# AGRICULTURAL AND FOOD CHEMISTRY

# Effect of Packaging Materials and Storage on Major Volatile Compounds in Three Australian Native Herbs

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**ABSTRACT:** Lemon myrtle, anise myrtle, and Tasmanian pepper leaf are commercial Australian native herbs with a high volatile or essential oil content. Packaging of the herbs in high- or low-density polyethylene (HDPE and LDPE) has proven to be ineffective in preventing a significant loss of volatile components on storage. This study investigates and compares the effectiveness of alternate high-barrier property packaging materials, namely, polyvinylidene chloride coated polyethylene terephthalate/casted polypropylene (PVDC coated PET/CPP) and polyethylene terephthalate/polyethylene terephthalate/aluminum foil/linear low-density polyethylene (PET/PET/Foil/LLDPE), in prevention of volatile compound loss from the three native herbs stored at ambient temperature for 6 months. Concentrations of major volatiles were monitored using gas chromatography–mass spectrometry (GC-MS) techniques. 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 (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 <30%. This study clearly indicates the importance of selecting the correct packaging material to retain the quality of herbs with high volatile content.

KEYWORDS: Australian native herbs, lemon myrtle, anise myrtle, Tasmanian pepper leaves, GC-MS, storage, high-barrier packaging

# INTRODUCTION

Australia's rich native flora, comprising more than 25,000 plants, have been used as food and medicine by the indigenous population for centuries.<sup>1</sup> Australian endemic plants have gained significant attention over recent years due to their increased use in pharmacy, medicine, food, beverages, cosmetics, perfumery, and aromatherapy.<sup>2</sup> This has resulted in 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 industries.<sup>3</sup> 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).

Three Australian native herbs that have entered commercialscale production, lemon myrtle, anise myrtle, and Tasmanian pepper, were included in this study.

Lemon myrtle (*Backhousia citriodora*), which falls under the family Myrtaceae, is an important and highly aromatic shrub endemic to eastern Australia.<sup>4</sup> The dominant (95%) volatile compound in lemon myrtle (chemotype *B. citriodora* F. Mueller) essential oil is citral (3,7-dimethylocta-2,6-dienal), which is an isomeric mix of two aldehydes, neral (*Z*-isomer) and geranial (*E*-isomer).<sup>5–8</sup> Citral has significant antimicrobial activity against a range of Gram-positive and Gram-negative bacteria, yeast, and mold.<sup>2,4</sup> As neat compounds, both neral and citral exhibit a lemon aroma.<sup>9</sup> Due to their strong lemon flavor,<sup>10</sup> the leaves and flowers of lemon myrtle are used in tea blends and beverages, dairy, cookies, breads, confectionery,

pasta, syrups, liqueurs, flavored oils, packaged fish (salmon), and dipping and simmer sauces.<sup>11</sup>

Anise myrtle (*Syzygium anisatum*, Myrtaceae) is a rare Australian rainforest tree from northeastern New South Wales and Queensland. The leaves are used as a herb in sweet and savory dishes as well as in cosmetics and provide an aniseed flavor.<sup>10</sup> A major volatile component of anise myrtle is (*E*)-anethole ((*E*)-1-methoxy-4-(10-propenyl)benzene) (79–90%).<sup>7</sup>

Leaves of Tasmanian pepper (*Tasmannia lanceolata*, Winteraceae) are used as a herb, whereas the berries are used as a spice. Both leaf and berry have a strong heat and pungent flavor on the palate.<sup>10</sup> The spicy character of Tasmanian pepper has been associated with the sesquiterpene polygodial.<sup>12</sup>

Low molecular weight volatile organic compounds are important constituents of food products as they influence the flavor.<sup>13</sup> Loss of quality in terms of aroma, taste, color, and texture is well-known in dried products.<sup>14</sup> Deterioration of food products takes place progressively during storage, and the loss of 'freshness' parameters such as aroma is immediately recognized by the consumer.<sup>15</sup> The human nose can detect volatile changes in products even at very low concentrations.<sup>16</sup> The aroma of food products must therefore be maintained during storage, and quality retention in dried products must be improved by altering processing and storage conditions and/or

