

It is unlikely that other claims about reduced risk of cancer or cardiovascular disease could be made without substantial further research.

4.2 Part 1: Overview of Current Australian Regulations for Labelling and Advertising

1) Food Standards Australia New Zealand

Nutrition, health, and related claims for food (both on labels and in advertising) are regulated in Australia through an integrated regulatory system involving both National and State agencies as well as voluntary industry codes of practice. Food standards are developed by the bi-national statutory agency FSANZ (Australia New Zealand Food Authority, 2002) and these standards are adopted by State governments who have the role of compliance enforcement.

Interpretive guides on the standards are also available from FSANZ (Food Standards Australia New Zealand, 2008a). Among other things, these food standards cover:

- general labelling provisions about the ingredients in foods – Standard 1.2.4
- the elements in the mandatory nutrition information panel (NIP) that must appear on all packaged food as well as regulations on additional voluntary and claims about the presence or absence of nutrients – Standard 1.2.8
- the special labelling requirements for foods requiring pre-market approval (eg novel foods, foods with genetically modified ingredients, or irradiated foods) – Standards 1.5.1-3
- health claims for foods - Standard 1.1A.2

At present in Australia and New Zealand nutrient content claims are allowed (*e.g. 'this food is a source of vitamin C'*) provided the food provides at least 10% of recommended dietary intake (RDI) per serve for a 'source' claim, or 25% RDI per serve for a 'good source/high' claim. (Note: for labelling purposes FSANZ defines RDI values in Standard 1.1.1, which in some cases are different to those defined by the National Health and Medical Research Council. FSANZ is planning to commence a review of these claimable levels at the end of this year and it is possible that in the next few years the values for some nutrients, like folate, will be revised upwards, but for the moment all claims must conform with current regulations). Some general level health-maintenance claims (nutrient function claims that describe the normal role of nutrients in the maintenance of health) are also permitted.

The predecessor of FSANZ developed guidance material on what sort of nutrient function claims were acceptable, and developed a list of examples – e.g., ' *fibre helps eliminate waste products from the body'* (National Food Authority, 1993). However, other types of health claims about the potential health benefits of foods are prohibited, with the sole exception of claims about the benefit of maternal consumption of folate, to reduce the risk of foetal neural tube defects. That claim is permitted for a defined range of foods listed in Standard 1.1A.2 – which includes a range of leafy vegetables including English Spinach. To be eligible, a food must contain at least 40μg folate per serve and not more than 14g fat, 500mg sodium or 10g added sugar.

FSANZ is currently developing a new standard which will permit scientifically substantiated health claims for foods that meet certain nutrient profiling criteria. The new Nutrition, Health and Related Claims Standard will encompass two levels of health claims: general level health claims and high level health claims. The level of a claim will determine how the claim is regulated, including the evidence required for substantiation (Food Standards Australia New Zealand, 2008b).

General Level Health Claims - do not reference a biomarker (eg, blood cholesterol or blood pressure) or a serious disease or condition and include content claims, function claims, enhanced function claims and risk reduction claims that reference a non-serious disease or non-serious condition. There are three types of general level claims:

a) Nutrient Function Claims – which describe the biological role of a food or energy or a nutrient [or a biologically active substance] in normal growth, development, maintenance and other like functions in the body (e.g. "*Iron helps transport oxygen around the body")*

b) Enhanced Function Claims - which describe the biological role of a food or energy or a nutrient [or a biologically active substance] beyond normal growth, development, maintenance and other like functions of the body (e.g., "*XX can help improve concentration")*

C) Risk Reduction Claims in relation to a non-serious disease or condition - which describe the biological role of a food or energy or a nutrient [or a biologically active substance] in significantly reducing the risk of developing a non-serious disease or condition (e.g., "*XX may help reduce the risk of acne")*

In the original proposed new framework, food companies would not need pre-approval to make general level claims but must use either a FSANZ model list of approved statements, or provide suitable scientific texts or dietary guidelines to support the claim, or must hold scientific evidence to substantiate such claims and produce this evidence, on request, for enforcement agencies.
The following sites provide guidance as to the sort of general level claims that are likely to be acceptable:

Generic Health Claims
Joint Health Claims Initiative (JHCI), United Kingdom
http://www.jhci.org.uk/

Health Claims in the Labelling and Marketing of Food Products, The Food Sector's Code of Practice
Swedish Nutrition Foundation.
http://www.snf.ideon.se/snf/en/rh/Healh_claims_FF.htm

Health Claims that meet Significant Scientific Agreement (SSA)
Food and Drug Administration, USA
http://www.cfsan.fda.gov/~dms/lab-ssa.html

Health Claims based on an Authoritative Statement of a Scientific Body
Food and Drug Administration, USA
http://www.cfsan.fda.gov/~dms/labfdama.html

Dietary Guidelines for Australian Adults
National Health and Medical Research Council.
http://www.nhmrc.gov.au/publications/synopses/_files/n33.pdf

High level claims – are claims which reference a biomarker or a serious disease or condition and include biomarker maintenance claims, biomarker enhancement claims and risk reduction claims which reference a serious disease or condition

a) Biomarker Maintenance Claims – describe the biological role of a food or energy or a nutrient [or a biologically active substance] in maintaining a normal level of a recognized biomarker (e.g., *helps maintain normal blood cholesterol*)

b) Biomarker Enhancement Claims – describe the biological role of a food or energy or a nutrient [or a biologically active substance] in reducing or increasing the level of a recognized biomarker (e.g., *helps reduce cholesterol*)

c) Risk Reduction Claims which reference a serious disease or condition - describe the biological role of a food or energy or a nutrient [or a biologically active substance] in significantly reducing the risk of developing a serious disease or condition (e.g., *helps reduce the risk of cardiovascular disease*)

FSANZ commissioned a series of scientific reviews of the evidence for a number of proposed pre-approved health claims to include in the new standard (Food Standards Australia New Zealand, 2007). After these reviews, only eight have ultimately been accepted for inclusion in the new standard as high level claims, relating to the following food or nutrient-disease relationships:

- Sodium (with or without potassium) and hypertension;
- Fruit and vegetables and coronary heart disease;
- Saturated fat (with or without trans fat) and elevated serum cholesterol and heart disease;
- Calcium (with or without vitamin D) and osteoporosis;
- Folate and neural tube defects.

In the future, if the new Standard is approved, manufacturers would be able to make applications for approval of other claims, which will need to be scientifically substantiated. FSANZ has developed a substantiation framework, to be used by manufacturers wanting to apply for approval to use a health claim on a product (Food Standards Australia New Zealand, 2008b).

The Final Assessment Report for Proposal P293, including draft Standard 1.2.7 – Nutrition, Health and Related Claims, was approved by the FSANZ Board in March 2008 and notified to the Australia and New Zealand Food Regulation Ministerial Council. The Council considered the draft and requested FSANZ undertake a review to consider a range of concerns about operation of the standard.

On 20 March 2009, FSANZ released a Consultation Paper which summarises proposed changes to address two of the key issues raised in the First Review Request, and is seeking public comments by 15 May:
http://www.foodstandards.gov.au/standardsdevelopment/proposals/proposalp293nutritionhealthandrelatedclaims/index.cfm

This main proposed difference is for the regulation of general level health claims be changed from industry self-substantiation, to an approach where such claims are only permitted if they refer to a food-health relationship listed in the Food Standard Code

The proposed new standard lists a number of ***proposed nutrient function claims*** that would be permissible, including the following that might be relevant to baby spinach or rocket:

Property of food	**General claim conditions**	**Specific descriptor**	**Specific descriptor conditions**
Dietary fibre	Contributes to regular laxation	The food meets the general conditions for making a nutrition content claim about dietary fibre.	
Vitamin C	Enhances absorption of iron from food	The food meets the general conditions for making a nutrition content claim about vitamin C.	
	Necessary for normal structure and/or function of connective tissue	The food meets the general conditions for making a nutrition content claim about vitamin C.	
	Necessary for normal structure and function of blood vessels		
	Contributes to cell protection from the damage caused by free radicals		
	Necessary for normal neurological function		
	Contributes to normal growth and development		For children
Vitamin A	Necessary for normal vision	The food meets the general conditions for making a nutrition content claim about vitamin A	
	Necessary for normal structure and function of the skin and mucous membranes		
	Necessary for normal cell differentiation		
	Contributes to normal growth and development		For children
Folate	Necessary for blood formation	The food meets the general conditions for a nutrition content claim about folate.	
	Necessary for normal cell division		
	Necessary for normal growth and development		For children

It also list the following ***proposed pre-approved high level health claims:***

Property of food	**General claim conditions**	**Specific descriptor**	**Specific descriptor conditions**
Folic acid	Reduced risk of foetal neural tube defects	(a) the food contains no less than 40 μg folic acid per serving; and (b) the food is not – (i) soft cheese; or (ii) pâté; or (iii) liver or liver product; or (iv) foods containing added phytosterol esters or added tall oil phytosterols; or (v) food standardised in Standard 2.6.4; or (vi) food standardised in Part 2.7; or (vii) food standardised in standards 2.9.2 and 2.9.4; or (viii) a formulated meal replacement standardised in Division 2 of Standard 2.9.3.	(a) the population group is women of child bearing age; and (b) a varied diet including food sources of folate and a recommendation that women consume at least 400 μg of folic acid per day, at least the month before and three months after conception.
Increased intake of fruit and vegetables	Reduced risk of coronary heart disease	(a) claims are not permitted on – (i) fruit juice or vegetable juice standardised under Standard 2.6.1; or (ii) non alcoholic beverages and brewed soft drinks standardised under Standard 2.6.2; and (b) the food contains no less than 90% fruit or vegetable by weight.	Healthy diet with an increased intake of both fruit and vegetables and consisting of a variety of foods.
A high intake of fruit and vegetables	Reduced risk of coronary heart disease	(a) claims are not permitted on – (i) fruit juice or vegetable juice standardised under Standard 2.6.1; or (ii) non alcoholic beverages and brewed soft drinks standardised under Standard 2.6.2; and (b) the food contains no less than 90% fruit or vegetable by weight.	Healthy diet high in both fruit and vegetables and consisting of a variety of foods.
Fruits and vegetables	Contributes to heart health	(a) the food is not – (i) fruit juice or vegetable juice as standardised by Standard 2.6.1; or (ii) a food standardised by Standard 2.6.2; and (b) the food contains no less than 90% fruit or vegetable by weight.	(a) Healthy diet with an increased intake of both fruit and vegetables and consisting of a variety of foods *or* (b) Healthy diet with a high intake of both fruit and vegetables and consisting of a variety of foods.

Communication of Health Messages to Consumers

Food labels are an important tool to assist consumers in making healthy food choices. In addition to mandatory nutritional labelling information, manufacturers have a variety of options on food packages to communicate the nutrition and health benefits of their products (Williams & Ghosh, 2008). However, consumers often express concern that health claims are just another sales tool, and the use of poorly substantiated claims could increase the current levels of consumer scepticism about all attempts to communicate the health benefits of food (Williams, 2005). Recent trends in the USA and the EU indicate that in the future regulators may require further research to test how consumers are likely to interpret and use any health claim.

Nutrient profiling

Nutrient profiling of foods is the science of categorizing foods based on their nutrient composition. For regulatory agencies, nutrient profiles can be the basis for disallowing nutrition or health claims and for regulating advertising to children. The EU and Australia/New Zealand have adopted nutrient profiling as the basis for regulating which foods will be permitted to carry health claims. The Australian approach proposes a mixed scoring system of disqualifying nutrients (energy, saturated fat, sodium, sugar) balanced with positive scores for protein, fibre, fruit and vegetable content (Food Standards Australia New Zealand, 2008b). In general, plain vegetables (such as leafy green vegetables) will have little difficulty meeting the nutrient profiling criteria, but those with excessive levels of added sodium or sugar may not be eligible to make claims.

2) Australian Competition & Consumer Commission (ACCC)

The ACCC has responsibility for enforcement of the Trades Practices Act, which generally prohibits any form of false or misleading claims in relation to all products on sale or in advertising, including food. The ACCC has developed guidelines related to a number of issues concerning food in their publication *Food descriptors guideline to the Trade Practices Act* (Australian Competition and Consumer Commission, 2006). However, the ACCC takes a wide view of evidence as to whether a claim on labels or in advertising is misleading, and impressions provided by graphics and design can be taken into account, not just the accuracy of specific text. The ACCC does not necessarily accept guidelines from FSANZ when deciding what are false or misleading representations. For example, previously FSANZ had allowed claims of 'fat free' on products that contained minimal levels of fat (<0.15%). The ACCC has taken a more rigorous interpretation, and decided that unless a product contains zero fat, claims of 'fat free' would be false and misleading.

3) Australian Food and Grocery Council (AFGC)

The AFGC has produced a voluntary code of practice on the provision of information on food products (Australian Food and Grocery Council, 1995). This incorporates a code of practice on nutrient claims that was earlier developed by the National Food Authority, which sets the levels of nutrients required in foods to be able to make claims about high, low, reduced or increased levels of nutrients, or comparisons between products (National Food Authority, 1995). In general products need to have a difference of at least 25% from comparison products to be able to make claims of nutritional superiority.

The fact that this guide is only voluntary has led to a considerable amount of non-compliance – particularly for claims about "% fat free". As a result, it is proposed that all claims for nutrient content will be included in black letter law of the new food standards on nutrition, health and related claims.

4.3 Part 2: Comment on Current Claims in the Market

Despite the current prohibition on health claims, there are a number of claims on products at present that appear to be non-compliant with current regulations. The prevalence of health claims on labels, in magazines and on the internet, and the level of compliance of these claims with existing regulations, has been studied recently in a series of studies from the Smart Foods Centre (Dragicevich *et al*, 2006; Williams *et al*, 2006; Williams *et al*, 2007). These data show that many of the claims in the Australia food market at present are high level claims or therapeutic claims which are not legally permitted by current food standards. In some cases this may be due to lack of awareness of the regulations, or in some cases the claims are carefully worded to be a *Nutrient Function Claim* (which is permitted) rather than a *Health Claim*.

Claims about antioxidants are not regulated within current Food Standards or the voluntary Code of Practice on Nutrient Claims (because “antioxidant” is not a specific nutrient), but they would fall under the category of claims about “bioactive substances” that will be referred to in Clause 11 of the proposed new standard on health claims. In this standard it is proposed that manufacturers would need to define for themselves the daily reference quantity of the substance that is recommended, and then products would need to contain at least 10% of that level per serve to justify a claim.

The table below shows how antioxidant ingredients are being claimed in the Nutrition Information Panel on a variety of packaged food products in the market today.

Brand	**Product**	**Entry in NIP**	**Quantity and Units**
Lipton	Iced Tea	Flavonoid antioxidants	26mg/100mL
Pokka	Iced Tea	Catechins (antioxidants)	10mg/mL
Leggos	Tomato Paste	Lycopene	43mg/100g
Twinings	Tea	Flavonoid antioxidants	25-60mg/100mL
Ocean Spray	Cranberry Juice	Phenolic antioxidants	60mg/100mL
Nestle	Noir Chocolate	Total polyphenols (epicatechin equivalents)	Min 1000mg/100g
Nestle	Nescafe	Natural coffee polyphenols and melanoidin antioxidants	210mg/100mL
Uncle Toby’s	Plus Antioxidant Lift (cereal)	Vitamin A Vitamin E	200µg/100g (25% RDI) 2.5mg/100g (25% RDI)
Woolworths	Dark berry blend muesli	Antioxidant	1190TE* (*Trolox equivalent)

Other claims in the market

There are many other nutrient and health claims currently in the market. The following table gives some examples of different types of health claims on the market that are permissible and those that breach current regulations (Williams et al 2007).

Permissible	*Not permissible*
Assists in maintaining digestive health	Beta-glucan from oats can help the body control cholesterol levels
With calcium for strong bones and teeth	A diet rich in phytoestrogens may decrease the risk of breast cancer
Zinc is an important mineral that helps support kid's healthy growth	Can help with weight loss by keeping you fuller for longer
Provides sustained energy release	A low GI diet can help diabetics manage their blood glucose levels

Enforcement of Food Standards

Enforcement of the food regulations is the responsibility of the Health Departments in individual States. Generally the level of auditing of compliance is variable, and labelling breaches are not given a high priority for action, compared to food safety issues. A recent survey on the labelling of 7850 products in 47 different food categories found that more than 5% of health claims were not complying with the current regulations and that the standards were not being fully enforced (Williams et al, 2006). However, if consumers or competitors notify the departments of apparent breaches of standards, they will usually follow up these cases for action.

4.4 Part 3: Opportunities for Claims

NUTRIENT CLAIMS

The current Food Standards Code summarises the regulations on nutrient claims in Standards 1.2.8 (FSANZ 2002) with explanatory guidance about this in the Users Guide (FSANZ 2008a). The definition of a **nutrition claim** is:
a representation that states, suggests, or implies that a food has a nutritional property whether general or specific and whether expressed affirmatively or negatively, and includes a reference to:

a) energy, or
b) salt, sodium or potassium, or
c) amino acids, carbohydrate, cholesterol, fat, fatty acids, fibre, protein, starch, or sugars, or
d) vitamins or minerals, or
e) any other nutrient, or
f) a biologically active substance.

A nutrient claim (and values declared in the Nutrition Information Panel) must be based on the "average" level of the component in that product. This can be determined in a variety of ways:

a) Laboratory analysis
b) Food composition tables or databases, or
c) Calculation from the actual ingredients used in a product (eg a recipe)

In the case of fresh produce like vegetables this means the choice is between analysis of products or published data. Detailed discussion of these options is provided in the Users Guide to Standard 1.2.8 (FSANZ 2008a). However, given the likely variation in nutrient content by season and growing region, it would probably be unwise to rely on overseas published data alone, especially if nutrient function or health claims were to be made.

Of the nutrients found in leafy vegetables, the following are likely to be worth consideration for claims. However claims can only be made if a serve contains at least 10% of the RDI in Standard 1.1.1 - and it is up to the manufacturer to define a declare what is a serve size, and to have good data on the nutrient content.

Vitamin	**RDI**
Vitamin C	40mg
Vitamin A	750μg retinol equivalents (= 4500 μg beta carotene)
Folate	200 μg
Vitamin K	80 μg

It is likely that other nutrients (such a fibre or calcium would be unable to get to the level needed to make a claim. For example to be a source of fibre, foods need to contain 2g/serve. Leafy vegetables contain around 2g/100g, but a serve would normally be much less than this (1 cup = approx 20g).

Antioxidants

- *Baby spinach/rocket are a natural source of antioxidants*

Aside from specific vitamin and mineral claims, a number of products in the market are now making general antioxidant claims (eg, "*rich in antioxidants*". Such claims are not regulated within current Food Standards or the voluntary Code of Practice on Nutrient Claims, but they would fall under the category of claims about "bioactive substances" that will be referred to in the proposed new standard on health claims. In this standard it is proposed that manufacturers would need to define for themselves which components are antioxidants and the daily reference quantity of each substance that is recommended, and then products would need to contain at least 10% of that level per serve to justify a claim. If claims were to be made that a foods was a source of bioactive substances such as glucosinolates then One Harvest would need to be able to have scientific evidence for a recommended daily intake.

HEALTH CLAIMS

The following comments summarise the status of some potential types of claims that might be made for baby spinach and rocket, although many claims would need to be able to be substantiated with nutritional analysis information.

Folate

One of the pre-approved FSANZ nutrient function claims is that "Folate is necessary for normal blood formation", and this could be used on salad greens now, provided the folate level was declared in the NIP and contained more than 20 μg/serve (10% RDI in Food Standards Code). It would also be permissible to make a high level claim for the role of folate in the reducing the risk of neural tube defects, but this may not necessarily be an appealing message to put on a product targeted at a wide population. To make this high level claim, One Harvest would first need to apply to FSANZ to have their products added to the permitted list of products in Standard 1.1A.2 of the Food Standards Code.

