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Innovative Products from Australian Native Foods

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by M. Forbes-Smith and J.E. Paton

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Foreword

Australian native foods have traditionally played an important role in the diet of Aboriginal people. With the introduction of the image of native foods as a unique Australian cuisine in the 1980s, interest in native foods by the commercial restaurant sector has developed steadily both in Australia and overseas. However, the Australian native food industry is now at a critical stage. Unless sales are increased, an over supply of some native foods may occur. Establishing a market focus and developing new market sectors will be the key to ensure a profitable future of the native food industry.

The aim of the study was to (1) improve shelf-life, quality and safety of existing native foods and to identify and develop novel products from native foods and (2) to develop applications across the food industry leading to significant increase in demand for the raw material with subsequent growth of the native food industry.

This report covers the following research findings:

- Expanded range of traditional foods containing native food ingredients
- Novel flavours, fragrances and extracts from native foods for use as conventional flavours, flavour enhancers and food ingredients
- Improved raw material quality and microbiological assessment of native foods
- Potential application of natural antioxidants and preservatives to improve food products

This project was funded from RIRDC Core Funds which are provided by the Federal Government.

This report, a new addition to RIRDC's diverse range of over 800 research publications, forms part of our of our New Plants Products R & D program, which aims to facilitate the development of new industries based on plants or plant products that have commercial potential for Australia.

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Simon Hearn
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We thank Cryovac Australia Pty Ltd for supplying the modified atmosphere packaging materials.

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Abbreviations

Aw	water activity
BHT	butylatedhydroxytoluene
BU	consistency values
cfu	colony forming units
CO_2	carbon dioxide
CRC	Cooperative Research Centre
Da	Daltons
DRBCA	dichloran rose bengal chloramphenicol agar
FAME	fatty acid methyl ester
GC-MS	gas chromatography-mass spectrometry
GC-O	gas chromatography-olfactometry
HPLC	high performance liquid chromatography
id	internal diameter
LC-MS	liquid chromatography-mass spectrometry
LDPE	low density polyethylene
LMO	lemon myrtle oil
LSD	least significant difference
m wt	molecular weight
m/z	mass to charge ration
MA	modified atmosphere
MEA	malt extract agar
nd	not detected
ns	not significant
O_2	oxygen
OTR	oxygen transmission rate
P	probability
PCA	plate count agar
ppm	parts per million
Rf	retention fraction
rt	retention time
SDE	simultaneous distillation and extraction
SPME	solid phase micro extraction
TLC	Thin Layer Chromatography
Tr	trace
UV	ultra-violet

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Executive Summary

In recent years, there has been a rising interest in novel food products and in particular those products containing Australian native foods. A range of native herbs, spices, nuts, essential oils and fruits are currently marketed for their distinctive flavour and functional qualities both in Australia and overseas. Most native food products are developed as condiments in that their particular tastes are so strong, a little goes a long way, hence their common use in sauces, syrups, preserves, seasonings etc. However, small quantities of fresh herbs and spices are also demanded by the food service and catering industry.

The Australian native food industry now encounters marketing challenges. There has been a marked interest in plantation produced or wild harvested native foods by land holders and indigenous groups, and unless sales are increased, an over supply of some native foods may occur. An effective strategy to advance growth of the Australian native food industry is to develop new market areas.

The purpose of this study was to:

- Improve shelf-life, quality and safety of existing native foods and to identify and develop novel products from native foods
- Develop applications across the food industry potentially leading to significant increase in demand for the raw material with subsequent growth of the Australian native food industry.

In reference to these objectives, the project consisted of the following investigations.

Extension of postharvest shelf-life: Speciality culinary indigenous herbs such as warrigal greens, lemon myrtle and mountain pepperleaf are sought by the commercial food retail and restaurant sectors. However, quality of fresh native herbs during marketing can only be provided through proper temperature management and postharvest packaging. The aim of this study was to determine the postharvest requirements of native herbs using novel modified atmosphere (MA) packaging systems.

Storage at 0-5ºC together with selected MA packaging significantly improved postharvest quality of warrigal greens, lemon myrtle and mountain pepperleaf. Higher storage temperature and inappropriate packaging promoted respiration rates, weight loss and characteristics of senescence. The presence of ethylene in some MA packages may have contributed to the deterioration of native herbs (eg. warrigal greens), although application of Purafil, an ethylene absorbent, virtually had no effect in extending postharvest shelf-life. However, ethylene production and rate of ethylene response of plant tissue would probably differ with various batches of native herbs.

The recommended postharvest requirements specified in this report should increase the value of postharvest warrigal greens, lemon myrtle and mountain pepperleaf, and provide a better return for growers and distributors.

Native food flavour research: The main economic value of Australian native foods is their unique and intense flavours. Research at the CRC has focussed on identifying flavour/aroma components and evaluating the importance of the flavours, fragrances and extracts from selected Australian native foods for use as conventional flavour enhancers in foods and other potential food ingredients. Native food flavours are currently well recognised by consumers and the attraction of new native food essences in food products will help increase demand.

Lemon myrtle oil: This study assessed the sensory nature of citral isomers neral and geranial, and the flavour contributions of the non-citral components of lemon myrtle oil. The main sources of the lemon and sweet aromas of lemon myrtle were neral and geranial, citronella and linalool. Non-lemon aromas included α-pinene, eucalyptol and aromadendrene-allo. Product development analyses showed that consumers consider lemon myrtle flavoured sorbet acceptable and desirable. Incorporation of less

sugar and/or higher amounts of acidulants such as citric acid would probably further advance the sweetness acceptability of lemon myrtle sorbet.

Lemon aspen: From the volatile compounds identified in lemon aspen fruit, the most abundant was limonene, followed by 3-carene, terpinolene and santalene. Components possessing a lemon aspen/lemon odour included α-terpineol, α-bergamotene (cis), santalene, limonene and β-myrcene. The acids (eg. citric acid) and sugars (eg. fructose) also contribute to the unique flavour quality of lemon aspen.

A new sorbet product incorporating lemon aspen and citrus juices was found desirable by consumers but a beverage developed with pineapple and lemon aspen flavours was not particularly well received due to bitterness intensity and inadequate sweetness. Inclusion of low levels of salt to reduce bitterness intensity and sugar to increase sweetness are likely to improve flavour characteristics of lemon aspen beverages.

Wild lime: In general, there was no appreciable differences in the types and levels of volatile compounds (eg. limonene, β-pinene, γ-terpinene), acids (eg. citric) and sugars (eg. fructose) in wild and Mexican limes. Furthermore, GC-olfactometry (GC-O) and sensory analysis determined little distinction between wild lime and Mexican lime flavour attributes. However, consumers considered wild lime flavouring in a cheesecake product highly desirable and acceptable. Wild lime is has yet to reach its capacity in the native foods niche market and this is possibly the best target area for the future.

Wattleseed: Various extraction methods (eg. simultaneous distillation) were used to isolate flavour components from roasted wattleseed. Pyrazines, which were the main compounds present, provided the dominant nutty, coffee and roasted aromas of wattleseed. The caffeine and quinolines detected may have contributed to the bitterness found in wattleseed. The presence of sugars (fructose, glucose), organic acids (eg. citric acid) and free amino acids suggest they play a part in the overall flavour of wattleseed.

A bitter aftertaste limited the overall liking of wattle flavours in blended chocolate beverage products. Sensory analysis of an instant wattleseed/chocolate beverage recipe using milk (to balance bitterness intensity) and sugar indicated superior consumer acceptability, although additional tests are needed to confirm this.

Preservation of lemon myrtle oil: Lemon myrtle oil (LMO) loses its unique flavour characteristics over time when added to products such as carbonated beverages, mustards and mayonnaises. Our investigations demonstrated the advantage of selected gums (eg. sodium alginate), pH regulation at 3.5 and microencapsulation of LMO to preserve fresh flavour of lemon myrtle in carbonated beverages. However, further shelf-life extension of LMO is required before realistic application in commercial soft drinks, which are marketed under ambient conditions. In mustard and mayonnaise however, encapsulation is potentially an effective technique for preserving lemon myrtle flavour.

Improvement of bread formulations containing akudjura: Incorporation of akudjura into established food products, such as bread, would assist to increase use of this spice and hence expand its market prospects. However, addition of akudjura to bread formulations has a detrimental effect on the character of the final baked product. Although akudjura in doughs had little influence on yeast activity during proofing, akudjura doughs appeared web-like and the 3% akudjura dough produced large holes. Extensograph and farinograph tests showed that dough strength, stability and development times declined with increasing akudjura content.

The decline of α and β-gliadins in akudjura doughs during proofing suggested that naturally occurring enzymes impair wheat proteins and prevent proper development of the gluten matrix. With mild heat

treatment (40°C for 5 min) of akudjura to inhibit possible enzymic activity, bread doughs containing the treated akudjura rose and held their shape over proofing.

Microbiological issues of dried native foods: The aims of this study were to (a) ascertain the microbiological status of selected dried native foods and if appropriate (b) investigate natural strategies to improve microbiological quality, thus minimising transfer of micro-organisms to further processed food products.

In general, microbial loads in native herbs and spices were fairly low ($\leq 10^3$ cfu/g), except aniseed myrtle, akudjura and Red Desert Seasoning (10^4-10^5 cfu/g). There were no coliforms, *Escherichia coli*, *Staphylococcus aureus* or *Bacillus cereus* present in different batches of these native foods.

Fumigation of spices with acetic acid effectively reduced microbial numbers but tended to cause tainting and caking of the product. Application of potassium permanganate after fumigation adequately removed acetic acid odour from Red Desert Seasoning but caking reduction with calcium chloride counteracted the antimicrobial efficacy of acetic acid fumigation.

Novel antioxidants: Major opportunity exists to increase the industry potential of Australian native foods by identifying natural additives that improve food products. In our studies, various native foods demonstrated a positive antioxidative response to conserve the intensity of orange/red pigment of β-carotene and slow formation of conjugated diene compounds in linoleic acid reaction tests.

Considerable antioxidant action of native food antioxidants/extracts was measured when incorporated into traditional foods. Native thyme extract significantly reduced surface browning of guacamole while lemon aspen extract minimised the rate of browning in freshly prepared apple juice. An antioxidant compound isolated from native thyme was determined as eugenol, whereas lemon aspen contained an antioxidant with a molecular mass calculated tentatively at 408 Da. Some antioxidant effect was observed in guacamole and apple juice when challenged to the purified forms of the native thyme antioxidant (eugenol) and lemon aspen antioxidant.

Novel antimicrobial compounds: Compounds from native pepperberry demonstrating antimicrobial activity were isolated and characterised using mass spectral techniques. One antimicrobial compound was tentatively identified as benzene, 1,1'-(3-methyl-1,3-butadienylidene)bis-, the other compound indicated a molecular mass of 490 da.

Upon application in foods, pepperberry extract minimised spoilage of tomatoes by *Botrytis cinerea* and Penicillium infection of apples. Shredded lettuce treated with pepperberry also contained lower viable counts of micro-organisms. The potential of this as a natural preservative should be investigated.

Implications: Growth of the native food industry would be of a major economic benefit for both local and international markets to develop from a uniquely Australian endeavour. The enhanced penetration of novel bushfood ingredients from our research findings into the general food industry will indirectly contribute to a wider appreciation of our native resources as foods and food adjuncts. The social impact has relevance to collectors and producers of bushfoods, value adders and end-users. Beneficiaries could include wild harvesters such as Aboriginal communities, specialist industry nurseries, gourmet food processors and manufacturers, restaurants ranging from the novel to the sophisticated upmarket sector, and speciality food service groups and distributors.

1. Introduction

1.1 Background

Australian native foods are presently marketed for their fresh, wild qualities both in Australia and overseas. The majority of native foods are prepared as preserves, sauces, chutneys and other condiments, although fresh herbs and spices are also used by the food service and catering industry. There is increasing national and international demand for these native food products particularly with a growing Australian identity. However, there is some concern that unless demand is further increased the industry might be in risk of an over supply of some native foods. Establishing an industry focus and developing new market sectors will facilitate a sustainable industry growth of indigenous Australian foods.

Major opportunities exist to further develop industrial outcomes that are uniquely Australian. These include:

- **Quantification of the unique flavour components and promotion of their use in conventional food products**

  The commercial importance of native foods is essentially associated to the distinctiveness and intensity of flavours imparted to food preparations. Flavours, fragrances and extracts add character and depth to foods and beverages and there is a major market demand for flavourings obtained from natural resources, especially those that are with novel qualities. Australia is in a unique position as few other countries can offer such a range of indigenous food flavours, fragrances and extracts, particularly products which have been unexploited. Determination of the chemical entities responsible for the unique flavours of native foods is necessary from a basic scientific knowledge aspect in respect to that of quality, especially during processing and storage. Enhancement of flavours and development of novel micro-application techniques can make existing small scale supplies cost-effective and commercially feasible. As the consumer acceptance of native food flavours in food products is well established, continued appeal of native food essences in food flavourings, sauces, beverages, etc will help increase demand.

- **Improvement of quality, shelf-life and microbiological safety of native food products leading to better acceptance and performance in products**

  In order to encourage greater sales of native food products to the mainstream retail and wholesale food markets, they must be of agreeable appearance, meet minimum food quality standards and have established food microbiological standards. It is also likely that avoiding the use of synthetic fumigants, such as ethylene oxide, and promoting natural means of microbial control would enhance market opportunities. Quality and food safety standards for fresh and dried native foods required to ensure quality, acceptability and maximisation of shelf-life have not been defined and formalised on an industry wide basis. Developing products with acceptable, attractive appearance, which meet high food quality standards, and implementing safe food microbiological standards would encourage larger food manufacturers to utilise native foods in the mainstream retail and wholesale food markets.

- **Identification and development of food ingredient components from native foods such as natural antioxidants and antimicrobial compounds and their potentially nutraceutical extracts/products for incorporation into conventional and functional foods**

  A plethora of opportunity exists to maximise the industry potential of Australian native foods by formulating new nutraceutical products that offer both functional and health benefits, and natural additives that improve food products. Sales of food products with health claims have been an

important growth area in the food industry. Natural antioxidants and components of Australian native foods may also fulfil similar functions. In addition, there is considerable scope to identify and assess antimicrobial compounds in native foods that may be potentially beneficial as food preservatives. Utilisation of native foods as natural preservatives would have significant consumer appeal.

This RIRDC sponsored project was initiated by the rising number of expressions of interest from the broader food industry to the CRC for Food Industry Innovation and to Vic Cherikoff, over the opportunities presented by indigenous Australian ingredients.

1.2 Objectives

The general objectives of this research were to:

- Improve shelf-life, quality and safety of existing native foods and to identify and develop novel products from native foods
- Develop applications across the food industry leading to significant increase in demand for the raw material with subsequent growth of the Australian native food industry.

In relation to these objectives, the project consisted of following individual studies:

- Extension of postharvest shelf-life of warrigal greens, mountain pepperleaf and lemon myrtle leaf
- Analysis and development of flavour components of lemon myrtle, wattleseed, wild lime and lemon aspen, and assess novel opportunities for commercialisation
- Application/product development, including preservation of lemon myrtle oil and improved bread formulations containing akudjura
- Microbiological analysis of dried native spice and herb products and investigation of strategies to improve microbiological safety
- Evaluation of antioxidant activity of potential native food sources and determination of active antioxidant component(s) in the most active preparation identified
- Evaluation antimicrobial activity of potential native food sources and determination of active antimicrobial component(s) in the most active preparation identified

2. Extension of postharvest shelf-life

Leading fresh indigenous herbs marketed in Australia include warrigal greens (*Tetragonia tetragonoides*), lemon myrtle (*Backhousia citriodora*) and mountain pepperleaf (*Tasmannia lanceolata*). However, the postharvest specifications for these herbs are ambiguous and at the time of study, Cherikoff indicated they were experiencing swelling of some of their packages of mountain pepperleaf, possibly due to inappropriate packaging materials. The aims of this study were to evaluate postharvest storage requirements of warrigal greens, lemon myrtle and mountain pepperleaf to maintain quality (appearance, fragrance, flavour) and maximise shelf-life using innovative packaging systems.

2.1 Methods

2.1.1 Respiration rates

An airflow ventilation system was utilised to measure respiration rates of native foods. Samples of exit air (flow rate: ca. 1 l/h) from receptacles containing the leaves (ca. 50 g) were collected with a 1 ml syringe and measured for carbon dioxide using Gowmac GC equipped with a stainless steel column (180 x 0.002 cm id, packed with Porapak® type Q, 80-100 mesh) and a thermal conductivity detector operated at 60ºC. Samples were replicated 4-5 times at 5 and 20ºC.

2.1.2 Storage temperature

Native herbs (ca. 25 g) placed in 28 l drums were stored at 0, 5, 10 and 20ºC. Moistened tissue was included to provide a high relative humidity (RH) during storage. To avoid accumulation of stale air, drums were opened daily for 5-10 min. Leaf quality parameters assessed included weight loss, appearance and presence of off-odours.

2.1.3 Packaging

The following packaging materials were investigated to extend postharvest shelf-life of fresh lemon myrtle, warrigal greens and mountain pepper leaf:

- Low density polyethylene (LDPE) - 25, 50, 75, 100 µm gauge
- Rob's long life vegetable bags (Gelpack Enterprises)
- Nylon (7 µm gauge) laminated with LDPE (15 µm) (Cryovac Sealed Air Corporation)
- Plastic films (Cryovac Sealed Air Corporation) with the following oxygen transmission rates (OTR): 2400, 6000, 7000 cm^3m^{-2} over 24 hr at 23ºC

Storage trials were conducted at 5ºC. Carbon dioxide content in packages was measured using the GC equipment described in section 2.1.1. Oxygen levels were sampled with a Gowmac GC equipped with a stainless steel column (90 x 0.002 id cm) packed with molecular sieve (80-100 mesh, Alltech) and electroconductivity detection at 60ºC. Determination of ethylene concentration was achieved using a Varian FID GC with a stainless steel column (90 x 0.002 cm id, packed with Porapak® type Q, 80-100 mesh). For ethanol quantification, headspace samples (5 µl) were injected on to a DB-Wax capillary column (30 m, 0.25 mm id) in a 3400 Varian GC.

With some MA trials of warrigal greens, Purafil® (2, 5 g/kg leaves), an ethylene absorbent, was investigated for its ability to remove ethylene within packages and extend postharvest shelf-life.

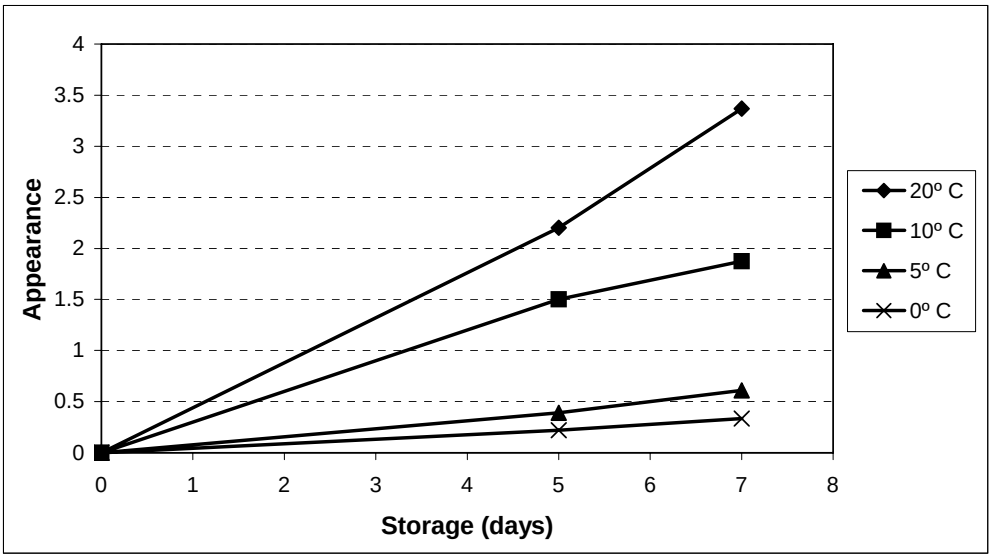
2.2 Results and discussion

2.2.2 Temperature

Appropriate temperature control is essential for optimal preservation of fresh horticultural produce. This usually occurs just above the temperatures that cause chilling or freezing injury. In this study, the ideal storage temperature for warrigal greens and lemon myrtle leaf occurred between 0-5ºC (Fig. 2.1). Increasing temperature accelerated characteristics of senescence, which included:

- leaf chlorosis (yellowing), physiological breakdown (decomposition) of tissue and production of off-odours in warrigals; and
- loss of leaf gloss followed by tissue deterioration in lemon myrtle

(A)



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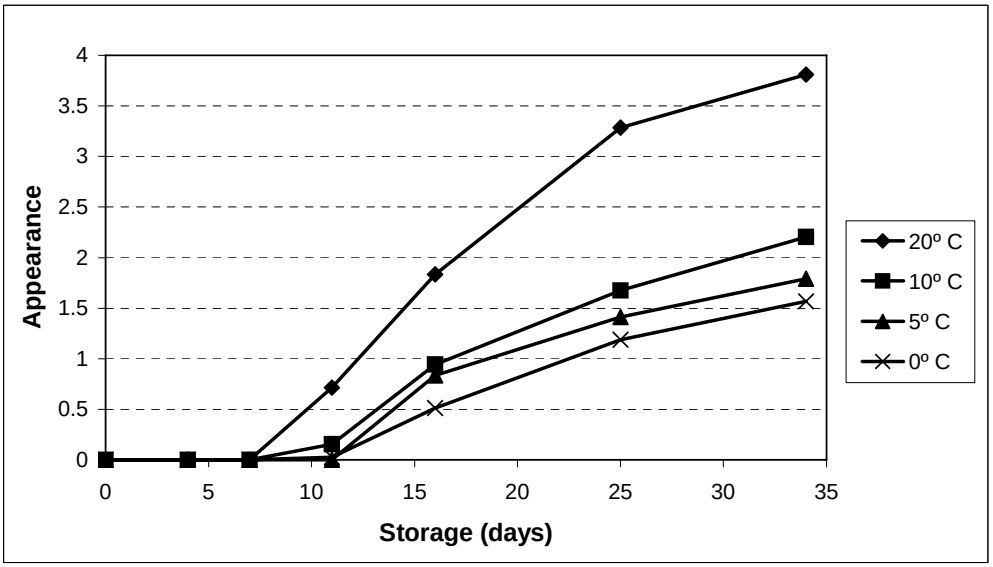


Figure 2.1 Effect of temperature on visual quality of (A) warrigal greens and (B) lemon myrtle. Appearance index: 0=excellent, 5=complete deterioration.

The rate of respiration is considered a good indicator of the potential postharvest longevity of horticultural commodities. In general, the higher the rate of respiration, the shorter the postharvest shelf-life of produce. Exposure of commodities to higher temperatures often results in increased respiration and a reduced shelf-life. For warrigal greens, the rate of respiration characteristically increased as temperature increased. At 5ºC, the respiration rate of warrigals stored for 6 days was 33 ml/kg/hr. When stored at 20ºC, the respiration rate increased approximately three fold to 110 ml/kg/hr for the same storage period (Fig. 2.2).

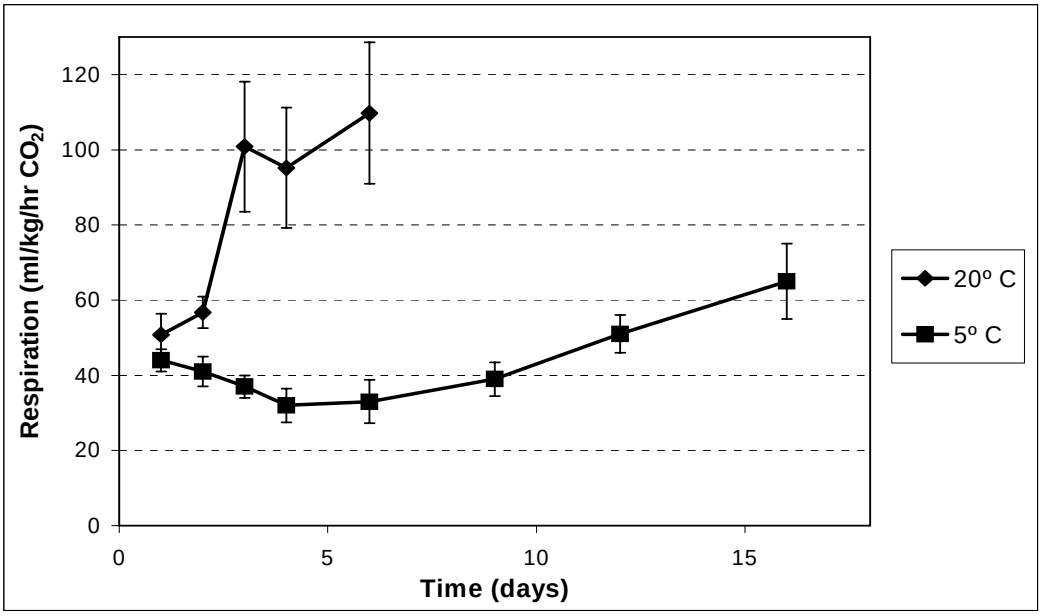
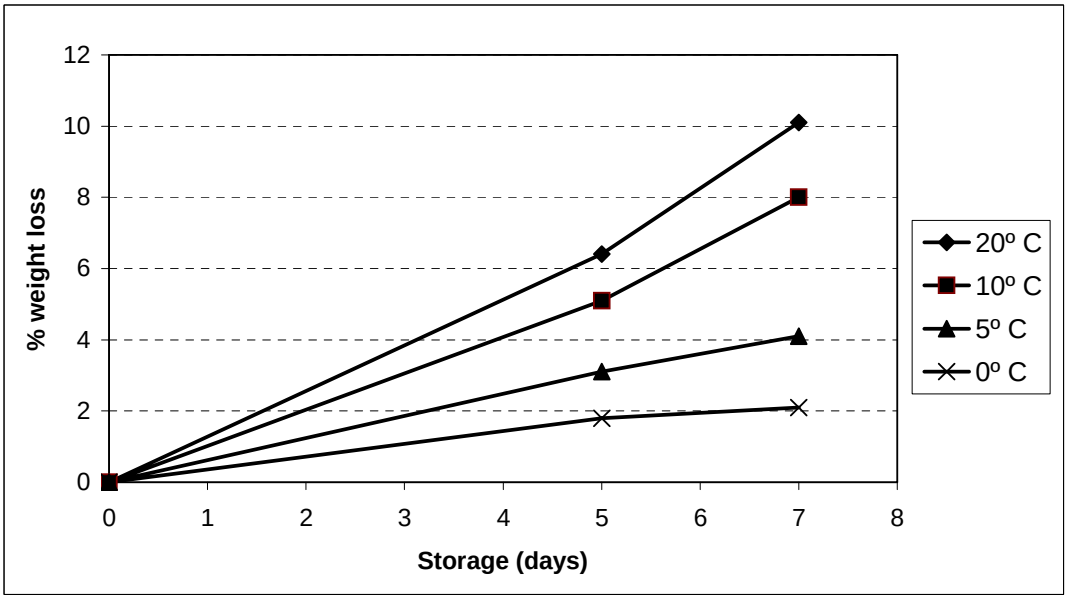


Figure 2.2 Respiration patterns of warrigal greens stored at 5 and 20ºC.