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pretreatments. This has been a major research area in recent years.  $^{17}\!$ 

The development of improved packaging materials for food and agricultural industries is a rapidly growing and important area of research and development. Packaging fulfills a number of purposes including preventing contamination during distribution, preserving product integrity, and maintaining the desired flavor profile of the product.<sup>13,16,18</sup> Selection of packaging type is a crucial factor in maintaining the quality and stability of any food product. Migration of water, oxygen, and aroma volatiles can take place through packaging materials, which can change the quality of the food product and result in decreased flavor intensity or modification of the sensory profile. Furthermore, flavor "scalping" can occur, whereby volatiles are removed directly from foods via adsorption by the packaging material itself.<sup>19'</sup> Ideally, packaging materials will maintain the product integrity and allow cost reduction. Arguably the most crucial factor in a suitable packaging material for bulk commercial food ingredients is its barrier properties, which can have the greatest impact on product quality. Modern packaging materials are typically developed by combining a number of different materials through lamination, coextrusion, or coating that offer products with better barrier properties than the individual materials alone.<sup>18</sup> Combinations of layers with different types of foil, plastics, such as polyethylene (PE), polypropylene (PP), polyethylene terephthlate (PET), nylon, and ethylene vinyl alcohol (EVOH), paper, and adhesives are commercially available.

The Australian native food industry reports that retaining aroma and flavors of dried milled leaves of native herbs is a major challenge even after only 1 month of storage in PE packages. This is presumably due to significant losses of volatile aroma components. Moreover, certain volatile compounds such as citral migrate into the packaging material, causing rapid disintegration of the packaging material itself (personal correspondence with supplier). Most of the active components of the herbs are volatiles themselves, and therefore effective high barrier property packaging is critical to maintain the quality of products.

To date, the Australian native food industry has been unable to provide shelf life information together with the commercially available dried herb products as there is no information on the retention of active volatiles when stored at room temperature. Although the major volatiles present in lemon myrtle, anise myrtle, and Tasmanian pepper berry have been identified,<sup>6,7</sup> the effects of packaging material on these volatiles during storage have not been studied. In this study, the effectiveness of three packaging materials with various high-barrier properties was investigated in the prevention of volatile loss in three native Australian herbs stored at room temperature for 6 months.

# MATERIALS AND METHODS

**Samples.** Three native Australian herb samples selected by the Australian Native Food Industry Ltd. (ANFIL) were analyzed in this study. Samples of lemon myrtle (*B. citriodora* F. Muell) and anise myrtle (*S. anisatum* (Vickery, Craven & Biffen) were obtained from Australian Rainforest Products (NSW, Australia). Tasmanian pepper (*T. lanceolata*) leaf samples were supplied by the Diemen Pepper Co. (Tasmania, Australia).

The herb samples were supplied in the commercially available formats, dried and milled. The suppliers also provided fresh samples of 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 unmilled (for lemon myrtle and Tasmanian pepper). These samples are referred to as fresh and month = 0 samples throughout the paper.

**Chemicals.** A preliminary GC-MS 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 anethole (1-methoxy-4-(1-propenyl)benzene) in anise myrtle, and eucalyptol (1,3,3-trimethyl-2-oxabicyclo[2.2.2]octane) and eugenol (4-allyl-2-methoxyphenol) in Tasmanian pepper. Standards of each of these compounds were purchased from Sigma-Aldrich, Australia. Hexadecane (Sigma-Aldrich) was used as the internal standard (IS). All of the reagents and solvents used were of analytical quality and used without further purification. All aqueous solutions were prepared using deionized water. Stock standard solutions were stored at -20 °C.

