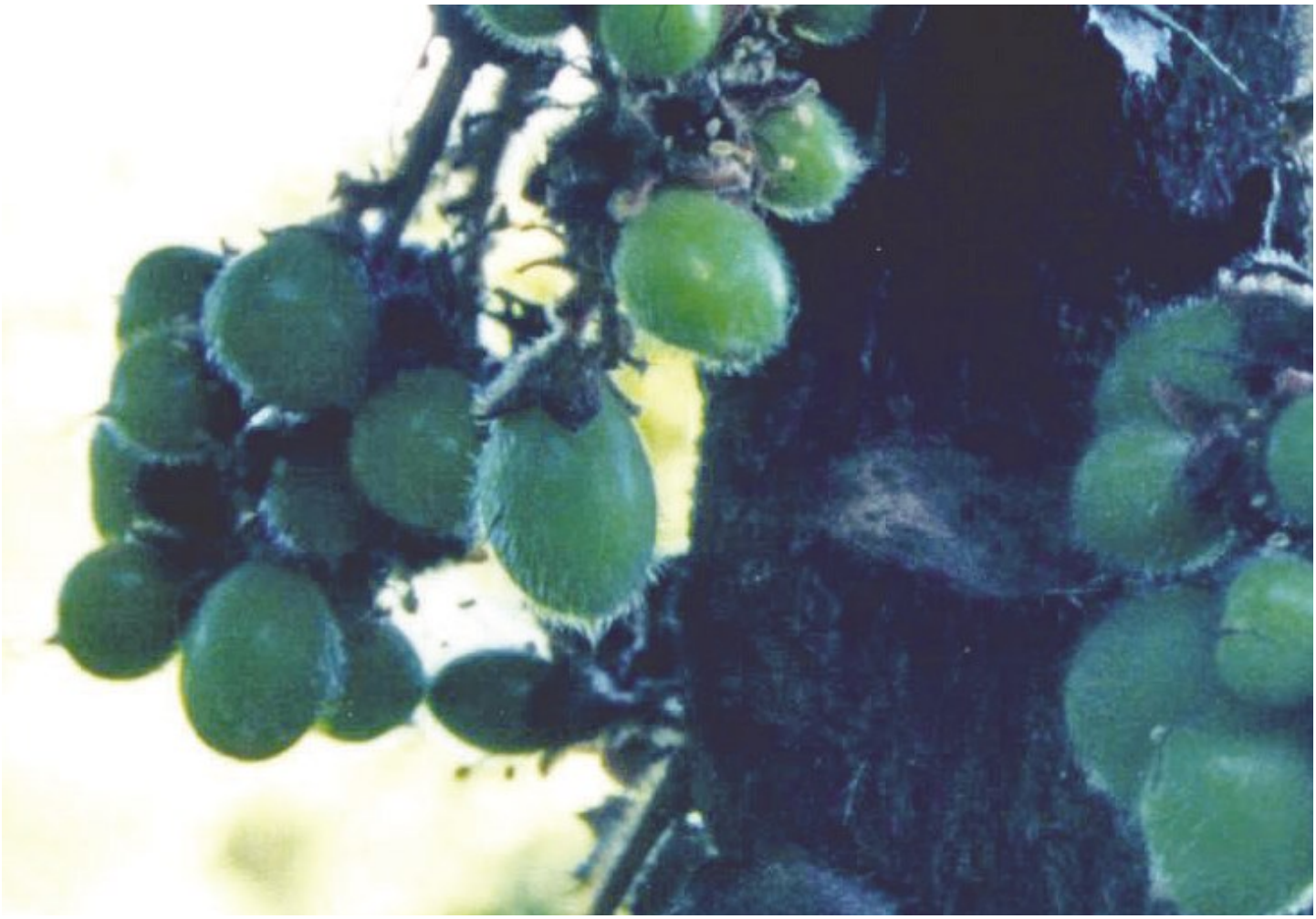
Australian Government
Rural Industries Research and Development Corporation

RURAL INDUSTRIES
Research & Development Corporation

Changes in Quality and Bioactivity of Native Foods during Storage



February 2015

RIRDC Publication No. 15/010

Australian Government
Rural Industries Research and Development Corporation

Changes in Quality and Bioactivity of Native Foods during Storage

by Y. Sultanbawa, D. Williams, M. Chaliha, I. Konczak, and H. Smyth

February 2015

RIRDC Publication No 15/010
RIRDC Project No PJR-005135

ISBN 978-1-74254-754-1
ISSN 1440-6845

Changes in quality and bioactivity of native foods during storage
Publication No. 15/010
Project No. PJR-005135

Researcher Contact Details

Dr Yasmina Sultanbawa
Queensland Alliance for Agriculture and Food Innovation
University of Queensland
Health and Food Sciences Precinct
Block 10, 39 Kessels Road
Coopers Plains QLD 4108

Phone: 07 32766037
Fax: 07 32169591
Email: y.sultanbawa@uq.edu.au

In submitting this report, the researcher has agreed to RIRDC publishing this material in its edited form.

RIRDC Contact Details

Rural Industries Research and Development Corporation
Level 2, 15 National Circuit
BARTON ACT 2600

PO Box 4776
KINGSTON ACT 2604

Phone: 02 6271 4100
Fax: 02 6271 4199
Email: rirdc@rirdc.gov.au.
Web: http://www.rirdc.gov.au

Electronically published by RIRDC in February 2015
Print-on-demand by Union Offset Printing, Canberra at www.rirdc.gov.au
or phone 1300 634 313

Foreword

Australian native foods have long been consumed by the Indigenous people of Australia. There is growing interest in the application of these foods in the functional food and complementary health care industries. Recent studies have provided information on the health properties of native foods but systematic study of changes in flavour and health components during processing and storage has not been done.

It is well known that processing technologies such as packaging, drying and freezing can significantly alter the levels of health and flavour compounds. However, losses in compounds responsible for quality and bioactivity can be minimised by improving production practices.

This report outlines research developed to provide the native food industry with reliable information on the retention of bioactive compounds during processing and storage to enable the development of product standards which in turn will provide the industry with scientific evidence to expand and explore new market opportunities globally.

Data on the composition of flavours, phytochemicals and vitamins will be an important tool to both optimise the quality and promote the benefits of native foods. Additionally, these parameters are needed for establishing quality assurance processes that can validate quality and bioactivity across different batches of the same plant source and throughout the storage life of the labelled product.

This report represents the first systematic evaluation of changes in quality and bioactivity of native herbs and fruits during processing, packaging and storage and the generation of information on key chemical markers that could be used to determine the storage life of processed products.

Finally, the report provides information on processing and packaging improvements which will ensure the quality and safety of the product over extended storage periods. The results from this study can be used in developing product standards for the selected native herbs and fruits. This will enable the development of functional ingredients and open new markets for the native food industry in Australia.

This report was funded from the RIRDC core funds provided by the Australian Government and also from industry through Australian Native Food Industry Limited (ANFIL) and the Coles Indigenous Food Fund.

It is an addition to RIRDC's diverse range of over 2000 research publications and is part of the New and Emerging Plant Industries program which aims to facilitate the development of new rural industries based on plants or plant products that have commercial potential for Australia.

Most of RIRDC's publications are available for viewing, free downloading or purchasing online at www.rirdc.gov.au. Purchases can also be made by phoning 1300 634 313.

Craig Burns
Managing Director
Rural Industries Research and Development Corporation

Acknowledgments

This project was conducted with industry partners from the Australian Native Food Industry Limited (ANFIL).

We would like especially to thank the following industries for supplying samples and patiently answering our questions during one-on-one discussions and for filling in the questionnaire to understand current industry practices: Tasmanian pepper leaf – Diemen Pepper Supplies (Chris Read); lemon myrtle and anise myrtle – Australian Rainforest Products Pty Ltd (Gary Mazzorana, Sibyilla Hess-Buschmann); Davidson's plum (*Davidsonia pruriens)* – Rainforest Bounty Pty Ltd (Margo Watkins) and Ooray Orchards (Kris Kupsch); quandong – Outback Pride (Mike and Gayle Quarmby); Kakadu plum – Coradji Pty Ltd (Robert Forbes, Robert Dean).

A special thanks to Alison Saunders (Senior Research Manager, New Rural Industries, RIRDC) for her support during this project.

The project team from Department of Agriculture, Fisheries and Forestry is gratefully acknowledged for their contribution towards this project: Jessica Sanderson, David Edwards, Sharon Pun, Andrew Cusack, Margaret Currie, Paul Burt, Kevin Matikinyidze, Jimmy Baker, Kerrie Abberton .

Abbreviations

AA	Ascorbic acid
ANFIL	Australian Native Food Industry Limited
AOAC	Association of Analytical Communities
ASE	Accelerated solvent extraction
BO	Biaxially oriented
CPP	Casted polypropylene
DHAA	Dehydroascorbic acid
DW	Dry weight
EA	Ellagic acid
EDTA	Ethylenediamine tetraacetic acid
EG	Ellagic acid glycoside
ET	Ellagitannin
FRAP	Ferric reducing antioxidant power
FW	Fresh weight
GAE	Gallic acid equivalents

GCMS	Gas chromatography mass spectrometry
HDPE	High density polyethylene
HPLC	High performance liquid chromatography
LDPE	Low density polyethylene
LLDPE	Linear low-density polyethylene
LLE	Liquid-liquid extraction
NCTC	National Collection of Type Cultures
PE	Polyethylene
PET	Polyethylene terephthalate
PVDC	Polyvinylidene chloride
RH	Relative humidity
RIRDC	Rural Industries Research and Development Corporation
RT	Retention time
SIM	Selected ion monitoring
TFA	Tri-fluoroacetic acid
TP	Total phenolics
TSYEB	Tryptone soya yeast extract broth

Contents

Tables

Figures

Executive Summary

What the report is about

This report addresses changes in quality and bioactivity at critical steps along the production chains of three native herbs (lemon myrtle, anise myrtle and Tasmanian pepper leaf) and three native fruits (Kakadu plum, Davidson's plum and quandongs). This is the first systematic study to evaluate and monitor such changes. Processing, packaging and storage stages are investigated. Current industry practices are evaluated and alternative methods to minimise losses in bioactivity and flavour are recommended.

This research will enable the industry to select processing and storage options that will maximise product quality and meet shelf life expectations of customers. The measurement of bioactive and flavour components will enable quality specifications to be set as well as provide information on the composition of antioxidants and health components that can be used for marketing purposes.

Who is the report targeted at?

The report is targeted at:

- the Australian native food industry
- the general food and beverage industry
- extraction companies interested in developing functional ingredients
- food standards and regulating authorities.

Background

Consumers desire food products that are healthy but also innovative and authentic. As a consequence, food and beverage manufacturers are looking for ingredients derived from natural sources and new functional foods that can provide a point of difference in the market. They are also increasingly calling for improved manufacturing guidelines, accurate labelling and higher levels of consistency and safety. The functional food market is evolving into a more mature industry with scientific evidence to support product claims. The emerging Australian native food industry with its diverse and rich flora has enormous potential to contribute to this market of natural ingredients with the advantage of its native foods being consumed traditionally, having a unique flavour diversity and possessing significant health-promoting and other functional properties.

However, the industry is still challenged in delivering native foods (whole and/or processed product) with consistent quality through the entire value chain. Retaining and ensuring the stability of bioactive compounds during handling, processing, packaging and storage will greatly benefit this industry and open new markets.

Recent studies on native foods have revealed promising antioxidant and antimicrobial activities that would be of value in commercial applications. However, more studies are required on the systematic identification of compounds in native foods that contribute to functional properties like health benefits, food preservation and flavour changes.

With the current information available on the bioactivity of native foods, this study is timely and fulfils a need of the native food industry to produce products with assured quality throughout the production chain. The issues that have to be addressed include processing conditions, packaging and storage, and identification of suitable key components for standardisation of product.

Aims/objectives

The aim of this study was to use chemical analyses to track changes in quality and bioactivity of six native foods (three herbs and three fruits) during processing, packaging and storage and to identify possible improvements for these stages in the production lines for native foods.

Methods used

A set of six native plant species were investigated: three herbs (lemon myrtle, anise myrtle, Tasmanian pepper leaf) and three fruits (Kakadu plum, Davidson's plum (*Davidsonia pruriens*) and quandong).

Changes in the composition of key health and flavour compounds were monitored during storage periods of varying length. A series of trials were conducted to determine if improvements such as use of packaging with high-barrier properties, different milling processes and improved freezing and drying techniques would minimise changes in bioactivity and flavour.

Commercial and processed native herb and fruit products were subjected to the following analyses:

- volatile analysis, using SIM-GCMS
- anti-oxidant capacity assays, using the ferric reducing antioxidant power(FRAP) assay
- total phenolic (TP) content, quantified using the Folin-Ciocalteu assay
- anti-microbial activity, using broth dilution assays
- analysis of vitamin C
- analysis of phytochemicals, using HPLC.

Results/key findings

This research has yielded a range of significant findings, suitable for immediate adoption by the industry at various stages in the production line and for the future development of product standards to provide producers and customers with a reference point for product quality and safety.

- High-barrier packaging material was evaluated for its ability to retain the major volatiles of lemon myrtle, anise myrtle and Tasmanian pepper leaf. The packaging was shown to extend the shelf life of these native herbs from 1 month to 12 months at ambient temperature. The industry now has the opportunity to store products for a longer period of time and increase export potential.
- Milling of native herbs can have a significant effect on volatiles as conventional milling generates heat and losses of up to 30% of characteristic volatiles can occur. On the other hand, cryogenic milling significantly reduces volatile loss due to its low temperature which absorbs heat generated during the grinding operation and stabilises volatile oils within the herbs. This presents another opportunity for the industry to improve product quality for targeted, high-value niche markets.
- Antimicrobial and antioxidant bioactive compounds of blast-frozen Davidson's plum halves and puree can retain efficacy for a period of 18 months under frozen storage (–20°C). However, when frozen, the puree showed a greater loss of antioxidant activity compared to the frozen halves. Davidson's plum extracts have indicated broad-spectrum antimicrobial activity against Gram-positive and Gram-negative bacteria, making the fruit very attractive as a natural food preservative.

- Selected bioactive compounds in whole and pureed Kakadu plum can retain efficacy for a period of 18 months under frozen storage (–20°C). The high free ellagic acid content in Kakadu plum enhances its application in the functional food market. Kakadu plum is more potent as an antimicrobial agent against Gram-positive bacteria than against Gram-negative bacteria.

- Drying at higher temperatures (ranging from 40°C to 60°C) did not affect antioxidant and antimicrobial activity in quandong. The current industry drying practices are acceptable as a processing standard.

- Chemical markers to determine end-of-storage life have been identified for the native herbs and Kakadu plum. These include volatile markers for lemon myrtle (citral which is the combination of two isomers neral and geranial), anise myrtle (estragole and anethol) and Tasmanian pepper leaf (eucalyptol and eugenol) and non-volatile markers for Kakadu plum, lemon myrtle and anise myrtle (ellagic acid) and Tasmanian pepper leaf (chlorogenic acid).

- Polyphenol-rich extracts obtained from the native herbs or residues of leaves after extraction of essential oils, have valuable bioactive components that can be used for further value addition in the functional food ingredient and health markets.

Implications for relevant stakeholders for:

If adopted, this research will enable the development of individual product standards which will provide producers and customers with a reference point for product quality and safety. The benefits of extended storage life through improvements to processing and packaging will open new markets for the native food industry. Implications of the research include:

- enhanced ability of the industry to meet appropriate product standards and increase export opportunities

- improved product information, including information on stability of bioactivity in native foods and identification of chemical markers during storage, will support market access and growth

- market expansion through product innovation and scientific evidence to support product claims

- improved manufacturing guidelines for products and accurate information for labelling

- sustainable growth for the Australian native foods industry based on an increased understanding of their protocols and products.

Recommendations

Information obtained from this project can be used to promote native plant products as functional ingredients with credible scientific data. The economic benefit of getting access to global markets and gaining customer confidence is very significant and could increase sales exponentially. Opportunities for cross-industry applications of native fruit and herb extracts as natural preservatives and antioxidants in other food systems present innovative uses of native plant ingredients. Such applications could be considered only the beginning of potential uses of these ingredients, not only in food and beverages but also in the complementary health care and cosmetic industries.

Growth of the native food industry will increase cultivation, harvesting and processing of native foods in rural communities, which will create much-needed employment in remote areas. In most Indigenous communities it is the women who cultivate and harvest native foods and growth in this industry would give economic power to women.

1. Native herbs and fruits

1.1 Industry needs for quality improvements through processing

1.1.1 Introduction

The Indigenous population of Australia has been using Australian endemic plants as food and medicine for centuries. The rich Australian native flora comprises more than 25 000 plants (Cooper 2004). Australian endemic plants have gained significant attention over recent years due to their use in pharmacy, medicine, food, beverages, cosmetics, perfumery and aromatherapy. There has been an increasing national and international demand for Australian native foods such as native herbs, spices, nuts, essential oils and fruits. Most of the native foods are used as preserves, sauces, chutneys and other condiments, although fresh herbs and spices are also used by the food service and catering industry. These herbs are usually incorporated as the essential oil (e.g. essential oils of lemon myrtle and anise myrtle) or as a milled form of the dried leaves (e.g. Tasmanian pepper leaves). The following products and their growers/processors were selected as project participants in consultation with ANFIL and RIRDC:

- lemon myrtle and anise myrtle – Australian Rainforest Products Pty Ltd (Gary Mazzarona)
- Tasmanian pepper leaf – Diemen Pepper Supplies (Chris Read)
- Davidson's plum (*Davidsonia pruriens*) – Rainforest Bounty Pty Ltd (Margo Watkins) and Ooray Orchards (Kris Kupsch)
- quandong – Outback Pride (Mike and Gayle Quarmby)
- Kakadu plum – Coradji Pty Ltd (Robert Dean).

1.1.1.1 Lemon myrtle (*Backhousia citriodora*)

Lemon myrtle *(Backhousia citriodora*, family Myrtaceae) is an important and highly aromatic shrub endemic to eastern Australia (Hayes and Markovic 2002). Lemon myrtle leaves are used to extract the essential oil which is incorporated into food products, cosmetics and toiletries and also used as an aromatherapy oil. The predominant (95%) volatile aromatic compound in the essential oil from lemon myrtle (chemotype B. citriodora F. Mueller) is citral (3,7-dimethyl-2-6-octadienal) which is an isomeric mix of two aldehydes – neral and geranial. Citral has significant antimicrobial activity against a range of Gram-positive and Gram-negative bacteria, yeast and mould (Konczak et al. 2010a; Hayes and Markovic 2002). Due to their strong lemon flavour, the leaves and flowers are used in tea blends and beverages, dairy products, biscuits, breads, confectionery, pasta, syrups, liqueurs, flavoured oils, packaged fish (salmon), dipping and simmer sauces (Konczak et al. 2010a).

1.1.1.2 Anise myrtle (*Syzygium anisatum*)

Anise myrtle (*Syzygium anisatum*, family Myrtaceae) is a rare Australian rainforest tree. The large anise myrtle trees (20–45 m in height) are restricted to rainforests in north-east New South Wales and Queensland. The leaves provide an aniseed flavour and are used as herbs in sweet and savoury dishes as well as in cosmetics. The major volatile component of anise myrtle is (E)-anethol (79.4–90%). Another variety of anise myrtle contains 4.4–10.1% methyl chavicol (Southwell et al. 1996).

1.1.1.3 Tasmanian pepper leaf (*Tasmannia lanceolata*)

Tasmanian pepper (*Tasmannia lanceolata* R. Br., family Winteraceae) is associated with the humid Antarctic flora of the southern hemisphere. The plant is a shrub (height up to 5 m) with dark green leaves and distinctive crimson young stems (Netzel et al. 2006). Most Tasmanian pepper leaf is supplied from Tasmania and Victoria. Both the pepper-flavoured berry (5–7 mm diameter) and leaves are sold commercially and used as a condiment, giving an unusual fragrant, spicy taste and a 'bushy' rainforest feel (Ahmed and Johnson 2000). Leaves of Tasmanian pepper are used as a herb whereas berries are used as a spice. The warm, pungent and spicy character of Tasmanian pepper has been associated with a sesquiterpene polygodial (Konczak et al. 2010a).

1.1.1.4 Davidson's plum (*Davidsonia pruriens* var. pruriens and var. jerseyana)

The Davidson's plum (*Davidsonia pruriens*, family Davidsoniaceae) is a small rainforest tree with a height of up to 15 m. Davidson's plum production occurs in the sub-tropical coastal regions of New South Wales and Queensland. The plum-like, sour fruit can be used for jams, sauces, vinegars, dressings, ice creams, drinks, and stewing. Davidson's plums have a high anti-oxidant capacity and a high ratio of potassium to sodium (Clarke 2012; Konczak et al. 2009). There are three species of *Davidsonia*:

- *Davidsonia jerseyana* – also known as the New South Wales Davidson's plum
- *Davidsonia johnsonii* – the smooth-leaved Davidson's plum, is native to New South Wales, rarely cultivated and endangered in the wild
- *Davidsonia pruriens* – the Queensland Davidson's plum, grows to a height of 12 m and is native to tropical north-east Queensland.

1.1.1.5 Quandong (*Santalum acuminatum*)

The quandong (*Santalum acuminatum*, family Santalaceae) is a small tree that grows in semi-arid areas of Australia, producing an edible fruit which can be used in sweet or savoury dishes. The major volatile identified in quandong is methyl benzoate (Ahmed and Johnson 2000).

1.1.1.6 Kakadu plum (*Terminalia ferdinandiana*)

The Kakadu plum (*Terminalia ferdinandiana*, family Combretaceae) is a small tree found in the Northern Territory and Western Australia. The edible Kakadu plum fruit are used in a variety of food products, and also in cosmetics. Kakadu plum is known to have an exceptionally high level of ascorbic acid with 71.3 µmol/g fresh weight (FW) compared to blueberry which has a vitamin C content of 0.076 µmol/g FW (Netzel et al. 2007). Kakadu plum is further value added in the forms of freeze dried powder, liquid extract, frozen whole and pureed Kakadu plum.

1.1.2 Objective

The aim of this study is to track quality changes, specifically bioactivity, of a selection of key commercial native foods during storage.

1.1.3 Methods

One-on-one discussion was carried out and a questionnaire was sent to industry partners to understand current production practices and identify industry needs.

1.1.4 Results

A summary of the production line for each native herb or fruit studied is given below.

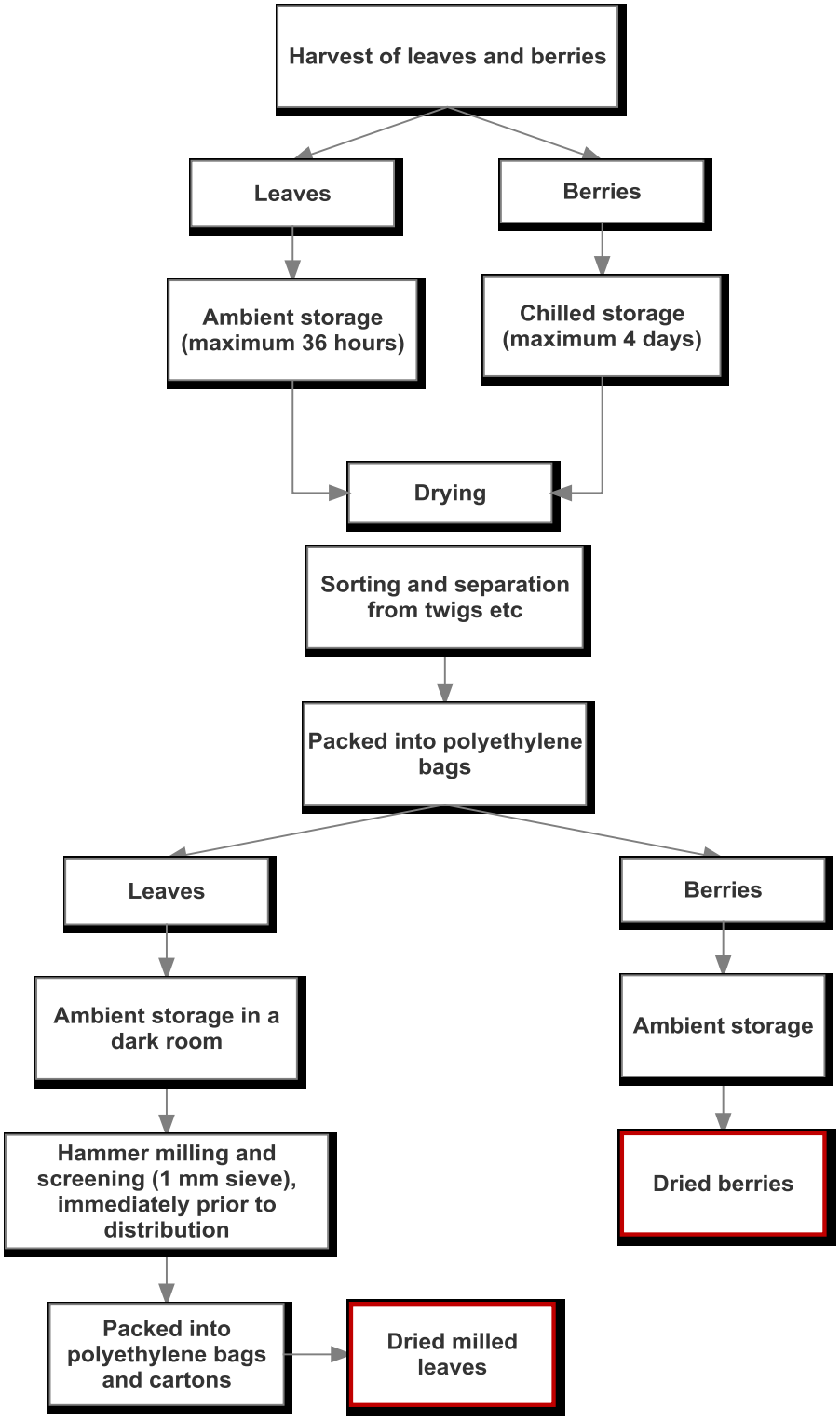


Figure 1.1 Processing flowchart for Tasmanian pepper leaves and berries

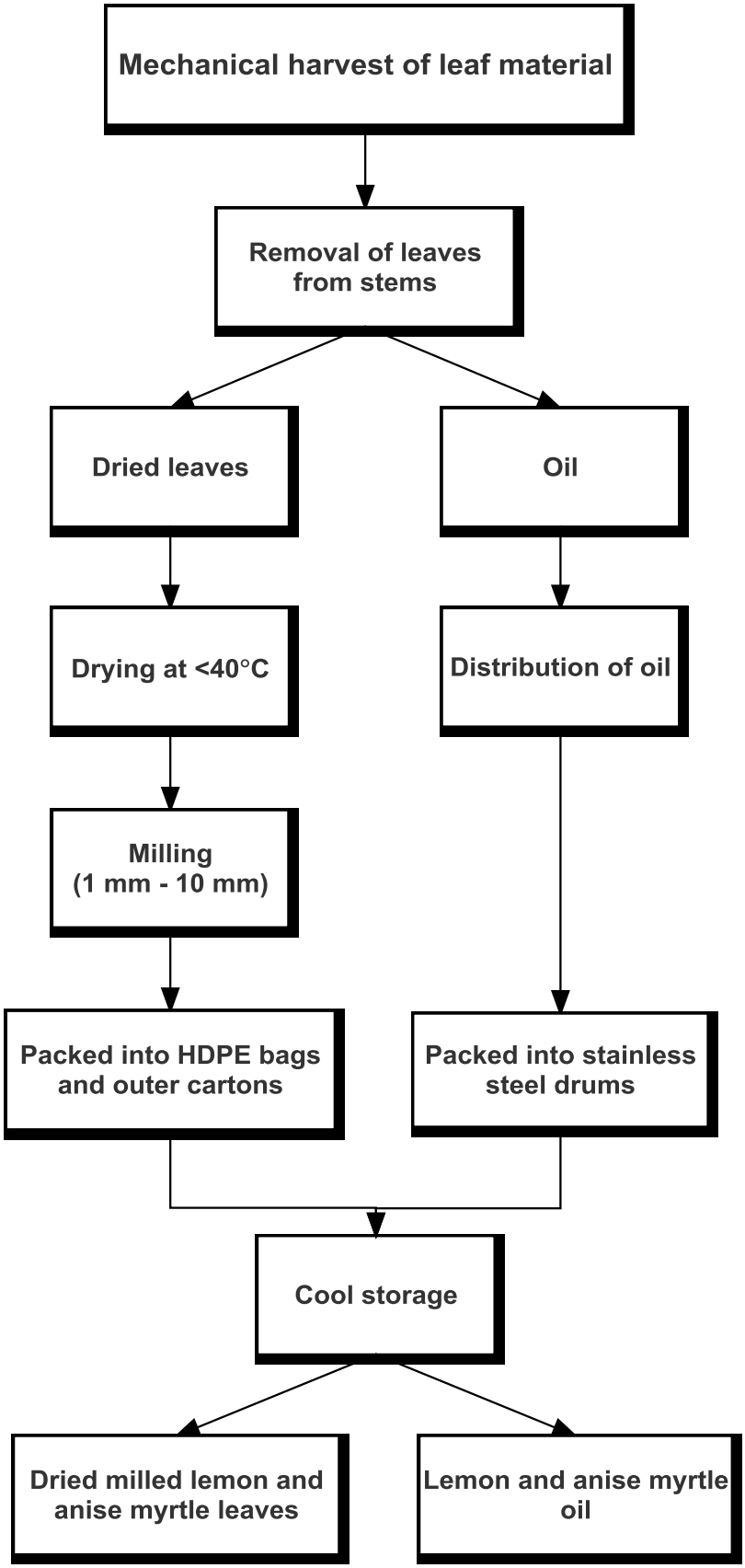


Figure 1.2 Processing flowchart for lemon and anise myrtle

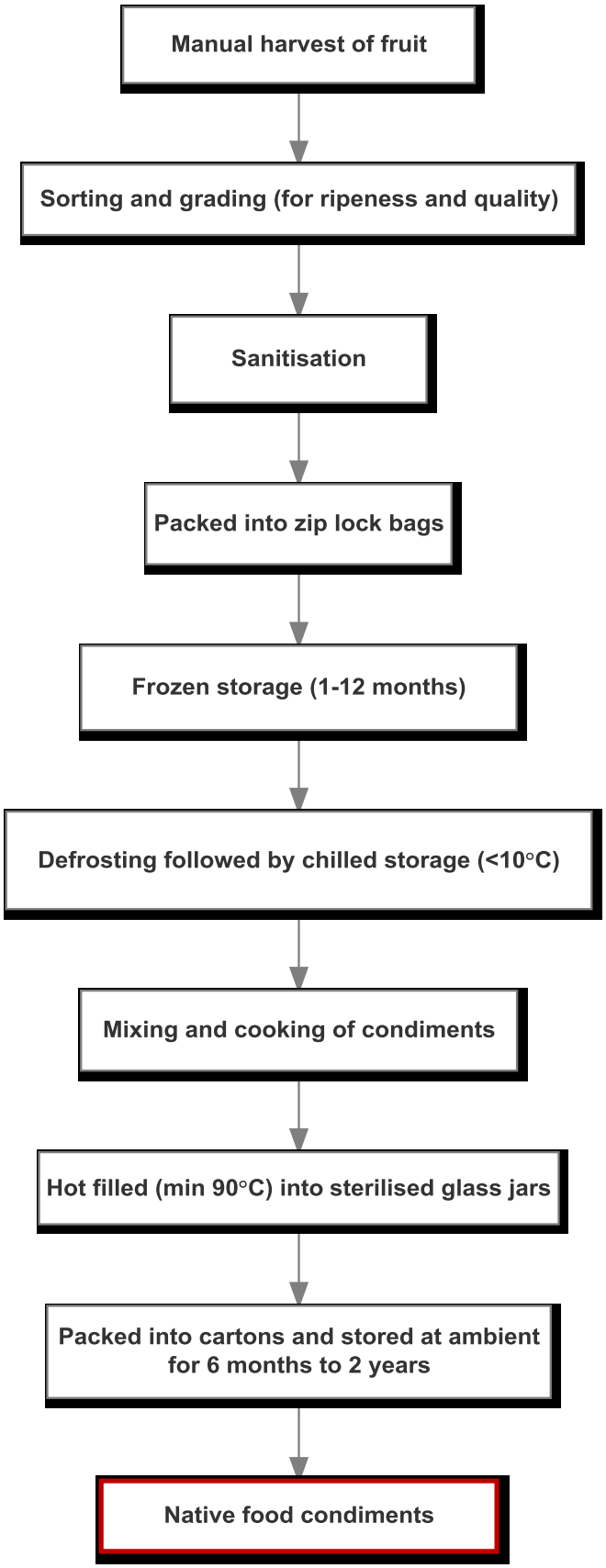


Figure 1.3 Processing flowchart for Davidson's plum

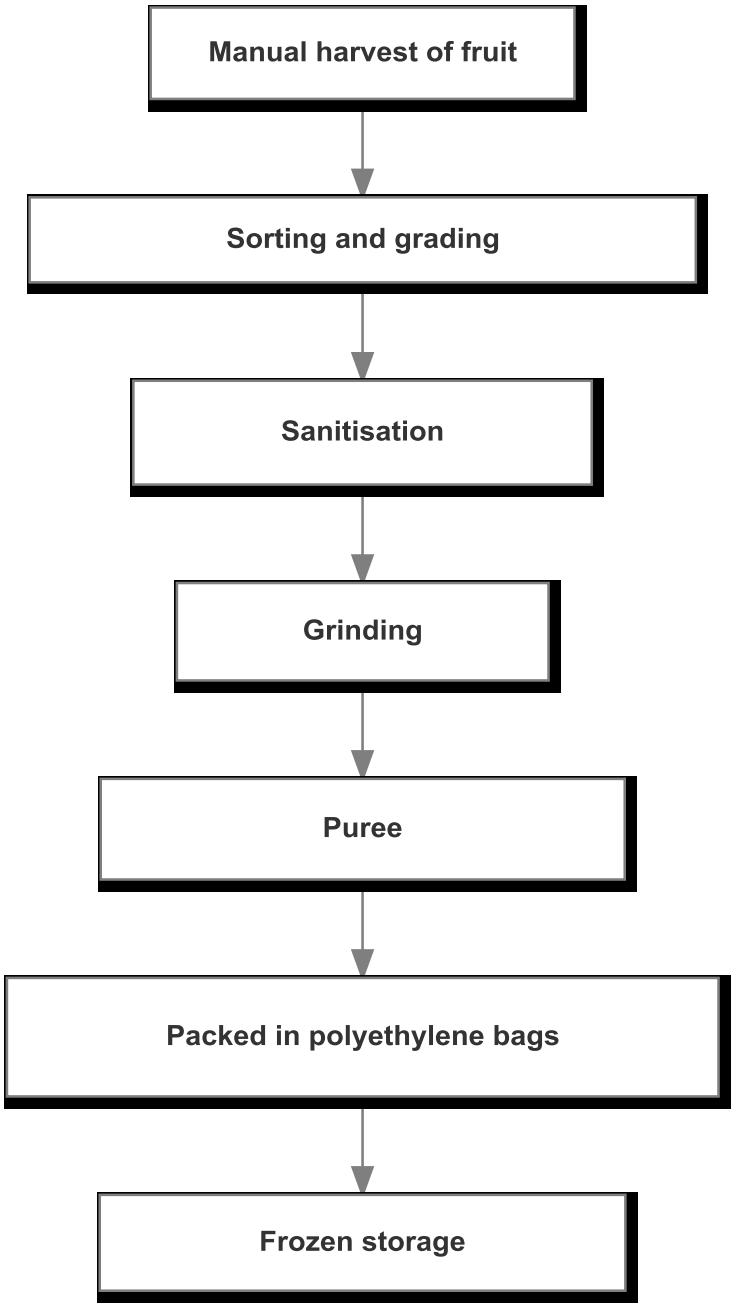


Figure 1.4 Processing flowchart for Kakadu plum

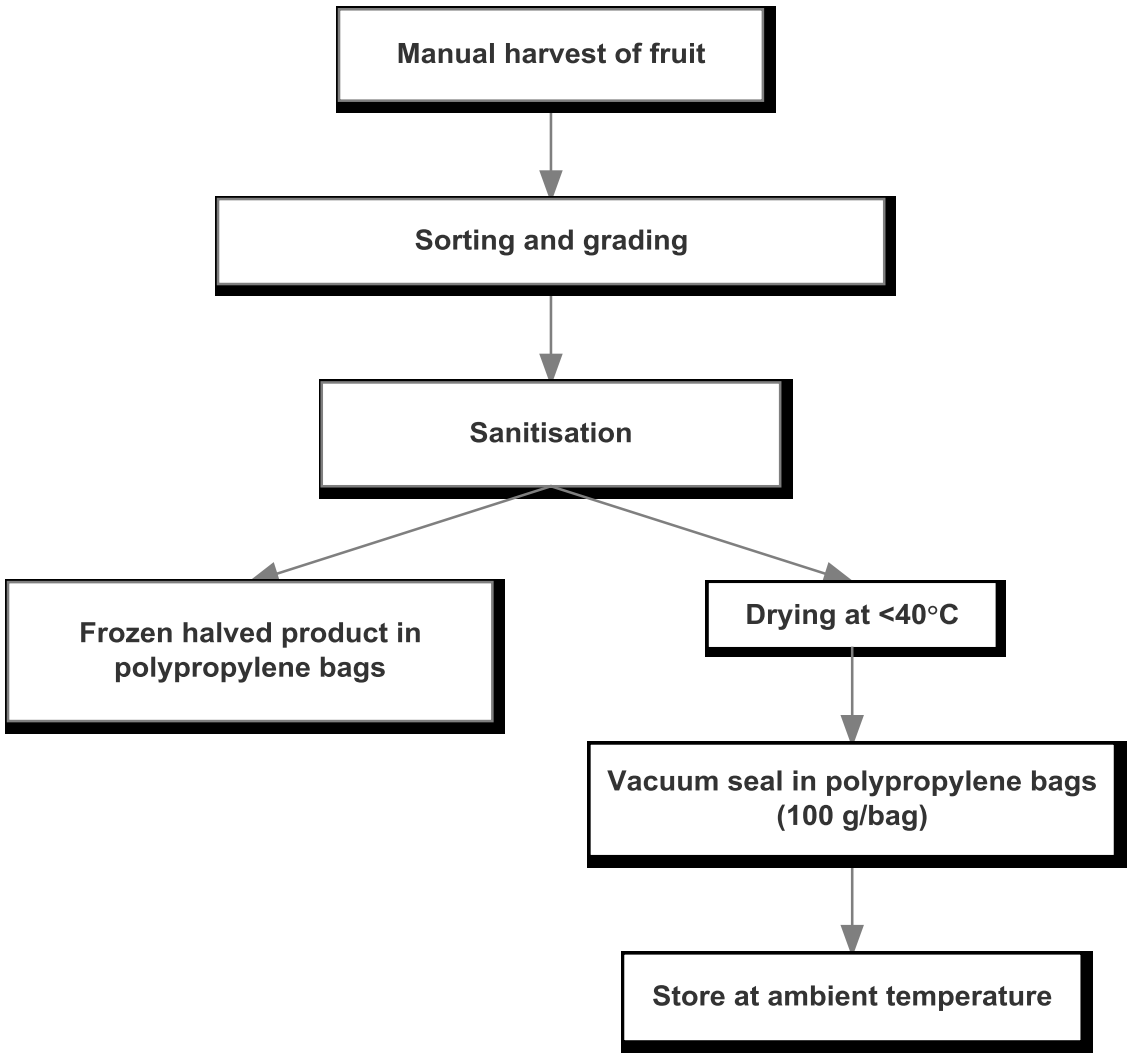


Figure 1.5 Processing flowchart for quandong

Table 1.1 Details of the commercial products used for the first phase of the storage study

Product	Supplier	Quantity and harvest period	Packaging material	Date of packaging and storage
Tasmania pepper leaf	Diemen Pepper Supplies	100 g per package (12 packs); harvest of March/June 2010	High density polyethylene packaging (HDPE Qenos)	26/08/2010
Lemon myrtle	Australian Rainforest Products Pty Ltd	200 g per package (12 packs); harvest of June/July 2010	Polyethylene packaging (Alkathene Qenos)	31/08/2010
Anise myrtle	Australian Rainforest Products Pty Ltd	125 g per package (12 packs); harvest of June/July 2010	Polyethylene packaging (Alkathene Qenos)	31/08/2010
Davidson's plum	Rainforest Bounty Pty Ltd	1 kg per package (12 packs); harvest of August 2010	Polyethylene (Ziploc bags)	27/08/2010
Kakadu plum	Anne Osborne Australian Produce Company	1 kg per package (12 packs); stock from 2008 NT harvest (March–June) stored at –18°C	Polyethylene	24/08/2010
Quandong	Outback Pride	100 g per package (12 packs)	Polypropylene	8/10/2010

1.1.4.1 Industry needs

One-on-one discussion and a questionnaire was sent to the industry partners to understand current production practices and a summary of the improvements identified within each production line is given below:

- Respondents represented the main commercial producers of six key native food species (lemon and anise myrtle, Tasmanian pepper leaf, Kakadu plum, quandong and Davidson's plum).
- They are all involved in multiple aspects of the supply chain, i.e. growing, processing, marketing etc.
- There is no scientific basis used for quality assessments. Currently it is largely a subjective assessment of visual quality, taste and aroma intensity.
- There was agreement between all respondents on the need for future research; that being 1) appropriate parameters for determining acceptable shelf life, 2) optimal storage conditions to maximise shelf life and eating quality and 3) identification of protective, environmentally friendly and convenient packaging.

1.1.4.1.1 Lemon myrtle, anise myrtle and Tasmanian pepper leaf

An immediate and important issue that needs to be addressed for these herbs is packaging. Most of the active components are in the volatile form and therefore packaging with a high-barrier property is urgently required. At the moment the industry is unable to assign a shelf life to the product as there is

no information on the retention of active volatiles when stored at room temperature. In addition there is no information on the loss of active volatiles during drying and milling.

1.1.4.1.2 Davidson's plum

The processors identified the need for value addition of the fresh fruit to an intermediate product that could be utilised as the starting material for further value addition when there is a large harvest. Improved frozen fruit should be similar to fresh fruit for flavour, colour and nutritional value when thawed. The intermediate products identified by the industry included frozen halves and puree with a possible frozen storage life of 18–24 months.

1.1.4.1.3 Quandong

The processors identified drying as an area that needs further improvement. At present, the drying process is not optimised and changes in bioactivity after drying and during storage is an area that needs to be studied.

1.*1.4.1.4 Kakadu plum*

Kakadu plum is further value added as freeze-dried powder, liquid extract, whole fruit and frozen pureed form. Changes in bioactivity of whole Kakadu plum and puree during frozen storage needs to be evaluated.