Another important factor affecting fresh produce is moisture loss. Desiccation caused by moisture loss adversely affects the appearance (eg. wilting) and weight of produce. With warrigal greens and lemon myrtle, lower storage temperatures produced lower weight losses than the higher temperatures (Fig. 2.3). For example, weight loss of warrigal leaves was minimal at 0 and 5ºC (2.1 and 4.1%, respectively), as opposed to 8-10 % weight loss at 10-20ºC after 7 days storage. Lower storage temperature, together with high RH (*ca.* 90-95% recommended), typically decreases moisture loss from produce because the vapour pressure deficit between the produce and surrounding air is reduced (Wills *et al*., 1998).

(A)



(B)

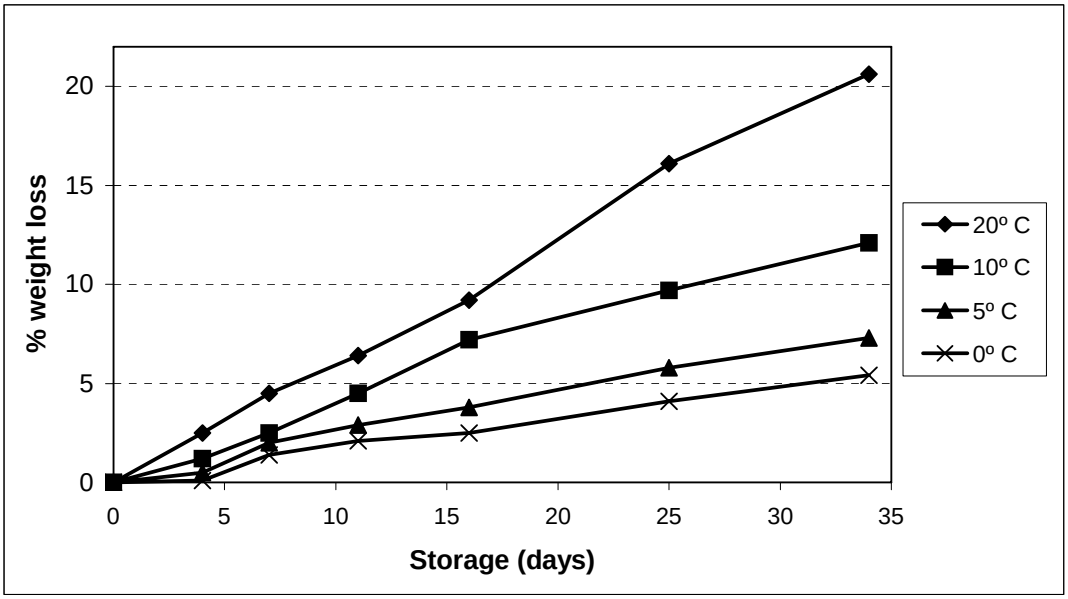


Figure 2.3 Effect of temperature on weight loss of (A) warrigal greens and (B) lemon myrtle

2.2.3 MA packaging

Use of packaging films is an effective method in extending shelf-life of fresh commodities because:

- Films restrict the movement of water vapour and produce a high RH atmosphere within the package, thus preventing excessive water loss from the produce
- Modified atmospheres advantageous to shelf-life can be generated

The key element in MA packaging is that the increased CO_2 or decreased O_2 produced by respiration by the commodity reduces tissue respiratory rate. The energy available for biochemical changes that take place in the produce is reduced, which can result in increased storage life. The actual concentration of CO_2 and O_2 within the MA package is mainly dependent on crop characteristics such as mass, temperature, maturity etc, and the permeability of the film. If film of correct gas permeability is selected, a desirable MA is established when rates of CO_2 and O_2 transmission through the package are equal to the respiration rate of the produce (Thompson, 1996). With warrigal greens, lemon myrtle and mountain pepperleaf, MA packaging showed a significant effect in maintaining postharvest quality.

2.2.3.1 Warrigal greens

Preliminary packaging studies indicated that film type and thickness influenced shelf-life quality of warrigals. Higher gauged LDPE films (75, 100 µm) tended to preserve leaf freshness more effectively than 50 and 25 µm LDPE, and Rob's vegetable bags, after 20 days storage at 5ºC (Fig. 2.4).

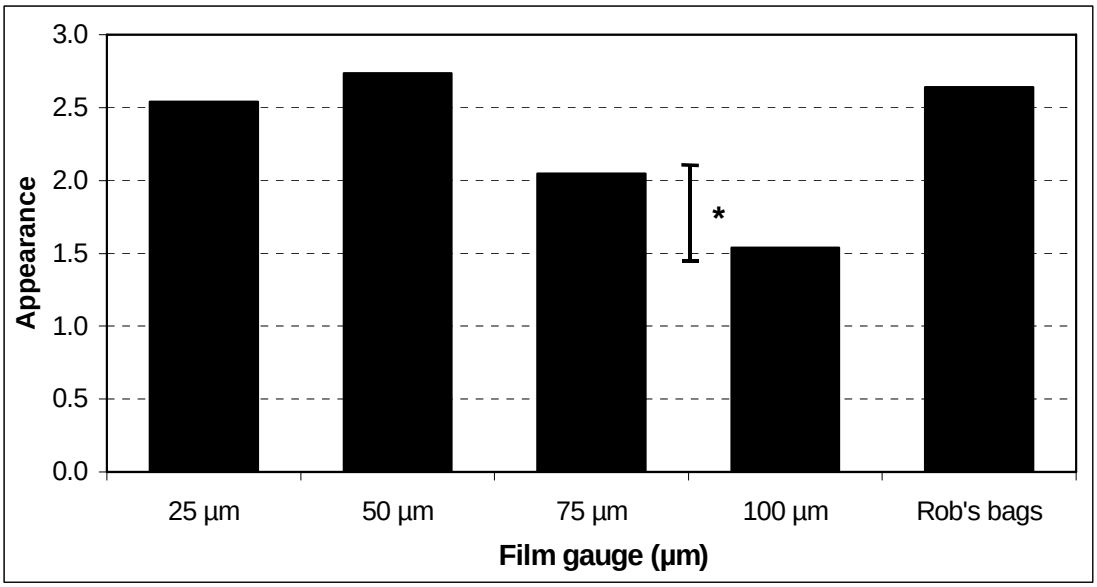


Figure 2.4 Effect of differently gauged bags on postharvest quality of warrigal greens stored at 5ºC for 20 days. Appearance index: 0=excellent, 5=complete deterioration. * represents LSD at 0.05 level

To optimise MA packaging of warrigals, 'smart' plastic films that contain specific OTRs were evaluated and compared to the Rob's bags which, at the time were used routinely by Cherikoff. Warrigals (30 g) packed in films (12 x 19 cm pack) with OTRs of 2400, 6000 and 7000 cm^3m^{-2} over 24 hr at 23ºC had significantly less tissue deterioration to those held in the Rob's bags at 5ºC after 25 days ($P<0.05$) (Fig. 2.5). Overall, leaves packaged in the '2400' and '6000' films were preferred because:

- over 65% of their leaves were marketable (index 1 or less signified a high quality product), as opposed to 57% and 42% of leaves in the '7000' and Rob's bags, respectively
- organic off-odours were detected upon opening the Rob's bags and to a lesser extent, the '7000' bags.

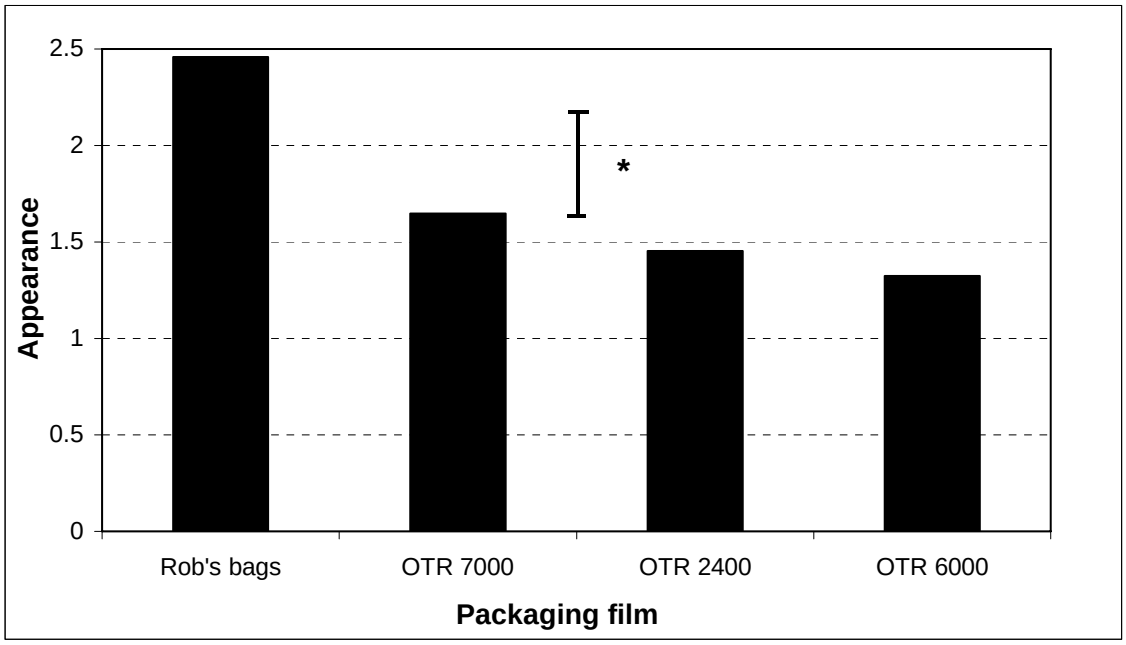


Figure 2.5 Effect of packaging type on quality of warrigal greens after 25 days at 5ºC. Appearance index: 0=excellent, 5=complete deterioration. * represents LSD at 0.05 level

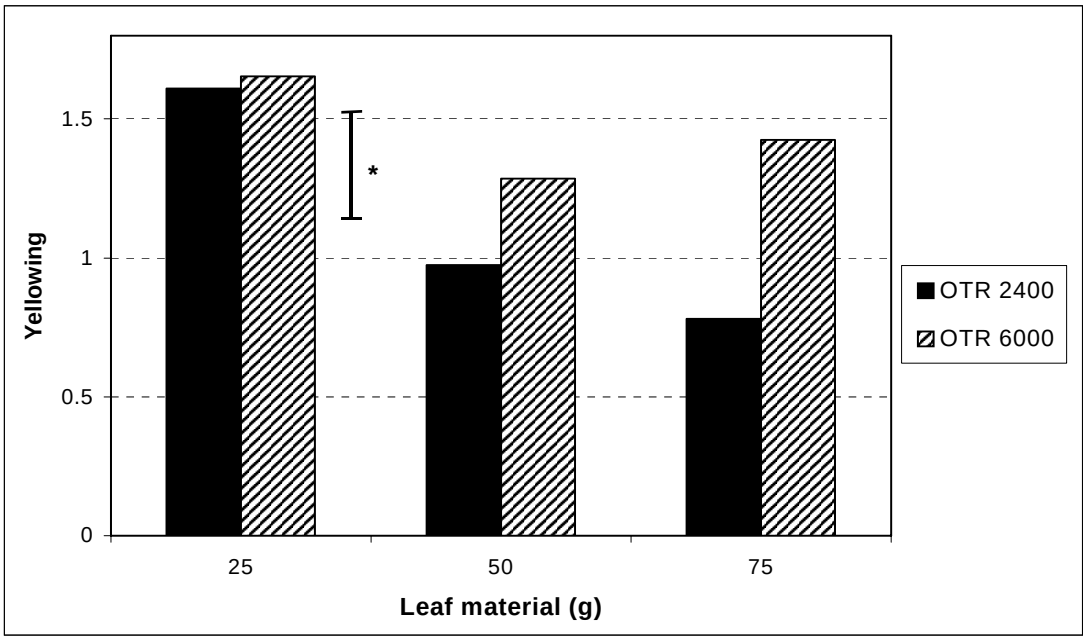
Although O_2 levels between packets were similar (around 11%), the slightly higher CO_2 concentrations (*ca.* 2%) detected in '2400' and '6000' packages (Rob's bags and '7000' both contained *ca*. 0.9% CO_2) perhaps contributed to the superior leaf quality. Weight loss of warrigals was low for all packaging films (*ca.* 0.3% average loss after 25 days) and therefore unlikely to be a major cause of leaf degradation.

Further improvement in leaf quality was achieved by increasing the mass of warrigal greens in packages. In particular, 75 g samples in the '2400' packages (17.5 x 20 cm) showed less leaf yellowing and tissue degradation than 25 and/or 50 g samples after 24 days at 5ºC ($P<0.05$) (Fig. 2.6). Varying the sample size of warrigals in the '6000 'packs had less influence on leaf freshness and in comparison to the '2400' packs, produced lower quality warrigals ($P<0.01$).

The atmospheric composition which favoured preservation of warrigals (75 g) in '2400' packages (mean values of 7.4% CO_2 and 3.4% O_2 at 15 days storage) differed considerably to those in bags with 50 g (5.8% CO_2; 6.2% O_2) and 25 g samples (3.5% CO_2; 10.9% O_2). In addition, ethylene, a plant growth regulator, was detected in package atmospheres (0.4-1.8 µl/l) and its concentration was positively related to severity of yellowing in leaves after 24 days ($P<0.05$).

Ethylene sensitivity was confirmed in a different batch of warrigal greens, where exposure of leaves to exogenous ethylene (ca. 2 µl/l for 24 hr at 20ºC) resulted in a 3-fold increase in yellowing after 7 days at 20ºC. In general, ethylene in postharvest storage environments has a negative impact on non-climacteric produce owing to promotion of senescence and loss of quality.

(A)



(B)

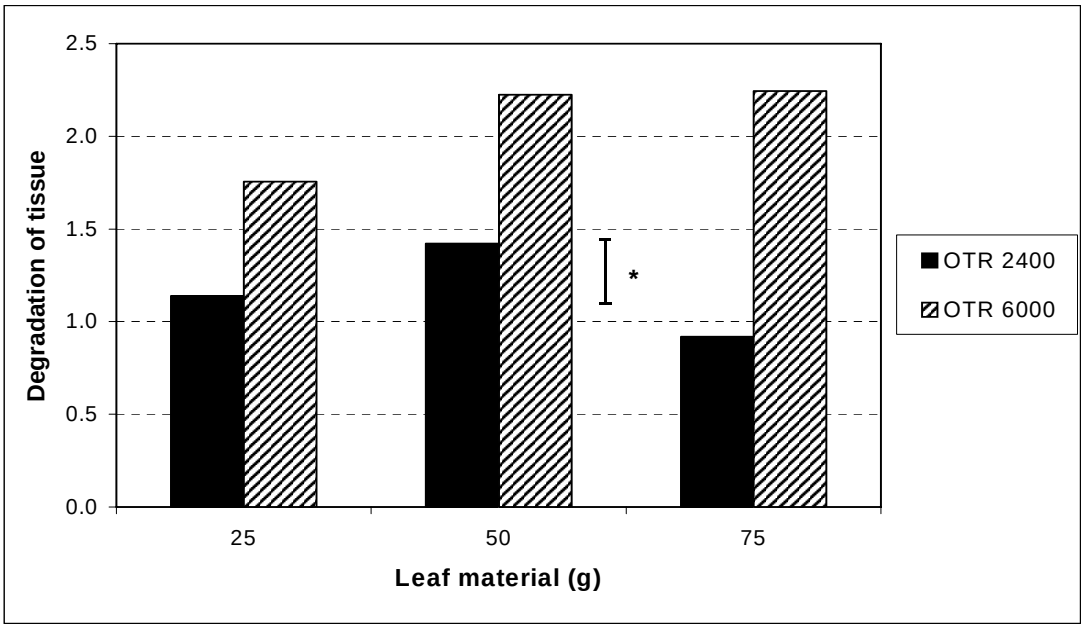


Figure 2.6 Leaf quality of warrigal greens in MA packaging at 5ºC for 25 days. (A) Yellowing index: 0=none , 5=very severe. (B) Degradation index: 0=none, 5=very severe. Index 1 or less signifies saleable product. * represents LSD at 0.05 level

A study was subsequently conducted using Purafil (2 & 5 g/kg leaves) with warrigal greens (75 g) in '2400' packets (17.5 x 20 cm) stored at 5ºC. However, at 3 weeks storage no obvious difference in yellowing and leaf decomposition occurred between leaves treated with Purafil and the controls, and all products were suitable for consumption. Although no ethylene was detected in the Purafil-treated packets, ethylene levels found in the controls (*ca*. 0.05 µl/l) were lower than those in the previous packaging experiment (0.4-1.8 µl/l) and possibly insufficient to accelerate leaf yellowing and/or

decomposition. Even when warrigals were stored for a further 3 weeks (and therefore beyond their marketable shelf-life), Purafil had no significant effect in reducing leaf yellowing and decomposition.

Production of ethylene from produce is influenced by environmental and physiological factors. It seems that for this crop of warrigals, low storage temperature at 5ºC diminished the accumulation of ethylene within packages and possibly the rate of response of the tissue to ethylene.

In light of these results, the recommended postharvest requirements for warrigals follow:

◊ MA storage using a film OTR of 2400 cm^3m^{-2} over 24 hr at 23ºC, *ca*. 0.107 g leaves/cm^2 film (eg. 75 g leaves in the 17.5 x 20 cm packet used in this study) at 0-5ºC for up to 3 weeks

The extra cost of employing Purafil with MA packaging of warrigal greens cannot be justified as Purafil had very little effect in maintaining postharvest quality of the leaves.

2.2.3.2 Lemon myrtle

Further studies demonstrated that application of MA packaging systems was advantageous in sustaining postharvest quality of lemon myrtle leaves. Films with QTRs of 6000 or 7000 cm^3m^{-2} over 24 hr at 23ºC were superior to '2400' film and a nylon/LDPE laminate that was used by Cherikoff for packaging and distribution of lemon myrtle ($P<0.001$) (Table 2.1).

Table 2.1 Effect of MA packaging on the appearance and saleability of lemon myrtle stored for 11.5 weeks at 5ºC. Appearance index: 0 = green, shiny, no tissue deterioration; 1 = slight (<5%) deterioration present on leaf; 2 = browning and dulling present, 6-25% deterioration; 3 = 26-50% deterioration; 4 = 51-75% deterioration; 5 = 76-100% deterioration. Percent marketable product was derived on the proportion of leaves having an appearance index of 0 to 1.

Film	Atmosphere *+ (% CO_2 : % O_2)	Appearance *# (index units)	Marketable*# leaves (%)
Nylon/LDPE laminate	19.4 : 1.0	3.5 **b** ^	0
OTR 2400	3.3 : 2.4	3.7 **b**	1
OTR 6000	0.9 : 4.6	0.5 **a**	91
OTR 7000	1.6 : 6.0	0.4 **a**	93

* Data are mean values of two replicates
\+ At 7 weeks storage
\# At 11.5 weeks storage
^ Values within the column followed by the same letter are not significantly different ($P=0.05$)

After 10.5 weeks storage at 5ºC, 91-93% of leaves (50 g) held in packets (13 x 17.5 cm) made with '6000' and '7000' films were of excellent quality ie. shiny, bright green and virtually free (> 95% of leaf surface area) of deterioration. A fresh, herbaceous odour was present upon opening the packets and leaves, after crushing, immediately released a strong, pleasant fragrance of lemon myrtle. In comparison, no lemon myrtle leaves were commercially acceptable in '2400' and 'nylon/LDPE' bags under the same storage conditions (5ºC; 10.5 weeks). Leaves were dull, brown and necrotic, an alcoholic off-odour was present (particularly in the nylon/LDPE packets), and minimal lemon myrtle aroma was detected from the crushed leaves.

It appears that the elevated levels of CO_2 in the '2400' and 'nylon/LDPE' packets (3.3 and 19.4% at 7 weeks storage, respectively) and low O_2 (2.4 and 1.0 %, respectively) were detrimental to lemon myrtle quality. When oxygen levels fall to very low concentrations, anaerobic respiration will initiate in produce. Anaerobic respiration is generally associated with undesirable off-odours and flavours owing to the accumulation of ethanol, acetaldehyde and organic acids, and the deterioration of produce quality (White and Roberts, 1992). Ethylene also detected in the '2400' and 'nylon/LDPE' bags (0.4 and 1.4 ppm, respectively at 7 weeks) possibly assisted leaf degradation. The atmospheres produced in the '6000' packages (0.9% CO_2; 4.6% O_2; no ethylene detected) and '7000' packages (1.6% CO_2; 6.0% O_2; no ethylene) clearly benefited extension of postharvest shelf life.

Optimum postharvest conditions for lemon myrtle are as follows:

◊ *ca*. 0.110 g leaves/cm^2 of '6000' or '7000' film, stored at 0-5ºC for 11 weeks

2.2.3.3 Mountain pepperleaf

Inadequate packaging of mountain pepperleaf in nylon/LDPE film has been reported by Vic Cherikoff due to swelling of packages and alcoholic off-odours. In our trials with mountain pepperleaf packaged in nylon/LDPE film (25 g/pack; 12 x 19 cm pack size), browning and loss of gloss occurred within 2 weeks at 5ºC, and leaves were totally unacceptable for purchase at 5 weeks (Table 2.2). The high CO_2 (22.3%) and low O_2 (2.3%) atmosphere in the bags, and the presence of alcoholic off-odours and ethanol (*ca.* 0.0012% w/v) indicated active anaerobic respiration by the mountain pepperleaf.

When compared to the other types of MA packaging, approximately 88% of leaves held in '2400' and '6000' packets for 5 weeks were of high quality ie. shiny, green and virtually free (< 5% of leaf surface area) of deterioration with no obvious off-odours. At 11 weeks storage, around 51-59% of leaves was suitable for consumption.

The following postharvest storage specifications for mountain pepperleaf are recommended:

◊ Packaging in '2400' or '6000' film (*ca*. 0.055 g leaves/cm^2), stored at 0-5ºC for 5 weeks.

Table 2.2 Effect of MA packaging on the appearance and saleability of mountain pepperleaf stored at 5ºC. Appearance index: 0 = green, shiny, no tissue deterioration; 1 = slight (<5%) deterioration present on leaf; 2 = browning and dulling present, 6-25% deterioration; 3 = 26-50% deterioration; 4 = 51-75% deterioration; 5 = 76-100%. deterioration. Percent marketable product was derived on the proportion of leaves having an appearance index of 0 to 1.

Film	Atmosphere * + (% CO_2 : % O_2)	5 weeks storage		11 weeks storage	
		Appearance (index units)	Marketable product (%)	Appearance (index units)	Marketable product (%)
Nylon/LDPE laminate	22.3 : 2.3	4.9	0	5	0
OTR 2400	4.6 : 14.4	0.7	88	2.3	51
OTR 6000	2.3 : 12.4	0.7	88	1.9	59
OTR 7000	1.6 : 13.6	1.1	81	1.7	59

* Data are mean values of two replicates at 5 weeks storage

2.3 Summary

The ideal temperature range for postharvest native herbs occurred around 0-5ºC. Higher storage temperatures significantly promoted respiration rates, weight loss and characteristics of senescence. Further improvement in postharvest quality was achieved by application of selected MA packaging systems. The recommended postharvest requirements for selected native herbs are as follow:

- ***Warrigal greens***: Packaged in '2400' film (*ca*. 0.107 g leaves/cm^2), stored at 0-5ºC for up to 3 weeks
- ***Lemon myrtle***: Packaged in '6000' or '7000' film (*ca*. 0.110 g leaves/cm^2), stored at 0-5ºC for up to 11 weeks
- ***Mountain pepperleaf***: Packaging in '2400' or '6000' film (*ca*. 0.055 g leaves/cm^2), stored at 0-5ºC for up to 5 weeks.

The presence of ethylene possibly contributed to the degradation of native herbs in packages although Purafil, an ethylene absorbent, had little effect in maintaining postharvest quality when incorporated with warrigal greens.

3. Native food flavour research

The main commercial values of indigenous Australian foods are their unique and intense flavours, which can be minor ingredients in mixtures yet contribute to distinctly different food preparations. However, there is a dearth of knowledge concerning the exact nature of the components in Australian native foods responsible for their attractive flavour characteristics. Subsequently, research at the CRC has been directed towards evaluating the importance of the flavours, fragrances and extracts from selected Australian native foods, namely lemon myrtle, lemon aspen, wild lime and wattleseed, for use as conventional flavour enhancers in foods and other potential food ingredients. GC-MS, GC-O and HPLC techniques were used to investigate the volatile components that contribute to the aroma of the product, as well as sugars and acids, but sensory evaluation and product development also played an important aspect of the research.

3.1 Material and methods

3.1.1 Raw materials

Lemon myrtle (*Backhousia citriodora*) oil, lemon aspen (*Acronychia acidula*), wild lime (C*itrus australis)* and wattleseed (*Acacia* sp., in the form of ground roasted wattleseed) were provided by Vic Cherikoff. Miss Thai Ann Chorr donated cultivated (Mexican) lime.

3.1.2 Extraction

Extraction of volatile components from native foods involved the following:

- Solvent extraction using iso-octane, dichloromethane, ethanol (95%) and/or water, at various temperatures and intervals
- SDE with dichloromethane for 1 hour using the Likens and Nickerson apparatus
- Absorption of volatiles (from wattleseed) by SPME. Exposure of the syringe unit containing the SPME fibre (non-bonded polydimethylsiloxane) to wattleseed samples proceeded at ambient temperature (ca. 25ºC) for 45 min. and 20 hours, and 60ºC for 10 min

A derivatisation procedure similar to the method described by Russell and Kenyon (1955) was used to extract citral from lemon myrtle oil. Rather than reacting pure oil with sodium bicarbonate/sodium sulphite solution to isolate citral, lemon myrtle oil was extracted 1:1 (wt/vol) with iso-octane before derivatisation. This technique was applied to minimise possible side-reactions with minor non-aldehydic compounds during the citral reaction, as the non-aldehydic components are retained in the iso-octane phase and thus more likely to be protected. After completion of the reaction, the iso-octane layer was removed and the derivatised citral (ie. the labile disulphonate) remaining in the aqueous reaction solution (the labile disulphonate is water soluble) was regenerated on gradual addition of sodium hydroxide (2.5 M). Fresh iso-octane was used to re-extract the citral during the regeneration process.

Solvent extracts (iso-octane, dichloromethane) containing volatile components from native foods were dried over sodium sulphate and concentrated as 'gently' as possible by using a stream of cool nitrogen.

Sugars and organic acids in wattleseed samples were extracted using the methods of Wills, Balmer & Greenfield (1980) and Wills, Scriven & Greenfield (1983), respectively. Wattleseed extractions and juices of lemon aspen and wild lime filtered through 0.22 µm membranes were analysed for sugars and organic acids by HPLC (see section 3.1.3 for details). Ninhydrin reagent prepared by the method of Craske (1963) was used to detect free amino acids in wattleseed extracts spotted on filter paper.

3.1.3 Analysis

3.1.3.1 Volatile components

A Hewlett Packard GC model 5890 coupled to a HP mass spectrometer (model 5972) was used to analyse the volatile components of native foods. The GC was fitted with a J&W capillary column (30 m x 0.25 mm id) coated with DB5MS (5% phenyl [equiv.] polysilphenylene-siloxane) with a film thickness of 0.25 µm. To validate results, the mass spectra from the volatile components matched by the mass spectral software library (NBS75K) were also compared to mass spectra and retention times of compounds published by Adams (1995), and to external standards. Internal standards (eg. dodecane, tridecane, n-nonane) were used to aid quantification of extracted compounds.