**Conditions for Storage Trial.** The low- and high-density polyethylene (HDPE and LDPE) packaging material currently used in commercial practice was provided by Australian Rainforest Products and Diemen Pepper, respectively. The low-density polyethylene (LDPE, Alkathene, LDH215, Qenos Pty Ltd., Victoria, Australia) is used commercially for packaging lemon and anise myrtle, whereas Tasmanian pepper is commercially packaged in high-density polyethylene material (HDPE, GM4755F, Qenos Pty Ltd.). Two laminated packaging films, namely, polyvinylidene chloride coated polyethylene terephthalate/casted polypropylene (PVDC coated PET/CPP) and polyethylene terephthalate/polyethylene terephthalate/aluminum foil/linear low-density polyethylene (PET/PET/Foil/ LLDPE), were supplied by Amcor Flexibles Australasia and Detmold Flexibles, respectively. Properties of these packaging materials are described in Table 1. For the storage experiment, dried, milled leaves

 Table 1. Packaging Material Gas and Moisture Barrier

 Properties

	transmission rate				
film <sup><i>a</i></sup> (layer thickness, $\mu$ m)	water [g/(m²/24 h)]	oxygen [ cm <sup>3</sup> /(m <sup>2</sup> /24 h atm)]			
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)	0.5–1at 38 °C (RH 90%)	2-4 cm <sup>3</sup> m <sup>-2</sup> /24 h 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.

( $\sim$ 200 g per bag for lemon myrtle and Tasmanian pepper leaves,  $\sim$ 125 g per bag for anise myrtle) were packed in bags made of three types of packaging materials.

Filled bags were sealed under vacuum using a Multivac Chamber machine C 500 (Multivac Sepp Haggenmüller GmbH & Co. KG, Germany). All bags containing herb sample were packed into cardboard boxes for the duration of the trial (as per standard commercial practice).

Sampling for analysis occurred at time zero (before packaging, hereafter referred to as month = 0) and once each month for a total of 6 months, labeled months 1, 2, 3, 4, 5, and 6. Duplicate bags were opened for sampling at each time point, each of which were subsampled in duplicate, resulting in a total of four replicates per sampling point. Once sampled, the herbs were immediately subjected to volatile extraction as detailed below, and the extracts were stored in amber vials and stored at -80 °C before being instrumentally analyzed.

**Preparation of Samples for Analysis.** Fresh samples (labeled as month = 0) of native herbs 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 1 min. The cells were then inserted into the cryomill and ground for 30 s at a speed of 30 Hz. These samples were analyzed to obtain baseline information regarding the concentration of volatiles. These samples were analyzed at the beginning of the storage trial at month = 0.

The volatile component of the dried, premilled, native herb samples was extracted using liquid–liquid extraction (LLE). For LLE, 0.5 g of prepared herb sample was weighed into a 25 mL clear glass vial, and pentane/diethyl ether (2:1 ratio, 5 mL) was added to each sample along with deionized water (5 mL) and the internal standard (IS) solution (50  $\mu$ L of 1000 mg/L hexadecane in ethanol). The sample was shaken for 5 min in a vortex and allowed to sit at room temperature until two clear solvent layers could be seen. The top organic layer was carefully transferred to a new amber vial and dried with magnesium sulfate. The resulting extract was reduced to 1 mL under continuous N<sub>2</sub> flow. A volume of 100  $\mu$ L of the resulting concentrated extract was added to a 2 mL glass vial and made to volume with dicholoromethane ready for instrumental analysis.