1.2 Initial chemical and physical composition of native herbs and fruits

1.2.1 Introduction

Food quality in this report will refer to the changes in physicochemical parameters (e.g. colour, volatiles) and bioactivity (e.g. antimicrobial activity, antioxidant activity) during processing, packaging and storage of native fruits and herbs. This study will also assess the changes in bioactive compounds (or phytochemicals) and selection of phytochemical markers to standardise product quality. These bioactive compounds are defined as 'inherent non-nutrient constituents of food plants with anticipated health promoting and beneficial effects when ingested' (Gry 2007).

The physicochemical property of the starting raw material and the processed product supplied by the native food industry is assessed in this section. This information is useful for the development of individual product standards which will provide producers and customers with a reference point for product quality and safety.

Table 1.2 Details of the fresh products used for the first phase of the storage study

Product	Supplier	Quantity and harvest period
Tasmanian pepper leaf	Diemen Pepper Supplies	Fresh whole leaves (harvested in August 2010) and dried whole leaves
Lemon myrtle	Australian Rainforest Products Pty Ltd	Fresh whole leaves, with and without stems (harvested in August 2010) and dried whole leaves
Anise myrtle	Australian Rainforest Products Pty Ltd	Fresh leaves with and without stems (harvested in August 2010)
Davidson's plum	Rainforest Bounty Pty Ltd	*Davidsonia pruriens* harvested in August 2010
	Ooray Orchards	Two types of *Davidsonia pruriens* were sent by client called lowland and highland hairy Davidson's plum, harvested from 16/08/2010 to 23/08/2010
Quandong	Outback Pride	Fresh fruits expected in January 2011

1.2.2 Methods

The overall experimental approach is depicted in Figure 1.6. Methods were developed to determine changes in volatiles and antimicrobial and antioxidant activities and are described briefly here with details for each method found in following sections of the report.

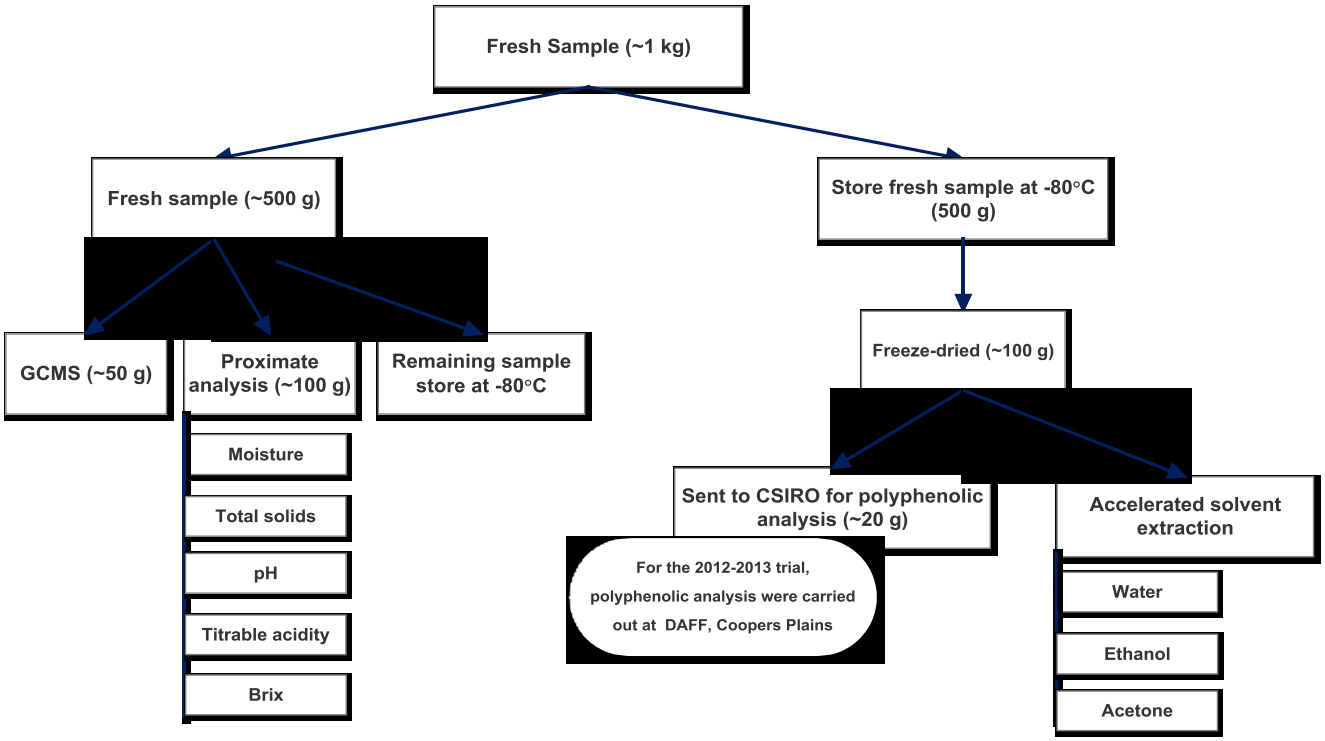


Figure 1.6 Overall approach to experiments

1.2.2.1 Determination of antioxidant activity

1.2.2.1.1 Total phenolic (TP) content

The method for determining total phenolic (TP) content of native herbs and fruits was done using the Folin-Ciocalteu assay (Singleton and Rossi 1965) and is described in detail in Section 3.2.2.3.1.

1.2.2.1.2 Ferric reducing antioxidant power (FRAP) assay

The method for assaying FRAP of native herbs and fruits was according to Benzie and Strain (1996) and is described in detail in Section 3.2.2.3.2.

1.2.2.1.3 Vitamin C

The measurement of vitamin C was carried out according to Konczak et al. (2010a).

1.2.2.2 Determination of antimicrobial activity

The method for assaying antimicrobial activity of native herbs and fruits is described in detail in Section 3.2.2.4.2.

1.2.2.2.2 Microtitre assay method

Antimicrobial activity was determined using a microtitre assay method (Sultanbawa et al. 2009) and described in Section 3.2.2.4.2.

1.2.2.3 Quantification of volatile compounds

1.2.2.3.1 Extraction of volatile compounds

The method for volatile extraction from native food samples is described in detail in Section 2.1.3.5.

1.2.2.3.2 Gas chromatography mass spectrometry (GCMS)

The method for GCMS analysis for quantification of major volatile compounds in native herb samples is described in detail in Section 2.1.3.6.

1.2.2.4 Measurement of colour

A Minolta Chroma Meter CR 400 (Konica Minolta Sensing, Inc, Japan) was used to measure colour (Figure 1.7) in the stored herbs (lemon myrtle, anise myrtle and Tasmanian pepper leaf). The following procedure was performed:

- 5 g of the stored herb sample was placed in a polypropylene Petri dish (50 mm i.d. x 12 mm)
- colour of the sample was measured using a Minolta Chroma Meter CR 400
- three replicates of the colour measurement were made for each sample and reported data were the means of the three measurements.

Figure 1.7 Steps involved in the measurement of colour

1.2.3 Results

1.2.3.1 Results from chemical analysis of native fruits and herbs

Table 1.3 Summary of the chemical analysis in native fruits

Sample	pH	TA*	Moisture	Total solids	Brix	Water activity	Colour
Highland Qld Davidson's plum (Ooray Orchards; Kris Kupsch)	3.10	4.82	90.87%	9.13%	6.5	0.991	L=30.71, a=35.54, b=35.81
Lowland Qld Davidson's plum (Ooray Orchards; Kris Kupsch)	2.76	5.14	92.50%	7.49%	5	0.993	-
Fresh Qld Davidson's plum (Rainforest Bounty Pty Ltd; Cairns)	2.63	4.58	92.14%	7.86%	4.5	0.994	L=31.52,a=35.73, b=14.396
Qld Davidson's plum frozen (Rainforest Bounty Pty Ltd; by client)	2.82	4.62	92.50%	7.49%	4.7	0.992	L=27.55, a=34.68, b=10.92
Qld Davidson's plum cooked and pureed (Rainforest Bounty Pty Ltd)	-	-	80.21%	19.79%	-	-	-
Kakadu plum	-	-	84.56%	15.44%	-	-	-
Fresh quandong halves	3.74	2.76	79.22%	20.78%	20.31%	0.9791	-
Dried quandong halves commercial	-	-	17.86%	82.14%	-	-	-

*Titrable acidity (TA) was expressed as malic acid.

Table 1.4 Summary of the chemical analysis in native herbs

Sample	Moisture
Lemon myrtle fresh leaves (with stem)	60.04%
Lemon myrtle fresh leaves (without stem)	55.75%
Lemon myrtle dried whole leaves	11.30%
Lemon myrtle leaves dried, milled	8.89%
Anise myrtle fresh leaves (with stem)	53.55%
Anise myrtle fresh leaves (without stem)	55.98%
Anise myrtle leaves dried, milled	6.25%
Tasmanian pepper fresh leaves	65.00%
Tasmanian pepper dried leaves	12.29%
Tasmanian pepper leaves dried, milled	11.15%

All dried samples had a moisture content less than 12 %.

1.2.3.2 Results from microbiological assessment of raw and finished native food samples

Table1.5 Microbiological qualities of raw and finished products

Product description		CFU*/gram		
		Standard plate count	Yeast	Mould
Quandong	Fresh	2.10E+05	1.00E+04	1.30E+04
Davidson's plum	Highland, NSW	3.00E+01	<100	1.00E+02
	Lowland, NSW	1.00E+01	<100	<100
	Lowland, Cairns	1.20E+02	1.00E+02	2.00E+02
	Pureed fruit, Cairns	3.30E+03	3.20E+03	2.00E+02
Kakadu plum	Whole (frozen 2008 batch)	8.10E+03	<100	1.00E+02
	Minced (frozen 2008 batch)	1.40E+04	<100	<100
Tasmanian pepper leaf	Fresh unmilled leaves	7.50E+02	<100	2.00E+02
	Dried leaves	2.00E+02	<100	2.00E+02
	Commercially milled	1.10E+02	<100	3.00E+02
Lemon myrtle	Fresh leaves with stems	1.30E+05	>25000	8.40E+03
	Fresh leaves without stem	7.40E+04	1.00E+03	2.90E+03
	Dried whole leaves	1.50E+04	7.00E+02	3.00E+03
	Commercially milled (1.6 mm)	1.00E+04	<100	4.80E+03
Anise myrtle	Fresh leaves with stems	8.80E+04	>25000	4.20E+03
	Fresh leaves without stem	>250000	>25000	3.30E+03
	Commercially milled (1.6 mm)	1.20E+04	3.00E+02	1.60E+03

*CFU – colony forming units.

It is recommended that an effective sanitation step be included for the raw materials (fruits and herbs) that have high loads of microorganisms.

2. Effect of processing, packaging and storage on the quality and bioactivity of native herbs – aroma volatiles, non-volatile bioactive compounds and colour

2.1 Quality changes in native herbs during storage at room temperature for a period of six months

2.1.1 Introduction

Packaging is an immediate and important issue that needs to be addressed for the processors of native herbs (in particular lemon myrtle, anise myrtle and Tasmanian pepper leaf).

Low molecular weight organic volatile compounds are important constituents of food products as they influence the flavor of the food products (Mohney et al. 1988). Moreover, many of the active components are in the volatile form. Loss of quality in terms of aroma, taste, colour and texture is well known in dried products (Nijhuis et al. 1998). Deterioration of food products takes place progressively during storage and loss of the freshness parameters such as aroma are immediately recognised by consumers (Rizzo and Muratore 2009) as the human nose can detect olfactory sensation even at a very low concentration (Quezada-Gallo et al. 2000). Therefore the maintenance of aroma of food products during storage and the improvement of quality retention in dried products via altered processing, storage conditions and/or pretreatments, has been a major research area in recent years (Cohen and Yang 1995).

2.1.1.1 Current challenges faced by the native food industry

At the moment the industry is unable to give a shelf life to the product as there is no information on the retention of active volatiles when stored at room temperature. In addition there is no information on the loss of active volatiles during drying and milling. Currently, the Australian native food industry faces a challenge in retaining the aroma and flavours in dried, milled leaves as there are significant losses of volatile aroma compounds after 1 month of storage in high density polyethylene (as reported by the industry). Moreover, certain volatile compounds such as citral migrate into and cause disintegration of the packaging material.

Packaging materials for food and agricultural industries is a rapidly growing and important area. Packaging fulfils a number of purposes from preventing contamination during distribution, through to preserving product integrity and maintaining the desired flavour profile of the product (Risch 2000). Packaging materials are crucial factors in maintaining the quality and stability of food products. Migration of water, oxygen and aroma can take place through packaging materials which can change the quality of the food product. Loss of aroma leads to a decrease in the flavour intensity or modification of the aroma profile of the food. The food industry therefore tries to prevent or control the transfer of micro molecules between food products and the surrounding media by incorporating an appropriate packaging material (Mohney et al. 1988; Quezada-Gallo et al. 2000).

Although a few studies have reported on the major volatiles present in Australian native plants (Brophy et al. 1995; Southwell et al. 1996), the effect of packaging material on the stored native herbs of Australia has not been studied.

2.1.2 Objective

In this study, we investigated the effectiveness of two packaging materials with a high-barrier property in preventing the loss of aroma volatiles in three native Australian herbs, stored at room temperature for six months.

2.1.3 Materials and methods

2.1.3.1 Plant materials

Three native Australian herb samples selected by Australian Native Food Industry Ltd. (ANFIL), were analysed in this study. Samples for lemon myrtle (*Backhousia citriodora* F. Muell) and anise myrtle (*Syzygium anisatum* Vickery, Craven & Biffen) were obtained from Australian Rainforest Products (New South Wales, Australia). Leaf samples of Tasmanian pepper (*Tasmannia lanceolata)* were supplied by Diemen Pepper (Tasmania, Australia).

The herb samples were supplied in the commercially available dried, milled form. The suppliers also provided fresh samples (Figure 2.1) for each of the herbs, which included: fresh whole leaves with stem (for lemon myrtle), fresh whole leaves separated from stem (for lemon myrtle, anise myrtle and Tasmanian pepper), and whole dried leaves before milling (for lemon myrtle and Tasmanian pepper). These samples are referred to as fresh, month = 0, samples herein.

2.1.3.2 Chemicals

A preliminary gas chromatography mass spectrometry (GCMS) analysis was carried out to identify the major volatiles in each of the selected plant samples which included neral and geranial (Z- and E-isomers of citral respectively) in lemon myrtle, estragole (1-allyl-4-methoxybenzene) and anethol (1-methoxy-4-(1-propenyl)benzene) in anise myrtle, eucalyptol (1,3,3-trimethyl- 2-oxabicyclo[2,2,2] octane) and eugenol (4-allyl-2-methoxyphenol) in Tasmanian pepper. All these reagents were purchased from Sigma-Aldrich, Australia. Hexadecane (Sigma-Aldrich, Australia), was used as an internal standard. All the reagents and solvents used were purchased at the analytical quality, and used without further purification. All aqueous solutions were prepared using deionised water. Stock standard solutions were stored at –20ºC.

2.1.3.3 Conditions for storage trial

For the storage experiment, dried, milled leaves (~200 g per bag for lemon myrtle and Tasmanian pepper leaves, ~125 g per bag for anise myrtle) were packed in bags made from one of three different types of packaging materials (Table 2.1). Figure 2.2 represents the processing and sampling plan of the native herbs for the 6-month storage. The packaging was done in duplicate for reproducibility. Filled bags were sealed under vacuum using a Multivac Chamber machine C 500 (Multivac Sepp Haggenmüller GmbH & Co. KG, Germany). The storage trial was conducted at 22°C for six months. All the bags with herb samples were placed in cardboard boxes for the duration of the trial (as per standard commercial practice). Sampling for analysis occurred at time zero (before packaging, here after referred as month = 0) and on each month for a total of six months, labelled as month 1, 2, 3, 4, 5 and 6. At each time point, two bags of each packaging type were opened for sampling, which were further sub-sampled into two replicates per bag (resulting in four replicates overall at each time point). Once sampled, the herbs were immediately subjected to volatile extraction as described below and the extracts were stored at –85°C before volatile analysis.

Table 2.1 Packaging material gas and moisture barrier properties

Material (layer thickness in µm)	Transmission rate	
	Water [g/m^2/24 h]	Oxygen [cm^3/m^2/24 h]
LDPE (80)	10–20 at 38°C (RH 90%)	6500–8500 at 38°C (RH 90%)
HDPE (80)	7–10 at 38°C (RH 90%)	1600–2000 at 38°C (RH 90%)
PVDC coated PET (12)/ CPP (20)	0.5–1 at 38°C (RH 90%)	2–4 at 23°C (RH 50%)
PET (12)/ PET (12)/ Foil (9)/ LLDPE (65)	0.25 at 37°C (RH 98%)	0.02 at 25°C (RH 95%)

HDPE – high density polyethylene; PET – polyethylene terephthalate; PVDC – polyvinylidene chloride; CPP – casted polypropylene; LLDPE – linear low-density polyethylene; RH – relative humidity.

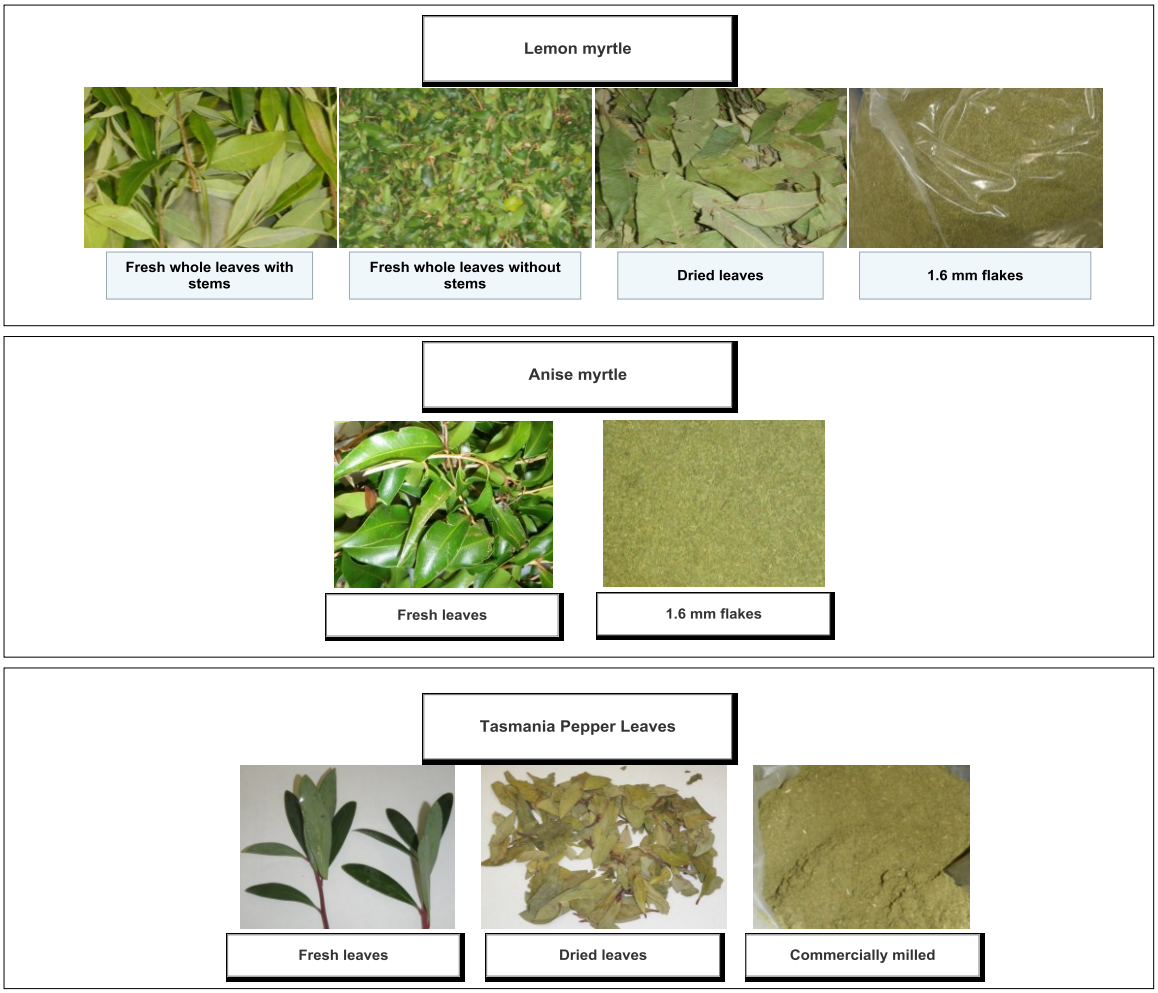
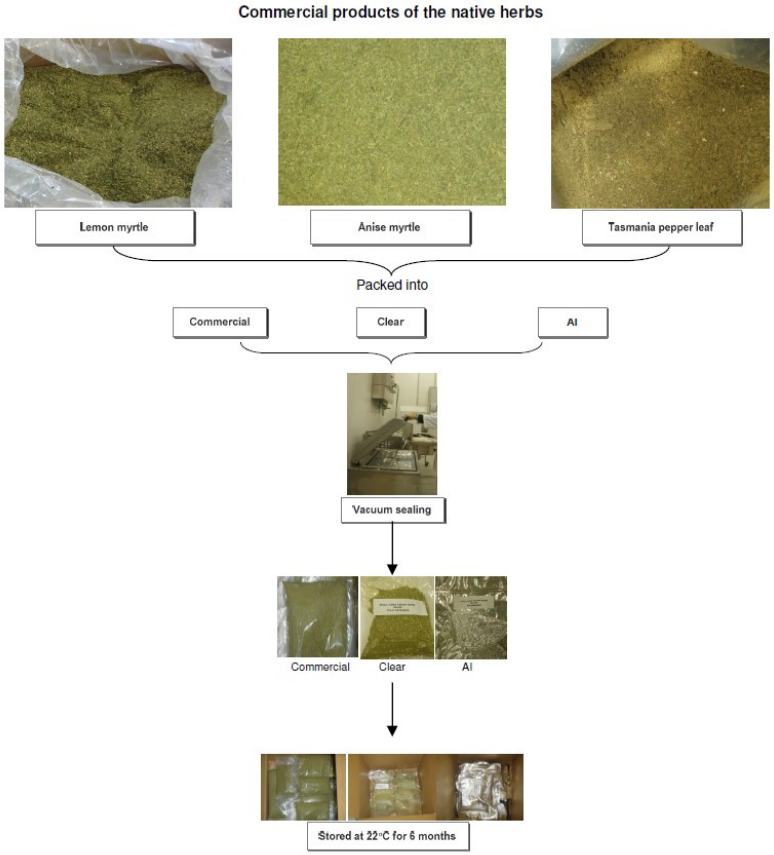


Figure 2.1 Samples used for collecting baseline data on proximate analysis and bioactivity and proof-of-concept studies on processing and packaging for native herbs



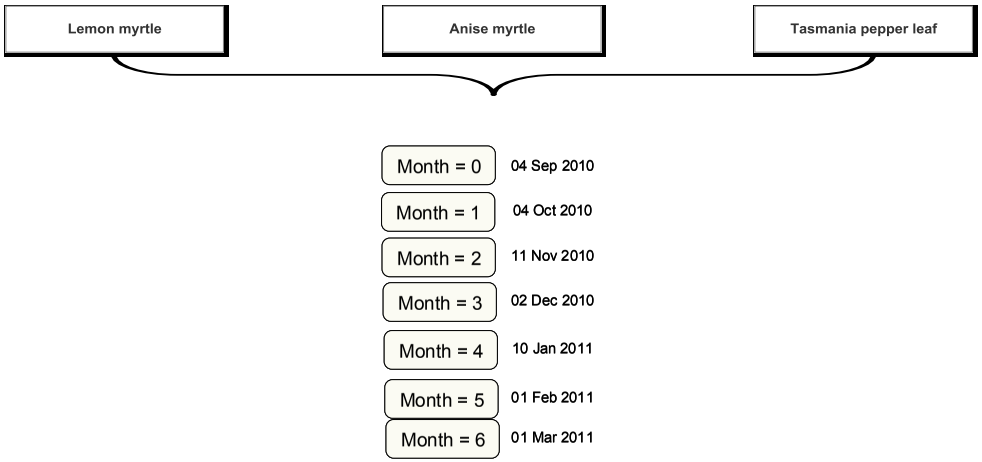


Figure 2.2 Processing flowchart and sampling plan for the 6-month storage of native herbs

2.1.3.4 Cryomilling of fresh samples

All the fresh samples of native herbs (labelled as month = 0) were cryogenically milled using a Mixer Mill MM 200 (Retsch, Germany). Approximately 1 g of leaf material was weighed into stainless steel cells, sealed and immediately immersed in liquid N_2 for a minute. The cells were than inserted into the cryomill and ground for 30 sec at a speed of 300 rpm/sec. These samples (month = 0) provided the baseline information regarding the concentration of volatiles in the material at the beginning of the storage trial.

2.1.3.5 Extraction of volatiles from herb samples

Liquid-liquid extraction (LLE) was employed for extracting the volatile compounds from the native herb samples as it has been reported as an adequate extraction technique for simple matrices like fruits and vegetables (Plutowska and Wardencki 2007). Figure 2.3 shows the schematic representation of the protocol involved in LLE. All dried, pre-milled native herb samples were directly subjected to LLE. For LLE, 0.5 g of prepared herb sample was weighed immediately in a 25 mL clear glass vial. The extraction solvent used was pentane-diethyl ether (2:1 ratio) and 5 mL of this extraction solution was added to each of the samples along with 5 mL of deionised water and 50 µL of the internal standard (hexadecane, concentration 1000 mg/L) was also added. The samples were shaken for 5 min in a vortex mixer and allowed to sit at room temperature until two clear layers of solvent could be seen. The top organic layer was carefully transferred to new amber vials. A small volume of magnesium sulphate was added to each of the vials to get rid of any aqueous phase left behind. The liquid extract in theses vials were blown down to 1 ml under continuous and gentle N_2 flow.

2.1.3.6 Gas chromatography mass spectrometry (GCMS)

Samples were analysed with a 6890N gas chromatograph equipped with an MSD 5975 mass spectrometric detector (Agilent Technologies, Palo Alto, CA, USA). The gas chromatograph was fitted with a DB-WAX column (J&W Science, 253.00 µm i.d., length 30.0 m, film thickness 0.25 µm). Helium (BOC gasses, ultra high purity), was used as a carrier gas at a linear velocity of 56 cm/min and at a flow rate of 2.4 mL/min.

The native herb samples prepared by LLE were analysed with the help of liquid injection. For the liquid injections, the samples were allowed to come back to room temperature. From the concentrated extracts, 100 µL was added to 2 mL clear glass vials with the help of a glass syringe, and topped up with dicholoro methane (as organic compounds for GCMS must be in an organic solution for injection into the gas chromatograph). The oven temperature was started at 50°C for 1 min then increased at 20°C per min to 240°C and held for 4 min. The extracts were injected with the help of a 10 µL automated MPS liquid injection syringe (GERSTEL, Germany). The fill volume of the syringe was 10 µL and injection volume was 3 µL at 5 µL/s speed. The syringe had two wash cycles in 2-propanol and the extraction solvent in between samples. Solvent delay was 3 min.

The mass spectrometry ion source was kept at 250°C. Mass spectra in the electron impact (EI) mode were generated at 70 eV and ran at specific ion monitoring (SIM) mode. Data analysis was carried out with the help of the MSD ChemStation Data Analysis software (Agilent Technologies, Palo Alto, CA, USA). Compounds were identified by comparison with the mass spectra library (NIST 05).

All target volatiles were analysed in the selected ion monitoring (SIM) mode in mass spectrometry. Peak identification was achieved by comparing the retention times and matching the area ratios of three characteristic ions of each compound. The characteristic ions for each target volatile in SIM mode are listed in Table 2.2. Ions used for SIM and quantitation were m/z 57 for hexadecane.

Table 2.2 GCMS retention times, selected ions, calibration range, and linearity of target volatile compounds

Compound	Ions selected (m/z)[a]	Relative retention time (min)	Calibration range (mg/l)	Linearity (r^2)
Eucalyptol	81, 108, 139	4.260	0.625–50	0.941
Estragole	133, 147,148	7.619	0.625–50	0.958
Neral	69, 94, 109	7.700	0.625–50	0.986
Geranial	69, 109, 137	8.008	0.625–50	0.983
Anethol	133, 147, 148	8.610	0.625–50	0.975
Eugenol	137, 149, 164	10.482	0.625–50	0.807

[a] Quantitative ions are underlined.

2.1.3.7 Quantitative analysis

Calibration graphs were obtained by injecting five standard solutions containing a mixture of the target compounds eucalyptol, estragole, neral, geranial, anethol and eugenol in concentrations of 0.0, 0.625, 12.5, 25.0 and 50.0 mg/L respectively and a constant concentration of the internal standard hexadecane of 1000 mg/L. The calibration curves were obtained by plotting the ratio of the peak area of the target compounds to the peak area of the internal standard against the corresponding concentration. Five linear calibration graphs were obtained. The calibration range and linearity of the curves are summarised in Table 2.2.

2.1.3.8 Statistical analysis

All statistical analysis was performed using the JMP statistical package (JMP 6, SAS Institute). The analysis of variance (ANOVA) with respect to each analytical compound detected in the herbs; the effect of storage and the type of packaging material were assessed. Pair-wise comparison was determined with the help of a student's t test.

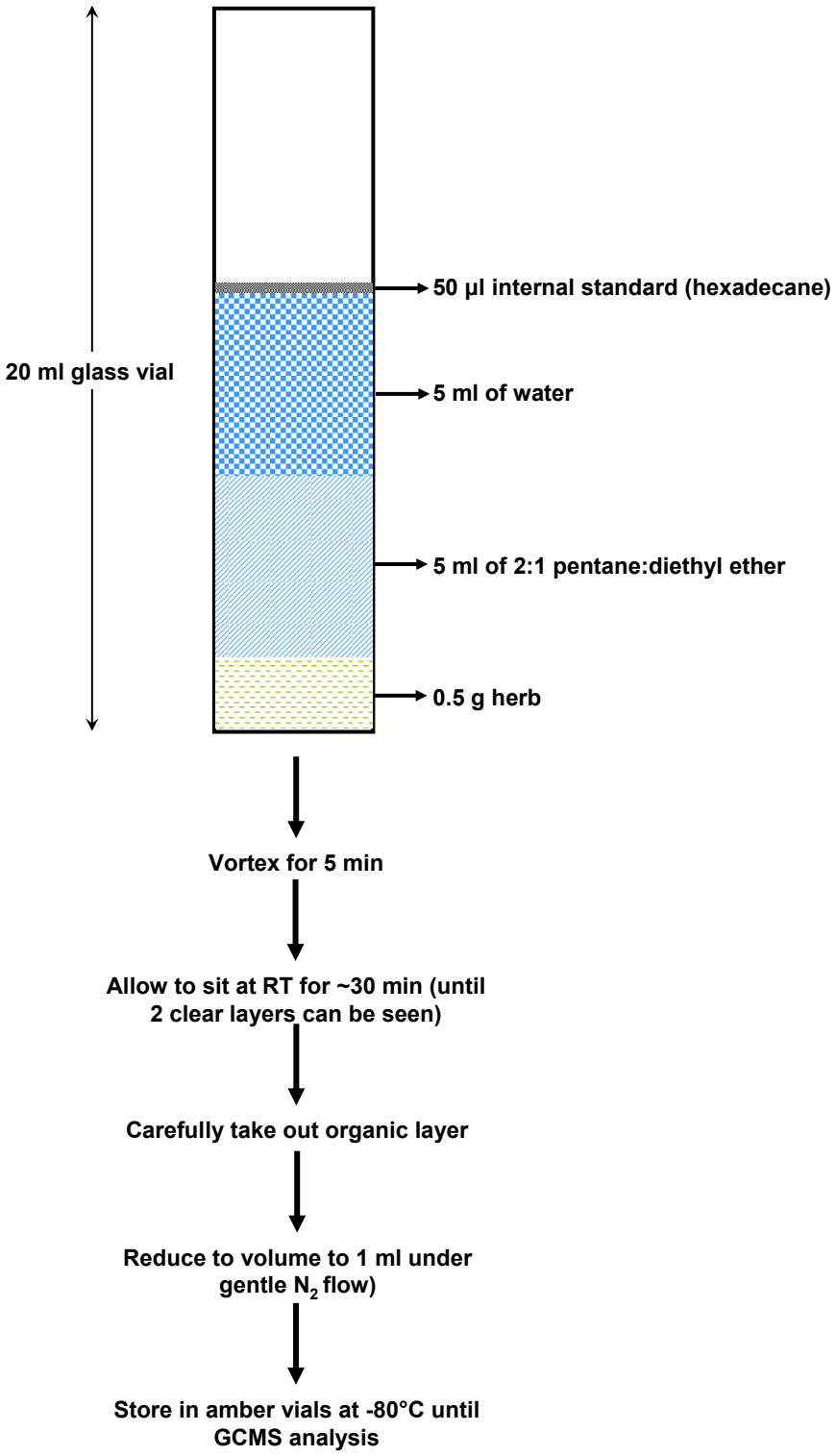


Figure 2.3 Schematic representation of liquid-liquid extraction of volatiles from native herbs

2.1.4 Results and discussion

2.1.4.1 Concentration of major volatiles in month = 0 samples

Interesting comparisons could be made between the fresh samples of lemon myrtle and anise myrtle with and without leaves. While similar levels and ratios of the two major volatile components were found in lemon myrtle with and without stems (Figure 2.4 [A]), quite different ratios between the two major components were found for anise myrtle with and without stems (Figure 2.4 [B]). The fresh leaves of anise myrtle appear to be much richer in anethol, the character impact aroma component of anise, and have a reduced level of estragole compared to the sample containing both stems and leaves. A sample of Tasmanian pepper leaf without stems was not available for comparison.

In lemon myrtle and Tasmanian pepper leaf, the concentration of major volatiles was highest in the dried leaves before milling, (Figure 2.4 [A] and [C]). By comparison, Braja et al. (1989) reported a three-fold increase in linalool concentration in coriander (*Coriander sativum)* seeds upon drying. After milling the concentrations of major volatiles for lemon myrtle and Tasmanian pepper leaf decreased, indicating herb quality loss caused by the process of milling. This reduction in volatiles after processing (milling) should be investigated in future work with the view to reduce the impact of processing on product quality. For anise myrtle there were no samples available of dried herb pre-milling. When compared to the fresh herb without stems, both estragole and anethol were observed to increase in concentration after drying and milling (Figure 2.4 [B]).

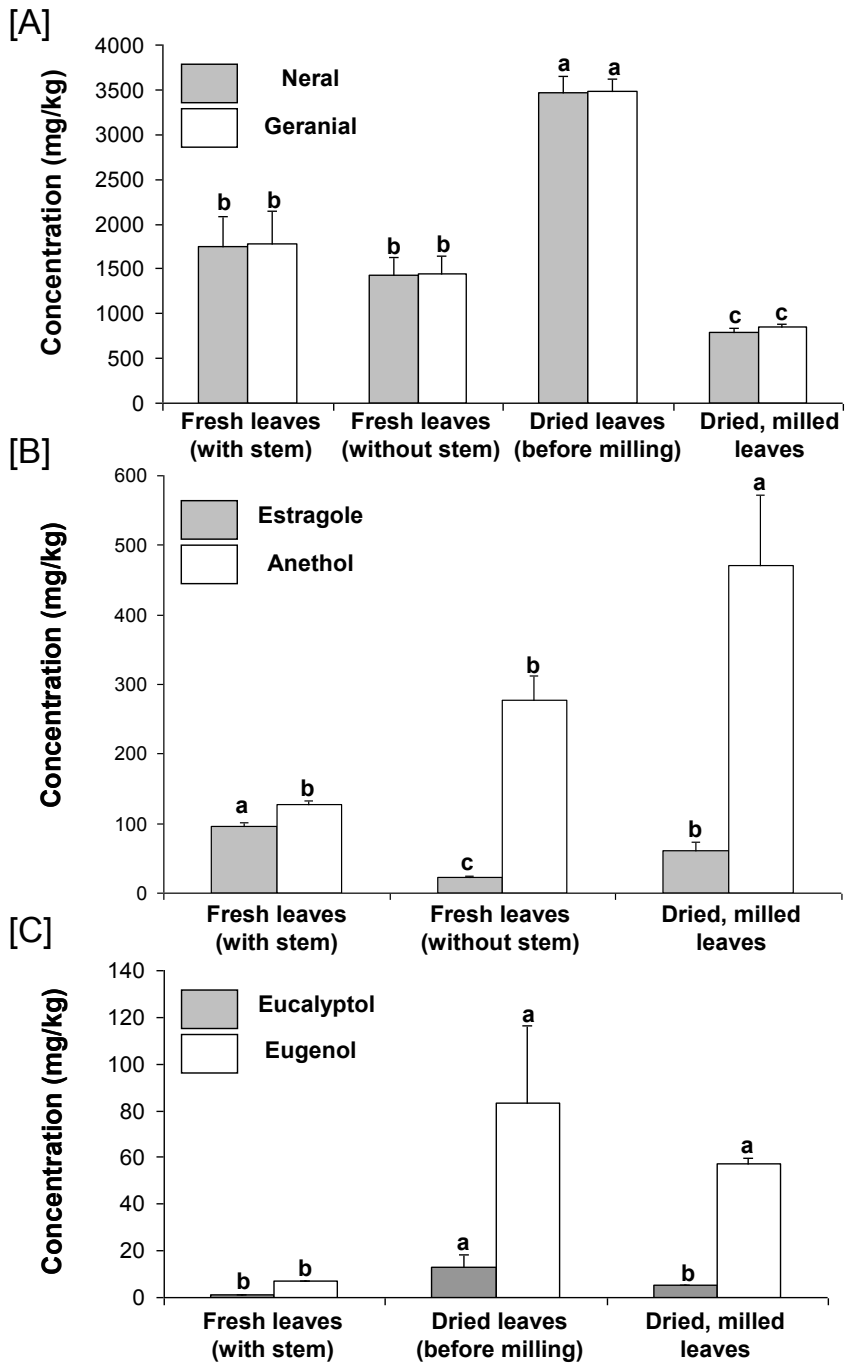


Figure 2.4 Concentrations of major volatiles (mg/kg) present in native herb samples at the beginning of the storage trial (month = 0) for [A] neral and geranial in lemon myrtle, [B] estragole and anetholin anise myrtle and [C] eucalyptol and eugenol in Tasmanian pepper leaves. (n=4). Average concentrations analysed with a student's t test. Different letters (i.e. a,b,c) across sample types for each volatile denote significant differences between mean concentrations according to a Tukey-Kramer HSD.

2.1.4.2 Concentration of major volatiles in commercial samples during storage

As shown in Table 2.3, the average concentration (n = 4) of major volatiles found in samples of dried milled leaves prior to packaging and storage were: neral (795 mg/kg) and geranial (844 mg/kg) in lemon myrtle; estragole (60 mg/kg) and anethol (471 mg/kg) in anise myrtle; and eucalyptol (5.2 mg/kg) and eugenol (57 mg/kg) in Tasmanian pepper leaves.

For herbs, the most important factors in preserving quality are the water and oxygen transmission rates of packaging material. The recommended transmission rates for water and oxygen in packaging material for herbs are <1 g/m^2/day, (38°C, 90% RH) and <1 cm^3/m^2/day respectively (Anonymous 2012). The two high-barrier packaging materials selected for inclusion in this study were PET/CPP and PET/PET/Foil/PE based on their transmission rates for water and oxygen (refer to Table 2.1). The standard HDPE packaging used commercially for lemon myrtle, anise myrtle, and LDPE packaging used commercially for Tasmanian pepper berry, were also included in the storage trial for comparison.

Samples packed in the PVDC-coated PET/CPP and PET/PET/Foil/PE materials showed a substantial and significant retention of the major volatiles compared to those packed in the commercial LDPE and HDPE packages (Table 2.3) for all three herbs studied. The most rapid decline in the concentration of key volatiles over the storage period was observed in samples stored in the LDPE (or HDPE) packing material (Figures 2.5 to 2.7). This can be explained by the fact that LDPE and HDPE materials have a relatively high gas permeability rate (Table 2.1) which allows the volatiles to migrate out of the sample matrix. By comparison, the higher barrier properties of the PVDC coated PET/CPP and the PET/PET/Foil/PE materials have very low water and oxygen permeability (Table 2.1) which prevents the loss of volatiles. For all three herbs, a gradual decline in the concentration of major volatiles was observed with increased time in storage irrespective of the packaging type

Table 2.3 Concentration of volatiles (mg/kg)

	Volatile	Packaging type	Storage time (months)							
			0	1	2	3	4	5	6	LSD
Lemon myrtle	Neral	LDPE	795	627 a	548 c	319 d	275 e	221 f	182 f	42
		PVDC coated PET/ CPP		930 a	860 b	766 d	816 bc	644 e	578 f	49
		PET/PET/Foil/PE		1142 a	1089 a	963 b	1175 a	1106 a	1082 ab	142
	Geranial	LDPE	844	683 b	618 c	367 d	317 e	257 f	215 f	48
		PVDC coated PET/ CPP		941 a	868 b	766 d	814 cd	650 e	588 f	50
		PET/PET/Foil/PE		1173 a	1098 a	961 bc	1164 a	1109 a	1087 ab	148
Anise myrtle	Estragole	LDPE	60	48 b	26 c	15 d	9 de	7 e	4 e	8
		PVDC coated PET/ CPP		115 a	105 ab	92 b	114 a	107 ab	97 b	18
		PET/PET/Foil/PE		110 a	105 a	96 a	99 a	102 a	80 b	16
	Anethol	LDPE	471	525 a	376 b	295 c	187 d	153 de	93 e	68
		PVDC coated PET/ CPP		800 a	726 abc	639 c	750 ab	728 abc	668 bc	119
		PET/PET/Foil/PE		762 a	727 a	660 a	676 a	716 a	561 b	112
Tasmanian pepper leaf	Eucalyptol	HDPE	5.2	1.6 b	0.7 c	0.5 d	0.4 d	0.2 e	0.2 e	0.1
		PVDC coated PET/ CPP		9 a	8 ab	7 b	5 c	4 c	5 c	1.2
		PET/PET/Foil/PE		7.4 a	7.5	7.6 a	8.3 a	6.4 ab	7.5 a	2.3
	Eugenol	HDPE	57	69 a	57 b	52 bc	46 cd	42 d	32 e	7
		PVDC coated PET/ CPP		85 a	68 b	60 b	40 c	41 c	45 c	11
		PET/PET/Foil/PE		78 a	69 b	67 b	70 b	63 bc	65 bc	9

Different letters within a row (i.e. a, b, c) denote significant differences between means according to a Tukey-Kramer HSD.