Olfactory gas chromatography (HP model 5890 series GC) was utilised for assignment of odour characteristics to specific volatile components. The outlet stream from the capillary column (30 m x 0.25 mm id; DB5-MS) was split 1:1 between the FID and olfactory port. Aroma descriptions were recorded with retention time of compounds.

3.1.3.2 Non-volatile components

Sugars from native food were identified and quantified by injecting samples into a Waters HPLC (Model ALC/GPC 244) equipped with a differential refractive index detector. The HPLC was fitted with a silica Rad-Pak, 100 x 8 mm id column, using the mobile phase acetonitrile:water (80:20) with 0.1% SAM at the flow rate of 4 ml/min.

Analysis of organic acids was accomplished using a Waters HPLC system fitted with a tuneable UV detector (Model 486) set at λ 214 nm. The HPLC was fitted with an ion exclusion column (Bio-Rad Aminex HPX-87H, 300 x 7.8 mm id) heated to 55ºC. The mobile phase consisted of 0.06% orthophosphoric acid in water at a flow rate of 0.5 ml/min.

3.1.4 Product development

Sensory evaluation of products flavoured with native food extracts was conducted to determine consumer acceptability of the product formulation, as well as consumer acceptability of native food extracts as flavouring agents. The following products were developed:

- Sorbet containing lemon myrtle oil (and compared to lemon oil flavoured sorbet)
- Citrus/lemon aspen and pineapple/lemon aspen beverages
- Cheesecakes incorporating wild lime and Mexican lime extracts
- Wattleseed/chocolate beverages

Panellists evaluated properties of products such as intensity and liking of appearance, aroma, taste and texture using quantitative descriptive or discriminative (duo trio) methods. Correlation matrices and ANOVA of data were used to compare perceptions of product attributes.

3.2 Results and discussion

3.2.1 Lemon myrtle

3.2.1.1 Analysis of compounds

The citral isomers neral (37.1%) and geranial (45.3%) comprised 82.4% of lemon myrtle oil (Table 3.1). In another batch of lemon myrtle oil, citral content was higher at 89.01% with 39.74% of neral and 49.27% of geranial (results not shown). Clearly, citral is the main component in lemon myrtle oil, although the levels recorded were slightly lower than citral concentrations (90-95%) published by other workers (Southwell *et al.*, 2000; Taylor, 1996).

Minor components detected in the initial sample of lemon myrtle oil included 5-hepten-2-one 6-methyl (1.54%), geraniol (1.26%) and two unidentifiable compounds (peaks 13 and 14) at 2.55% and 4.32%, respectively (see Table 3.1). Remaining compounds occurred in trace (<1%) amounts.

Reduction of neral and geranial content using the derivatisation reaction described in section 3.1.2 permitted easier identification of minor compounds. At three hours of derivatisation, only small amounts of neral (2.17%) and geranial (2.59%) were present in the lemon myrtle oil extract (Table 3.2). Virtually all the citral isomers were derivatised within half the reaction time stated by Russell and Kenyon (1955). Additional compounds identified included linalool (5.85%), β-myrcene (4.39%) and 2,3-dehydro-1,8-cineole (3.52%).

Upon recovery of citral (95.06% yield) from the aqueous extract, a low concentration (4.94%) of non-citral components was present, suggesting loss of non-citral compounds during the derivatisation process. This loss may be due to the formation of sulphonates by ketones and other aldehydes, which were extracted with the citral.

3.2.1.2 Aroma analysis

Determination of aromas of the volatile compounds by GC-O provided important information on the flavour description of the compounds in lemon myrtle oil. Compounds that contributed strong lemon/lemonade aroma typical of lemon myrtle included neral, geranial and citronellal (Table 3.1). The concentration of citronellal was low (0.25%) in lemon myrtle oil, which suggests it also has a low threshold at which detection by smell is possible. Other compounds recording lemon/lemonade/citrus odours included linalool, 5-hepten-2-one 6-methyl, β-myrcene, β-elemene, germacrene B and unidentified peaks 13 and 24, which all occurred at low concentrations.

Similar results were obtained after most of the citral was removed by derivatisation, except more non-lemon aromas were detected probably because the odour threshold of minor components increased (Table 3.2). The components α-pinene, eucalyptol, aromadendrene-allo, although minimal contributors of aroma, formed the dry notes of the oil particularly after exposure at room temperature. Citronellal again provided a strong lemon odour consistent with lemon myrtle, however nerol and unidentified peaks 18 and 19 caused a rosy and softer aroma. The lemonade aroma typical of lemon myrtle was contributed by β-elemene. Reduction in citral concentration by derivatisation also allowed more detailed characterisation of its isomers, where neral was found sweeter.

Although GC-O is a valuable tool in determining particular odours present in a food product, it is dependent on the ability of the analyst to effectively characterise aromas. It can be influenced by factors such as range and threshold limits that the analyst can sense, recognition and labelling of specific odours, and the concentration of the odour. In addition, the time required for the aroma to be detected by the human nose might coincide with other aromas eluting immediately afterwards.

Table 3.1 Concentration (%) and aroma descriptions of compounds present in lemon myrtle oil before citral derivatisation

Peak No.	rt (min)	Quality NBS (%)	Identity	Concentration* (%)	Odour description
1	7.61	91	5-Hepten-2-one,6-methyl	1.54	orange, floral
2	7.77	94	β-Myrcene	0.75	lemony
3	7.84	58	1,2,4,4-Tetramethylcyclopentene	#	nd
4	9.02	93	1,3,8-p-Menthatriene	#	grassy
5	9.31	97	Eucalyptol	#	camphor, eucalyptus
6	9.79	94	Ocimene Z β	#	sweet
7	11.56		unidentified	#	watermelon, sweet
8	11.95	90	Linalool	0.65	faint lemon
9	12.44		unidentified	#	nd
10	12.88		unidentified	#	nd
11	13.71		unidentified	0.80	nd
12	14.08	97	Citronellal	0.25	crushed lemon leaves
13	14.51		unidentified	2.55	fresh lemon
14	15.33		unidentified	4.32	nd
15	15.42		unidentified	#	nd
16	16.16		unidentified	#	nd
17	16.51		unidentified	#	nd
18	17.40	64	2,6-Octadien-1-ol,2,7-dimethyl	0.32	nd
19	18.09	97	Neral	37.12	very strong lemon
20	18.55	90	Geraniol	1.26	fruity, orange
21	19.46	93	Geranial	45.31	grassy lemon
22	20.61	98	Tridecane	*	acetone
23	21.42		unidentified	#	faint lemon
24	21.99		unidentified	#	strong lemon
25	23.54		unidentified	#	nd
26	25.29	78	β-Elemene	#	strong lemonade
27	28.62	76	Germacrene B	0.20	lemonade
			Total	95.06	

* internal standard is excluded from the compounds present in the oil
\# peak area on the chromatogram too small to quantify
nd not detected

Table 3.2 Concentration (%) and aroma descriptions of compounds present in lemon myrtle oil after derivatisation of citral for 3 hours

Peak No.	rt (min)	Quality NBS (%)	Identity	Concentration* (%)	Odour description
1	6.13	91	α-Pinene	#	eucalyptus, pine
2	7.60	93	5-Hepten-2-one,6-methyl	13.82	nd
3	7.75	91	β-Myrcene	4.39	nd
4	7.83	56	2,3-Dehydro-1,8-cineole	3.52	nd
5	8.35		unidentified	#	nd
6	9.02	62	1,3,8-p-Menthatriene	1.12	grassy
7	9.29	96	Eucalyptol	#	mint
8	9.77	90	Ocimene Z β	#	nd
9	11.58		unidentified	#	sweet, pineapple
10	11.92	90	Linalool	5.85	rose, floral
11	12.44		unidentified	#	nd
12	12.88		unidentified	#	nd
13	13.51		unidentified	2.61	lemon
14	13.86		unidentified	#	nd
15	14.06	95	Citronellal	2.19	crushed lemon leaves
16	14.51		unidentified	17.45	faint lemon
17	15.03		unidentified	#	slight rosy lemon
18	15.33		unidentified	22.03	leafy, rose
19	15.43		unidentified	3.35	strong rose
20	16.16		unidentified	#	faint rose
21	16.30		unidentified	#	faint rose
22	16.52		unidentified	2.67	nd
23	16.80		unidentified	#	nd
24	17.22	94	Nerol	2.66	rose
25	17.31		unidentified	#	nd
26	17.77	91	Neral	2.17	sweet lemon
27	18.36	94	Geraniol	8.28	grass, lemon
28	19.06	94	Geranial	2.59	lemon
29	20.59	98	Tridecane	*	acetone
30	24.30		unidentified	1.62	herb
31	25.25	87	β-Elemene	1.51	faint lemonade
32	25.95		unidentified	#	nd
33	26.30		unidentified	#	nd
34	27.12	99	Aromadedrene allo	#	grassy
35	28.59	72	Germacrene B	2.18	faint floral
			Total	100	

* internal standard is excluded from the compounds present in the oil
\# peak area on the chromatogram too small to quantify
nd not detected

3.2.1.3 Lemon myrtle sorbet

A sensory evaluation was conducted to determine consumer acceptance of lemon myrtle oil as a flavouring agent by comparison with lemon oil in sorbet. Overall, lemon myrtle flavoured sorbet was found acceptable and desirable by consumers, although both lemon flavours were liked equally (Table 3.3). The lemon flavour and aftertaste of the lemon myrtle oil sorbet were considered the right strength, whereas the lemon flavour and aftertaste in lemon oil sorbet were reported as being slightly weak ($P<0.05$). However, this difference did not affect the liking of lemon flavour and aftertaste, which occurred towards the 'extremely like' end of the scale (ie. values > 6.0) for both sorbets.

Consumers preferred the sweetness intensity of lemon oil sorbet, which probably relates back to types and concentrations of compounds present in the oils. Neral and geranial, which are highly concentrated in lemon myrtle oil (see Table 3.1 for percentages), have sweet lemon aroma/flavours. In contrast, lemon oil contains little neral and geranial (1.15 and 2.0%, respectively), and consists mainly of limonene (*ca*. 60%), which has a citrus lemon aroma/flavour and is not described as being sweet (results not shown). Incorporating less sugar and/or more acid (eg. citric acid) would most likely improve the sweetness acceptability of lemon myrtle oil sorbet.

3.1.2.4 Summary

The study has been useful in evaluating the sensory nature of citral isomers neral and geranial, and the flavour contributions of the non-citral components of lemon myrtle oil. Overall, 34 compounds were revealed in lemon myrtle oil with 16 positive identifications. The major contributors of the lemon and sweet aromas of lemon myrtle were neral and geranial, and to a lesser extent citronella and linalool. Non-lemon aromas that contributed to the fragrance of lemon myrtle included α-pinene, eucalyptol and aromadendrene-allo.

Product development studies showed that consumers found lemon myrtle flavoured sorbet acceptable and desirable. The potential to use lemon myrtle oil as flavourants in further valued foods such as sorbet is significant, yet the dilemma of maintaining lemon myrtle oil distinctiveness in unchilled food products needs to be resolved, as discussed in Section 4.1.

Table 3.3 Mean scores for sorbet attributes. The scores are values out of 10 and the greater the score, the greater the degree of liking or intensity.

ATTRIBUTES	Lemon Myrtle	Lemon
Appeal of Appearance	6.4 a *	6.5 a
Liking of Appearance	6.3 a	6.4 a
Degree of Sweetness	6.3 a	5.6 a
Liking of Sweetness	**5.9 a**	**7.1 b**
Degree of Sourness	4.5 a	4.0 a
Liking of Sourness	6.1 a	6.3 a
Strength of Lemon Flavour	**5.4 a**	**4.5 b**
Liking of Lemon Flavour	6.0 a	6.4 a
Degree of Thickness	5.3 a	5.2 a
Liking of Thickness	6.3 a	6.9 a
Strength of Aftertaste	**6.4 a**	**4.7 b**
Liking of Aftertaste	6.3 a	6.9 a
Overall liking	6.2 a	6.8 a

* Values within the row followed by the same letter are not significantly different (P=0.05)

3.2.2 Lemon aspen

3.2.2.1 Volatile components

Of the 44 volatile components detected from lemon aspen juice, 20 were positively identified by mass spectral analysis and retention time. In general, lemon aspen juice consisted predominantly of monoterpenes and sequiterpenes. Major monoterpenes identified included limonene, which was the most abundant volatile compound (38.9% of total area of peaks), followed by:

- 3-carene (23.1%)
- terpinolene (11.7%)
- β-myrcene (3.1%)
- α-pinene (1.8%)
- γ-terpinene (1.0%)

Main sequiterpenes detected in lemon aspen were santalene (5.1% of total peak area) and α-bergamotene (cis) (1.0%). Alcohols found included 3 cyclohexan-1-ol (1.8%), α-terpineol (1.0%), spathulenol (0.6%), and linalool (<0.2%), as well as the benzenoid aromatic structured compounds ρ-cymene (0.4%) and ρ-cymenene (0.2%).

Quantification of compounds in lemon aspen was calculated by inclusion of an internal standard to a sample extracted three times with dichloromethane (Table 3.4). Of the most abundant compounds identified, limonene was present at 186 ppm and 3-carene at 115 ppm, followed by terpinolene (56 ppm), santalene (22 ppm), β-myrcene (13 ppm) and 3 cyclohexan-1-ol (8 ppm). Identifications for the 24 components were unobtainable as their concentrations were probably too low for reliable matching (except unidentified peak 19, which was 24 ppm), although these compounds cannot be dismissed as unimportant as they may contribute to the aroma and taste of the fruit.

Lemon aspen fruit contains volatile compounds that are regarded important in the flavour of citrus fruits, including lemon (eg. limonene, γ-terpinene, bergamotene), lime (limonene, α-pinene), mandarin (limonene, α-terpineol, linalool) and orange (limonene, α-pinene, linalool, α-terpineol) (Shaw, 1991). In addition, limonene is present in citrus fruit at high concentration, where for example lime and grapefruit have been reported to contain 47-64% and 86-95% limonene, respectively (Shaw, 1979). Perhaps this accounts for the view that lemon aspen flavour is similar to a blend of lime and grapefruit (Anon., 1999a)

3.2.2.2 Aroma analysis

Results from GC-O indicated that α-terpineol, and unidentified compounds 23 and 24 express a powerful lemon aspen aroma and to a lesser extent, α-bergamotene (cis), santalene and unknown peak 26. These compounds, except santalene, were present in small amounts (< 5 ppm), which suggests that volatile components at low concentrations contribute to the aroma of the fruit (see Table 3.4 for concentrations).

Table 3.4 Concentration of compounds present in lemon aspen juice

Peak no.	Quality NBS (%)	Identity	Concentration (ppm)
1		unidentified	Tr *
2	94	α-Pinene	6
3		unidentified	Tr
4	94	β-myrcene	13
5	95	3-carene	110
6	90	α-terpinene	Tr
7	91	ρ-cymene	Tr
8	97	limonene	175
9	91	β-phellandrene	Tr
10		unidentified	Tr
11		unidentified	Tr
12		unidentified	Tr
13	94	γ-terpinene	2
14		unidentified	Tr
15	98	terpinolene	56
16	91	ρ-cymenene	Tr
17	72	linalool	Tr
18		unidentified	Tr
19		unidentified	24
20	96	3 cyclohexan-1-ol	8
21		unidentified	6
22	86	α-terpineol	4
23		unidentified	Tr
24		unidentified	3
25		unidentified	Tr
26		unidentified	Tr
27		unidentified	Tr
28		unidentified	1
29	93	α-bergamotene (cis)	4
30	98	santalene	22
32	99	aromadendrene	Tr
33		unidentified	Tr
34		unidentified	Tr
35		unidentified	Tr
36		unidentified	Tr
37		unidentified	Tr
38	81	(E,E) β-farnesene	Tr
39	93	β-bisbolene	Tr
40		unidentified	Tr
41		unidentified	Tr
42	98	δ-cadinene	Tr
43		unidentified	Tr
44	95	spathulenol	2

* Tr = < 1.0 ppm

The compounds listed below form the overall aroma of the lemon aspen fruit.

- lemon (limonene, β-myrcene, α-terpinene, γ-terpinene, unidentified peaks 28, 33, 34);
- pineapple (1);
- burnt rubber (unidentified peak 3);
- paint (ρ-cymene);
- herb (unidentified peak 10);
- light pine (linalool);
- grass (unidentified peak 19); and
- formic acid (unidentified peak 25)

Certain odour descriptions (eg. lemon) did not relate to retention times of compounds identified by GC-MS.

3.2.2.3 Non-volatile components

Sweetness and sourness are the major taste contributors, and they are determined by the amount of organic acids and sugars in the juice. Organic acids determined in lemon aspen included:

- citric acid (concentration: 5.3 %)
- malic acid (0.59 %)
- succinic acid (0.51 %)
- lactic acid (0.21 %)
- acetic acid (0.20 %)
- tartaric acid (0.03 %)

These organic acids are common in fruits except lactic acid, although lactic acid presence has been reported in apples, grapes and bananas (Ulrich, 1970). The sugars identified in lemon aspen were fructose (0.08 %) and glucose (0.04 %).

Acid and sugar contents in lemon aspen would fluctuate in response to extrinsic factors (eg. climate and soil type during growth) and intrinsic factors, such as stage of maturity of the fruit.

3.2.2.4 Lemon aspen products

Sorbet: A sensory evaluation (duo-trio test) was conducted on sorbets, one flavoured with lemon and orange juice, and the other with lemon, orange and lemon aspen juice. The recipe for the lemon-orange sorbet consisted of freshly squeezed lemon juice (125 ml) and orange juice (20 ml), sugar (200 g), one egg white and 200 ml water. The lemon-orange-lemon aspen sorbet included lemon juice (123 ml), orange juice (18 ml) and lemon aspen juice (2.5 ml) with the same amounts of sugar, egg white and water.

Most (69%) assessors were able to distinguish between the two types of sorbets ($P<0.05$). Of those who detected a difference, 68% preferred the sorbet containing lemon aspen mainly because of its tangy, unique flavour. The other 32% of assessors liked the sorbet without lemon aspen because it had a nice citrus flavour.

Incorporation of lemon aspen flavour with citrus products is not recommended as it negates the flavour of the lemon aspen (Anon., 1999a). Although our finding does not determine whether lemon aspen flavour remains unchanged, it does indicate the development of a significantly different flavour, which in the case of sorbet is desirable.

Beverage: A beverage was developed incorporating canned pineapple juice (500 ml) and lemon aspen juice (2.6. g), and compared to pineapple juice only. Sensory tests showed that addition of lemon

aspen juice decreases the sweetness and increases the bitterness of the beverage ($P<0.05$) (Table 3.5). The panellist liking for sourness, bitterness and aftertaste attributes was less for the pineapple/lemon aspen beverage than for the pineapple beverage. This was sustained by the fact that all positive correlations were associated with attributes related to sweetness and negative correlations with bitterness and sourness.

Table 3.5 Mean scores for beverage attributes. The scale values are out of 10, greater values correspond to a greater degree of strength and liking.

Attributes	Pineapple	Pineapple/ lemon aspen
Strength of sweetness	**6.57 a**	**5.06 b***
Like of sweetness	5.56 a	4.70 a
Strength of sourness	4.07 a	5.09 a
Like of sourness	**6.60 a**	**5.35 b**
Strength of bitterness	**1.67 a**	**3.38 b**
Like of bitterness	**6.86 a**	**4.88 b**
Strength of aftertaste	4.81 a	4.97 a
Like of aftertaste	**6.06 a**	**4.52 b**
Overall liking	**6.27 a**	**4.60 b**

* Values within the row followed by the same letter are not significantly different ($P=0.05$)

The lemon aspen beverage tested was not overly well received and this may be because the recipes used were more a contributing factor than the flavours themselves. Sweetness of beverages can be simply increased by adding sugar although the main obstacle for lemon aspen/pineapple beverage is the presence of bitter aftertaste. A practical method for lowering bitterness intensity and increasing flavour acceptance in beverages is addition of salt. Less addition of lemon aspen to the beverage may also retract the effects of bitterness and insufficient sweetness while still maintaining the flavour contribution.

3.2.2.5 Summary

From the 44 volatile compounds revealed in lemon aspen fruit, 20 were positively identified by GC-MS. The most abundant volatile was limonene, followed by 3-carene, terpinolene, unidentified compound 19 and santalene. The presence of acids (eg. citric acid) and sugars (eg. fructose) indicated that they contribute to the distinctive flavour character of lemon aspen. Combining lemon aspen with citrus in sorbet was considered desirable by consumers but pineapple/lemon aspen beverages need to be sweeter and less bitter.

3.2.3 Wild lime

Wild lime fruit are slightly bitter with a very tart lime flavour. They are used to garnish main dishes or desserts, and as an ingredient in marmalades and savoury sauces. However, little is known about the nature of the components (volatiles, sugars and acids) that give wild limes their distinctive flavour character.

Oil and juice analyses were an integral part of this study. The volatile components that produce the citrus aroma are mainly found in the oil of the rind, whereas the source of taste is the juice. The flavour profile of wild lime was compared to the Mexican lime.

3.2.3.1 Volatile components

Vigorous mixing of grated rind in dichloromethane at room temperature was an effective method to extract volatile components from wild and Mexican limes. Overall, the identities of the volatile components in the oil of wild lime and Mexican lime were similar but less volatiles were detected in the wild lime (32 compounds) than the Mexican lime (35) (Tables 3.6 and 3.7). Major compounds identified in wild lime included limonene, followed by β-pinene and γ-terpinene. Wild lime contained higher amounts of limonene, β-pinene, geranial and neral (38.2, 14.3, 7.9 and 4.9%, respectively) than those in Mexican lime (31.7, 8.4, 6.3 and 4.0%, respectively), but less γ-terpinene (12.2% and 20.4% in wild and Mexican lime, respectively). Although several compounds were unique to wild lime (eg. E-β-ocimene) and to Mexican lime (eg. 1,8-cineole, α-bisabolol), they were only present at trace levels.

Many of the compounds identified were similar to aroma constituents reported in other limes (Azzous and Reineccins, 1976; Pino and Tapanes, 1983), although amounts of limonene determined in this study (31-38%) were lower than values published by Shaw (1979) (47-64%). The coumarin compounds (eg. 7-dimethoxy coumarin) and psoralens (eg. bergaptene) found in the wild lime oil have also been reported in cold-pressed oils from cultivated limes (*Citrus aurantifolia, C. latifolia*) (Shaw, 1976; Dugo *et al.* 1997). Major components in wild lime oil that corresponded to components in wild Russel River lime (*Microcitrus inodora*) included limonene, α--pinene, myrcene, sabinene, terpinolene, linalool, neral and geranial (Shaw, Moshonas and Bowman, 2000).

3.2.3.2 Aroma analysis

Subjective analyses by GC-O concluded there were no major differences between aroma attributes of wild lime and Mexican lime (Tables 3.6 and 3.7). Aromas of wild lime were usually described in terms of citrus-like odours, for example:

- faint lemon (identified component: sabinene)
- rose, strong lemon (α-terpinene)
- faint orange (geranial)
- β-pinene (lime)

Other aroma descriptors included spicy (α-thujene), grassy/leafy (limonene) and menthol (γ-terpinene).

Not all compounds found by GC-MS were detectable by an aroma, particularly the higher molecular weight compounds which eluted late (>30 min.) during GC-O. Certain odours (eg. lemon) did not match retention times of compounds identified by GC-MS.

Table 3.6 Concentration (%) and aroma descriptions of compounds present in wild lime

Peak No.	Quality NBS (%)	Identity	Concentration (%)	Odour description
1	90	α-Thujene	<0.5	sweet, spicy
2	97	α--pinene	1.41	nd
3	97	sabinene	2.45	faint lemon
4	95	β-pinene	14.28	lime
5	91	β-myrcene	0.99	nd
6	90	α-terpinene	<0.5	rose, lemon
7	96	D-limonene	38.22	grassy, leafy
8	83	(E) β-ocimene	<0.5	nd
9	97	γ-terpinene	12.17	menthol
10	91	terpinolene	<0.5	nd
11	72	linalool	<0.5	nd
12	91	α-terpineol	<0.5	rose, lemon grass
13	91	decanal	<0.5	nd
14	90	neral	4.91	nd
15	95	geranial	7.93	faint orange
16	98	δ-elemene	1.25	nd
17		neryl acetate	<0.5	nd
18	83	geranyl acetate	<0.5	nd
19	80	β- elemene	<0.5	nd
20		unidentified	<0.5	nd
21		E-caryophyllene	<0.5	nd
22		γ-elemene	<0.5	nd
23	80	α-trans bergamotene	1.76	nd
24	91	germacrene D	<0.5	nd
25	86	α-farnescene (E,E)	3.57	nd
26	98	β-bisabolene	3.04	nd
27	93	germacrene B	<0.5	nd
28	96	7-dimethoxy coumarin	<0.5	nd
29	68	5,7-dimethoxy coumarin	3.66	nd
30	91	iso-bergaptene	<0.5	nd
31	83	bergaptene	<0.5	nd
32	93	isopimpinellin	3.78	nd

nd = not detected

Table 3.7 Concentration (%) and aroma descriptions of compounds present in Mexican lime

Peak No.	Quality NBS (%)	Identity	Concentration (%)	Odour description
1	91	α-Thujene	<0.5	sweet, rose
2	95	α--pinene	1.45	lemon
3	97	sabinene	1.41	off-lemon
4	96	β-pinene	8.4	lime
5	91	myrcene	0.97	nd
6	95	α-terpinene	<0.5	nd
7	96	limonene	31.65	grassy, leafy
8	95	1,8-cineole	<0.5	menthol
9	96	γ-terpinene	20.4	nd
10	98	terpinolene	0.87	plastic
11	83	linalool	<0.5	lime
12		isoborneol	<0.5	floral
13	86	terpineol	<0.5	lemon orange
14	64	decanal	<0.5	nd
15	96	neral	4.02	nd
16	94	geranial	6.31	nd
17	93	δ-elemene	<0.5	faint lemon grass
18	91	neryl acetate	2.3	nd
19	90	geranyl acetate	<0.5	grassy
20	62	β-elemene	<0.5	nd
21	99	E-caryophyllene	<0.5	nd
22		γ-elemene	<0.5	nd
23		α-trans bergamotene	1.84	nd
24	81	germacrene D	<0.5	nd
25	64	Z-α- bisabolene	<0.5	faint lemon
26		unidentified	<0.5	nd
27	95	β-bisabolene	2.99	nd
28	93	germacrene B	<0.5	nd
29	72	α-bisabolol	<0.5	nd
30	96	7-dimethoxy coumarin	3.48	nd
31		unidentified	<0.5	nd
32	94	5,7-dimethoxy coumarin	7.08	nd
33	91	iso-bergaptene	3.04	nd
34		bergaptene	<0.5	nd
35	97	isopimpinellin	1.94	nd

nd = not detected

3.2.3.3 Non-volatile components

Organic acid and sugar analyses showed that wild lime juice had slightly higher levels of acids but a lower sugar content than the Mexican lime juice. The main organic acid present in the wild and Mexican limes was citric (5.03 and 4.14%, respectively), followed by malic (1.20 and 1.86%, respectively) and succinic (0.22 and 0.11%, respectively). Citric, malic and succinic acids are suggested as the main organic acids present in lime juice (Vandercook, 1977; Kale and Adsule, 1995).