**Instrumental Analysis.** Samples were analyzed with a 6890N gas chromatograph (GC) equipped with a 5975 mass spectrometric detector (MSD) (Agilent Technologies, Palo Alto, CA, USA). The GC was fitted with a DB-WAX column (J&W Science, i.d. = 0.25  $\mu$ m, length = 30.0 m, film thickness = 0.25  $\mu$ m), and helium (BOC gases, ultrahigh 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 GC oven temperature started at 50 °C for 1 min, was increased at 20 °C/min to 240 °C, and was held for 4 min. The native herb extracts were injected with a 10  $\mu$ L liquid injection syringe using an automated Multi Purpose Sampler (MPS2) (Gerstel, Germany). The injection volume was 3  $\mu$ L at a speed of 5  $\mu$ L/s in splitless mode and with a solvent delay of 3 min.

The MSD ion source was kept at 250 °C. Positive ion electron impact spectra were recorded in selected ion monitoring (SIM) mode. Data analysis was carried out with MSD ChemStation Data Analysis software (Agilent Technologies). Peak identification was achieved by comparison of spectra and retention times with authentic reference standards. The characteristic ions selected for each target volatile in SIM mode are listed in Table 2. For the internal standard hexadecane

 Table 2. GC-MS 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 <sup>2</sup> )
eucalyptol	<u>81</u> , 108, 139	4.260	0, 0.625-100	0.941
estragole	133, 147 <u>,148</u>	7.619	0, 0.625-100	0.958
neral	<u>69</u> , 94, 109	7.700	0, 0.625-100	0.986
geranial	<u>69</u> , 109, 137	8.008	0, 0.625-100	0.983
anethol	133, 147, <u>148</u>	8.610	0, 0.625-100	0.975
eugenol	137, 149, <u>164</u>	10.482	0, 0.625-100	0.807
<sup>a</sup> Quantitativ	e ions are under	lined.		

m/z 57 was used for quantitation. Due to the high concentrations of components in the herbs, SIM was not a necessary advantage, although it ensured that components could be measured even if very low levels were observed at the end of the storage trial.

**Development of Calibration Equations.** Standard addition calibrations were obtained by duplicate injection of five standard solutions containing a mixture of the target compounds eucalyptol, estragole, neral, geranial, anethole, and eugenol at concentrations of 0, 0.625, 12.5, 25.0, 50.0, and 100.0 mg/L and a constant concentration of the internal standard hexadecane of 1000 mg/L. The calibration equations for each compound were obtained by plotting the peak area response ratio (target compound/internal standard) versus the corresponding concentration ratio (target compound/internal standard) ard). Linearity was found throughout the range for each component, and the performance of each calibration is summarized in Table 2.

**Statistical Analysis.** All statistical analysis was performed using the JMP statistical package (JMP 6, SAS Institute Inc., Cary, NC, USA). One-way analysis of variance (ANOVA) and Student's *t* test were used to determine statistical differences in volatile compound concentrations across storage duration and across packaging materials and sample formats (i.e., fresh or dried). Mean values were considered to be significantly different when *p* < 0.05. Means were compared and ranked using a Tukey–Kramer honestly significant difference (HSD), and the LSD was used to determine absolute difference between means.

# RESULTS AND DISCUSSION

Volatile compounds contribute to the characteristic aroma profile of herbs and spices. Their concentration determines the presence or absence of pleasant or off-flavors in the herbs and thus are important contributors of quality. The volatile content of commercially available dried, milled leaf material from three Australian native plants, lemon myrtle, anise myrtle, and



**Figure 1.** 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 anethole in anise myrtle, and (C) eucalyptol and eugenol in Tasmanian pepper leaves (n = 4). Average concentrations analyzed with 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.

Table 3.	Concentrations	of Volatiles in	Native	Herbs after	Storage in	Different	Packaging	Materials	(n =	4)
					0				•	