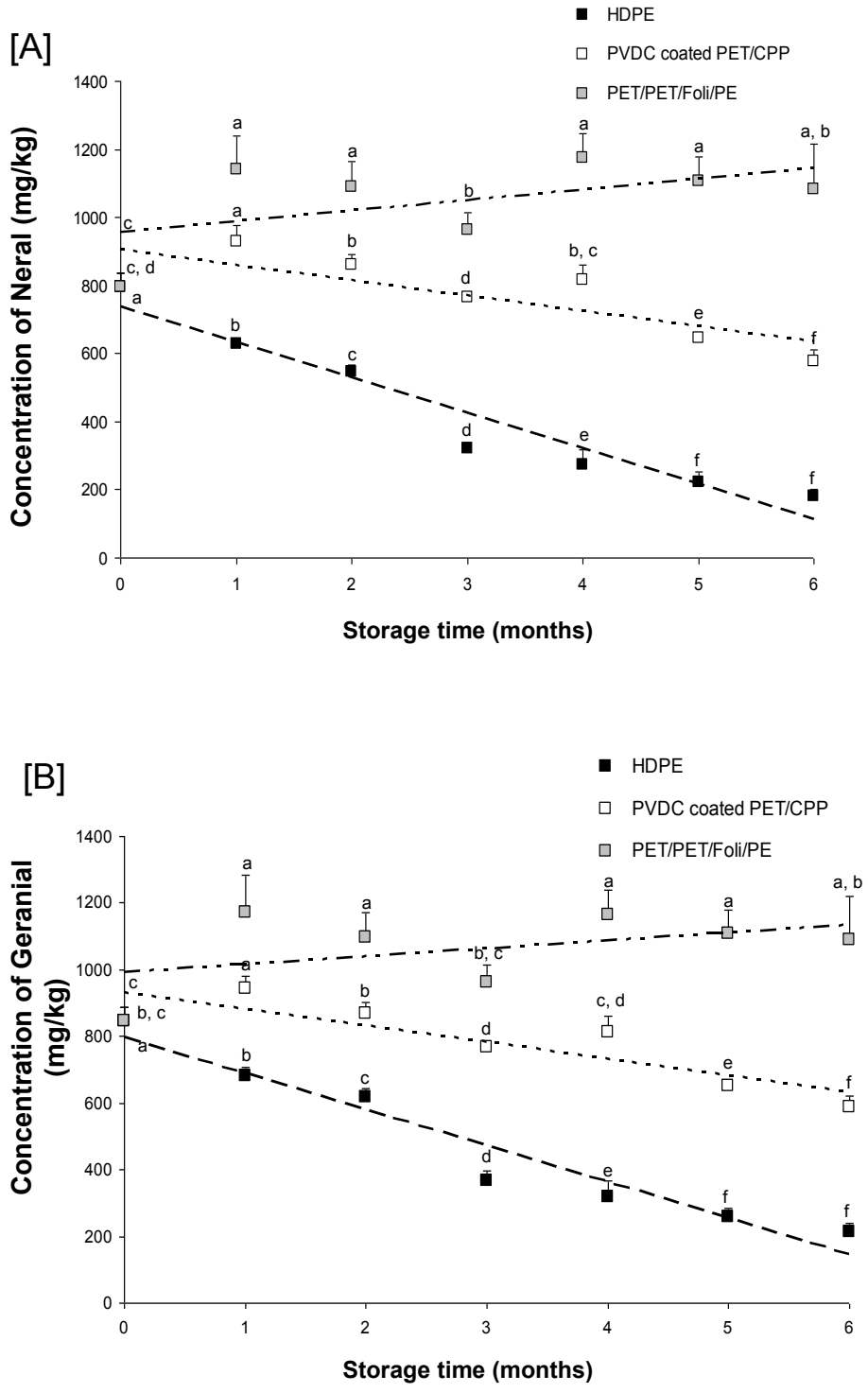


Figure 2.5 Change in concentration of volatiles (mg/kg) during six months of storage for lemon myrtle (dried, milled leaves) in packaging materials – LDPE, PVDC coated PET/CPP and PET/PET/Foil/PE of [A] neral and [B] geranial (n=4). Average concentrations analysed with a student's t test. Different letters (i.e. a, b, c) within a storage month denote significant differences between mean concentrations according to a Tukey-Kramer HSD.

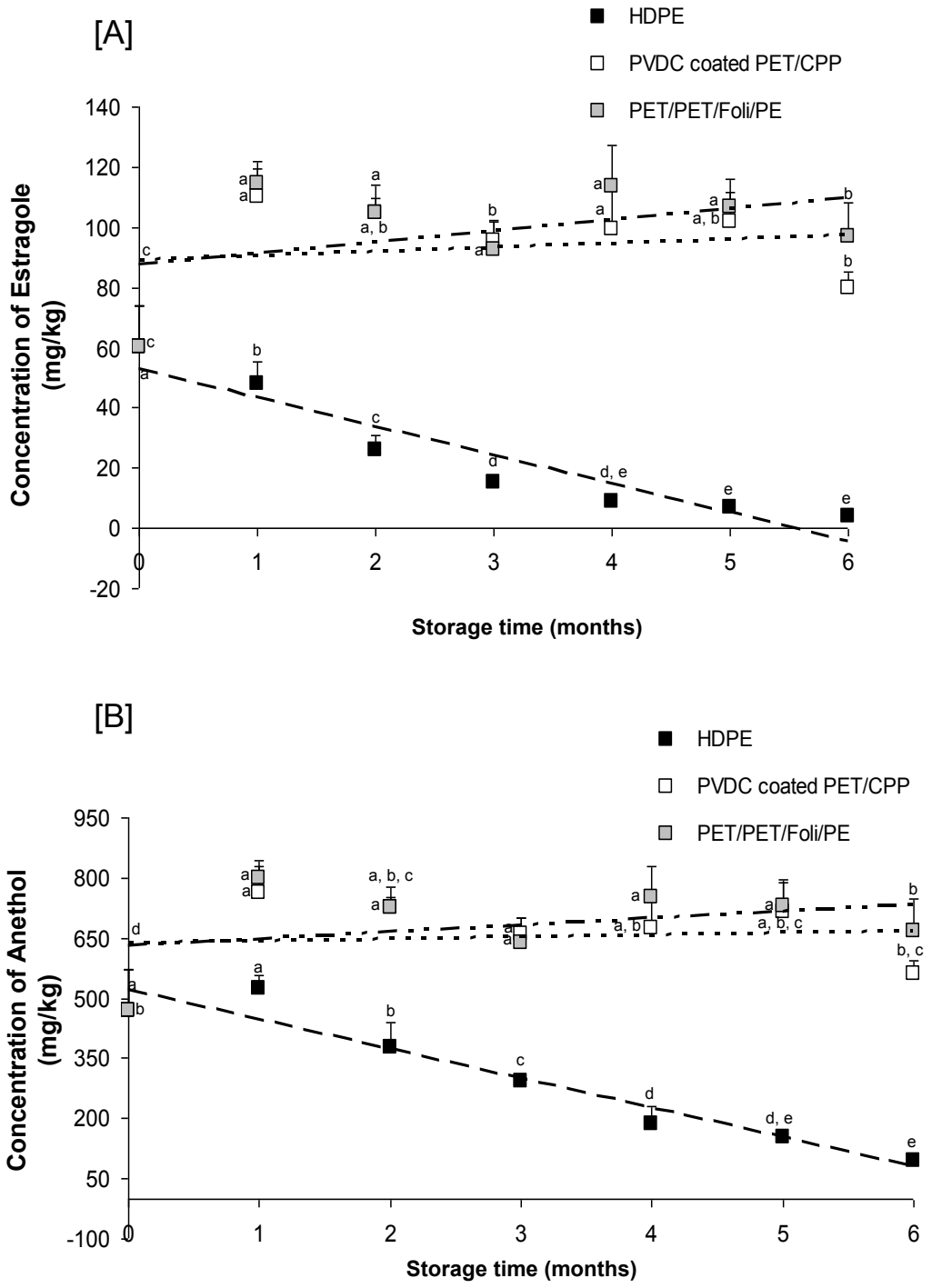


Figure 2.6 Change in concentrations of volatiles (mg/kg) during six months of storage for anise myrtle (dried, milled leaves) in packaging materials – LDPE, PVDC coated PET/CPP and PET/PET/Foil/PE of [A] estragole and [B] anethol (n=4). Average concentrations analysed with a student's t test. Different letters (i.e. a, b, c) within a storage month denote significant differences between mean concentrations according to a Tukey-Kramer HSD.

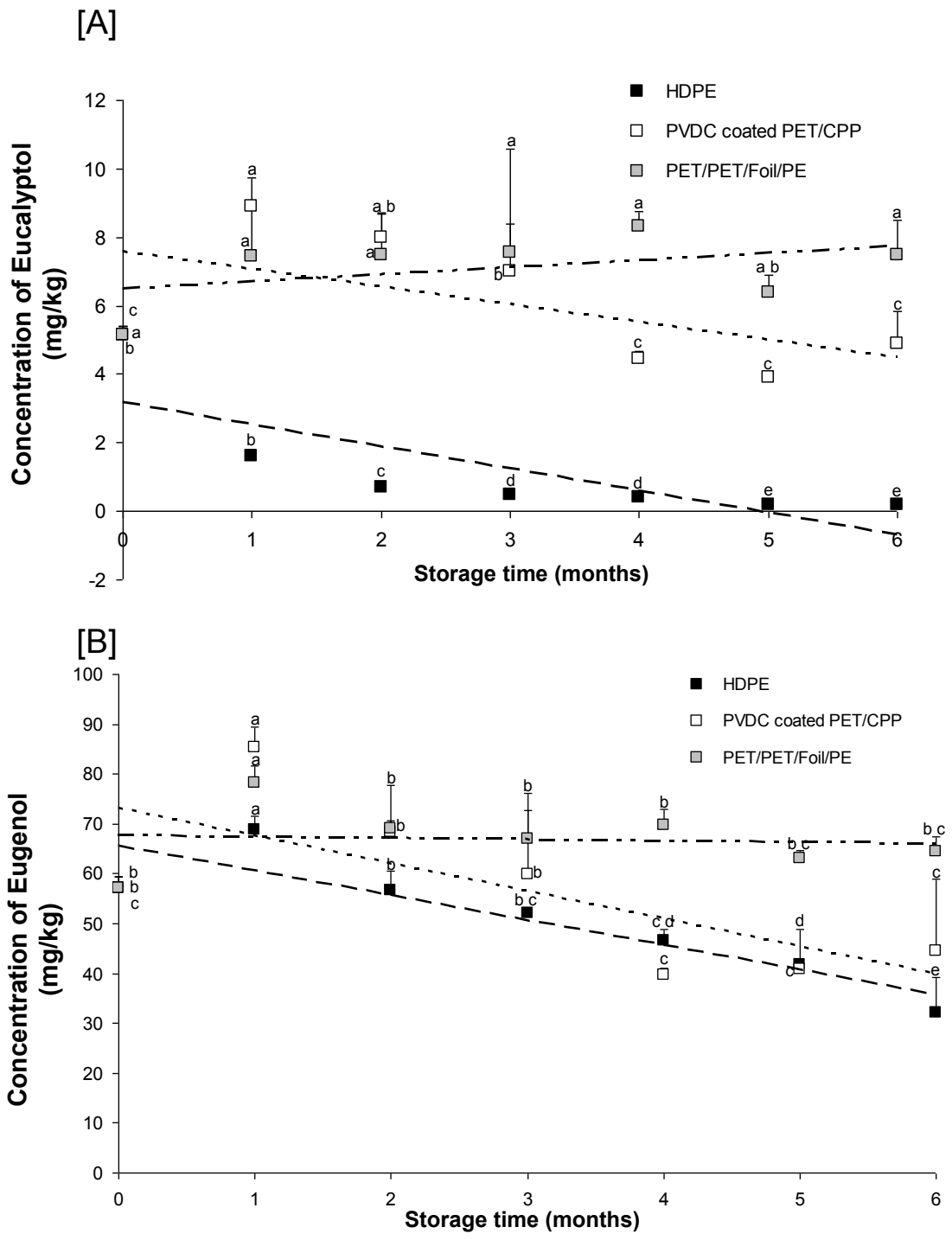


Figure 2.7 Change in concentration of volatiles (mg/kg) during six months of storage for Tasmanian pepper leaves (dried, milled) stored in three different packaging materials – HDPE, PVDC coated PET/CPP and PET/PET/Foil/PE of [A] eucalyptol and [B] eugenol (n=4). Average concentrations analysed with a student's t test. Different letters (i.e. a, b, c) within a storage month denote significant differences between mean concentrations according to a Tukey-Kramer HSD.

2.1.4.3 Conclusion

The findings suggest that the native food industry in Australia has an opportunity to significantly improve the quality and shelf life of stored herb products by using alternate packaging materials with high-barrier properties.

The packing with the best performance in retaining key volatiles neral and geranial in lemon myrtle was PET/PET/Al foil/PE bags (Table 2.3 and Figure 2.5). Thus, using PET/PET/Al foil/PE packaging material would be the preferred option to improve product quality and shelf life for the packaging of lemon myrtle product intended for storage.

Interestingly, for anise myrtle and Tasmanian pepper leaf, there was no significant difference between samples stored in PET/PET/Al foil/PE bags and those stored in PVDC coated PET/CPP bags, in regards to the retention of key volatiles (Table 2.3, Figures 2.6 and 2.7). Either of these two materials would be preferred compared to conventional LDPE or HDPE materials for packaging anise myrtle or Tasmanian pepper leaf to minimise volatile loss and increase storage life.

2.2 Quality changes in lemon and anise myrtle during long-term storage at room temperature

2.2.1 Introduction

Following the preliminary study done in 2010–2011, which consisted of a shorter storage trial of six months, this study aimed to investigate the effectiveness of high barrier packaging materials in preventing the loss of major volatiles during longer-term storage. The earlier study revealed that after 6 months of storage the greatest loss of volatiles from lemon myrtle was observed in traditional LDPE packaging (87% loss) followed by storage in PVDC coated PET/CPP (58% loss) and PET/PET/Foil/LLDPE a loss of 23%. The volatile loss from anise myrtle and Tasmanian pepper leaf stored in PVDC coated PET/CPP and PET/PET/Foil/LLDPE packaging was less than 30% (Chaliha et al. 2013). Thus high-barrier packaging materials were much more efficient in retaining volatiles during storage than the commercial low-barrier packaging used by the native food industry.

2.2.2 Objective

In this phase, the effectiveness of high-barrier packaging materials in preventing loss of volatiles in herbs stored for a long storage period of 12 months was investigated (Figure 2.8). In addition, the patterns of volatile loss among the top, middle and bottom layer of lemon myrtle and anise myrtle flakes (1.6 mm) stored in 10 kg bags were also determined (Figure 2.9).

Lemon myrtle

Anise myrtle

Supplied by Australian Rainforest Products, NSW

Stored at 22°C for 12 months

Sampled every 2 months

Sampling plan for Lemon and Anise myrtle under long storage

Month 0	22-02-2012
Month 2	23-04-2012
Month 4	22-06-2012
Month 6	22-08-2012
Month 8	22-10-2012
Month 10	21-12-2012
Month 12	22-02-2013

Figure 2.8 Flowchart depicting the longer storage trial of lemon myrtle and anise myrtle with sampling dates

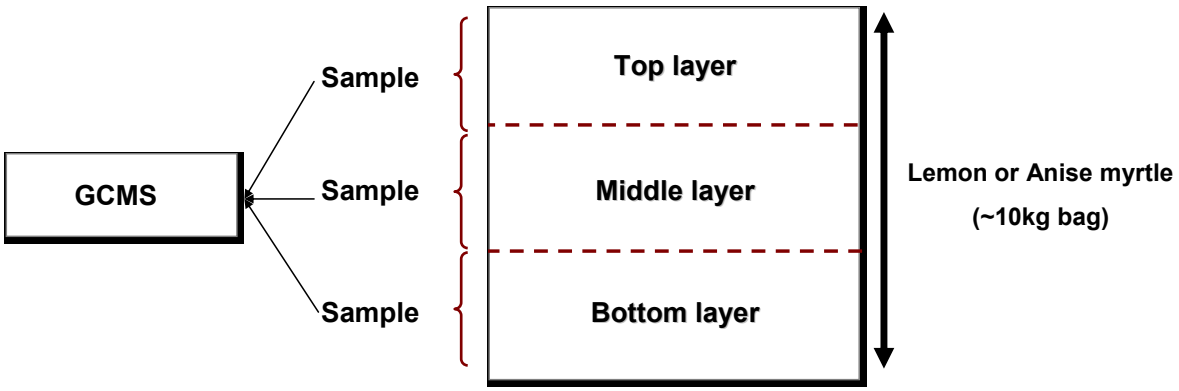


Figure 2.9 Plan for volatile analysis in lemon myrtle and anise myrtle under longer storage

2.2.3 Materials and methods

2.2.3.1 Sampling of the herbs for volatile analysis

The lemon myrtle and anise myrtle flakes were stored in 10 kg bags made of high-barrier packaging material. The high-barrier packaging material used had the following specifications: inner layer PET (12 µm), middle layer metalised PET (12 µm) and outer layer LDPE (80 µm). For sampling, approximately 200 g of flakes were carefully taken from the top, middle and bottom layer of each of the bags and immediately placed at –80°C until further processing. Table 2.4 gives barrier properties of metalized PET. Lemon myrtle and anise myrtle flakes were sampled once every two months. The storage dates are given in Figure 2.8.

Table 2.4 Packaging material gas and moisture barrier properties

Material (layer thickness in µm)	Transmission rate	
	Water [g/m^2/24 h]	Oxygen [mlm^2/24 h]
metalised PET	0.8	1.2

2.2.3.2 Extraction of volatiles from herb samples

LLE of the volatiles was carried out as per the method described in Section 2.1.3.5.

2.2.3.3 Gas chromatography mass spectrometry (GCMS)

GCMS techniques were employed to analyse the major volatiles as described in Section 2.1.3.6.

2.2.3.4 Quantitative analysis

Calibration graphs were obtained as described in Section 2.1.3.7.

2.2.3.5 Statistical analysis

All statistical analysis was performed using the XLSTAT Pro statistical package (Version 2013.5.04, Addinsoft, France). The analysis of variance (ANOVA) with respect to each analytical compound detected in the herbs, the effect storage and the type of packaging material was assessed. Pair-wise comparison was determined with the help of a student's t test.

2.2.4 Results and discussion

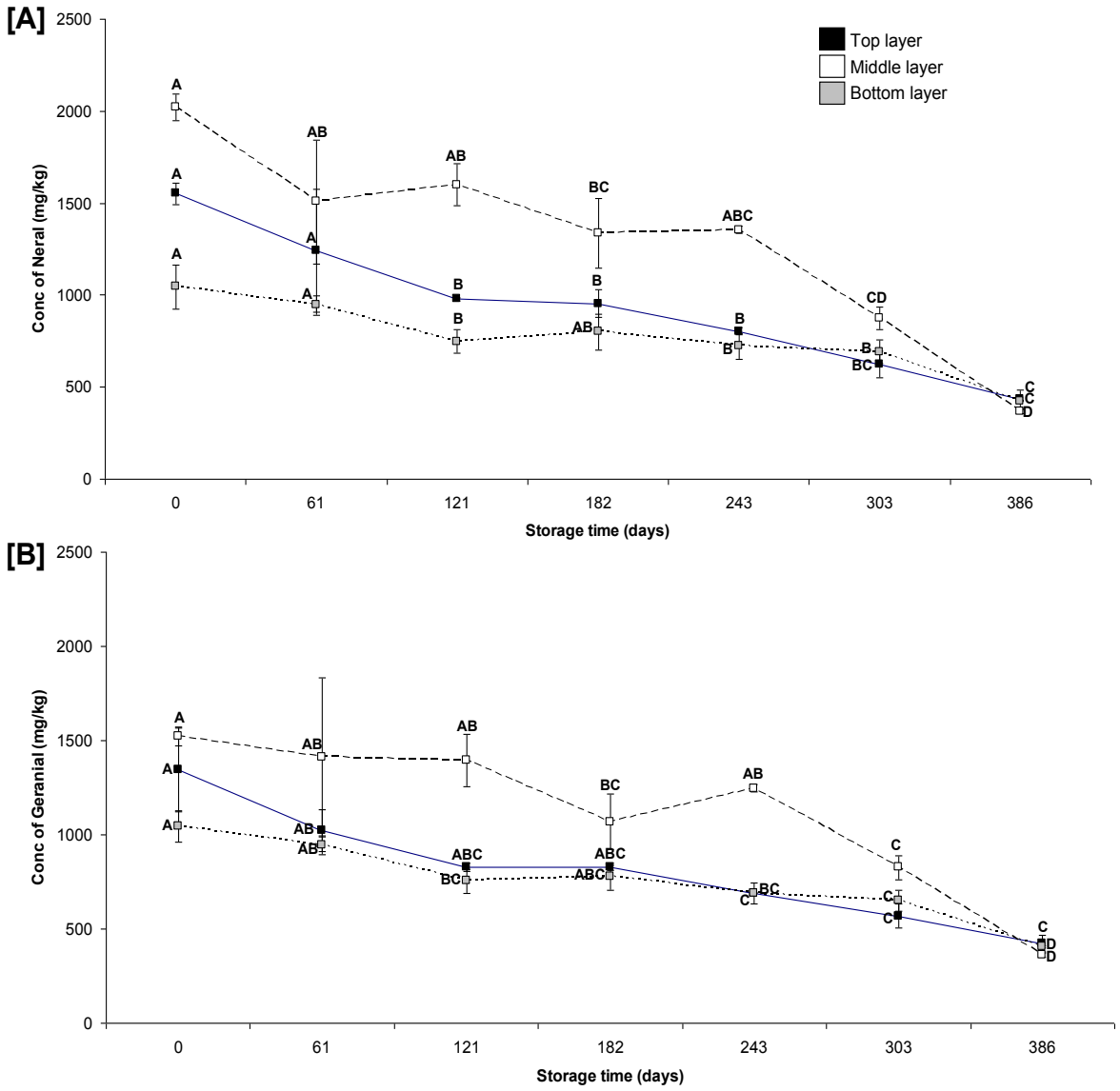


Figure 2.10 Change in concentration of major volatiles [A] neral and [B] geranial (mg/kg) of lemon myrtle (dried, milled leaves) during 12 months of storage (n=4). Average concentrations analysed with a student's t test. Different letters (i.e. A, B, C) within a storage time (days) denote significant differences between mean concentrations according to a Tukey-Kramer HSD.

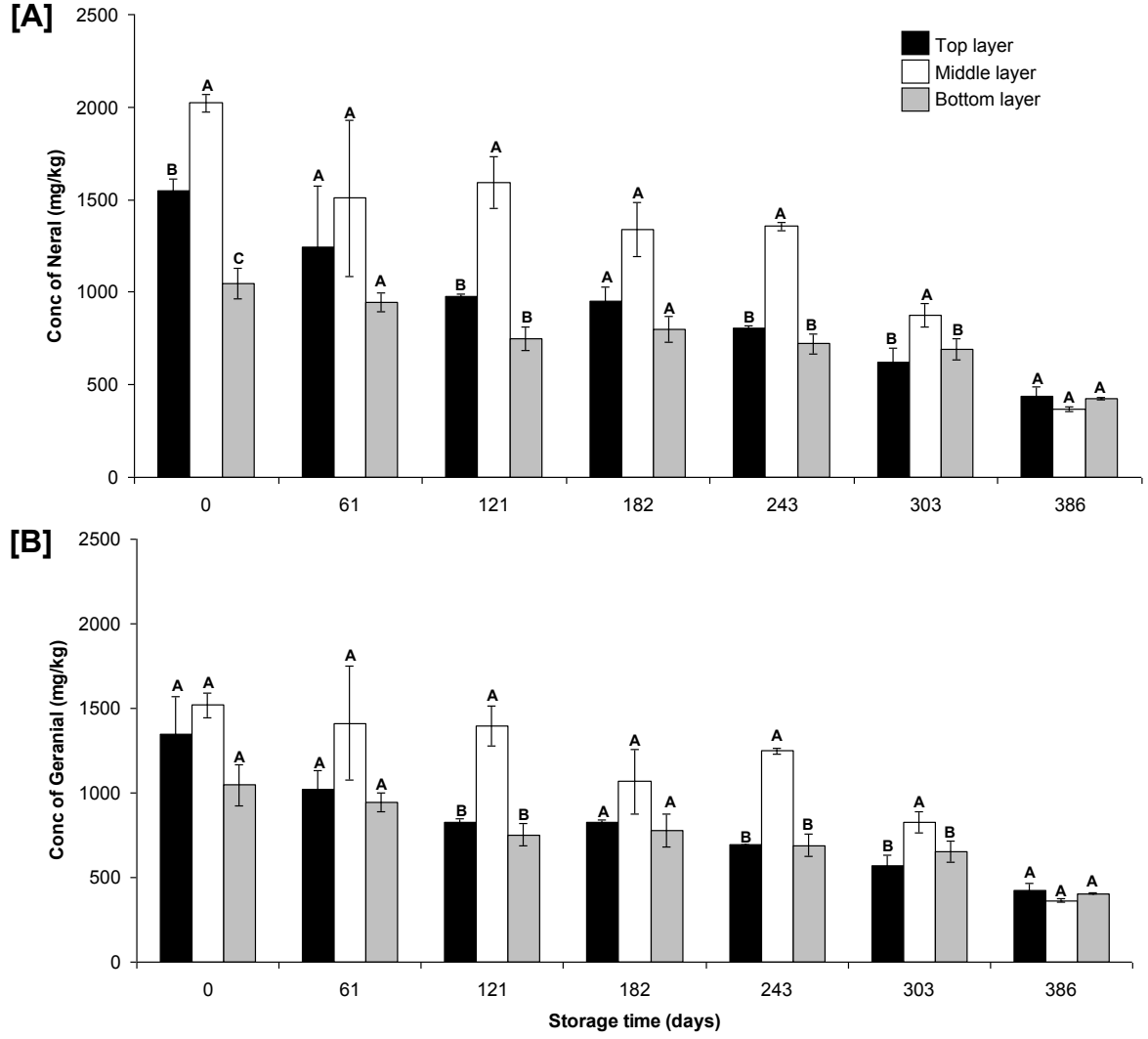


Figure 2.11 Change in concentration of major volatiles [A] neral and [B] geranial mg/kg) in top, middle and bottom layers in lemon myrtle (dried, milled leaves) during 12 months of storage (n=4). Average concentrations analysed with a student's t test. Different letters (i.e. A, B, C) within a storage time (days) denote significant differences between mean concentrations in the top, middle and bottom layers according to a Tukey-Kramer HSD.

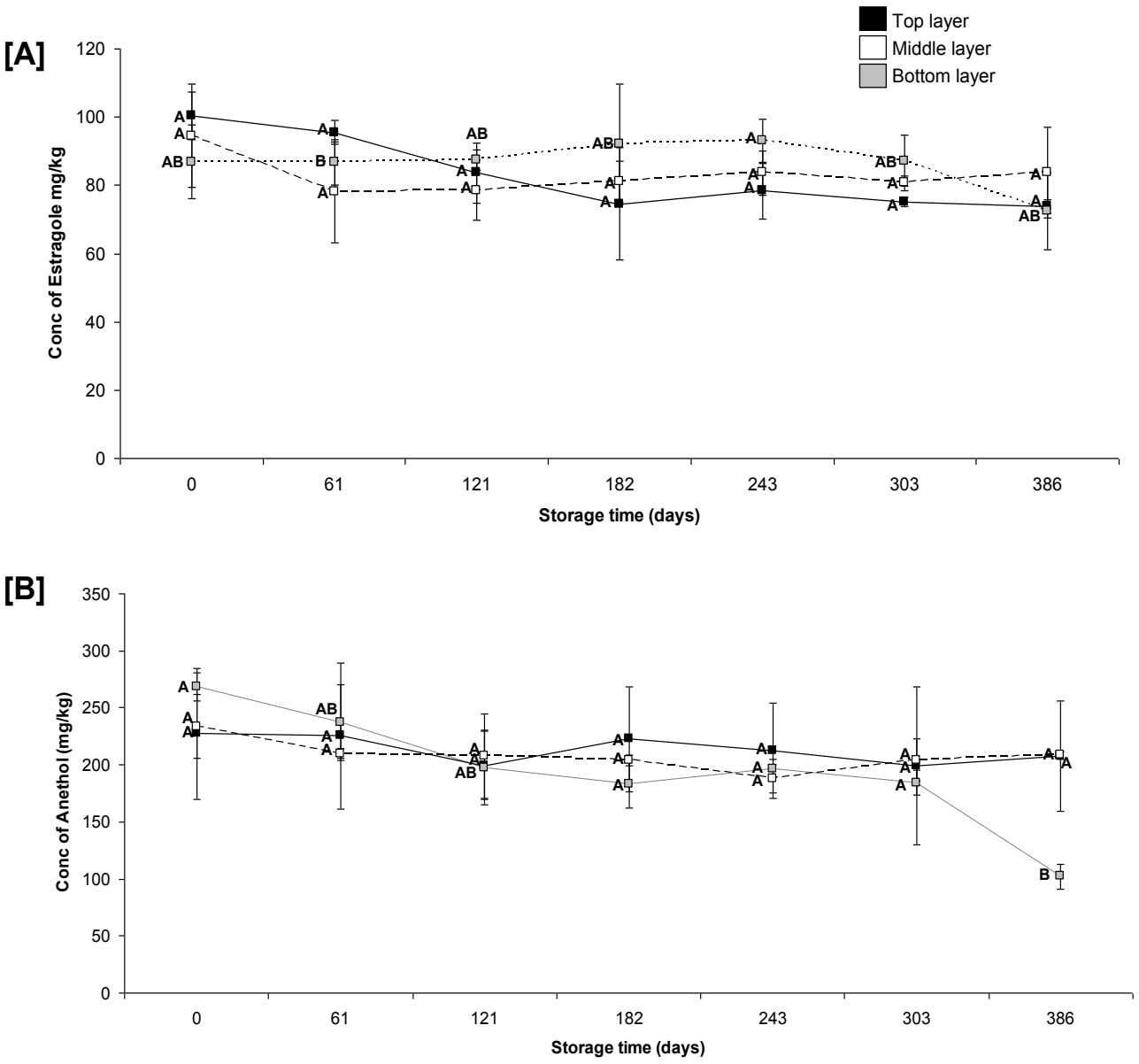


Figure 2.12 Change in concentration of major volatiles [A] estragole and [B] anethol (mg/kg) of anise myrtle (dried, milled leaves) during 12 months of storage (n=4). Average concentrations analysed with a student's t test. Different letters (i.e. A, B, C) within a storage time (days) denote significant differences between mean concentrations according to a Tukey-Kramer HSD.

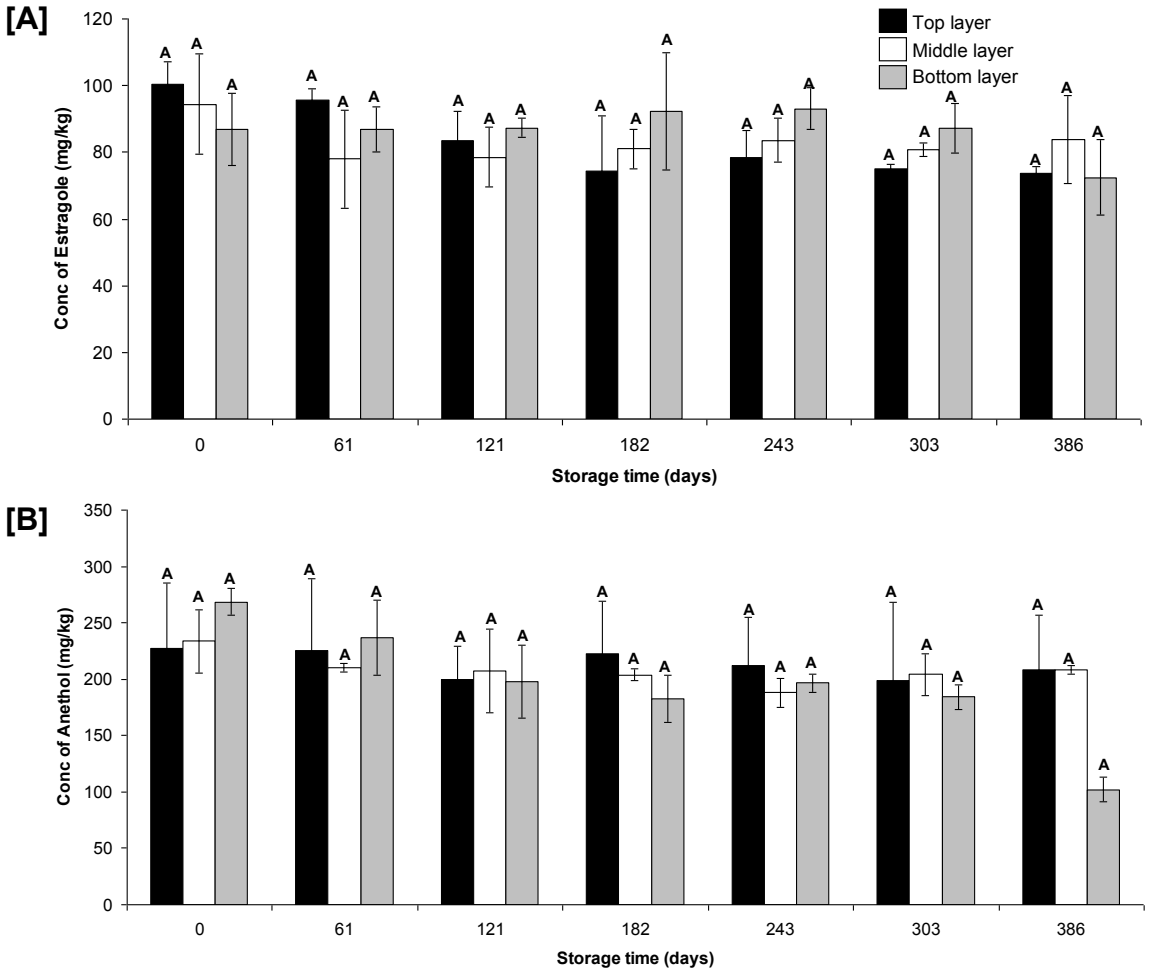


Figure 2.13 Change in concentration of major volatiles [A] estragole and [B] anethol (mg/kg) in top, middle and bottom layers in anise myrtle (dried, milled leaves) during 12 months of storage (n=4). Average concentrations analysed with a student's t test. Different letters (i.e. A, B, C) within a storage time (days) denote significant differences between mean concentrations in the top, middle and bottom layers according to a Tukey-Kramer HSD.

Table 2.5 Concentration of neral and geranial in top, middle and bottom layer of stored lemon myrtle flakes during a storage period of 12 months

Storage time (days)	Top layer		Middle layer		Bottom layer	
	Conc. mg/kg					
	Neral	Geranial	Neral	Geranial	Neral	Geranial
0	1550.81	1346.25	2021.70	1520.60	1044.86	1045.14
61	1241.29	1022.55	1508.09	1412.82	944.52	944.71
121	979.28	826.74	1594.48	1394.70	747.64	753.10
182	952.90	825.37	1336.25	1067.96	800.33	777.41
243	803.61	691.05	1355.26	1246.56	721.45	689.86
303	623.56	568.27	874.79	826.54	692.93	650.88
386	435.16	420.55	368.30	361.85	424.54	405.04

Table 2.6 Concentration of estragole and anethol in top, middle and bottom layer of stored anise myrtle flakes during a storage period of 12 months

Storage time (days)	Top layer		Middle layer		Bottom layer	
	Conc. mg/kg					
	Estragole	Anethol	Estragole	Anethol	Estragole	Anethol
0	100.38	227.23	94.46	233.80	86.90	268.36
61	95.51	225.32	78.01	210.07	86.84	236.95
121	83.62	199.62	78.58	207.58	87.38	197.59
182	74.53	222.59	81.00	203.97	92.20	182.81
243	78.34	212.50	83.63	187.97	93.04	196.70
303	75.18	199.06	80.70	204.25	87.18	184.28
386	73.78	207.92	83.83	208.45	72.45	101.99

A gradual decrease was observed in the concentration of major volatiles neral and geranial in the lemon myrtle herbs, over the long-term storage trial (Figure 2.10). Interestingly, differences in the concentration of the major volatiles (neral and geranial) in the top, middle and bottom layers of the stored lemon myrtle samples were revealed (Figure 2.11, Table 2.5). The middle layer contained a significantly higher concentration of major volatiles compared to the top and bottom layers for both of these volatile compounds. However, toward the end of the 12-month storage period the concentration in all the three layers seemed to reach uniformity.

The significantly lower levels of volatiles in the top and the bottom layer might be due to the 'scalping' effect. Scalping is a phenomenon where flavour compounds are absorbed by the packaging material and may lead to an alteration in the quality of the food product during storage (Roland and Hotchkiss 1991). Aroma sorption may significantly affect the organoleptic quality of packaged food. The packaging industry considers scalping a critical factor that contributes to the loss of quality in packaged food products (Lebosse´ et al. 1997). It is possible that the flavour compounds present in the lemon myrtle products have undergone scalping as the top and the bottom layers of the sample are in direct contact with the packaging material and had a slightly reduced volatile concentration during the storage period.

The packaging material used for this phase of the study was different to the packaging material in the earlier study (Table 2.1). The high-barrier packaging material used in this study has LDPE as the innermost layer which is in constant contact with the food product. LDPE materials are widely used as the inner surface of the packaging materials by the food industry due to their inertness to most food products, their good barrier properties and thermostability. However, they are known to absorb large quantities of nonpolar compounds such as most of the aroma compounds due to their highly lipophilic nature, thereby causing an imbalance in the aroma profile of the food product (Sajilata et al. 2007). The packaging material seemed more prone to scalping than the four-layered high-barrier packaging used in the preliminary study.

The breakdown of citral might be another reason for change in the aroma profile. Citral is known to undergo degradation when affected by conditions such as pH, temperature, light and availability of oxygen (Liang et al. 2004). In our study the bags of lemon myrtle leaves (10 kg per bag) were not sealed under vacuum which may lead to oxidation of the citral compounds. Lebosse´ et al. (1997) reported previously that any degradation in neral and geranial plays a major role in the change of flavour profile of citrus juice stored in plastic containers. Nguyen et al (2009) have shown that citral compounds in lemon oil are susceptible to oxidation, leading to a decrease in the overall concentration of citral in lemon oil.

The new packaging material worked satisfactorily with the anise myrtle samples and effectively prevented drastic loss in the volatiles over the storage period. There was no statistically significant difference in the concentration of the major volatiles in anise myrtle during the storage period (Figure 2.12, Table 2.6). No significant difference was observed between the volatile concentration among the top, middle and bottom layers of the packaged anise myrtle leaves (Figure 2.13). One possible explanation why scalping is not so prominent in the anise myrtle could be the selective nature of the scalping phenomenon (Lebosse´ et al. 1997).

In September 2012, a layered material consisting of biaxially oriented (BO) nylon/Al foil/Nat LLDPE (specifications are given in Table 2.7) was used to conduct a packaging trial in order to overcome the issues faced with volatile loss in lemon myrtle flakes. However, BO nylon/Al foil/Nat LLDPE packaging was not efficient in retaining the major volatile constituents of lemon myrtle and the shipment was not accepted.

Another packaging trial was conducted with PET(12)/ PET(12)/Foil(9)/ LLDPE(65) packaging material. A total of four bags were made, each containing 5 kg of lemon myrtle (1.5 mm flakes). Bags were sealed under vacuum and were sent to Germany in October 2013, through a shipment from

Australian Rainforest Products Pty Ltd (figure 2.14).This shipment has been accepted and the client is now in a position to export larger quantities of lemon myrtle in the future.

Table 2.7 Gas and moisture barrier properties of the packaging material

Material (layer thickness in µm)	Transmission rate	
	Water [g/m^2/24 h]	Oxygen [cm^3/m^2/24 h]
BO Nylon (15)/ Al foil (12)/Foil(9)/NAT LLDPE(80)	<0.1 (at 38^{o}C, RH 100%)	<0.1 (at 23^{o}C, RH 50%)

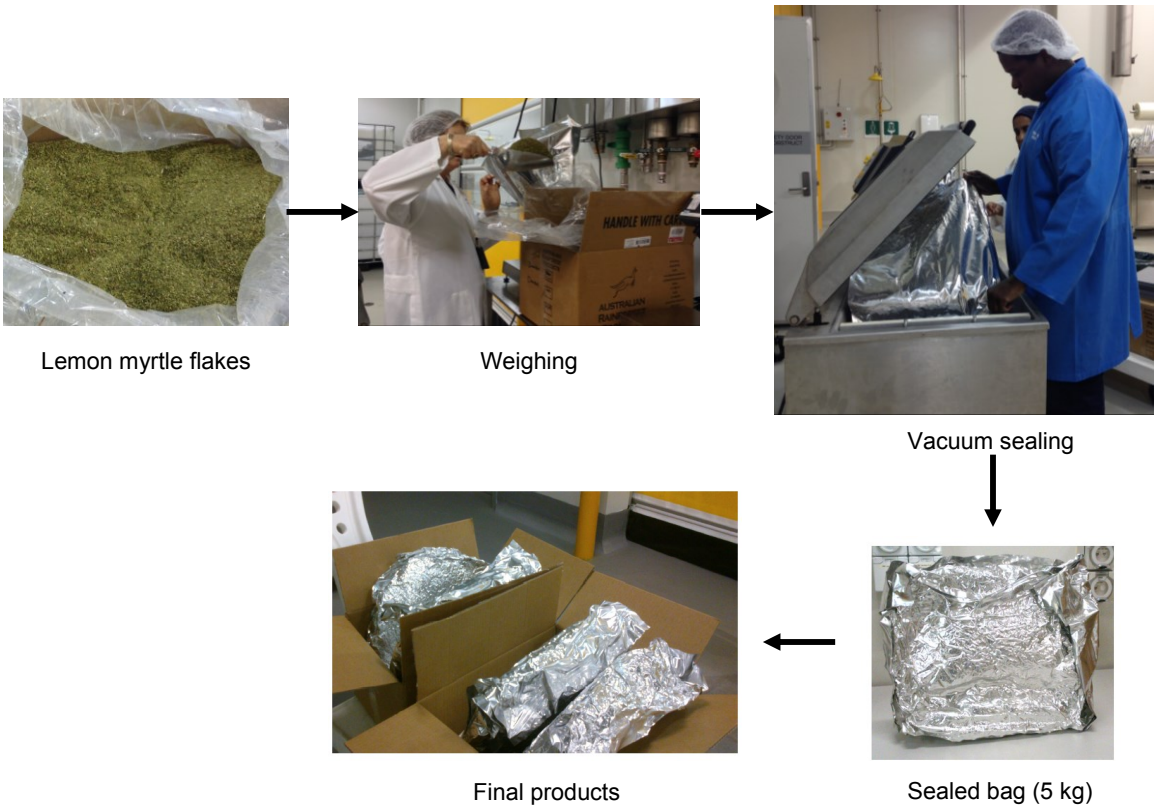


Figure 2.14 Flowchart depicting the trial conducted in October 2013

2.3 Effect of milling on volatile phytochemicals in Tasmanian pepper leaf during storage

2.3.1 Introduction

The first phase of the study with native herbs indicated that the current milling practices contribute hugely to loss of volatiles in the native herbs (Figure 2.4). To further understand the process of volatile loss during room storage, the effect of milling on Tasmanian pepper leaf was investigated.