Sugars in wild and Mexican lime were fructose (0.43 and 0.58%, respectively), glucose (0.42 and 0.74%, respectively) and sucrose (0.24 and 0.52%, respectively). McCready (1977) indicated that the content of fructose, glucose and sucrose in lime was slightly higher at 0.9%, 0.9% and 0.3%, although factors such as climate during growth and maturity of fruit would influence the content of sugar in lime.

3.2.3.4 Product development

A sensory evaluation was conducted on the wild and Mexican lime, using baked cheesecake as the food matrix. The recipe utilised both lime rind (oil) and juice, and the recipe was duplicated for each lime type. In general, there was no obvious difference in intensity and liking of any of the attributes (aroma, sweetness, sourness, bitterness, aftertaste, texture) surveyed. Panellists could not significantly differentiate between samples of cheesecake made from wild or Mexican lime, and this may be because the chemical compositions of wild lime and Mexican lime are quite similar. However, the sensory test indicated that wild lime is an acceptable flavour substitute for Mexican lime in cheesecake and possibly different food products as well.

3.2.3.5 Summary

In general, there were no significant dissimilarities in the types and levels of volatile components (eg. limonene, β-pinene), acids (eg. citric) and sugars (eg. fructose) between wild and Mexican limes. Aroma attributes of wild lime, mostly described in terms of citrus-like odours, were comparable to those of Mexican lime. Consumers found cheesecakes prepared with either wild lime or Mexican lime favourable but detected no apparent differences in sensory characteristics between the lime types.

3.2.4 Wattleseed

Wattleseed is an Australian native food flavouring, initially developed by Vic Cherikoff in 1984. It is being increasingly used to enhance food products including beverages, breads and dairy foods (ice cream, cream and yoghurt). The roasted seed, which is ground to a free-flowing product that looks like coffee grounds, has a coffee-chocolate-hazelnut flavour. The major thrust of this project was to characterise the volatile components of wattleseed and to determine their sensory significance.

3.2.4.1 Volatile components

Isolation of volatile flavour compounds from wattleseed was accomplished using a number of extraction techniques, including solvent (dichloromethane) extraction at ambient temperature and 40ºC, and SPME (45 min and 20 hr exposures at ambient temperature and 10 min exposure at 60ºC). The SDE method proved to be the most efficient extraction technique with 145 compounds being detected by GC-MS, compared to solvent extraction at 40ºC (59 compounds) and ambient temperature (17 compounds), and SPME (up to 56 compounds) (Tables 3.8 - 3.11). Major compound groups determined from wattleseed included:

- pyrazines
- aldehydes/ketones
- fatty acid methyl esters (FAME)
- furans
- pyridines
- terpenes
- pyrroles
- amines
- quinolines
- hydrocarbons
- fatty acids

Unlike lemon myrtle, lemon aspen and wild lime, wattleseed contained pyrazines, which are nitrogenous compounds. Pyrazines were the major compound class found using SPME (16 pyrazine compounds detected) and SDE (22 compounds, which gave a total concentration of 20.6 ppm). A lower number (9) of pyrazine compounds were identified using solvent extraction at 40ºC and none with solvent extraction at ambient temperature.

Pyrazines most likely resulted from the roasting process of wattleseed by reactions such as the Maillard reaction and Strecker degradation (Shibamoto, 1989). They are described as having sweet, bitter and corn-like aromas, although alkyl substitution of pyrazines can lead to aromas of burnt, grassy, roasted, pungent and nutty character (Dart and Nursten, 1985). Major pyrazines identified using SDE included 2,5-dimethyl pyrazine (4.91 ppm), trimethyl pyrazine (4.65 ppm), 3-ethyl 2,5-dimethyl pyrazine (2.79 ppm) and 2-ethyl-6-methyl pyrazine (2.76 ppm).

Aldehydes/ketones were the largest group of compounds extracted by solvent at 40ºC (5 compounds yielding a total of 13.69 ppm total) and the second largest group by SDE (12 compounds; 1.94 ppm). The Maillard reaction and Strecker degradation, as well as oxidation of phospholipids and polyunsaturated fatty acids can produce aldehyde/ketone compounds (Manley, 1989). Major aldehydes/ketones detected in this study included 3,7-dimethyl-2,6-octadienal, 1-(6-methyl-2-pyrazinyl)-1-ethanone and 4-cyclopentene-1,3-dione.

Table 3.8 Characterisation of wattleseed components extracted by SDE

Peak	rt (min)	Identity	Quality NBS (%)	Quantity (ppm)	Odour description
1	5.54	Furfuryl alcohol	96	0.75	burnt
2	5.69	Pyridine, 3-methyl-	97	2.63	fried onion
3	5.74	2-Propanone, 1-(acetyloxy)-	59	trace	nd
4	5.80	2(3H)-Furanone, 5-methyl-	58	trace	burnt meat
5	6.10	4-Cyclopentene-1,3-dione	64	0.21	nd
6	6.24	Methyl amyl ketone	64	0.15	strawberry
7	6.80	Ethanone, 1, (2-furanyl)-(2-acetylfuran)	62	trace	nd
8	6.86	Pyrazine, 2,5-dimethyl-	91	4.91	fried smell
9	6.94	Pyrazine, ethyl-	53	trace	vegetables
10	7.00	Pyrazine, 2,3-dimethyl	91	trace	plastic
11	7.36	Pyrazine, ethenyl-	81	0.30	smoke
12	8.09	Pyridine, 3-ethyl-	95	2.73	soil
13	8.47	5-Methyl furfural	96	16.34	fried food
14	8.57	unidentified		trace	nutty
15	8.79	Furancarboxylic acid, methyl ester	94	6.96	burnt
16	8.93	unidentified		0.11	burnt meat
17	9.06	Pentanoic acid, 4-oxo, methylester	78	0.13	corn cheese chips
18	9.16	unidentified		0.44	nd
19	9.27	unidentified		0.32	nd
20	9.46	Pyrazine, 2-ethyl-6-methyl-	91	2.76	burnt leaves
21	9.62	Pyrazine, trimethyl	87	4.65	medicine
22	9.71	1H-pyrrole-2-carboxaldehyde, 1-methyl-	90	trace	burnt
23	9.77	1-Propanone, 1-(2-furanyl)-	80	trace	nd
24	9.83	Pyrazine, propyl-	91	trace	burnt
25	10.13	unidentified		1.06	soil
26	10.29	unidentified		0.50	vegetables
27	10.44	unidentified		trace	coffee
28	10.58	Limonene	96	1.55	lemon
29	10.64	unidentified		trace	coffee
30	10.88	unidentified		trace	nd
31	11.02	unidentified		trace	nd
32	11.14	Benzeneacetaldehyde	92	0.37	nd
33	11.28	2-Pyrimidineamine, N,N-dimethyl-	72	0.16	chocolate
34	11.36	Pyridine, 3-propyl-	81	0.14	medicine
35	11.55	unidentified		trace	nd
36	11.66	Terpinene <gamma>	90	0.13	burnt leaves
37	11.76	unidentified		0.14	nd
38	11.87	unidentified		0.32	nd
39	12.00	unidentified		trace	stir-fry vegetables
40	12.20	unidentified		0.45	nd
41	12.38	Pyrazine, 3-ethyl-2,5-dimethyl-	95	2.79	barbecue
42	12.49	Pyrazine, 2,3-diethyl-	93	trace	leaves
43	12.61	Pyrazine, 2-ethyl-3,5-dimethyl-	91	0.75	burnt
44	12.73	Pyrazine, 5-ethyl-2,3-dimethyl-	91	1.21	burnt wood
45	12.88	3-Furancarboxylic acid, 2-methyl-, methyl ester	72	trace	popcorn
46	12.95	unidentified		0.81	nutty
47	13.11	Furan, 2,2-methylenebis-	58	0.39	burnt
48	13.25	Pyrazine, 2-methyl-5-(1-propenyl)-, (E)-	59	0.72	strong burnt

trace = <0.1 ppm nd = not detected

Table 3.8 (cont) Characterisation of wattleseed components extracted by SDE

Peak no.	rt (min)	Identity	Quality NBS (%)	Quantity (ppm)	Odour description
49	13.56	Nonanal	80	0.81	nd
50	13.60	unidentified		trace	strong burnt
51	13.69	unidentified		trace	nd
52	13.75	unidentified		0.51	nd
53	13.84	unidentified		trace	burnt wood
54	13.90	1-(5-Methyl-2-pyrazinyl)-1-ethanone	94	0.16	smoke
55	14.07	unidentified		trace	faint burnt
56	14.16	1-(6-Methyl-2-pyrazinyl)-1-ethanone	81	0.43	strong burnt
57	14.26	unidentified		trace	nd
58	14.37	unidentified		trace	nd
59	14.45	unidentified		trace	nd
60	14.60	unidentified		trace	fried beans
61	14.76	Pyrazine, 2-methyl-3-propyl-	52	trace	faint burnt
62	14.82	unidentified		0.22	faint burnt
63	14.96	Pyrazine, 5H-5-methyl-6,7-dihydrocyclopenta-	91	0.60	faint burnt
64	15.04	unidentified		trace	nd
65	15.22	unidentified		trace	fried tofu
66	15.33	unidentified		trace	nd
67	15.45	Pyrazine, 2,3-diethyl-5-methyl-	62	trace	stir-fry smell
68	15.51	unidentified		trace	coffee
69	15.58	Pyrazine, 3,5-diethyl-2-methyl-	93	1.01	stir-fry smell
70	15.68	Pyrazine, 2,3,5-trimethyl-6-ethyl-	90	0.30	peanut
71	15.74	Pyrazine, 2,5-dimethyl-3-propyl-	91	trace	peanut
72	15.88	unidentified		0.37	peanut
73	16.02	2,4,6-trimethyl-1,3-phenylenediamine	87	0.75	peanut
74	16.10	unidentified		trace	bean cake
75	16.33	unidentified		trace	nutty
76	16.43	unidentified		trace	nd
77	16.54	unidentified		0.45	sweet nutty
78	16.67	1H-pyrrole, 1-(2-furanylmethyl)-	64	0.69	yellow beans
79	16.76	unidentified		trace	banana leaf
80	16.87	unidentified		trace	banana leaf
81	16.98	unidentified		0.35	banana leaf
82	17.06	unidentified		trace	grassy
83	17.18	unidentified		trace	strong nutty
84	17.32	unidentified		0.58	nd
85	17.50	unidentified		trace	nutty
86	17.63	Dodecane *	96		
87	17.69	Pyrazine, 1-methylpyrrolo[1,2-a]-	72	trace	faint nutty
88	17.81	unidentified		trace	coffee
89	17.90	unidentified		0.11	nd
91	18.39	1-(3,5-dimethyl-2-pyrazinyl)-1-ethanone	83	trace	nutty
92	18.48	unidentified		0.47	banana leaf
93	18.63	unidentified		0.26	banana leaf
94	18.71	unidentified		trace	nutty
95	18.97	unidentified		trace	nutty
96	19.30	unidentified		trace	fried foods
97	19.38	unidentified		0.35	nd
98	19.64	unidentified		0.22	nd
99	19.79	unidentified		0.25	strong nutty

trace = <0.1 ppm nd = not detected * designates internal standard

Table 3.8 (cont.) Characterisation of wattleseed components extracted by SDE

Peak no.	rt (min)	Identity	Quality NBS (%)	Quantity (ppm)	Odour description
100	19.99	unidentified		trace	roasted sesame seeds
101	20.21	unidentified		0.15	roasted sesame seeds
102	20.35	unidentified		trace	could not be detected
103	20.56	unidentified		trace	faint sesame seeds
104	20.75	unidentified		trace	nutty
105	20.97	unidentified		trace	nd
106	21.14	unidentified		trace	nd
107	21.26	unidentified		trace	faint nutty
108	21.40	Benzene, 1-methoxy-4-(1-propenyl)-	96	0.30	nutty
109	21.54	unidentified		trace	strong nutty
110	21.67	unidentified		trace	strong nutty
111	21.78	unidentified		0.15	nd
112	21.90	unidentified		trace	nd
113	22.20	unidentified		trace	fried foods
114	22.34	unidentified		trace	nd
115	22.49	Pyrazine, 2-butyl-3,5-dimethyl-	64	trace	burnt
116	22.64	unidentified		trace	nutty
117	22.81	unidentified		0.33	strong nutty
118	23.02	unidentified		trace	woody
119	23.12	unidentified		0.10	nutty
120	23.84	2-Methyl-6-hydroxyquinoline	80	0.58	coffee
121	24.15	unidentified		0.26	nutty
122	24.27	unidentified		0.30	coffee
123	26.70	unidentified		0.73	weak burnt
124	27.74	unidentified		0.60	nd
125	28.21	unidentified		0.19	nd
126	28.41	unidentified		trace	nd
127	29.35	unidentified		0.21	fried beans
128	30.42	unidentified		trace	nd
129	30.80	unidentified		0.21	nd
130	31.01	unidentified		0.22	nutty
131	31.19	unidentified		0.11	nd
132	34.84	Tetradecanal	87	trace	weak nutty
133	38.00	2-Pentadecanone	87	0.12	nd
134	38.63	Pentadecanal	91	0.34	nd
135	38.95	unidentified		trace	weak nutty
136	42.46	unidentified		trace	nd
137	43.05	2-Pentadecanone, 6,10,14-trimethyl-	91	0.16	nd
138	43.85	unidentified		0.19	nutty
139	44.16	unidentified		0.11	coffee, nutty
140	44.54	unidentified		trace	coffee
141	44.81	unidentified		trace	slightly burnt
142	45.07	unidentified		trace	nd
143	45.89	Hexadecanoic acid, methyl ester	98	0.59	nutty
144	51.13	Methyl linoleate	99	0.83	nd
145	51.35	Methyl oleate	99	0.58	nd
146	51.52	unidentified		trace	nd

trace = <0.1 ppm nd = not detected

Table 3.9 Identification of wattleseed components extracted by dichloromethane at 40ºC

Peak	rt (min)	Identity	Quantity (ppm)	Quality NBS (%)
1	5.70	2-furanmethanol	10.06	96
2	5.93	2-propanone, 1-(acetyloxy)-	13.69	64
3	7.12	Pyrazine, 2,5-dimethyl-	6.88	83
4	7.26	Pyrazine, 2,3-dimethyl-	trace	72
5	8.44	Pyridine, 3-ethyl-	trace	90
6	8.57	2-Furancarboxaldehyde, 5-methyl- (furfurfal, 5 methyl)	20.81	91
7	8.97	Furancarboxylic acid, methyl ester	trace	80
8	9.79	Pyrazine, 2-ethyl-6-methyl-	trace	91
9	9.94	Pyrazine, 2-ethyl-3-methyl-	trace	39
10	10.52	1H-pyrrole-2-carboxaldehyde	9.07	87
11	10.68	2-butenedioic acid (E)-, dimethyl ester	trace	42
12	11.01	Butanedioic acid, dimethyl ester	trace	40
13	12.38	Ethanone, 1-(1H-pyrrol-2-yl)-	trace	91
14	12.81	Pyrazine, 3-ethyl-2,5-dimethyl-	trace	90
15	13.17	Pyrazine, 2-ethyl-3,5-dimethyl-	trace	80
16	13.39	Cyclopentanone, 2,4,4-trimethyl-	trace	32
17	13.70	unidentified	trace	
18	14.09	Pyrazine, (1-methylethenyl)-	trace	64
19	14.36	Cyclohexene, 1-methyl-5-(1-methylethenyl)-, (R)-	trace	36
20	14.65	1-(6-methyl-2-pyrazinyl)-1-ethanone	trace	83
21	14.79	Butanedioic acid, hydroxy-, dimethyl ester	trace	72
22	14.90	4-hydroxy-5-oxohexanoic acid lactone	trace	64
23	15.09	unidentified	trace	
24	15.47	Ethanone, 1-(4-methyphenyl)-	trace	33
25	15.63	unidentified	trace	
26	16.65	2,6-octadien-1-ol, 3,7-dimethyl-, acetate, (E)-	21.42	93
27	18.19	Dodecane *		96
28	19.46	2-furancarboxaldehyde, 5-(hydroxymethyl)-	trace	60
29	20.82	Pyrazine, 1-methylpyrrolo[1,2-a]-	trace	90
30	21.12	unidentified	trace	
31	22.55	Hydroxylamine, o-decyl-	trace	64
32	22.90	Cyclohexane, 1,1-dimethyl-2-propyl-	trace	49
33	23.26	unidentified	trace	
34	23.43	Bicyclo[2,2,2]oct-2-ene, 1,2,3,6-tetramethyl-	trace	38
35	23.57	Pyrazine, 4-methylpyrrolo[1,2-a]-	trace	87
36	24.42	2-methyl-6-hydroxyquinoline	trace	80
37	24.76	unidentified	trace	
38	27.66	Butylated hydroxytoluene	15.47	98
39	27.94	unidentified	trace	
40	29.15	Hexadecane, 7-methyl-	trace	38

trace = <6 ppm * designates internal standard

Table 3.9 (cont.) **Identification of wattleseed components extracted by dichloromethane at 40°C**

Peak	rt (min)	Identity	Quantity (ppm)	Quality NBS (%)
41	31.07	Butylated hydroxytoluene (phenol, 2,6-bis-(1,1-dimethylethyl)-4-methyl)	19.93	98
42	31.39	Phenol, 2,4-bis(1,2-dimethylethyl)-	trace	80
43	31.57	unidentified	trace	
44	31.81	6-(3-methyl-3-cyclohexenyl)-2-methyl-2,6-heptadienol	trace	32
45	32.25	unidentified	trace	
46	32.66	unidentified	trace	
47	33.05	Octadecane, 1-(ethenyloxy)-	trace	64
48	39.94	unidentified	trace	
49	40.50	unidentified	trace	
50	41.38	unidentified	trace	
51	41.81	1-nonadecanol	trace	42
52	41.90	unidentified	trace	
53	42.28	Hexadecane	trace	78
54	51.69	9,12-octadecadienoic acid (Z,Z)-, methyl ester	37.42	99
55	51.94	9-octadecenoic acid (Z)-, methyl ester or 8-octadecenoic acid (Z)-, methyl ester	26.07	99
56	53.61	Oleic acid (cis-9-octadecenoic acid)	193	89
57	54.10	Octadecanoic acid	trace	52
58	62.50	unidentified	trace	
59	62.65	unidentified	trace	
60	63.23	unidentified	trace	

trace = <6 ppm

Table 3.10 Identification of wattleseed components extracted by dichloromethane at ambient temperature

Peak	rt (min)	Identity	Quantity (ppm)	Quality NBS (%)
1	8.04	Pyridine, 2-ethyl	trace	4
2	8.15	unidentified	trace	
3	10.53	Limonene (cyclohexene,1-methyl-4-(1-methyl ethenyl))	11.72	95
4	11.63	1-methyl-4-(1-methylethyl)-1,4-cyclohexadiene (gamma-terpinene)	trace	64
5	17.64	Dodecane *		97
6	19.27	3,3,6-trimethyl-1,4-heptadiene	trace	33
7	20.56	3,7-dimethyl-,(E)-2,6-octadienal	4.35	91
8	30.46	Butylated hydroxytoluene (phenol, 2,6-bis-(1,1-dimethylethyl)-4-methyl)	4.85	91
9	42.82	Caffeine	5.55	91
10	45.10	4,5-dimethyl-nonane	trace	53
11	47.19	Hexadecanoic acid (palmitic acid)	trace	87
12	51.12	7-hexadecyne or 9-octadecyne	trace	
13	51.35	Oleic acid (cis-9-octadecenoic acid)	trace	47
14	52.46	9,12-octadecadienoic acid (Z,Z)-	13.45	99
15	52.65	9,17-octadecadienoic acid (Z,Z)-	15.75	55
16	57.16	5-octadecene,(E)- or 9-octadecene, (E)-	9.89	50
17	61.69	Cyclododecyne	9.48	43
18	61.85	9-octadecenal (Z)-	7.28	38

trace = < 4 ppm

* designates internal standard

Table 3.11 Wattleseed components from SPME identified by GC-MS. SPME1 = 45 min exposure at ambient temperature; SPME2 = 10 min at 60ºC; SPME3 = 20 hr at ambient temperature.

Identity	SPME1		SPME2		SPME3	
	rt	Quality NBS	rt	Quality NBS	rt	Quality NBS
Pyridine	3.77	94	3.52	96	3.76	96
Pyrazine, methyl-	5.03	91	4.87	91	5.02	91
2-Furancarboxaldehyde	-	-	5.04	91	-	-
unidentified	-	-	-	-	5.17	
unidentified	-	-	5.51		-	-
unidentified	-	-	5.75		-	-
Pyridine, 3-methyl-	-	-	-	-	5.79	91
unidentified	5.86		-	-	5.84	
Butyrolactone	7.04	91	-	-	-	-
Pyrazine, 2,5-dimethyl	-	-	6.99	91	7.07	91
Pyrazine, 2,6-dimethyl	7.10	87	-	-	-	-
unidentified	7.20	-	-		-	-
Pyrazine, 2,3-dimethyl-	7.25	91	7.15	94	7.21	94
Pyridine, 3-ethyl-	8.46	94	8.36	95	8.40	95
2-Furancarboxaldehyde, 5-methyl-	-	-	8.49	86	-	-
unidentified	8.57					
from SPME fibre	9.17				9.13	
Pyrazine, 2-ethyl-6-methyl-	9.82	91	9.77	91	9.78	90
Pyrazine, trimethyl-	9.97	87	9.92	87	9.92	90
unidentified	10.25		-	-	-	-
Pyrazine, 2-ethenyl-6-methyl-	-	-	10.50	72	-	-
Pyrazine, 3-ethyl-2,5-dimethyl-	12.86	95	12.82	95	12.8	95
Pyrazine, 2-ethyl-3,5-dimethyl-	13.11	86	-	-	13.1	91
Pyrazine, 5-ethyl-2,3-dimethyl-	13.23	91	13.20	91	13.2	91
Pyrazine, 2-allyl-3-methyl-	13.75	50	-	-	13.70	45
unidentified	14.17		14.12		14.1	
from SPME fibre	15.33		-	-	15.3	
Pyrazine, 5H-5-methyl-6,7-dihydrocyclopenta-	15.52	90	-	-	15.5	94
Pyrazine, 2,3-diethyl-5-methyl-	-	-	-	-	16	90
Pyrazine, 3,5-diethyl-2-methyl-	16.15	87			16.10	90
unidentified			16.14			
Pyrazine, 2,3,5-trimethyl-6-ethyl-	-	-	-	-	16.2	91
Phenylene-1,3-diamine, 2,4,6-trimethyl-	-	-	-	-	16.5	86
Decanal	17.12	64	-	-		
Pyrazine, 2-methyl-5-trans-propenyl-	-	-	-	-	17.9	80

- not applicable

Table 3.11 (cont) Wattleseed components from SPME identified by GC-MS. SPME1 = 45 min exposure at ambient temperature; SPME2 = 10 min at 60ºC; SPME3 = 20 hr at ambient temperature

Identity	SPME1		SPME2		SPME3	
	rt	Quality NBS	rt	Quality NBS	rt	Quality NBS
unidentified	-	-	17.91		-	-
Pyridine, 3-(2-methyl propyl)-	-	-	19.07	43	-	-
unidentified	-	-	-	-	19.2	
2-Decenal, (E)-	20.12	78	-	-	-	-
Pyrazine, 1-methylpyrrolo[1,2-a]-	-	-	20.86	83	20.8	83
fibre	22.71		-	-	22.7	
2,4-decadienal (E,E)	22.91	96	-	-	-	-
Pyrazine, 2,6-dimethyl-5-isopentyl-	-	-	24.46	87	23.1	83
2-methyl-6-hydroxyquinoline	-	-	-	-	24.4	86
unidentified	-	-	-	-	24.7	
unidentified	-	-	-	-	24.8	
Undecenal <(E)-2->	25.09	80	-	-	-	-
unidentified	27.13		-	-	-	-
unidentified	-	-	-	-	28.3	
2(1H)-quinoline, 3,4-dimethyl-	-	-	28.37	36	-	-
unidentified (fibre)	29.72				29.7	
Diethyl phthalate (fibre)	34.37	97	-	-	-	-
unidentified	-	-	-	-	36	
unidentified	-	-	47.81		47.7	
Furan, 3-ethyl-2,5-dihydro-	-	-	52.01	22	-	-

- not applicable

In the SDE extract, 6 FAMEs (eg. hexadecanoic acid, methyl ester) were discovered in low amounts (9.09 ppm total) whereas by solvent extraction (40ºC), 5 FAMEs (eg. 9-octadecenoic acid (Z)-methyl ester) yielded a total of 63.49 ppm. Positively identified furans, which are also generated by the Maillard reaction and generally responsible for the caramel-like odour of thermally processed carbohydrates, included 2-furanmethanol, 5-methyl furfural and 2,2-methylenebis-furan.

Pyridines identified in wattleseed (*eg*. pyridine, 3-methyl pyridine) generally occurred in trace amounts upon extraction with solvent at ambient temperature and 40ºC, and amounted to 5.5 ppm using SDE. Most frequent aromas observed in pyridines include bitter, green, astringent, roasted aroma and coffee-like, even fruity (Dart and Nursten, 1985). Pyridines have an unusual aroma that can play both positive and negative roles in thermally treated foods. For instance, a high level of pyridine can produce unpleasant and pungent odour but at low concentrations provide a tallow-like or fatty aroma (Shibamoto, 1989).

The main terpene identified in wattleseed was limonene (maximum level achieved - 1.72 ppm using solvent extraction at ambient temperature). Pyrroles (*eg.* 1-methyl-1H-pyrrole-2-carboxaldehyde), amines, (*eg.* 2,4,6-trimethyl-1,3-phenylenediamine), quinolines (*eg.* 2-methyl-6-hydroxyquinoline) and hydrocarbons (*eg.* 7-methyl hexadecane) occurred at trace levels. Caffeine detected at 5.55 ppm could contribute to the bitter aftertaste characteristic of wattleseed.

3.2.4.2 Flavour analysis

In order to reveal if both taste and aroma contribute to the overall flavour of wattleseed, samples were extracted with polar solvents (water, 95% food-grade ethanol) and dichloromethane, which is non-polar. Water extracts contained a coffee and nutty aroma with a bitter, coffee taste, whereas the wattleseed residue after water extraction was tasteless, suggesting flavour components (volatiles, sugars, acids, etc) were extracted with the water. A coffee, hazelnut and woody aroma was detected in ethanolic extracts of wattleseed and contained a sweet, coffee woody taste. The seed residue was moderately bitter in taste. Dichloromethane extracts after drying revealed a light coffee, slightly nutty, woody aroma.

From GC-O analysis, pyrazines have been recognised as a significant contributor to the aroma profile of wattleseed (Table 3.8). Overall, burnt, coffee and nutty aromas dominated the wattleseed aroma. Some citrus and fruity odours were detected through the presence of terpenes and aldehydes/ketones, but FAMEs had little to contribute to wattleseed aroma.

3.2.4.3 Non-volatile components

HPLC analyses of wattleseed detected the following organic acids:

- Citric (concentration: 0.86%)
- Succinic (0.65%)
- Malic (0.54%)
- Butyric (0.36%)
- Acetic (0.25%)
- Lactic (0.01%)

Concentrations of organic acids in wattleseed were lower than those found in lemon aspen and wild lime, suggesting they are less likely to influence the overall taste of wattleseed. Sugars quantified by HPLC included fructose (2.04%) and glucose (2.03%) but no sucrose, as found previously in wattleseed by Jermyn (1985). In addition, free amino acids, which were detected in aqueous wattleseed extracts by ninhydrin, might play an important part of flavour development in wattleseed.