If the analytical results show at least 20 μg/serve of folate in a product then One Harvest could make both content and nutrient function claims:

- *A source of folate*
- *Folate is necessary for normal blood formation*
- *Folate is a B group vitamin that is essential for normal cell growth and development*

Dietary guideline claims

- *Leafy salad vegetables form part of a healthy diet of fruits and vegetables. Eating a diet rich in fruits and vegetables as part of an overall healthy diet may reduce risk for stroke and perhaps other cardiovascular diseases*

The first sentence is fine; it is consistent with Dietary Guidelines for Australians (NH&MRC, 2003) and is permitted now as normal nutritional advice of a general nature.

The second sentence contravenes current regulations, even though it is a well established fact. However it is one of the claims that has been pre-approved for use in the proposed new health claims standards and could be used once the new standard came into force.

Antioxidant Function Claims

- *Baby spinach/rocket are a natural source of antioxidants which may reduce the risk of cancer*

Any claim about reducing the risk of a serious disease (like cancer or cardiovascular disease) is currently prohibited and would require pre-approval by FSANZ when the new standard comes into law. It is unlikely that there is sufficient clinical evidence yet to substantiate these claims adequately, although there are some promising animal research studies. Claims about antioxidants and their normal function in the body would be permissible, provided there was analytical data to demonstrate a reasonable amount present in a single serve. Such claims could be worded as Nutrient Function Claims as follows:

- *Spinach is a natural source of antioxidants vitamins A and C that help protect the cells from oxidative damage*
- *A natural source of the antioxidants – to help keep your body healthy*
- *Antioxidants in Spinach help protect the body against free radical damage.*

[*Note:* According to published data, Spinach, but *not* Rocket, would provide more than 10%RDI of vitamin A per 20g serve. Any claim for Rocket would therefore have to rely solely on vitamin C as an antioxidant, and this is probably insufficient to substantiate an antioxidant claim]

General health benefit claim

- *Baby spinach/rocket are a source of vitamin C, carotenoids and glucosinolates/isothiocyanates, which have many health benefits*

These types of Nutrient Content Claims are normally permissible, provided they are substantiated. The proposed standards for nutrient claims in the P293 are slightly different to the current voluntary standards and it would be prudent to ensure these standards are met before making such nutrient claims.

Comparative Claim

- *Baby spinach has twice as much beta-carotene as lettuce*

Comparative claims such as this are currently covered under the voluntary Code of Practice on Nutrient Claims (NFA, 1995). Normally there needs to be at least a 25% difference before a claim can be made *and* claims are meant to be restricted to comparisons between foods in the same food group. Thus a comparison between a vegetables and meat or dairy foods would normally be discouraged. As with all nutrient content claims, comparisons should take into account the amount per serve, not just the amount per 100g.

Other Claims

There are a number of possible nutrients or nutrient function claims that could be relevant to salad vegetables within current regulations, for example:

- All the goodness of one serve of vegetables
- All natural

Furthermore, although most vegetables are naturally low in fat, such claims are compelling for consumers (Chan et al, 2005) and there is no reason not to highlight the fact with claims such as:

- *Low fat*
- *Low in saturated fat*

Final Note

This review has not assessed whether any of these potential claims have sufficient scientific substantiation to support the claim. In the case of function and health claims this would involve a substantial body of work to search for and summarise all the relevant scientific studies. It is no longer sufficient to rely on single studies to support such claims; a food company has to demonstrate they have followed the FSANZ substantiation guidelines, which require a defined literature search strategy, evaluation of the quality of each study, and an overall assessment of the balance of evidence, with particular emphasis placed on human clinical trials.

4.5 References

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5 Scientific Review

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5.1 Introduction

Epidemiological evidence supports increased consumption of vegetables in the reduction of a number of chronic diseases including various cancers, obesity and cardiovascular disease (1-4). Baby leaf rocket and spinach are vegetables that have gained in popularity among consumers in the recent years and they contain important minerals, vitamins and bioactive compounds which may promote health.

The nutrient composition of vegetables is very intricate. The level of plant metabolites is influenced by genetic and environmental factors as well as storage and transportation. Other factors that may influence the level of metabolites are growth factors including soil, temperature, light, humidity and application of fertilizers and pesticides (5).

Vegetables, as well as fruits, contain a large number of phytochemicals, including glucosinolates, flavonoids and other polyphenols, phenolic acids, isothiocyanates, dietary fibre, phytosterols and monoterpenes, and these affect health with different mechanisms. Some phytochemicals have antioxidant properties like carotenoids and flavonoids. Vitamin C (ascorbic acid), Vitamin E and some enzymes also have antioxidant properties. Other modes of action of phytochemicals include antibacterial and hormonal actions as well as stimulating the immune system (6). Phytochemicals are generated in plants to protect them from damage and some of them also give plant foods their colour and flavour (7). Antioxidants protect against detrimental effects of free radicals and other reactive oxygen species. For example, the process of atherosclerosis involves the action of free radicals and oxidized low density lipoproteins (LDL) and leads eventually to cardiovascular diseases. Phytochemicals may also have anti-inflammatory activity and this may play a role for the healthy heart, as well as having anti-carcinogenic effects by minimising oxidative stress caused by free radicals and leads to DNA damage (8).

Other advantages associated with eating a diet high in vegetables are that it has a lower energy density, mainly due to the high water content of vegetables. The content of fibre may also influence this. The lower energy density of a diet high in vegetables enables people to eat more food at a given caloric level. It is also known that increasing the water content of a meal but maintaining the energy content, increases satiety. This means that a diet high in vegetables may play an important role in weight management (3).

Antioxidant capacity of foods

There are several ways to measure the total antioxidant capacity of food stuffs. The assays are divided into two main groups the hydrogen atom transfer (HAT) assays and the single electron transfer (SET) assays. HAT and SET reactions always occurs at the same time in a sample and all methods have their weaknesses and strengths.

The hydrogen atom transfer assays include: ORAC (Oxygen Radical Absorbance Capacity), TRAP (Total Trapping Antioxidant Parameter), TOSC (Total Oxidant Scavenging Capacity) and crocin bleaching assays. These assays measure the ability of an antioxidant to quench free radicals by hydrogen donation and can be completed fast, often within seconds. ORAC is one of the most common ways of measuring the antioxidant capacity of food stuffs.

The single electron transfer assays include: FRAP (Ferric Reducing Antioxidant Power), TEAC (trolox equivalence antioxidant capacity), 'total antioxidant capacity potential' using a cupper complex as an antioxidant (DPPH) and the Folin-Ciocalteu or total phenolics assay. These assays detect the possible ability of an antioxidant to transfer one electron to reduce any compound including metals and radicals. The electron transfer reactions are usually slow and require long times to be completed. (9, 10)

Rocket salad

- Total phenolics expressed as mg gallic acid/ 100 grams fresh weight for Rocket: 208.11 ± 18.32 and Wild Rocket: 100.08 ±10.63 (11).
- The antioxidant capacity measured by the TEAC assay: Glucsinolate: 1100 Trolox equivalents nM in high light while between 650-700 Trolox equivalents nM in low light. Flavonoid: 1200 Trolox equivalents nM. (12).

Spinach

- The antioxidant capacity measured by the ORAC assay was 2640 μmol of trolox unit equivalents per 100 gram fresh weight. In comparison total antioxidant capacity for strawberries is 3577 and for blueberries 6220 (7).
- Total phenolics expressed as mg gallic acid/ 100 grams fresh weight for spinach: ~90 ± 9. In comparison total phenolics expressed as mg gallic acid/ 100 fresh weight for broccoli 101.6 ± 1.24 (highest in study), red pepper 66 ± 5.7, cabbage 55 ± 4.3 and cucumber 19.5 ± 1.61 (lowest in the study) (13)
- Total antioxidant capacity measured by the TOSC assay: 42.2 ± 0.71 μmol vitamin C equivalents/ grams vegetable.

Heimler et al pointed out that various mixed salads, including different lettuce, chicory and rocket, can be regarded as a functional food when used fresh and in quantities of about 100 grams per serving (11). However, this statement should be considered in the context of what an actual serving size is. According to 'Australian Guide to healthy Eating' (14) one serve of a salad vegetables is one cup. One cup baby rocket and spinach weighs 20 grams and 25 grams, respectively.

5.2 Method for Scientific Review

A PubMed search using defined search terms was performed. All searches was limited to the English language and between 1996- 2009.

The following search terms were used: spinacia, diplotaxis and eruca. The search with spinacia was limited to humans since the original search without this limit rendered: 1789 articles. The searches rendered together 51+32 (rocket) 157 (spinacia) articles. The abstracts from these articles were then reviewed and 37 were then collected for review. All of these articles did not end up in this report since they were not found of interest. For nutritional information the United States (USDA), the Australian (NUTTAB) and Swedish Food Composition databases were accessed.

5.3 Phytochemicals

SPINACH

Spinach (*spinacia oleracea* L) is a leafy vegetable that belongs to the goosefoot family (Chenopodiaceae) and probably originated from Southwest Asia and the Western Himalayas, but wild varieties also grow in North Africa and Iran (15). Baby spinach is sown at closer density and is harvested earlier, than other varieties (6). Anti-proliferative activity of 10 commonly consumed vegetables (spinach, broccoli, red pepper, carrot, onion, cabbage, potato, celery, lettuce and cucumber) was studied in an *in vitro* model using $HepG_2$ human liver cancer cells. This study showed that spinach had the highest anti-cancer effect *in vitro*, followed by cabbage, red pepper, onion and broccoli (13) .

Glycolipids

A research group from Kobe-Gakuin University in Japan has in a series of experiments investigated glycolipids purified from Spinach. Plant glycolipids such as monogalactosyl diacylglycerol (MGDG), digalactosyl diacylglycerol (DGDG) and sulfoquinovosyl diacylglycerol (SQDG) have anticancer functions. Spinach is especially high in SQDG. (16-19). One recent paper showed that 20 mg/day of an orally administered spinach glycolipid fraction effectively suppressed colon tumour growth in mice. This fraction also suppressed the growth of cancer cells from human and mice in vitro. The authors suggested that this spinach fraction has the potential to become a functional food with anti-cancer effects (18).

Carotenoids

The carotenoids are a class of naturally occurring phytochemicals with biological activity and more than 600 natural caroteinoids exist. Carotenoids are divided into oxygenated xanthophylls like lutein and zeaxanthin and the hydrocarbon carotenes like α- and β-carotene and lycopene (20). Spinach contains carotenoids from both groups. Carotenoids have antioxidant properties and are converted to retinol and retinoids in the body. Lutein for example prevent macular degeneration and zeaxanthin may prevent macular degeneration and certain cancer types (7).

In a small dietary controlled intervention nine female subjects consumed a basal diet low in carotenoids. This diet was then supplemented with spinach for three weeks, followed by a wash-out of two weeks and subsequently followed by an additional three week period with a basal diet supplemented with both spinach and tomato. The diets influence on oxidative stress, measured as DNA damage in lymphocytes, was investigated. The study showed that DNA damage was reduced both during the only spinach and the spinach and tomato period, but the latter period did not confer an additional protection. It was not possible to determine which carotenoid (or any of the other bioactive compounds in the vegetables) that mediated the effects (21).

In the US health professionals' study the intake of carotenoids were prospectively evaluated in relation to the risk of extraction of cataracts. Intakes of lutein and zeaxanthin, but not other carotenoids, were associated with a 19% lower risk (in the highest vs. lowest quintile) of cataract extraction. Broccoli and spinach was most consistently associated with a lower risk of cataract in this study (22).

In a population based case-control study (6888 cases and 9428 controls) in the US, the association between the intake of β-carotene and Vitamin A on the risk of breast cancer was investigated. Consumption of carrots or spinach more than twice weekly was associated with a significant lower risk (~40 %) of breast cancer. Total vitamin A was also associated with a deceased risk of breast cancer and the intakes of spinach and carrots contributed mostly to total vitamin A intake (23).

Flavonoids

At least 10 flavonoid glycosides have been reported in spinach. Flavonoids act as antioxidants by means of free-radical scavenging. Spinach looses some of its antioxidant capacity when stored and boiling reduces the total content of flavonoids (50%) (24).

The natural antioxidant mixture (NAO) in spinach leafs is a potent inhibitor of lipid peroxidation. The NAO mixture is made up by flavonoids and coumaric acid and has antioxidant capacity. NAO may have antitumor and anti-inflammatory effects. Studies in experimental animals have shown beneficial effects of NAO on prostate (25) and skin cancer. (24)

Prospective study of prostate cancer and intake of spinach

The association of prostate cancer risk and intake of fruits and vegetables was studied in a prospective study consisting of 29 362 men. 1338 of the participants were diagnosed with prostate cancer. The mean follow-up time was 4.6 years. The study participants completed a food frequency questionnaire at baseline and special emphasis on groups of vegetables was undertaken. The intakes of vegetables and fruits were categorized into quintiles. The study showed some evidence that aggressive prostate cancer decreased with increasing spinach intake but the relation was non-significant when restricted to extraprostatic disease (non-organ-confined prostate cancer).(26)

ROCKET SALAD

Rocket salads belong to the cruciferae or brassicaceae family (crucifer vegetables) and are a close relative of mustard. Other vegetables in the cruciferae family are: cabbage, broccoli, cauliflower, turnip, rapeseed, mustard, radish, horseradish, cress and watercress. In ancient Egypt and the Roman Empire rocket leaves were considered to be aphrodisiac (15). Both salad rocket (*Eruca vesicaria*) and wild rocket (*Diplotaxis tenuifolia*) exists. Rocket is called arugula in American English.

<u>Antioxidants</u>

Flavonoids

Many flavonoid compounds have been detected in rocket. The main flavonoids are quercetin, kaempferol and isorhamnetin, but there is also significant accumulation of cyanidin in high light (12). Quercetin derivatives and kaempferol were the main flavonoid compounds in wild rocket and salad rocket, respectively. Isorhamnetin derivatives were found in both types of rocket (27).

The content of flavonoids is influenced by growing conditions. For example, there was a 15 fold higher production of flavonoids in rocket when grown in sunlight compared to when grown in shaded areas. Thus, this means that flavonoids are produced in the plant in response to stress, for example exposure to sun light (12)

Polyphenols and Vitamin C

The content of polyphenols in rocket salads was higher than in green lettuce, some broccoli, cabbage, chinese cabbage and brussel sprouts. The content of polyphenols may be influenced by many factors such as genetic factors, environmental influences, growing period and maturity stage at harvest. The content of vitamin C was higher in wild rocket 103mg/100g than in salad rocket 80mg / 100g, which is much higher than indicated in the food composition databases on page 11. The anti-oxidant activity of salad rocket was found to be 88mg/100g (27).

One study investigated the potential gastric anti-ulcer properties of rocket in an experimental rat study. There was a significant dose-dependent reduction in basal gastric acid secretion, titratable acidity and ruminal ulceration in rats after receiving rocket extract. The extract had antioxidant effects and appeared to strengthen the mucosal barrier. Phytochemical screening of the extract indicated presence of flavonoids, sterols and/or triterpenes (28).

Glucosinolates

Both *Eruca* and *Diplotaxis* have characteristic flavour and odour due to the presence of the thiol-containing glucosinolate, glucostavin (4-mercaptobutylglucosinolate) and the isothiocyanate derived from this glucosinolate. One study found that Italian *Eruca sativa* had consistently high total glucosinolate content and highest percentage (29). Gluconsinolates may protect against degenerative disease and many intact gluconsinolates have a bitter taste. Further, some isothiocyanates produce a burning sensation in the mouth. Thus, the higher the content of gluconsinolates in the rocket salad is, the less acceptable it is in taste. One experiment showed that none of the rockets salads high in gluconsinolates belonged to the sensorial pleasant categories.(30)

In a review article from 2007, Higdon et al (31), presents the epidemiological evidence and mechanistic basis for the association between intake of cruciferous vegetables and various types of cancer. Cruciferous vegetables are rich in a variety of nutrients and phytochemicals that may act in concert to help prevent cancer. Especially, cruciferous vegetables are rich sources of glucosinolates. When cruciferous vegetables are chopped or chewed bioactive glucosinolate hydrolysis products, such as isothiocyanates and indole-3-carbinol, are formed. Although some prospective cohort studies have reported individuals with a higher intake of cruciferous vegetables to have less cancer, the evidence for an inverse association between cruciferous vegetables and cancers of the lung, bowel, breast and prostate are not conclusive. There is evidence that genetic variation in genes that affects the metabolism of glucosinolate hydrolysis products may influence the effects on cancer risk.

Erucin

Erucin (ER) is a dietary isothiocyanate (ITC) found in cruciferous vegetables. ER is obtained from hydrolysis of glucoerucin, a glucosinolate found in rocket, but also through reduction *in vivo* of sulforaphane (SF) derived from broccoli. The *in vivo* inter-conversion and similar structures suggest similar biological activity (32).

One study investigated the chemopreventive effects of ER in a lung cancer model. This study showed that ER was able to up-regulate the protein expression of p53 and p21, which inhibits proliferation of human lung cancer cells. Both isothiocyanate significantly increaseed p21 levels at high concentrations, while SF was a stronger inducer of p21 protein than ER. However, ER increased levels at a lower concentration (but not statistically significant) (32) .

Conclusion Phytochemicals

Both spinach and rocket are sources of many phytochemicals including glucosinolates, flavonoids, glycolipids and carotenoids with potential health effects. There is evidence that a high intake of spinach and cruciferous vegetables may be beneficial for cardiovascular diseases and several cancers, although the evidence is not conclusive. In addition, spinach had the highest anti-proliferative activity *in vitro* among 10 commonly consumed vegetables. Many phytochemicals have antioxidant properties that confer health promoting effects. It is, however, difficult to report the exact total antioxidant capacity of baby rocket and spinach, since there are several methods to measure the antioxidant capacity of food stuffs and this is also influenced by growing conditions.

5.4 Nutrients

Nutrient data

The most complete nutritional information on raw spinach (spinacia oleracea) and rocket (Eruca sativa) was found in the United States USDA national nutrient database for standard reference (release 21, 2008 (33)) and the Swedish Food composition database (Swedish Food Administration, version 2009-05-19 (34)). The nutritional content of rocket was the same in the two databases but differed to some extent for spinach. The nutrient value for spinach was 97 KJ vs. 73 KJ per 100 grams in the American and Swedish databases, respectively. However, for many micronutrients the values were comparable. In the Australian nutrient database (NUTTAB 2006 (35)) only information on spinach was found and the nutrient values were comparable to the American values.

To be able to compare the nutrient content between databases for rocket and spinach nutrient values from the US, Australian and Swedish nutrient tables are provided below. Also included in the tables are the recommended dietary intakes (RDI) and average intakes (AI) or estimated safe and adequate daily dietary intakes (ESADDI) according to the NHMRC (36) and the Food Standard 1.1.1 (37). The Recommended Dietary Intake (RDI) is the average daily intake set to be sufficient to meet the nutrient requirements of nearly all (97-98%) healthy individuals in a particular life stage and gender group. The Adequate Intake (AI) gives the average daily nutrient intake when RDIs cannot be determined (38).

A nutrient content claim can at present be made if the food provides 10% (source claim) or 25% (good source/high claim) of RDI set by the Food Standard 1.1.1(37) by Food Standards Australia New Zealand (FSANZ). One portion of baby spinach and rocket was based on the dry fresh weight of a cup of vegetables (measured on scales). A cup is the suggested serving size in the ‘Australian Guide to healthy Eating’ (14) for salad vegetables. One cup of baby spinach weighed 25 grams and one cup of baby rocket weighed 20 grams. The manufacturer gives the portion sizes of 30 grams and 50 grams for baby rocket and spinach on the packages, respectively. We believe that these are a bit big, since very few people would consume such big portions of fresh salad.