Milling or grinding of spices is an age-old technique. The main aim of spice grinding is to obtain smaller particle size with good product quality in terms of flavour and colour. In the normal grinding process, heat is generated when energy is used to fracture a particle into a smaller size. This generated heat usually is detrimental to the product and results in some loss of flavour and quality. The fat in spices generally poses extra problems and is an important consideration in grinding. During grinding, the temperature of the product rises to a level in the range of 42 to 95°C (Singh and Goswami 1999), which varies with the oil and moisture content of the spices, but spices lose a significant fraction of their volatile oil or flavouring components due to this temperature rise.

2.3.2 Objective

To determine the effect of milling on the major volatile constituents of Tasmanian pepper leaves, commercially milled samples were compared with hammer milling and cryogenic milling.

2.3.3 Materials and methods

2.3.3.1 Plant materials

Samples of Tasmanian pepper (*T. lanceolata)* leaf samples were supplied by Diemen Pepper (Tasmania, Australia). The supplier provided commercially milled samples and dried Tasmanian pepper leaves before milling.

2.3.3.2 Packaging

The packaging was done in duplicate for reproducibility. All the bags were made of the high-barrier material PET(12)/ PET(12)/Foil(9)/ LLDPE(65), the properties of which are shown in Table 2.1.

2.3.3.3 Sampling of herbs for volatile analysis

For the commercially milled Tasmanian pepper leaves, 250 g of the sample was weighed and placed in the high-barrier packaging material bag. For the dried leaves, 750 g of the material was weighed and placed in the high-barrier packaging material bag. Filled bags were sealed under vacuum using a Multivac Chamber machine C 500 (Multivac Sepp Haggenmüller GmbH & Co. KG, Germany). The storage trial was conducted at 22°C for 18 months. All the bags with herb samples were placed in cardboard boxes for the duration of the trial (as per standard commercial practice), as shown in Figure 2.15. A single bag was drawn at every sampling point. The sampling dates are given in Figure 2.16. The commercially milled samples were immediately stored at –80°C until further analysis. After sampling, the dried milled leaves were subjected to hammer and cryogenic milling. For hammer milling, approximately 200 g of the dried leaves was milled using a 1 mm screen/sieve (Lab mill, 8000 RFM, Christry and Norris, Chelmsford, UK). For the cryogenic milling, approximately 50 g of the dried whole leaves were milled using a Mixer Mill MM 200 (Retsch, Germany). Approximately 1 g of each of the samples was weighed into stainless steel cells, sealed and immediately immersed in liquid nitrogen (N_2) for a minute. The cells were than inserted into the cryomill and the samples were

ground for 1 min at a speed of 300 rpm/sec, at chilled temperature. This process was repeated until a volume of ~50 g was obtained.

2.3.3.4 Extraction of volatiles from herb samples

Liquid-liquid extraction of volatiles was carried out as per the method described in Section 2.1.3.5.

2.3.3.5 Gas chromatography mass spectrometry (GCMS)

GCMS techniques were employed to analyse the major volatiles as described in Section 2.1.3.6.

2.3.3.6 Quantitative analysis

Calibration graphs were obtained as described in Section 2.1.3.7.

2.3.3.7 Statistical analysis:

All statistical analysis was performed as described in Section 2.1.3.8.

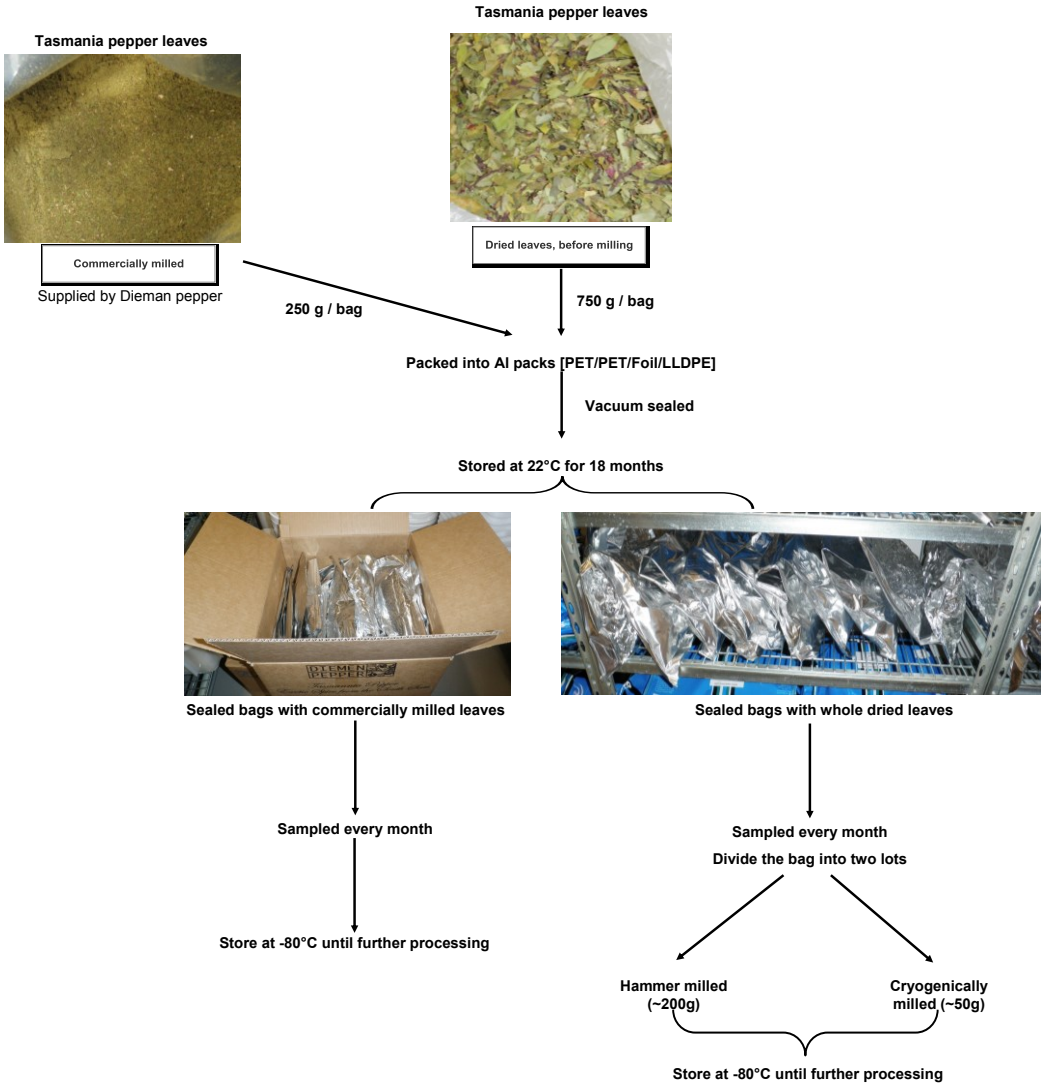


Figure 2.15 Flowchart depicting the storage and milling trial of Tasmanian pepper leaves

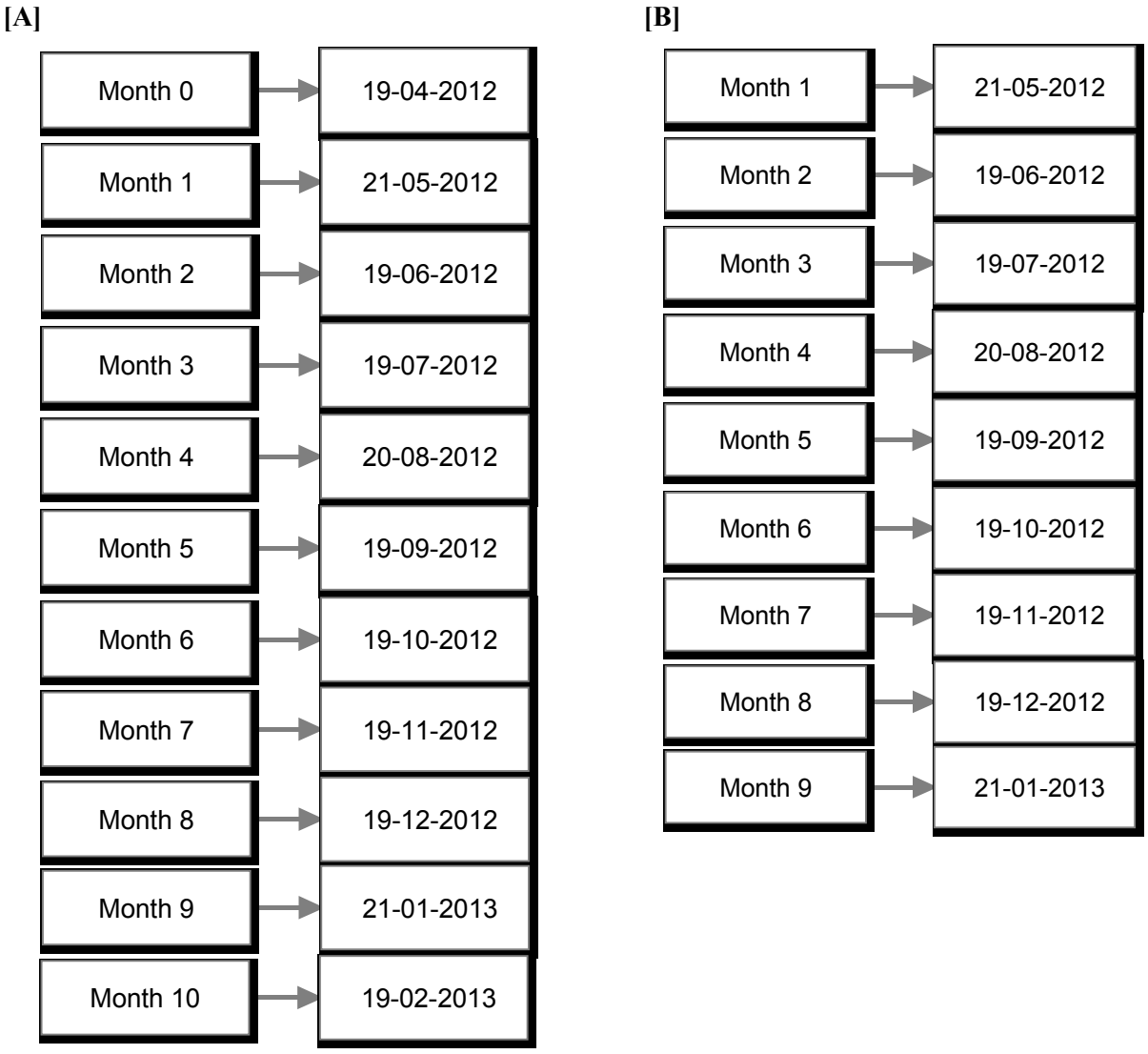


Figure 2.16 Sampling plan for Tasmanian pepper leaves [A] dried unmilled leaves and [B] commercially milled samples

2.3.4 Results and discussion

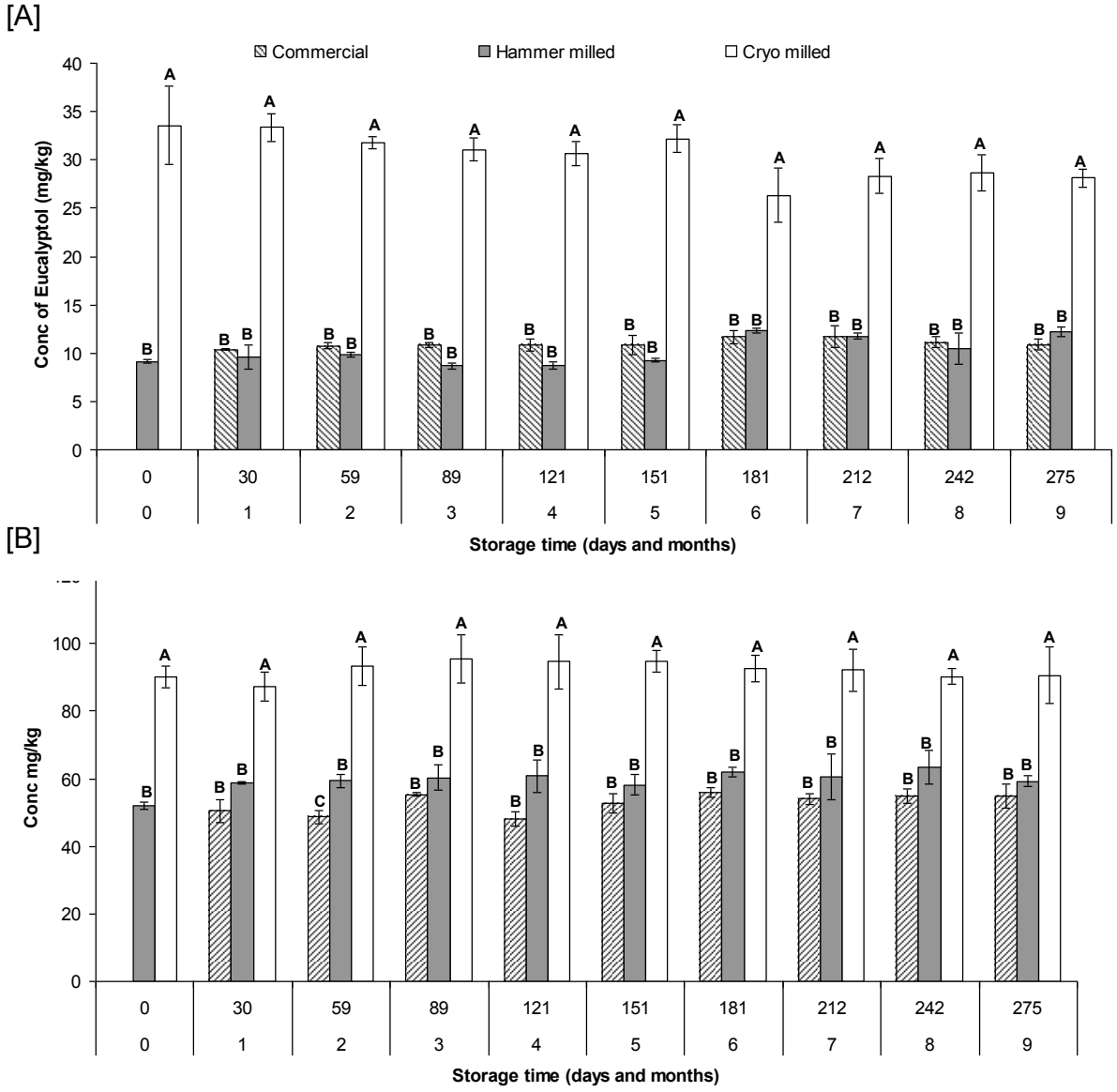


Figure 2.17 Change in concentration of major volatiles [A] eucalyptol and [B] eugenol (mg/kg) in Tasmanian pepper leaves during storage (n = 4) and with different milling treatments. Average concentrations analysed with a student's t test. Different letters (i.e. A, B, C) within a storage time (days) denote significant differences between mean concentrations in different sampling points according to a Tukey-Kramer HSD.

The dried whole Tasmanian pepper leaves were ground by hammer milling and cryogenic milling and compared with the commercially milled samples.

As expected, the cryogenic milling was found to be the most effective milling process in preventing loss of major volatiles. Both the commercial and hammer milling showed similar results and were less effective in preventing volatile loss compared to cryogenic milling (Figure 2.17).

The cryogenic milling technique is well known to be very effective in retaining volatiles during milling. Use of liquid nitrogen (N_2) during cryogenic milling provides the refrigeration needed to pre-cool the spices and maintain a low temperature by absorbing the heat generated during the grinding operation (Singh and Goswami 1999). Low temperature during milling reduces the loss of volatile oils and moisture thereby retaining most of the flavour strength per unit mass of spice.

Cryogenic temperature helps the sample particles to solidify so that they become more brittle and thus they crumble easily to a fine and consistent size. The flavour is uniformly spread throughout the finely ground product (Sowbhagya et al. 2007).

Throughout the storage trial we used the high-barrier packaging material (as specified in Table 2.1) to store the leaf samples and observed that there was no statistical difference in the concentration of the major volatiles from one sampling point to the next (Figure 2.17). The extremely low transmission rates for water and oxygen of the packaging material used in the experiment effectively prevented any drastic loss of volatiles from the herb.

2.4 Antimicrobial and antioxidant activity of native herbs

2.4.1 Introduction

The samples analysed in this study have been described in detail in Section 2.1.3.1. Here we are evaluating the bioactivity of the native herbs to determine if packaging has an effect on the non-volatile bioactive compounds and colour.

2.4.2 Materials and Methods

2.4.2.1 Measurement of antioxidant activity during storage

As described in Section 3.2.2.3.

2.4.2.2 Measurement of antimicrobial activity during storage

As described in Section 3.2.2.4.

2.4.2.3 Measurement of colour

As described in Section 1.2.2.4.

2.4.3 Results and discussion

2.4.3.1 Changes in antimicrobial activity in native herbs during 6 months of storage

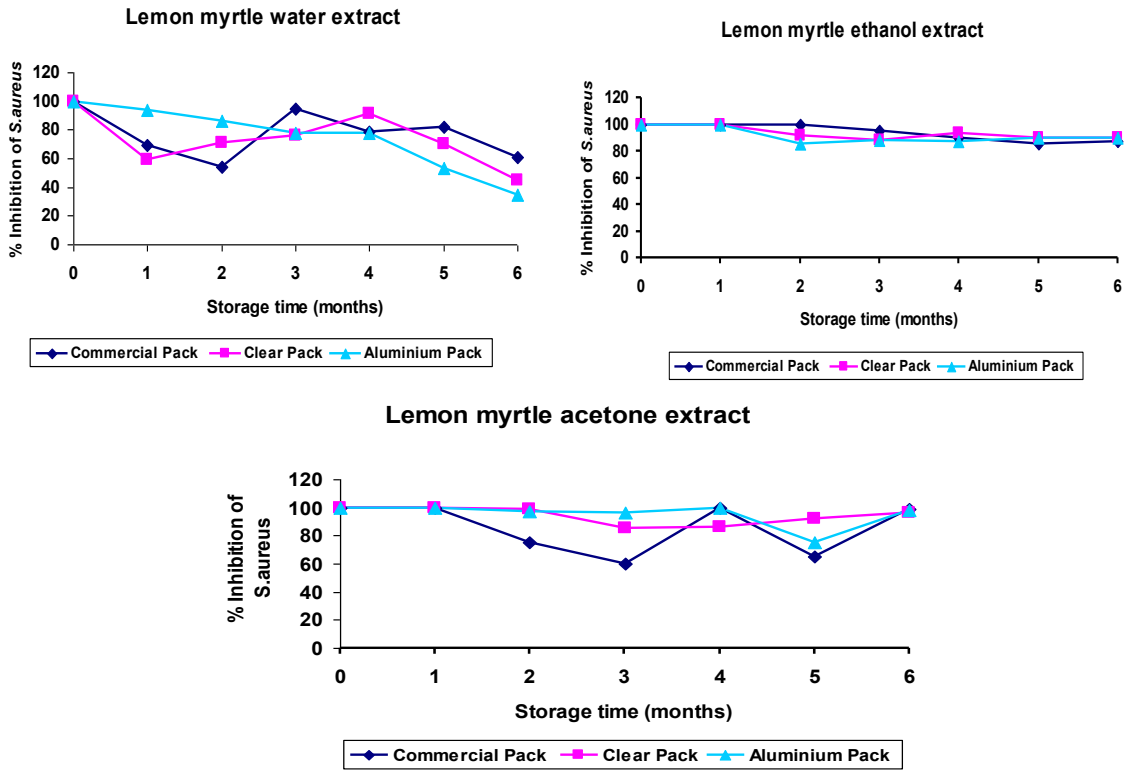


Figure 2.18 Changes in antimicrobial activity of different extracts of lemon myrtle (at a concentration of 8.75% v/v) over 6 months of storage in different packaging, as measured by inhibition of *Stahylococcus aureus*

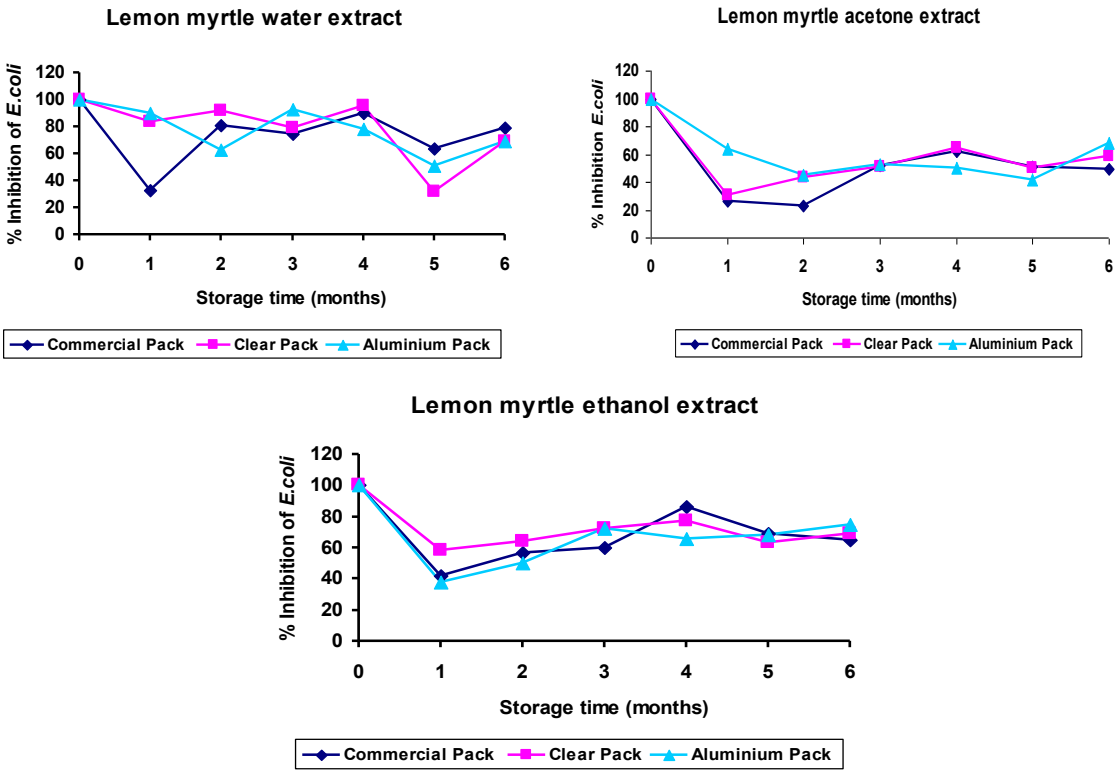


Figure 2.19 Changes in antimicrobial activity of different extracts of lemon myrtle (at concentration of 8.75% v/v) over 6 months of storage in different packaging, as measured by inhibition of *E.coli*

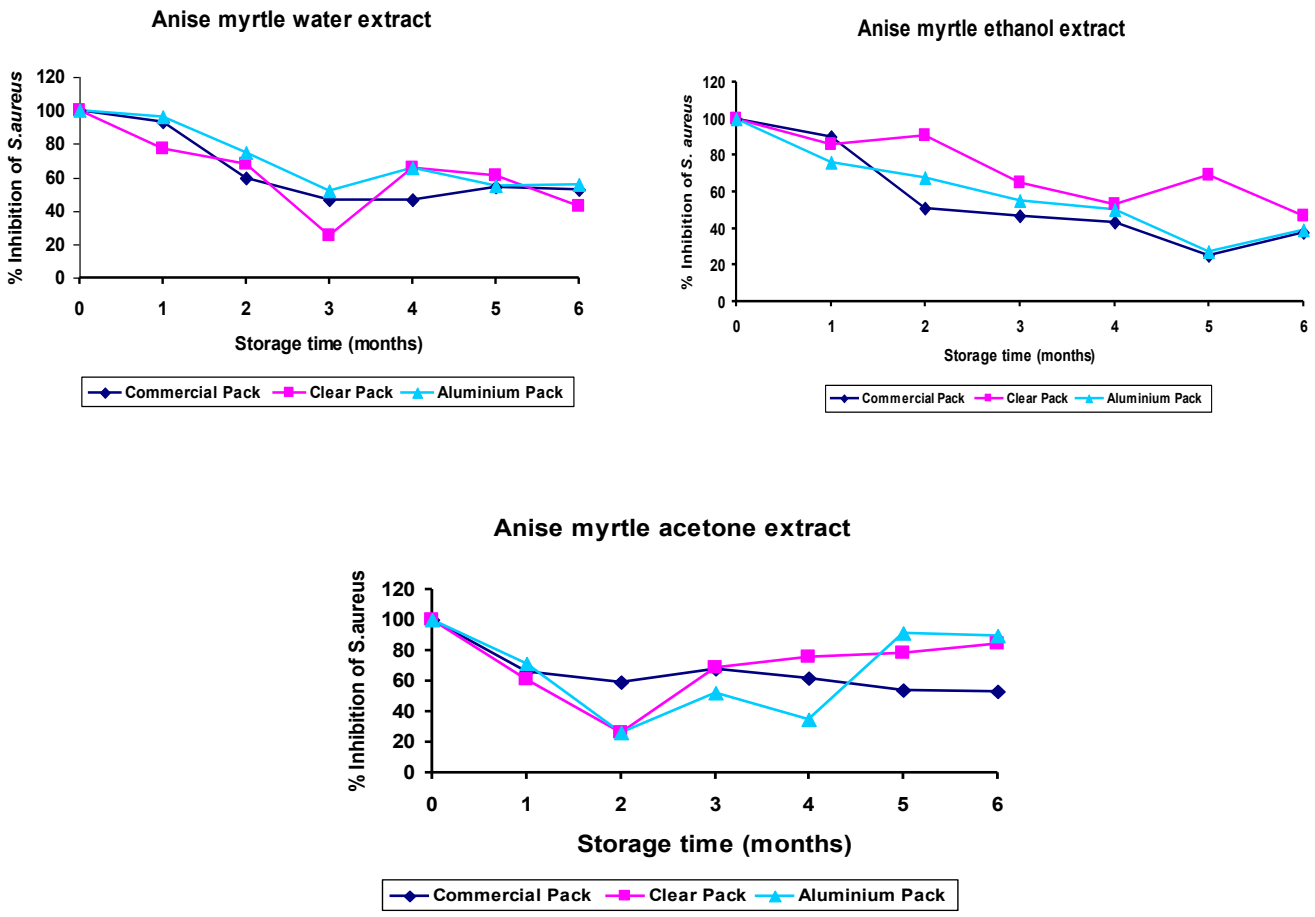


Figure 2.20 Changes in antimicrobial activity of different extracts of anise myrtle (at a concentration of 8.75% v/v) over 6 months of storage in different packaging, as measured by inhibition of *Stahylococcus aureus*

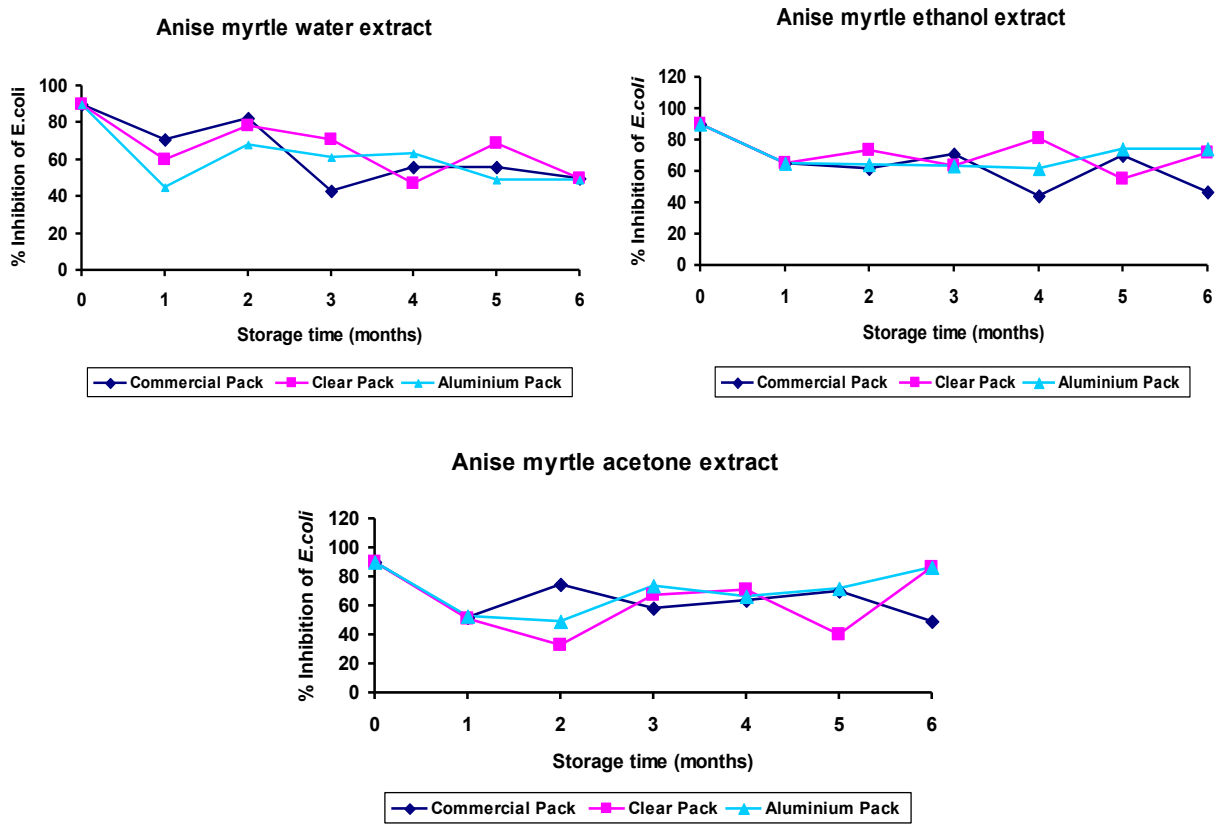


Figure 2.21 Changes in antimicrobial activity of different extracts of anise myrtle (at concentration of 8.75% v/v) over 6 months of storage in different packaging, as measured by inhibition of *E.coli*

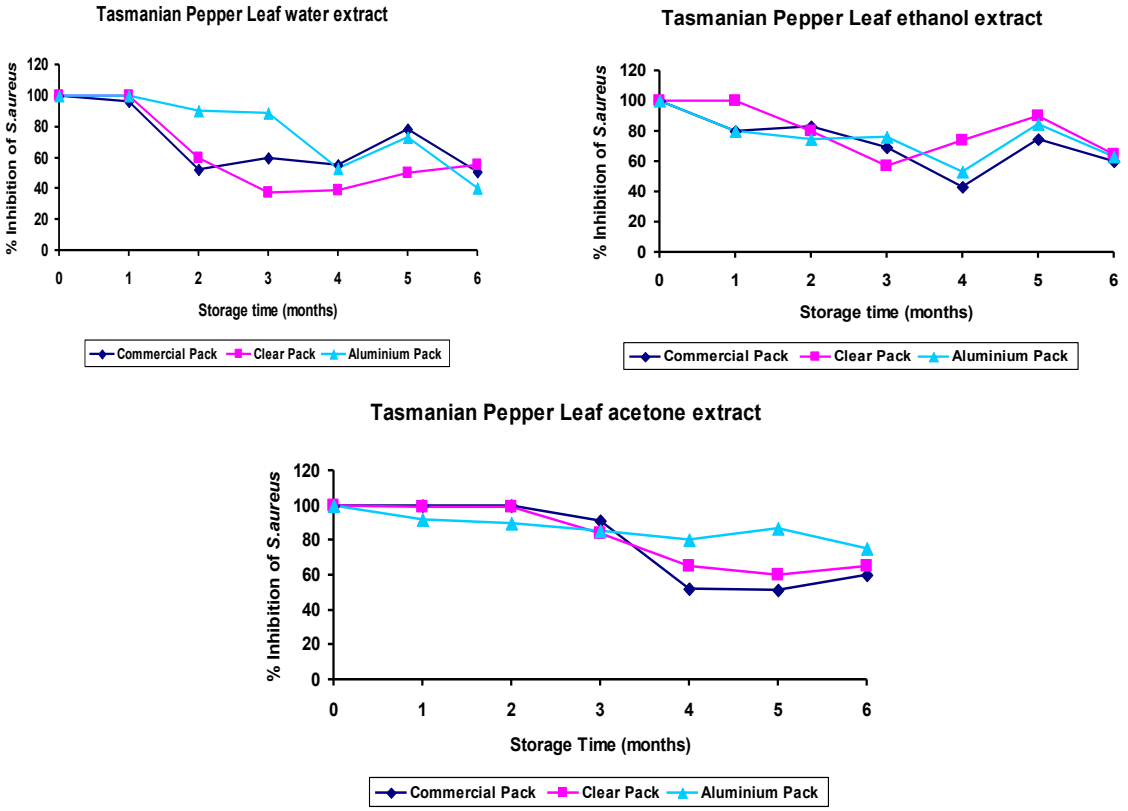


Figure 2.22 Changes in antimicrobial activity of different extracts of Tasmanian pepper leaf (at concentration of 8.75% v/v) over 6 months of storage in different packaging, as measured by inhibition of *Stahylococcus aureus*

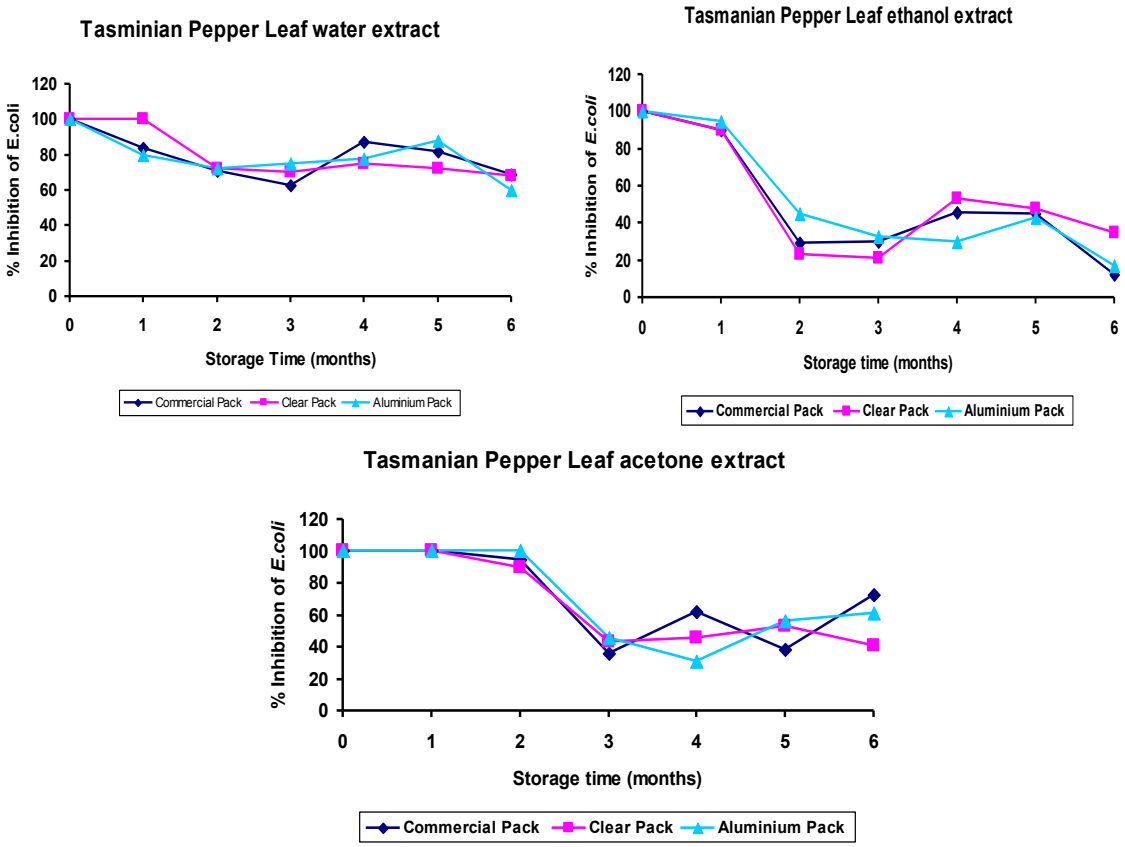


Figure 2.23 Changes in antimicrobial activity of different extracts of Tasmanian pepper leaf (at concentration of 8.75% v/v) over 6 months of storage in different packaging, as measured by inhibition of *E. coli*

2.4.3.2 Changes in antioxidant activity in native herbs over 6 months of storage

Table 2.8 Antioxidant activity of fresh and dried native herbs

Sample (received 3 November 2010)	Total phenolics (GA Eq/g DW)		FRAP (umol Fe^{+2} Eq/g DW)	
	Average	SD	Average	SD
Lemon myrtle, fresh whole	134.6	9.03	1970.9	96.0
Lemon myrtle, dried, milled 1.6mm	100.3	2.77	1918.7	144.9
Anise myrtle, fresh whole	90.1	2.30	2479.8	33.7
Anise myrtle, dried, milled 1.6mm	100.4	11.92	2476.2	9.3
Tasmanian pepper leaf, fresh whole	15.6	0.85	205.5	66.9
Tasmanian pepper leaf, dried, milled 1.6mm	85.8	7.06	1155.7	30.2
Tasmanian pepper leaf, freeze-dried fresh leaf	31.59	2.73	481.86	35.67

2.4.3.3 Changes in antioxidant activity of lemon myrtle with different packaging material during storage

Table 2.9 Changes in antioxidant activity of lemon myrtle with different packaging material during storage

Month	Commercial pack		Clear pack		Aluminium pack	
	TP	FRAP	TP	FRAP	TP	FRAP
0	117.9±2.8	1918.6±144.9	117.9±2.8	1918.65±144.9	117.9±2.8	1918.6±144.9
1	114.2±8.6	1680.8±112.1	94.1±2.4	1574.74±104.6	98.2±5.4	1740.2±98.7
2	90.4±11.9	1028.1±84.8	73.5±8.8	934.76±114.1	82.6±2.8	828.8±185.1
3	84.2±5.7	1055.0±100.9	82.4±2.6	1113.84±70.2	86.5±6.8	835.32±185.1
4	84.5±4.8	998.7±124.9	80.5±3.8	901.99±34.7	81.8±1.9	922.2±170.1
5	84.9±8.1	1178.7±51.0	78.5±2.5	1159.07±105.3	78.2±9.4	1100.8±75.1
6	91.6±7.6	1248.3±108.7	84.6±2.9	1063.35±129.7	80.5±8.5	1042.9±137.9

TP – total phenolics (mg GA Eq/g DW); FRAP – ferric reducing antioxidant power (µmol Fe^{+2} Eq/g DW).

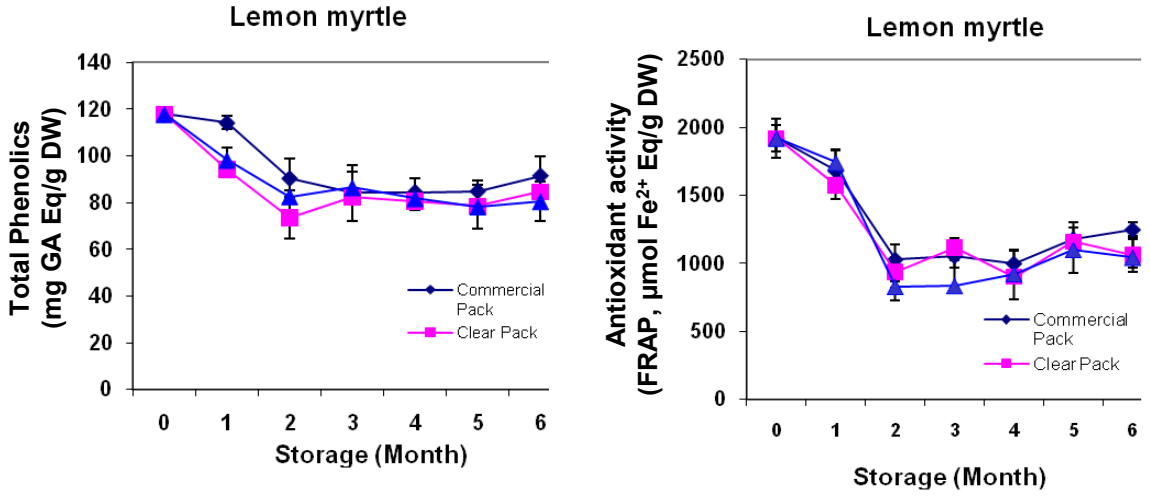


Figure 2.24 Changes in antioxidant activity of lemon myrtle with different packaging material during storage

2.4.3.4 Changes in antioxidant activity of anise myrtle with different packaging material during storage

Table 2.10 Changes in antioxidant activity of anise myrtle with different packaging material during storage

Month	Commercial pack		Clear pack		Aluminium pack	
	TP	FRAP	TP	FRAP	TP	FRAP
0	118.1±11.9	2476.2±9.3	118.1±11.9	2476.2±9.3	118.1±11.9	2476.2±9.3
1	86.2±3.7	2426.4±64.7	85.5±5.6	2392.3±77.7	88.8±5.1	2559.0±92.4
2	86.9±5.2	1987.4±193.2	89.4±1.7	1942.7±50.5	89.6±6.6	1913.2±116.0
3	86.4±3.7	2000.8±214.6	82.1±1.4	2168.9±34.2	86.6±5.0	1972.5±91.2
4	83.4±6.2	2142.7±115.3	77.8±3.7	1863.7±185.9	76.4±4.2	1962.3±84.5
5	80.1±1.1	2119.2±±25.1	70.7±2.4	2116.9±133.2	82.1±2.5	2059.9±207.1
6	76.5±3.8	2053.2±50.3	69.9±4.1	2016.8±63.65	80.9±2.8	2156.0±190.9

TP – total phenolics (mg GA Eq/g DW); FRAP – ferric reducing antioxidant power (µmol Fe^{+2} Eq/g DW).