3.2.4.4 Wattleseed-chocolate beverage

The following products were developed and evaluated for aroma, taste and wholesomeness and compared to 100% chocolate drink.

- 50% wattleseed-50% chocolate drink
- 15% wattleseed-85% chocolate drink

Overall, consumers preferred the 100% chocolate beverage, followed by 15% wattleseed-85% chocolate and 50% wattleseed-50% chocolate (Table 3.12). Although people liked the aroma and nutty flavour of 15% wattleseed-85% chocolate, they objected to the bitterness, which affected the strength of aftertaste and overall liking of wattleseed flavoured beverages. Increasing bitterness and decreasing sweetness were positively related to increasing wattleseed content in the beverage (results not shown).

Table 3.12 Mean scores for wattleseed/chocolate beverage attributes. The scores are values out of 10 and the greater the score, the greater the degree of liking or intensity.

Attributes	50% wattleseed 50% chocolate	15% wattleseed 85% chocolate	100% chocolate
Intensity of aroma [ns]	55.39	51.04	49.18
Liking of aroma	41.71 a	59.57 b	57.96 b*
Intensity of sweetness	17.07 a	27.93 b	58.79 c
Liking of sweetness	19.82 a	40.21 b	61.29 c
Intensity of bitterness	69.89 b	39.43 a	28.29 a
Liking of bitterness	23.32 a	54.25 b	61.39 b
Intensity of chocolate	22.57 a	46.32 b	64.18 c
Liking of chocolate	23.64 a	51.39 b	65.0 c
Intensity of nutty flavour	71.04 c	44.71 b	32.32 a
Liking of nutty flavour	28.86 a	54.5 b	53.04 b
Intensity of aftertaste	27.11 a	52.14 b	62.31 b
Liking of aftertaste	26.36 a	52.04 b	63.32 c
Overall liking	22.85 a	49.07 b	68.07 c

ns not significant
* values within the row followed by the same letter are not significantly different (P=0.05)

Information on the product glossary of Vic Cherikoff Food Services Pty Ltd (www.cherikoff.net) recommends wattleseed usage at 3-5% addition rate for premium economy and taste suggesting that the recipes used may have been the primary contributing factor to taste acceptance. Methods to enhance flavour acceptance of food and beverage products include additions of sugar to increase sweetness and salt and/or milk to mask bitterness. In preliminary studies concerning wattleseed beverages, consumers favoured an 'instant' (freeze-dried) wattleseed and chocolate mixture (1:2) to wattleseed/chocolate (1:1) and 100% chocolate, when taken with milk and sugar.

3.2.4.5 Summary

Simultaneous distillation proved to be the most proficient extraction method (145 compounds detected by GC-MS), followed by solvent extraction at 40ºC (59 compounds), SPME (up to 56 compounds) and solvent extraction at ambient temperature (17 compounds).

Pyrazines, which essentially were the major compound class present, provided the dominant nutty, coffee and burnt aromas of wattleseed. Other major compound groups included aldehydes/ketones (eg. 3,7-dimethyl-2,6-octadienal), FAMEs (eg. hexadecanoic acid, methyl ester), furans (eg. 2-furanmethanol) and pyridines (eg. 3-methyl pyridine). Caffeine and quinolines also detected may contribute to the bitterness in wattleseed. The presence of sugars (fructose, glucose), organic acids (eg. citric acid) and free amino acids suggest they play a role in the overall taste of wattleseed.

Bitter aftertaste is the main impediment to overall liking of wattle flavours in mixed chocolate beverage products but concentration of wattleseed in recipes is important to acceptance. However, consumers considered an instant wattleseed/chocolate beverage with milk and sugar desirable. Further sensory tests are required to establish these results.

4. Application/product development

4.1 Preservation of lemon myrtle oil

Lemon myrtle oil (LMO) loses its distinctiveness during storage when incorporated into added valued products, such as carbonated beverages, mustards and mayonnaises. Storage temperatures, acidity and/or other intrinsic components in these products appear to have a detrimental effect on the quality of lemon myrtle. Depending on the cause of degradation, various methods will be examined to maintain the distinctiveness of the native food flavour, including the addition natural antioxidants, plant/herbal extracts and acidulants. Encapsulation of lemon myrtle may also be a feasible method in preserving flavour uniqueness in these products with different coatings suited to particular applications.

4.1.1 Materials and methods

Bottled carbonated beverages, mustards and mayonnaises flavoured with LMO emulsion and encapsulated LMO (both oil products received from Vic Cherikoff) were tested with antioxidants, stabilisers, acidulants, plant/herbal extracts and other compounds (eg. limonene) for potential ability to preserve flavour quality. Lemon myrtle flavour in mustard and mayonnaise was determined by sensory analysis (triangle and paired comparison tests), whereas neral and geranial, the key aroma compounds of lemon myrtle (see section 3.2.1), were used as an indicator of flavour stability in carbonated beverages stored at various temperatures (5, 20, 25, 40ºC).

A solid phase extraction procedure using C18 cartridges was developed to purify and concentrate neral and geranial from the beverages. Quantification of neral and geranial was obtained using a Varian GC (3400 series) equipped with a DB-Wax column (30 m x 0.25 mm id). The column oven was temperature programmed at 80° C for 3 min, 12ºC/min to 200ºC and then 2 min at 200ºC. A GC-MS fitted with a DB-Wax column (60 m x 0.25 mm id) was used to identify degradation products of citral. Due to the commercial sensitivity of the product, formulations of lemon myrtle oil used to flavour carbonated beverages cannot be disclosed.

4.1.2 Results and discussion

4.1.2.1 Carbonated beverages

Shelf-life studies incorporating supplements to preserve neral and geranial

Initial experiments involved incorporation of high amounts (100, 300 µl/l) of antioxidants, including ascorbic acid, erythorbic acid, butylatedhydroxytoluene (BHT), tocopherols (α and δ isomers applied singly or as a mixed concentrate), vitamin E and lecithin, and the thickening/stabilising agent acacia gum (0.5, 1.5% w/v) to beverages flavoured with LMO emulsion. However, after 4 days at 40ºC, none of the beverages contained neral and geranial components, and off-odours were released on opening the bottles. Neral and geranial, which undergo a series of cyclisation and oxidation reactions particularly at low pH, are know to produce potent odourants which may cause off-flavours (Charalambous, 1992; Grein, 1994). The metabolite p-mentha-1,5-dien-8-ol, a degradation product of citral (Kimura *et al*.,1983), was identified by GC-MS in the lemon myrtle beverages.

When shelf-life trials of beverages were repeated at 25ºC only acacia gum slowed degradation of neral and geranial (Table 4.1). To illustrate, beverages without acacia gum lost 80-91% of neral and geranial within 7 days at 25ºC. In beverages containing 1.5% acacia gum, 64-70% of neral and geranial was lost after the same storage period.

Table 4.1 Percent loss of neral and geranial in LMO flavoured carbonated beverages after storage at 25ºC for 7 days.

Additive	% loss	
	neral	geranial
acacia gum (1.5%)	**69.6**	**64.1**
acacia gum (0.5%)	76.4	72.5
δ-tocopherol (100 µl/l)	81.7	80.3
mixed (α,δ) tocopherols (300 µl/l)	83.3	79.5
control (no additives)	**85.0**	**80.2**
δ-tocopherol (300 µl/l)	86.1	82.8
ascorbic acid (100 µl/l)	87.9	84.2
vitamin E (300 µ/l)	87.9	85.6
mixed (α,δ) tocopherols (100 µl/l)	87.9	85.1
erythorbic acid (300 µl/l)	88.0	84.9
BHT (100 µl/l)	88.4	82.4
vitamin E (100 µ/l)	88.5	85.7
BHT (300 µl/l)	88.6	83.3
erythorbic acid (100 µl/l)	88.9	86.5
lecithin (100 µl/l)	89.8	86.7
ascorbic acid (300 µl/l)	89.9	84.7
lecithin (300 µl/l)	90.9	88.7

Acacia gum is unique among the food gums because of its high solubility, low viscosity and good stabilising properties for flavour oil-in-water emulsions. Further studies found that increasing concentration of acacia gum (0, 1, 2, 3%) in carbonated beverages was positively related to stability of neral and geranial ($P<0.01$), although beverages with high gum content (2, 3%) tended to have unacceptable mouthfeel (eg. less carbonation) (Fig 4.1).

Ascorbic acid is often added to beverages because of its antioxidant activity. However, addition of ascorbic acid (100 µl/l) to beverages containing acacia gum (0.5, 1, 2% w/v), instead of stabilising lemon myrtle oil, was found to increase the rate of neral and geranial degradation when stored at 25ºC for 8 days ($P<0.001$) (Table 4.2). This enhanced loss of neral and geranial may be due to the fact that ascorbic acid is a redox agent, which means it is antioxidant in some circumstances, and prooxidant in others (Giese, 1995). Furthermore, ascorbic acid possibly reduced the pH of the lemon myrtle beverages. Neral and geranial degradation is pH dependent, degradation being higher at lower pH values (Freeburg *et al.,* 1994). Studies concerning pH influence on neral and geranial stability is detailed in following section, 'Effect of thickeners/stabilisers'.

Figure 4.1 Effect of acacia gum on stability of neral and geranial in LMO flavoured carbonated beverages stored at 25ºC for 8 days

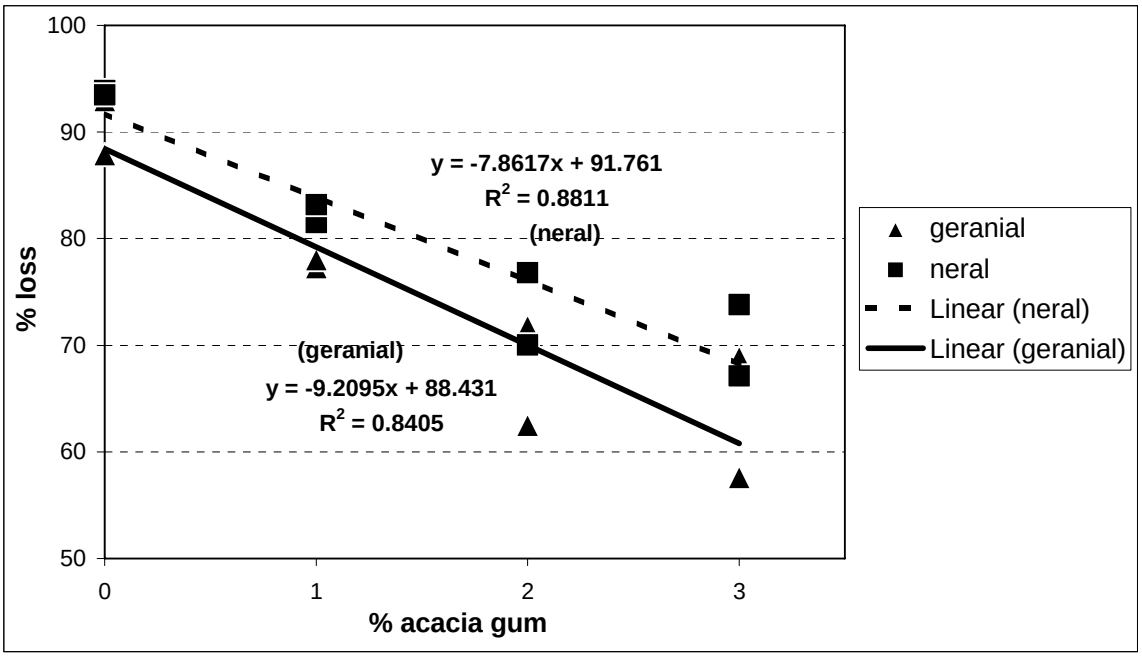


Table 4.2 Percent loss of neral and geranial in LMO flavoured carbonated beverages after 8 days at 25ºC

Acacia gum (%)	% loss of neral		% loss of geranial	
	no ascorbic acid	ascorbic acid (100 µl/l)	no ascorbic acid	ascorbic acid (100 µl/l)
0	90.63 c *	95.73 d	85.07 c #	93.31 d
0.5	80.66 b	91.75 c	77.08 b	88.83 cd
1.0	78.01 b	88.41 c	75.95 b	86.48 cd
2.0	68.27 a	80.13 b	63.41 a	77.18 b

* Values for % loss of neral followed by the same letter are not significantly different (P=0.05)

Values for % loss of geranial followed by the same letter are not significantly different (P=0.05)

Further investigations were carried out using additives at lower concentrations (4, 40 µl/l) to delay degradation of geranial and neral in carbonated beverages. The following substances were tested in LMO flavoured carbonated beverages stored at 25 ºC for 7 days:

- ◊ limonene, ascorbyl palmitate, grapeseed, BHT, ginseng (Siberian), ascorbic acid, flavone, erythorbic acid, catechin, α-tocopherol, glutathione (reduced form), ginkgo biloba leaf, β-carotene, pinus radiata bark, bilberry leaf, co-enzyme Q10 (mixed in salmon and primose oils), cold pressed lemon oil, sodium selenite, sodium metabisulphite, rosemary oil, guiacic gum, gallic acid, ferulic acid, akudjura, fruit salad herb

In comparison to control samples, none of these supplements effectively prevented loss of neral and geranial in beverages, although less off-odour was detected in beverages containing cold pressed lemon oil. Mixed antioxidants often show a synergistic action in providing protection in foods, but various combinations of compounds tested in this study - ascorbyl palmitate and α-tocopherol (eg. 4 µl/l each); ascorbyl palmitate and ascorbic acid; fruit salad herb and akudjura; fruit salad herb and ferulic acid; akudjura and ferulic acid - were unproductive in preventing loss of geranial and neral, and production of unfavourable odourants. In addition, higher concentrations (300, 400 µl/l) of selected substances (ascorbic acid, glutathione, sodium metabisulphite, gallic acid, cold pressed lemon oil and ferulic acid) had no effect in improving neral and geranial stability. Limonene is reported to have a stabilising effect on neral and geranial in beverages (Friedrich and Gubler, 1979) but this was not observed in our study.

Effect of thickeners/stabilisers

As acacia gum had an advantageous effect in preserving neral and geranial in carbonated beverages (see Table 4.1), it was envisaged that other types of thickeners/stabilisers may be as or more effective than acacia in conserving neral and geranial (Table 4.3).

In general, decreasing concentration of thickeners/stabilisers lowered efficacy to preserve lemon myrtle oil flavour, whereas high concentrations (0.25, 1%) increased the viscosity of the beverage and reduced beverage quality (eg. mouthfeel). The most effective thickener/stabiliser, which delayed degradation of neral and geranial while not adversely affecting the quality of the beverage, was sodium alginate (0.1% w/v), followed by tragacanth gum (0.1%), acacia gum (1%), carboxymethylcellulose (0.1%) and gelatine (0.25%). Xanthan, guar, gellan and carrageen gums, pectin, chitosan and starch showed minimal stabilisation of neral and geranial.

The ability of certain thickeners/stabilisers, including sodium alginate, to slow neral and geranial degradation may be partly due to their influence on the pH of the beverage. At low pH values, the concentrations of neral and geranial decrease readily by a series of cyclisation and oxidation reactions (Charalambous, 1992; Grein *et al*., 1994). In this study, the typical pH of lemon myrtle flavoured beverages without additives was 2.9 after carbonation. On addition of, for example, 0.1% sodium alginate, the pH of beverages increased to 3.2.

Further studies showed that when the pH of LMO beverages was adjusted to 3.5 (using potassium citrate), the loss of neral and geranial was around one third less than that occurring in beverages at pH 2.9, after 8 days storage at 25ºC (Table 4.4). Significant stability of neral and geranial was achieved at pH 4.0, although at this acidity beverages may have potential problems with growth of spoilage micro-organisms and a detrimental effect on beverage sensory quality.

Table 4.3 Effect of gums/stabilisers on % loss of (A) neral and (B) geranial in LMO flavoured carbonated beverages after 8 days at 25ºC. Percent losses of neral and geranial in control beverages were 95.8% and 95.1%, respectively

(A) Loss of neral (%)						
Thickener / stabiliser	1.0%	0.25%	0.1%	0.075%	0.05%	0.025%
xanthan gum	+	78.4	93.0	-	-	-
guar gum	+	91.4	96.0	-	-	-
acacia gum	81.5	93.0	93.4	-	-	-
gelatine	74.7	92.2	92.3	-	-	-
pectin	97.1	96.8	94.3	-	-	-
gellan gum	+	87.2	93.0	-	-	-
carboxymethylcellulose	+	69.2	87.2	91.1	93.0	94.5
carrageen gum	+	94.6	95.4	-	-	-
chitosan (high m wt)	94.8	93.6	97.2	-	-	-
starch	94.5	96.3	95.8	-	-	-
sodium alginate	59.1	69.4	74.7	77.5	87.9	92.8
tragacanth gum	+	67.4	77.7	84.7	95.5	95.2

(B) Loss of geranial (%)						
Thickener / stabiliser	1.0%	0.25%	0.1%	0.075%	0.05%	0.025%
xanthan gum	+	71.0	91.7	-	-	-
guar gum	+	91.5	94.9	-	-	-
acacia gum	74.0	91.8	92.7	-	-	-
gelatine	75.1	90.1	90.0	-	-	-
pectin	96.4	95.6	93.1	-	-	-
gellan gum	+	84.5	91.2	-	-	-
carboxymethylcellulose	+	61.3	86.6	90.4	92.6	93.2
carrageen gum	+	95.2	94.9	-	-	-
chitosan (high m wt)	94.2	97.0	95.6	-	-	-
starch	94.1	95.9	94.9	-	-	-
sodium alginate	59.1	61.8	68.2	73.8	86.6	92.2
tragacanth gum	+	59.3	69.9	81.7	94.4	94.2

\+ samples too viscous for solid phase purification
\- not evaluated

Table 4.4 Effect of pH on % loss of neral and geranial in LMO flavoured carbonated beverages after 8 days at 25ºC.

pH	% loss of neral	% loss of geranial
2.9	95.5	95.4
3.5	62.1	53.4
4.0	33.0	22.2

Use of encapsulated LMO

Extensive shelf-life trials using LMO emulsion and encapsulated LMO as flavourants at approximately the same concentration of LMO were conducted in beverages with the following compositions:

- pH 2.9; no added sodium alginate
- pH 3.2; 0.1% sodium alginate
- pH 3.5; no added sodium alginate (pH adjusted with potassium citrate)
- pH 3.5; 0.1% sodium alginate (pH adjusted with potassium citrate)

Major aims of the study were to determine:

- ability of encapsulated LMO to maintain lemon myrtle flavour, in comparison to emulsified LMO flavouring
- if sodium alginate in beverages (pHs 3.2, 3.5) is more effective in maintaining lemon myrtle flavour than beverages without sodium alginate (pH 3.5)

Treatments were held at 20ºC and 5ºC for 17 and 46 days, respectively, in triplicate.

<u>20ºC trial</u>

The use of encapsulated LMO to flavour beverages had no apparent advantage over emulsified LMO flavouring in maintaining neral and geranial levels at 20ºC ($P>0.05$) (Table 4.5). Virtually all (*ca*. 94-97 %) neral and geranial components were depleted in beverages at a pH of 2.9 and to a lesser extent in beverages at pH 3.2 containing 0.1% sodium alginate. Degradation of neral and geranial was least in beverages at pH 3.5 ($P=0.05$) but off-odours were still present. Addition of 0.1% sodium alginate to beverages maintained at pH 3.5 was of no benefit in preserving neral and geranial.

Table 4.5 Percent loss of neral and geranial in LMO flavoured carbonated beverages after (A) 17 days at 20ºC and (B) 46 days at 5ºC

(A)

	% loss of neral			% loss of geranial	
Beverage conditions	LMO emulsion	LMO encapsulation		LMO emulsion	LMO encapsulation
pH 2.9; no alginate	96.78 c *	96.93 c		96.36 c #	94.35 c
pH 3.2; 0.1% alginate	81.39 b	80.86 b		78.48 b	75.06 b
pH 3.5; 0.1% alginate +	53.29 a	53.95 a		44.81 a	41.74 a
pH 3.5; no alginate +	50.86 a	46.73 a		41.74 a	37.70 a

(B)

	% loss of neral			% loss of geranial	
Beverage conditions	LMO emulsion	LMO encapsulation		LMO emulsion	LMO encapsulation
pH 2.9; no alginate	86.47 e *	83.25 e		86.84 f #	80.75 e
pH 3.2; 0.1% alginate	68.33 d	60.80 c		67.35 d	47.17 c
pH 3.5; 0.1% alginate +	49.33 b	37.34 a		48.07 b	26.54 a
pH 3.5; no alginate +	46.72 b	34.05 a		43.48 b	21.91 a

* Values for % loss of neral followed by the same letter are not significantly different (P=0.05)
# Values for % loss of geranial followed by the same letter are not significantly different (P=0.05)
\+ pH adjusted with potassium citrate

5ºC trial:

In general, the rate of neral and geranial degradation was slower at 5ºC than at 20ºC (Table 4.5). The highest losses of neral and geranial occurred in beverages at pH 2.9, followed by pH 3.2 and pH 3.5 (P=0.05). However, unlike samples held at 20ºC, beverages flavoured with encapsulated LMO were less prone to neral and geranial degradation than emulsified LMO flavoured beverages (P<0.0001). This may be because the membranes that encapsulate LMO (capsule walls consisted of acacia gum) were less soluble at 5ºC and provided better protection. Encapsulated LMO in beverages at pH 3.5, with or without 0.1% sodium alginate, was preferred in terms of both neral and geranial protection (P=0.05), and taste quality.

4.1.2.2 Summary

Only few of the additives (eg. sodium alginate) effectively maintained LMO flavour in beverages. However, acidity played an important role in determining shelf-life of beverages, where pH 3.5 was preferable to pH 2.9 and 3.2 for protecting LMO uniqueness. Although beverages at pH 4 had less neral and geranial degradation, they may have problems with growth of spoilage micro-organisms and inferior beverage sensory quality (eg. mouthfeel). Sodium alginate in beverages at pH 3.2 or 3.5 had no obvious advantage to beverages without sodium alginate at pH 3.5.

Encapsulation of LMO facilitated flavour retention in beverages at 5° C but not at 20ºC. Consequently, flavouring with the encapsulated LMO formulated for this study is unlikely to benefit commercial soft drinks, which are typically marketed without refrigeration for extended periods. Further development of encapsulation procedures, such as using alternative materials to produce a more protective, outer capsule membrane, may assist shelf-life extension of LMO flavour at ambient storage temperatures.

4.1.2.3 Mustards and mayonnaises

Triangle and paired sensory tests were used to evaluate diversity in lemon myrtle flavour of mustard and mayonnaise containing lemon myrtle oil or encapsulated lemon myrtle oil, with or without antioxidants (ascorbic acid, mixed concentrate of tocopherols at 100µl/l). In triangle tests, each presentation involved three samples, two of which were the same. Panelists were requested to choose which sample was different. In paired comparison tests, panelists were asked to choose which sample they preferred.

When mayonnaise and mustard containing lemon myrtle oil (0.2% v/w) were incubated under accelerated shelf-life conditions at 40ºC for 4 days, panelists were unable to determine a significant difference in lemon myrtle odour between control samples and samples containing the antioxidants. In another sensory trial, consumers could not consistently taste differences between samples containing encapsulated lemon myrtle oil (0.1% v/w) with or without antioxidants, stored at 25ºC for 8 days. However, the panel reported no off-flavour development of lemon myrtle, which suggests encapsulation alone is potentially an effective method for preserving lemon myrtle flavour in mustard and mayonnaise.

4.2 Improvement of bread formulations containing akudjura

Akudjura, which is produced from the drying and grinding of the bush tomato (*Solanum* spp.), has a flavour described as being tamarillo and caramel like, similar to concentrated sun dried tomatoes and quite strong in its intensity. Incorporation of akudjura into established food products, such as bread, is a mechanism to increase consumption of this material and thus expand its market potentiality. However, addition of akudjura to bread formulations has a negative effect on the quality of the final baked product. It was proposed that this might be due to either i) inactivation of the yeast by the akudjura or ii) breakdown of the gluten network of the dough.

4.2.1 Materials and methods

Akudjura from the bush tomato, *S. centrale*, was acquired from Vic Cherikoff. Bread was made using a standard test baking recipe. Dough pieces were proofed for 3 hours at 35ºC and baked at 220ºC for 20 min. Akudjura was included at 0, 1 and 3% of flour weight.

Yeast population was monitored during proofing using standard microbiological sampling procedures on malt extract agar. A simple water displacement system measured the gas producing capabilities of the yeasts in samples of dough. Submerging samples in paraffin allowed the gas retaining properties of the dough to be determined.

Bread dough rheology was measured with extensograph and farinograph systems. The extensograph testing required stretching of the dough by means of a moving arm. The arm slowly pulls the dough down between two clamps that are holding the dough in place at a steady rate. The maximum resistance is indicative of the dough strength, while extensibility reflects the ability of the dough to stretch before breaking.

he farinograph is used to determine the optimal dough development time and assess the stability of the dough. The mechanical abuse from the farinograph equates to that which occurs during fermentation. The maximum dough consistency is indicative of the maximum resistance of the dough to this abuse. The development time is the time taken to reach the maximum height of the curve and provides a measure of dough strength. Dough breakdown is the difference between the maximum and final consistency values (BU) after the ten minute recording period.

Gliadin components of the wheat proteins in the dough were extracted with 30% ethanol and analysed on a Biofocus capillary electrophoresis system (Model 3000, Biorad) equipped with a 24 cm column (50 µm id) at 12.5 kV and 40ºC with an injection pressure of 1 psi. The buffer system used was iminodiacetic acid (50 mM) in aqueous acetonitrile (20%).

Quality of bread containing akudjura was determined by sensory evaluation.

4.2.2 Results and discussion

4.2.2.1 Yeast growth kinetics and gas production capabilities

Yeast growth kinetics and the gas producing capabilities of the yeast were monitored during proofing. Yeast counts from the control loaves and the akudjura loaves were comparable however the akudjura loaves required longer proofing times to attain the same degree of expansion as the control loaves. Gas production rates were measured with a simple water displacement system and all loaves (control, 1% and 3% akudjura) demonstrated comparable gas production rates. These results indicated that yeast activity was not appreciably affected by the addition of the akudjura, although longer proofing times were required.

4.2.2.2 Gas retention of doughs

Control doughs and doughs prepared with 1% and 3% akudjura were submerged in liquid paraffin to determine gas retention of the dough. Measurement of the change in the volume of the paraffin due to the expansion of the dough was inconclusive, however, what was of interest was the behaviour of akudjura containing doughs in the latter stages of the experiment. The control dough remained compact and intact throughout the experiment. In contrast, the akudjura doughs appeared web-like in appearance due to tearing and breaking apart of the dough, with the 3% dough displaying large holes where the structure had totally disintegrated.

4.2.2.3 Dough rheology - extensograph and farinograph measurements

Table 4.6 indicates the decreasing dough strength with increasing akudjura concentration and a decrease in extensibility with the addition of 3% akudjura.

Table 4.6 Extensograph measurements for flour/akudjura mixtures.