			concn of volat	$lles^a$ (mg/kg) aft	er storage for		
packaging type	1 month	2 months	3 months	4 months	5 months	6 months	LSD
		Lemon N	Ayrtle				
LDPE	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
LDPE	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 M	lyrtle				
LDPE	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
LDPE	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 Pe	pper Leaves				
HDPE	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 a	7.6 a	8.3 a	6.4 ab	7.5 a	2.3
HDPE	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
	LDPE         PVDC coated PET/CPP         PET/PET/Foil/PE         HDPE         PVDC coated PET/CPP         PET/PET/Foil/PE	packaging typeI monthLDPE627 aPVDC coated PET/CPP930 aPET/PET/Foil/PE1142 aLDPE683 bPVDC coated PET/CPP941 aPET/PET/Foil/PE1173 aLDPE48 bPVDC coated PET/CPP115 aPET/PET/Foil/PE110 aLDPE525 aPVDC coated PET/CPP800 aPET/PET/Foil/PE762 aHDPE1.6 bPVDC coated PET/CPP9 aPET/PET/Foil/PE7.4 aHDPE69 aPVDC coated PET/CPP85 aPET/PET/Foil/PE7.8 a	packaging type         1 month         2 months           LDPE         627 a         548 c           PVDC coated PET/CPP         930 a         860 b           PET/PET/Foil/PE         1142 a         1089 a           LDPE         683 b         618 c           PVDC coated PET/CPP         941 a         868 b           PET/PET/Foil/PE         1173 a         1098 a           LDPE         48 b         26 c           PVDC coated PET/CPP         115 a         105 ab           PET/PET/Foil/PE         110 a         105 a           LDPE         48 b         26 c           PVDC coated PET/CPP         115 a         105 ab           PET/PET/Foil/PE         110 a         105 a           LDPE         525 a         376 b           PVDC coated PET/CPP         800 a         726 abc           PET/PET/Foil/PE         762 a         727 a           HDPE         1.6 b         0.7 c           PVDC coated PET/CPP         9 a         8 ab           PET/PET/Foil/PE         7.4 a         7.5 a           HDPE         69 a         57 b           PVDC coated PET/CPP         85 a         68 b           PET/PET/Foil/	packaging type         1 month         2 months         3 months           LDPE         627 a         548 c         319 d           PVDC coated PET/CPP         930 a         860 b         766 d           PET/PET/Foil/PE         1142 a         1089 a         963 b           LDPE         683 b         618 c         367 d           PVDC coated PET/CPP         941 a         868 b         766 d           PET/PET/Foil/PE         1173 a         1098 a         961 bc           DPE         683 b         618 c         367 d           PVDC coated PET/CPP         941 a         868 b         766 d           PET/PET/Foil/PE         1173 a         1098 a         961 bc           LDPE         48 b         26 c         15 d           PVDC coated PET/CPP         115 a         105 ab         92 b           PET/PET/Foil/PE         110 a         105 a         96 a           LDPE         525 a         376 b         295 c           PVDC coated PET/CPP         800 a         726 abc         639 c           PET/PET/Foil/PE         762 a         727 a         660 a           LDPE         1.6 b         0.7 c         0.5 d	packaging type         1 month         2 months         3 months         4 months           LDPE         627 a         548 c         319 d         275 e           PVDC coated PET/CPP         930 a         860 b         766 d         816 bc           PET/PET/Foil/PE         1142 a         1089 a         963 b         1175 a           LDPE         683 b         618 c         367 d         317 e           PVDC coated PET/CPP         941 a         868 b         766 d         814 cd           PET/PET/Foil/PE         1173 a         1098 a         961 bc         1164 a           PUDC coated PET/CPP         941 a         868 b         766 d         814 cd           PET/PET/Foil/PE         1173 a         1098 a         961 bc         1164 a           DPE         48 b         26 c         15 d         9 de           PVDC coated PET/CPP         115 a         105 ab         92 b         114 a           PET/PET/Foil/PE         110 a         105 a         96 a         99 a           LDPE         525 a         376 b         295 c         187 d           PVDC coated PET/CPP         800 a         726 abc         639 c         750 ab           PET/PET/Foi	packaging type         1 month         2 months         3 months         4 months         5 months           LDPE         627 a         548 c         319 d         275 e         221 f           PVDC coated PET/CPP         930 a         860 b         766 d         816 bc         644 e           PET/PET/Foil/PE         1142 a         1089 a         963 b         1175 a         1106 a           LDPE         683 b         618 c         367 d         317 e         257 f           PVDC coated PET/CPP         941 a         868 b         766 d         814 cd         650 e           PET/PET/Foil/PE         1173 a         1098 a         961 bc         1164 a         1109 a           DPE         48 b         26 c         15 d         9 de         7 e           PVDC coated PET/CPP         115 a         105 ab         92 b         114 a         107 ab           PET/PET/Foil/PE         110 a         105 a         96 a         99 a         102 a           LDPE         48 b         26 cc         15 d         9 de         7 e           PVDC coated PET/CPP         110 a         105 a         96 a         99 a         102 a           LDPE         525 a	packaging type         1 month         2 months         3 months         4 months         5 months         6 months           Lope         627 a         548 c         319 d         275 e         221 f         182 f           PVDC coated PET/CPP         930 a         860 b         766 d         816 bc         644 e         578 f           PET/PET/Foil/PE         1142 a         1089 a         963 b         1175 a         1106 a         1082 ab           LDPE         683 b         618 c         367 d         317 e         257 f         215 f           PVDC coated PET/CPP         941 a         868 b         766 d         814 cd         650 e         588 f           PET/PET/Foil/PE         1173 a         1098 a         961 bc         1164 a         1109 a         1087 ab           LDPE         48 b         26 c         15 d         9 de         7 e         4 e           PVDC coated PET/CPP         115 a         105 ab         92 b         114 a         107 ab         97 b           PET/PET/Foil/PE         110 a         105 a         96 a         99 a         102 a         80 b           LDPE         525 a         376 b         295 c         187 d <t< td=""></t<>