SPINACH (*spinacia oleracea*)

USDA United states national nutrient database for standard reference, NUTTAB Australian nutrient database
SWE FCT Swedish Food composition tables, RDI recommended dietary intakes, AI average intakes, ESADDI estimated safe and adequate daily dietary intakes. * Meets the requirement for a source claim-10% of RDI/ ESADDI set out by FSANZ(37)

Nutrient	*USDA*	*NUT TAB*	*SWE FCT*	*Unit*	*Per serving (1cup= 25 g)*	*RDI and AI for adults 19-50 years NHMRC (36)*		*RDI and ESADDI FSANZ (37)*	*Meets the requirement for a source claim-10% of RDI**
						Men	Women		
Fiber	2.2		1.3	g	0.55	30	25		
Energy	97	68	73	kJ					
Energy	23		18	kcal					
Protein	2.86	2.6	1.9	g	0.72	64	46		
Carbohydrate (Sugars)	0.42	0.7	0.7	g					
Total Fat	0.39	0.3	0.5	g					
SFA	0.063		0.1	g					
MUFA	0.010		0.0	g					
PUFA	0.165		0.3	g					
C18:2	0.026		0.1	g					
C18:3	0.138		0.2	g					
Monosaccharides			0.2	g					
Disaccharides			0.1	g					
Sucrose	0.07		0.1	g					
Glucose (dextrose)	0.11								
Fructose	0.15	0.2							
Galactose	0.1								
Retinol equiv.	469	336	275		117	900	700	750	X-16%
Retinol	0	0	0	µg					
Vitamin A (IU)	9377			IU					
Vitamin E		1.2	2.90	mg					
a-Tocopherol	2.03	1.2	2.90	mg	0.51	10 AI	7 AI	10	
γ-Tocopherol	0.18			mg					
Carotene (β)	5626	2018	3 300	µg					
Carotene (α)		60		µg					
Thiamin	0.078	0.07	0.10	mg	0.02	1.2	1.1	1.1	
Riboflavin	0.189	0.18	0.28	mg	0.05	1.3	1.1	1.7	
Vitamin C (ascorbic acid)	28.1	29	46	mg	7.0	45	45	40	X-17.5%
Niacin equiv		1.1	1.2			16	14		
Niacin	0.724	0.4	0.9	mg	0.18			10	
Vitamin B6	0.195	0.15	0.20	mg	0.05	1.3	1.3	1.6	
Folate	194	120	194	µg	49	400	400	200	X- 24.5%

SPINACH (*spinacia oleracea*) continued

USDA United states national nutrient database for standard reference, NUTTAB Australian nutrient database

Nutrient	*USDA*	*NUT TAB*	*SWE FCT*	*Unit*	*Per serving (1cup= 25 g)*	*RDI and AI for adults 19-50 years NHMRC (36)*		*RDI and ESADDI FSANZ (37)*	*Meets the requirement for a source claim-10% of RDI**
	Per 100 grams					Men	Women		
Biotin		2.3		μg	0.58	30	25	30	
P	49	43	30	mg	12	1000	1000	1000	
Fe	2.71	3.5	2.00	mg	0.68	8	18	12	
Cu	0.130	0.041		mg	0.033	1.7 AI	1.3 AI	3.0	
K	558	623	560	mg	140	3800	2800		
Ca	99	58	93	mg	25	1000	1000	800	
Mg	79	74	79	mg	20	420	320	320	
Na	79	23	6	mg	20	2300	2300	8	
Mn	0.897	0.729		mg	0.22	5.5 AI	5.0 AI	5.0	
Se	1.0	0.0	0.5	μg	0.25	70	60	70	
Zn	0.53	0.7	0.90	mg	0.13	14	8	12	
Ash	1.72	1.7	1.6	g					
Water	91.4	92.9	94.0	g					
Vitamin K	482.9			μg	121	70 AI	60 AI	80 AI	X- 151%
I		9.0	1.0	μg		150	150	150	
Lutein+ zeaxanthin	12198			μg	3050				
Crypyoxanthin		38		μg					
Choline	18.0			mg	4.5	550	425		
Betaine	550.4			mg	138				
Phytosterols	9			mg	2.25				
Malic acid		0.1		g					
Oxalic acid		0.3		g					
Alanine	142	136		mg					
Arginine		132		mg					
Aspartic Acid	240	254		mg					
Cystine + Cysteine		44		mg					
Glutamic Acid	343	268		mg					
Glycine	134	137		mg					
Histidine	64	56		mg					
Isoleucine	147	104		mg					
Leucine	223	195		mg					
Lysine	174	140		mg					
Methionine	53	39		mg					
Phenylalanine	129	119		mg					
Proline	112	100		mg					
Serine	104	117		mg					
Threonine	122	118		mg					
Tryptophan	39	38		mg					
Tyrosine	108	93		mg					
Valine	161	138		mg					

SWE FCT Swedish Food composition tables, RDI recommended dietary intakes, AI average intakes, ESADDI estimated safe and adequate daily dietary intakes. * Meets the requirement for a source claim-10% of RDI/ ESADDI set out by FSANZ(37)

RAW ROCKET (*Eruca sativa*)

Nutrient	*USDA*	*Swedish food comp tables*	*Unit*	*Per serving (1 Cup=20 grams)*	*RDI and AI for adults 19-50 years NHMRC (36)*		*RDI and ESADDI FSANZ (37)*	*Meets the requirement for a source claim-10% of RDI**
	Per 100 grams				Men	Women		
Fiber	1.6	1.7	g	0.32	30	25		
Energy	105	115	kJ					
Energy	25	28	kcal					
Protein	2.58	2.6	g	0.52	64	46		
Carbohydrate (Sugars)	2.05	2.0	g		NA	NA		
Total Fat	0.66	0.7	g					
SFA	0.086	0.1	g					
MUFA	0.049	0.1	g					
PUFA	0.319	0.3	g					
C16:0	0.072	0.1	g					
C18:1	0.046	0.1	g					
C18:2	0.13	0.1	g					
C18:3	0.17	0.2	g					
Retinol equiv.	119	118		23.8	900	700	750	
Retinol		0	µg					
Vitamin A, IU	2373		IU					
Carotene (β)	1424	1 424	µg					
Vitamin E		0.43	mg					
a-Tocopherol	0.43	0.43	mg	0.09	10 AI	7 AI	10	
Thiamin	0.044	0.04	mg	0.009	1.2	1.1	1.1	
Riboflavin	0.086	0.09	mg	0.017	1.3	1.1	1.7	
Vitamin C (ascorbic acid)	15.0	15	mg	5	45	45	40	X-12.5%
Niacin equiv		0.7			16	14		
Niacin	0.305	0.3	mg					
Vitamin B6	0.073	0.07	mg	0.015	1.3	1.3	1.6	
Vitamin B12	0.00	0.00	µg	-	2.4	2.4	2.0	
Folate	97	97	µg	19.4	400	400	200	X-9.7%
Pantothenic acid	0.437		mg	0.09	6.0	4.0	5.0	
P	52	52	mg	10	1000	1000	1000	
Fe	1.46	1.46	mg	0.3	8	18	12	
Cu	0.076		mg	0.015	1.7 AI	1.3 AI	3.0	
K	369	369	mg	73.8	3800	2800		
Ca	160	160	mg	32	1000	1000	800	
Mg	47	47	mg	9.4	420	320	320	
Na	27	27	mg	5.4	2300	2300		
Se	0.3	0.3	µg	0.06	70	60	70	
Zn	0.47	0.47	mg	0.09	14	8	12	
Mn	0.321		mg	0.06	5.5 AI	5.0 AI	5.0	
Ash	1.4	1.4	g					
Water	91.7	91.7	g					
Vitamin K	108.6		µg	22	70 AI	60 AI	80	X-27.5%
Lutein+ zeaxanthin	3555		µg	711				
Choline	15.3		mg		550	425		
Betaine	0.1		mg					

USDA United states national nutrient database for standard reference, NUTTAB Australian nutrient database
SWE FCT Swedish Food composition tables, RDI recommended dietary intakes, AI average intakes, ESADDI estimated safe and adequate daily dietary intakes. * Meets the requirement for a source claim-10% of RDI/ ESADDI set out by FSANZ(37)

One serving of baby spinach and rocket provides more than 10% of the RDI set up by FSANZ for the following nutrients:

	Serving Size:
Baby Spinach	25 grams
• retinol equivalents-Vitamin A	16%
• Vitamin C	17.5%
• Folate	24.5%
• Vitamin K	151%
Baby Rocket	20 grams
• Vitamin C	12.5%
• Folate	9.7%
• Vitamin K	27.5%

Even if the serving sizes used by the manufacturer would be used, the nutrients provided in larger quantities than 10% would not be much different. The only nutrient that would be added for spinach would be α-tocopherol. For baby rocket there would be no change. Also, all the nutrients provided in spinach, except α-tocopherol, would meet the requirement of 25% of the RDIs for a 'good source/high' claim. Below follows a short description of the functions of nutrients provided by baby spinach and rocket in quantities larger than 10%.

Vitamin A

Vitamin A is a generic term for several substances that has vitamin A activity and is a fat soluble vitamin. Retinol and β-carotene are the main sources of vitamin A in animal and vegetable foods, respectively. Vitamin A is needed for our eye sight, reproduction and for the normal differentiation and stability of the mucosal production of mucus. Vitamin A is also important for the maintenance of the immune system. (39)

Vitamin C

Vitamin C is the collective term for ascorbic acid and its derivates. Vitamin C is a water soluble vitamin and is an antioxidant. Up to an intake of 60 mg per day, no vitamin C is excreted in the urine. However when the intake is about 100 mg per day, 25% is excreted. Vitamin C is needed for the synthesis of collagen, which is an important connective tissue in the skin, skeleton, teeth and blood vessels. Ascorbic acid is involved when cholesterol is converted to bile salts and when the amino acid tryptophan is converted to serotonin. Absorption of iron is more effective in the presence of vitamin C rich foods. (39)

Vitamin K

Vitamin K is the generic name of several quinone derivates, which are dived into two main groups. The phylloquinones which are found in green plants and the menoquinones which is synthesized by bacteria in the small intestine and to a smaller extent exist in animal tissue. About 50% of the vitamin K need is provided by the endogenous production in the small intestine. Vitamin K is needed for blood coagulation and participates in chemical reactions (carboxylation) in several tissues. (39)

Folate

Folate exists in several forms, which all have folic acid activity and are known under the generic name folacin. Folacin is a provitamin that has to be converted to its biologically active form in the body. Folate in plant foods are present in the conjugated form that needs to by hydrolysed to exert its biological effect. Folate is needed for cell division and protein synthesis. Without folate, DNA synthesis slows and cells division halts. The first sign of folate deficiency is megaloblastic anaemia. Neural tube effects may also be caused by folic acid deficiency. (39)

α-tocopherol

Vitamin E is the generic form of eight similar structures divided into the tocopherol and tocotrienol forms. α-tocopherol is the most common form of vitamin E in nature and has also the highest biological activity. Vitamin E is a fat soluble antioxidant and helps to protect unsaturated fatty acids in cellular membranes from oxidation. Vitamin E also protects low-density-lipoprotein (LDL) from oxidation. Vitamin E is needed for our immune system as well as for the protection of the nervous system, skeletal muscles and the retina of the eye from oxidative damage. (39)

5.5 Conclusions about Nutrients

Baby spinach is a good source of Vitamin A, Vitamin C, Folate and Vitamin K, at a serving size of 25 grams. If the serving size is increased to 50 grams, one additional nutrient, α-tocopherol is provided in a quantity more than 10% of RDI set by FSANZ.

Baby rocket is a good source of Vitamin C, Folate and Vitamin K and if the serving size is increased from 20 grams to 30 grams, this does not add any extra nutrients that would be s provided in a quantity more than 10% of RDI set by FSANZ.

5.6 Recommendations about Nutrients

Based on the literature review and evaluation of the nutrient content for spinach and rocket the following nutrients and phytochemicals are advised to be analysed:

Spinach: energy content, vitamin A, Vitamin C, Folate, Vitamin K, total antioxidant capacity.

Rocket: energy content, vitamin C, Folate, Vitamin K, total antioxidant capacity and potentially characterisation of other phytochemicals such as glucosinolates.

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6 Consumer Focus Groups to Determine Baseline Knowledge about Nutritional Value of Baby Leaf Rocket and Spinach

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6.1 Summary of Results

To obtain as broad a picture as possible of aspects that might impact the sales and marketing of leafy green vegetables (baby leaf spinach and rocket) three age related focus groups were conducted with twenty three participants: 50 +, 35-5- and 25-50. Participants were asked to comment on a range of areas impacting food shopping, vegetable selection and nutrition knowledge about these products.

Overall consumers viewed vegetables in particular leafy green vegetables as critical in supporting a healthy diet. Three key themes emerged that provided opportunities and hurdles for baby leaf spinach and rocket to be included in consumers dietary repertoires. They related to typical shopping behaviours; variety of vegetables normally selected; and perceptions of nutrition benefits of these products.

Recommendations to enhance the sales and marketing for baby leaf spinach and rocket include:

1. Promote quality management protocols for pre-packaged baby leaf spinach and rocket to ensure consumer confidence

2. Market nutritional profile of baby leaf spinach and rocket specifically vitamin (Vitamin A pre-cursers – beta-carotene, Vitamin C, Vitamin K and folate) and other phyto-nutrients with antioxidant activity and contribution to the diet.

3. Promote the versatility of baby spinach and rocket by highlighting opportunity for use within hot and cold dishes.

6.2 Materials and Methods

This research aimed to clarify consumer understanding of the nutritional quality of leafy green vegetables (baby spinach and rocket), their roles in the diet and perceptions of their use, convenience and nutritional contribution to the diet.

Twenty three females representing main grocery buyers attended three age related focus groups: 50 + (F.G.1), 35 - 50 (F.G. 2), 25 - 35 (F.G. 3) (n=10, 6 and 7 respectively). Participants were asked to comment on a range of areas including: key consideration points for food shopping; how vegetables specifically leafy green vegetables (baby spinach and rocket) featured as part of their food choice selection; any perceived benefits to leafy green vegetables (baby spinach and rocket) consumption; any issues that may prevent selection and their responses to nutrition messages focusing on nutrient content, nutrient function and health benefits.

6.3 Key findings

Overall consumers clearly viewed vegetables as critical in supporting a healthy diet and in particularly green vegetables. Leafy green vegetables were generically ranked as having priority as part of vegetable intake. Consumers were familiar with, and reported regularly incorporating leafy green vegetables particularly baby spinach as part of their food selection. Variations to self reported intake and acceptability were evident related to life-stage and needs.

A number of hurdles and opportunities were identified in relation to the selection of leafy green vegetables (baby spinach and rocket) which pertained to three key themes: shopping behaviours; variety of vegetables in consumers' repertoires; and perceptions of nutritional value of baby spinach and rocked.

1. *Shopping behaviours*

These shopping behaviours were dictated by time constraints for many consumers which inherently dictated use of retail supermarket chains or shopping malls for convenience. However there was a strong reported demand for access to quality produce from price competitive suppliers. This created tension for consumers who reported a higher level of trust in speciality green grocer suppliers being able to meet the need for both quality and price. Specifically consumers reported being more confident with quality of baby spinach and rocket from speciality green grocers when they were able to select their own from loose leaf containers. Negative comments about the unreliability of quality in pre-packaged baby spinach and rocket particularly from retail supermarket chains and the associated cost were reported by all focus groups. This was linked to perceptions of wastage with pre-packaged baby leaf spinach and rocket for the 50 + age groups due to the large one size that was generally available.

2. *The variety of vegetables*

The variety of vegetables consumers included in their dietary repertoires was linked to a number of important factors including: understanding of cuisines – familiarity and skills in producing a range of meals; food allergies both real and perceived; and the acceptance by significant others. Consumers became engaged and eager when sharing ideas about the versatility of baby spinach and rocket in range of meals and embraced ideas to provide for significant others with or without their overt acceptance.

3. *Perceptions of nutritional value for baby leafy spinach and rocket*

Nutritional values for baby leaf spinach and rocket was invariably linked a number of key factors. This included consumers' knowledge of the role and requirement in the diet; awareness of current social trends in food selections; acceptability of new nutrition claims about their attributes and relationship with health and disease management and finally taste preferences. Rocket was notably reported as being less regularly used by most consumers with the exception of those who included more Asian style recipes and were regular consumers of mixed leaf salads. Consumers often commented on the more powerful flavour of rocket which a number of consumers reported being too strong for their own and their family's palate.

The following sections will explore these three themes in-depth and provides commentary from focus group participants to substantiate the findings.

Shopping Behaviour

Time poor consumers reflected that the vegetable suppliers accessed can be dictated by convenience rather than any other driver.

> " It's more , it's convenient and I don't have time to be running up to Shell Harbour Square or somewhere else just to get fruit and vegies" F.G. 1

Consumers who indicated that they were impulse buyers also highlighted that convenience was often a driver.

> "I'm not much of a pre-planner when it comes to cooking so usually it's an hour before or the day before or something so yeah the one stop shop suits me" F.G. 3

This offers a specific recognised benefit for baby spinach and rocket to be included as a healthy vegetable meal option due to the ease with incorporating baby spinach and rocket into meals with minimal preparation. This was particularly recognised by the 25-35 and the 35 -50 years age group with comments like;

> "The fact is (it's) relatively easy to play with, it's not a complicated vegetable" F.G. 3

> "Really easier to prepare, its in the bag, you just pull it out!" F.G. 2

However there was a strong emphasis on quality over convenience and price reflected in all groups. Consumers indicated that trusted suppliers was a major consideration to obtain quality produce and was coupled with the belief that trusted suppliers were still able to be price competitive. One participant commenting on her green grocer stated:

> "I find their products come in better than Woolworths and even price wise they're actually a grocery store that can keep their prices below Woolworths…its a lot fresher…stuff in Woolworths you find it goes off after a day or two you know and you're paying high prices for it" F.G. 1

Trust and familiarity with the suppliers management of product and turnover was also something mentioned across all groups. Baby leafy greens particularly the pre-packaged variety was a product that had reported issues. Most consumers reported a preference for loose leaf spinach due to the concern about lack of consistency in quality. Participants highlighted this issue with comments like:

> "How long will it last? (*pre-packaged from the supermarkets)* And if I go to Crinis (*local green grocer*) I can just buy a little or a lot depending on when I'm going back, and we do tend to use it, use it a lot" F.G. 2

> "I bought a packet once from Coles and it wasn't really pleasant" F.G. 1

This issue of quality was inherently tied to price acceptability with pre-packaged baby leafy greens (spinach and rocket) and broader issues of portion size and wastage.

> "Woollies is alright except it has those big bags of spinach…and they are nearly four bucks and they are far more spinach than I want to eat so that's a bit of a crime" F.G. 1

> "It's nearly $4.00 I think for packaged salad….and they are rotten and you can't tell" F.G. 2

However one participant particularly mentioned concerns about aspects of food safety and texture was the main reason for her choosing pre-packaged baby spinach which was clearly not considered by other participants.

> " I am one of those ones that buy pre-packaged because usually it is washed, I only buy it if its washed, I don't like loose leaf stuff somehow I don't think its as crunchy" F.G. 2

This prompted other participants to recognise food safety as an issue for loose leaf vegetables

> "you don't know what other people are doing with it" F.G. 2

The younger participants 25-35 specifically raised confusion about food safety for pre-packaged salad vegetables for pregnant women with comments like:

> "Pre-packed contains something too doesn't it, when your pregnant you're not to buy pre-packed salads, there is something in it"

Other themes of interest raised about shopping behaviour included consumers who had a specific ethical stance related to retail monopolies and scepticism about pricing structures with comments like:

> "I wouldn't mind paying for it if I knew the farmers were getting it but they're not and I wonder where the profit is going?"