Sample	Extensibility (cm)	Maximum Resistance (BU)
0% akudjura (flour only)	20.4	600
1% akudjura	24.2	160
3% akudjura	7.9	95

The farinograph for the control dough indicated a high-quality dough; the strength of the dough was maintained for a long period of time and the dough is highly stable (Fig. 4.2). The 1% and 3% akudjura doughs are much weaker, less stable doughs with reduced dough development times and maximum resistance values and increased dough breakdown values, with the changes in the dough properties being more extensive at the 3% level (Figs. 4.3 and 4.4).

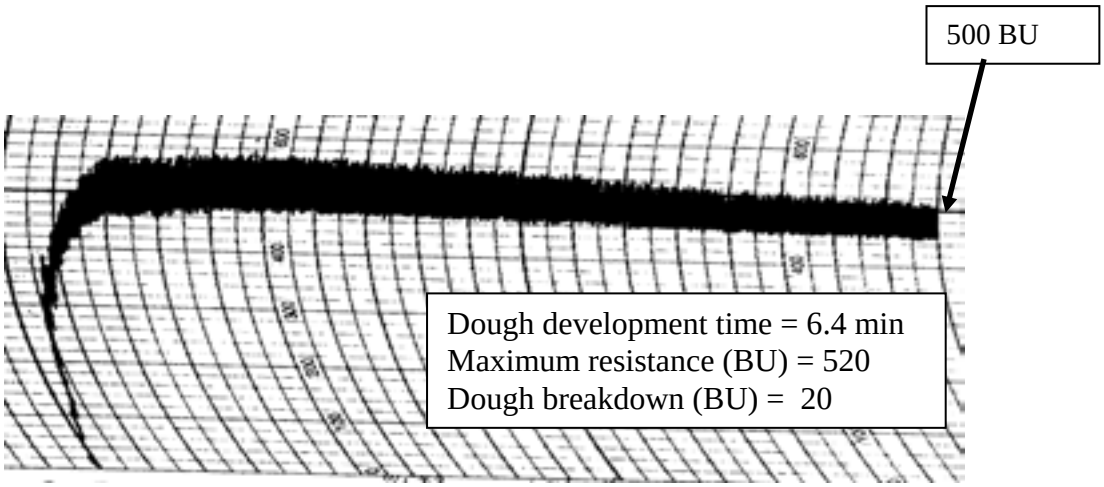


Figure 4.2. Farinograph of the control dough

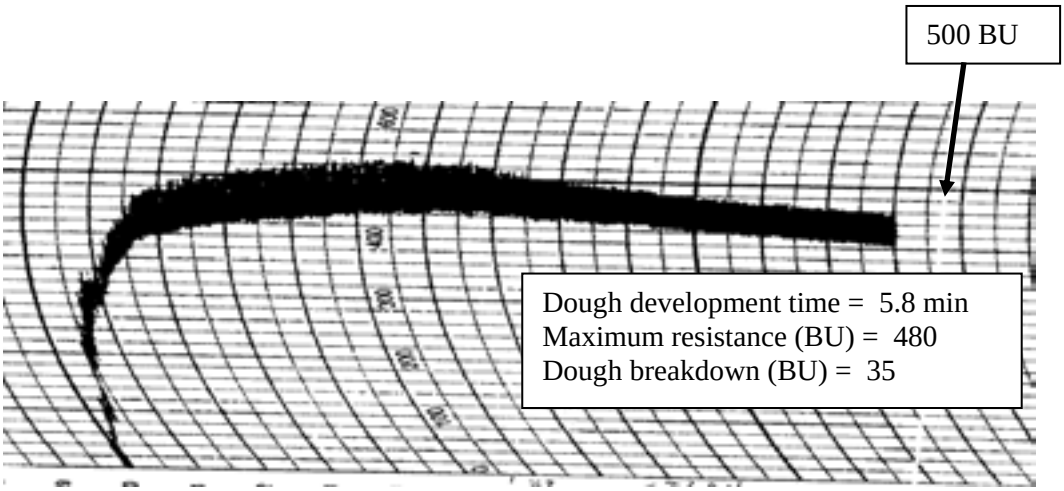


Figure 4.3 Farinograph of the 1% akudjura dough

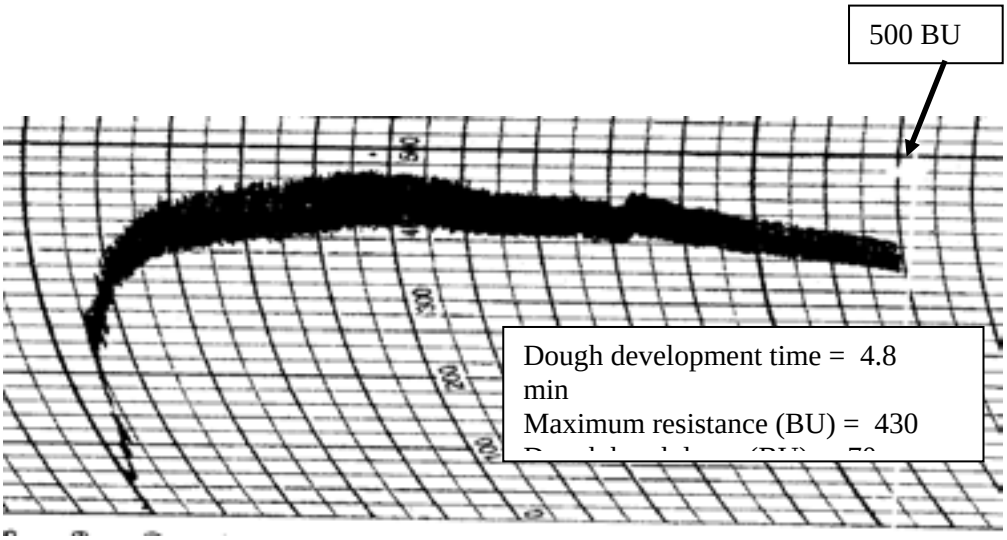


Figure 4.4 Farinograph of the 3% akudjura dough

4.2.2.4 Capillary electrophoresis

The breakdown of the dough as indicated by visual observation and dough rheology measurements suggested the possible presence of naturally occurring enzymes with proteolytic activity in the akudjura meal, which may undermine gluten structure of the dough during proofing. This was investigated by analysis of the dough using capillary electrophoresis to determine whether the wheat proteins were being broken down during proofing. Constraints of time and developmental nature of the work meant that only the gliadin components of the wheat proteins were analysed.

The gliadin profile obtained from the control dough at time 0 and 2 h proofing was compared with those of the akudjura dough (1 and 3%) at the same times. The electropherograms for the control and 3% akudjura doughs at 0 h are shown in Figs 4.5 and 4.6, respectively. The profile for 1% akudjura was very similar to the 3% dough and there were few differences between the profiles at 0 and 2 h.

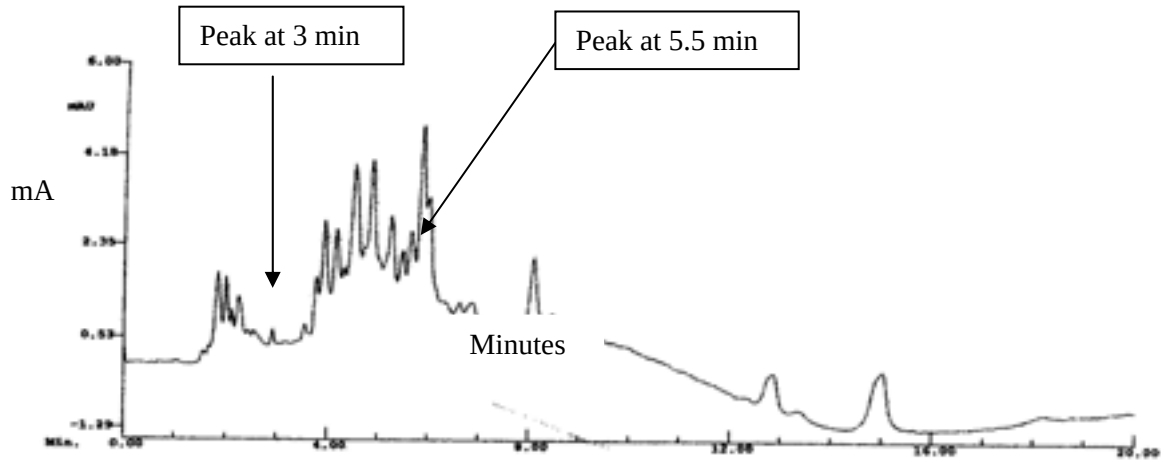


Figure 4.5 Electropherogram of the control dough

A small peak eluting at 3 minutes in the control dough gliadin extract, was barely perceptible in the 1% akudjura dough extract and practically not existent in the 3% akudjura dough extract. The disappearance of a peak in this region of the graph indicated the breakdown of a α-gliadin. The peak for a β-gliadin eluting at approximately 5.5 min. in the control dough gliadin extract was also lower in both doughs containing akudjura but more so for the 3% akudjura dough. The similarity in the profiles at 0 h and 2 h indicates that the changes occurring very quickly and commence during mixing.

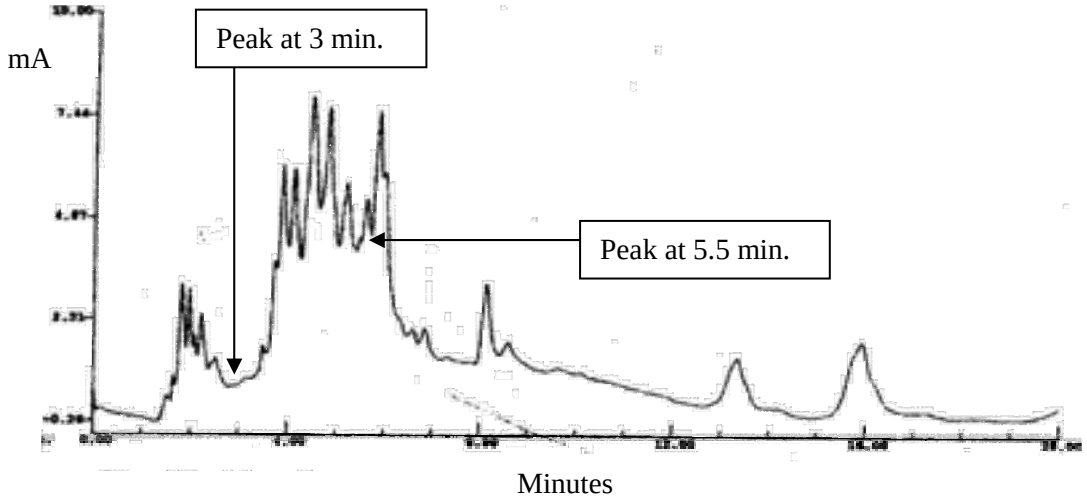


Figure 4.6 Electropherogram of the 3% akudjura dough

These results support the proposal of naturally occurring enzymes that are attacking the wheat protein structure and preventing proper formation of the gluten network that provides elasticity, extensibility and strength to the bread dough. This allows the dough to expand through the action of the carbon dioxide produced by the yeast, while retaining the leavening gas, and prevents collapse of the dough during proofing and baking.

4.2.2.5 Treatment of akudjura to inhibit enzymic activity

A mild heat was considered the most appropriate method of inhibiting the enzymic activity. Different time and temperature combinations were evaluated and the method selected was heating the akudjura at 40°C for 5 min. This proved to be successful as the doughs containing the treated akudjura rose and held their form throughout proofing and baking.

4.2.2.6 Product development and evaluation

The bread containing 3% akudjura did not appear at all different from normal loaves of bread, nor was the taste distinctive so higher levels of 4.5% and 6% akudjura were also trialed using consumer taste testing. Increasing the concentration of akudjura did not significantly ($P>0.05$) alter the liking of the appearance of the bread, nor did it affect the saltiness or the textural properties (softness, chewiness) with the exception of the 6% akudjura loaf which was significantly ($P<0.05$) less desirable than the 3% akudjura. This could indicate that the heat treatment used only reduced enzymic activity rather than eliminating it totally, which in the 3 and 4.5% akudjura loaves was sufficient to prevent deterioration of the dough properties, however, at 6% akudjura there was sufficient residual enzymic activity to affect the dough slightly. Longer heating times may be required to result in complete enzyme inactivation.

The attributes related to flavour, aftertaste and overall liking were found to change significantly as the concentration of akudjura increased. Liking of overall flavour was significantly different ($P<0.05$) between the 3 and 6% akudjura loaves. Liking of the 6% akudjura was low (4.26 out of a score of 10) but a decrease in concentration to 3% significantly ($P<0.05$) increased the liking of the bread (score = 6). The higher score for the 3% akudjura loaf suggests that a more subtle akudjura flavour is preferable to a strong akudjura flavour.

Tomato flavour was significantly ($P<0.05$) stronger in the 6% akudjura loaf compared to the 3% akudjura loaf, however the tomato flavour was undesirable (scores ranged from 3.8 to .9), irrespective of the perceived strength.

As the concentration of akudjura increased the strength of the aftertaste increased significantly ($P<0.05$) and in the 6% akudjura loaf the aftertaste was perceived as being quite strong (score = 7.01). This intense aftertaste was highly undesirable (liking = 3.94) and again the lower concentration of 3% was more desirable.

4.2.3 Summary

Incorporation of akudjura into doughs did not appreciably affect yeast activity during proofing. However, akudjura doughs became web-like in appearance and the 3% dough displayed large holes. Extensograph and farinograph measurements indicated that dough strength, stability and development times decreased with increasing akudjura concentration.

The decrease of α and β-gliadins in akudjura doughs during proofing suggested that naturally occurring enzymes attack the wheat protein structure and prevent proper development of the gluten network. Mild heat treatment (40°C for 5 min) of akudjura to inhibit enzymic activity appeared to be successful as the doughs containing the treated akudjura rose and held their form throughout proofing and baking.
The attributes related to tomato flavour, aftertaste and overall liking were found to change significantly as the concentration of akudjura increased. In general, a mild akudjura flavour was preferred to strong akudjura flavour.

5. Microbiological issues of dried native foods

Spices and herbs may present a microbiological problem to foods. They potentially pose a risk to public health because they are often added to foods which undergo no further processing or which are eaten raw. In addition, spices and herbs may introduce food spoilage organisms to a range of foodstuffs.

Although information regarding types and numbers of micro-organisms present in traditional dried herbs and spices (eg. pepper, paprika) is well established, little is known about the microbiological quality and safety of Australian native herbs and spices. The objectives of this study were to (a) determine the microbiological status of selected dried native foods and if appropriate (b) investigate natural strategies to improve microbiological quality, thus preventing transfer of micro-organisms to further processed food products. Use of natural means of microbial control and avoiding the use synthetic fumigants, such as ethylene oxide, would favour market opportunities for native foods.

It should be noted that this section of the project was commenced prior to gamma irradiation being accepted as a means to reduce the bacterial load in culinary herbs and spices. This is now the norm rather than the exception. However, non-irradiation techniques still have their applications, particularly for organic markets which are a growing segment of the food industry.

5.1 Materials and methods

5.1.1 Raw materials

Vic Cherikoff provided dried native herbs and spices, including forestberry herb, pepperberry, native or Australian mint, aniseed myrtle, lemon myrtle, mountain pepper, native peppermint, akudjura and Red Desert Seasoning, which is a blend of native and traditional herbs/spices (eg. pepperberry and paprika, respectively). Water activity of samples was determined using a Vaisala HMII RH probe.

5.1.2 Microbiological analysis

Incidence of micro-organisms in native herbs and spices was measured according to Australian Standard Methods. Serial dilutions of herb/spice homogenates in 0.1% bacteriological peptone were spread-plated in duplicate on PCA for total viable counts of bacteria, and on MEA (with 100 µl/l oxytetracycline) and DRBCA for yeast/mould populations. Microscopic examination and colony morphology characterisation on selective agars were used to identify and enumerate potential spoilage micro-organisms and food-borne pathogens.

5.1.3 Disinfection strategies

Natural strategies investigated to eliminate micro-organisms from native spices included UV-C irradiation and fumigation with natural plant volatiles. Exposure to UV-C light was carried out by placing selected spice samples approximately 70 cm from a UV source (2 x 15 W UV-C lamps) for various periods. A UV probe/radiometer (International Light Inc.) was used to measure the dose of UV.

Fumigation of spices with natural plant volatiles was conducted at different doses, temperatures and air pressures for up to 18 hrs. The fumigation process involved applying the volatile compound as a liquid to absorbent tissue, which was placed with the spice inside a sealed fumigation chamber.

5.2 Results and discussion

5.2.1 Water activity

The water activity of the native spices/herbs (Aw=0.59-0.68) is in the lower part of the intermediate-moisture food range, which is susceptible to growth of xerophilic (osmophilic) yeasts and fungi, and survival of heat resistant bacterial spores (Table 5.1). Little variation in Aw occurred between batches of mountain pepper, lemon myrtle, aniseed myrtle and akudjura.

Table 5.1 Water activity of selected native herbs and spices.

Herbs/spices	Water activity (Aw)
Native mint [a]	0.68
Mountain pepper [a]	0.67
Mountain pepper [b]	0.66
Native pepperberry [a]	0.66
Lemon myrtle [a]	0.65
Lemon myrtle [b]	0.64
Native peppermint [a]	0.65
Aniseed myrtle [a]	0.62
Aniseed myrtle [b]	0.61
Forestberry herb [a]	0.60
Red Desert seasoning [a]	0.59
Akudjura [a]	0.61
Akudjura [b]	0.59

a 1st batch of samples received August 1999
b 2nd batch of samples received October 1999

5.2.2 Microbiological assessment

Tests were conducted on two batches of spices/herbs and results were consistent for each batch (Table 5.2). There were no yeasts present in the samples except akudjura. All samples but mountain pepper contained moulds. The highest mould counts (10^2 cfu/g) were observed for aniseed myrtle, lemon myrtle and native mint, and akudjura contained mainly yeasts (10^4 cfu/g). The highest total bacterial counts occurred in aniseed myrtle and Red Desert Seasoning (10^4 cfu/g each). Native mint and lemon myrtle contained up to 10^3 cfu/g, and native peppermint and pepperberry contained 10^2 cfu/g.

Mountain pepper, akudjura and Red Desert Seasoning were chosen for further microbiological analysis to determine if they contained micro-organisms that would spoil foods or bacteria that could cause food poisoning. Spore-forming organisms are of particular importance because (1) they may survive the actual drying process given to the spice/herb, and (2) if the spice/herb is added to a food product that may be subjected to a heat process, the spore can germinate thus allowing the vegetative form of the bacterium to grow in the cooked food.

All products were assessed for total microbial counts and for the presence of lactic acid bacteria. Akudjura was also examined for the presence of spoilage yeasts. The predominant individual types were isolated and tested further for identification. Selective isolation techniques were used for the presence of:

- coliform bacteria and *Escherichia coli* (presence of these organisms in foods indicates the possibility of contamination with faecal material)
- the spore-forming, food-borne pathogen, *Bacillus cereus*
- *Staphylococcus aureus*, which may arise in foods through handling and processing.

Table 5.2 Microbiological assessment of selected native herbs and spices

Herbs/Spices	Microbial population (cfu/g)		
	Total Bacterial Count (PCA)	Yeast and Mould Count (MEA)	(DRBC)
Aniseed myrtle [a]	7.1×10^4	7.0×10^2 *	1.6×10^4 *
Aniseed myrtle [b]	1.4×10^3	2.2×10^2 *	1.2×10^3 *
Red Desert Seasoning [b]	2.7×10^4	85 *	50 *
Native mint [a]	2.9×10^2	1.8×10^2 *	2.5×10^2 *
Lemon myrtle [a]	65	1.1×10^2 *	1.4×10^2 *
Lemon myrtle [b]	-	1.2×10^2 *	1.7×10^2 *
Forest berry herb [a]	65	15 *	50 *
Pepperberry [a]	40	0	0
Peppermint [a]	20	5 *	10 *
Mountain pepper [a]	-	0	50 *
Mountain pepper [b]	1.5×10^2	0	0
Akudjura [a]	1.4×10^4	1.3×10^4 +	-

1 1st batch
2 2nd batch
* no yeasts detected
+ yeasts mainly present
- not assessed

The total microbial populations ranged from 10^3 cfu/g (akudjura and mountain pepper) to 10^5 cfu/g (Red Desert Seasoning). The three products were tested twice and results were consistent. The populations in the Red Desert Seasoning in particular are of concern. Red Desert Seasoning is a blend of traditional and native spices and it is probable that the pepper and paprika in this mix make an important contribution to the microbial population. Microbial counts of around 10^6-10^7 cfu/g spice are reported in the literature for these products (Pafumi, 1986).

The total yeast population present in akudjura was around 10^4cells/ g. Three types of yeasts were isolated. One species is a mould-like yeast, *Aureobasidium pullulans*, commonly present on fruits and vegetables. The other isolates were tentatively identified by ATB as *Saccharomyces exiguus*, also commonly found on fruits and in fermented products.
Lactic acid bacteria were detected in both Red Desert Seasoning and akudjura. Populations of 10^2-10^3 cfu/g were observed on an agar medium that is designed to encourage the growth of these organisms. However, the identity of the organisms was not determined conclusively. Yeasts such as *S. exiguus* and lactic acid bacteria are fermentative organisms that often grow in the presence of carbohydrates

such as glucose and sucrose. If present in herbs/spices, they may contribute to the spoilage of foods containing these products.

Tests for food-borne pathogens concluded that there were no coliforms, *E. coli*, *S. aureus* or *B. cereus* present in the three products.

Isolates that were selected for identification were first divided up on the basis of the Gram reaction test. All Gram negatives microscopically observed were rods. Further analysis (eg. biochemical tests, diagnostic strips such as ATB ID 32 GN) would have to be used to give definitive results where these organisms are concerned. Fifteen isolates were found to be Gram positive and 12 of these isolates were presumptively identified as *Bacillus* spp. One of the Bacillus isolates was given the preliminary identification of *B. lichenformis*, which has been implicated in the spoilage of foods and human illness (Jenson, Jenson and Hyde, 2001). No *B. cereus* was isolated in the course of this examination. The colony and cell morphology of the remaining three isolates suggested that they were lactic acid bacteria.

Another batch of Red Desert Seasoning was re-examined to confirm microbiological status. No *S. aureus* and *Clostridium* spp. were present in the sample and a number of isolates were again presumptively identified as *Bacillus* spp.

5.2.3 Control of microbial contaminants

The microbial populations (10^4-10^5 cfu/g) found in the Red Desert Seasoning are of importance. Red Desert Seasoning is a blend of traditional and native spices and it is likely that the pepper and paprika in this mix make a significant contribution to the microbial population. Natural treatments investigated to reduce microbial populations in Red Desert Seasoning included irradiation with UV-C and fumigation with natural plant volatiles.

5.2.3.1 UV-C irradiation

The initial experimental design for UV-C treatment was to disperse the Red Desert Seasoning sample thinly on a transparent plastic film fixed above a reflective medium, thus allowing UV rays to reach both the upper and underside regions of the spice particles. The outcome showed that low dose UV-C light (20-100 mJ/cm^2) had a minor effect in the lowering microbial content (up to approximately 0.25 log reduction). Higher UV doses (800-3000 mJ/cm^2) achieved a 0.5 log reduction but the results were often irregular and tended to cause undesirable changes to the appearance (bleaching) and aroma of the spice.

To improve the disinfection proficiency of UV irradiation, Red Desert Seasoning was vibrated rapidly on a flat surface whilst being treated with low dose UV-C (100-900 mJ/cm^2). Continuous agitation of the spice during UV treatment showed an improvement in the reduction of micro-organisms (eg. 0.7 log reduction with 900 mJ/cm^2 dose), presumably because the surfaces of the spice particles were more uniformly exposed to the UV rays. Bleaching and loss of aroma were detected in samples but less severely than when Red Desert Seasoning was irradiated without agitation.

5.2.3.1 Fumigation with natural plant volatiles

A range of volatile compounds was examined for antimicrobial efficacy using Red Desert Seasoning as the test product. The volatiles tested included:

- limonene, anethole, tea tree oil, propionic acid, lemon myrtle oil, acetic acid, ethyl pyruvate, methyl pyruvate, propionaldehyde, iso-propanol, ethanol, acetaldehyde, methyl propionate, linalool, methyl caproate, ethyl isovalerate, methyl benzoate, ethyl sorbate, 1-pentanol.

Total plate counts were taken from spice samples to measure fumigation proficiency of volatiles. At the rate 0.5 g/l at 30ºC for 18 hr, volatile compounds that showed consistent antimicrobial activity included propionic acid, acetic acid, acetaldehyde and limonene (ca. 0.6, 0.6, 0.4 and 0.3 log reduction, respectively) (Table 5.3). Fumigation of Red Desert Seasoning at lower temperature (20ºC) had no effect in enhancing antimicrobial efficacy, whereas higher fumigation temperature (40ºC) increased antimicrobial effectiveness in most volatile compounds (eg. 1.0, 1.2, 0.5 and 0.4 log reductions of micro-organisms resulted from propionic acid, acetic acid, acetaldehyde and propionaldehyde fumigation, respectively). Certain combinations of volatile compounds (eg. acetic acid/ethyl pyruvate 0.5 g/l each at 40ºC for 18 hr) were also trialed but these had no accumulative/synergistic effect on antimicrobial capability.

Table 5.3 Log reduction of micro-organisms in Red Desert Seasoning fumigated with natural plant volatiles for 18 hr at 20, 30 and 40ºC. Data are mean values of two replicates. Numbers of micro-organisms enumerated in untreated samples (controls) at 20, 30 and 40ºC were log 5.27, 5.37 and 5.31 cfu/g, respectively.

Volatile compound	Fumigation temperature (ºC)		
	20ºC	30ºC	40ºC
propionic acid	0.41	0.62	0.99
acetic acid	0.49	0.55	1.17
acetaldehyde	0.18	0.38	0.52
limonene	0.14	0.30	0.19
lemon myrtle oil	-0.03*	0.24	0.26
iso-propanol	0.12	0.20	0.32
ethyl pyruvate	0.08	0.15	0.28
tea tree oil	0.09	0.12	0.19
methyl pyruvate	0.19	0.11	0.25
propionaldehyde	0.18	0.09	0.37
anethole	0.07	-0.05*	0.20
ethanol	0.10	-0.05*	0.02
1-pentanol	-	-	0.17
ethyl sorbate	-	-	0.05
methyl caproate	-	-	0.11
ethyl isovalerate	-	-	0.33
linalool	-	-	0.06
methyl	-	-	0.07
propionate	-	-	0.18
methyl benzoate			

* negative values indicate increase in microbial numbers
\- not assessed

With the prospect of creating a 'hurdle' effect in reducing microbial load in Red Desert Seasoning, a 4 hour fumigation treatment with acetic acid (0.5 g/l) was subdivided into four temperature regimes of 1 hour each. Red Desert Seasoning was first fumigated at 30ºC for 1 hour, followed by hourly fumigation intervals at 0ºC, -20ºC and 20ºC. However, fumigation capability with a sequence of different temperatures was no better than fumigating samples constantly at 30ºC.

To increase the volatility and possible antimicrobial activity of acetic acid, fumigation of Red Desert Seasoning mix was conducted for 4 hours under low atmospheric pressure (*ca*. -80 kPa). No obvious enhancement of antimicrobial behaviour of acetic acid was observed.