Tasmanian pepper, was analyzed after storage under "commercial" conditions. Although the major volatile components of a range of Australian native plants are known,<sup>6,7</sup> no previous studies have determined the effect of packaging material and storage on the volatile profile of Australian native plants.

An analytical method was developed to determine the concentration of major volatiles present in the three selected native herb samples using gas chromatography-mass spectrometry. Two of the most predominant volatiles were selected, per species, to monitor analytically. The compounds selected for each species are listed in Table 2. Preliminary analysis by GC-MS confirmed these target volatiles as major volatiles present in each species.

Prior to commencement of the storage trial, commercial samples of fresh leaves (with or without stems) and dried leaves (before and after milling) were collected to assess the effect of processing on the volatile composition of the products. The dried and powdered form of the herb is more favorable than fresh herb for export as it retains its quality over time, making storage and transport easier.<sup>20</sup> Commercial processing of lemon myrtle, anise myrtle, and Tasmanian pepper leaf differs to some extent, and the exact method of drying is treated as confidential information by the industry. Nevertheless, in the case of all three herbs, the freshly harvested raw materials (leaves) are subjected to drying and milling. The results of the volatile composition comparison between fresh leaves (with and without stems) and dried milled leaves are shown in Figure 1.

Comparisons could be made between the fresh samples of lemon myrtle and anise myrtle leaves with and without stems. Similar levels and ratios of the two major volatile components were found in lemon myrtle leaves both with and without stems (Figure 1A). This indicates that inclusion or exclusion of stems with the leaves will not change the overall aroma quality of the herb product. For anise myrtle, quite different ratios between the two major components were found for leaves with and without stems (Figure 1B). The results indicate that the stems are relatively low in anethole content, the character impact component of anise, and comparatively higher in estragole compared to the leaves alone. It may be important for industry to note that inclusion or removal of stems from anise myrtle leaf sample will influence the volatile profile and potential aroma quality of the herb product. A sample of Tasmanian pepper leaf without stems was not available for comparison.