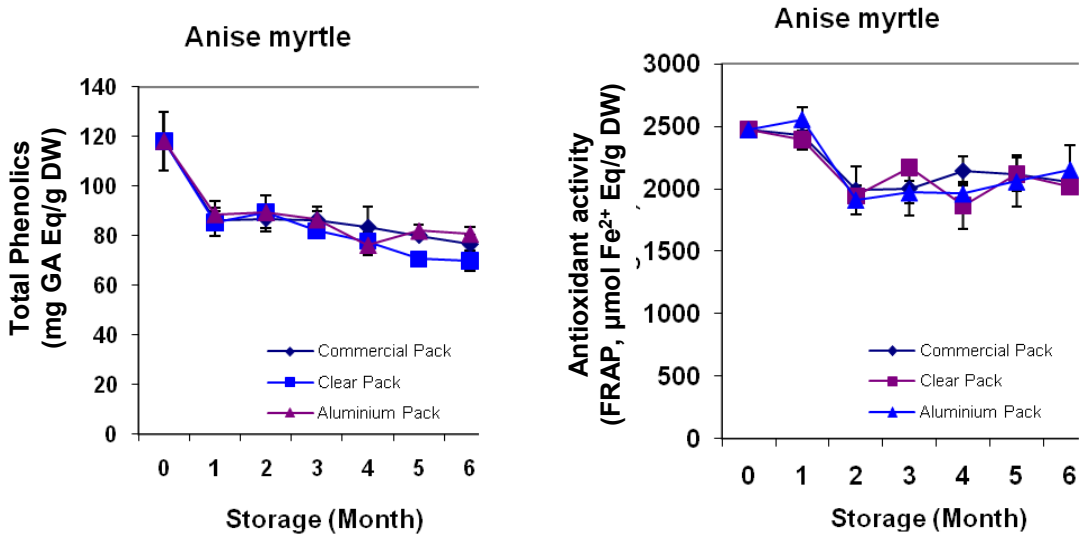


Figure 2.25 Changes in antioxidant activity of anise myrtle with different packaging material during storage

2.4.3.5 Changes in antioxidant activity of Tasmanian pepper leaf with different packaging material during storage

Table 2.11 Changes in antioxidant activity of Tasmanian pepper leaf with different packaging material during storage

Month	Commercial pack		Clear pack		Aluminium pack	
	TP	FRAP	TP	FRAP	TP	FRAP
0	85.8±7.1	1237.8±8.5	85.8±7.1	1237.8±8.6	85.8±7.1	1237.8±8.6
1	85.0±9.9	1124.5±164.0	81.2±8.2	1271.7±51.2	85.4±5.7	1192.1±79.3
2	73.7±1.0	913.7±102.8	65.6±4.0	904.1±58.8	65.3±5.6	1016.2±34.9
3	68.0±4.6	990.4±74.9	68.4±4.0	999.1±97.5	69.7±4.1	900.2±64.9
4	70.1±3.6	937.3±74.0	68.7±1.5	880.6±88.9	66.7±4.5	1012.1±93.4
5	75.1±1.6	1013.9±29.3	68.9±9.9	960.7±108.7	64.9±5.1	954.6±90.0
6	75.9±2.3	1027.7±80.4	75.2±6.1	1081.7±63.2	71.5±3.1	997.0±70.3

TP – total phenolics (mg GA Eq/g DW); FRAP – ferric reducing antioxidant power (µmol Fe^{+2} Eq/g DW).

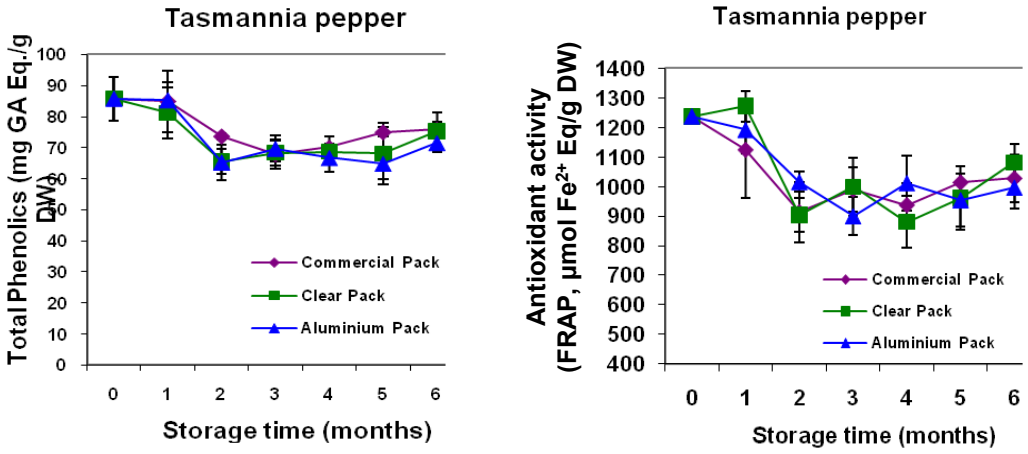


Figure 2.26 Changes in antioxidant activity of Tasmanian pepper leaf with different packaging material during storage

2.4.3.6 Variation in colour in native herbs during longer storage

In Tables 2.12 to 2.15, the L* represents lightness or darkness on a scale of 0 to 100 (100 being white and 0 being back), a* represents the greenness or redness of the sample (–50 being green to +50 being red), b* represents blueness or yellowness of the sample (–50 being blue and +50 being yellow).

Table 2.12 Effect of storage on the colour of lemon myrtle 1.6 mm flakes

Storage month	Sampling date	L*	a*	b*	Chroma
0	22-Feb-12	45.67±0.49	-3.22 ± 0.69	23.38 ± 0.59	23.60 ± 0.64
2	23-Apr-12	46.60±0.43	-2.57 ± 0.97	23.32 ± 0.21	23.48 ± 0.29
4	22-Jun-12	42.51±0.45	-1.56 ± 0.82	22.12 ± 0.27	22.18 ± 0.26
6	22-Aug-12	44.40±1.36	-1.17 ± 0.42	22.08 ± 0.58	22.12 ± 0.60
8	22-Oct-12	45.42±0.98	-0.56 ± 1.12	22.85 ± 0.46	22.88 ± 0.48

Table 2.13 Effect of storage on the colour of anise myrtle 1.6 mm flakes

Storage month	Sampling date	L*	a*	b*	Chroma
0	22-Feb-12	46.55 ± 1.34	-3.01 ± 0.75	22.54 ± 0.41	22.75 ± 0.44
2	23-Apr-12	47.70 ± 1.29	-3.22 ± 0.15	22.81 ± 0.45	23.04 ± 0.46
4	22-Jun-12	46.27 ± 1.23	-2.83 ± 0.73	22.87 ± 0.83	23.05 ± 0.91
6	22-Aug-12	47.49 ± 0.26	-2.74 ± 0.85	22.87 ± 0.41	23.04 ± 0.44
8	22-Oct-12	47.80 ± 1.62	-2.87 ± 0.11	23.14 ± 0.36	23.31 ± 0.37

Table 2.14 Effect of storage on the colour of Tasmanian pepper leaf commercially milled samples

Storage month	Sampling date	L*	a*	b*	Chroma
1	19-Apr-12	43.98±1.59	-1.26±0.13	27.13±0.58	27.16±0.58
2	21-May-12	42.08±0.04	-0.34±1.07	27.06±0.27	27.08±0.27
3	19-Jun-12	46.49±0.78	-1.13±0.27	28.14±0.48	28.16±0.47
4	19-Jul-12	44.60±0.72	-0.71±0.78	26.99±0.66	27.00±0.68
5	20-Aug-12	43.56±1.10	-1.03±0.17	27.44±0.24	27.46±0.58
6	19-Sep-12	44.29±1.42	-1.02±0.31	26.86±0.57	26.88±0.58
7	19-Oct-12	42.91±0.47	-2.63±0.16	25.55±0.09	25.69±0.10

Table 2.15 Effect of storage on the colour of Tasmanian pepper leaf dried leaves

Storage month	Sampling date	L*	a*	b*	Chroma
0	21-May-12	40.92±1.35	-2.94±0.32	24.42±0.32	24.60±0.32
1	19-Jun-12	43.37±1.18	-2.48±0.30	26.25±0.48	26.36±0.50
2	19-Jul-12	42.85±0.19	-2.21±0.30	25.42±0.45	25.51±0.47
3	20-Aug-12	43.86±2.14	-2.70±0.09	25.90±0.46	26.04±0.45
4	19-Sep-12	42.49±1.35	-2.67±0.16	26.26±0.43	26.40±0.43

Unlike in the Kakadu and Davidson's plum (see Chapter 3) the antimicrobial and antioxidant activity of the native herbs decreased in all three packaging material in the 6 months of storage at ambient temperature of 22±2°C (Tables 2.8 to 2.11, Figures 2.18 to 2.26). This indicates that the bioactive compounds contributing to antimicrobial activity are reduced during the storage period.

The green colour of both anise myrtle (stored in high barrier packaging, specifications-inner layer PET (12 μm), middle layer metallised PET (12 μm) and outer layer LDPE (80 μm), refer to Table 2.4), and Tasmanian pepper leaf (packed in PET(12 μm)/ PET(12 μm)/Foil(9 μm)/LLDPE(65 μm), refer to Table 2.1) has been maintained without significant differences in the L*, a*, b* values over the 7 to 8 months of storage of the milled samples and 4 months of storage of the whole Tasmania pepper leaves (Tables 2.13 to 2.15). For the lemon myrtle (Table 2.12), there was a significant reduction in the negative a* value from –3.22 to –0.56, indicating a loss in green colour and this was visually observed when taking the samples every month for volatile analysis. The high-barrier packaging bag PET/PET/LDPE is not suitable for the storage of lemon myrtle but is successful in retaining the quality in anise myrtle and the PET/ PET/Foil/LLDPE is suitable for Tasmania pepper leaf.

3. Effects of processing and storage on the changes in quality and bioactivity of native fruits – value addition through freezing and drying

3.1 An introduction to freezing and some literature on changes in quality and bioactivity

Frozen fruits are an important processed food product in today's society. Freezing enables year-round processing of frozen whole fruit, slices or pulps into jams, juices and syrups. The quality of frozen fruits or frozen fruit products affects the value-added end product. If the frozen fruit is to be consumed as a whole fruit without further processing, then texture of the fruit is critical; if it is to be processed to a juice then the retention of flavour and colour are more important. The effect of freezing, frozen storage and thawing on fruit quality has been extensively investigated and these studies have included the influence of temperature on ice crystal formation, the effects of cell rupture on tissue texture and the effects of enzymatic reactions on odour and flavour as well as ascorbic acid and colour deterioration (Skrede 1996).

Fruits are processed to pulps, purees, juices and nectars using various processing operations prior to freezing. The advantage of these products is that they can be protected from oxidation but have the disadvantage of losing some of the nutritional and bioactive compounds during the production process (Venning et al. 1989). Fruits exposed to oxygen are subject to oxidative degradation, resulting in browning and reduced storage life of the products. Packaging of frozen fruits excludes air from the fruits tissue by the removal of oxygen using a vacuum and oxygen-impermeable film as the packaging material which retards degradation due to oxidation (Bissett et al. 1975).

Ice crystallisation can cause extensive microstructural changes to tissue foods like fruits during freezing. Rapid freezing of plant tissues results in the formation of small ice crystals which are uniformly distributed within the tissue and this minimises the formation of large ice crystals as experienced in slow freezing. Drip loss which occurs during thawing after frozen storage of the fruit is affected by the rate of freezing. Blast freezing which is a rapid form of freezing is recommended for retaining quality and reducing drip loss (Douglas Goff 1992).

In this study, both the native fruit products Davidson's plum and Kakadu plum the frozen whole fruit and puree is considered as an intermediate product for further value addition to functional ingredients like powders or composite food products such as jams, cordials and sauces. Further evaluations of the changes in quality and bioactivity of the whole fruit and pureed product will be determined during long-term frozen storage.

3.2 Effects of freezing and frozen storage on Davidson's plum

3.2.1 Introduction

Processors of Davidson's plum identified the need for value addition of fresh fruit to an intermediate product that could be used as the starting material for further value adding when there is a large harvest. For example, 2010 was a year when excess fruit was available for processing, and during such periods it is critical to produce intermediate products with extended storage life. Intermediate products identified by the industry were frozen halves and puree with a possible frozen storage life of 12–18 months. Frozen fruit should be similar to the fresh fruit with regards to chemical composition and bioactivity when thawed. The parts of the native food industry that value add by making sauces were interested in the effect cooking had on bioactivity, therefore cooking of the puree at 100°C for 20 minutes was included in this study to evaluate the effect of heat treatment on bioactivity.

3.2.2 Materials and methods

Davidson's plum (*Davidsonia pruriens*) was sourced from Rainforest Bounty Pty Ltd from far north Queensland. A batch of 25 kg of whole Davidson's plum harvested in August 2010 was transported by truck to Brisbane under refrigerated storage in plastic tubs. Commercial product of destoned halved fruit harvested in August 2010 packed in polyethylene bags of 1 kg weight was also transported to Brisbane under refrigerated storage. The commercial product was immediately frozen, in a storage freezer at a temperature of –20±2°C. The fresh whole fruit was immediately processed (see Section 3.2.2.1 below) and stored. There was a variation in the size and maturity of the fruit that were sent by the Rainforest Bounty Pty Ltd and this could affect the results of the study. Ooray Orchards also supplied two cultivars of Davidson's plum (*Davidsonia pruriens*) called 'highland' and 'lowland'.

3.2.2.1 Processing of Davidson's plum – halves and puree

Whole fruit was washed in potable water and cut into halves after destoning. The cut halves were placed on stainless steel trays and blast frozen (Blast freezer, CSK Climateck, Australia) at a temperature of –35°C for 30 minutes till the centre point on fruit half reached a temperature of –35°C, this was monitored using thermocouples.

The blast-frozen halves were divided into 1 kg lots (12) and placed in bags made of barrier packaging material polyethylene/nylon with 115 µm thickness, vacuum packed (Easy Vac Inc. USA) and frozen at –20±2°C. Some of the cut halves were processed to a puree using a juicing machine (model no-3600, Brown International Corp., USA) and blended again with a mixer (Dynamic mixer, France). The puree (500 g) was placed in plastic boxes and blast frozen at –35°C for 1 hour till the centre point reached a temperature of –35°C, this was monitored using thermocouples. The frozen puree block was then removed from the plastic box, placed in bags made of barrier packaging as given above and placed at –20±2°C.

During frozen storage, samples of Davidson's plum fruit (halves and puree) were assessed every month for antioxidant and antimicrobial activity over a 6-month period. Davidson's plum puree (2 kg) was placed in a steam jacketed kettle (Atherton, Australia) and cooked at 100°C for 20 minutes to determine the effect on bioactivity.

3.2.2.2 Processing of Davidson's plum – freeze drying

All fruits were delivered chilled. The chilled samples were destoned and immediately frozen at –80°C and freeze dried (freeze dryer Christ Beta 1-8 LD, Italy). The freeze-dried samples were finely ground using the cryomill and stored at –20°C until analysed (Figure 3.1).

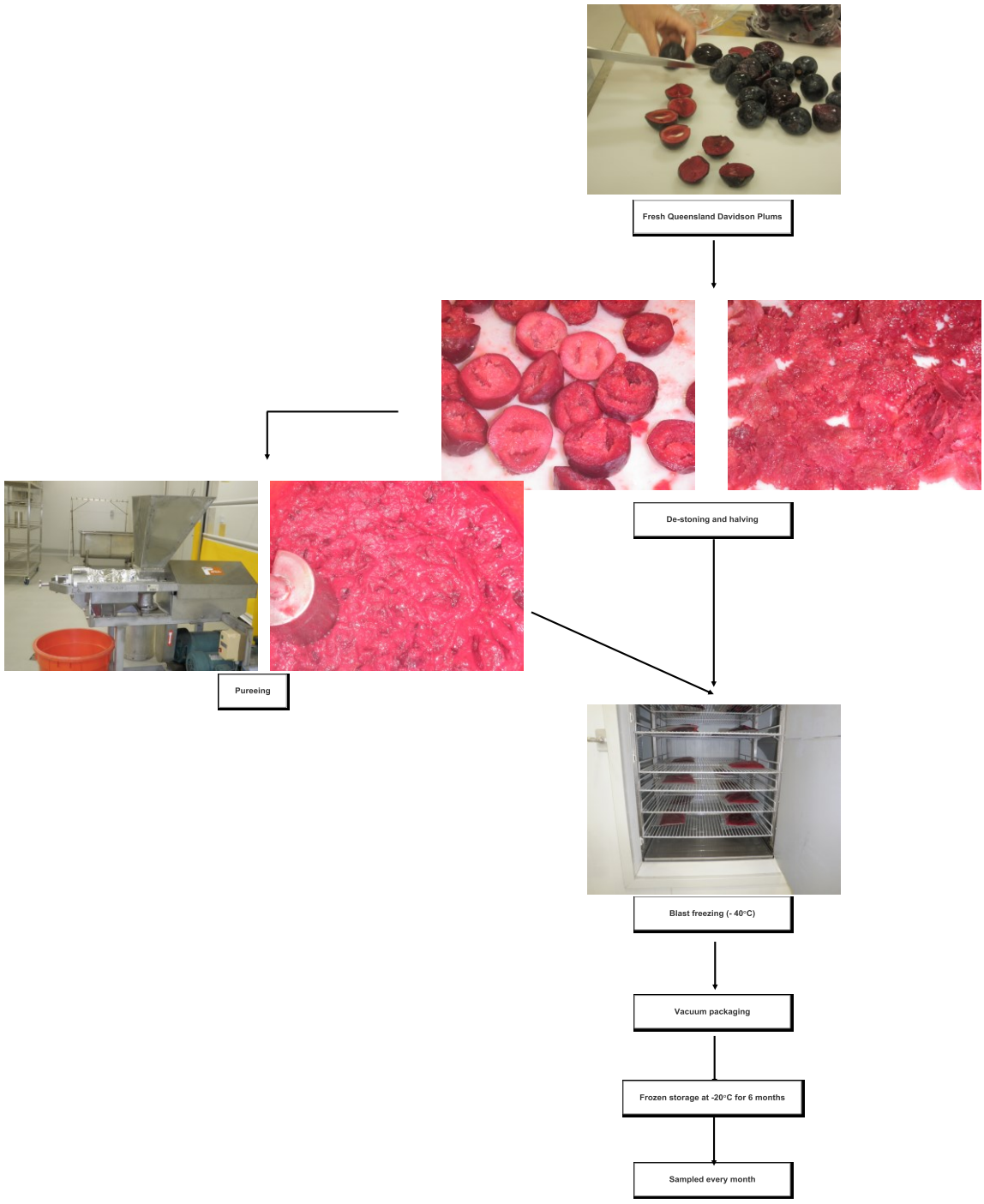


Figure 3.1 Flowchart depicting the frozen storage trial of Queensland Davidson's plum

3.2.2.3 Measurement of antioxidant activity during storage

3.2.2.3.1 Total phenolic content (TP)

The total phenolic content was determined using the Folin-Ciocalteu assay (Singleton & Rossi 1965). Diluted extracts were directly assayed at 600 nm using a spectrophotometer (Wallac, Labsystems Multiskan MS; Thermo Fisher Scientific, Waltham, MA, USA) with gallic acid as a standard. The analysis was conducted in 96-well flat-bottom microplates in triplicate and the results were corrected for vitamin C. Results were expressed as milligrams of total phenolics (gallic acid equivalents) per gram dry weight (mg GA Eq/g DW).

3.2.2.3.2 FRAP (ferric reducing antioxidant power) assay

The assay was conducted according to Benzie and Strain (1996) with minor modifications. Thirty µL of water and 10 µL extracts were placed in 96-well flat-bottom microplates and 200 µL FRAP reagent (ferric chloride and 2,4,6-tripyridyl-s-triazine) were added. The microplate was shaken for 60 seconds. Absorbance was measured after 4 min at 600 nm using a spectrophotometer (Wallac, Labsystems Multiskan MS; Thermo Fisher Scientific, Waltham, MA, USA). Reducing capacity was calculated using the absorbance difference between the sample and a blank and a further parallel Fe(II) standard solution. Results were expressed as micromoles of Fe^{2+} equivalents per gram dry weight (µmol Fe^{2+} Eq/g DW).

3.2.2.4 Measurement of antimicrobial activity during storage

3.2.2.4.1 Accelerated solvent extraction (ASE) method

An accelerated solvent extraction (Dionex ASE 200 (Dionex Corp., Sunnyvale, CA) system was used. Aliquots of freeze-dried Davidson's plum samples (1.0 g) were mixed with diatomaceous earth and placed in an 11 mL stainless steel extraction cell. The use of a dispersion agent, such as diatomaceous earth, is recommended to reduce the solvent volume used for the extraction. The cell containing the sample was prefilled with the extraction solvent, pressurised, and then heated (preheating period = 5 min). The sample was extracted by six extraction cycles with acetone and ethanol at 60°C and water at 80°C at 1000 psi. Then, the cell was rinsed with fresh extraction solvent (60% of the extraction cell volume) and purged with a flow of nitrogen (150 psi during 90 s). The extract was collected into 60 mL amber glass vials. The solvent used was previously degassed to avoid the oxidation of the analytes under the operating conditions. The extracts were stored at –20 °C in darkness until antimicrobial analysis. The collected extracts were concentrated in a miVac sample concentrator (GeneVac Inc., NY, USA) at 45°C (for acetone and ethanol extracts) and 65°C (for water extracts). Concentrated water extracts were reconstituted in 2 mL distilled water. Ethanol and acetone extracts were reconstituted using 5 mL of 20% ethanol. The reconstituted extracts were used for determining antimicrobial activity.

3.2.2.4.2 Measurement of microbial inhibition (antimicrobial assay)

Food related bacteria strains *S. aureus* strain 6571 (NCTC – National Collection of Type Cultures, Health Protection Agency Centre for Infection, London, UK) and *E. coli* strain 9001 (NCTC) were used for the screening tests. These organisms were grown in tryptone soya yeast extract broth (TSYEB) (CM0129 with the addition of 6g/L yeast LP0021, Oxoid, Basingstoke, UK) for 24 h at 37ºC. The overnight growth of the culture was quantified to an absorbance reading of 0.5 at 540 nm using a spectrophotometer (Unicam, Helios alpha, UK) by diluting with TSYEB to get an inoculum having 105 CFU/mL for use in the assay.

3.2.2.5 Measurement of drip loss

Drip loss was calculated as:

Percentage drip loss = [Weight of the whole sample (halved fruits + liquid exudate)/weight of the halved fruits] X 100

3.2.3 Results and discussion

3.2.3.1 Changes in antioxidant activity

Table 3.1 Average levels of total phenolic compounds and antioxidant capacity (FRAP values) of Davidson's plum samples

	Total phenolics (GA Eq/g DW)		FRAP (µmol Fe^{+2} Eq/g DW)	
Sample	Average	SD	Average	SD
Qld Davidson's plum, highland (Ooray Orchard, NSW) fresh	61.2	2.26	1011.0	107.8
Qld Davidson's plum, lowland (Ooray Orchard, NSW) fresh	37.3	2.66	762.1	42.1
Qld Davidson's plum, lowland (Cairns) fresh fruit	40.0	1.47	788.3	18.3
Qld Davidson's plum, comm. sample (Cairns) frozen by client at –20ºC	33.1	2.46	650.3	55.1
Qld Davidson's plum, lowland (Cairns) fresh fruit, puree, cooked	38.7	1.09	921.3	218.7

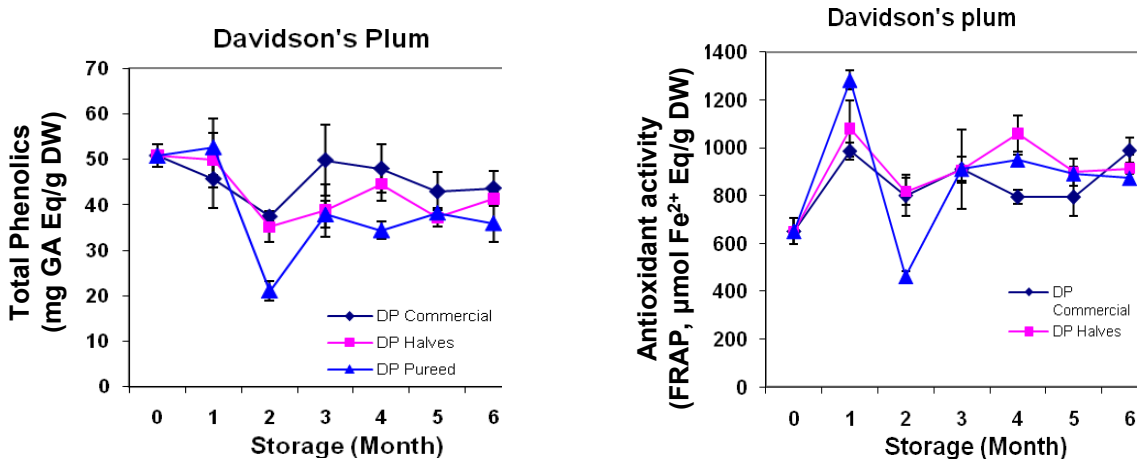


Figure 3.2 Changes in antioxidant capacities of Davidson's plum samples during storage

Table 3.2 Changes in antioxidant activity during storage of Davidson's plum after different processing

Month	Qld D. plum, commercial		Qld D. plum, halves frozen		Qld D. plum, puree frozen	
	TP	FRAP	TP	FRAP	TP	FRAP
1	45.71±6.3	986.42±36.2	49.82±5.9	1080.06±117.8	52.72±6.1	1282.14±39.8
2	37.44±1.1	800.54±87.6	35.15±3.4	816.71±56.3	21.07±2.2	461.66±20.4
3	49.79±7.7	910.84±165.1	38.80±5.8	905.69±54.6	37.87±3.0	910.28±50.47
4	47.96±5.4	794.77±28.6	44.54±3.7	1058.77±75.1	34.40±1.9	948.82±21.1
5	42.93±4.2	794.33±81.1	37.14±1.9	897.93±56.1	38.26±1.1	891.66±28.0
6	43.68±3.7	988.94±53.8	41.33±1.5	913.44±56.6	35.99±4.1	872.43±17.2

3.2.3.2 Changes in antimicrobial activity

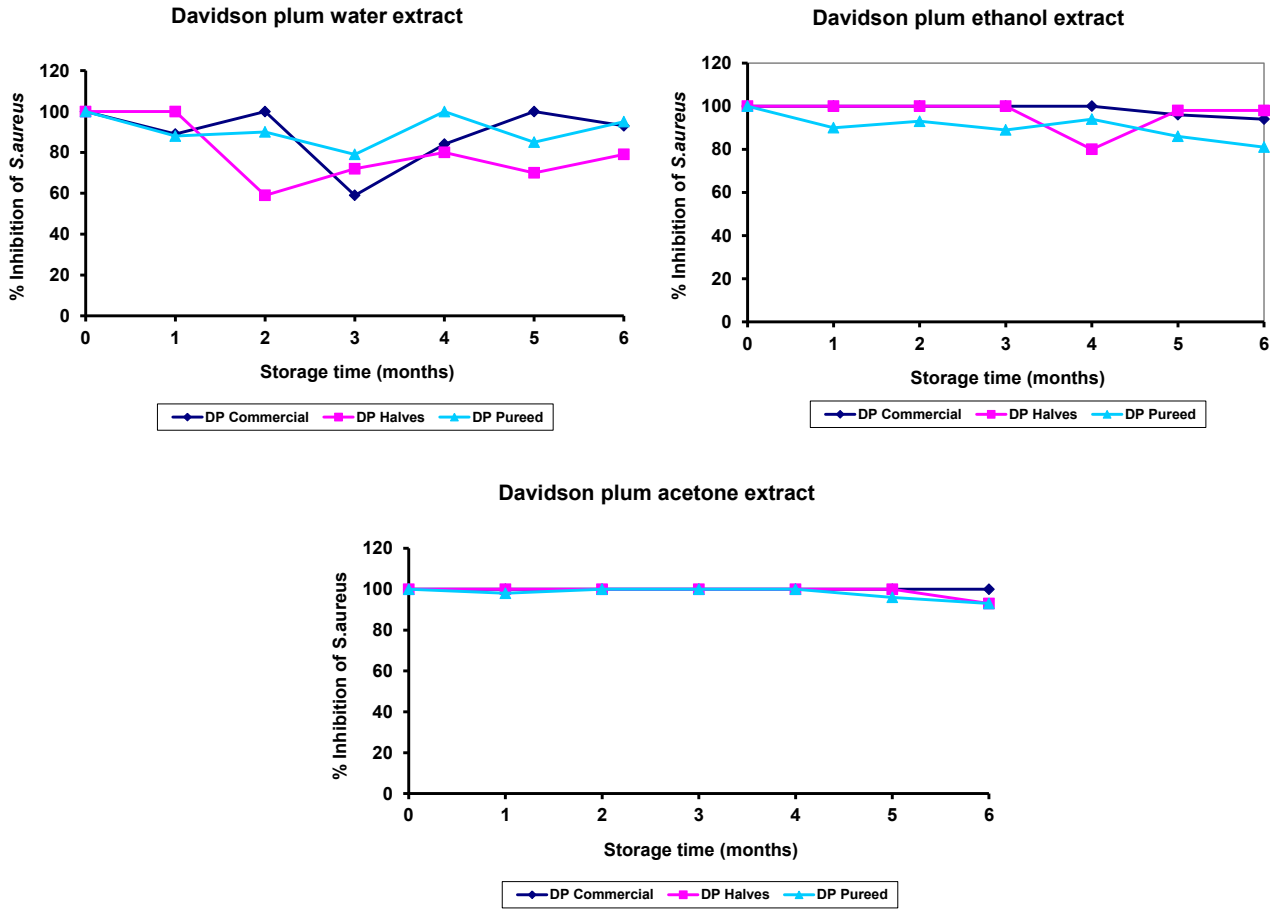


Figure 3.3 Changes in antimicrobial activity of different extracts of Davidson's plum (at a concentration of 8.75% v/v) over 6 months of storage, as measured by inhibition of *Stahylococcus aureus*

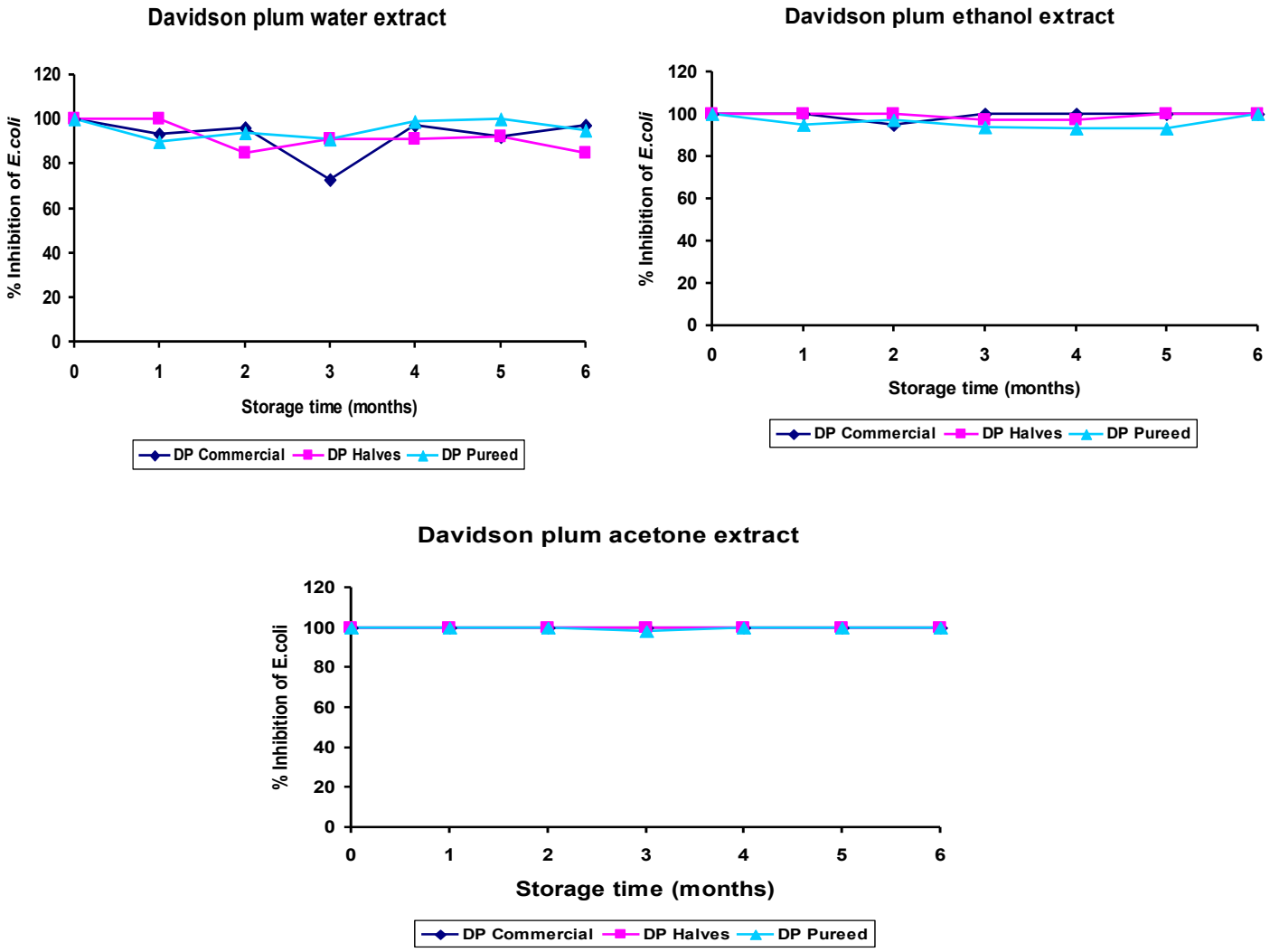


Figure 3.4 Changes in antimicrobial activity of different extracts of Davidson's plum (at a concentration of 8.75% v/v) over 6 months of storage, as measured by inhibition of *Escherichia coli*

3.2.3.3 Drip loss

Drip loss during month 3 and month 4

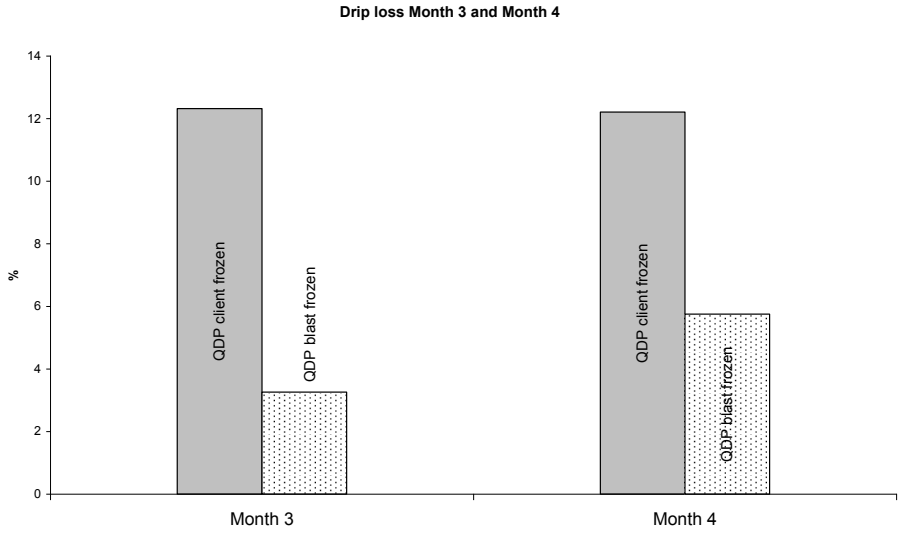


Figure 3.5 Drip loss during month 3 and month 4 for blast-frozen or client frozen Queensland Davidson's plum

Davidson's plum 'highland' from New South Wales had significantly higher total phenolics levels and antioxidant capacity (FRAP values, Table 3.1, Figure 3.2) than all other samples. The 'lowland' Davidson's plum from northern Queensland (Cairns) and New South Wales had similar total phenolics levels and FRAP values. The differences between the highland and lowland Davidson's plums indicate the differences in antioxidant capacities among the cultivars. Similarly, significant differences in total phenolics and antioxidant capacities have been reported for four cultivars of red raspberry fruit (Krüger et al. 2011). Further studies are needed to determine the physicochemical differences and health attributes of Davidson's plum in order to identify the most suitable cultivars to supply to a range of end-user markets.

Cooking of the Davidson's plum puree increased the FRAP values by 16.8% (from 788.3 to 921.3 µmol Fe^{+2} Eq/g DW) (Table 3.1). This increase could be due to degradation during heat treatment of complex phenolics present in Davidson's plum into monomers (which exhibit higher total antioxidant capacity than the original polymeric compounds) or to an accumulation of Maillard reaction products. In agreement, Dini et al. (2013) reported an increase in the FRAP values when pumpkin pulp was steamed and boiled and this increase was attributed to the production of redox-active secondary metabolites.

The retention of total phenolics and FRAP activities during storage in both the commercial and processed plum halves was over 80%, while in the puree it was only 68%, indicating a greater loss of antioxidant activity in the frozen puree samples (Table 3.2). This result suggests that the level of physical injury to the skin, which is the natural protective barrier, and to the fruit tissue during processing are negatively correlated with the retention of phytochemicals and their antioxidant activities. Similarly Patthamakanokporn et al. (2008) reported a continuous decrease in total phenolics levels and oxygen radical absorbance capacity (ORAC) in homogenised guava (*Psidium guajava*). They found that at the end of 3 months frozen storage at –20°C, the retention of polyphenols and

ORAC was 69% and 61% respectively, and there was a 100% retention in FRAP values. A possible explanation for the decrease in total polyphenols is the reaction of the endogenous polyphenol oxidase during frozen storage at –20°C. This could result in the observed gradual reduction of the level of phenolic compounds in guava during storage in the freezer; this homogenisation process had an effect on ORAC values but not the FRAP values. Phenolic compounds are also water-soluble and oxygen-labile. It has been reported previously that frozen products lose fewer nutrients initially because of the short heating time in blanching, but they lose more nutrients during storage owing to oxidation. (Rickman et al. 2007). A similar effect could have occurred here when the Davidson's plum was pureed and stored.

This study indicates that pureeing the Davidson's plum and then freezing it resulted in a greater loss of antioxidant activity in comparison to the frozen halves of the fruit. Antioxidant values between the commercial and processed Davidson's plum samples could not be compared due to the variation in maturity of the fruits.

The ethanol and acetone extracts of Davidson's plum applied at a concentration of 8.75% (v/v) showed complete inhibition of *S. aureus* (Figure 3.3) and *E.coli* (Figure 3.4). A greater than 90% inhibition was observed in the water extract at the same concentration. The antimicrobial activity of the fruit halves and puree were stable during the 6-month storage period. This indicates the stability of the bioactive compounds contributing to the antimicrobial activity and the potential of using Davidson's plum as a natural antimicrobial in food applications.

There was significant drip loss from the commercial sample in comparison to the blast-frozen samples during frozen storage (Figure 3.5); this could have an effect if the frozen halves are to be dehydrated. The effect would be minimised in a pureed product if all the drip loss is incorporated into the puree. Blast freezing, which is a rapid form of freezing, has been successful in retaining quality and reducing drip loss.

3.3 Effects of freezing and frozen storage on Kakadu plum

3.3.1 Introduction

Kakadu plum has been identified as an excellent source of natural vitamin C and high antioxidant activity (Konczak et al. 2010b). The following value-added products of Kakadu plum are commercially available: freeze-dried powder; liquid extract and frozen puree. The Kakadu plum processing industry is interested in understanding the changes in bioactivity of whole Kakadu plum and puree during frozen storage to develop these as intermediate frozen products for further value addition. At present the industry is unable to estimate a frozen storage life for these products. Having intermediate frozen products with extended storage life will be of benefit to the industry as it will minimise post-harvest losses and will enable value addition throughout the year. This study will focus on the changes in bioactivity of whole Kakadu plum and puree during frozen storage.

3.3.2 Materials and methods

Kakadu plum was sourced from an Australian produce company, samples were from the Northern Territory harvest (March–June) from a 2008 batch of fruit stored at –18°C. The samples of Kakadu plum were packed in 1 kg polyethylene bags and sent to Brisbane under frozen storage. On arrival the samples were immediately transferred to a –20±2°C freezer and samples taken out for analysis every month for a period of 6 months, this storage trial commenced in August 2010. The Kakadu plum harvesting period is in January and since the project commenced after the harvesting period it was decided to source fruit in January 2011for the processing trial. Due to the floods affecting Queensland during this period it was not possible to source fresh whole Kakadu plum samples and thus processing was postponed until March 2012. Samples of wild harvest, whole Kakadu plum fruits (10 kg) from the 2012 harvest were transported chilled to Brisbane from the Northern Territory and Kakadu plum puree (4 kg) also from the Northern Territory was transported under frozen storage to Brisbane.

3.3.2.1 Processing of Kakadu plum

Both the fresh whole fruit and puree (sourced from Coradji Pty Ltd) were divided into 800 g lots (12) of whole fruit and 300 g lots (12) of Kakadu plum puree. The whole fruit and puree was frozen as described in Section 3.2.2.1 (see also Figure 3.6). The blast-frozen samples were stored at –20 ± 2°C and samples removed every month to evaluate bioactivity and phytochemical changes. Samples were stored for a period of 10 months.

3.3.2.2 Measurement of antioxidant and antimicrobial activity

Measurement of antioxidant activity was carried out as described in Section 3.2.2.3 and antimicrobial analysis was carried out as described in Section 3.2.2.4.