This was followed up by a few participants commenting on the pricing and declaring that they would try to grow their own to meet their specific supply needs, quality expectations and price concerns.

> "I eat so much of it and I find it's quite expensive, rocket. It can be so, yeah I'm going to grow it" F.G. 2

Some consumers indicating they bought their selection of vegetables out of habit and the discussion itself highlighted the option to incorporate foods like baby spinach and rocket more regularly within their diet. Others mentioned that they were committed to buying vegetables in season and often not considering baby spinach and rocket considering them more as salad vegetables.

"I find in winter I don't really feel like salad and I think it's great if you are trying to lose weight, I just find I gravitate to the root vegetables more" F.G. 1

Variety of Vegetables in Consumers' Repertoire

From the role as the main grocery buyer and often a carer's responsibility for the general wellbeing of the household there were a number of themes identified that linked the variety of vegetables that appeared within the dietary repertoire. The role of significant others more often husbands or partners for the 50 + age group and children for the younger age groups 25-35 and 35- 50 played a critical role in what vegetables were considered overtly for meals.

For instance:

"I have a husband who doesn't like a lot of vegetables, give him his peas, beans, and carrots….I love any vegetable…but I'm kind of sneaky like I'll grate some (in) when I make minestrone" F.G. 1

"Alana she is pretty good but there's a lot of veggies that she doesn't like before she has even eaten them because she doesn't like the flavour or the texture…so you have to get pretty creative at hiding your veggies and stuff" F.G. 3

Single consumers also reflected on the influence of flatmates and friends on types of vegetables consumed.

"My friends and I eat a lot of Asian food, like if we go out that's what we're eating, it's always a veggie dish, veggies and tofu" F.G. 3

Allergies were reported as a reason for eliminating certain vegetables from the diet with a broad spectrum reported including capsicum, onions, celery, Brussels sprouts, avocados and garlic. However one participant reported:

"My grand-daughter she's allergic to peas, beans, she can't have cabbage and she can't have spinach, she's allergic to every green vegetable" F.G. 1

However there was genuine confusion about allergies for vegetables with one participant declaring:

"I wouldn't think of vegetables would been a high allergy" F.G. 3

"It seems weird to be allergic to vegetables from my perspective… because they're natural"

This natural whole food perspective ensured that consumers responded particularly well to loose leaf baby spinach and rocket.

"Well it's natural and it doesn't come out of a can or a plastic bag…all the nutrients aren't taken out in the process"

Familiarity, experience and knowledge about cuisines were themes that were clearly inherently linked to the types of vegetables incorporated within the diet. The older age groups 50 + were clearly more familiar with silver beet (often referred to as spinach) and acknowledged that the changing cuisines within Australia had broadened the types of green leafy vegetables consumed.

"But if you go back years and years ago like I'm going back about twenty years now you wouldn't have heard of rocket, you only had spinach(*meaning silver beet)* and that's all you got" F.G. 1

"And I had a recipe with baby spinach in it and I thought what the hell is baby spinach" F.G. 1

Discussions in all groups even the 25-35 years age group indicated confusion regarding the varieties of leafy green vegetables and may add to confusion in marketing messages.

"Spinach and silver beet - are not the same thing?" F.G. 3

"Silver beet it is tall too - isn't it but not as tall?" F.G. 1
'That's a language thing isn't it - silver beet and baby spinach"

Experience and skills in cooking were clear hurdles for some in understanding how to incorporate a broader variety of fresh produce and limited ideas on how to incorporate baby spinach and rocket in the diet.

"I look at all these wonderful array of things in the fruit market and I think, what do you do with that you know and I know that is sounds really stupid" F.G. 1

"I feel intimidated by vegetable…but my mother cooked the same veggies and it was like potato, things you're not supposed to have a huge amount of" F. G. 1

"I'm a pretty basic cook so it's salad with baby spinach and rocket" F.G. 3

However others were more experienced and adventurous with their cooking

"I love cooking green vegetables and (making) different…concoctions as I call it" F. G. 1

"And I like cooking a lot of Asian style foods so I prefer rocket" F.G. 2

"I eat rocket and spinach quite a lot especially in hot dishes I always put it through risottos for a bit of colour" F.G 3

The more other participants talked about uses for baby spinach and rocket and tips on how to add to recipes the more engaged other participants indicating an inherent interest with increasing their use in the diet offerings.

"Yeah I hadn't thought about it into stir frys, and things like that, just thought of it more as a salad" F.G. 3

"I'm moving in with you, you can cook for me, I'm moving house" F.G. 1

The discussion opened up the versatility of baby spinach and rocket and the option for incorporating into hot dishes

"I think its one of those things that people just don't know how much you can use it…they think of salad and you just use it for salad…I didn't know until now..(baby spinach and rocket) was going in this and that and it was like ooh that's a great idea, yeah" F.G. 3

The role that baby spinach and rocket can play to enhance a meals appearance, taste and texture was also identified in the discussion across all groups

> "Colour and appearance is really important too. So I like the look of rocket and spinach in salads" F.G. 2

> "Appearance …even in restaurants you see them used and on the plate" F.G. 3

Perceptions of Nutritional Value of Baby Spinach and Rocket

The nutritional value of baby spinach and rocket was clearly supported by the general halo effect of goodness of vegetables in general and particularly in relation to green vegetables overall. General knowledge about nutrients contained within baby spinach and rocket was variable and cautiously linked to fibre and generally to vitamins with one specific reference to folate.

> "I'd like to think that there's fibre in there, I'm not sure if there is but I would like to think it is there" F.G. 2

Two participants cited them as a source of antioxidants without elaborating on their role. However the response to claims including reference to antioxidant content did meet with some mixed reaction particularly in the 35-50 and 50 + age group who were more sceptical about their role and value in the diet related to its perceived 'faddish' use within marketing and media commentary.

> "The anti-oxidant, to me it sounds like a trend because I have heard about that about ten years ago about the free radicals and all that, and I still don't know if that is just a trendy thought pattern that going through" F.G. 2

> "You hear so much about anti-oxidants and that other thing, the free radicals and whatnot and its actually having an understanding of what the hell are they talking about. Like they get on the ad and they sprout all this stuff and you think oh yeah" F.G. 1

The 25-35 who were more comfortable with the antioxidants and their role were far more supportive.

> "There is a lot of study done probably in the last twenty years even and I like to know what I'm eating, I like to, if it says it rich in an anti-oxidant because I know what they are, how it works so I'll tend to buy it and I don't waste my time eating vegetables that don't have any nutritional value" F.G. 3

However it was evident that supportive evidence might sway those who were sceptical.

> "I'd want to be reading the labels a bit more…for evidence… the labels in the supermarket, I want more evidence of those anti-oxidants" F.G. 2

Baby leaf spinach and rocket were discussed as alternative that have grown in acceptability in modern cuisine related to Asian influences, use by celebrity chefs, cooking shows and up market restaurants which also cast a shadow about the cost benefit particularly for the 50+ age group.

> "But does anyone feel because restaurants made it such a big thing out of it you know rocket and all that, and for the price that you pay for salads that it's over-rated, you know I reckon they charge to much"

This clearly leaves an opportunity to support the nutritional superiority to alternatives which was highlighted by substitution of baby spinach for iceberg lettuce within the 25-35 year age group.

> "We started using baby spinach at our place to iceberg lettuce because it has more nutrients in it" F.G. 3

Vegetables were also acknowledged to be satisfying: and were critical in management of disease particularly for the 50 + year age group.

> "fills the plate up and fills your up more too" F.G. 1

> "I'm a diabetic, I try to have vegetables everyday at least four or five" F.G. 1

There was also an inherent bias as the consumers taking part in the focus groups were clearly regularly vegetable eaters.

> "I love veggies. I eat lots of veggies every day" F.G. 1

However taste preferences clearly dictated whether baby spinach or rocket was included regularly or not across all groups with some consumers being particularly vocal in their preference.

> " I absolutely hate rocket" F.G. 1

> "I can't eat rocket but I'll eat spinach" F.G. 2

> "Rocket makes good mixes. On its own it's a bit strong" F.G. 3

6.4 Recommendations

1. Identify quality management protocols for pre-packaged baby leaf spinach and rocket to promote to consumers to ensure confidence in pre-packaged products. Options for smaller serving pre-packaged portions might be considered for individual or small households.

2. Market nutritional profile of baby leaf spinach and rocket highlighting specific vitamin (Vitamin A pre-cursers – beta-carotene, Vitamin C, Vitamin K and folate) and other phyto-nutrients with antioxidant activity and contribution to the diet. Additional supportive evidence provided to identify contribution to daily requirements should be made available for example through shelf talkers or accompanying leaflets.

3. Promote the versatility of baby spinach and rocket by highlighting opportunity for use within hot and cold dishes. For instance in demonstrations within appropriate cooking shows, provision of recipes and suggested uses at point of sale or competitions for sharing ideas on use of baby leaf spinach and rocket.

7 DETERMINATION OF THE AVERAGE LEVEL OF VITAMIN C AND FOLATE IN BABY LEAF SPINACH AND ROCKET

Nutrient labelling is quite common on many processed foods such as breakfast cereals and fruit juices. However it is a new concept for fresh produce. The processed salad lines have a unique opportunity in the fresh produce section to take advantage of this type of labelling as they are one of the few products that have packaging that would visually support such a label. Consumers of semi-processed fresh produce are also known to want a convenient healthy choice. A nutrient label would support their healthy choice.

In order for OneHarvest to confidently put a nutrient claim on their packaging they need to have enough data to support a claim. For fresh produce this means a choice between the analysis of fresh products or published data. It was recommended in the Regulatory Review (Chapter 4) that because of the likely variation in nutrient content by season and growing region, it would be unwise to rely on overseas published data alone. As a result this study aimed to collect data for the level of vitamin C, folate and beta-carotene in baby leaf rocket and spinach in relation to growing season, district, storage temperature and time in order to understand the variability of these nutrients.

7.1 Outline of the Experiments

This experiment began in January 2010 and was completed in October 2010. Although an additional set of samples was analysed for the summer season for beta-carotene (Vitamin A precursor) in February 2011.

7.1.1 Treatments

1. Season: Summer and Winter harvest from all districts. Summer starting January 2010.

2. District: 3 districts Qld – Stanthorpe, Gympie and Gatton

   3 districts VIC – Bacchus Marsh, Lindenow and Clyde

   1 district NSW – Camden

   2 districts WA – Rockingham and Baldivis

3. Variety: A range of varieties were received for both rocket and spinach. We were unable to determine specific inter variety variations from this data set. It is likely that the effect of variety would be small compared to the effect of growing season and district.

4. Storage temperature: Samples were stored at either 0°C, 5°C or 4 days at 5°C + 12 days at 7°C (OneHarvest storage protocol).

5. Storage time: Samples were analysed after 0, 4 and 12 days in storage.

7.1.2 Sampling

For each harvest (per district, per season, per variety) 4 samples of 100g each were received. The leaves were harvested at commercial maturity as determined by the OneHarvest protocol and where possible the samples within a season were of similar age (Days to harvest).

7.1.3 Storage sampling times

The samples were analysed on the day of arrival and a sub-sample was analysed after the following OneHarvest postharvest protocol; 4 days at 5°C + 12 days at 7°C. Samples were also analysed after 4 days and 12 days at 0 and 5°C. This meant there was data for continuous storage at 0 and 5°C and storage using the OneHarvest protocol.

7.1.4 Nutrient analysis

It is important to note that the AHR methods for Vitamin C and folate analysis were cross checked with parallel samples from a NATA accredited laboratory (Silkier, Sydney). The results for folate were the same and the results from the NATA lab were lower for Vitamin C. The NATA lab had < 1mg/100 of Vitamin C for spinach and rocket which is incorrect as all references to Vitamin C in spinach and rocket have levels higher than that and our data was also higher (more than 40 mg/100g). When the matter was pursued, Silkier were unwilling to disclose their method or to explain the error in their results.

I. Beta –carotene (precursor to Vitamin A) analysis

The level of beta-carotene was analysed by NMI (National Measuring Institute, NATA accredited) in Melbourne. Due to physical constraints of this commercial laboratory the samples were analysed on arrival and after 9 days at 4°C. As a result, the OneHarvest protocol could not be tested for these samples.

II. Vitamin C analysis

Vitamin C was analysed according to the method described by Carnevale (1980) with some modifications. In brief, 5 g of baby spinach or rocket leaves were homogenized in 50 ml of 5% meta-phosphoric acid. The samples were centrifuged at 13,000 rpm for 15 min at 4°C. The supernatant was diluted 10 times in deionised water and filtered through a Millipore 0.45 µm membrane. The extracted samples were analysed using an Agilent 1200 series HPLC. Samples were injected (25 µl) through a C18 column fitted with a guard column. The flow rate was 1 ml per minute, and the mobile phase was 0.375% metaphosphoric acid in deionised water. Peaks were detected using a UV detector set at 245 nm with Vitamin C being a single peak eluting at around 4.7 minutes. The concentration of Vitamin C was calculated from a standard curve of ascorbic acid in 0.5% metaphosphoric acid in deionised water (Vitamin C).

III. Folate analysis

The level of Folate was analysed using the AOAC method 2004.05 with some modifications. In brief, 5 g of fresh rocket or spinach leaves were homogenized in a blender with 100ml of 0.1 M Na-phosphate and 1% ascorbic buffer pH 7.8 (extraction buffer). An aliquot of 5 ml of the homogenate was transferred to a 125 ml Erlenmeyer flask and 7.5 ml of deionised water was added, and the flask was covered with aluminium foil. The flask and its contents were then autoclaved at 121°C for 15 min. After cooling, 2.5 ml of the extraction buffer was added to the flask and then 0.25 ml of protease enzyme (2 mg/ml) was added. The flask was incubated in a water bath for 3 hours at 37°C. After 3 hours of incubation, the reaction of the enzyme was stopped by placing the flask in boiling water for 3 min. After cooling, 0.25 ml of α-amylase was added (20 mg/ml) and the flask was again incubated for 2 hours at 37°C in a water bath. After 2 hours of incubation 1 ml of chicken pancreas (5 mg/ml) was added and incubated for 16 hours at 37°C in a water bath in order to deconjugate the folates. After 16 hours of incubation, the reaction was stopped by placing the flask in boiling water for 3 min.

The deconjugated extract was made up to a 25 ml volume with deionised water and the pH was adjusted to 4.5. The extract was then centrifuged at 10,000 rpm for 15 min at 4°C. The supernatant was collected in an amber bottle. A blank sample using only the extraction buffer was run with the sample extracts.

The deconjugated extracts were then diluted 20 times with a dilution buffer (0.05 M Na-phosphate and 0.15% ascorbic acid pH 6.8).

For the assay, 0.5 ml of the diluted extract in the test tube was taken and made up to a 1.5 ml volume with dilution buffer. Then 1.5 ml Folic acid *L. casei* medium was added to the test tube to make the assay volume 3 ml. The test tube was then autoclaved at 121° C for 5 min, then cooled immediately in a water bath. Then 50 µl of diluted inoculum (200 µl of cryoprotected *Lactobacillus casei* in 50 ml of 0.85% NaCl solution) was added to the test tube and incubate in a water bath at 37° C for 16-18 h. After incubation the test tubes were placed in ice, vortexed and the absorbance was measured using a spectrophotometer at 540 nm after zeroing with an un-inoculated blank sample.

7.2 Results and Discussion

7.2.1 What claims can be confidently made?

The following tables and figures illustrate the changes in vitamin C, folate and vitamin A (beta-carotene) in baby leaf spinach and rocket. In the tables and on the figures there is an “lsd” value. This value is the Least Significant Difference (lsd) which is a statistical measure of whether the difference between the average values for a treatment is greater than the natural variation between the samples. If the difference between two averages is greater than the lsd then the difference is the result of the treatment or factor being compared.

In these experiments there were many of factors that affected the level of vitamin C and folate in baby leaf spinach and rocket. However, it is important to restate the main aim of this work;

“Do the levels of vitamin C, folate and vitamin A (beta-carotene) in baby leaf spinach and rocket stay above the threshold level based on the FSANZ (Food Standards Australia and New Zealand) guidelines to confidently allow a nutrient claim to be put on the packaging of these products”

To clarify this, a “source claim” can be made when one serve of the product contains more than 10% of the recommended daily intakes (RDI) set by FSANZ for that nutrient and a “good source claim” can be made if one serve contains more that 25% of the RDI.

The answer to the main project aim can be summarised as follows;

- Rocket can be confidently labelled as a source of folate, Vitamin C and beta-carotene (Vitamin A).

  In most cases it could be labelled as a good source of Vitamin C but the WA summer samples fell below the required threshold for this claim after 12 days in storage.

- Spinach can be labelled as a source of folate and beta-carotene (Vitamin A) and winter samples could be labelled as a source of vitamin C.

  For spinach the summer samples from Victoria and WA fell below the threshold level for a source claim for vitamin C after 12 days in storage.

The following types of claims could therefore be used for baby spinach/rocket:

- *Baby Leaf Spinach/Rocket form part of a healthy diet of fruits and vegetables*
- *Baby Leaf Rocket is a source of vitamin C, folate and Vitamin A (beta-carotene)*
- *Baby Leaf Spinach is a source of folate and Vitamin A (beta carotene)*

7.3 What are the main factors that affect the level of vitamin C, folate and Vitamin A (beta-carotene) in baby leaf spinach and rocket?

7.3.1 Vitamin C

The data for vitamin C in spinach and rocket are summarised in Tables 7.1 and 7.2 and in Figures 7.1 to 7.3. The main points to be made are;

- Rocket contains more vitamin C than spinach
- For both crops the level of vitamin C is higher in winter than in summer
- The level declines in storage after harvest, irrespective of storage conditions
- The levels remain higher when stored at 0^oC compared to storage under the OneHarvest shelf life protocol (4 days at 5^oC + 12 days at 7^oC).
- For both crops the level of vitamin C was lowest in the WA samples but this may have been the result of longer transport times.
- Figure 7.2 shows the difference in vitamin C level between rocket and spinach and it is also an example of how, in some samples the level of vitamin C in spinach dropped below the threshold for a source claim after 12 days in storage under the OneHarvest protocol.