In lemon myrtle and Tasmanian pepper leaves, the concentration of major volatiles was highest in the dried leaves before milling (Figure 1A,C). By comparison, Braja et al. reported a 3-fold increase in linalool concentration in coriander (*Coriander sativum*) seeds upon drying.<sup>21</sup> Similarly, basil has been reported to increase in volatile composition after drying.<sup>22,23</sup> After milling, the concentrations of major volatiles for both lemon myrtle and Tasmanian pepper leaves 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 a view to reduce the impact of processing on product quality.

For anise myrtle there were no samples available of dried herb premilling. When compared to the fresh herb without stems, both estragole and anethole were observed to increase in concentration after drying and milling (Figure 1B).

As shown in Table 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 anethole (471 mg/kg) in anise myrtle, and eucalyptol (5.2 mg/kg) and eugenol (57 mg/kg) in Tasmanian pepper leaves.

The main variables that affect quality of a stored product<sup>24</sup> include water activity or available water in product; availability of oxygen; presence of preservatives such as salts or antioxidants; and temperature during storage. For herbs the most important factors in preserving quality are water and oxygen transmission rates. The recommended transmission



**Figure 2.** Change in concentration of volatiles (mg/kg) during 6 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 were analyzed with 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.

rates for water and oxygen in herbs are <1 g/m<sup>2</sup>/day (38 °C, 90% RH) and <1 cm<sup>3</sup>/m<sup>2</sup>/day, respectively.<sup>25</sup> The two highbarrier packaging materials selected for inclusion in this study were PET/CPP and PET/PET/Foil/PE on the basis of their transmission rates for water and oxygen (refer to Table 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 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 2–4). This can be explained by the fact that LDPE and HDPE materials have a relatively high gas permeability rate (Table 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 1), which prevents the loss of volatiles. For all three herbs, a gradual decline in the concentration of major



**Figure 3.** Change in concentrations of volatiles (mg/kg) during 6 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) anethole (n = 4). Average concentrations were analyzed with 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.

volatiles was observed with increased time in storage irrespective of the packaging type.

These results suggest the native food industry in Australia has an opportunity to significantly improve the quality and longevity of their stored herb products by using alternate packaging materials.

The packaging with the best performance in retaining key volatiles neral and geranial in lemon myrtle was PET/PET/ Foil/PE bags (Table 3 and Figure 2). The PET/PET/Foil/PE packaging material performed significantly better than the PVDC coated PET/CPP bags over storage time in retaining key volatiles of lemon myrtle. Using PET/PET/Foil/PE packaging material would be the preferred option for the packaging of lemon myrtle product intended for storage to improve product quality and shelf life.

Interestingly, for anise myrtle and Tasmanian pepper leaf, there was no significant difference between samples stored in PET/PET/Foil/PE bags and those stored in PVDC coated PET/CPP bags in the retention of key volatiles (Table 3; Figures 3 and 4). Either of these two materials would be preferred for packaging anise myrtle or Tasmanian pepper leaf to minimize volatile loss and increase storage life compared to conventional LDPE or HDPE materials.

The loss of volatiles in the commercial polyethylene packaging could also be attributed to "flavor scalping". Scalping is the process by which volatile flavors from the food products are absorbed by the packaging material.<sup>19</sup> Polyethylene materials are well-known absorbers of flavor volatiles, especially aldehyde compounds.<sup>26</sup> This could partly explain the  $\sim 70\%$ reduction in aldehydes neral and gernial from lemon myrtle samples stored in LDPE over 6 months. Flavor scalping has been previously reported in Apulia table wine stored in plastic packages, which affected the quality of the wine.<sup>27</sup> Sadler and Braddock reported rapid absorption of citrus flavors, including limonene, ethyl butyrate, myrcene, and  $\alpha$ -pinene, from citrus stored in LDPE materials.<sup>28</sup> Significant losses of volatile components have also been reported in fruit juice stored at 4  $^\circ C$  and packed in PE packaging.  $^{29,30}$  In addition to affecting the volatile profile of a food product, scalping can also change the efficacy of the storage material itself, including increasing the permeability to aroma compounds and oxygen.<sup>26,27</sup> Migration of volatiles from the packaging material to the food matrix can be another issue in loss of important aroma volatiles. Migration of undesired volatiles from the packaging material can affect product quality and food safety.<sup>24</sup>