It is apparent that acetic acid is a promising fumigant for reducing microbial populations in Red Desert Seasoning mix. Even at higher rates (1 and 2 g/l for 18 hr at 40ºC) acetic acid further reduced microbial loads in the Red Desert Seasoning (2.0 and 2.6 log reductions, respectively). However, acetic acid vapour also caused tainting and caking of the product. Less dosage of acetic acid (0.25 g/L for 2 hours fumigation at 40ºC) eliminated the caking and darkening problem and reduced product tainting, but with significant decreased antimicrobial action.

Subsequent work focussed on developing methods to minimise caking and tainting of spices during/after the fumigation procedure. Effective deodorisation of Red Desert Seasoning tainted with acetic acid was accomplished with addition of potassium permanganate to the chamber after fumigation. With sufficient potassium permanganate, acetic acid can be completely oxidised to carbon dioxide and water. Use of sodium bicarbonate also reduced acetic acid taint but less efficiently than potassium permanganate.

A low humidity environment during acetic acid fumigation is likely to reduce caking of spices. Use of calcium chloride, which under saturated conditions provides a low humidity atmosphere (*ca.* 32% RH at 20ºC), prevented caking of Red Desert Seasoning during fumigation but appreciably decreased the antimicrobial action of acetic acid. Possibly, calcium chloride rapidly absorbed acetic acid in the chamber, thus preventing the vapour penetrating and killing micro-organisms in the spice. Lower amounts (0.5, 1 g/l) of calcium chloride permitted greater microcidal action of acetic acid while minimising caking, but fumigation without calcium chloride continued to be the most efficient procedure in eliminating micro-organisms from Red Desert Seasoning.

5.3 Summary

Native herbs and spice products containing the highest microbial counts included akudjura, aniseed myrtle and Red Desert Seasoning (up to 10^5 cfu/g). The total yeast population present in akudjura was around 10^4cells/ g, whereas 10^2-10^3 cfu/g of lactic acid bacteria were detected in both Red Desert Seasoning and akudjura. There were no coliforms, *E. coli, S. aureus* or *B. cereus*, contaminating the three products.

Low dose UV-C light moderately reduced micro-organisms present in Red Desert Seasoning although tended to cause bleaching and undesirable changes to the aroma of the spice. With fumigation of Red Desert Seasoning with natural plant volatiles at 30ºC, compounds that showed consistent antimicrobial action included acetic acid, propionic acid, acetaldehyde and limonene. Higher fumigation temperature (40ºC) increased antimicrobial capability in many of the volatile compounds tested.

Acetic acid was determined the most effective fumigant for reducing microbial populations in Red Desert Seasoning but caused tainting and caking of the product. Use of potassium permanganate effectively deodorised Red Desert Seasoning after fumigation although caking reduction with calcium chloride adversely affected the antimicrobial efficacy of acetic acid during fumigation.

6. Novel antioxidants

Certain Australian native food sources have been identified as potential sources of antioxidants. For example, lemon aspen juice, even under diluted and sweetened conditions, keeps fresh for extended periods. Blue cypress oil was also reported to have a preservative effect on mushroom stems. Roasted wattleseed extract has natural emulsifying properties and when incorporated with cream prevents spoilage. Components of native foods may be utilised as novel antioxidants for preservation of foods and have applications for human health.

This study involved screening of potential native food sources for antioxidant activity, and determination of active antioxidant component(s) in the most two active preparations identified.

6.1 Materials and methods

Vic Cherikoff provided native foods for antioxidant evaluation. Native foods were extracted with solvents (water, methanol, acetone, dichloromethane) overnight at 20ºC, except lemon myrtle and blue cypress oils and sugarbag, which were mixed with methanol (10% v/v). After removing undissolved solids by centrifugation and filtering, the extracts were concentrated to dryness with a cold stream of nitrogen. The material obtained was made up to 0.1 g/ml solvent and stored at -80ºC until required for analysis.

A β-carotene bleaching agar diffusion test based on the method of (Dapkevicius *et al*., 1998) was selected for initial screening of a large number of samples because of its simplicity and visual evidence of results. Sample extracts applied as 20µl aliquots to assay discs on the surface of a β-carotene/linoleic agar, which contained 0.5% Tween 80 to aid dispersion of potential antioxidant substances, were incubated at 40ºC for up to 48 hr.

Appraisal of the formation of conjugated diene compounds (linoleic acid hydroperoxides) was also used to evaluate antioxidative effect of native food extracts, under accelerated shelf-life conditions (Lingnert, Vallentin and Eriksson, 1979). Using emulsified linoleic acid reaction mixture as the substrate, the formation of conjugated dienes during oxidation at 37ºC was determined by spectrophotometric measurement of absorption at λ234 nm.

Two native food preparations showing the most active antioxidant potential were fractionated using suitable chromatographic techniques, including TLC (silica Kieselgel) and HPLC with diode array detection. Fractions were tested for antioxidant activity using the linoleic acid reaction test described above to identify specific components responsible for the antioxidant activity. GC-MS (Hewitt Packard GC model 5890 coupled to a HP MS model 5972) and LC-MS (Agilent 1100 LC-MS ion trap) were employed to identify antioxidant compounds. Incorporating extracts/compounds in apple juice and blended avocado (guacamole) tested for potential antioxidant action in foods.

6.2 Results and discussion

6.2.1 β-carotene bleaching agar diffusion test

The following native ingredient sources (24) were evaluated for antioxidant activity by the β-carotene bleaching agar diffusion test:

- aniseed myrtle, mountain pepper, forestberry herb, native peppermint, native thyme, wild rosella, lemon aspen, akudjura, wattleseed, eremophila, bunya nut kernel, bunya nut shell, Illawarra plum, fruit salad herb, quandong kernel, quandong shell, wild lime rind, wild lime juice/pith, munthari berry, Davidson plum, lemon myrtle oil, blue cypress oil, warrigal greens, sugarbag

The bright orange/red background colour around the untreated discs and discs with solvent only (controls) bleached to light pink within 24 hours at 40ºC. Discs containing known antioxidants (1.0, 0.1 g/L BHT in methanol; pure rosemary oil) impeded bleaching of the agar around the disc for up to 48 hr. Samples were tested in duplicate.

Although no samples showed the capacity of BHT (1.0 g/l) to conserve the intensity of orange/red pigment of β–carotene in the agar after 24 hours, many native foods showed a positive antioxidative response (Table 6.1). However, a limitation of the β-carotene bleaching agar diffusion test was that pigments characteristically present in some bushfood extracts (eg. a brown/green colour in the methanolic extract of eremophila) darkened the agar medium around the disc containing the extract. Possible bleaching caused by the oxidation of β-carotene could have been obscured by the diffusion of extract pigments into the β-carotene test agar, and therefore indicate false positives or incorrectly show an increase in the antioxidant effect of some samples. Extracts that effectively retained orange/red colour of the β-carotene medium around the paper disc with low to moderate colouring of the agar by diffusion of extract pigments included:

- water (wattleseed, mountain pepper, akudjura)
- methanol (quandong shell, lemon aspen)
- acetone (native thyme, mountain pepper, fruit salad herb)
- dichloromethane (native thyme)

Samples that exhibited no antioxidant behaviour (Index 0) after 24 hours included:

- munthari (water and methanol extracts)
- sugarbag (water, methanol, acetone, dichloromethane)
- bunya nut kernel (methanol)
- Davidson plum (methanol)
- quandong shell (dichloromethane)
- wild lime juice/pith (dichloromethane)

Table 6.1 Evaluation of antioxidant activity in native food extracts using the β-carotene bleaching agar diffusion test. Data are mean values of two replicates.
Antioxidant index: 0=no obvious activity (bleached pink background) to 5=high activity (brilliant orange/red background)

	Extraction solvent							
	methanol		acetone		dichloromethane		water	
Incubation period (hr)	24	48	24	48	24	48	24	48
Native food								
native thyme	4.5	3.5	4.5	2.5	4.5	2.5	3.5	3.0
aniseed myrtle	4.0	3.5	2.5	1.5	2.5	2.0	4.5	4.5
lemon aspen (dried)	4.0	3.0	1.5	1.0	2.0	0.5	2.5	0.5
eremophila	4.0	3.0	3.0	2.0	2.0	1.5	4.5	2.5
fruit salad herb	4.0	3.5	4.0	3.5	3.0	3.0	-	-
quandong shell	4.0	3.0	2.0	2.0	0.0	0.0	-	-
akudjura	3.0	0.0	3.0	0.5	1.5	0.0	4.0	2.0
wattleseed	3.0	0.5	2.0	0.5	3.0	0.5	4.5	2.0
bunya nut shell	3.0	2.0	2.5	1.5	1.0	0.0	-	-
wild lime rind	2.5	1.0	3.0	1.5	3.0	1.0	-	-
lemon myrtle oil	2.5	0.0						
warrigal greens	2.5	0.5	3.0	2.0	3.5	3.0	-	-
native peppermint	2.0	2.0	4.5	3.5	2.5	1.0	4.0	3.0
quandong kernel	2.0	1.0	0.5	0.0	1.0	0.5	4.0	3.5
mountain pepper	1.5	1.5	4.0	2.5	3.0	0.5	4.5	3.5
forest berry herb	1.5	1.5	3.5	2.0	1.0	1.0	3.5	3.0
Illawarra plum	1.5	0.0	3.0	1.5	2.5	1.5	-	-
wild lime juice/pith	1.5	0.0	0.5	0.0	0.0	0.0	1.5	0.0
wild rosella	1.0	0.0	0.5	0.0	0.5	0.0	2.5	0.5
blue cypress oil	1.0	0.5						
bunya nut kernel	0.0	0.0	2.0	0.5	1.5	0.5	3.5	0.5
munthari	0.0	0.0	2.0	2.0	1.0	0.5	0.0	0.0
Davidson plum	0.0	0.0	1.0	1.0	1.0	0.0	1.5	0.0
sugar bag	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
solvent controls	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
BHT 1.0 g/l	5.0	3.5						
BHT 0.1 g/l	2.0	0.5						
rosemary oil *	2.5	1.0						

- not assessed
* 100% oil

6.2.2 Linoleic acid reaction test

6.2.2.1 Crude extracts

Native food extracts were originally tested at 2.0 and 0.2 mg/ml linoleic acid reaction substrate. However, in many of the preparations (eg. those containing lemon myrtle oil, fruit salad herb, mountain pepper), initial (time zero) spectrophotometric absorbency readings at λ 234 nm were quite high, which consequently may have reduced detection sensitivity of conjugated dienes formation. Further linoleic acid reaction tests with native food extracts at 0.02 mg/ml substrate showed low initial absorbency measurements while also permitting sensitive monitoring of the formation (or more importantly the lack of formation) of conjugated dienes.

Overall, antioxidant activity of native foods was less prevalent with polar solvent extracts (water, methanol) than non-polar ones (dichloromethane, acetone). Dichloromethane applied singly also reduced the rate of formation of conjugated dienes, which may have enhanced the antioxidant efficacy of some native foods extracted with dichloromethane (Table 6.2). From 25 native food types examined, 13 showed moderate to high antioxidant activity (< 0.5 relative antioxidant effect to controls). Native ingredients with antioxidant potential included:

Dichloromethane extracts:
lemon aspen, warrigal greens, Illawarra plum, wild lime rind, fruit salad herb, eremophila, native thyme, wild rosella

Acetone extracts:
native thyme, eremophila, fruit salad herb, lemon aspen, mountain pepper, warrigal greens, native peppermint, munthari

Methanol extracts:
eremophila, fruit salad herb, lemon aspen, mountain pepper, native thyme

Water extracts:
mountain pepper, forestberry herb, native peppermint

Absorbance of sample extracts that possessed none or little antioxidant activity (eg. wattleseed) increased rapidly after 24 hr, which indicated the production of conjugated diene compounds. Further incubation to 2-3 days often caused a decrease in absorbency values, suggesting that the hydroperoxides formed were rather unstable and receded by further reactions. No native food extracts (methanol, acetone, water) showed an oxidative effect similar to 0.002 mM BHT (positive control).

When comparing the linoleic acid reaction results to the β-carotene bleaching agar diffusion test, the highest activity was associated with native thyme (acetone and dichloromethane extracts), lemon aspen (methanol) and mountain pepper (water).

Table 6.2 Linoleic acid reaction test measuring the relative antioxidant effect of native food extracts (0.02 mg/ml reaction substrate). Data are mean values of two replicates.

Native food	Extraction solvent			
	methanol*	acetone*	water*	dichloro+ methane
eremophila	0.31	0.20	0.61	0.19
fruit salad herb	0.37	0.23	-	0.20
dried lemon aspen	0.43	0.29	1.10	0.12
native thyme	0.44	0.19	1.12	0.36
mountain pepper	0.47	0.30	0.18	0.92
native peppermint	0.51	0.34	0.32	0.65
aniseed myrtle	0.51	0.40	0.55	0.70
munthari	0.52	0.36	1.10	-
Davidson plum	0.66	0.98	1.12	-
warrigal greens	0.71	0.31	1.10	0.13
quandong shell	0.80	0.71	-	-
lemon myrtle oil	0.81			
forest berry herb	0.83	0.47	0.25	1.12
bunya nut shell	0.84	0.69	-	-
Illawarra plum	0.84	0.96	-	0.14
pepper berry	0.91	0.66	1.07	1.20
wild lime (juice/pith)	0.93	1.11	1.14	-
blue cypress oil	0.95			
quandong kernel	0.96	1.13	1.09	-
bunya nut kernel	0.98	1.16	1.09	-
wild rosella	1.00	0.81	0.61	0.26
sugar bag	1.01	0.90	-	-
ground wattleseed	1.03	0.84	1.08	0.71
wild lime rind	1.03	0.52	-	0.15
akudjura	1.10	0.80	1.13	0.56
controls	1.00	1.00	1.00	1.00
BHT (0.002 mM)	0.12	0.09	0.13	0.18

* absorbance values measured after 24 hr
\+ absorbance values measured after 48 hr
\- not analysed

$$\text{Relative antioxidant effect} = \frac{\text{increase of absorption at } \lambda \text{ 234 nm during the incubation time}}{\text{corresponding increase of the control}}$$

6.2.2.2 Fractionation of extracts

Thin Layer Chromatography was used as the first phase to isolate the components responsible for the antioxidant behaviour in native food extracts that showed the most active antioxidant potential. Extracts selected for testing included:

- eremophila (methanol, acetone, dichloromethane extracts)
- fruit salad herb (methanol, acetone, dichloromethane)
- lemon aspen (methanol, acetone, dichloromethane)
- native thyme (methanol, acetone, dichloromethane)
- warrigal greens (acetone)

Extracts (0.1 g/ml) applied as 10µl spots were developed using the solvent system, methanol : dichloromethane (1:9). Four regions (*ie*. Rfs 0-0.25, 0.25-0.50, 0.50-0.75, 0.75-1.0) were taken from the chromatographed spots and evaluated for antioxidant potential in duplicate using the linoleic acid reaction test.

Table 6.3 shows that antioxidant activity was most prominent in the Rf region 0.75 - 1.0, in particular from:

- eremophila (methanol and acetone extracts)
- lemon aspen (methanol, acetone)
- native thyme (methanol, dichloromethane)
- warrigal greens (acetone)

A higher degree of fractionation was employed (*ie*. Rfs 1.0-0.95; 0.95-0.90, 0.90-0.85, etc) to optimise isolation of antioxidant components from TLC. Samples that consistently demonstrated high antioxidant potential after TLC purification were methanolic extracts of lemon aspen and native thyme. Maximum antioxidant activity for lemon aspen occurred around Rf 0.90-0.95 (solvent system - methanol:dichloromethane, 1:9) and at Rf 0.75-0.80 for native thyme (solvent system - hexane:dichloromethane, 1:9). A spot that quenched short wave (λ 254 nm) UV-light was detected in that Rf region and was thought to be the compound/s causing the antioxidant activity in the native thyme extract.

Native Thyme:

Regions taken from the TLC separation of methanolic extract of native thyme were analysed by HPLC using a 150 x 3.9 mm i.d. reverse phase C18 column and mobile phase 30% water and 70% methanol at 0.8 ml/min. In the region of antioxidant activity (Rf 0.75-0.8), two major peaks were present, each having virtually identical UV spectrums (λUV_{max}: 230, 280 nm). The larger peak (compound B) occurring at the retention time (rt) of 5.7 min represented 94% of total peak area at λ280 nm; the smaller peak (compound A; rt 4.0 min) around 5.4% of total peak area (Fig. 6.1).

Fractions of the HPLC mobile phase containing the compounds were concentrated over dry nitrogen to remove the methanol. An attempt to remove water from fractions by further drying with nitrogen or with freeze-drying tended to remove the targeted compound/s.

Using the linoleic acid reaction test, the fraction containing compound A retarded production of conjugated dienes (0.17 relative antioxidant activity), whereas compound B and other fractions produced very little antioxidant activity (0.89-0.94).

Table 6.3 Linoleic acid reaction test measuring the relative antioxidant effect of TLC fractions of native food extracts. Data are mean values of two replicates. TLC was developed using the solvent system, methanol:dichloromethane (1:9)

Native Food	Extraction solvent	Region taken from TLC (Rfs)			
		0-0.25	0.25-0.5	0.5-0.75	0.75-1.0
eremophila	methanol acetone dichloromethane	0.63 0.83 1.10	0.68 0.94 0.98	0.80 0.93 1.00	0.15 0.18 0.83
fruit salad herb	methanol acetone dichloromethane	0.64 0.76 0.96	0.49 0.45 1.00	0.54 0.42 0.92	0.25 0.29 0.81
lemon aspen (dried)	methanol acetone dichloromethane	0.62 0.89 0.70	0.42 0.46 0.69	0.29 0.55 0.83	0.13 0.12 0.60
native thyme	methanol acetone dichloromethane	1.00 0.93 0.56	0.85 0.95 0.84	0.34 0.98 0.70	0.13 0.13 0.20
warrigal greens	acetone dichloromethane	0.82 0.54	0.94 0.54	0.58 0.82	0.13 0.29

$$\text{Relative antioxidant effect} = \frac{\text{increase of absorption at } \lambda \text{ 234 nm during the incubation time}}{\text{corresponding increase of the control}}$$

HPLC fractions were injected into GC-MS to identify compounds. Compound A displaying the antioxidant activity was matched as eugenol (97% quality) by the NBS compound software library and by retention time of external standard; compound B was 1,2-dimethoxy-4-(2-propenyl) benzene (96% match).

The antioxidant activity of eugenol is well established (Farag *et al.*, 1989; Imark, Kneubuhl and Bodmer, 2001; Lee and Shibamoto, 2001). The antioxidant activity of eugenol could be related to the inhibition of the hydroperoxide formation. In general, eugenol acts as a hydrogen donor to the peroxy radicals, thus retarding the autoxidation of linoleic acid by chain radical termination.

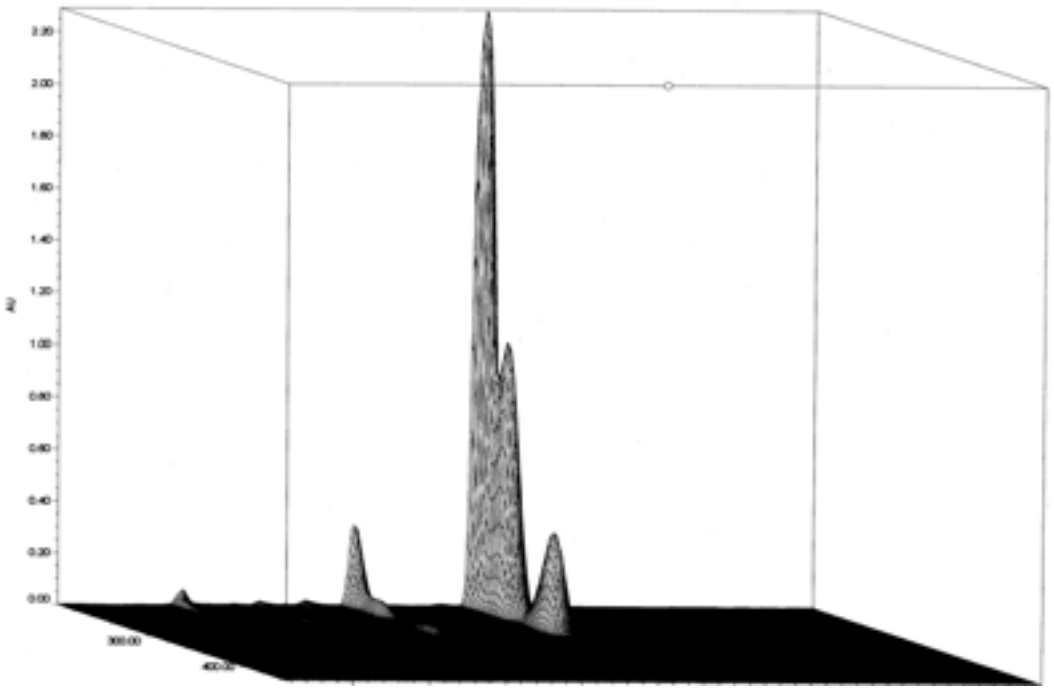


Figure 6.1 HPLC chromascan of native thyme. The larger peak (compound B) occurring at rt 5.7 min represents 94% of total peak area at λ 280 nm; the smaller peak (compound A at rt 4.0 min) around 5.4% of total area.

Lemon thyme:

The TLC region that showed maximum antioxidant activity (Rf 0.9-0.95) was analysed by HPLC using the C18 column described previously. Major components were ideally separated using the mobile phase 85% methanol : 15% water with 0.1% acetic acid at the rate of 0.8 ml/min. Fractions were collected and evaluated using the following steps:

1. Solvent-solvent extraction at –20ºC (placing samples at –20ºC separated the acetonitrile and aqueous solutions into two discrete phases and subsequently permitted the water to freeze)
2. Evaporation of the collected acetonitrile phase with dry nitrogen

Linoleic acid oxidation tests showed that a fraction containing a compound (λUV_{max}: 200, 282 nm, shoulder c. 320 nm) at rt 5.4 min reduced production of conjugated dienes (0.10 relative antioxidant activity). Other fractions collected from the HPLC run co-eluted little antioxidant behaviour and in some cases promoted oxidation of linoleic acid (0.8-1.2 activity).

Examination of the antioxidant fraction by GC-MS produced no observable peaks suggesting that the component in question has a non-volatile nature. When the active compound was injected into an Agilent 1100 LC-MS ion trap, three major ions (205.0, 409.3, 817.5 m/z) all singly charged were presented in the full (all MS) spectrum. A small peak displayed at 839.6 m/z suggests the presence of sodium (Fig. 6.2).

LC-MS/MS studies of the antioxidant showed that 817.5 m/z peak generally fragmented to 409.3 m/z, and the 409.3 m/z peak to 205 m/z, suggesting a close relationship between the ions (monomer unit, dimer unit etc). Since the 409.3 m/z ion generated in the full (all MS) scan clearly had the greatest intensity, it is probable that the molecular mass of the antioxidant compound is 408 Da.

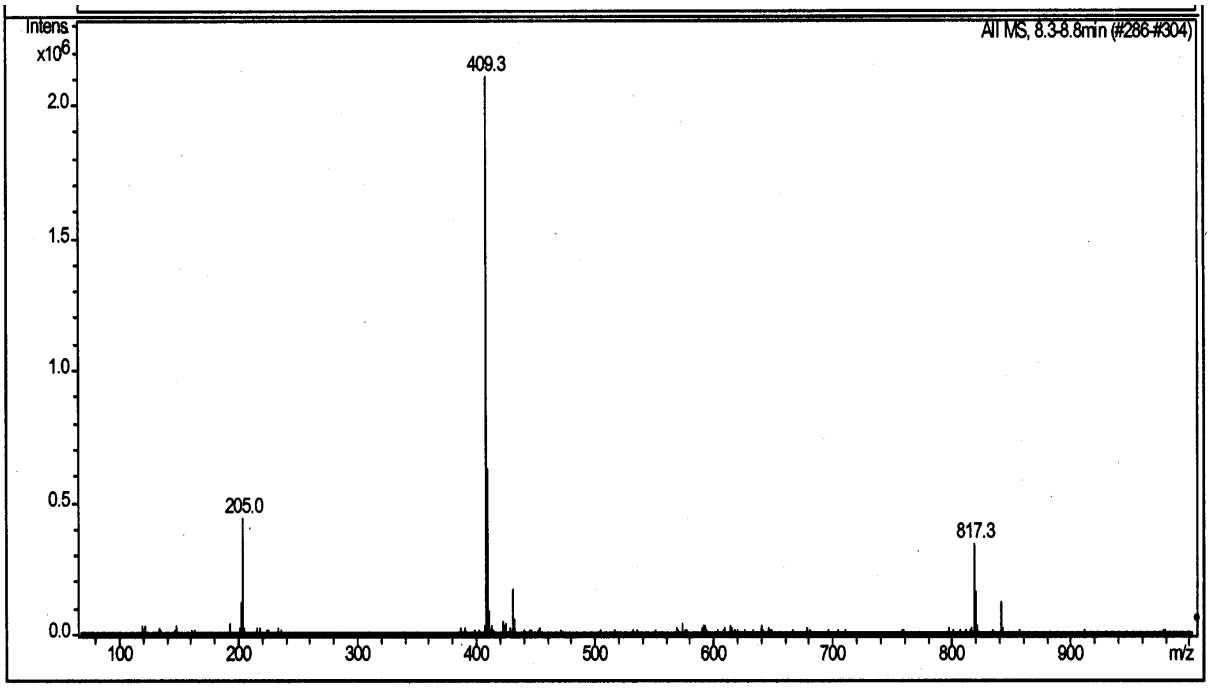


Figure 6.2 LCMS full scan (all MS) of antioxidant compound extracted from lemon aspen.

6.2.3 Antioxidant activity in foods

Ethanolic extracts of lemon aspen and native thyme (0.1g/ml) were tested in foods to inspect for possible antioxidant action. The first test involved application of extracts (8 drops) to the surface of guacamole. Ascorbic acid (0.5% in ethanol) was used as a positive control. After 5 hours at 25ºC, native thyme extract significantly reduced browning on the guacamole surface almost as proficiently as ascorbic acid. Guacamole treated with lemon aspen extract browned rapidly together with that of the control (ethanol only). Conversely, the HPLC fraction containing the antioxidant from lemon aspen (1 drop) reduced browning slightly in guacamole but the HPLC purified fraction of the native thyme antioxidant (eugenol) had no inhibitory effect.

In freshly prepared apple juice, lemon aspen extract (1% in juice) impeded browning but not as effectively as ascorbic acid (0.5%), which completely maintained green colouring of the juice greater than 5 min at 20ºC. Addition of HPLC purified antioxidants from lemon aspen and native thyme to apple juice had no inhibitory effect on browning. Higher concentration of extracts and antioxidants may reduce browning of guacamole and apple juice, although one cannot exclude the likelihood of other compounds in the crude extracts of lemon aspen and native thyme contributing to overall antioxidant impact.

6.3 Summary

Various native foods (eg. fruit salad herb, lemon aspen) demonstrated a positive antioxidative response to conserve the intensity of orange/red pigment of β–carotene in agar. In addition, linoleic acid reaction tests showed that around half of the 25 native ingredients, including eremophila, fruit salad herb, lemon aspen and native thyme, tested moderate to high antioxidant activity. Samples that constantly produced high antioxidant effect after TLC purification were methanolic extracts of lemon aspen and native thyme.