Figure 3.6 Flowchart depicting frozen storage trial of Kakadu plum

3.3.3 Results and discussion

3.3.3.1 Changes in antioxidant activity

Table 3.3 Changes in antioxidant activity of commercial whole Kakadu plum during frozen storage

Month	Total phenolics (µmol GA Eq/g DW)	Antioxidant activity (µmol Fe^{+2} Eq/g DW)
0	230.5±10.1	4583.0±23.4
1	188.1±6.8	4289.5±91.6
2	190.3±20.6	4040.1±132.8
3	183.9±6.2	4042.1±131.4
4	191.2±19.2	4074.0±131.8
5	194.8±9.8	3443.5±178.0
6	191.7±12.1	3432.1±225.3

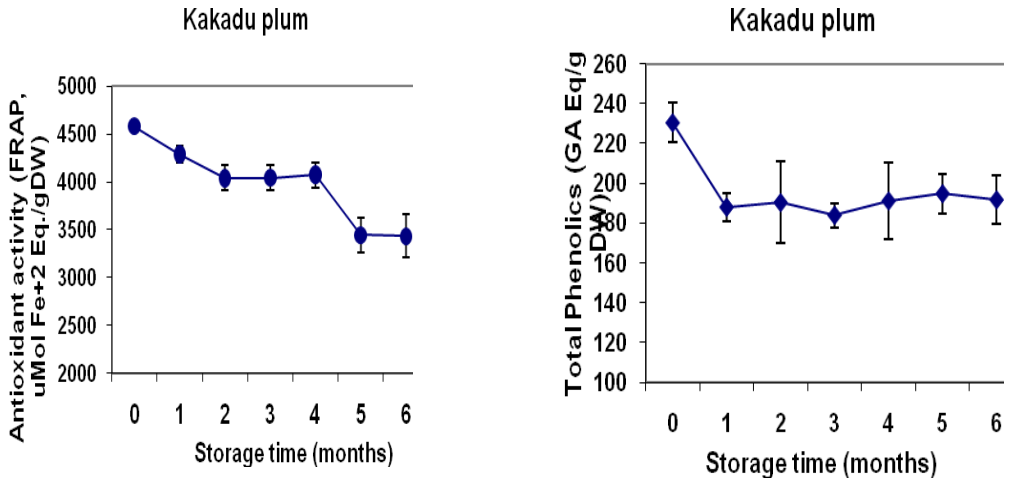


Figure 3.7 Changes in antioxidant activity of commercial whole Kakadu plum during frozen storage

3.3.3.2 Changes in antimicrobial activity

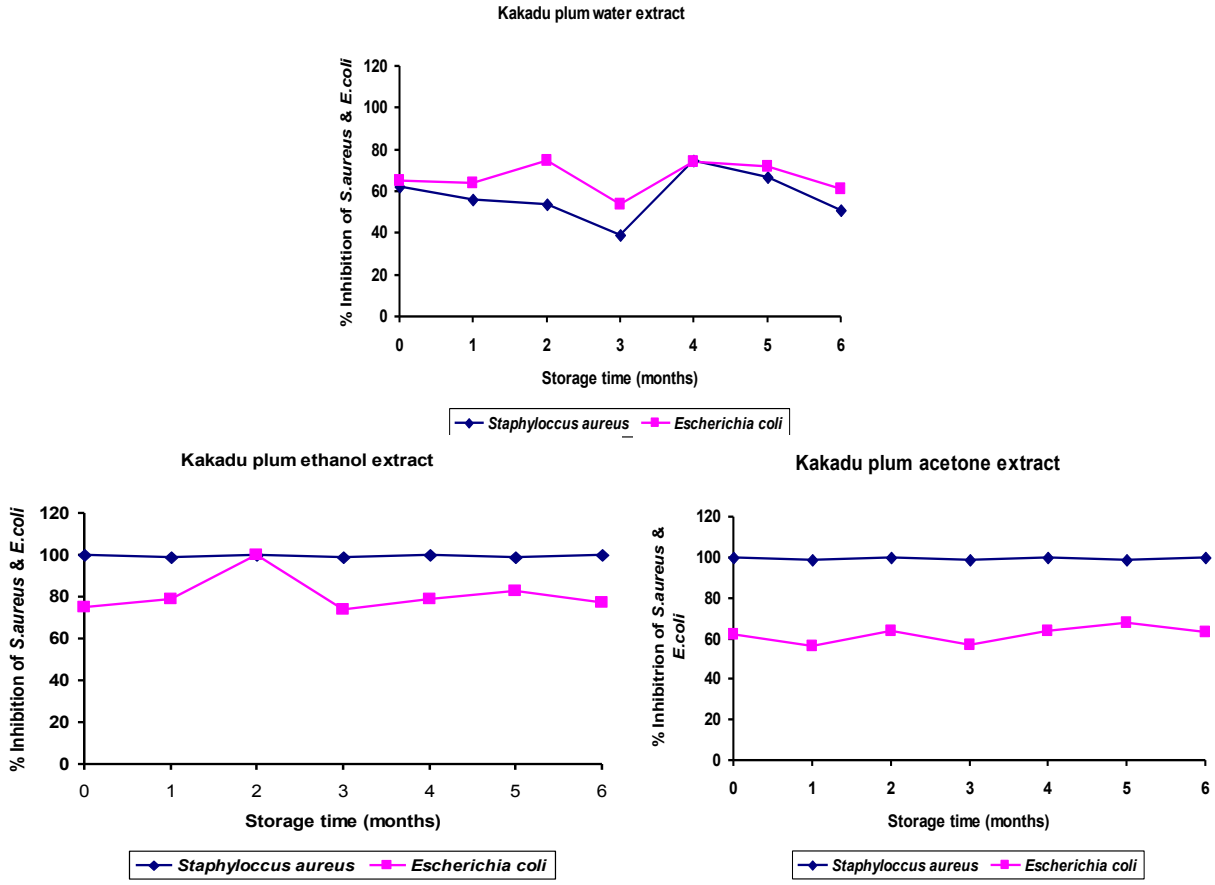


Figure 3.8 Changes in antimicrobial activity of different extracts of Kakadu plum (at a concentration of 8.75% v/v) during 6 months of storage, as measured by inhibition of *Staphylococcus aureus* and *Escherichia coli*

3.3.3.3 Changes in antimicrobial activity of whole and pureed Kakadu plum over 10 months of storage

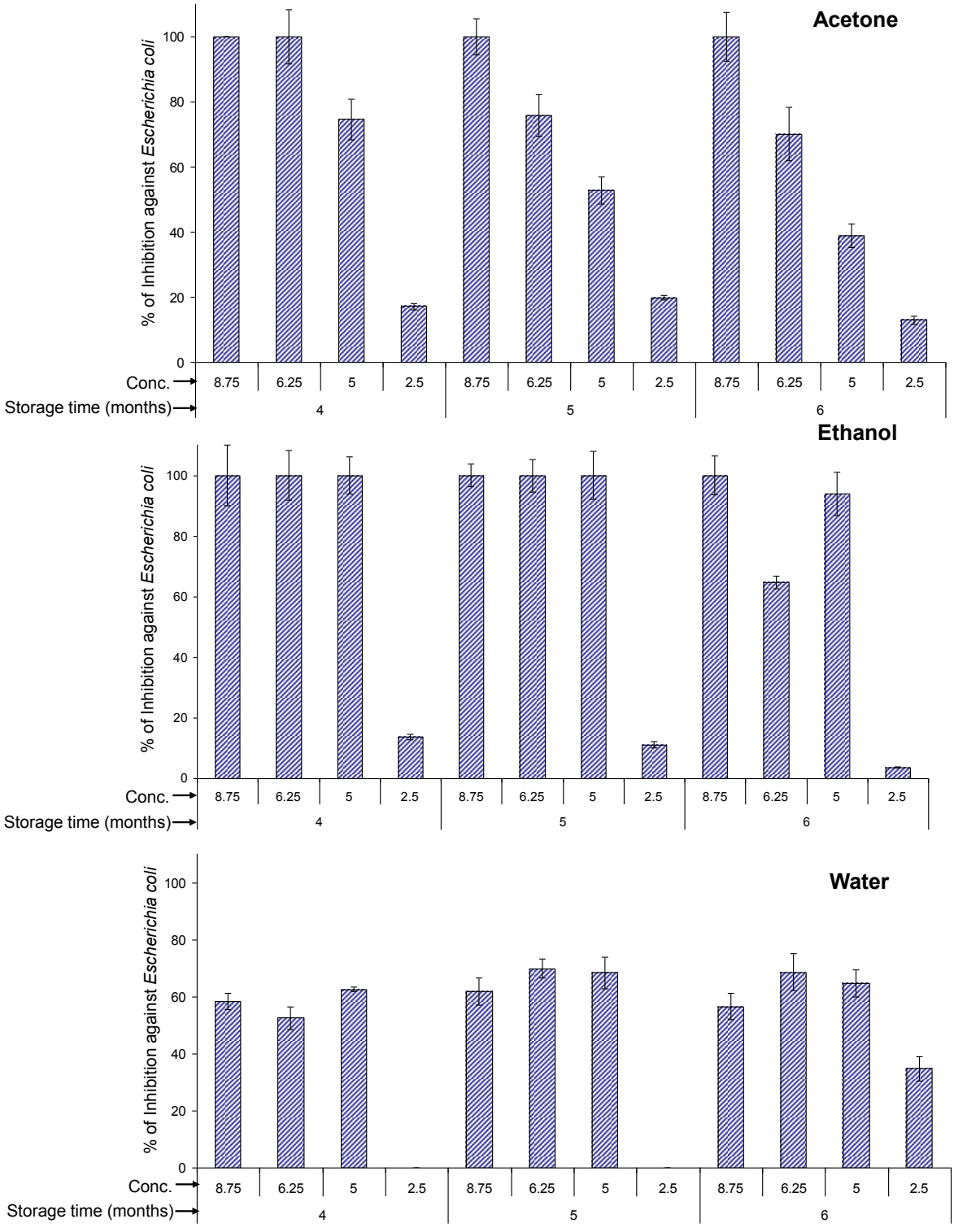


Figure 3.9 Antimicrobial activity of Kakadu puree extracts against *Escherichia coli* during 4, 5 and 6 months of storage at a concentration ranging from 2.5 to 8.75% (v/v)

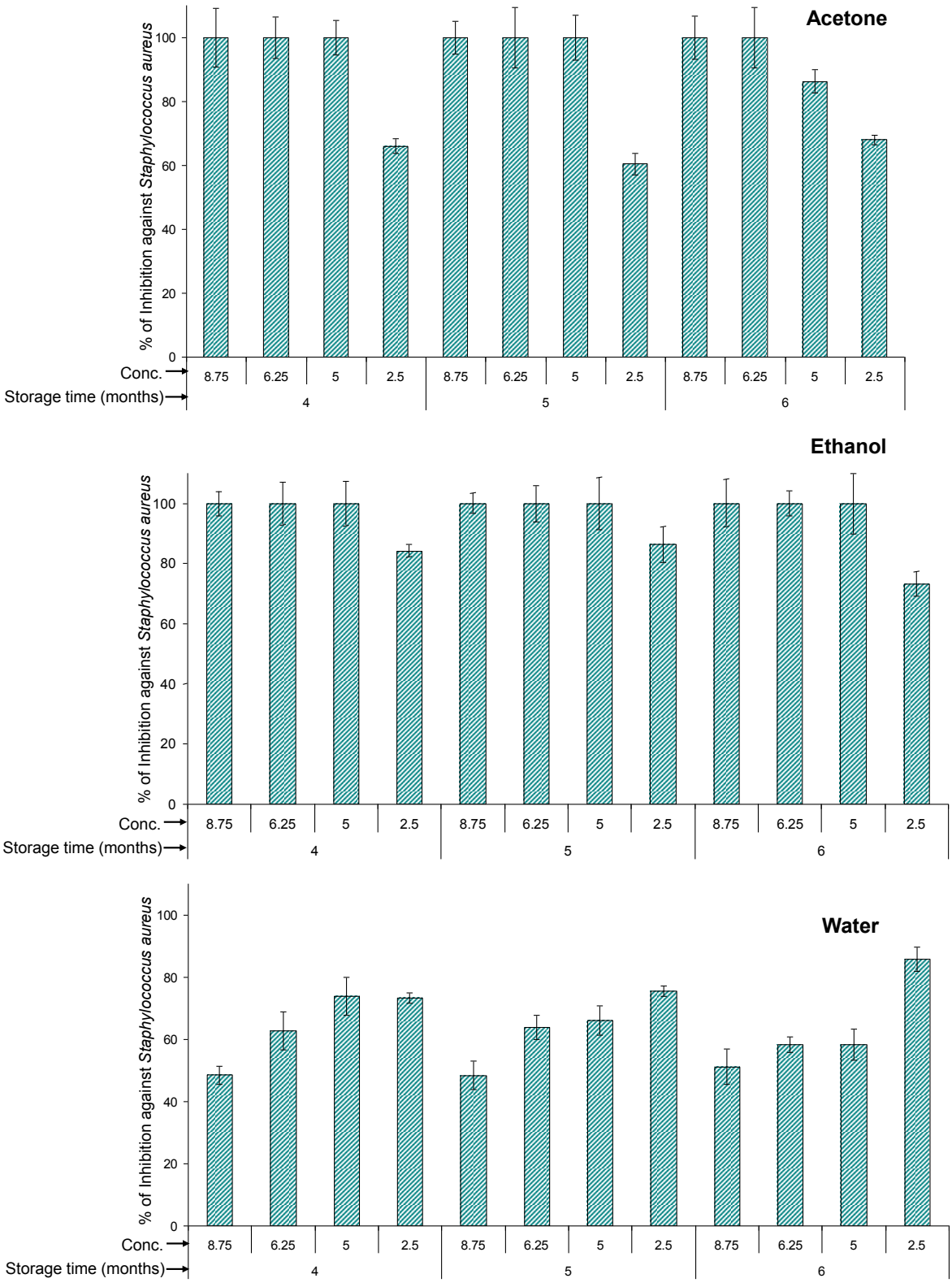


Figure 3.10 Antimicrobial activity of Kakadu puree extracts against *Staphylococcus aureus* during 4, 5 and 6 months of storage at a concentration ranging from 2.5 to 8.75% (v/v).

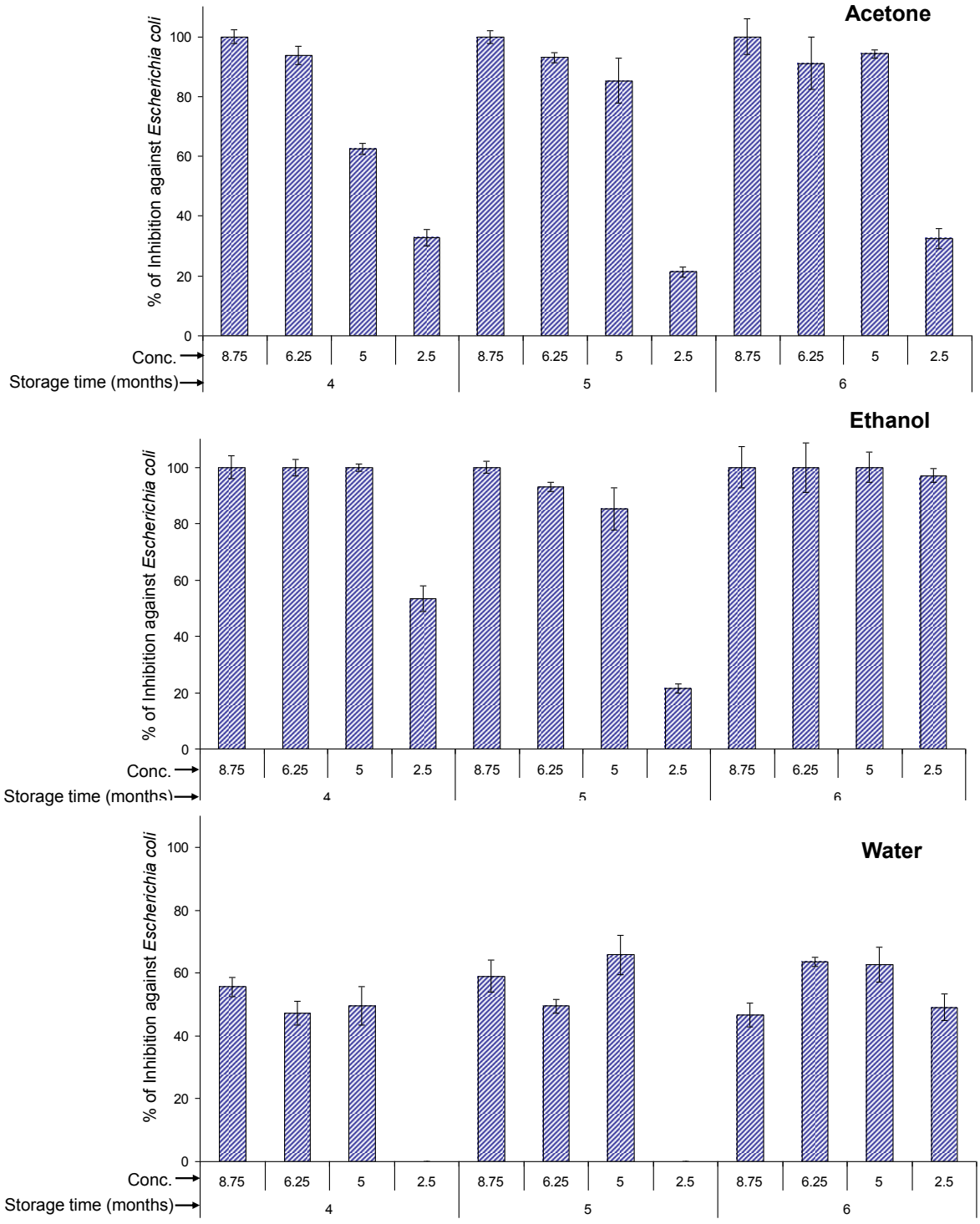


Figure 3.11 Antimicrobial activity of Kakadu whole extracts against *Escherichia coli* during 4, 5 and 6 months of storage at a concentration ranging from 2.5 to 8.75% (v/v)

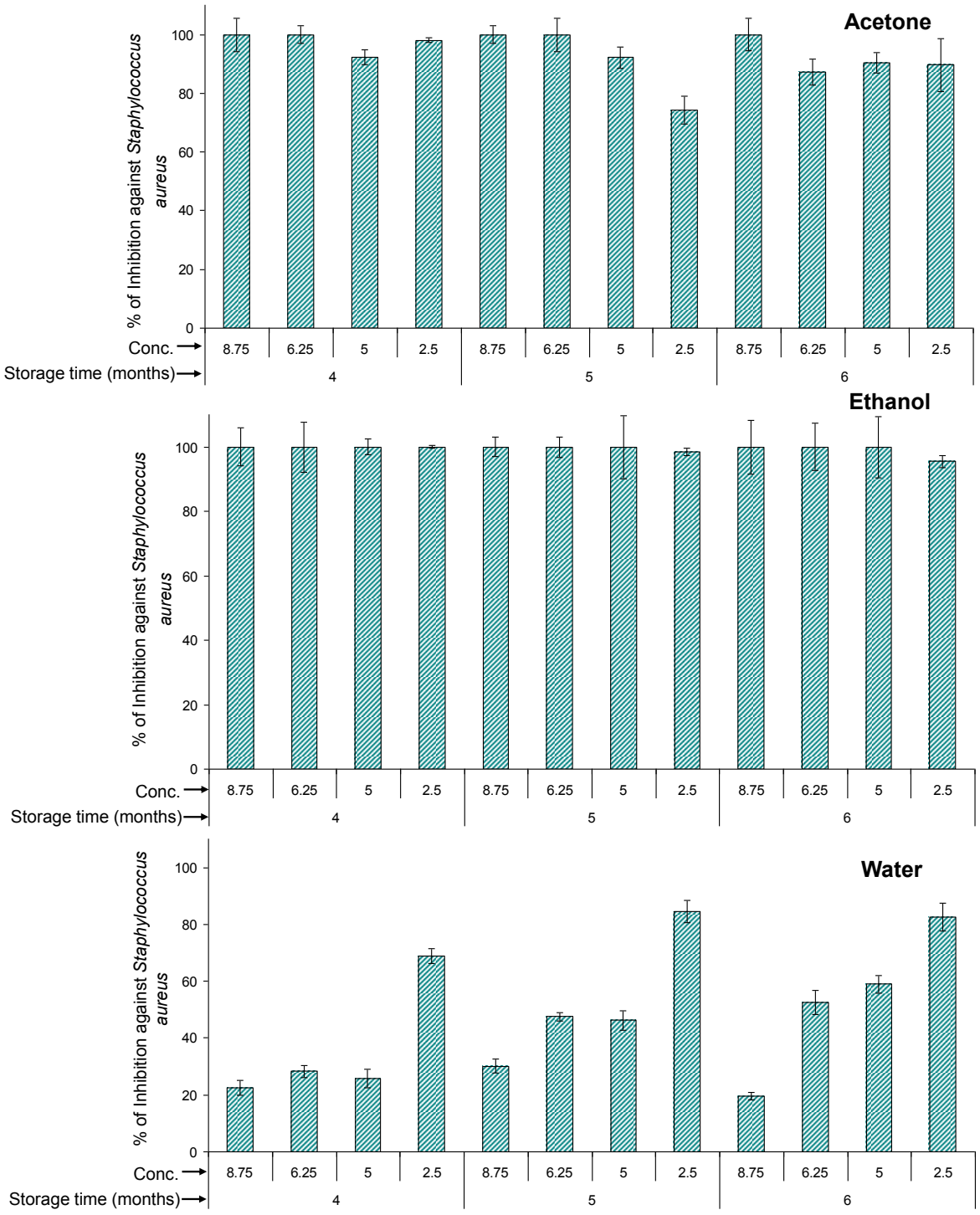


Figure 3.12 Antimicrobial activity of Kakadu whole extracts against *Staphylococcus aureus* during 4, 5 and 6 months of storage at a concentration ranging from 2.5 to 8.75% (v/v)

Retention of total phenolics in the commercial whole Kakadu plum over a 6-month frozen storage period was greater than 80% and correlated well with the retention of antioxidant capacity (FRAP values, retention of 75%; Table 3.3, Figure 3.7). Overall, a reduction in antioxidant activity of the whole fruit occurred during frozen storage. This reduction was lower than that observed in the frozen halves of Davidson's plum. These results clearly showed that the stability and retention of total phenolics and antioxidant activity in Kakadu plum is better than that in Davidson's plum after 6 months of frozen storage. The high vitamin C content in Kakadu plum and the low pH value of the fruit may have a positive effect on the stability of the phytochemicals.

Vitamin C was one of the first compounds to be studied in relation to the quality of frozen fruits. As ascorbic acid is a reactive compound, it also serves as an indicator substance for chemical reactions taking place in the product. The oxidation of ascorbic acid can be enzymatic or nonenzymatic and occurs in the presence of oxygen (Skrede 1996). A study by Sahari et al. (2004) indicated a loss of ascorbic acid when strawberries were stored at different temperatures. According to this study the major losses of ascorbic acid occurred during the first 15 days of storage and the percentage were 64.5, 10.7 and 8.9 at –12,–18 and –24°C respectively. No statistical significant differences were observed between –18 and –24°C. The total vitamin C content off Kakadu plum whole fruits and puree was assessed as part of the storage trial of 10 months and those findings are presented and discussed in Section 4.2.

The ethanol and acetone extracts of Kakadu plum blast-frozen whole fruit and puree at a concentration of 8.75% (v/v) showed complete inhibition of *S. aureus* (refer to Figure 3.8). The antimicrobial properties of both ethanol and acetone extracts of Kakadu plum were more effective against the Gram-positive *S. aureus* than the Gram-negative *E.coli*. The Kakadu plum water extract showed a much lower inhibition of both *S. aureus* and *E. coli* at the same concentration. The antimicrobial activity of the whole fruit and puree were stable during the 10-month storage period, (as indicated by Figures 3.9 to 3.12). This indicates the stability of bioactive compounds contributing to antimicrobial activity and the potential of using Kakadu plum as a natural antimicrobial in food applications.

3.3.4.2 Conclusion

The freezing performances of both Davidson's plum and Kakadu plum have indicated a loss in total phenolics and antioxidant activity during the 6-month storage period. In Davidson's plum the loss in total phenolics and antioxidant activity was higher in the puree than in the frozen halves. The antimicrobial activity of both Kakadu plum and Davidson's plum was stable over the frozen storage period, making it an attractive intermediate product for value addition to functional ingredients with antimicrobial activity.

3.4 Effect of drying, packaging and storage on the bioactivity of quandong

3.4.1 Introduction

Drying is one of the oldest methods of preserving food and is an essential process in the preservation of agricultural products. The drying process reduces the food's moisture content to a level which allows storage of the dehydrated product for a long period of time at ambient temperature. Drying also reduces the weight of the product and the cost of packaging, storage and transportation. The removal of moisture prevents the growth of microorganisms and minimises the degradation of product quality(Wankhade et al. 2013). There are many methods used for drying fruits and vegetables, currently the most popular is hot air drying, which is a simple convective method for drying (Bazyma et al. 2006). The drying temperatures used in hot air drying can result in phenol degradation but this can give a better product with a higher antioxidant and polyphenol content as demonstrated in the drying of Cafona cultivar of apricot (Madrau et al. 2009). Packaging is critical in retaining the quality of the dried product, and barrier packaging is recommended for the storage of these products to prevent oxidation and the re-absorption of moisture into the product from the atmosphere.

The aim of this study was to assess the effect of drying temperature on polyphenolic content and antioxidant and antimicrobial activity of quandongs. These native fruits are highly perishable and must be stabilised immediately after harvesting by further processing to reduce post-harvest losses. The native food industry has been using drying as a technique to increase the shelf life of the product and processors of quandong identified drying as an area that needs further improvement. At the present time the drying process is not optimised and changes in bioactivity after drying and during storage is an area that needs further investigation. The hot air drying method was used to assess the effect on bioactivity of quandongs and the study was not targeted at improving the colour of the end product.

3.4.2 Materials and methods

Quandongs (25 kg) were sent as destoned halves under chilled storage from Outback Pride (South Australia) in October 2010. A fair amount of discolouration (browning) had taken place when the samples arrived in Brisbane. The product was washed in potable water and immediately blast frozen as showed in Figure 3.13 and then processed from 22–26 November 2010 and stored at ambient temperature.

3.4.2.1 Processing of quandongs

A pilot-scale hot air dryer (Lindner + May, Queensland, Australia) was used for drying the quandongs. A sample weight of halved quandongs (550–575 g) was placed on each tray of the dryer and dried at an air temperature of 40, 50 and 60°C. The average thickness was 3.2 mm and the average diameter was 28.1 mm. The air velocity was 2 m/s and relative humidity (RH) was between 21 and 25%. Each tray was removed at 2-hour intervals and the weight recorded manually; drying was continued until a constant weight was reached. This was repeated for 5 kg of fresh product at each temperature. The final yield at each temperature was about 25% and the moisture of the dried product was less than 10%. The commercial sample moisture content was 17.86%. The final product 12 packages (150 g) from each temperature was packed in barrier packaging material polyethylene/nylon with 115 μm thickness and vacuum packed (Easy Vac Inc. USA) and stored at 22±1°C (Figure 3.13).

3.4.2.2 Measurement of antioxidant and antimicrobial activity during storage

Measurement of antioxidant activity was carried out as described in Section 3.2.2.3, and antimicrobial analysis was carried out as described in Section 3.2.2.4.

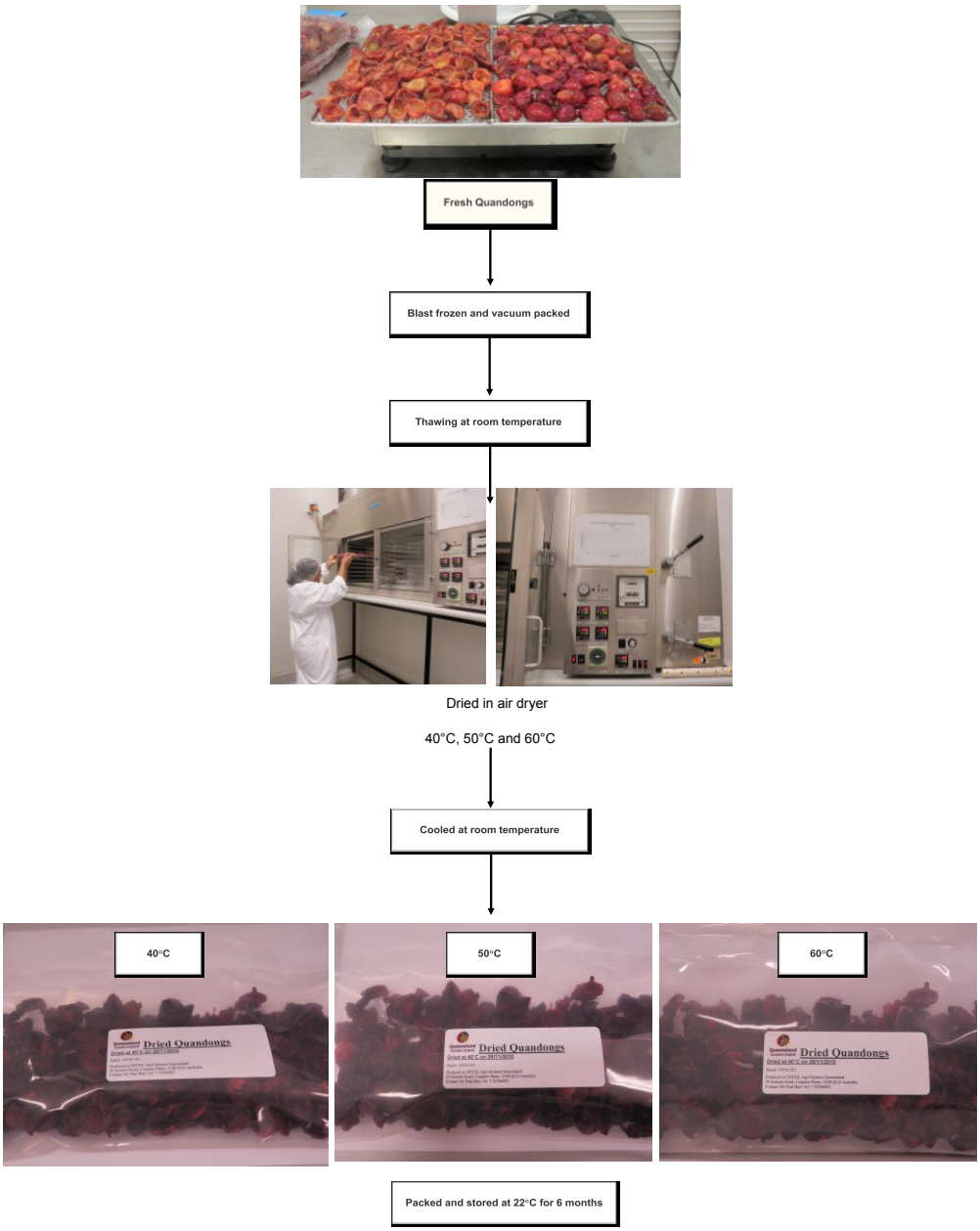


Figure 3.13 Flowchart depicting the dehydration trial for quandongs

3.4.3 Results and discussion

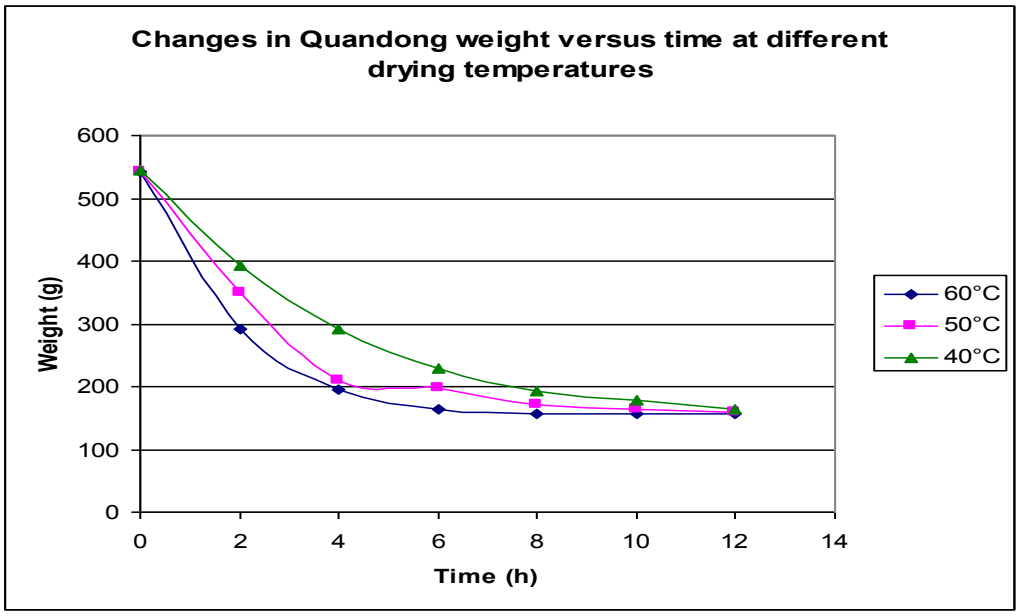


Figure 3.14 Changes in quandong weight versus time at different drying temperatures

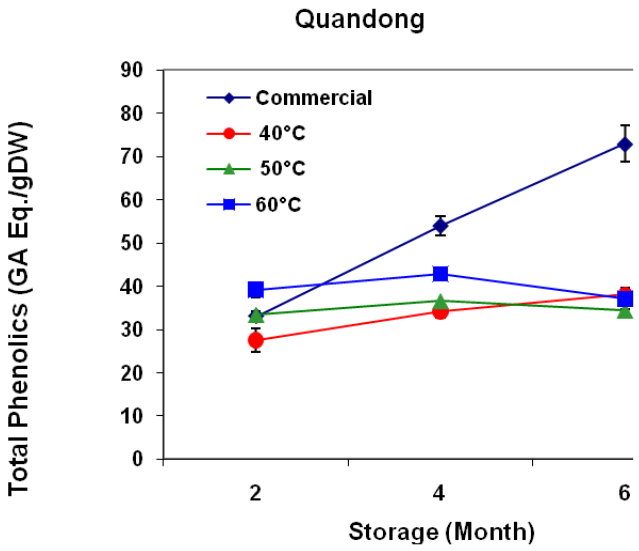


Figure 3.15 Changes in total phenolics (mg GA Eq/g DW) over storage time of quandong dried at different drying temperatures

Table 3.4 Changes in total phenolics (mg GA Eq/g DW) over storage time of quandong dried at different drying temperatures

Treatment	Month		
	2	4	6
Commercial	33.1±1.0	54.0±2.2	72.9±4.2
40°C	27.6±2.6	34.2±1.5	38.1±1.7
50°C	33.4±3.7	36.6±1.0	34.4±2.1
60°C	39.2±1.8	42.8±1.5	37.1±2.4

Table 3.5 Changes in FRAP (µmol Fe^{+2} Eq/g DW) over storage time of quandong dried at different drying temperatures

Treatment	Month		
	2	4	6
Commercial	552.0±24.7	962.5±29.1	1223.8±10.3
40°C	417.3±47.2	502.6±21.2	541.9±28.9
50°C	545.9±66.2	551.4±45.8	522.3±35.6
60°C	581.1±38.0	624.4±43.1	556.7±20.6

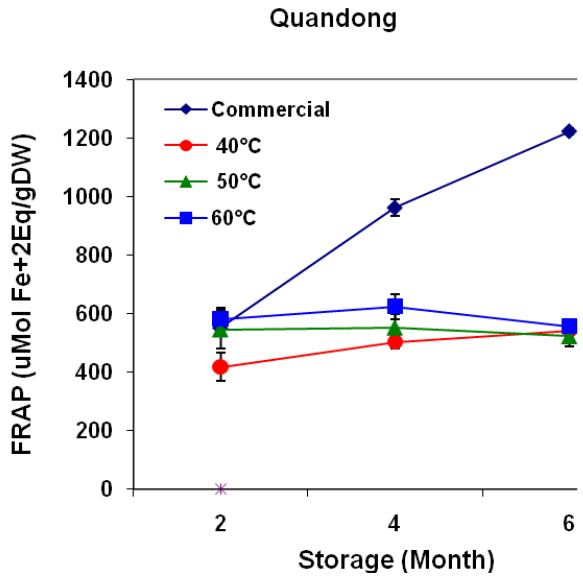


Figure 3.16 Changes in FRAP (µmol Fe^{+2} Eq/g DW) over storage time of quandong dried at different drying temperatures

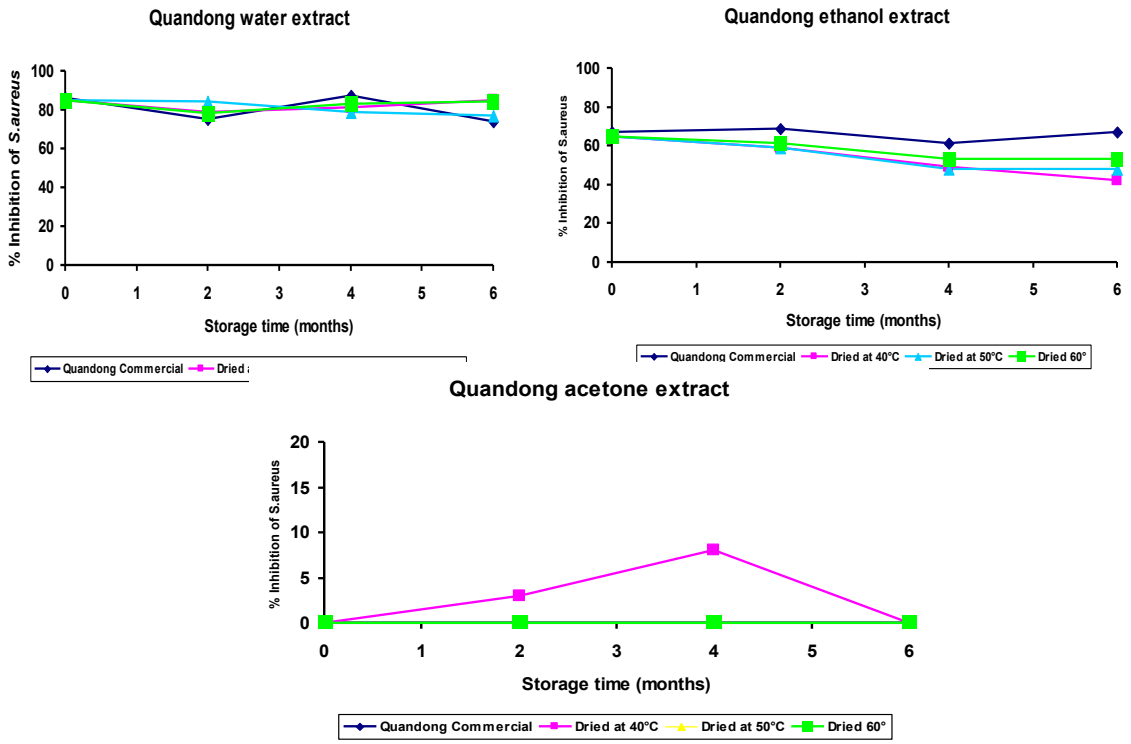


Figure 3.17 Changes in antimicrobial activity of different extracts of quandong (at a concentration of 8.75% v/v) during 6 months of storage, as measured by inhibition of *Staphylococcus aureus*

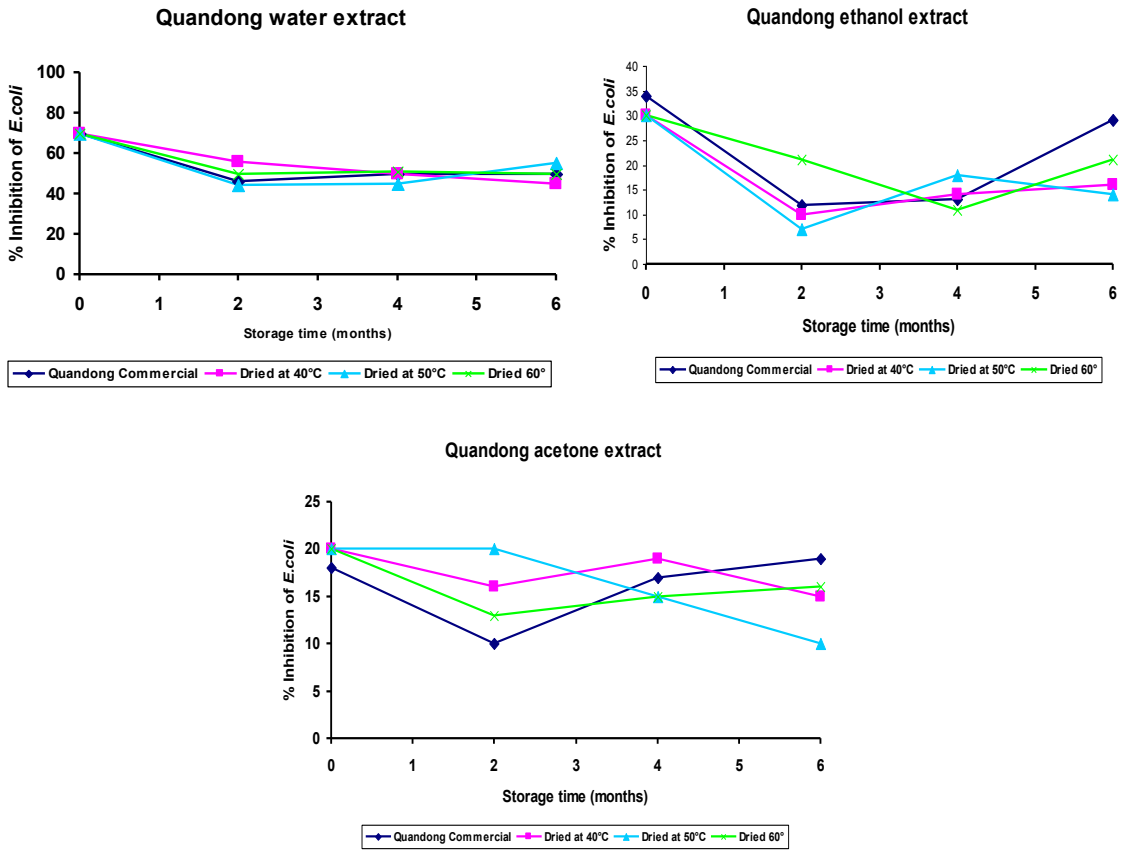


Figure 3.18 Changes in antimicrobial activity of different extracts of quandong (at a concentration of 8.75% v/v) during 6 months of storage, as measured by inhibition of *Escherichia coli*

It is recommended that the quandongs are sent as whole fruits under chilled storage or sent as blast-frozen halves during transportation for further processing. Since the discolouration is quite rapid the application of an anti-browning treatment as soon as the fruit is destoned and halved is recommended before further processing.

From the drying curves it is clear that the total phenolics and the FRAP values were stable for quandong samples dried at 40, 50 and 60°C, during the 6-month storage period. In contrast, in the commercial sample the total phenolics and FRAP values increased significantly during storage (refer to Figures 3.15 and 3.16) and a possible explanation could be the increased amounts of phytochemicals released from the matrix by thermal processing (Orikasa et al. 2014). The difference between the commercial and pilot-scale drying methods was the period of time and the low temperature (40–45°C). The commercial sample was dried for a much longer period of time (in excess of 24 hours) than the pilot scale method (where the drying was completed within 6–12 hours) (refer to Figure 3.14). In the drying of apricots, an improved microwave method gave a higher phenolic content particularly for chlorogenic acid, however, the microwaved sample had a similar antioxidant value to the air-dried apricots as the identified phenolic compounds did not significantly contribute to the antioxidant activity (Igual et al. 2012). Al-Weshahy et al. (2013) reported on the levels polyphenolic compounds during storage of freeze-dried potato peels at different storage temperatures of –20, 4 and 25°C, where a maximum loss was observed at the highest storage temperature. Storage time caused a decline in the levels of polyphenolic compounds up to 4 weeks at all temperatures followed by a significant increase at the end of week 8. A similar scenario could have occurred during the storage of the commercial samples of quandong.