It should be noted that for all three herbs studied, the concentrations of volatiles in the samples prepackaging (i.e., month = 0) were observed to be lower than the concentrations of volatiles measured in samples after 1 month of storage (month = 1). This was a curiosity that could not be explained by the authors and may be due to handling issues. All of the samples were supplied by industry in bulk format already packed in PE material. The bulk packs were subsequently opened, subsampled for volatiles extraction (month = 0), and repackaged into new packaging materials for storage. Potentially there may have been liberated volatiles trapped in the headspace of the high-barrier bags that, after sealing in the new packages, were readsorbed by the herbs during 1 month of storage, thus inflating the concentration data obtained. The authors are currently undertaking a new storage trial with Australian native herbs over a longer storage period and will be investigating this issue further.

The findings from our study can be extended to other aromatic herbs and spices as the six target volatiles studied here are also the major volatile constituent of other herbs. For example, sweet basil (Ocimum basilicum) contains neral, geranial, estragole, and eugenol as major volatile constituents;<sup>31</sup> neral and geranial are the major constituents of lemon grass (Cymbopogon citratus) and ginger (Zingiber cassumunar Roxb.);<sup>32</sup> estragole is the major constituent in tarragon (Artemisia dracunculus);<sup>33</sup> anethole is the major volatile compound in Turkish bitter fennel (Foeniculum vulgare var. vulgare)<sup>34</sup> and rocket leaves (Eruca sativa);<sup>35</sup> eucalyptol is found in wild thyme (Thymus serpyllum L.),<sup>36</sup> bay leaf (Laurus nobilis L.),<sup>37</sup> and rosemary (Rosmarinus officinalis L.);<sup>20</sup> and eugenol is a major volatile component of clove oil (Eugenia caryophyllata L.),<sup>38</sup> nutmeg pericarp (Myristica fragrans),<sup>39</sup> and a range of cinnamon species.<sup>40</sup> These and other herbs and spices would be likely to benefit from packaging in either PET/ PET/Foil/PE or PVDC coated PET/CPP materials to minimize volatile loss during storage.

The interplay between packaging material, food product, and the environment can have an adverse effect on both the food product and the packaging material. Many food products lose their quality during the storage process due to factors such as moisture absorption, undesirable odor absorption, and



**Figure 4.** Change in concentration of volatiles (mg/kg) during 6 months of storage for Tasmanian pepper dried, milled leaves 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 were analyzed with 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.

migration of packaging components into the food.<sup>26,41</sup> Not surprisingly, the development of new packaging materials with high barrier properties is a continuing focus in food technology. Suitable packaging materials provide cost reduction in handling and product distribution and transportation, but also helps in reducing waste. Barrier properties of packaging materials, which include permeability of gases (oxygen, carbon dioxide, nitrogen, ethylene, etc.), water, vapor, aromas, and light, are key factors in maintaining the quality of herbs and spices under storage.<sup>42</sup> A significantly improved retention of key volatiles from three Australian native herbs was observed in those packed in highbarrier packaging materials such as PVDC coated PET/CPP and PET/PET/Foil/PE compared to conventional HDPE and LDPE packaging materials over 6 months of storage. The laminated packaging films provide excellent protection against volatile loss during storage of dried, milled native herbs.

Furthermore, this packaging type is ideal for storage and transport of bulk herbs intended for commercial purposes where the nontransparent material does not present a problem for consumers who might prefer to see the herb product through the package. The improved packaging suggested in this study can be used by the native food industry to target export markets, where a longer shelf quality is required when bulk herb products are shipped and transported.

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## Notes

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