Table 7.1. Summary of the main factors affecting the level of Vitamin C in rocket

Main Comparisons	**Vitamin C in Rocket**				
District	**NSW**	**QLD**	**VIC**	**WA**	**Lsd**
	103.6	95.4	86.7	49.0	1.68
Season	**Winter**	**Summer**			**Lsd**
	89.5	77.9			1.191
Storage Time	**0 days**	**4 days**	**12 days**		**Lsd**
	96.8	90.8	63.4		1.46
Storage Regime	**0 °C**	**5 °C**	**Oneharvest commerical protocol**		**Lsd**
	89.5	82.7	78.8		1.46

Table 7.2. Summary of the main factors affecting the level of Vitamin C in spinach

Main Comparisons	**Vitamin C in Spinach**				
District	**NSW**	**QLD**	**VIC**	**WA**	**Lsd**
	48.34	35.03	34.76	26.14	0.9
Season	**Winter**	**Summer**			**Lsd**
	38.7	33.43			0.63
Storage Time	**0 days**	**4 days**	**12 days**		**Lsd**
	43.17	38.6	26.4		0.78
Storage Regime	**0 °C**	**5 °C**	**Oneharvest commerical protocol**		**Lsd**
	39.29	35.51	33.4		0.78

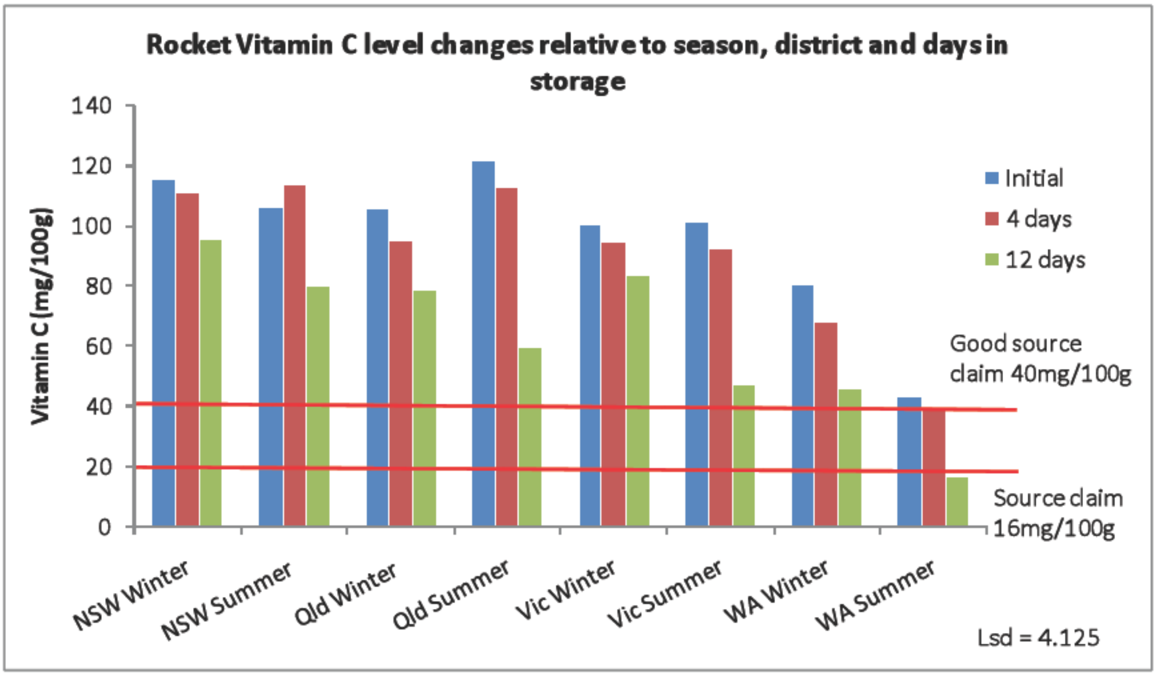

Figure 7.1. Changes in the level of vitamin C in rocket relative to district, growing season and time in storage.

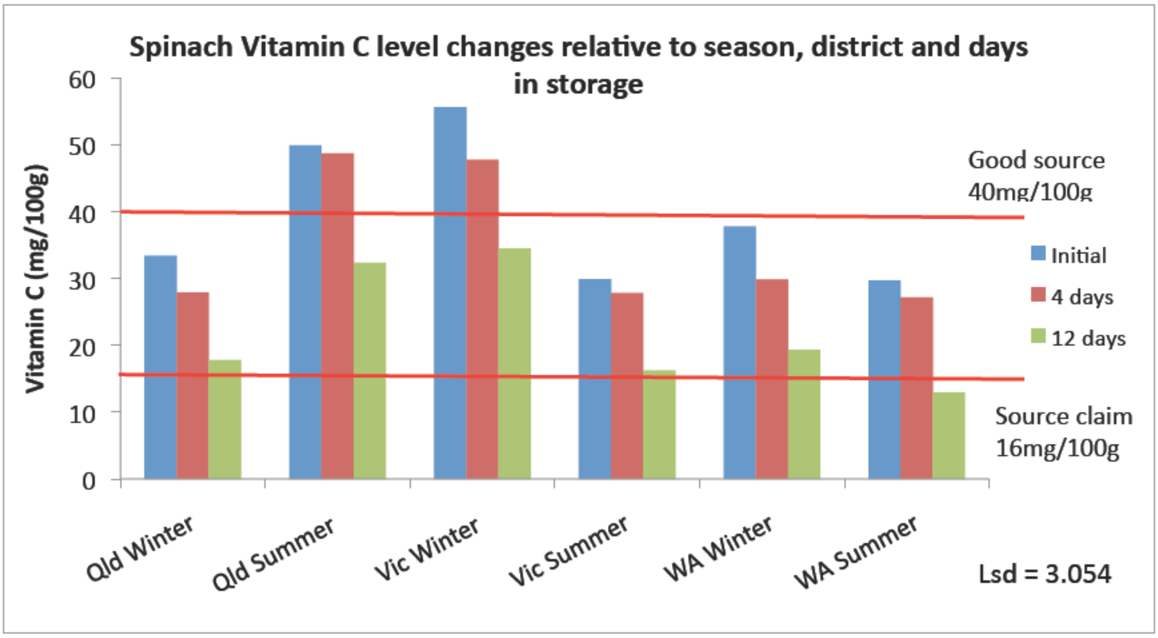

Figure 7.2. Changes in the level of vitamin C in rocket relative to district, growing season and time in storage.

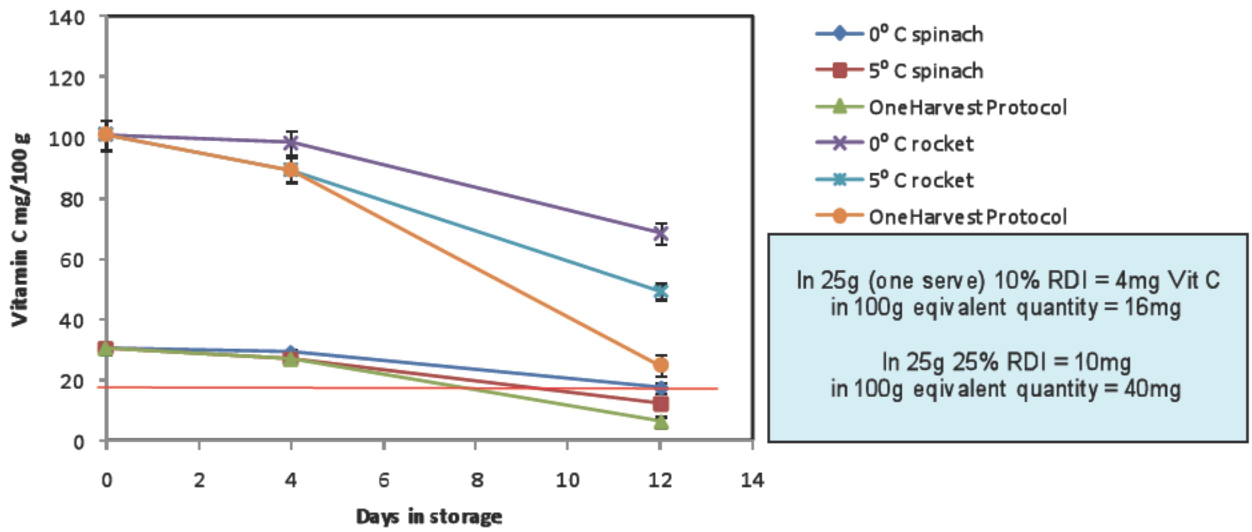

Figure 7.3. Changes in the level of Vitamin C in baby leaf spinach and rocket (Victorian samples) after harvest when stored at different temperatures.

Figure 7.3 shows the rate of vitamin C loss in baby leaf spinach and rocket from Victoria. This data is an example of how the concentration changes with time in storage at different temperatures for rocket and spinach; the lower the storage temperature the slower the rate of vitamin C loss. The OneHarvest commercial protocol is a worst case storage scenario and resulted in the highest rate of vitamin C loss in both spinach and rocket.

7.3.2 Total folate

The data for total folate in spinach and rocket is summarised in Table 7.3, 7.4 and Figures 7.3 and 7.4. The main points to be made are;

- The level of folate in rocket is more consistent across the states than it is in spinach
- The levels of folate in both crops is higher in winter than in summer
- In both crops the level was higher at harvest than after storage (irrespective of storage protocol)
- The level was higher in both crops when they were stored at 0°C compared to the OneHarvest protocol.

Table 7.3. Summary of the main factors affecting the level of total folate in rocket

Main Comparisons		Tota folate in Rocket				
	District	NSW	QLD	VIC	WA	Lsd
		163.1	174.1	148.5	196.1	3.12
	Season	Winter	Summer			Lsd
		189.2	151.7			2.21
	Storage Time	0 days	4 days	12 days		Lsd
		187	174.6	149.8		2.71
	Storage Regime	0 °C	5 °C	Oneharvest commerical protocol		Lsd
		175.3	169.9	166.1		2.71

Table 7.4. Summary of the main factors affecting the level of total folate in spinach

Main Comparisons		Total folate in spinach				
	District	NSW	QLD	VIC	WA	Lsd
		204	154.5	124.7	90.5	2.28
	Season	Winter	Summer			Lsd
		144.9	141.9			1.61
	Storage Time	0 days	4 days	12 days		Lsd
		152.3	146.1	131.9		1.97
	Storage Regime	0 °C	5 °C	Oneharvest commerical protocol		Lsd
		146.1	142.8	141.4		1.97

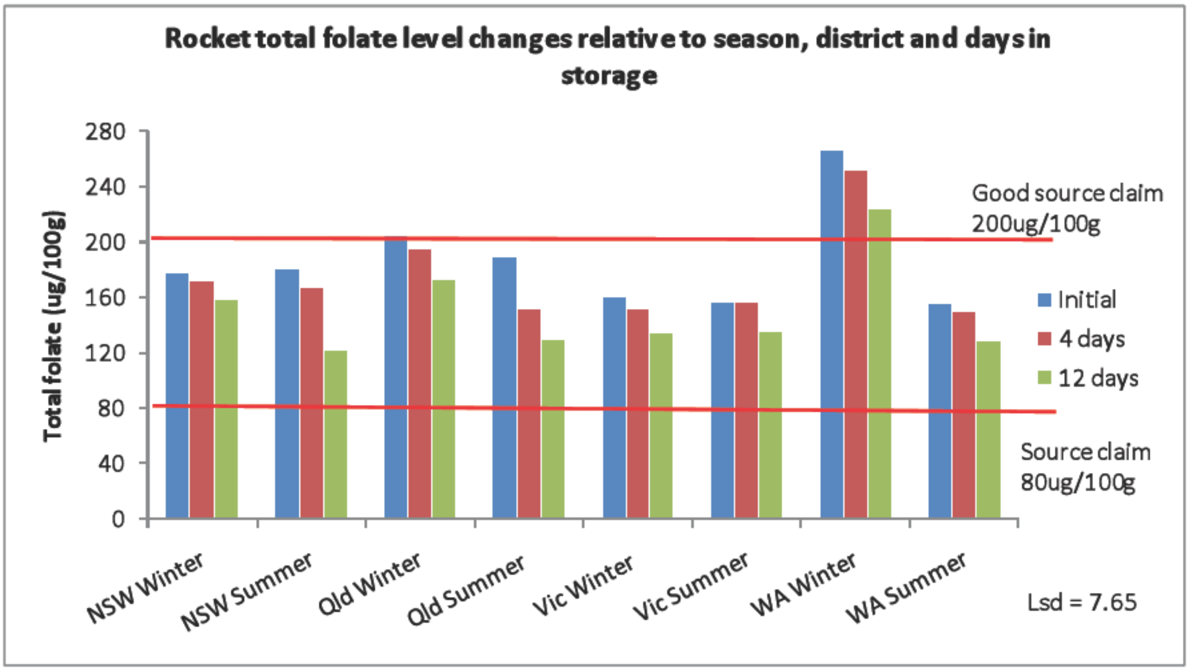

Figure 7.3. Changes in the level of total folate in rocket relative to district, growing season and time in storage.

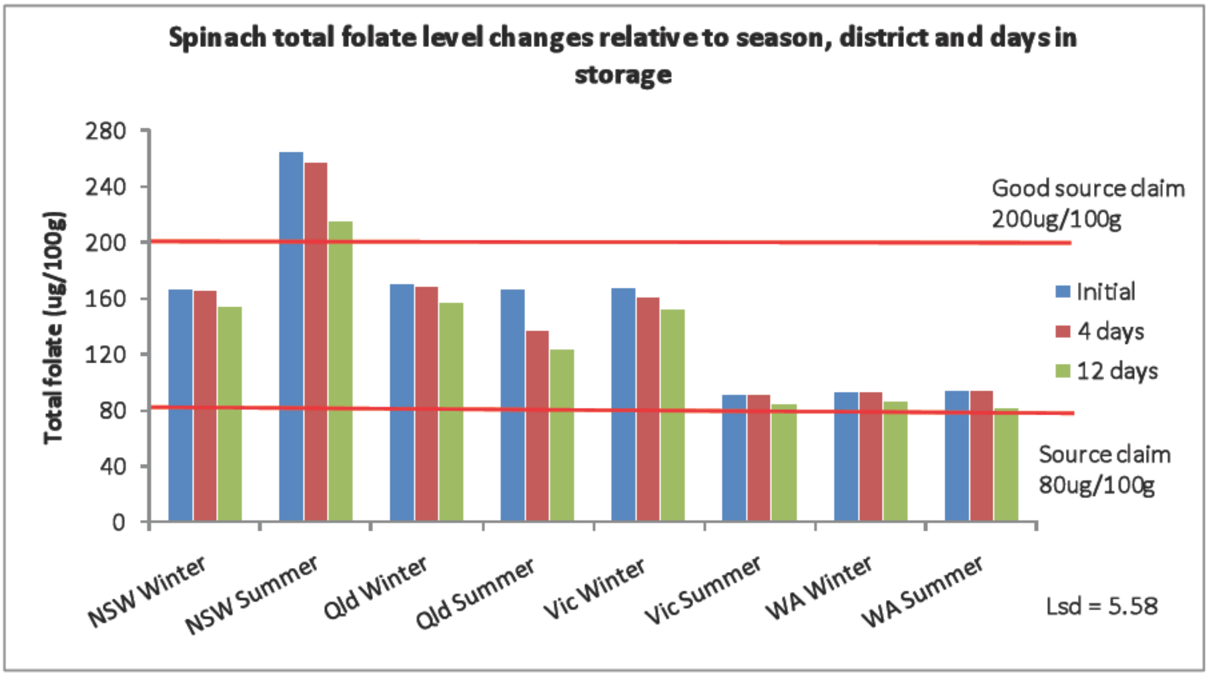

Figure 7.4. Changes in the level of total folate in rocket relative to district, growing season and time in storage.

7.3.3 Vitamin A (beta carotene)

The level of beta carotene has only been measured from one district in the winter. In each case 4 samples were measured on receipt at the National Measuring Institute in Melbourne and after 9 days at 4°C.

The results are summarised in Tables 7.5 and 76. The data shows that;

- The level of beta-carotene increases during storage for both rocket and spinach
- In both crops the level is maintained well above the threshold for a content claim for this nutrient

Table 7.5. Changes in the level of beta-carotene in baby leaf spinach after 9 days in storage at 4°C.

Spinach	**Beta-carotene (Vitamin A precursor) ug/100g**		
Grower	**Variety**	**4° C Initial**	**4°C after 9 days**
Winter - VIC 1	Black glove	3425	4050
Winter - VIC 2	RZ51-117	2900	2925
Summer - Young	Spinach (4 reps)	1900	2055
Minimum level for source claim	1800 ug/100g		

Table 7.6. Changes in the level of beta-carotene in baby leaf rocket after 9 days in storage at 4°C.

Rocket	**Beta-carotene (Vitamin A precursor) ug/100g**		
Grower	**Variety**	**4° C Initial**	**4°C after 9 days**
Winter - VIC 1	Nature	2125	2300
Winter - VIC 3	Nature	2950	3225
Summer - Borato	Rocket (4 reps)	1150	1250
Minimum level for source claim	1800 ug/100g		

The data supports the use of content claims on the packaging of baby leaf spinach. The data for rocket only supports a source claim for winter-grown rocket.

7.3.4 Nutrient calculation models for salad mixes

The data collected throughout 2010 showed that nutrient claims were possible for salad mixes that contained reasonable amounts of baby leaf rocket and spinach. The next step was to use that data in a commercial context.

Table 7.7 summarises the key data for deciding in a mix contains enough spinach or rocket to warrant a nutrient claim. The average level of vitamin C and folate for a sample of baby leaf spinach and was calculated from the data collected (288 samples analysed for each nutrient over the course of the study). These values have a standard deviation of between 29 to 52 mg/100g which is quite high. In discussions with OneHarvest it was decided that the average value was appropriate as the FSANZ guidelines refer to a nutrient claim having to be based on the "average" level of the components in that product (see Chapter 4 Regulatory Review).

The levels of vitamin C and folate required for a source claim or a good source claim were also summarised.

Table 7.7. Average level of vitamin C and folale in baby leaf spinach and rocket and the level of vitamin C and folate needed in a salad mix to warrant a nutrient claim.

	Average values from AHR/OneHarvest Data			
	Rocket Vit C	Spinach Vit C	Rocket folate	Spinach folate
Units	mg/100g	mg/100g	ug/100g	ug/100g
Source claim limit	4 mg/serve	4 mg/serve	20 ug/serve	20 ug/serve
Good source claim limit	10 mg/serve	10 mg/serve	50 ug/serve	50 ug/serve
Average value / 100g	84	36	170	143
Standard deviation	29	14	38	52
Minimum value / 100g	55	22	133	91
Number of samples analysed	288	288	288	288
Summary of samples taken	2 seasons			
	4 districts			
	3 storage times			
	3 storage temperatures			
	4 replicates			
	Requirements for packaged product labels			
Average quantity / gram	0.8	0.4	1.7	1.4
Source claim10% RDI / serve	4	4	20	20
Minimum serve size to meet 10% criteria	5	11	12	14
Good Source claim 25% RDI / serve	10	10	50	50
Minimum serve size to meet 25% criteria	12	28	29	35

* the RDI for Vitamin C is 40mg and the RDI for Folate is 200ug

The following Table 7.8 is an example of a bled of leaves in a salad mix.

Table 7.8. Salad mix recipe

		80g packs- 2 servings per pack
Folate Blend	**Recipe %**	40g serving size
Spinach	63%	25.0
Red Chard	5%	2.0
Rocket	33%	13.0
Folate - 80g - 50 g spinach, 4 gram red chard, 26 g rocket		

Using the weights of each type of leaf in a mix it is possible to estimate the total level of vitamin C and folate in the mix based on the average levels determined in the research work. Table 7.9 shows the analysis of the salad blend described in Table 7.8. This method of calculating the levels from the actual ingredients used in the product is described in the FSANZ guidelines (see Chapter 4 Regulatory Review).

Table 7.9. Table for estimating the level of vitamin C and folate in a salad blend.

	Requirements for packaged product labels					
	Rocket Vit C	**Spinach Vit C**	**Rocket folate**	**Spinach folate**		
Units	**mg**	**mg**	**ug**	**ug**		
RDI for Nutrient	**40**	**40**	**200**	**200**		
Amount required for 10% RDI / serve	**4 mg/serve**	**4 mg/serve**	**20 ug/serve**	**20 ug/serve**		
Amount required for 25% RDI/ serve	**10 mg/serve**	**10 mg/serve**	**50 ug/serve**	**50 ug/serve**		
Average quantity / gram*	0.8	0.4	1.7	1.4		
Minimum grams to meet 10% criteria	5	11	12	14		
* See values from research work summary on Nutrient claims page in this workbook						
Folate Blend	80 g packs, 40 g serve				Total Vitamin C (mg/serve)	Total Folate (ug/serve)
Quantity leaf in mix (g)	13.0	25.0	13.0	25.0		
Nutrient in mix (mg Vit C and ug Folate)	10.9	9.0	22.2	35.9	19.9	58.0
This mix meets the criteria for a good source of Vit C and folate.						