The total phenolics content in quandong (Table 3.4) is comparable to Davidson's plum (Table 3.1) but far less than Kakadu plum (refer to Table 3.3). FRAP values in quandong (Table 3.5) are less than Davidson's plum (Table 3.1) and Kakadu plum (Table 3.3). The solvent extracts of quandong did not completely inhibit *S. aureus* and *E. coli* at a concentration of 8.75% (v/v) (Figures 3.17 and 3.18) in comparison to Davidson's plum where both bacteria were completely inhibited at this concentration (Figures 3.3 and 3.4) and Kakadu plum (Figures 3.9 to 3.12). The results of the present study suggest that quandong would be most suitable as a value-added native fruit product such as a dehydrated fruit, or a frozen or glazed product to be consumed for its health benefits as a fruit or as an added ingredient like sultanas or glazed cherries.

3.4.4.1 Conclusion

The current drying, packaging and storage practice of the industry is preserving the total phenolics and antioxidant properties of the dried quandong during processing and storage. These manufacturing practices can be adopted as industry manufacturing guidelines and product standards can be based on this data.

4. Changes in quality and bioactivity of native herbs and fruits during storage – further investigations into antimicrobial and antioxidant activity

4.1 Phytochemical analysis of native herbs and fruit

4.1.1 Introduction

Antioxidants are compounds that inhibit the oxidation of lipids or other molecules by delaying the initiation of oxidising chain reactions (Zheng & Wang 2001). The antioxidant activity of these compounds is mainly due to their redox properties which play an important role in neutralising free radicals, quenching singlet and triplet oxygen or decomposing peroxides (Osawa 1994). Due to the perceived adverse health effects of synthetic antioxidants, interest has increased in finding naturally occurring antioxidants for use in foods or medicinal materials (Madsen & Bertelsen 1995). Studies on culinary and medicinal plants have assigned superior antioxidant capacities to berries, fruits, vegetables and nuts originating from the presence of high levels of vitamin C and/or phenolic compounds (Deutsch 2000; Zheng & Wang 2001).

The role of vitamin C in human metabolism is complex but its protecting action against the oxidising effect of free radicals is believed to be of crucial importance (Novakova et al. 2009). This role is considered so important that the content of vitamin C (sum of ascorbic acid and dehydroascorbic acid) is used as an index of the health-related quality of fruits (Odriozola-Serano et al. 2007).

The protective effects of many plant foods are also associated with the antioxidant activity of the class of compounds known as phenolics. Typical phenolic compounds that possess high antioxidant properties are the phenolic acids and flavonoids (Aaby et al. 2005; Kähkönen et al. 1999). Phenolic acids such as chlorogenic acid and the dimeric derivative of gallic acid (ellagic acid, EA), have been repeatedly implicated as the natural antioxidant agents in fruits, vegetables and other plants (Konczak et al. 2010a; Zheng & Wang 2001). In fact, several studies have shown the high antioxidant activity in several of these plants is due more to the high content of these phenolics than to vitamin C content (Guo et al. 2003; Robbins 2003).

Research over many years has delivered a vast amount of data indicating which plants are valuable sources of these compounds. However, little information on their levels in commercially grown native Australian plants was available until the publication of the comprehensive evaluations by Konczak et al. (2009, 2010a) and Sakulnarmrat and Konczak (2012). These authors presented values for certain native plants that possessed far higher antioxidant capacities than those previously reported with high antioxidant properties. They attributed the enhanced antioxidant capacities to the unusually high levels of vitamin C and phenolic compounds.

It has been widely reported that post-harvest operations such as storage conditions have a major influence on the levels of these natural antioxidants in plants and plant products. Conventional processing (thermal) and storage conditions (both domestic and industrial) are known to lower the levels of phytochemicals in plant products compared to the freshly harvested produce (Rawson et al. 2011; Tiwari & Cummins 2011; Volden et al. 2009). In order to retain phytochemicals during the storage of these products the processor or consumer must optimise processing and storage conditions to restrict their degradation.

To advance this field of research, accurate and reproducible methods for isolating and determining the amounts of these natural antioxidants are required. Firstly, a methodology is needed that measures both bioactive forms of vitamin C, i.e. ascorbic acid (AA) and dehydroascorbic acid (DHAA). Secondly, simple, rapid and robust methodologies are needed for the separation and quantification of the phenolics of interest, i.e. chlorogenic acid and ellagic acid as well as ellagitannins. The diverse chemical nature of these phenolic compounds complicates the extraction and possible hydrolysis steps required for their determination. Although numerous extraction methods for phenolics have been described in the literature, a common feature is their validation using only one plant material type and for only one specific class (reviewed in Nuutila et al. 2002). Some of the discrepancies in the literature between levels of individual phenolics from the same plant source could be attributed in part to the use of differing extraction and hydrolysis protocols (see Table 4.4).

The objectives of the current study were threefold: 1) to optimise extraction and high performance liquid chromatography (HPLC) conditions for the analysis of vitamin C and selected phenolics; 2) to evaluate these antioxidant constituents in samples of commercial native Australian plants; and 3) to determine the impact of storage on antioxidant constituents.

4.1.2 Materials and methods

4.1.2.1 Native herb and fruit samples

The following samples for detailed examination were provided by the Australian native food industry:

- anise myrtle: commercial samples (1.6mm flakes), obtained from Australian Rainforest Products Pty Ltd, sampled at 0, 2, 4, 6, 8, 10 and 12 months while undergoing room-temperature storage

- lemon myrtle: commercial samples (1.6mm flakes), obtained from Australian Rainforest Products Pty Ltd, sampled at 0, 2, 4, 6, 8, 10 and 12 months while undergoing room-temperature storage

- Tasmanian pepper leaf: (1) commercially milled samples, obtained from Diemen Pepper, sampled at 1, 2, 3, 4, 5, 6, 7, 8 and 9 months while undergoing room-temperature storage; and dried pepper leaves, obtained from Diemen Pepper and milled by two methods at the laboratory (2) hammer milled, sampled at 0, 1, 2, 3, 4, 5, 6, 7, 8 and 9 months while undergoing room-temperature storage and (3) cryomilled, sampled at 0, 1, 2, 3, 4, 5, 6, 7, 8 and 9 months while undergoing room-temperature storage

- Kakadu plum: puree including seed, obtained from Coradji Pty Ltd, sampled at 1, 2, 3, 4, 5, 6, 7, 8 and 10 months while undergoing freezer storage at –20^{o}C (frozen sub-samples after the specified storage duration were freeze dried prior to analysis)

- Kakadu plum: whole, obtained from Wild Harvest, Northern Territory, individual whole fruit placed in polyethylene bags, vacuum sealed and blast frozen, sampled at 0, 1, 2, 3, 4, 5, 6 and 8 months while undergoing storage at –20^{o}C (sub-samples after the specified storage duration were freeze-dried and then deseeded prior to analysis).

4.1.2.2 Chemicals

The phenolic acid, chlorogenic acid and the phenolic acid derivative, ellagic acid as well as ascorbic acid and DL-homocysteine were purchased from Sigma-Aldrich (Sydney, Australia). The HPLC-grade methanol, formic acid, 2-propanol and acetonitrile were purchased from Thermo Fisher Scientific (Victoria, Australia). All other chemicals were of analytical grade.

4.1.2.3 Methods

4.1.2.3.1 Moisture content

The moisture content of the dried and freeze-dried samples was determined according to AOAC (1984), official method 964.22. Briefly, each sample (1 g) was dried for approximately 16 h to a constant weight at 70°C in a vacuum oven. The difference between initial weight and constant weight after drying was taken as moisture lost and hence moisture content of the sample.

4.1.2.3.2 Extraction of total vitamin C and ascorbic acid

To determine both forms of vitamin C that are biologically active a two-step subtraction approach was employed. Firstly, the content of ascorbic acid (AA) was determined by HPLC with UV-detection of the original sample to obtain the initial concentration. Reduction of DHAA to AA was performed by the addition of DL-homocysteine. After this conversion the samples were analysed for total vitamin C again by HPLC with UV-detection. The method adopted in this study was based on those outlined by Dennison et al. (1981), Gökmen et al. (2000) and Hoare et al. (1993).

Ascorbic acid was determined by weighing 100 mg of Kakadu plum sample into a 15 mL centrifuge tube followed by 10 mL of extracting solution consisting of 1% (m/v) citric acid containing 0.05% (m/v) ethylenediamine tetraacetic acid (EDTA) as the disodium salt in 50% (v/v) methanol. After being shaken by hand the tubes were centrifuged at ≈3220 *g* for 5 min and 1 mL of clear supernatant was added to a 50 mL volumetric flask and made to volume with extracting solution. An aliquot of this solution was filtered through a 0.45 µm syringe filter prior to HPLC analysis.

Total vitamin C was determined by weighing 100 mg of sample followed by adding 4 mL 0.8 % DL-homocysteine (m/v) to a 15 mL centrifuge tube and adjusting the pH to 7 with 45% K_2HPO_4 (m/v) solution. After 15 min the solution was made to approximately 10 mL with extracting solution. Again the tubes were mixed and centrifuged at ≈3220 *g* for 5 min and 1 mL of clear supernatant was added to a 50 mL volumetric flask and made to volume with extracting solution. An aliquot of this solution was filtered through a 0.45 µm syringe filter prior to HPLC analysis.

4.1.2.3.3 Identification and quantification of vitamin C and ascorbic acid

Separation of AA was achieved with a Waters HPLC system (Waters Associates, Rydalmere, NSW, Australia) consisting of a pump (LC-515), auto-sampler (Plus 717), and UV-visible detector (model 481) linked to Varian Star software (Version 6.41). A 5 µm Supelcosil LC-NH_2, 4.6 x 250 mm column (Supelco, Sigma Aldrich, Sydney, Australia) efficiently separated ascorbic acid isocratically by using a solution of 40:60 (v/v) methanol: 0.25% K_2HPO_4 (m/v) buffer (adjusted to pH 3.5 with phosphoric acid) as mobile phase. The flow rate was 1.0 mL/min. An aliquot of 10 µL of sample was injected and the ascorbic acid peak was detected at 245 nm and identified and quantified by comparison to a commercial standard.

Serial dilutions containing 10-100 mg/L were prepared by dissolving reference grade AA in extracting solution. A calibration curve of peak area versus standard AA concentration was plotted and the concentration of AA and total vitamin C was determined after applying the appropriate dilution factor. The DHAA concentration was calculated by subtracting the AA value from total vitamin C. The total vitamin C, AA and DHAA concentrations of the samples were expressed as mg/100 g DW.

For both types of Kakadu plum products, sub-samples after the specified storage duration were analysed in triplicate unless otherwise specified and were expressed as means ± SD.

4.1.2.3.4 Extraction of chlorogenic acid

Most phenolic acids in plants are present not as 'free acids' but linked through ester, ether or acetal bonds either to structural components of the plant, to larger phenolics (flavonoids) or smaller organic molecules (e.g. glucose, quinic or gallic acids). To release the phenolic acids for quantification, these attachments are cleaved by acidic, basic or enzymatic hydrolysis steps (reviewed in Robbins 2003).

Under most acidic conditions commonly used for phenolic compound extraction, chlorogenic acid is severely degraded thereby excluding simultaneous analysis with other phenolic classes (Nuutila et al. 2002). Under alkaline hydrolysis conditions and with the addition of antioxidants, most phenolic acids are more stable but chlorogenic acid is still converted to caffeic acid (Nardini et al. 2002). As quantification of chlorogenic acid will be impossible using alkaline hydrolysis, the procedure selected for extracting the acid has no acidic or alkaline hydrolysis steps and was based on that described by Padda and Picha (2008) but with several important modifications.

Duplicate samples (~0.1g) were accurately weighed into a 15 mL centrifuge tube and 3 mL (3 x extractions) of 80% aqueous methanol were added and vortexed for 30 sec. The tubes were capped and immersed in a shaking water bath at 80°C for 10 mins. The mixture was then vigorously shaken by hand, cooled and centrifuged (≈3220 *g*, 5 min). The clear supernatant was transferred to a 10 mL volumetric flask and made to volume with extracting solution. About 1.5 mL of the diluted supernatant was filtered through a 0.45 μm syringe filter into an HPLC vial to be stored at –80°C prior to analysis.

4.1.2.3.5 Identification and quantification of extraction of chlorogenic acid

Samples were analysed using a Shimadzu HPLC system (Shimadzu Co., Kyoto, Japan) consisting of a system controller (SCL-10Avp), degasser (GastorGT-104), pump (LC-10ADvp), auto-sampler (SIL-10ADvp), column oven (CTO-10Avp), and UV-visible detector (SPD-10AV) linked to Class VP software (Version 6.14 SP1). Optimal separation of the chlorogenic acid was achieved on a reversed-phase C_{18} Gemini, 5 μm, 4.6 x 150 mm column (Phenomenex, Lane Cove, NSW, Australia) with matching guard column. Both columns were maintained at 30°C. The best-suited mobile phase was 1% (v/v) formic acid in H_2O: acetonitrile: 2-propanol (70:22:8, v/v/v), pH 2.5 under isocratic conditions with a flow rate of 0.75 mL/min as it provided optimal separation with a reasonably low back pressure. An aliquot of 10 μL of sample was injected and peaks were detected at 320 nm and identified and quantified by comparison to commercial standards. The chlorogenic acid eluted at a retention time (RT) of ~2.9 min.

Five standard solutions containing chlorogenic acid (5-caffeoylquinic acid, 5-CQA) were prepared in 100% methanol at a concentration range of 2–200 μg/mL (Mattila & Hellstrom, 2007) and used to prepare a standard curve based on peak area.

Chlorogenic acid content was expressed as mg/g of sample or mg/g DW after moisture determinations as outlined by Mattila et al. (2007) and Nardini et al. (2002).

4.1.2.3.6 Extraction of free ellagic acid

Similar to chlorogenic acid extraction, quantification of free ellagic acid was impossible using alkaline hydrolysis. With this in mind the procedure selected for extracting free ellagic acid was based on that described by Amakura et al. (2000) minus the acidified methanol assisted SPE clean-up. No hydrolysis steps were necessary as only free EA was to be measured.

For this phenolic, duplicate samples (~0.2 g for anise myrtle samples; ~0.4 g for lemon myrtle samples; ~0.1 g for Kakadu plum samples) were accurately weighed into a 15 mL centrifuge tube and 3 mL or 5mL (for Kakadu plum samples) (3 x extractions) of 100% methanol were added and sonicated for 10 min. The mixture was centrifuged (≈3220 *g*, 5 min). The clear supernatant was

transferred to a 10 mL or 25 mL (for Kakadu plum samples) volumetric flask and made to volume with extracting solution. About 1.5 mL of the diluted supernatant was filtered through a 0.45 µm syringe filter into an HPLC vial to be stored at –80°C prior to analysis.

4.1.2.3.7 Extraction and hydrolysis of ellagitanins

An aliquot (2 mL) of the free ellagic–methanol extract was pipetted into 5 mL Reacti-Therm (Thermo Scientific, Rockford, USA) vial containing a stirring slug. The methanol was evaporated under nitrogen before 2 mL of 2N TFA (tri-fluoroacetic acid) was added to the vial which was then capped and mixed to dissolve the residue. The vial was placed into the Reacti-Therm heater/stirrer unit where the contents were hydrolysed at 120ºC for 120 min. After hydrolysis the vial was cooled and the contents transferred into a 5 mL volumetric flask with 100% methanol. About 1.5 mL of this solution was filtered through a 0.45 µm syringe filter into an HPLC vial to be stored at –80°C prior to analysis.

4.1.2.3.8 Identification and quantification of ellagic acid

Samples were analysed using a Shimadzu HPLC system (Shimadzu Co., Kyoto, Japan) consisting of a system controller (SCL-10Avp), degasser (DCU-14A), pump (LC-10ATvp), auto-sampler (SIL-20ATHT), column oven (CTO-10Avp) and a photo-diode array detector (SPD-M10Avp) linked to Labsolutions software. Optimal separation of the ellagic acid was achieved on a reversed-phase C_{18} Acclaim, 3 µm, 4.6 x 150 mm column (Thermo Fisher Scientific, Victoria, Australia) with matching guard column. Both columns were maintained at 30°C. Optimal separation required gradient elution. Mobile phases consisted of 0.1% formic acid (v/v) in water (Solvent A) and 0.1% formic acid (v/v) in acetonitrile (Solvent B). The gradient used the following conditions:

- 15% B for 1min
- 15–20% B in 20min
- 20–90% B in 2min
- 90% B for 4min

Followed by the re-equilibration steps:

- 90–15% B in 0.5min
- 15% B for 7.5min

A flow rate of 1.5 mL/min was maintained for each step.

An aliquot of 10 µL of sample was injected and the peak was monitored simultaneously at 250 and 365 nm; and identified and quantified by comparison to a commercial standard. Under these conditions the ellagic acid eluted at an RT ~10.5 min.

Five standard solutions containing ellagic acid were prepared in 100% methanol at a concentration range of 2–100 µg/mL (Mattila & Hellstrom, 2007) and used to prepare a standard curve. Due to the closeness of adjoining peaks, height rather than area of the ellagic acid peaks was used for quantification.

Ellagic acid content was expressed as mg/g of sample or mg/g DW after moisture determinations.

4.1.3 Results and discussion

4.1.3.1 Total vitamin C and ascorbic acid content

Ascorbic acid and DHAA are reduced and oxidised forms of vitamin C, which are ubiquitously found in various fruits and vegetables (Figure 4.1). Both are biologically active although their role in human metabolism is reported to be complex (Odriozola-Serrano et al. 2007). Various methods have been reported for the determination of vitamin C in foods including titration (AOAC 1984), fluorometry (AOAC 1984), and HPLC (Gökmen et al. 2000). Most of these methods, other than HPLC, are time-consuming and often produce overestimates due to the presence of interfering compounds and/or do not measure DHAA (Gökmen et al. 2000). As the sum of AA plus DHAA is used as an index of the potential health quality of fruits this prompted renewed interest in simplifying measurement of both active forms (Odriozola-Serrano et al. 2007**).**

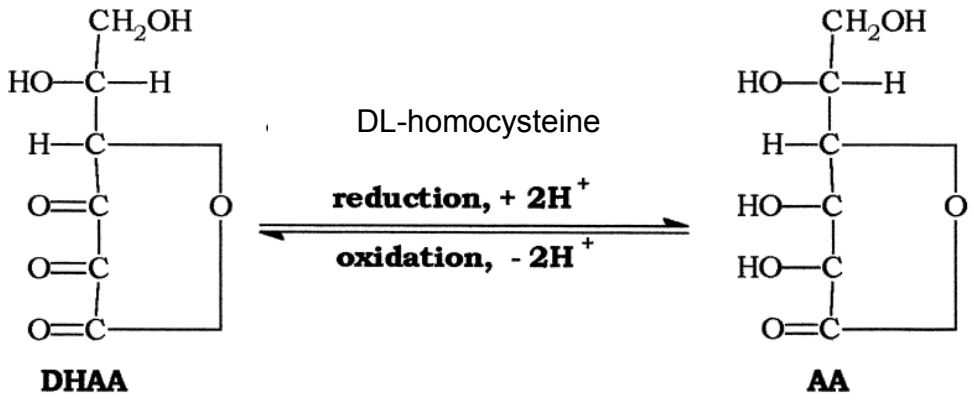


Figure 4.1 Reduction scheme of DHAA to AA using DL-homocysteine

In saying that, simultaneous direct detection of AA and DHAA provides a complicated analytical challenge to the researcher. In the current study, DHAA was determined as the difference between total vitamin C after DHAA reduction with homocysteine and the AA content of the original sample (Dennison et al. 1981; Hoare et al. 1993).

Table 4.1 Vitamin C content (mg/100g DW) of stored Kakadu plum puree

	Vitamin C (mg/100 g DW)		
Storage (months)	**Total**	**AA**	**DHAA**
1*	9106 ± 103	8273 ± 95	833
2*	11541 ± 77	8152 ± 65	2389
3*	13566 ± 203	11619 ± 179	1947
4	11621± 171	9894 ± 141	1727
5	12146 ± 183	10140 ± 167	2006
6	12368 ± 186	9278 ± 146	3090
7	12645 ± 234	7323 ± 154	5322
8	13306 ± 228	7732 ± 150	5574
10	13361 ± 245	9600 ± 163	3761

*Values are expressed as means ± SD for triplicates.
All other values are expressed as means ± SD for duplicates.

To avoid possible underestimation that may occur by reporting just AA levels, food regulatory bodies in Australia have stated that any testing procedures to determine vitamin C or AA should be able to determine total vitamin C, i.e. the sum of AA and DHAA (Standard 2.9.2, Australian & New Zealand Food Standards Code 2013).

The levels of total vitamin C, AA and DHAA in frozen stored Kakadu plum puree and whole fruit are presented in Tables 4.1 and 4.2 respectively.

The values for AA content of both sample types are comparable to those reported by Konczak et al. (2009) in the RIRDC report *Health Benefits of Australian Native Foods*, i.e. 7252 mg/100 g DW. As the authors comment this is a very rich source of AA, amounting to 7.2% of the dry weight of fruit. The values presented here in most cases are even higher.

Table 4.2 Vitamin C content (mg/100g DW) of stored individual whole Kakadu plum

	Vitamin C (mg/100g DW)		
Storage (months)	**Total**	**AA**	**DHAA**
0*	12801 ± 230	11604 ± 187	1197
1*	12041 ± 248	10496 ± 232	1545
2*	11954 ± 276	10606 ± 283	1348
3	13300± 327	9829 ± 255	1832
4	11660 ± 291	10306 ± 257	1354
5	10192 ± 203	8434 ± 188	1758
6	9851 ± 197	8547 ± 238	1304
8	9614 ± 192	7984 ± 179	1630

*Values are expressed as means ± SD for triplicates

All other values are expressed as means ± SD for duplicates

The AA/DHAA ratio provides a measure of the extent of AA to DHAA oxidation and is often used to monitor vitamin C losses during food processing, a necessity as the two forms have different resistances to thermal degradation and oxidation (Gökmen et al. 2000). Also the AA/DHAA ratio has a role in evaluating the redox state of biological materials (Novakova et al. 2009). Figure 4.2 illustrates variations in this ratio during the frozen storage of Kakadu plum samples.

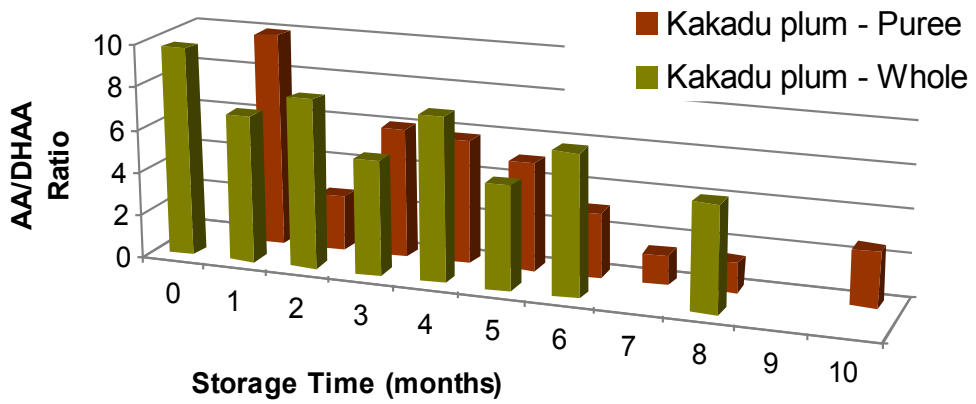


Figure 4.2 The AA/DHAA ratio of frozen stored Kakadu plum samples

These ratios highlight that AA is the predominant form of vitamin C in both Kakadu plum products agreeing with previous studies on citrus fruits by Gökmen et al. (2007) (orange – 12.4; grapefruit – 28.0) and Chebrolu et al. (2012) (lime – 2.37; grapefruit – 2.98). However, the latter study found an almost equal distribution in some orange varieties (1.30; 0.76). Interestingly both reported that while AA was predominant in fruits, DHAA was the major form in vegetables.

For the stored Kakadu plum puree samples the AA/DHAA ratio began to reduce noticeably after 6 months suggesting considerable oxidation of AA to DHAA. Excluding the total vitamin C values for months 1 and 2, total vitamin C content was stable, varying little during storage. There appears to be little in the way of scientific evaluation of changes in AA and DHAA content of fruit on storage. Hoare et al. (1993) investigated changes in both vitamin C forms during refrigerator and freezer storage of orange juice. They reported considerable conversion of AA to DHAA for opened orange juice cartons to the extent that after 35 days refrigerator storage all the vitamin C was in the DHAA form. They also noted that towards the end of the storage time the high DHAA content decreased due to DHAA degrading to other products. This may have occurred in the current study, note the slight increase in the AA/DHAA ratio at 10 months (Figure 4.2).

Results of vitamin C testing on the individual Kakadu plums showed a similar if less pronounced decrease in the AA/DHAA ratio with storage. This trend occurred in a backdrop of decreasing total vitamin C content, a decrease also noted by Hoare et al. (1993) in their orange juice study. Perhaps the rigorous processing of the Kakadu plum puree has facilitated a more rapid oxidation of the AA.

Finally, it is worthwhile to consider that vitamin C is one of the least-stable vitamins (Coultate, 1989) and that loss during sample processing and storage can occur by a number of different routes (Nisperos-Carriedo et al. 1992). To fully understand the relationship between total vitamin C, AA and DHAA content and the changes that occur on processing and storage of Kakadu plum more detailed investigations are necessary.

4.1.3.2 Chlorogenic acid content

Phenolic acids are naturally occurring antioxidant compounds that are widely spread throughout the plant kingdom. There has been renewed interest in sources of phenolic acids due to mounting evidence that their elevated antioxidant activity may bestow substantial health benefits (reviewed in Crozier et al. 2009). Although there are several mechanisms associated with this activity, the predominant one is believed to be radical scavenging via hydrogen atom donation (reviewed by Robbins 2003).

The phenolic acid composition of stored Tasmanian pepper leaf obtained in this study is given in Table 4.3. Chlorogenic acid was the major compound present in all samples. In fact not a trace of other frequently found related phenolic acids, i.e. caffeic acid and the three isomers of dicaffeoylquinic acid, were detected, even though considerable attention was paid in trying to identify them.

The values presented are close to those reported by Konczak et al. (2010a) for Tasmanian pepper leaf (30.0 mg/g DW) in their comprehensive report on antioxidant capacity and phenolic compounds in native Australian herbs and spices. This was not unexpected as their leaves were sourced from the same supplier as the current study.

Table 4.3 Chlorogenic acid (5-CQA) content (mg/g DW) of stored Tasmanian pepper leaf – milled by three different methods

	5-CQA content (mg/g DW)		
Storage (months)	**Commercial**	**Hammer**	**Cryo**
0	NT	20.9 ± 0.2	20.9 ± 0.0
1	18.6 ± 0.6	20.6 ± 0.2	21.2 ± 0.1
2	18.8 ± 0.6	21.0 ± 0.3	21.7 ± 0.2
3	18.0 ± 0.1	20.8 ± 0.5	21.7 ± 0.1
4	18.3± 0.2	20.8 ± 0.1	21.4 ± 0.1
5	18.8 ± 0.2	20.9 ± 0.3	21.3 ± 0.1
6	18.5 ± 0.4	20.5 ± 0.3	20.7 ± 0.1
7	18.5 ± 0.2	20.5 ± 0.3	22.4 ± 0.4
8	18.6 ± 0.2	20.5 ± 0.1	20.0 ± 0.1
9	18.9 ± 0.2	20.1 ± 0.2	21.0 ± 0.1
Av. Moisture %	5.3	5.8	6.2

All values are expressed as means ± SD for duplicates.

The chlorogenic acid levels of these leaves are certainly very high especially when compared to other well-reported vegetable sources (Table 4.4).

Storage at room temperature appears to have minimal effect on the chlorogenic acid content regardless of the type of milling performed. In contrast, different modes of milling seem to have a profound effect, with procedures generating little or no heat producing the highest values. The moisture levels in conjunction with the often-reported thermal instability of phenolic acids (reviewed in Williams et al. 2013; Takenaka et al. 2006) supports this supposition.

Table 4.4 Chlorogenic acid content (mg/kg) of vegetables (from Williams et al. 2013)

Vegetable	Chlorogenic acid + caffeoylquinic acids	Reference
Carrot	150–260[a]	Mattila and Hellstrom (2007)
Globe artichoke	2930	Ferracane et al. (2008)
Lettuce-red leafed-red tissue	1696[a]	Ferreres et al. (1997)
Lettuce-red leafed-green tissue	570[a]	Ferreres et al. (1997)
Lettuce-red leafed-midribs (white tissue)	213[a]	Ferreres et al. (1997)
Potato-small russet	133[a]	Friedman (1997)
Potato-large russet	142[a]	Friedman (1997)
Potato roots	260[a]	Friedman (1997)
Potato tubers	170[a]	Friedman (1997)
Sweetpotato-cream fleshed	31–46[a]	Rautenbach et al. (2010)
Sweetpotato-orange fleshed	190–580[b]	Padda and Picha (2008)
Sweetpotato-purple fleshed	1150[b]	Padda and Picha (2008)
Sweetpotato-white fleshed	93–910[b]	Padda and Picha (2008)
Sweetpotato leaves	4305–4630[a]	Truong et al. (2007)
Sweetpotato peel	585–1050[a]	Truong et al. (2007)
Sweetpotato whole root	80–320[a]	Truong et al. (2007)
Sweetpotato periderm	20–1825[b]	Harrison et al. (2008)
Sweetpotato cortex	1830–12,440[b]	Harrison et al. (2008)
Sweetpotato stele	505–12,205[b]	Harrison et al. (2008)
Sweetpotato leaves-immature	88,500[b]	Padda and Picha (2007)
Sweetpotato root-small	10,300[b]	Padda and Picha (2007)

[a] Data expressed as mg/kg fresh weight.
[b] Data expressed as mg/kg dry weight.

Chlorogenic acid is a highly bioactive molecule with well-known antioxidant capacity (reviewed by Gonzalez-Castejon & Rodriguez-Casado 2011). The very high levels as well as its stability on prolonged room temperature storage as shown by the current study suggests Tasmanian pepper leaf would be ideally suited as a functional food additive or ingredient.

4.1.3.3 Free ellagic acid content

Ellagic acid, a naturally occurring phenolic compound has aroused considerable interest because of its promising chemoprotective effects (da Silva Pinto et al. 2008; Maas et al. 1991). Fruit, particularly berries, nuts (Tomas-Barberan & Clifford 2000) and recently, some native Australian plants (Sakulnarmrat & Konczak 2012) are suggested as being rich sources of ellagic acid (EA). This compound can exist as the free form, glycoside or linked as ellagitannins esterified with glucose (da Silva Pinto et al. 2008; Maas et al. 1991). The free form according to these authors is rarely found, a statement supported by the results for anise myrtle and lemon myrtle in the current study (Table 4.5).

Table 4.5 Free ellagic acid content (mg/g DW) of stored anise myrtle, lemon myrtle and Kakadu plum

	Ellagic acid content (mg/g DW)			
Storage (months)	**Anise myrtle**	**Lemon myrtle**	**Kakadu plum puree**	**Kakadu plum whole**
0	0.29 ± 0.00	0.08 ± 0.00		9.8 ± 0.0
1			15.3 ± 0.1	9.7 ± 0.1
2	0.30 ± 0.01	0.08 ± 0.00	26.2 ± 0.2	8.2 ± 0.2
3			15.1 ± 0.1	10.0 ± 0.0
4	0.32 ± 0.03	0.07 ± 0.00	13.3 ± 0.1	9.2 ± 0.2
5			15.9 ± 0.1	10.6 ± 0.1
6	0.31 ± 0.03	0.08 ± 0.00	14.6 ± 0.1	11.8 ± 0.3
7			15.7 ± 0.1	
8	0.28 ± 0.05	0.08 ± 0.00	15.5 ± 0.5	10.8 ± 0.0
10	0.31 ± 0.08	0.09 ± 0.00	15.2 ± 0.0	
12	0.32 ± 0.06	0.07 ± 0.00		
SDR		0.18 ± 0.01		
Av. Moisture %	6.5	8.3	1.9	3.6
SDR Moisture%		2.6		

SDR – Steam distilled residue.

All values are expressed as means ± SD for duplicates.

In an investigation into the EA content of strawberries, da Silva Pinto et al. (2008) reported very low free EA levels (0.006–0.026 mg/g FW). These figures were duplicated by similarly low values presented by Aaby et al. (2005) for free EA content in strawberry flesh (0.002–0.026 mg/g FW). Interestingly these researchers found elevated free EA levels in the achenes (0.13–0.87 mg/g FW) which are comparable to those of anise myrtle found in the current study (Table 4.5). The values for anise myrtle and lemon myrtle free EA content presented by Sakulnarmrat and Konczak (2012) were consistently higher than our measurements (anise myrtle – 153 mg/g DW; lemon myrtle – 102 mg/g DW). However, it should be noted that in Sakulnarmrat and Konczak (2012) measurements were performed on enriched polyphenolic fractions.

The free EA content of the Kakadu plum products were very high, indeed being ~50-fold and ~180-fold greater than those presented for anise and lemon myrtle respectively.

Difficulties in comparing literature values of free EA were highlighted by Tomas-Barberan and Clifford (2000) when they observed that free EA content was often underestimated due to EA's insolubility in many of the extracting solvent combinations. The differing solubility of the commercial EA standard and its efficiency in extracting the EA from the plant samples by the adopted extraction solvent, i.e. 100% methanol in the current study testifies to these difficulties.

The far from rigorous storage regimes (i.e. room temperature for the anise myrtle and lemon myrtle; freezer for the Kakadu plum products) not unexpectedly had minimal effect on the free EA content. A study that evaluated processing to obtain raspberry jam and its subsequent room temperature storage on free EA content reported a three-fold increase (Zafrilla et al. 2000). The authors suggested that this

increase was due to the release of free EA from the abundant ellagitannins during processing. This enhanced release possibly accounts for the increased values of EA exhibited by the steam-distilled residue of lemon myrtle (Table 4.5).

4.1.3.4 Ellagitannin content

The detection and quantification of ellagitanins are based on the fact that when these compounds are exposed to acids, the ester bonds are hydrolysed and spontaneously rearrange into water-insoluble ellagic acid (Clifford & Scalbert, 2000). Preliminary analyses failed to detect any trace of ellagitanins in both the Kakadu plum puree or individual whole samples. The total EA contents of the anise myrtle and lemon myrtle are presented in Table 4.6.

Even taking into account the effect of expressing the concentrations as dry weight (DW) in the current study, values are far higher than those for strawberry cultivars reported by da Silva Pinto et al. (2008) (0.17–0.47 mg/g FW) and Aaby et al. (2005) for strawberry flesh puree (0.08–0.11 mg/g FW). The values presented for strawberry achenes in Aaby et al. (2005) are again comparable with those observed for anise myrtle and lemon myrtle in the current study.

Table 4.6 Total ellagic acid content (mg/g DW) of stored anise myrtle and lemon myrtle

	Ellagic acid content (mg/g DW)	
Storage (months)	**Anise myrtle**	**Lemon myrtle**
0	10.30 ± 0.26	4.68 ± 0.04
2	9.85 ± 0.59	5.34 ± 0.09
4	9.58 ± 0.86	4.44 ± 0.02
6	9.13 ± 0.10	4.66 ± 0.12
8	9.04 ± 0.03	4.76 ± 0.01
10	8.48 ± 0.13	5.37 ± 0.07
12	8.79 ± 0.00	3.63 ± 0.14
SDR		1.24 ± 0.07
Av. Moisture %	6.5	5.8

All values are expressed as means ± SD for duplicates.

A cautionary note in comparing ellagitannin contents was introduced by Tomas-Barberan and Clifford (2000) and is certainly relevant here, that techniques for measuring these compounds although possessing good accuracy and reproduction, produce results that differ markedly depending on extraction method or whether hydrolysis was performed. Difficulties in further comparison of EA content are compounded with some workers reporting EA strictly as EA, while others combine it with EA glycosides and even ellagitannins (reviewed in Aaby et al. 2005).

Calculation of free EA/total EA content as a percentage indicated some release of EA from ellagitannins during the storage of anise myrtle samples, whereas the lemon myrtle ellagitannins appear more stable (Figure 4.3).

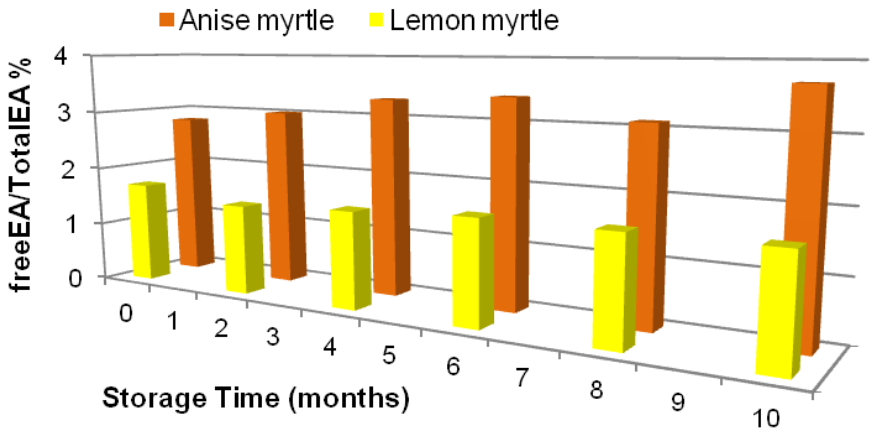


Figure 4.3 The free EA/total EA% of stored anise myrtle and lemon myrtle samples

The very high free EA/total EA % recorded for the steam distillate residue (14.5%) provided further evidence of enhanced free EA formation caused by the more rigorous sample extraction. Zaffrilla et al. (2001) postulated another reason for an increase in free EA on processing, that is the easier extractability of this compound due to the degradation of plant cell structures. This cannot be discounted in the current study.

As EA and its derivatives gain more popularity as highly bioactive compounds, rich sources such as anise myrtle and lemon mrytle extracts as well as Kakadu plum will ably fit the bill as functional food ingredients. Their relative stability during storage further encourages this application.

4.1.3.5 Conclusions

In recent years reports suggesting beneficial health-promoting properties of phenolic compounds most notably phenolic acids and vitamins such as vitamin C have increased interest in sources of these plant bioactives. A vast amount of literature, although little on Australian-native produce until recently, has emerged comparing different plants as possible rich sources of these compounds. However, limitations in many of the analysis methods used previously meant comparison with literature values was problematic. For this study we have had to optimise extraction and HPLC conditions in order to develop simplified and robust methodologies that firstly identified then subsequently quantified chlorogenic and ellagic acids (the latter as free and as ellagitannins) as well as both bioactive forms of vitamin C. The methods developed illustrated good linearity and accuracy. In saying that, the method developed for the analysis of free EA was still a compromise between solubility of the standard material and the extractability of the liberated EA from the plant samples.

The native Australian plants evaluated here confirmed previous reports that stated several native plants are very rich sources of antioxidant compounds such as vitamin C and phenolic compounds. Plant products such as those derived from Kakadu plum and Tasmanian pepper leaf were significantly higher in content of these potentially health-promoting compounds than other previously published sources. The finding here that Kakadu plum products contained only free EA with no ellagitannins, possibly enhances its application in the functional food/pharmaceutical industries. This enhanced applicability is due to the belief that EA monomers are more easily absorbed in the intestinal tract than the high molecular weight ellagitannins (Clifford & Scalbert 2000).

Previous studies suggest that processing procedures and storage conditions have a pronounced influence on the levels of these natural antioxidants, however, our data indicated minimal degradation occurred under the storage conditions used for these series of measurements although milling procedures for the Tasmanian pepper leaf that generated less heat were seen to better preserve their chlorogenic acid content. Admittedly the storage conditions used in the current study were far from rigorous but in saying that they still reflected common commercial and consumer practice. Perhaps testing a wider range of processing procedures and storage conditions would give a better understanding of the stability of these health-promoting compounds.

4.2 Antimicrobial properties and phenolic compounds in a polyphenol-rich extract of Davidson's plum

4.2.1 Introduction

The food industry has growing demand for plant-sourced natural antimicrobials. Native Australian Davidson's plum (*Davidsonia prurierns)* originating from north Queensland has limited information on its antimicrobial properties. Exploring Australian native plants as a source of phytochemicals offers opportunities for the development of novel functional ingredients for the food industry.

4.2.2 Methods

4.2.2.1 Extraction of polyphenol-rich extracts from native fruits

Briefly, raw fruit material was ground into a pulp and acidified methanol (80% methanol, 19.9% $H^2O$and 0.1% HCl, v/v/v) was added; the mixture was stirred for 15 min, centrifuged for 25 min at 11,000×g at 4°C and the supernatant collected. The extraction was conducted three times with supernatants pooled together. The solvent was evaporated to produce a concentrated crude methanolic extract. The extract was then purified twice on an XAD-7HP column (Sigma-Aldrich, St. Louis, MO, USA) and freeze-dried to produce a lyophilised powder representing a purified polyphenolic fruit fraction (Tan et al. 2011).

4.2.2.2 Measurement of antimicrobial activity of polyphenol-rich fractions

Phytochemicals from the freeze-dried powder of the whole plum were extracted using acetone, hexane, ethanol, methanol and water. The percentage inhibition of the solvent extracts at dilutions ranging from 10–35% against *Listeria monocytogenes*, *Shewanella putrefaciens*, *Acinetobacter baumannii*, *Enterobacter aerogenes*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Escherichia coli* and *Staphylococcus aureus* was determined using a microtitre plate assay as described in Section 3.2.2.4.2.

The polyphenol-rich fraction was evaluated for percentage inhibition at concentrations ranging from 0.25 to 0.75 µg/ml.