The antioxidant compound in native thyme extract was identified as eugenol. GC-MS examination of the antioxidant fraction from lemon aspen produced no observable peaks suggesting that the component in question be of a non-volatile nature. LC-MS analyses of lemon aspen showed three major ions (205.0, 409.3, 817.5 m/z) all singly charged in the full (all MS) scan. The 409.3 m/z ion generated in the full scan had the highest intensity, which suggests the molecular mass of the antioxidant compound is 408 Daltons (Da).

Considerable antioxidant effect was observed after incorporation of antioxidants/extracts into foods. Native thyme extract significantly reduced surface browning of guacamole while lemon aspen extract (1% in juice) minimised the rate of browning in freshly prepared apple juice. Less antioxidant effect was shown in guacamole and apple juice when challenged to the purified forms of the native thyme antioxidant (eugenol) and lemon aspen antioxidant.

7. Antimicrobial compounds

The use of naturally occurring antimicrobial substances to enhance the preservation and safety of fresh and processed foods is of great interest to the food industry. Although the antimicrobial activity of components from traditional herbs and spices is well recorded, there is considerable opportunity to identify and evaluate antimicrobial compounds in native foods that may be potentially beneficial as food preservatives.

The aims of this study were to evaluate the antimicrobial activity of native food sources and to determine the active antimicrobial component(s) in the most active preparation identified.

7.1 Materials and methods

Vic Cherikoff provided the native food sources. Food-borne and postharvest pathogens obtained from Department of Food Science and Technology UNSW included *Penicillium expansum, Saccharomyces cerevisiae, Erwinia carotovora, Listeria monocytogenes, Bacillus cereus* and *Salmonella enteritidis.*

The antimicrobial ability of native food extracts (see section 6.1 for extraction details) was determined *in vitro* using the disc diffusion assay technique. Sample extracts were applied as 20 µl aliquots to assay discs on the surface of agar medium (nutrient agar or potato dextrose agar) previously seeded with a spore/cell suspension of the target pathogen, and incubated at 25 or 37ºC for 24-48 hours. The sample exhibiting maximal antimicrobial activity was fractionated using TLC.

To detect the presence of antimicrobial metabolites after separation by TLC, a bioautographic TLC procedure was utilised where plates sprayed with a spore suspension of *P. expansum* in double strength Czapek Dox broth were incubated at 25ºC in a moist atmosphere. After 24-48 hr, region/s with antifungal activity indicated the presence of toxic metabolite/s. Regions of corresponding Rf values on a duplicate TLC plate (but not sprayed with *P. expansum*) were extracted with methanol and purified by HPLC. Gas chromatography coupled with mass spectrometry and LC-MS (Agilent 1100 LC-MS ion trap) were employed to characterise antimicrobial compounds from extract fractions.

The most promising native food source was evaluated as a preservative on selected postharvest horticultural commodities (tomatoes, apples, lettuce).

7.2 Results and discussion

7.2.1 Disc diffusion assay tests

All native food samples except warrigal greens prevented microbial growth and produced a distinct zone of inhibited growth (1-14 mm radius) around the site of application (Table 7.1). In general, *Saccharomyces cerevisiae* was most susceptible to the bushfood extracts, followed by *B. cereus, P. expansum, E. carotovora, L. monocytogenes* and *S. enteritidis*.

Wild lime juice/pith, lemon aspen and Davidson plum inhibited the growth of bacterial pathogens (2-6 mm radius) more effectively than fungi, possibly because of the acidic nature of their constituents (eg. presence of organic acids in wild lime and lemon aspen). Conversely, pepperberry extracts were more fungicidal than bactericidal, with zones of inhibition for *S. cerevisiae* and *L. monocytogenes* of 7-10 mm and 0-3 mm radius, respectively. Several reports have commented on the antimicrobial effects of extracts and distillates from native pepperberry (Costello, 1998; Anon., 1999b;).

Table 7.1 Antimicrobial activity of native food extracts using disc diffusion assay tests. Data are mean values of two replicates.

		Zone of inhibition from disc (mm)					
Native food source	Extraction solvent	*Penicillium expansum*	*Erwinia carotovora*	*Bacillus cereus*	*Listeria monocy-togenes*	*Salmonella enteritidis*	*Saccharo-myces cervisae*
aniseed myrtle	acetone	1.63	1.5	1	2	1	-
	methanol	2	-	-	-	1.63	2
mountain pepper	acetone	3	2	3.25	0.75	-	8
	methanol	1.5	-	2.5	0.75	2	6.5
forest berry herb	acetone	3.25	-	0.75	-	0.75	1.25
	methanol	2	0.75	1.75	0.5	1	-
native peppermint	acetone	-	4	-	0	2	-
	methanol	3	-	2	1.5	2	3
native thyme	acetone	-	-	-	-	-	-
	methanol	2	-	1	2	1	1.5
wild rosella	acetone	1.5	3.25	4.75	1.88	2	-
	methanol	1.5	2.75	3.25	1.75	0.5	-
dried lemon aspen	acetone	-	1.75	4	1.25	-	2
	methanol	1	1.75	3.5	2.13	-	1
akudjura	acetone	-	-	1.88	-	0.75	-
	methanol	-	1	-	2	-	3
eremophila	acetone	-	-	1	4.75	0.75	1
	methanol	2	-	1	-	1.25	-
bunya nut kernel	acetone	-	1	-	-	-	-
	methanol	-	2	2	-	-	2
fruit salad herb	acetone	6.63	-	2.38	-	0.5	2
	methanol	4.13		1.5	2	-	
wild lime rind	acetone	-	2	2.13	-	-	-
	methanol			2.25	-	1.5	2
wild lime juice/pith	acetone	1.5	4.25	5.75	2.75	2.75	-
	methanol	1	2.5	4.75	1.75	1.5	-
Davidson plum	acetone	-	5.5	5.5	2.88	1.75	1.5
	methanol	3	4	5	3	1.75	2
lemon myrtle oil	acetone	-	-	-	-	-	-
	methanol	8.25	4.5	10	6.25	4.75	13.8
blue cypress oil	acetone	-	-	-	-	-	-
	methanol	3	1.75	4	2.5	1.38	3.75
warrigal greens	acetone	0	-	0	0.5	0	0
	methanol	0	-	0	0	-	1
sugarbag	acetone	-	-	-	-	3	-
	methanol	2.5	5.75	0	6.25	3	-
pepperberry	acetone	5.25	2.5	1.88	0.75	0.25	10.3
	methanol	3.5	1.25	1.75	2.38	1.25	8.75
solvent (controls)	acetone	0	1	0.5	0.75	0	1
	methanol	0	1.75	0.75	0.75	1.25	1.5
citral standard	acetone	-	-	-	-	-	-
	methanol	10	7.75	22.5	3.75	-	15.3

- not assessed

Overall, native pepperberry and lemon myrtle oil were the most effective in inhibiting the growth of the test micro-organisms. Lemon myrtle oil had the greatest antimicrobial effect (5-14 mm radius) and

since neral and geranial (citral) are the primary components of lemon myrtle oil (see section 3.2.1.1), it is likely that they were responsible for the antimicrobial action. Application of a citral standard (10% v/v in methanol) purchased from Sigma Aldrich caused similar or larger zones of microbial inhibition, which supports this hypothesis.

7.2.2. Bioautographic TLC

Bioautographic TLC developed with the solvent systems, methanol : dichloromethane (1:9, 3:7, 5:5) showed that both methanol and acetone extracts of pepperberry produced a zone of antifungal activity to *P. expansum*. Higher proportions of methanol (ie. increased polarity) in the solvent system shifted antifungal activity from the solvent front to a lower Rf value, but the zone of inhibition was large and diffuse (eg. for methanol : dichloromethane 5:5, the antifungal region ranged from Rf 0.56 to 0.88) and required refinement.

Subsequently, a wide range of solvent systems was investigated with the aim of producing distinct region/s of antifungal activity on bioautographic TLC. Development of TLC plates with tetrahydrofuran:hexane (1:9) produced two prominent areas of antifungal activity from the methanolic extract of pepperberry - Rf 0.71 (designated as MP1) and Rf 0.29 (MP2).

7.2.3 Purification and characterisation of antimicrobial compounds

7.2.3.1 MP1 region

HPLC (mobile phase - 70% acetonitrile : 30% water at 0.8ml/min) of components extracted from the MP1 region produced a pronounced peak at rt 11.4 min. The compound had no unique UV profile and generally peaked in the lower UV wavelength range (ie. λ200 nm). Fractions collected from the mobile phase were concentrated using the solvent extraction and concentration procedures described in section 6.2.2.2 and spotted on bioautographic TLC to confirm antifungal activity. The compound at rt 11.4 min showed strong antifungal activity to *P. expansum*, whereas other HPLC fractions exhibited no obvious fungicidal effect.

GC-MS analysis of the antifungal compound showed a mass spectrum with major ions at 41, 111, 135, 205 and 220 m/z (Fig. 7.2). The GCMS software library (NBS75K) tentatively identified the spectrum of the compound (79% match) as benzene, 1,1'-(3-methyl-1,3-butadienylidene)bis-. Further GC-MS analyses showed that benzene,1,1'-(3-methyl-1,3-butadienylidene)bis- was not present in the other HPLC fractions.

7.2.3.2 MP2 region

HPLC optimisation of the mobile phase successfully separated compounds isolated from the MP2 region (Rf 0.29) of TLC. Ideal separation occurred using the solvent mixture 20% aqueous 0.1% acetic acid, 50% methanol and 30% acetonitrile at the rate of 0.8 ml/min on a 250 x 4.6 mm id C18 column. After collection of the fractions, antimicrobial activity was tested using the bioautographic TLC procedure described previously.

Strong antifungal activity was observed from a fraction containing a compound (λUV_{max}: 231 nm) occurring at rt 8.4 min. Since GC-MS analysis produced no definite identification of the compound, LC-MS was used for the characterisation. The antimicrobial compound showed major fragments at 257.1 and 491.1 m/z (singly charged) in the full scan (all MS), suggesting a molecular mass of 490 da (Fig.7.3). The 491.1 m/z peak generally fragmented to 257.1 m/z in the MS/MS mode.

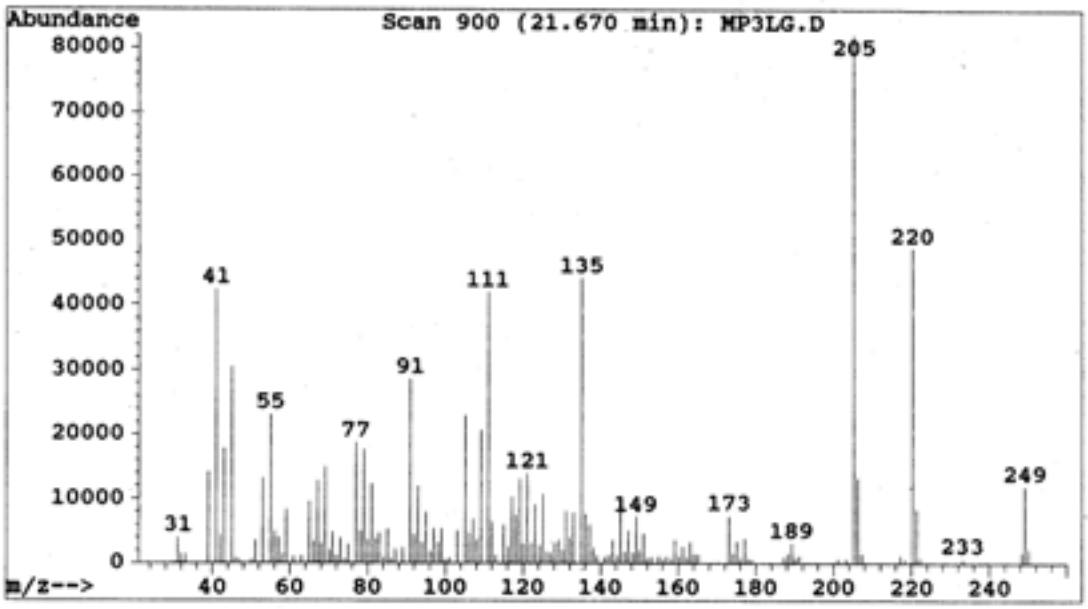


Figure 7.2 GCMS scan of the antimicrobial compound (MP1 region of TLC) extracted from native pepperberry

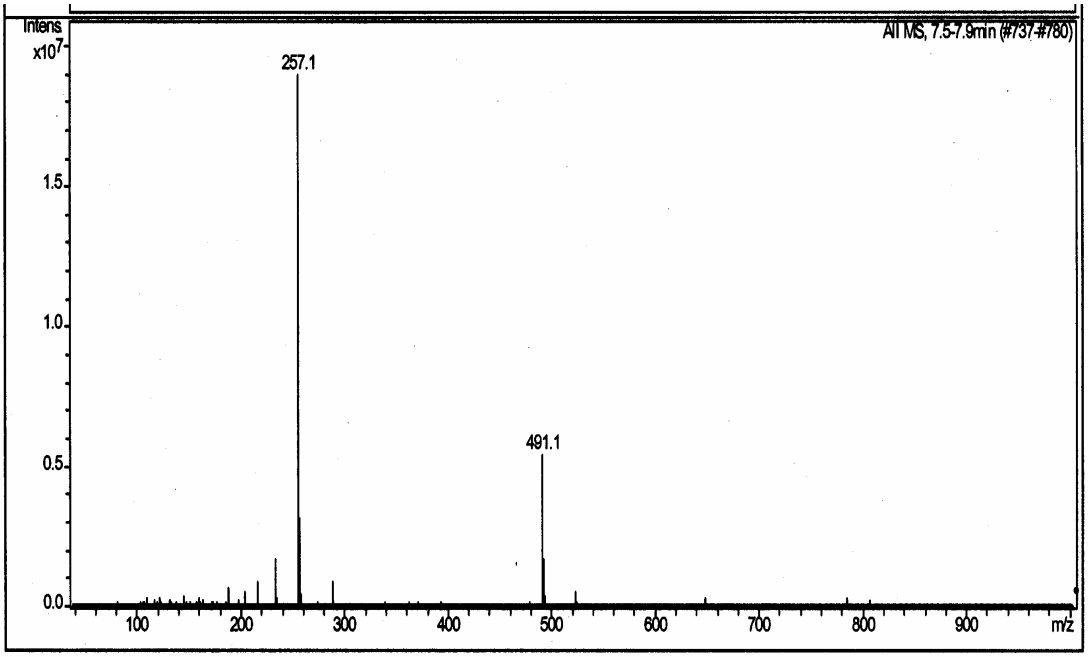


Figure 7.3 LCMS full scan (all MS) of antimicrobial compound (MP2 region of TLC) extracted from native pepperberry

Polygodial, a major component of pepperberry and considered to contribute to the pungent peppery taste, has potent antifungal activity (Dragar, Garland and Menary, 1998; Kubo, Fujita and Hwa Lee, 2001). The molecular weight and absorption maximum of polygodial is reported at 234.34 g and 231 nm, respectively. Although the λUV maximum of antimicrobial compound extracted from the MP2 region of TLC was also 231 nm, its molecular mass was higher at 490 da. Nevertheless, it is possible the structure of the antimicrobial compound is closely related to polygodial.

7.2.4. Control of microbial growth in horticultural produce

Ethanolic pepperberry extract (0.5g/ml for 24 hours at 20ºC) was diluted in water and applied to the following horticultural products:

- Tomatoes inoculated 2 hours previously with *Botrytis cinerea*
- Apples inoculated 2 hours previously with *P. expansum*
- Shredded lettuce salad

Antimicrobial efficacy of pepperberry treatments (1 and 10% extract in 10% ethanolic water) was evaluated by measuring the diameter of rots on tomatoes and apples, and plating homogenates of shredded lettuce on Tryptone Soya Agar for enumeration of total viable counts.

After 7 days at 20 C, 1% pepperberry extract reduced spoilage by *B. cinerea* in tomatoes ($P<0.05$). Increasing the concentration of pepperberry (10%) had no beneficial effect and tomatoes generally had Botrytis levels similar to the controls. This may be because the 10% pepperberry extract:

- caused chemical damage to the tomatoes, which facilitated infection of tomato tissue by Botrytis; and/or
- increased the availability of pepperberry components that nourished the growth of Botrytis

In contrast, there was no significant difference in spoilage by *P. expansum* between apples treated with pepperberry and the controls. However, the slightly lower mean levels of disease shown in apples with 1% pepperberry extract warrants further investigation.

Application of pepperberry extract to shredded lettuce moderately reduced microbial populations by around 0.4 log.

7.3 Summary

All native food samples except warrigal greens showed antimicrobial activity to target food spoilage micro-organisms and pathogens. Wild lime juice/pith, lemon aspen and Davidson plum were effective antibacterial sources, whereas pepperberry showed more fungicidal activity.

Bioautographic TLC (solvent system: 1 tetrahydrofuran: 9 hexane) of the methanolic extract of pepperberry produced two prominent areas of antifungal activity to *P. expansum*. A compound extracted from the antifungal region at Rf 0.71 (designated as MP1) was tentatively identified as benzene, 1,1'-(3-methyl-1,3-butadienylidene)bis-. LC-MS of the antimicrobial compound isolated from the MP2 region of TLC (Rf 0.29) showed major fragments at 257.1 and 491.1 m/z (singly charged) in the full scan (all MS) mode, suggesting a molecular mass of 490 da.

Pepperberry treatments (1%) significantly reduced spoilage of tomatoes by *B. cinerea* and to a lesser extent Penicillium infection of apples. Application of pepperberry extract to shredded lettuce reduced viable counts of micro-organisms by approximately 0.4 log.

8. Conclusions and outcomes

Shelf-life extension of fresh native herbs: Storage at 0-5ºC together with selected MA packaging systems showed a significant effect in maintaining postharvest leaf quality. Optimum storage specifications for native herbs are as follow:

- ***Warrigal greens***: Package in '2400' film (*ca*. 0.107 g leaves/cm^2 film), store at 0-5ºC for up to 3 weeks
- ***Lemon myrtle***: Package in '6000' or '7000' film (*ca*. 0.110 g leaves/cm^2), store at 0-5ºC for up to 11 weeks
- ***Mountain pepperleaf***: Package in '2400' or '6000' film (*ca*. 0.055 g leaves/cm^2), store at 0-5ºC for up to 5 weeks.

Although the presence of ethylene in some MA packages possibly contributed to the degradation of native herbs (eg. warrigal greens), use of Purafil as an ethylene absorbent had little effect in extending postharvest shelf-life. Nevertheless, the production of ethylene and rate of response of tissue to ethylene is likely to alter with different batches of native herbs. Further studies regarding the effects of ethylene on leaf quality in native herbs are necessary.

Using the postharvest storage specifications stated above increases the value of the native foods and provide a better return for growers and distributors. Close interaction with the horticultural and food industries is necessary to ensure that packaging of the native produce is applied under proper postharvest conditions.

Analysis and development of flavour components: Flavour components and extracts from lemon myrtle, lemon aspen, wild lime and wattleseed have been characterised and assessed for use as flavours in foods. It is envisaged that the appeal of these native food essences in food products will help increase demand.

***Lemon myrtle*:** Neral and geranial (isomers of citral) were clearly the dominant components in lemon myrtle oil. Major promoters of lemon and sweet aromas were neral and geranial and to a lesser extent citronella and linalool, whereas α-pinene, eucalyptol and aromadendrene-allo provided minor non-lemon aromas. Product development studies with lemon oil and lemon myrtle sorbets indicated that lemon myrtle compared favourably with lemon oil. Although consumers preferred the sweetness of lemon oil sorbet to lemon myrtle sorbet, formulation changes may be all that is required to enhance the sweetness of the lemon myrtle sorbet.

***Lemon aspen*:** GC-MS analyses revealed 44 compounds with limonene as the most abundant volatile, followed by 3-carene, terpinolene and santalene. Identified compounds which expressed lemon aspen/lemon aroma included α-terpineol, α-bergamotene (cis), santalene, limonene, β-myrcene and α and γ-terpinenes. Further work is required to identify a prominent compound (compound 19; 24 ppm) which contained a distinct, grassy overtone. The presence of acids (eg. citric acid) and sugars (eg. fructose) indicated that they contribute to the overall taste of lemon aspen.

Development of a sorbet product incorporating lemon aspen and citrus juices proved to be desirable by consumers. However, a beverage containing a blend of pineapple and lemon aspen was not particularly well received owing to bitterness intensity and insufficient sweetness. Recipes including more salt to mask bitterness and sugar to increase sweetness would be practical ways to promote flavour acceptance of lemon aspen beverages. Alternatively, decreasing the concentration of lemon aspen in the beverage may minimise bitterness and sweetness inadequacy, although this may result in little or no flavour perception.

***Wild lime*:** Analyses of the aroma and taste components did not show appreciable differences between wild and Mexican lime flavour profiles. In addition, GC-O and sensory analysis concluded that there

is a lack of unique or significantly different attributes between wild lime and Mexican lime. Nevertheless, consumers found wild lime flavour (in cheesecake) desirable and acceptable. Wild lime has yet to enter its full potential in the native foods niche market and this seems the best opportunity area for the future.

***Wattleseed*:** Compounds mainly responsible for roasted wattleseed aroma appear to be pyrazines, which have nutty, coffee and burnt odours. Caffeine and quinolines also detected may contribute to the bitterness found in wattleseed. Further analysis of volatile wattleseed components using, for example, a wider range of known external standards is suggested as a significant number of compounds were unidentifiable using the MS software library (NBS75K). The detection of sugars (fructose, glucose), organic acids (eg. citric acid) and free amino acids suggest they play a part of flavour development in wattleseed.

The main obstacle for wattle flavours in hot chocolate beverages is the presence of a bitter aftertaste. Development of an instant wattleseed/chocolate beverage with milk (to balance bitterness intensity) and sugar showed promise with consumer acceptability, although further sensory evaluation is required to substantiate this finding. Another viable option to overcome the aftertaste of wattleseed is to use it in food preparations containing coffee, which is also bitter but highly demanded by consumers.

Preservation of lemon myrtle oil: There is little question that LMO makes a significant contribution to the flavour value-added food products, such as carbonated beverages, however it is unstable and deteriorates rapidly during storage. Our studies demonstrated the benefit of selected gums (eg. sodium alginate), pH regulation at 3.5 and microencapsulation of LMO to maintain freshness of lemon myrtle flavour. Even so, shelf-life of lemon myrtle flavour needs to be extended considerably, particularly if food products are to be marketed at ambient temperature.

Further development of encapsulation procedures for improved protection of LMO, and/or incorporation of components or extracts (eg. limonene or lemon oil) that mask off-flavours caused by degradation products of neral and geranial may be effective methods in shelf-life extension. Additional screening of substances, such as antioxidants, for ability to maintain lemon myrtle flavour is recommended utilising model beverage systems buffered at stable pH.

Improvement of bread formulations containing akudjura: The reduction of α and β-gliadins in akudjura doughs during proofing suggested that naturally occurring enzymes weaken wheat proteins and prevent proper formation of the gluten matrix. Mild heat treatment of akudjura to inhibit possible enzymic activity was considered a simple and practical method as the bread doughs containing the treated akudjura rose and held their shape over proofing.

However, the addition of akudjura to a dough formulation did not produce a highly desirable product. Liking of the tomato flavour strength, overall flavour and aftertaste were found to among the drivers of acceptance of this product, so the flavour of the akudjura must be optimised. Lowering the concentration could be a consideration, however the effect would be limited if the flavour of the akudjura itself was the problem. This possibility is suggested by that even when the tomato flavour strength was not considered strong, the tomato flavour liking was still low. It could be that the poor flavour and aftertaste could be associated with the inclusion of the bush tomato seeds in the ground sample, so removal of the seeds before preparation could be investigated.

Another option is that akudjura could be incorporated into bread with other ingredients such as chilli, olives, cheese, herbs such as basil, rosemary and thyme so that tomato flavour enhances the natural flavours of these ingredients whilst they mask the undesirable aftertaste of the akudjura. Development of a sour dough bread recipe may be a more desirable product than the straight bread dough, given that sourdough is renowned for its sour flavour, which may overcome the aftertaste of the akudjura.

Microbiological issues of dried native foods: In general, microbial numbers in the native herbs and spices tested were quite low ($\leq 10^3$ cfu/g), except aniseed myrtle, akudjura and Red Desert Seasoning (10^4, 10^4 and 10^5 cfu/g, respectively). It may be concluded that the predominant microflora of the products tested are spoilage organisms. Although no food-borne pathogens were detected, pathogens such as *B. cereus* could possibly occur. These pathogens are all spore formers and may be found in dried food products. A broader investigation of the microbiology of native products is recommended.

Germicidal effect of UV irradiation on Red Desert Seasoning was lower than envisaged but perhaps this was because the UV rays could not penetrate and eradicate micro-organisms enmeshed around the spice particles. Furthermore, UV light at doses required for microbial decline caused undesirable changes to the appearance (bleaching) and aroma of the spice.

Fumigation by acetic acid vapour is an effective method in removing micro-organisms from native food spices but unfortunately causes detrimental effects to the sensory quality of the product, including acetic acid taint and caking. Exposure of Red Desert Seasoning to potassium permanganate after fumigation satisfactorily removed acetic acid taint but the cost effectiveness of this procedure requires further evaluation. The presence of calcium chloride to provide low RH during fumigation minimised caking of spices but unfortunately was associated with limited antimicrobial capability of acetic acid. Incorporation of other humectants (eg. silicon dioxide) during acetic acid fumigation.is a suggested approach for mitigating caking in dried native foods.

Even though sterilisation of herbs and spices by λ-irradiation is now accepted as a mainstream alternative to synthetic fumigants, it is still banned by many countries so microbial reduction using plant volatiles is still a viable option if it can be commercialised and standardised for those companies wishing to avoid the use of radioactive isotopes. A realistic scenario would see low dose acetic acid fumigation offered as a potential hurdle technology, together with efficient management and processing practices to ensure microbiological integrity in dried native herbs and spices.

Novel antioxidants: Visual assessment of the β-carotene bleaching (agar diffusion) model system and spectrophotometric determination of the formation of conjugated diene compounds demonstrated varying degrees of antioxidant activity exist in Australian native foods. Highest antioxidant activity was exhibited in extracts of eremophila, fruit salad herb, lemon aspen and native thyme.

In native thyme, the antioxidant activity was associated with the presence of eugenol. Lemon aspen contained an antioxidant compound with a molecular mass calculated tentatively at 408 Da. Both native foods showed ability to reduce browning in guacamole or apple juice, although further work is required for a better understanding of the protective mechanisms involved and possible application in foods.

Oxidation is one of the main degenerative processes that food products undergo during storage. Undoubtedly, application of natural antioxidants from Australian natives for improvement of foodstuffs, as well as formulating new nutraceutical products that offer both functional and health benefits, would be an important growth area in the Australian native food industry.

Novel antimicrobial compounds: Compounds from native pepperberry containing antimicrobial activity have been isolated and characterised using MS techniques. However, further studies are required to confirm the identities of these compounds, including benzene, 1,1'-(3-methyl-1,3-butadienylidene)bis- and the other having major mass spectral ions at 257.1 and 491.1 m/z (singly charged), suggesting a molecular mass of 490 da.
Pepperberry extract showed capacity to reduce microbial growth in horticultural commodities. It is envisaged that utilisation of antimicrobial pepperberry components/extracts in foods (eg. meat products) would be potentially beneficial for the control of spoilage micro-organisms and to improve food microbiological safety. Natural preservatives from native pepperberry, from a marketing perspective, would have significant public appeal.

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