It is possible to change the quantities of spinach and rocket leaves in a mix to make sure it meets the requirements for a nutrient claim. It is then possible to have the sample analysed for the level of vitamin C and folate to validate the models used in the estimation.

7.4 Conclusions

The data shows that it is possible to make vitamin C, folate and vitamin A nutrient claims on baby leaf spinach and rocket salads and salad mixes that contain appropriate quantities of these leaves. In relation to each the three bioactives studied, the following conclusions apply:

Folate:

- The levels of folate in rocket and spinach are high enough for a source claim.
- Folate levels are generally higher in winter than in summer in both rocket and spinach.
- The levels of folate decline in storage.
- The level was of folate was higher when either rocket or spinach were stored at 0°C compared to 4°C or the OneHarvest protocol (4 days at 5oC + 12 days at 7°C).

Vitamin C:

- Rocket Vitamin C levels were high enough for a source claim, and almost high enough for a good source claim.
- Spinach Vitamin C levels high enough for a source claim in winter but too low for a source claim in summer, especially under OneHarvest protocol.
- The Western Australian samples may have lost Vitamin C prior to analysis and so there is a case for ignoring these results.
  Vitamin C is higher in winter than in summer for both spinach and rocket.
- The OneHarvest storage method results in levels below a source claim for spinach (4 days at 5°C + 12 days at 7°C).

Beta Carotene

- Spinach and Rocket Vitamin A levels high enough for a source claim except for summer rocket
- The level of beta-carotene increases during storage for both rocket and spinach

Supported source claims

Baby Leaf Spinach/Rocket form part of a healthy diet of fruits and vegetables

Baby Leaf Rocket is a source of vitamin C, folate and Vitamin A (beta-carotene)

Baby Leaf Spinach is a source of folate and Vitamin A (beta-carotene)

Models were developed to determine the estimated levels of vitamin C and folate in blends of salad mixes using the average values from these trials. Nutrient claim labels were developed for the Macro label for Woolworths.

7.5 References

AOAC Official method 2004.05 for total Folates in cereals and cereal foods.

Carnevale, J. 1980. Determination of ascorbic, sorbic and benzoic acids in citrus juices by high-performance liquid chromatography. Food technology in Australia, 32: 302-305.

8 BABY LEAF PHYTONUTRIENTS: CONSUMER RESEARCH EVALUATING THE NUTRITION INFORMATION

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8.1 Executive Summary

For the purpose of investigating recommendations regarding how to market the nutritional profile of baby leaf spinach and rocket specifically vitamin (Vitamin A precursers – beta-carotene, Vitamin C, Vitamin K and folate), OneHarvest conducted research to determine the role that nutritional claims made on packaging play in the consumer purchase decision with regards to leafy greens salads.

The research design consisted of 5 group discussions with women ages 20 – 45 years who are regular buyers and consumers of leafy greens.

The proposed recommendations for marketing the nutritional benefits of leafy greens include:

1. The use of a nutritional claim as the predominant message in marketing salad products was questioned by consumers, and deemed not as important as flavour or variety.
2. There was a moderate amount of interest shown for the vitamin claims information being purposeful as an educational message.
3. The vitamin information could be added as secondary messaging to the packaging (either on the front or back of pack).

8.2 Background

OneHarvest in conjunction with Applied Horticulture Research Pty Ltd would like to clarify the importance of including nutritional claim messages on leafy green salad products; and whether this proposition creates a point of difference (POD) to support launching a range of products into the market under this pretence.

Forty females representing main grocery buyers were interviewed over five life-stage focus groups; where all women were aged 20 – 45 years and are regular buyers and consumers of leafy greens. Three group discussions included women who predominantly buy their leafy greens at supermarkets (e.g. Woolworths, Coles); two group discussions included women who predominantly buy their leafy greens at green grocer stores (e.g. Harris Farms, Thomas Dux). The groups were a combination of SINKS/DINKS (single and double income, no kids) and young families.

Participants were asked to comment on the following topics: are there perceived benefits to the consumption of leafy greens (in the context of leafy green salad leaves); how do they feel about leafy greens marketing nutritional claim messages on product packaging; and how do they respond to concept stimulus material displaying the nutritional claims (refer 8.5 Appendix).

8.3 Project Objective

To obtain the importance of including nutritional claim messages on leafy green salad products.

8.4 Key Findings

8.4.1 Salad is a Health Choice

Leafy greens are widely accepted as a very healthy choice. Their health status is undisputed. Findings from the research project revealed the following mindset to leafy greens (in a physical sense):

What is healthy?

- "Green". General consensus that the darker the green, the healthier it is
- (more chlorophyll)
- Fresh
- Natural, simple, unprocessed, and retain all their nutrients.
- Fibre rich
- Low in fat
- No cholesterol, sugar, salt

A satisfying (light) meal

- Feel satisfied without feeling bloated/weighted down
- Can be sustaining by adding extra ingredients

Good compliment/balance to heavier, fattier foods

- "Lightens" a heavier meal. Relieves the "guilt" of eating fattier foods.

Good for weight control

- Low in calories

Improves digestion

- Easily digested, good roughage

Regular and heavy users of leafy greens express a strong attachment to the concept of fresh food and healthy eating; they enjoy food that is healthy, simple and fresh. This mindset is captured by the below quotations:

> "I love my greens"
>
> "It just seems healthy… its fresh… and light. There's no goodness taken out. It's retained its natural state"
>
> "I love leafy greens and vegetables. I'm trying to educate my husband and child to be the same way"
>
> "You know you're providing something good for your family when you serve greens"
>
> "I just feel happy with myself when I've eaten salad. I know the food I've eaten is healthy. And I know I haven't gained kilos by eating it"
>
> "You just don't feel as guilty when you eat salad!"

8.4.2 Potential for a Nutritional Claim Platform

While heavy users of leafy greens are health motivated, the concept stimulus material that was viewed (refer Appendix A on page 10) does not act as a strong, compelling POD for launching a range of products into the market under the nutritional claim platform. Respondents were more interested in the ingredient mixes, such as the leaf mix, ingredient inclusions and flavour profiles, than the nutritional claims.

Three key points supporting this view are:

1. A familiar but over-used marketing strategy. While it has been successful in other categories (e.g. bread, cereal) there is a clear indication that respondents have become blasé and dismissive of variant claims such as these.

"It's just a bandwagon people jump on all the time. I feel like I'm being talked down to"

"It's ridiculous, I already know that salads have vitamins in them"

"I don't get the purpose of Folate and Vitamin A and C. It just looks like salad!"

"I think with leafy greens you get folate anyway"

2. It complicates the purchase decision by introducing another layer of decision for the respondents. Most respondents simply deflected to the appeal of the ingredients to make their choice.

"Salad is one of the last things we have where we don't have to read the contents on it. It's true! You can just pick up salad and not think that there's something added"

"It takes away from what you're buying – just nice, simple salad"

"Salad is good for you… don't need to tell me"

"It's just another thing to think about"

"It's making something simple technical"

3. The platform is perceived as promoting a vitamin deficiency that respondents (with the exception of pregnant women) are unaware of. Respondents don't want to choose their salad on the basis of a single vitamin. They want – and expect – their salads to a multi-vitamin.

"I don't know realistically whether I'd walk in and think I need some Vitamin A"
"I'd want a multi one, one with A, C and F"

(The Vitamin reference) "does not sell the salad to me"

With regards to the graphics of this particular stimulus material, it was consistently rejected among respondents. This finding is captured by the below words used to describe the graphics:

"Looks more like a vitamin supplement"

"Overpowering"

"In your face"

"Overwhelming"

(Looks) "mass produced", "commercialised", and "industrial"

8.4.3 Educational Value

Although the nutritional claim platform was not supported (refer Appendix A on page 10), some respondents liked the educational value of this concept, especially for kids.

> “It’s good. You can point out to your kids that it’s your Vitamin A and C”
>
> “It’s interesting, but it’s not ultra important”

8.4.4 Recommendations from consumer studies

1. The use of a nutritional claim as the predominant message in marketing salad products was questioned by consumers, and deemed not as important as flavour or variety. While heavy users of leafy greens are health motivated, the proposed nutritional claim platform does not act as a strong, compelling POD for launching a range of products into the market.

2. There was a moderate amount of interest shown for the vitamin claims information being purposeful as an educational message. The place for vitamin references could be relevant when developing the overall marketing strategy via the format of health facts (for instance, including this information on a vitamin education webpage and interactive social media).

3. Additionally, due to the moderate amount of interest shown for this information as an educational purpose, the vitamin information could be added as secondary messaging to the packaging (either on the front or back of pack).

8.5 The Stimulus Material

OneHarvest developed three concept salad blends where vitamin claims could be made. The concept salad blends were packaged in tubs and labelled in a similar way as you might see when shopping in-store (refer below). The packaging artwork was designed in the form of a label and was developed

Recipes for the Concept Salad Blends are detailed below.

No.	Salad Name	Nutritional Claim	Weight	Ingredients
1	Vitamin A Blend	Good Source of Vitamin A	90g	Baby Spinach (54%), Baby Rocket (22%), Carrot Shred (17%), Broccoli Stem Shred (7%)
2	Vitamin C Blend	Good Source of Vitamin C	100g	Baby Spinach (50%), Green Capsicum (20%), White Cabbage (13%), Carrot Shred (10%), Red Cabbage Shred (7%)
3	Folate Blend	Good Source of Folate	90g	Beetroot Shred (39%), Baby Spinach (35%), Tatsoi (15%), Red Chard (11%)

9 TECHNOLOGY TRANSFER

9.1 Abstract for the Nutrition Society of Australia and New Zealand Annual Scientific Meeting December, 2009.

The focus group results were presented at the Nutrition Society of Australia & Nutrition Society of New Zealand Joint 2009 Annual Scientific Meeting, 8 - 11 December 2009

<u>Abstract</u>

Understanding consumer knowledge of the potential health benefits of leafy green vegetables (baby spinach and rocket).

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Background - Epidemiological evidence supports increased consumption of vegetables to reduce a number of chronic health diseases. However dietary consumption of vegetables continues to be below recommendations in most developed countries. Working with producers and the food supply chain more broadly is a critical component to encourage an array of new forms of vegetables suited to current lifestyle trends. Baby leaf salad products, especially spinach and rocket, have become very popular alternatives in recent years and contain vitamins, minerals and antioxidants, which may promote health. Understanding how consumers might perceive less traditional forms of vegetables in their diet may lead to developing more appropriate delivery forms and effective communications.

Objective – This study aimed to describe consumer knowledge and understanding of the potential health benefits of leafy green vegetables (baby spinach and rocket).

Design - Three semi-structured focus groups were conducted using a purposeful non-probability sample including females representing main grocery buyers from three age groups(n= 23): 25-35. 35-50, and 50 +. Two coders examined each script and conducted thematic content analysis and constant comparison to identify main themes.

Outcomes – Preliminary analysis of the data reveals key themes pertaining to vegetable choice and consumption relate to usual shopping habits(influenced by time issues, convenience and access to quality and price competitive suppliers); understanding of cuisines(influenced by skills and understanding of modern and alternative cuisines); acceptability by household members; and perceptions of nutritional values of vegetables(especially leafy greens). Variations were evident related to life-stage in terms of level of importance for the themes.

Conclusion – Australian consumers generally view leafy green vegetables as healthy and embrace their use in the diet. Issues raised have implications for designing communications to further promote nutritional value and identify broad range of use in various cuisines to meet life-stage needs.

Funding source: Horticulture Australia Limited and Oneharvest Ltd

9.2 Article for Vegetables Australia Magazine May/June 2010

The following article appeared in Vegetables Australia Magazine May/June 2010, and was a requirement for milestone 103.

Nutrient labelling for fruit and vegetables: what's required?

By Dr Jenny Jobling, Applied Horticultural Research

A diet rich in fruit and vegetables is good for our health – of course, we all know that. But how many of us actually eat the recommended two serves of fruit and five serves of vegetables every day? The reality is, most of us don't.

Many in the horticultural industry would like to encourage people to eat more fresh produce by introducing labelling that highlights the key health-promoting nutrients found in fruit and vegetables. It could be that consumers need to be reminded why fruit and vegetables are good for them.

There are regulations defining what may be put on a food label. FSANZ (Food Standards Australia and New Zealand http://www.foodstandards.gov.au/) is the organisation that develops these guidelines and regulations. They are important as they ensure that labelling is consistent and that consumers are protected against people making misleading claims in relation to a food product. The guidelines are enforced by State Government Food Authorities who investigate complaints about food labels and food safety.

What type of food labelling is allowed for fruit and vegetables?

At present, nutrient content and nutrient function claims for foods are generally permitted, but claims about health enhancement or reduction of risk of diseases are not permitted (with one exception about folate and birth defects).

For example, a general statement such as "*This product is a natural source of antioxidants which may reduce the risk of cancer"* is not allowed to be used. However, the statement "*This product is a natural source of the antioxidants vitamins A and C that help protect cells from oxidative damage*" is allowed to be used.

The difference between these two statements is that the first one is not specific and there is not enough evidence to prove that it is unconditionally true. The second statement is true as the vitamins A and C do serve the biological role of being antioxidants that protect against oxidative cell damage.

It is very difficult to collect enough evidence to substantiate a health claim related specifically to a reduction or improvement in a disease outcome. Any claim that is made about a specific disease marker or health outcome must be approved by FSANZ before it can be used.

It is therefore more likely that labels for fruit and vegetables will highlight the nutritional content of the product. Nutritionists have established nutrition tables for the amounts of specific foods we need for a balanced diet. These levels are known as Nutrient Reference Values and are available on the Australian Government's National Health and Medical Research Council (NHMRC) website http://www.nrv.gov.au/nutrients/index.htm. The tables outline the recommended daily intake (RDI) for a balanced diet and include, for example, such things as the amount of energy (Kilojoules; kj) we need, the amount of carbohydrate, protein, vitamin C, vitamin A, minerals and other nutrients.

Food labels that make nutrient claims must base the claim on the amount of a specific nutrient that one serve of that food provides at the time of consumption. Fruit and vegetables can be "sources of" or "good sources of" specific vitamins, for example. To be a "source of", one average serve of the product must contain at least 10% of the recommended daily intake of the vitamin (10% RDI). To be a "good source of", the product must contain at least 25% of the RDI.

There is a database of nutrient information on the FSANZ website called NUTTAB 2006 http://www.foodstandards.gov.au/consumerinformation/nuttab2006/. This database contains information on the nutrient content of approximately 2600 foods and up to 169 nutrients per food. Many of the foods in the database are fruit and vegetables. It is therefore possible to find out how much of a specific nutrient a fruit or vegetable contains and then compare that value to the RDI requirements. You can then see if a fruit or vegetable contains more than 10% or more than 25% of the RDI for a particular nutrient, and in turn whether a "source of" or "good source of" claim can be made for that product.

The only downside is that much of the information in the NUTTAB 2006 database for fruit and vegetables was collected between 1983 and 1998, and so the amount of vitamins and minerals might be higher now, with new varieties and better handling. It is important to remember that the claims relate to the level of nutrients at the time of consumption and these levels do decline after harvest and with time in storage.

What about antioxidants?

There is a lot of promotion at the moment relating to antioxidants in foods and the protective role they have in relation to our health. Fruit and vegetables are known natural sources of antioxidants.

Claims such as "*A natural source of antioxidants – to help keep your body healthy*" or *"is a source of antioxidants which will help protect the body against free radical damage"* are currently permitted. However, when the new FSANZ guidelines are introduced towards the end of 2010 these claims will no longer be able to be made. The new guidelines for "Biologically Active Substances" require the manufacturer/producer to define the daily reference quantity (RDI) of the bioactive substance (for example, specific antioxidant) being claimed. They must also provide evidence that the level of that substance in the food is above 10% of the recommended level.

In the new guidelines, biologically active substances refer to dietary substances that are not traditionally recognised as 'nutrients', but which are associated with health effects (for example, flavonoids, carotenoids, co-enzymes, phytoestrogens, 'antioxidants'). Currently these substances do not have officially recommended reference intakes (RDIs). FSANZ states that "To demonstrate the required reference intake for a biologically active substance requires a structured scientific approach, including evidence of suitable quality and evidence substantiating the amount of substance required to be consumed per day to achieve the specific health effect". The new guidelines will mean that it will be very difficult to substantiate general claims related to antioxidants if the antioxidant is not already a component of the recommended daily intake tables.

There is a lot of potential for nutrient labeling for fresh fruit and vegetables. By using the resources and guidelines outlined in this article it would be possible to develop nutrient claim labels for many horticultural products. These new health messages could encourage the consumption of more fruit and vegetables for the benefit of the consumer's health, as well as the industry's!

Acknowledgement: This article has been partly funded through current HAL Project VG08148: *Developing a nutrient and/or health claim label for packaged baby leaf spinach and rocket*. Funds were provided by HAL and Harvest Freshcuts, part of the OneHarvest group.

9.3 Abstract for Poster Presented at the International Horticultural Congress, Lisbon August 2010

This abstract was submitted to the IHC Lisbon 2010 and was accepted as a poster presentation.

Health Claim Labelling for Baby Leaf Spinach and Rocket relative to Vitamin C and Folate.

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Consumers want food to be healthy and more recently some consumers want food to add "more" to their health. Marketing can take advantage of these desires and mislead consumers by making broad unsubstantiated claims about just what eating a certain food can do relative to health outcomes. For example there are many claims being made about antioxidants which are very popular in the media at the moment. Under new food labelling guidelines currently being developed by FSANZ (Food standards Australia and New Zealand) these broad antioxidant claims will no longer be able to be made. However health claims relative to essential nutrients can and will be able to be made as long as there is data to support that claim.

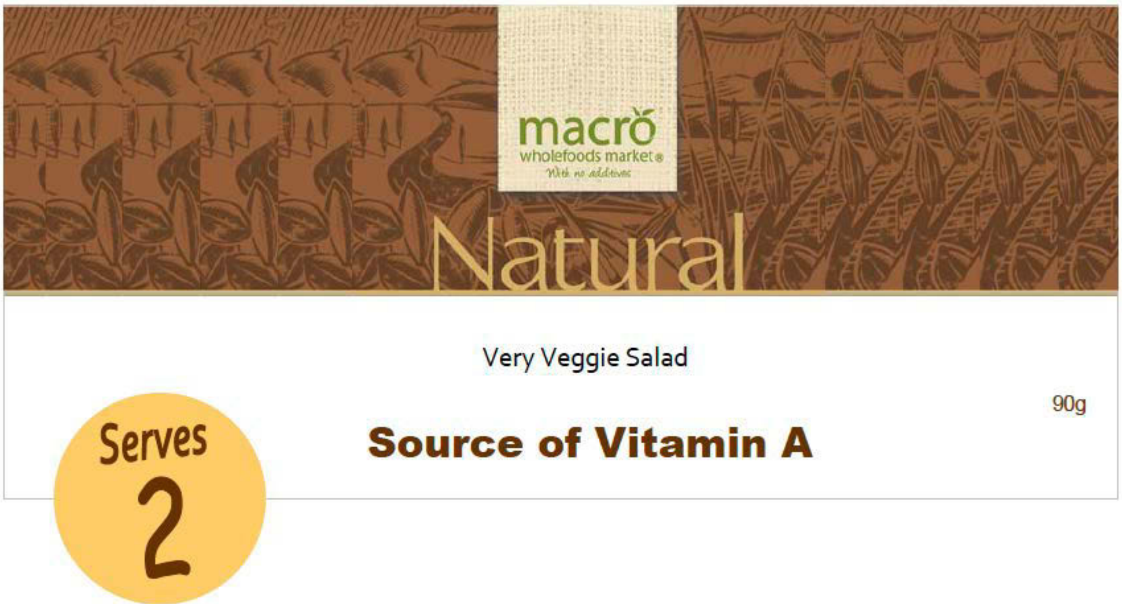
Our experiment collected data on the concentration of Vitamin C and Folate in baby leaf spinach (*Spinacia oleracea* L) and rocket (*Diplotaxis tenuifolia* (wild rocket) and *Eruca sativa* (cultivated rocket) grown in different districts and seasons in Australia. The aim of this was to collect data that would substantiate a health claim label on the packaging of fresh cut baby leaf spinach and rocket. Preliminary results show that baby leaf spinach and rocket could be labelled as sources of Vitamin C and Folate (more than 10% RDI (recommended daily intake) per serve) although rocket could be labelled as a good source of Vitamin C as it has more than 25% of the RDI per serve. The concentration of Vitamin C and Folate were maintained above the critical limit throughout the postharvest supply chain even during a poor postharvest handling scenario.