4.2.3 Results and discussion

4.2.3.1 Major phenolic compounds in polyphenol-rich fraction of Davidson's plum

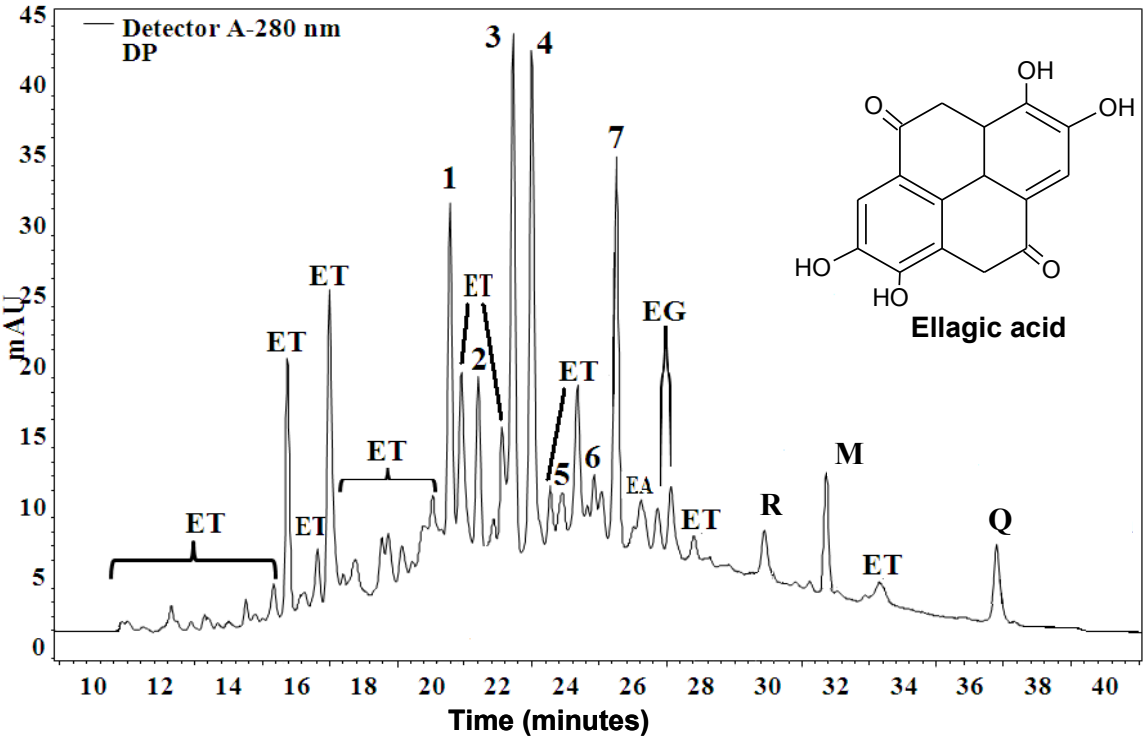


Figure 4.4 HPLC chromatogram of major phenolic compounds identified in Davidson's plum polyphenolic-rich fraction before acid hydrolysis. 1 delphinidin 3-sambubioside; 2 cyanidin 3-sambubioside; 3 pelargonidin 3-sambubioside; 4 peonidin 3-sambubioside; 5 and 6 unknown anthocyanins; 7 malvidin 3-sambubioside, 8 unknown anthocyanin, ET ellagitannin; EA ellagic acid; EG ellagic acid glycoside; R rutin; M myricetin; Q quercetin.

Table 4.7 Major phenolic compounds identified in polyphenol-rich fraction of Davidson's plum at 250 nm after acid hydrolysis

Compounds	Conc. (mg/g DW)
Ellagic acid	36 ± 5.0
Ellagic acid derivative	145 ± 7.2
Quercetin /quercetin derivatives	6 ± 0.4
Rutin	6 ± 0.4
Myricetin	10 ± 0.6
Delphinidin sambubioside	9 ± 0.0
Cyanidin sambubioside	4 ± 0.2
Pelargonidin sambubioside	13 ± 0.6
Peonidin sambubioside	13 ± 0.6
Malvidin sambubioside	7 ± 0.4

4.2.3.2 Antimicrobial activity of the polyphenol-rich fraction of Davidson's plum

Table 4.8 Antimicrobial activity (% inhibition) of solvent extracts of polyphenol-rich fraction of Davidson's plum (conc. of 8.75%)

	Acetone	Hexane	Methanol	Water	Ethanol
Geotritichum candidum	1.27±0.09	46.67±4.28	13.04±2.81	0	8.53±1.24
Listeria monocytogenes (Gram +ve)	91.58±9.14	0	84.60±5.43	100±7.54	77.68±5.47
Shewanella putrefaciens (Gram –ve)	100±3.67	43.18±3.71	96.47±9.95	100±11.81	83.54±7.78
Acinetobacter baumannii (Gram –ve)	83.41±2.06	0	98.15±9.59	100±10.75	94.53±4.83
Enterobacter aerogenes (Gram –ve)	60.62±6.61	14.54±1.48	96.79±3.45	100±6.25	97.82±6.99
Pseudomonas aeruginosa (Gram- ve)	100±4.54	13.21±0.10	90.69±7.67	100±3.43	97.23±1.73
Proteus vulgaris (Gram –ve)	94.06±4.86	8.25±0.54	92.43±2.11	100±4.17	97.50±1.29
Escherichia coli (Gram –ve)	69.01±4.94	5.45±0.20	89.79±7.72	100±10.49	98.32±2.00
Staphylococcus aureus (Gram +ve)	92.48±6.07	0	92.83±1.60	100±7.03	100±5.63

Table 4.9 Antimicrobial activity (% inhibition) of solvent extracts of polyphenol-rich fraction of Davidson's plum *

Microorganism	Davidson's plum	Southern high bush blueberries
Escherichia coli	64.73±1.98	65.71±1.35
Staphylococcus aureus	100.00±0.61	100.00±2.84

***** Conc of 0.75 µg/mL.

The polyphenol-rich fraction showed complete inhibition of *S. aureus* and 65% inhibition of *E. coli* and was comparable to blueberry (refer to Table 4.9). The high levels of ellagic acid could have contributed to the antimicrobial activity of the polyphenol-rich fraction.

The combined effect of malic acid and phenolic compounds in the aqueous extract could also have contributed to complete inhibition.

The highest antibacterial activity was observed in the water, acetone, methanol and ethanol extracts of Davidson's plum (Table 4.8). These extracts were equally effective against the Gram-negative and Gram-positive bacteria.

The hexane extract was not effective in inhibiting the growth of the bacteria. All the Davidson's plum extracts did not inhibit the growth of the *Geotritichum candidum* fungus. In general most natural antimicrobials such as essential oils are more effective against Gram-positive bacteria than Gram-negative bacteria and this can limit their application as a natural preservative.

The Davidson's plum extracts were effective against the selected range of food spoilage and pathogenic bacteria. This broad spectrum activity clearly indicates its potential as a functional ingredient with antimicrobial activity.

4.3 Antimicrobial properties and phenolic compounds in polyphenol-rich extract of commercially grown native Australian herbs

4.3.1 Introduction

Although a number of native Australian herbs have been commercialized, information regarding their antimicrobial properties is limited. Polyphenol-rich fractions obtained from three native Australian herbs (Tasmanian pepper leaf, anise myrtle and lemon myrtle) were characterised with regards to their composition and antimicrobial capacities using an *in vitro* assay. These polyphenol-rich extracts can be extracted from fresh leaves or the residue after extraction of essential oils.

4.3.2 Materials and methods

4.3.2.1 Extraction of polyphenol-rich extracts from native herbs

Dry herbs were weighed and finely ground. A five-fold volume of acidified methanol (80% methanol, 19% H2O and 1% acetic acid, v/v/v) was added, and the mixture was stirred for 2 h at a cool temperature (4°C) and centrifuged for 20 min at 15 320 *g* (10 000 rpm) at 4ºC (Sorvall RC-5B; DuPont, Wilmington, DE, USA; rotor Beckman JA14 (137 mm) serial No. 02U8152, USA). The supernatant was collected and the extraction was repeated twice. The third extraction was carried out overnight (16 h). The supernatants from the consecutive extractions were combined and the solvent evaporated under reduced pressure at 40°C using a rotary evaporator (Rotavapor R-205; Buchi, Switzerland). The concentrated alcoholic extracts were purified using an XAD-16 resin column (300x60 mm i.d.). The extracts were dissolved in acidified water (99% H_2O, 1% acetic acid, v/v), applied to the column, washed with acidified water and eluted with 80% ethanol (80% ethanol, 19.9% H2O, 0.1% trifluoroacetic acid, v/v/v). The eluate was collected and evaporated under reduced pressure at 37°C using a rotary evaporator. The purification was repeated and the resulting fraction was dissolved in purified water and freeze-dried under vacuum to obtain a fine lyophilised powder representing a polyphenolic-rich fraction. The extraction yield was calculated as a percentage of the original raw plant material according to the formula: Yield (%) = (LFX100)/DL, where LF was the weight of lyophilised fraction (g) and DL was the weight of the extracted sample (g) (Sakulnarmrat & Konczak 2012).

4.3.2.2 Measurement of antimicrobial activity of polyphenol-rich fractions

Antimicrobial activity measurement was done as described in Section 3.2.2.4.2. The concentrations of the polyphenol powders used for the three native herbs and by leaves were 0.25, 0.5, 0.625 and 0.75 µg/mL. The extracts were dissolved in sterile water and diluted before testing against the following food spoilage and pathogenic organisms: *Escherichia coli*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Shewanella putrefaciens*, *Acinetobacter baumannii*, *Enterobacter aerogenes*, *Pseudomonas aeruginosa* and *Proteus vulgaris*. *Geotritichum candidum* were tested again.

4.3.3 Results and discussion

4.3.3.1 Major phenolic compounds in polyphenol-rich fractions of native herbs

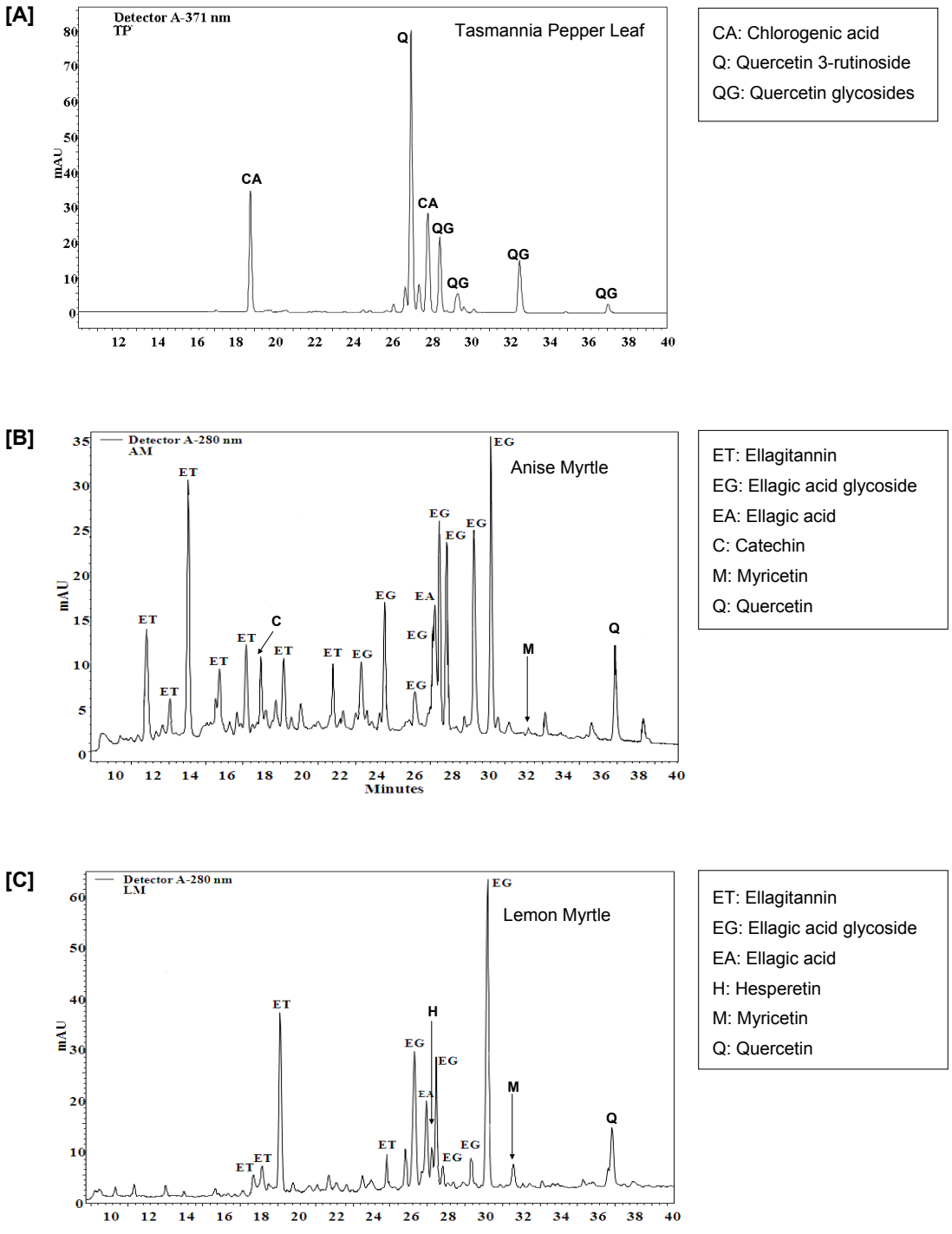


Figure 4.5 HPLC chromatograms of polyphenolic-rich fractions obtained from: [A] Tasmanian pepper leaf (TP), [B] anise myrtle and [C] lemon myrtle. Major polyphenols detected are shown in the chromatograms and their names are expanded in the right hand side panel.

4.3.3.2 Antimicrobial activity of the polyphenol-rich fraction of Australian herbs

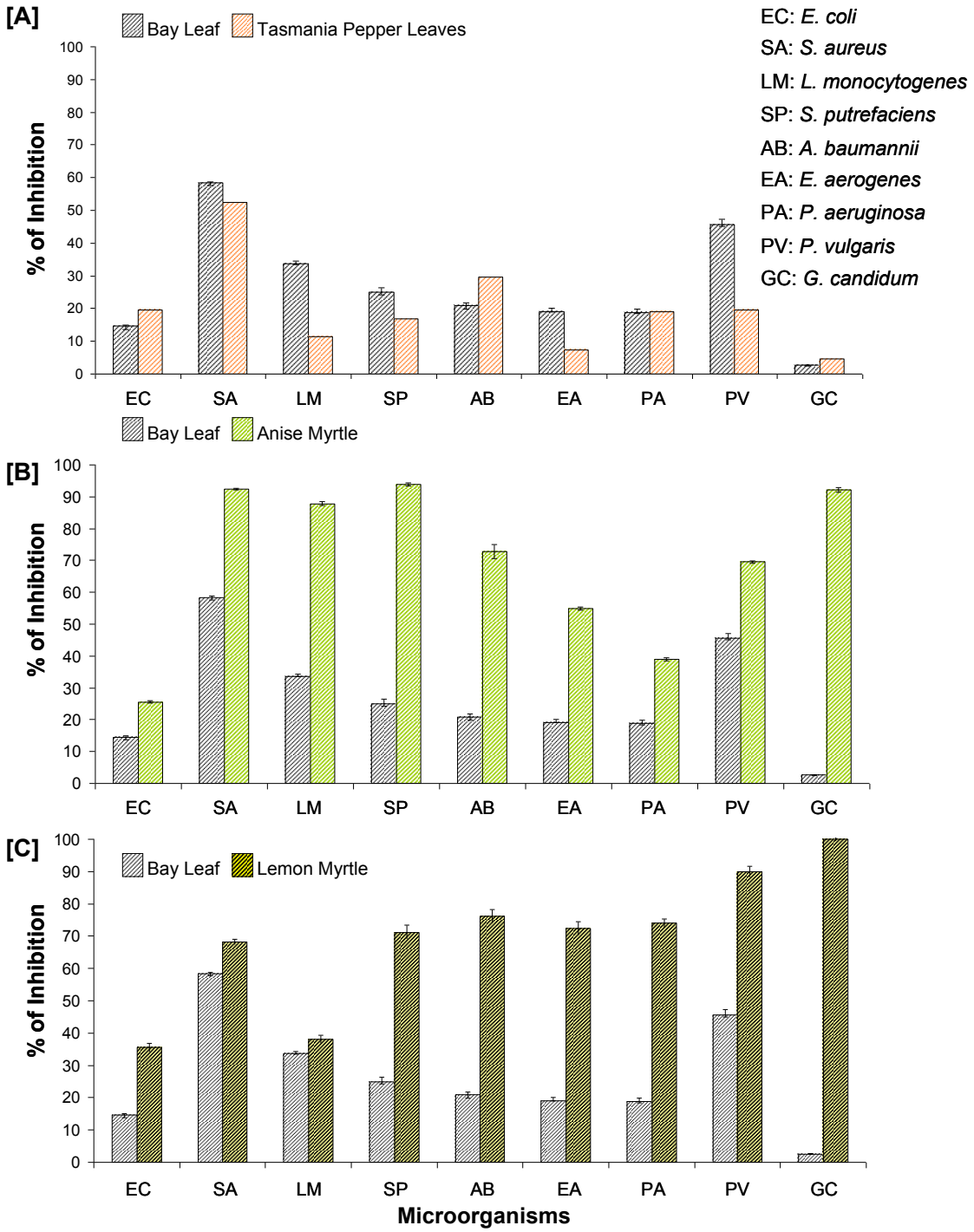


Figure 4.6 Antimicrobial activity of 0.75 µg/mL polyphenol-rich fractions of Australian native herbs [A] Tasmania pepper leaves, [B] anise myrtle and [C] lemon myrtle against a range of spoilage and pathogenic organisms. Polyphenol-rich fraction of bay leaf was used as a reference sample. The data represent an average value of six replicates.

Ellagic acid and derivatives were the dominant compounds of anise myrtle and lemon myrtle fractions, followed by flavanoids (catchin, myricetin, hespertetin and quercetin). The Tasmanian pepper leaf fraction was comprised of chloregenic acid and quercetin derivatives. Refer to Figure 4.5.

The percent inhibition determined across six replicates showed at a concentration of 0.75 μg/mL, anise myrtle and lemon had the highest growth inhibition for the tested organisms ranging from 26 to 100% and Tasmanian pepper leaf from 5 to 52% in comparison to a polyphenol-rich bay leaf fraction of 3 to 58% (Figure 4.6). The high levels of ellagic acid, ellagic acid derivatives and quercetin in anise myrtle and lemon myrtle and the chlorogenic acid, quercetin and quercetin 3-glucopside could have contributed to the antimicrobial activity.

Further research is needed to determine the potential use of native herbs as natural antimicrobials in food.

Appendix

Questionnaire

About you and your business

1. Please indicate the activities that your business is currently involved in.

☐ Cultivation ☐ Marketing ☐ Exporting
☐ Preliminary processing ☐ Value added processing

Other _____

2. Tell us a bit about your customers or product end-users. Please indicate which industry sector/s your business currently supplies. Tick as many as are relevant.

☐ Food industry (food manufacturers, retailers, distributors etc)
☐ Pharmaceutical or nutraceutical industry
☐ Flavour and fragrance industry
☐ Other. Please specify _____

3. In what markets is your products being sold and distributed?

☐ Domestic only ☐ Mostly domestic with some export
☐ Export only ☐ Mostly export with some domestic

About your Cultivation

4. Please list the species of native foods you currently grow, harvest and process in the table below.

Species		Processed Format/s
Common Name	**Botanical Name**	
1.		
2.		
3.		
4.		

5. Please provide an estimate of the annual production for each species.

Species (common name)	Annual Production Volume	
	Fresh	**Processed**
1.		
2.		
3.		
4.		

5. Would you indentify your company as one of the major producers of the native foods you grow? If you are aware of any other commercial growers in Australia please list them in the following table.

Species (common name)	Major Commercial Producer	Other Producers
1.	☐ Yes ☐ No. ► If no who is: _____	
2.	☐ Yes ☐ No. ► If no who is: _____	
3.	☐ Yes ☐ No. ► If no who is: _____	
4.	☐ Yes ☐ No. ► If no who is: _____	

6. Do you grow selected cultivars of plant species with defined characteristics or is your plant stock genetically variable.

Species (common name)	Specific Cultivar
1.	☐ Yes ► Cultivar _____ ☐ No
2.	☐ Yes ► Cultivar _____ ☐ No
3.	☐ Yes ► Cultivar _____ ☐ No
4.	☐ Yes ► Cultivar _____ ☐ No

About your Harvesting and Processing

7. Do you collect any products through wild harvest techniques?

☐ YES ► If so, please complete the table below
☐ NO ► Continue to next question

Species (common name)	Annual Collected Volume
1.	
2.	
3.	
4.	

8. What is the harvesting schedule for each of your cultivated species?

Species (common name)	Harvest Time		
	All year round	Mostly in one season or period	Only in one season or period
1.	↑	☐ Season/month: _____	☐ Season/month: _____
2.	↑	☐ Season/month: _____	☐ Season/month: _____
3.	↑	☐ Season/month: _____	☐ Season/month: _____
4.	↑	☐ Season/month: _____	☐ Season/month: _____

9. Does the overall product quality, composition or certain attributes change according to seasonal effects or weather conditions?

☐ YES ► If so, please complete the table below
☐ NO ► Continue to next question

Species (common name)	Product Changes
1.	
2.	
3.	
4.	

10. How do you determine your product is mature or ready to be harvested? An example may be when the fruit is a particular size or colour.

Species (common name)	Signs of Maturity/Readiness for Harvest
1.	
2.	
3.	
4.	

11. Please provide details of your post-harvest treatment for each of your species.

a) How the fresh product stored immediately post-harvest (e.g. refrigerated, ambient) while waiting to be processed?

_____

_____

_____

_____

b) Is the product washed or sanitised before storage or before processing?

☐ YES ► If so, please provide details below
☐ NO ► Continue to next question

_____

_____

_____

_____

c) Is the product harvested using mechanical or manual techniques?

☐☐ Mechanical

☐ Manual

d) Is product sorted or graded before processing?

☐ YES ► If so, please provide details below
☐NO ► Continue to next question

_____

_____

_____

_____

e) What is the approximate time between harvesting and processing?

_____

_____

_____

_____

12. Please provide a detailed description of the processing protocol for each of your end product formats.

An example for lemon myrtle could be:

1. *Mechanical harvesting of leaves*
2. *Sorting of leaves from stem*
3. *Drying in a convection oven at a temp below 40 deg C and a moisture content less that 10%,*
4. *Cooling and then packaging in HDPE pouches*

_____

_____

_____

_____

_____

_____

_____

_____

_____

13. Please describe the current packaging materials and formats used for each end product. If possible include details of pack sizes and dimensions.

14. Under what conditions is each processed product stored?

_____

_____

_____

_____

_____

_____

15. Please list any technical information that is available for your finished products (e.g. moisture content, nutritional information, recommended shelf life and storage conditions).
Where possible please also attach a copy of your current technical data sheet or product specification that you supply to customers.

_____

_____

_____

_____

_____

_____

_____

_____

_____

16. Please identify a step in your production practices that you would like improved during the first year of this RIRDC/ANFIL funded project. For example in the case of lemon myrtle this might be the improvement of packaging to minimise the loss of volatile components during storage.

_____

_____

_____

_____

_____

17. Are you able to provide any details of the shelf life for whole or processed products in terms of their bioactivity (e.g. antioxidant activity)?

_____

_____

_____

_____

_____

18. What are the microbial limits of your product?

a) Do you set a microbial specification for your processed product?

☐ YES ► If so what are the limits? TPC:_____cfu/g Yeast and moulds_____cfu/g

☐NO ► Continue to next question

b) What is the shelf life of your product in terms of microbial quality (e.g. 24 months at 20ºC with a final microbial count of $<10^3$cfu/g).

_____

_____

_____

_____

19. What product attribute/s do you and/or the industry believe are the most widely accepted indicator of finished product quality. For example in the case of lemon myrtle flakes this may be leaf colour.

Finished Product Format	Quality Indicator
1.	
2.	
3.	
4.	

20. Can you provide any further information that might be important when considering the processing or storage of your products?

21. Any comments?

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

Thank you for your time!

References

Aaby, K, Skrede, G & Wrolstad, RE 2005, 'Phenolic composition and antioxidant activities in flesh and achenes of strawberries', *Journal of Agricultural and Food Chemistry,* vol. 53, pp. 4032–4040.

Anonymous 2012, 'Different dry foods WVTR* and O2TR* demands for packaging materials', *[Online]. StoraEnso*. Available: http://81.209.16.114/Aineistopankki/PDF/Dry%20Foods/different4.pdf [Accessed 04/09/12 2012].

Ahmed, AK & Johnson, KA 2000, 'Turner review no. 3. Horticultural development of Australian native edible plants', *Australian Journal of Botany*, vol. 48, pp. 417–426.

Al-Weshahy, A, El-Nokety, M, Bakhete, M & Rao, V 2013, 'Effect of storage on antioxidant activity of freeze-dried potato peels', *Food Research International*, vol. 50, pp. 507–512.

Amakura, Y, Okada, M, Tsuji, S & Tonogai, Y 2000, 'High-performance liquid chromatographic determination with photodiode array detection of ellagic acid in fresh and processed fruits', *Journal of Chromatography A ,*vol. 896, pp. 87–93.

AOAC 1984, *AOAC Official Methods of Analysis*, 14th edn. Methods 43.064; 43.076.

Australian New Zealand Food Standards Code 2013, Standard 2.9.2.

Bazyma, LA, Guskov, VP, Basteev, AV, Lyashenko, AM, Lyakhno, V & Kutovoy, VA 2006, 'The investigation of low temperature vacuum drying processes of agricultural materials', *Journal of Food Engineering,* vol. 74, pp. 410–415.

Benzie, IFF & Strain, JJ 1996, 'The ferric reducing ability of plasma (FRAP) as a measurement of "antioxidant power": the FRAP assay', *Analytical Biochemistry*, vol. 239, pp. 70–76.

Bissett, OW & Berry, RE 1975, 'Ascorbic acid retention in orange juice as related to container type', *Journal of Food Science*, vol. 40, no. 1, pp. 178–180.

Braja, DM, Richard, AW, Robert, WT, Michael, JZ & Keith, PS 1989, *New Dimensions in Flavor Research*, American Chemical Society, Washington, DC.

Brophy, JJ, Goldsack, RJ, Fookes, CJR & Forster, PI 1995, 'Leaf oils of the genus *Backhousia* (Myrtaceae)', *Journal of Essential Oil Research*, vol. 7, no. 3, pp. 237–254.

Chaliha, M, Cusack, A, Currie, M, Sultanbawa, Y & Smyth, H 2013, 'Effect of packaging materials and storage on major volatile compounds in three Australian native herbs', *Journal of Agricultural and Food Chemistry,* vol. 61, no. 24, pp. 5738–5745.

Chebrolua, KK, Jayaprakashaa, GK, Yooa, KS, Jiona, JL & Patil, BS 2012, 'An improved method for quantification of ascorbic and dehydroascorbic acids by HPLC', *Food Science and Technology*, vol. 47, pp. 443–449.

Clark, M 2012, *Australian Native Food Industry Stocktake*, RIRDC Pub. No. 12/066, Rural Industries Research and Development Corporation, Canberra.

Clifford, MN & Scalbert, A 2000, 'Ellagitannins - nature, occurrence and dietary burden', *Journal of the Science of Food and Agriculture,* vol. 80**,** pp. 1118–1125.

Cohen, JS & Yang, TCS 1995, 'Progress in food dehydration', *Trends in Food Science & Technology*, vol. 6, pp. 20–25.

Cooper, W 2004, *Fruits of the Australian Tropical Forest*, Australia.

Coultate, TP 1989, *Food: the Chemistry of its Components*, 2nd edn, Royal Society of Chemistry Paperbacks, London.

Crozier, A, Jaganath, IB & Clifford, MN 2009, 'Dietary phenolics: chemistry, bioavailability and effects on health', *National Product Reports*, vol. 26, pp. 1001–1043.

da Silva Pinto, M, Lajolo, FM & da Silva Pinto, MI 2008, 'Bioactive compounds and quantification of total ellagic acid in strawberries', *Food Chemistry,* vol. 107**,** pp 1629–1635.

Dennison, DB, Brawley, TG & Hunter, GLK 1981, 'Rapid high performance liquid chromatographic determination of ascorbic acid and combined ascorbic acid – dehydroascorbic acid in beverages', *Journal of Agricultural and Food Chemistry,* vol. 29, pp. 927–929.

Deutsch, JC 2000, 'Dehydroascorbic acid', *Journal of Chromatography A*, vol. 881, pp. 299–307.

Dini, I, Tenore, GC & Dini, A 2013, 'Effect of industrial and domestic processing on antioxidant properties of pumpkin pulp', *LWT – Food Science and Technology*, vol. 53, no. 1, pp. 382–385.

Douglas Goff, H 1992, 'Low-temperature stability and the glassy state in frozen foods', *Food Research International*, vol. 25, no. 4, pp. 317–325.

Gökmen, V, Kahraman, N, Demir, L & Acer, J 2000, 'Enzymatically validated liquid chromatographic method for the determination of ascorbic and dehydroascorbic acids in fruit and vegetables', *Journal of Chromatography A*, vol. 881, pp. 309–316.

Gonzalez-Castejon, M & Rodriguez-Casado, A 2011, 'Dietary phytochemicals and their potential effects on obesity : a review', *Journal of Pharmacological Research*, vol. 64, pp. 438–455.

Gry, JR, Black, L, Eriksen, FD, Pilegaard, K, Plumb, J, Rhodes, M & Kroon, PA 2007, 'EuroFIR-BASIS – a combined composition and biological activity database for bioactive compounds in plant-based foods', *Food Science & Technology,* vol. 18, pp. 434–444.

Guo, C, Yang, J, Wei, J, Li, Y, Xu, J & Jiang, Y 2003, 'Antioxidant activities of peel, pulp and seed fractions from common fruits as determined by FRAP assay', *Nutrition Research*, vol. 23, pp. 1719–1726.

Hayes, AJ & Markovic, B 2002. 'Toxicity of Australian essential oil *Backhousia citriodora* (lemon myrtle). Part 1. Antimicrobial activity and *in vitro* cytotoxicity', *Food and Chemical Toxicology*, vol. 40, pp. 535–543.

Hoare, M, Jones, S, Lindsay, J 1993, 'Total vitamin C analysis of orange juice', *Food Australia*, vol. 45, pp. 341–345.

Igual, M, García-Martínez, E, Martín-Esparza, ME & Martínez-Navarrete, N 2012, 'Effect of processing on the drying kinetics and functional value of dried apricot', *Food Research International*, 47, pp. 284–290.

Kähkönen, MP, Hopia, AI, Vuorela, HJ, Rauha, J, Pihlaja, K, Kujala, TS & Heinonen, M 1999, 'Antioxidant activity of plant extracts containing phenolic compounds', *Journal of Agricultural and Food Chemistry,* vol. 47, pp. 3954–3962.

Konczak, I, Zabaras, D, Dunstan, M, Aguas, P, Roulfe, P & Pavan, A 2009, *Health Benefits of Australian Native Foods: an evaluation of health-enhancing compounds*, RIRDC Pub. No. 09/133. Rural Industries Research and Development Corporation, Canberra.

Konczak, I, Zabaras, D, Dunstan, M & Aguas, P 2010a, 'Antioxidant capacity and phenolic compounds in commercially grown native Australian herbs and spices' *Food Chemistry*, vol. 122, pp. 260–266.

Konczak, I, Zabaras, D, Dunstan, M & Aguas, P 2010b, 'Antioxidant capacity and hydrophilic phytochemicals in commercially grown native Australian fruits', *Food Chemistry*, vol. 123, no. 4, pp. 1048–1054.

Krüger, E, Dietrich, H, Schöpplein, E, Rasim, S & Kürbel, P 2011, 'Cultivar, storage conditions and ripening effects on physical and chemical qualities of red raspberry fruit', *Postharvest Biology and Technology*, vol. 60, 1, pp. 31–37.

Lebosse´, RG, Ducruet, V & Feigenbaum, A 1997, 'Interactions between reactive aroma compounds from model citrus juice with polypropylene packaging film', *Journal of Agricultural & Food Information*, vol. 45, pp. 2836–2842.

Liang, C-P, Wang, M, Simon, JE & Ho, C-T 2004, 'Antioxidant activity of plant extracts on the inhibition of citral off-odor formation', *Molecular Nutrition & Food Research*, vol. 48, pp. 308–317.

Maas, JL, Wang, SY & Galletta, GJ 1991, 'Evaluation of strawberry cultivars for ellagic acid content', *HortScience*, vol. 26, pp. 66–68.

Madrau, MA, Piscopo, A, Sanguinetti, AM, Del Caro, A, Poiana, M, Romeo, FV & Piga, A 2009, 'Effect of drying temperature on polyphenolic content and antioxidant activity of apricots', *European Food Research and Technology* , vol. 228, pp. 441–448.

Madsen, HL & Bertelsen, G 1995, 'Spices as antioxidants', *Trends in Food Science and Tecnology*, vol. 6, no. 8, pp. 271–277.

Mattila, P & Hellstrom, J 2007, 'Phenolic acids in potatoes, vegetables, and some of their products' *Journal of Food Composition and Analysis,* vol. 20**,** pp. 152–160.

Mohney, SM, Hernandez, RJ, Giacin, JR & Miltz, J 1988, 'Permeability and solubility of d-limonene vapor in cereal package liners', *Journal of Food Science*, vol. 53, no. 1, pp. 253–257.

Nardini, M, Cirillo, E, Natella, F, Mencarelli, D & Comisso, A 2002, 'Detection of bound phenolic acids: prevention by ascorbic acid and ethylenediaminetetraacetic acid of degradation of phenolic acids during alkaline hydrolysis', *Food Chemistry,* vol. 79, pp. 119–124.

Netzel, M, Netzel, G, Tian, Q, Schwartz, S & Konczak, I 2006, 'Sources of antioxidant activity in Australian native fruits. identification and quantification of anthocyanins', *Journal of Agricultural and Food Chemistry*, vol. 54, pp. 9820–9826.

Netzel, M, Netzel, G, Tian, Q, Schwartz, S & Konczak, I 2007, 'Native Australian fruits – a novel source of antioxidants for food', *Innovative Food Science and Emerging Technologies*, vol. 8, pp. 339–346.

Nguyen, H, Campi, EM, Jackson, WR & Patti, AF 2009, 'Effect of oxidative deterioration on flavour and aroma components of lemon oil', *Food Chemistry*, vol. 112, pp. 388–393.

Nijhuis, HH, Torringa, HM, Muresan, S, Yuksel, D, Leguijt, C & Kloek, W 1998, 'Approaches to improving the quality of dried fruit and vegetables', *Trends in Food Science & Technology*, vol. 9, pp. 13–20.

Nisperos-Carriedo, MO, Buslig, BS & Shaw, PE 1992, 'Simultaneous detection of dehydroascorbic acid, ascorbic acid and some organic acids in fruits and vegetables by HPLC', *Journal of Agricultural and Food Chemistry,* vol. 40, pp. 1127–1130.

Novakova, L, Solichová, D & Solich, P 2009. 'Hydrophillic interaction liquid chromatography - charged aerosol detection as a straightforward solution for simultaneous analysis of ascorbic acid and dehydroascorbic acid', *Journal of Chromatography A*, vol. 1216, pp. 4574–4581.

Nuutila, AM, Kammiovirta, K & Oksman-Caldentey, K 2002, 'Comparison of methods for the hydrolysis of flavonoids and phenolic acids from onion and spinach for HPLC analysis', *Food Chemistry*, vol. 76, pp. 519–525.

Odriozola-Serrano, I, Hernández-Jover, T & Martín-Belloso, O 2007, 'Comparative evaluation of UV-HPLC methods and reducing agents to determine vitamin C in fruits', *Food Chemistry*, vol. 105, pp. 1151–1158.

Orikasa, T, Koide, S, Okamoto, S, Imaizumi, T, Muramatsu, Y, Takeda, J, Shiina, T, Tagawa, A, 2014, 'Impacts of hot air and vacuum drying on the quality attributes of kiwifruit slices', *Journal of Food Engineering*, vol. 125, pp. 51–58.

Osawa, T 1994, 'Novel natural antioxidants for utilisation in food and biological systems', in Uritani, I (ed.), *Post Harvest Biotechnology of Plant Food Materials in the Tropics*, Japan Scientific Societies Press, Tokyo.

Padda, MS & Picha, DH 2008, 'Quantification of phenolic acids and antioxidant activity in sweetpotato genoyptes', *Scientia Horticulturae*, vol. 119, pp. 17–20.

Patthamakanokporn, O, Puwastien, P, Nitithamyong, A & Sirichakwal, PP 2008, 'Changes of antioxidant activity and total phenolic compounds during storage of selected fruits', *Journal of Food Composition and Analysis*, vol. 21, no. 3, pp. 241–248.

Plutowska, B & Wardencki, W 2007, 'Aromagrams – aromatic profiles in the appreciation of food quality', *Food Chemistry*, vol. 101, pp. 845–872.

Quezada-Gallo, JA, Debeaufor, F & Voille, A 2000, 'Mechanism of Aroma Transfer through Edible and Plastic Packagings', in Risch, S J (ed.), *Food Packaging Testing Methods and Applications*, American Chemical Society, Washington, DC.

Rawson, A, Patras, A, Tiwari, BK, Noci, F, Koutchma, T & Brunton, N 2011, 'Effect of thermal and non thermal processing technologies on the bioactive content of exotic fruits and their products: review of recent advances', *Food Research International*, vol. 44, pp. 1875–1887.

Rickman, JC, Barret, DM & Bruhn, CM, 2007, 'Nutritional comparison of fresh, frozen and canned fruits and vegetables. Part 1. Vitamins C and B and phenolic compounds', *Journal of the Science of Food and Agriculture*; vol. 87, pp. 930–944.

Risch, SJ 2000, 'New developments in packaging materials', in Risch SJ (ed.), *Food Packaging - Testing Methods and Applications,* American Chemical Society ,Washington, DC.

Rizzo, V & Muratore, G 2009, 'Effects of packaging on shelf life of fresh celery', *Journal of Food Engineering*, vol. 90, pp. 124–128.

Robbins, RJ 2003, 'Phenolic acids in foods: an overview of analytical methodology' *Journal of Agricultural and Food Chemistry,* vol. 51, pp. 2866–2887.

Roland, AM & Hotchkiss, JH 1991, *Determination of Flavor-polymer Interactions by Vacuummicrogravimetric Method*, American Chemical Society Washington, D.C.

Sahari, MA, Mohsen Boostani, F & Zohreh Hamidi, E 2004, 'Effect of low temperature on the ascorbic acid content and quality characteristics of frozen strawberry', Food Chemistry, vol. 86, no. 3, pp. 357–363.

Sajilata, MG, Savitha, K, Singhal, RS & Kanetkar, VR 2007, 'Scalping of flavors in packaged foods', *Compr. Rev. Food Sci. F.*, vol. 6, pp. 17–35.

Sakulnarmrat, K & Konczak, I 2012, 'Composition of native Australian herbs polyphenolic-rich fractions and *in vitro* inhibitory activities against key enzymes relevant to metabolic syndrome', *Food Chemistry*, vol. 134, pp. 1011–1019.

Sakulnarmrat, K & Konczak, I 2012, 'Composition of native Australian herbs polyphenolic-rich fractions and in-vitro inhibitory activities against key enzymes relevant to metabolic syndrome', *Food Chemistry*, vol. 134, pp. 1011–1019.

Singh, KK & Goswami, TK 1999, 'Design of a cryogenic grinding system for spices', *Journal of Food Engineering*, vol. 39, no. 4, pp. 359–368.

Singleton, VL & Rossi, JA 1965, 'Colorimetry of total phenolics with phosphor- molybdic phosphotungstic acid reagents', *American Journal of Enology and Viticulture*, vol. 16, pp. 144–158.

Skrede, G (ed.) 1996, *Fruits*, Marcel Dekker Inc., New York.

Southwell, I, Russel, M, Birmingham, E & Brophy, J 1996, 'Aniseed myrtle – leaf quality', *Australian Rainforest Bushfood Industry Association Newsletter*, vol. 4, pp. 13–15.

Sowbhagya, HB, Sampathu, SR & Krishnamurthy, N 2007, 'Evaluation of size reduction on the yield and quality of celery seed oil', *Journal of Food Engineering*, vol. 80, no. 4, pp. 1255–1260.

Sultanbawa Y, Cusack A, Currie M & Davis, C 2009, 'An innovative microplate assay to facilitate the detection of antimicrobial activity in plant extracts', *Journal of Rapid Methods & Automation in Microbiology*, vol. 17, pp. 519–534.

Takenaka, M, Nanayama, K, Isobe, S & Murata, M 2006, 'Changes in caffeic acid derivatives in sweetpotato (*Ipomoea batatas* L.) during cooking and processing', *Bioscience, Biotechnology, and Biochemistry,* vol. 70**,** pp. 172–177.

Tan, C, Hou, D, Konczak, I, Tanigawa, S, Ramzan, I & Sze, D, 'Native Australian fruit polyphenols inhibit COX-2 and iNOS expression in LPS-activated murine macrophages', *Food Research International,* vol. 44, pp. 2362–2367.

Tiwari, U & Cummins, E 2011, 'Factors influencing levels of phytochemicals in selected fruit and vegetables during pre- an post-harvest food processing operations', *Food Research International,* vol. 50, pp. 497–506.

Tomas-Barberan, FA & Clifford, MN 2000, 'Dietary hydroxybenzoic acid derivatives - nature, occurrence and dietary burden', *Journal of the Science of Food and Agriculture,* vol. 80**,** pp. 1024–1032.

Volden, J, Bengtsson, GB & Wicklund, T 2009, 'Glucosinolates, l-ascorbic acid, total phenols, anthocyanins, antioxidant capacities and colour in cauliflower (*Brassica oleracea L. ssp. botrytis*), effect and long-term freezer strorage', *Food Chemistry*, vol. 112, pp. 967–976.

Wankhade, PK, Sapkal, RS & Sapkal, VS 2013, 'Drying characteristics of okra slices on drying in hot air dryer', *Procedia Engineering*, vol. 51, pp. 371–374.

Williams, DJ, Edward, D, Hamernig, I, Jian, L, James, AP, Johnson, SK & Tapselle, LC 2013, 'Vegetables containing phytochemicals with potential anti-obesity properties: a review', *Food Research International*, vol. 52, pp. 323–333.

Venning, JA, Burns, DJW, Hoskin, KM, Nguyen, T & Stec, MGH 1989, 'Factors influencing the stability of frozen kiwifruit pulp', *Journal of Food Science*, vol. 54, no. 2, pp. 396–400.

Zafrilla, P, Ferreres, F, &Tomás-Barberán, FA 2001, 'Effect of processing and storage on the antioxidant ellagic acid derivatives and flavonoids of red raspberry jams', *Journal of Agricultural and Food Chemistry,* vol. 49, pp. 3651–3655.

Zheng, W & Wang, SY 2001, 'Antioxidant activity and phenolic compounds in selected herbs' *Journal of Agricultural and Food Chemistry,* vol. 49, pp. 5165–5170.

Changes in Quality and Bioactivity of Native Foods during Storage

By Y Sultanbawa, D Williams, M Chaliha, I Konczak and H Smyth

February 2015

Pub. No. 15/010

RURAL INDUSTRIES
Research & Development Corporation

Phone: 02 6271 4100
Fax: 02 6271 4199
Bookshop: 1300 634 313
Email: rirdc@rirdc.gov.au
Postal Address: PO Box 4776, Kingston ACT 2604
Street Address: Level 2, 15 National Circuit, Barton ACT 2600

www.rirdc.gov.au