This work also included a focus group study with consumers to gauge their knowledge and interest in health claim labelling on baby leaf spinach and rocket. An overview of this data will also be presented.

The critical consideration for fresh produce is the interaction between the environment and the cultivar in relation to the bioactive and nutrient content of the product. The concentration of the bioactive and nutrient can change during growth and during postharvest handling through the supply chain. Understanding how the physiology of a crop affects the bioactive and nutrient content of a product is essential if accurate and reliable nutrient and health claims are to be made about fresh products to consumers.

9.4 Development of the Macro range of salad mixes that have a nutrient claim label to encourage consumers to purchase these products

The nutrition label has been developed and the draft artwork for three babyleaf products is shown here.


macro
wholefoods market
With no additives
Natural
Very Veggie Salad
90g
Serves
2
Source of Vitamin A

10 RECOMMENDATIONS

The data shows that it is possible to make vitamin C, folate and vitamin A nutrient claims on baby leaf spinach and rocket salads and salad mixes that contain appropriate quantities of these leaves. In relation to each the three bioactives studied, the following claims are supported by this study:

- Baby Leaf Spinach/Rocket form part of a healthy diet of fruits and vegetables
- Baby Leaf Rocket is a source of vitamin C, folate and Vitamin A (beta-carotene)
- Baby Leaf Spinach is a source of folate and Vitamin A (beta-carotene)

Models were developed to determine the estimated levels of vitamin C and folate in blends of salad mixes using the average values from these trials, and nutrition claims could be made in relation to these salad mixes.

In relation to labelling as a marketing tool, at this stage nutritional claims package salads do not appear to be regarded by consumers a strong, compelling points of difference for launching a range of products into the market. There was a moderate amount of interest shown for the vitamin claims information being purposeful as an educational message.

The place for vitamin references could be relevant when developing the overall marketing strategy via the format of health facts (for instance, including this information on a vitamin education webpage and interactive social media). Additionally, due to the moderate amount of interest shown for this information as an educational purpose, the vitamin information could be added as secondary messaging to the packaging (either on the front or back of pack).

Further consumer research is recommended to confirm the value placed on nutrient labelling by consumers. It may be that is more that it is new and unexpected information rather than that is regarded as having limited value by consumers.

11 APPENDIX I. SCIENTIFIC PAPER PREPARED FOR: JOURNAL OF THE SCIENCE OF FOOD AND AGRICULTURE

Effect of growing region and storage conditions on the vitamin C and total folate content of baby leaf spinach (*Spinacea oleracea*) and wild rocket (*Diplotaxis tenuifolia*) grown in Australia.

Running title: Vitamin C and folate in baby leaf spinach and wild rocket

Anowarul I Bokshi[a], Jenny Jobling[a], Jayashree Arcot[b] and Gordon Rogers[a]

Abstract
Baby leaf spinach (*Spinacea oleracea*) and wild rocket (*Diplotaxis tenuifolia*) are popular minimally processed salad vegetables. These leafy vegetables are good sources of many vitamins and minerals important for human nutrition. However, little information is available on how the levels of these minerals, particularly Vitamin C and total folate, change relative to growing season, district and storage temperature and time.

The total folate and vitamin C content of baby spinach and wild rocket leaves were analysed from samples taken from different growing districts around Australia in summer and winter seasons. The samples were stored for 12 days at 0°C, 5°C or under a commercial protocol. The samples were analysed after 0, 4 and 12 days in storage. There was a significant variation in the levels of total folate and vitamin C content due to production region. The levels of these nutrients were higher in winter crops compared to summer crops. The levels of both folate and vitamin C diminished in storage; however, the reduction was less for leaves stored at 0°C compared to 5°C, or the commercial protocol of 5°C for 4 days and then 7°C for 8 days. Wild rocket leaves were higher in both vitamin C and total folate content compared to baby spinach but had a faster rate of nutrient loss during storage.

The levels of the total folate and vitamin C content in baby leaf spinach and rocket are affected by the growing region, season and storage temperature and time. The nutrient levels were higher in winter-grown crops and the nutrient levels were maintained at higher levels when the leaves were stored at 0°C.

Keywords: vitamin C, folate, baby spinach, wild rocket, postharvest storage
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INTRODUCTION

In recent years, baby leaf spinach (*Spinacea oleracea* L.) and baby leaf wild rocket (*Diplotaxis tenuifolia* (L.) DC.) have become popular ingredients for semi-processed salad mixes. The consumption of these fresh leafy salad vegetables can be a good source of vitamins and minerals in the human diet. Baby leaf spinach and rocket are considered to be good sources of vitamin C and total folate (NUTTAB 2010 database, FSANZ). The level of Vitamin C in baby leaf spinach and wild rocket leaves has been reported to be higher than in many other leafy salad ingredients.[1] However, a difference in their concentration has been observed as a result of leaf maturity, growing conditions and crop species.[2]

The growing environment plays a significant role in the biosynthesis and accumulation of secondary metabolites such as vitamin C and folate in vegetable crops. During plant development photosynthesis, respiration, accumulation of primary and secondary metabolites as well as plant hormones, are affected by the growing temperature.[3] Although temperate plants such as spinach and rocket are adapted to grow over a wide temperature range (between 0°C and 40°C) the cardinal temperature range is narrower for each individual crop.[4] Temperatures of around 15°C[5] and temperatures above or below this optimum may adversely affect the vitamin and nutrient content of spinach.[6] Light is another key factor as a source of energy for photosynthesis and a limiting factor controlling the photorespiration. As a result the concentration of vitamins and minerals is likely to vary with changes in temperature and photoperiod.[7]

Vitamins and phytonutrients concentrations in vegetables undergo changes after harvest. The rate of change is influenced by various postharvest conditions with temperature being the most influential factor.[8] The rate of degradation of phytonutrients such as vitamin C and total folate can be reduced during postharvest storage by maintaining light levels and by low temperature storage.[9] Higher storage temperatures increase the rate of respiration and catabolic metabolism. Storage at an optimum temperature can minimize metabolic rates, reducing phytonutrient loss and maximising shelf life of the fresh product.[10] Baby leaf spinach and rocket are recommended to be stored as close to 0°C for maximum shelf life, but in commercial supply chains and retail stores, storage temperatures are usually 5°C or higher.[8] However, the changes in phytonutrient content of leafy vegetables in storage do not follow a clear pattern; it is a complex process which is governed by several metabolic factors.[8]

The objectives of this study was to determine the change in concentrations of vitamin C and total folate in baby leaf spinach and rocket leaves grown in regions and seasons across Australia and to understand the effects of storage temperature and time on the rate of loss of these nutrients after harvest.

EXPERIMENTAL

Raw materials

The commercial varieties of baby leaf spinach (*S oleracea*) and baby leaf wild rocket (*D tenuifolia*) were grown on commercial farms around Australia. The growers were contracted to the commercial processing company OneHarvest™ (Brisbane, Australia) which coordinated collection of the leaf samples. The samples were sent via overnight by courier in cooled packaging to our laboratory at the University of Sydney for analysis and storage. Leaf samples from four replicates of spinach and rocket from Camden in New South Wales, Ballandean and Thulimba in Queensland, Bacchus Marsh and Lindenow in

Victoria and Baldivis and Rockingham in Western Australia were analysed for total folate and vitamin C content during storage at various temperatures. On arrival, samples were subdivided into three groups and stored as follows for 12 days. Samples of both crops were analysed at day 0 on arrival, and after 4 and 12 days in storage. The storage regimes were constant storage at 0^{o}C, constant storage at 5^{o}C and then according to a commercial protocol where the leaves were placed at 5^{o}C for 4 days and then transferred to 7^{o}C for 8 days. This is the commercial testing protocol for OneHarvest™ in house quality assurance.

Analysis of total folate

The analysis was conducted following the AOAC method 2004.05[11] and Arcot *et al.*[12], with some modifications. In brief, 5g of fresh leaf tissue was blended in a small blender with 100 ml of 0.1 M sodium phosphate (Sigma Aldrich) buffer and 1% ascorbic acid (Sigma Aldrich) pH 7.8. A sub-sample of 5 ml of homogenate was taken to a 125 ml conical flask, wrapped with aluminium foil and 7.5 ml of deionised water was added. The diluted homogenates were placed in boiling water for 15 min. The homogenate was then cooled immediately on ice and 2.5 ml of extraction buffer was added. To the homogenate 0.25 ml of 2 mg ml^{-1} protease (Sigma Aldrich, EC 232-752-2) was added and this was incubated at 37^{o}C in a water bath for 3 hours. At the end of incubation the reaction of the protease enzyme was stopped by placing the flasks in boiling water for 3 min. The flasks were cooled immediately and 0.25 ml of 20 mg ml^{-1} α-amylase (Sigma Aldrich, EC 232-565-6) was added and the flask placed in a 37^{o}C water bath for 2 hours. After 2 hours, 1 ml of chicken pancreas (5 mg ml^{-1}) (R-Biopharm AG, Darmstadt, Germany) was added and incubated for 16 ± 2 hours at 37^{o}C in a water bath. At the end of the incubation the reaction was stopped by heating the flask in boiling water for 3 min. The flasks were cooled immediately and 9 ml of deionised water was added. The pH of the extracts was then adjusted to 4.5 with 2N HCl. The extracts were filtered through Whatman filter paper #40. The filtrates were diluted for the assay of total folate or stored at 18^{o}C for later analysis.

The conjugated extracts were diluted 20 or 30 times with dilution buffer (0.05 M sodium phosphate, 0.15% ascorbic acid pH 6.8). An aliquot of 0.5 ml of the diluted extract was taken in a 25 ml glass vial wrapped with aluminium foil. To each vial was added 1 ml of dilution buffer and 1.5 ml of folic acid casei medium (94 mg ml^{-1}) (Becton, Dickinson and Company, USA) to make assay volume 3 ml. The assay vials were autoclaved at 121^{o}C for 5 min. The vials were cooled immediately and 50 µl of diluted inoculum was added (Glycero-cryoprotected *Lactobacillus casei*, obtained from Department of Microbiology, The University of New South Wales, Australia). A parallel set of blanks was run in triplicate for each analysis. The vials were vortexed and incubated in a water bath at 37^{o}C for 16 ± 1 hours and placed on ice. The absorbance of the medium with inoculum added was measured in a spectrophotometer (DU 800, Beckman Coulter, Nyon, Switzerland) at 540 nm using a 10 mm cell cuvette following vortexing of the tubes. The calculation of total folate was performed using a standard curve of folic acid run with the sample extracts and expressed as µg 100 g^{-1} of leaf tissue. A references material (BCR®- 485, European Commission Institute for Reference Materials and Measurements, Belgium) was analysed for recovery of total folate by using the method which has shown almost 100% (±5%) recovery.

Analysis of Vitamin C

Vitamin C content was analysed using an HPLC system (Agilent Technologies 1200 series) based on the method described by Carnevale.[13] In brief, 5g of fresh leaf was homogenised with 50 ml of stabilising solution (5% w/v metaphosphoric acid) using a food processing blender. The homogenate was centrifuged at 12,000 rpm at 4°C for 15 min. The supernatant was collected in a fresh centrifuge tube and diluted 10 times with deionised water for HPLC analysis or frozen at –80°C for later analysis. The diluted extract was syringe-filtered through a 0.45 µm filter (PTFE membrane) and 25 µl was injected into the HPLC. All the preparation for the vitamin C samples were done in ice and under defused light conditions to minimise loss of the nutrient.

The HPLC analyses was performed using a 250 mm x 4.6 mm, 5 µm C18 reverse-phase column together with a C18 guard column (12.5 mm x 4.6, 5 µm) with a flow rate of 1 mL min^{-1} of 0.375% metaphosphoric acid detecting at 245 nm on a UV detector as a single peak eluting at around 4.2 minutes. Calculation of vitamin C was performed from a standard curve derived from standard ascorbic acid and expressed as mg 100 g^{-1} of leaf tissue.

Climatic data

Average maximum and minimum temperatures, and mean photoperiod during each of the crop growth periods are presented in Table 1. Climatic data in the area of production were downloaded from the web pages of the Australian Bureau of Meteorology (http:://www.bom.gov.au/climate/data/). The photoperiod data were obtained from the daylight explorer web site tools of Astronomy Education of the Nebraska-Lincoln (http://astro.unl.edu/classaction/animations/ coordsmotion/daylighthoursexplorer.html).

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Table 1. Average temperature and photoperiod for baby spinach and rocket for the periods sampled in this study

	Maximum average daily temperature (°C)		Minimum average daily temperature (°C)		Photoperiod (d)	
Districts/State	Summer	Winter	Summer	Winter	Summer	Winter
Camden - NSW	26.0	17.2	15.1	3.0	13.8	9.9
Ballandean - Qld	27.4	21.5	15.6	6.4	13.3	10.4
Lindenow - Vic	25.6	16.4	13.4	6.1	13.8	9.5
Rockingham - WA	30.4	18.2	16.7	8.3	13.3	10.1

Statistical analysis

Data were statistically analysed by Analysis of Variance using a completely randomised design. There were significant interactions between districts, seasons, time of storage and storage temperature. Least significant differences (LSD) were used to compare treatment means. All statistical analysis was performed using the statistical software GenStat® (12th edition, VSN International Ltd., United Kingdom).

RESULTS
There was a significant difference between the vitamin C and total folate content of both the baby spinach and wild rocket samples grown in different districts around Australia (Table 2). There were also significant effects of growing season, storage temperature and duration of storage.

Table 2. Main effect of district, season, storage time and temperature on vitamin C* and total folate content in baby leaf

Factors	Vitamin C (mg 100 g^{-1})		Total folates (µg 100 g^{-1})	
	Wild rocket	Baby spinach	Wild rocket	Baby spinach
District effect				
NSW	103.6 d	48.3 c	163.1 b	204.0 d
QLD	95.4 c	35.0 b	174.1 c	154.5 c
VIC	86.7 b	34.8 b	148.5 a	124.7 b
WA	49.0 a	26.1 a	196.1 d	90.5 a
LSD	1.68	0.9	3.12	2.28
Season effect				
Winter	89.5 b	38.7 b	189.2 b	144.9 b
Summer	77.9 a	33.4 a	151.7 a	141.9 a
LSD	1.191	0.63	2.21	1.61
Storage time effect				
0 day	96.8 c	43.2 c	187.0 c	152.3 c
4 day	90.8 b	38.6 b	174.6 b	146.1 b
12 day	63.4 a	26.4 a	149.8 a	131.9 a
LSD	1.46	0.78	2.71	1.97
Temperature regime effect				
0° C	89.5 c	39.3 c	175.3 c	146.1 b
5° C	82.7 b	35.5 b	169.9 b	142.8 a
OneHarvest commercial protocol	78.8 a	33.4 a	166.1 a	141.4 a
LSD	1.46	0.78	2.71	1.97

Values are means of four replicates. Mean comparisons were made within the single effect of the district, season, storage time and temperature regime using LSD. Different letters indicate significant differences. ($P \leq 0.01$*).

There were significant differences in the levels of vitamin C and total folate in baby leaf spinach and rocket from the different growing regions. The highest levels of vitamin C and total folate were measured in crops from New South Wales. Levels of vitamin C and folate were higher in winter for both crops. Storage duration had a significant effect on the levels of vitamin C and folate in baby leaf spinach and rocket with the levels of both nutrients declining over time in storage. Storage temperature also had a significant effect on the levels of nutrients with storage at 0°C maintaining the highest level of nutrients (Table 2).

There was a significant interaction between district, growing season and storage time for the vitamin C content of wild rocket leaves (P<0.001) (Fig. 1). Vitamin C and folate levels were more stable in winter-grown crops. Across the season, the level of vitamin C was lowest in rocket leaves grown in Western Australia.

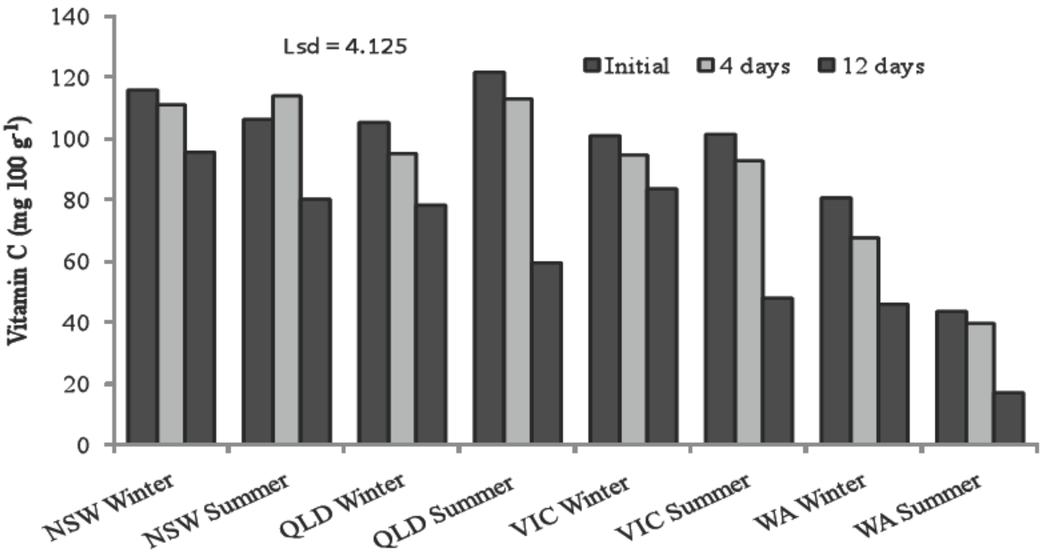

Figure 1. Vitamin C level changes in wild rocket relative to season, district and days in storage. Values are the means of three storage temperatures with four replicates (LSD calculated at the *P≤0.01*).

There were no significant interactions between district, growing season and storage time for the vitamin C content in baby spinach leaves. However, there was a significant interaction (*P<0.001*) between season and storage time and between the district and storage time (Fig 2). The highest level of initial vitamin C content was measured in baby leaf spinach grown in NSW during the winter and the lowest level in baby leaf spinach from WA during the summer. For baby leaf spinach the rate of vitamin C loss was greatest from summer-grown crops compared to winter-grown crops.

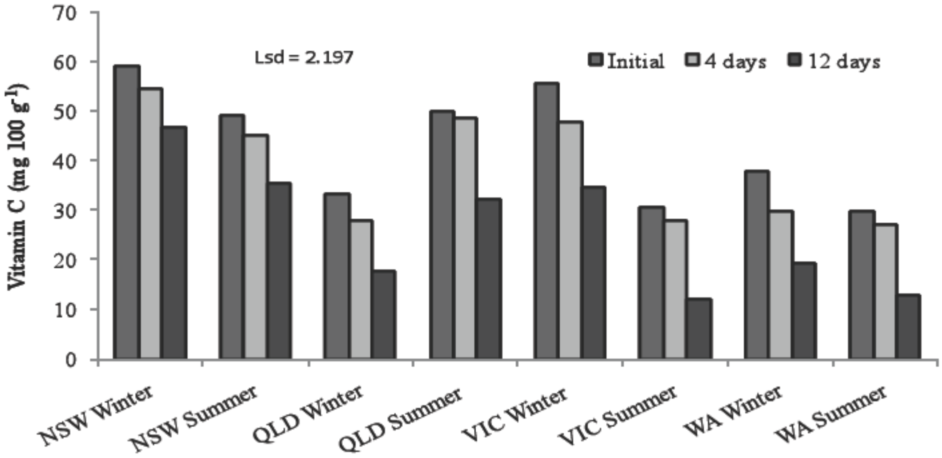

Figure 2. Vitamin C level changes in baby spinach relative to season, district and days in storage. Values are the means of three storage temperatures with four replicates (LSD calculated at the *P≤0.01*).

The levels of total folate in wild rocket leaves varied significantly (*P<0.001*) due to the interaction between growing district, harvest season and storage time (Fig 3). Wild rocket leaves harvested in WA during winter showed the highest level of total folate content compared to the other districts. However, during summer, the wild rocket from the same regions of WA showed the lowest level of total folate compared to the other districts. In general, the level of total folate in wild rocket was higher in winter compared to the summer-grown samples. The levels of folate were more stable in winter-grown samples.

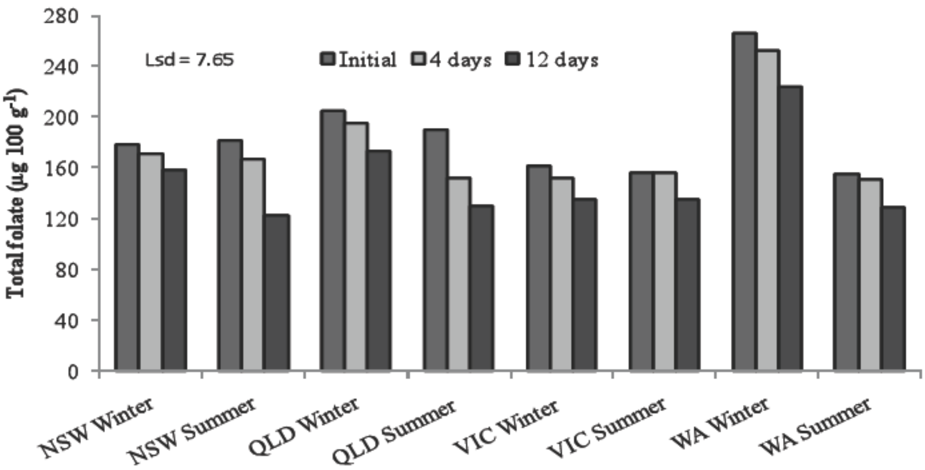

Figure 3. Level changes of total folate content in wild rocket relative to season, district and days in storage. Values are the means of three storage temperatures with four replicates (LSD calculated at the *P≤0.01*).

There was a significant interaction between district, season and storage time (*P<0.001*) for the total folate content of baby leaf spinach (Fig 4). The highest levels of total folate were found in baby leaf spinach leaves grown in NSW during summer (265 µg 100 g^{-1}).

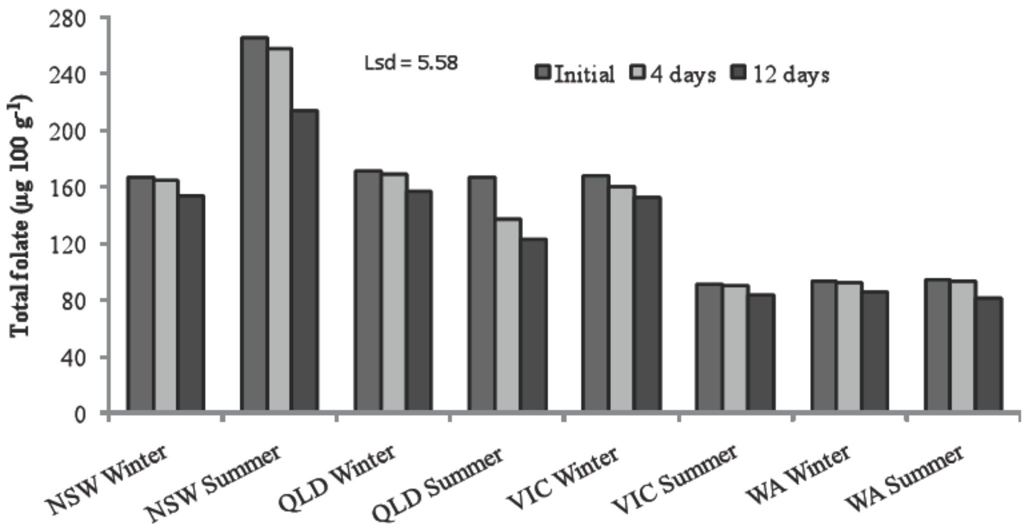

Figure 4. Level changes of total folate content in baby spinach relative to season, district and days in storage. Values are the means of three storage temperatures with four replicates (LSD calculated at the *P≤0.01*).

Vitamin C and total folate content were higher in wild rocket leaves compared to baby leaf spinach (Fig1, 2, 3 and 4) and the rate of nutrient loss during storage was higher when the initial levels were high.

DISCUSSION

The levels of total folate and vitamin C content varied in response to the district; generally they were higher in winter than summer. The samples were collected from regions with a range of different geographical and climatic conditions, which in turn influenced the growth and metabolic activities of the plants. Variable temperature and photoperiods in the different districts relative to their latitude and also seasonal difference (Table 1) may have contributed to the difference in vitamin C and total folate content of the baby spinach and rocket leaves.[14] The rate of biosynthesis of these nutrients is regulated by temperature and photoperiod during crop development.[3,9,14] However, variations in vitamin C and total folate content between district samples need further investigation to determine the cause of the differences. Possible factors include: soil type, agronomic management and transport conditions.

The higher concentrations of vitamin C and folate in winter-grown crops compared to summer-grown crops are likely to be the result of low temperature effect on physiology. A similar seasonal variation in vitamin C in tomato has been reported by Raffo *et al.*[15]. Spinach and wild rocket are essentially cool season crops[16]. The lower temperatures with an adequate 10 h photoperiod in winter may result in less oxidative stress but sufficient photosynthate supply to allow the plants to accumulate higher levels of vitamin C. Similar variation in rocket leaves was observed in a different study in our research laboratory (unpublished data), where rocket cultivars from winter contained higher levels of vitamin C compared to summer crops.

On the other hand, folates are synthesised in the growing tissues, they increase during leaf development and are stable in mature leaves.[18] Enhanced activities of vitamin C during winter that minimised the oxidative stress may have helped the plants to retain more folate in the winter baby leaves compared to summer-grown plants[19]. Folate is mostly found in the stroma of the chloroplasts.[4] Plants are folate autotrophs with a *de novo* mitochondrial pathway where light plays a role in the activity of the enzymes involved in the biosynthesis of folate.[20] Therefore, an adequate photoperiod at favourable temperatures during winter may have effectively increased photosynthate supply, resulting in a higher folate content in the plants compared to summer crops.

A summer crop that has suffered from some high temperature stress will also have a degree of oxidative stress, resulting in the degradation of vitamin C as well as total folate. This could also be a factor in explaining the higher levels of vitamin C and total folate in winter-grown crops. An average daily maximum temperature of 16-20^{o}C has been reported as the optimum for spinach biosynthetic processes including biosynthesis of vitamin C and folate[16, 6] and temperatures outside this range may cause a reduction in phytonutrient synthesis. The summer crops of baby spinach and wild rocket were grown at maximum daily average temperatures of 26-30^{o}C (Table 1) and this may have resulted in more free radical scavenging than in their winter counterparts, resulting in a greater demand for vitamin C and folate and less residual in these plants at harvest.

The reduction in the concentration of vitamin C and total folate during the 12 days of storage was a result of degradation of the compounds from metabolic activities in the leaves. Baby spinach and wild rocket were stored in the dark where no *de novo* biosynthesis of these nutrients would have occurred. However, catabolic activity of the stored metabolites would have continued as part of respiration and reduced the content of vitamin C and total folate in baby spinach and rocket leaves. The rate of reduction was slower during the first 4-6 days of storage (Table 2). Faster breakdown of vitamin C beyond 6 d of storage has previously been reported for baby spinach.[21] Martinez-Sanchez *et al.*[22], reported a similar effect for stored wild rocket where there was a greater reduction in the concentration of vitamin C after 6 d in storage in air at 4^{o}C. There was no clear explanation for the pattern of degradation of total folate during storage. Pandrangi and Laborde,[23] reported a 26% loss of folate in spinach leaves after 7 d storage at 4^{o}C, whereas, Scott *et al.*[24] reported a loss of 60% after 7 d at the same storage temperature. In this study, the slower reduction of vitamin C and total folate content between 0 and 4 d of storage may be because of an on-going biosynthesis of these vitamins after harvest.[8, 25]

At high storage temperatures, the higher respiration rate increases the rate of metabolic activity, which brings with it an increasing quantity of free radicals. The vitamin C and folate are used up in scavenging these free radicals and are therefore used up more quickly at higher storage temperatures.[8] In addition, harvested leaves stored at high temperature under starvation conditions when metabolites other than carbohydrates such as lipid and proteins become respiratory substrate, causes further oxidative stress and even more demand on the antioxidant pool.[26, 27] Therefore, for multiple reasons, storing baby spinach and wild rockets leaves at lower temperatures (5^{o}C or below) helps to minimize the loss of vitamin C and total folate [28].

This work has shown that wild rocket has a higher concentration of both vitamin C and total folate than baby leaf spinach. This difference in vitamin C content between baby leaf spinach and wild rocket may have occurred due to the variation in their nature of photosynthetic process, the carbon fixation cycle. Another possible factor could be that wild rocket has a C_3-C_4 intermediate pathway for the carbon fixation cycle. This means that photosynthesis is more efficient over a wider range of environmental conditions than the baby leaf spinach which uses C_3 photosynthesis[29,30,31]. The differences in photosynthetic and metabolic efficiencies may mean there is a greater accumulation of vitamin C and total folate in wild rocket compared to baby leaf spinach leaves grown under the same environmental conditions.

CONCLUSIONS

Vitamin C and total folate content in baby spinach and wild rocket are strongly influenced by season and growing region.

According to the NUTTAB 2010 FSANZ database, in baby spinach the average total folate and vitamin C contents are 120 µg 100 g^{-1} and 29 mg 100 g^{-1} respectively (no information is contained in the database for wild rocket). These values are supported by Martinez-Sanchez *et al*.[3] and Lester et al.[9] who reported the total folate content of baby leaf spinach to be in the range of 85 and 225 µg 100 g^{-1} and the concentration of free ascorbic acid to be between 26 and 54 mg 100 g^{-1} respectively. Whilst no data was reported for the concentration of total folate in wild rocket leaves, Pandrangi and Laborde[23] reported the concentration of vitamin C in wild rocket to be 73 mg vitamin C 100 g^{-1}, which support our results.

The results of this study show that the nutrient content of these popular leafy salad vegetables can be optimised by growing the crop under optimal environmental conditions and with correct postharvest storage at a lower temperature and for shorter periods.

ACKNOWLEDGEMENT

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12 APPENDIX II – BENEFIT COST ANALYSIS

Project Number: VG08148 Developing a health claim label for packaged baby leaf spinach and rocket

EXECUTIVE SUMMARY

Over the last decade the demand for baby leaf salads has been largely attributed to demand for convenience. However more recently, consumers are becoming increasingly interested in the health benefits of the foods they eat. It is proposed that more consumers would be encouraged to buy packaged baby leaf spinach and rocket if they were given more direct information about the health benefits of these products.

FSANZ is currently reviewing the food labelling guidelines and so before OneHarvest can label their salad lines with additional health information for consumers they must first understand the guidelines.

The results from this project will increase the interest and awareness of baby leaf spinach as a healthy food choice through new labeling that includes health information. However, there needs to be at least a 1% increase in sales as a result of including the information generated from this project on a package for there to be a 2 times benefit cost ratio. The reality is that the potential increase in sales is likely to be much more than that.

Comment from Ravi Hedge
Industry Analysis Manager, Horticulture Australia Limited
22nd May 2009

A BCA carried out, based on the information provided, concludes that the above project generates significant benefits over costs. It is found that, for the 4-year project period, every dollar invested would generate $3.40 worth of direct benefits by way of increase in sales. Sales are assumed to increase by 1% every year, on cumulative basis.

INTRODUCTION

Over the last decade the demand for baby leaf salads has been largely attributed to demand for convenience. However more recently, consumers are becoming increasingly interested in the health benefits of the foods they eat. It is proposed that more consumers would be encouraged to buy packaged baby leaf spinach and rocket if they were given more direct information about the health benefit of these products. FSANZ is currently reviewing the food labelling guidelines and so before OneHarvest can label their salad lines with additional health information for consumers they must first understand the guidelines.

This project aims to firstly provide a protocol for OneHarvest for what information is required to substantiate new health information on the package label, then to collect appropriate data to support new health information on the label as well as to run consumer focus groups at the beginning and end of the project to ensure that the information collected and presented to consumers is the information that they want.

Project Description

Research has shown that spinach and rocket are excellent sources of active phytonutrients compounds which could have specific health benefits, these include glucosinolates in rocket (Pappa, Strathmann, Lowinger et al., 2007) and antioxidants particularly flavenoids and phenols (Chu, Sun, Wu et al. 2002) as well as folate in spinach (Hart, Wright, Wolfe, et al 2006; FSANZ Standard 1.1 A.2 Transitional Standard – Health Claims).

This project will first clarify the revised food labelling laws, FSANZ (2008) as they relate to fresh fruit and vegetables, using packaged baby leaf spinach and rocket as a case study. From this review, a summary of how appropriate health and nutrient claims can be made for other fresh fruit and vegetables will be developed and published.

Once the labelling laws are clarified, a series of consumer focus groups will be run by the University of Wollongong's Smart Foods Centre to determine if consumers want that information on the label of packaged baby leaf spinach and rocket. If the answer is yes then the research will continue to collect the necessary data to substantiate a nutrient and/or health claim as outlined by FSANZ (2008) (Proposal P293).

One issue is that the nutrient claim data uses "average" values but it is not clear what these are for baby leaf spinach and rocket when factors such as growing season, variety and storage conditions have a significant impact on quality and shelf life (Ref: VG05068: Optimising crop management and postharvest handling for baby leaf salad vegetables). This project will therefore undertake agronomic and postharvest monitoring experiments to determine the variability of the nutrients, antioxidants, folate and glucosinolates respectively in spinach and rocket in relation to growing season, variety and storage conditions. This will help to ensure accurate information is provided on a label for consumers. The project expects FSANZ's specifications for what "average" values actually are will determine the number of years (of winter vs summer season) of agronomic/postharvest data sets needed to be undertaken for the nutritional label and health claim information. It is also expected that OneHarvest will ensure these "average" values are monitored on a regular basis to ensure that the rocket and baby spinach nutritional labels accurately reflect the content of these fresh salad products.

This project aims to add value to packaged baby leaf spinach and rocket by collecting reliable information on the components that make up a nutrient content information label and an appropriate health claim using the new FSANZ (2008) guidelines. This project will act as a case study for other horticultural industries who want to use the new FSANZ (2008) (Proposal P293) guidelines.

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Industry Description

Over the past decade, there has been a boom in the baby leaf salad industry worldwide, with it being the most rapidly expanding of all the fruit and vegetable sectors. Currently, the US market alone is valued in excess of US$2 billion (Zhang *et al.*, 2007). In Australia, spinach compromises 25% of the baby leaf vegetable market. With figures from November 2007, showing an estimated value of $10 million at the farm gate and $46 million in the retail sector (pers. comm Titley, 2009). This upward trend in the market is expected to continue for some time, with consumers increasingly opting for healthy and convenient food alternatives.

BASELINE FORECASTS AND ASSUMPTIONS

Price and Volume

OneHavests share of the baby leaf spinach and rocket segment of the retail market is in the order of $24 million per annum (pers comm. OneHarvest May 2009). This is equivalent to 1 million tonnes produced per year at an average farm gate price of $3.00 per kg to the grower and a retail price of about $25 per kg.

Growing Area and Industry Participants

This project is involves a voluntary contribution from OneHarest, one of the major commercial baby leaf manufacturers in Australia. OneHarvest sources Australian product year round and as a result product is sourced from commercial baby leaf farms from areas including Boisdale (Maffra), Victoria, Camden, New South Wales, Gympie, Queensland, Warwick, Queensland and Stanthorpe, Queensland.

Adoption

OneHarvest will label all of their packaged baby leaf spinach and rocket with new nutritional information as soon as this is approved by FSANZ in order to give OneHarvest the competitive market advantage over their competitors in the retail market.

It is expected that the novelty of this new label will attract new customers to these product lines. This means that after the initial launch of the new package the sales will jump and then it is likely that the sales will drop a little and settle to a new level that is higher than before the package was launched but not as high as the initial spike. It is envisaged that the new package will create new customers as the health message will help them change their purchasing habits.

FORECASTED BENEFITS

Direct Project Benefits

It is proposed that there will be an initial spike in interest in the new package but after 1 or 2 years the level of interest will drop back. However some new customers will have been created as a result of the new information and sales will continue to be higher than without the label.

The benefit cost analysis has been done using a scale of possible increases in sales. The analysis starts with a 1% increase in sales and is done for 5, 10 and 15% to take into account the possible variation in consumer demand.

Indirect Project Benefits

The indirect benefit will be that it will encourage more Australian shoppers to make healthy food choices. This is an important part of reducing the incidence of obesity and chronic disease.

COSTS

Project Costs

The project costs as outlined in the proposal are listed below.

Proposed Budget

Funds	***Amount***
Total funds from other sources (not managed by HAL)	$0.00
Total funds from voluntary contributions	$716,725.00
Total funds from industry levies	$0.00
Total funds for the project	$716,725.00
Total funds managed by HAL	$716,725.00

Proposed Budget details			
Source Type	**Source**	**Contributed Amount**	**Total Amount**
VC matchable	One Harvest		$716,725.00

Budget by Year

	2008/09		*2009/10*	
	Contributed Amount	***Total Amount***	***Contributed Amount***	***Total Amount***
One Harvest	$99,179.71	$171,088.00	$85,265.76	$147,086.00
Total VC Funds	**$99,179.71**	**$171,088.00**	**$85,265.76**	**$147,086.00**
Total Levy Funds	**$0.00**	**$0.00**	**$0.00**	**$0.00**
Total Funds Managed by HAL	**$99,179.71**	**$171,088.00**	**$85,265.76**	**$147,086.00**

	2010/11		*2011/12*	
	Contributed Amount	***Total Amount***	***Contributed Amount***	***Total Amount***
One Harvest	$95,161.23	$164,156.00	$118,502.28	$204,420.00
Total VC Funds	**$95,161.23**	**$164,156.00**	**$118,502.28**	**$204,420.00**
Total Levy Funds	**$0.00**	**$0.00**	**$0.00**	**$0.00**
Total Funds Managed by HAL	**$95,161.23**	**$164,156.00**	**$118,502.28**	**$204,420.00**

Implementation Costs

There will be no implementation costs as most of the label development will be done as part of the project. For example Focus groups will direct OneHarvest as to what information they want on a new label and how it should be presented.

The processed spinach and rocket is already packaged and labels are regularly changed to keep the customers interested.