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Flavour characterisation of fresh and processed pennywort (*Centella asiatica* L.) juices

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ABSTRACT

The flavour characteristics of fresh and processed pennywort juices treated by pasteurisation, sterilisation and high pressure processing (HPP) were investigated by using solid-phase micro-extraction combined with gas chromatography–mass spectrometry. Sesquiterpene hydrocarbons comprise the major class of volatile components present and the juices had a characteristic smell due to the presence of volatile compounds including β-caryophyllene, humulene, *E*-β-farnesene, α-copaene, alloaromadendrene and β-elemene. All processing operations caused a reduction in the total volatile concentration, but HPP caused more volatile acyclic alcohols, aldehydes and oxygenated monoterpenoids to be retained than pasteurisation and sterilisation. Ketones were not present in fresh pennywort juice, but 2-butanone and 3-nonen-2-one were generated in all processed juices, and 2-nonanone and 2-hexanone were present in pasteurised and sterilised juices. Other chemical changes including isomerisation were also reduced by HPP compared to pasteurisation and sterilisation.

1. Introduction

Asiatic pennywort (*C. asiatica*), also known as gotu kola and pegapa is one of the herbs grown in Thailand and other Asian countries that is claimed to possess various physiological effects. It has been used for hundreds of years as an anti-inflammatory and a treatment for leprosy and syphilis. It has been included in Thai traditional recipes as a poultice for wound healing (Farnsworth & Buryapraphatsara, 1992) and is commonly used as a vegetable and tonic (Pramongkit, 1995). Moreover, it has been reported that wound and ulcer healing are enhanced by its action in promoting fibroblast proliferation and collagen synthesis (Maquart, Bellon, Gillery, Wegrowski, & Borel, 1990).

Non-thermal methods for juice processing including high pressure have been applied more widely during the past few years. High pressure processing (HPP) is frequently applied in juice processing because it inactivates microorganisms with minimal damage to heat sensitive compounds. This method is capable of maintaining freshness and nutritive value (flavour, colour, vitamin content, biologically active components, etc.). The European Commission has included products processed by high pressure in the group of novel foods regulated by the Novel Foods Legislation.

Herbal plants have commanded special attention due to their value. However, quality control of raw herbs and their products is essential to ensure quality, safety and efficacy. The flavour of herbal plants is due to essential oils which are complex mixtures mainly containing volatile compounds. The most volatile are mono (C_{10}) and sesquiterpenoids (C_{15}) that represent the major components of essential oils. Monoterpenoids may have acyclic, monocyclic and bicyclic structures, and occur in nature as hydrocarbons, alcohols, ketones, aldehydes, ethers, etc. Many literature reports describe the importance of monoterpenoids for the flavour of foods (Perez-Cacho & Rouseff, 2008). However, monoterpenoid chemistry is complex, since they are distinguished by structural characteristics, including the presence of endocyclic and exocyclic carbon–carbon double bonds, which present steric limitations and contribute distinct reactivities; functionalised and substituted skeletons; the presence of highly tensioned rings, and the possibility of bicyclic ring rearrangements.

Chou (2005) identified nineteen compounds in the essential oil from *C. asiatica* in Taiwan as linalool, p-menth-1-en-8-ol, copaene, elemene, sesquiphellandrene, caryophyllene, thjosene, cadinene, acoradien, humulene, alloaromadendrene, farnesene, selinene, 1,5,5,8-tetramethyl-1,2-oxabicyclo [9,1,0] dodeca-3,7-diene, eudesmene, cuparene, caryophyllene oxide, phytol and hexahydrofarnesylacetone. Ali (2007) reported 23 compounds in this plant from Malaysia including α-pinene (1.2%), germacrene D (1.6%), (+)-cyclosativene (2.3%), β-farnesene (4.8%), β-cubebene (17%), α-caryophyllene (17%), α-humulene (22%), γ-murolene (22%) and various components with concentrations less than 1% including β-pinene, m-cymene, D-limonene, β-trans-ocimene, γ-terpinene,

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1-bromoallene, β-linalool, trans-3-nonen-2-one, terpinen-4-ol, o-menth-8-ene,4-isopropylidene-1-vinyl-α-cubebene, n-decyl acetate, β-cedrene, δ-cadinene and caryophyllene oxide. Oyedeji and Afolayan (2005) also reported that the essential oil extracted from *C. asiatica* grown in South Africa contained 11 monoterpenoid hydrocarbons (20.20%), 9 oxygenated monoterpenoids (5.46%), 14 sesquiterpenoid hydrocarbons (68.89%), 5 oxygenated sesquiterpenoids (3.90%) and 1 sulphide sesquiterpenoid (0.76%). Components present included sesquiterpenoid hydrocarbons including α-humulene (21.06%), β-caryophyllene (19.08%), bicyclogermacrene (11.22%), germacrene B (6.29%) and myrcene (6.55%). Other reports included trans-β-farnesene and germacrene D as prominent constituents of the essential oil.

The effect of heat treatment and HPP on pennywort juice flavour production has not been reported. In the present studies, we have analysed fresh and processed pennywort juice aroma by gas chromatography–mass spectrometry (GC–MS) using solid-phase micro-extraction (SPME).

2. Materials and methods

2.1. Raw material and preparation

The plant sample was freshly harvested, with commercial maturity (2–4 months) obtained from high land, Chiangmai, Thailand. Upon arrival at the laboratory, the sample was washed with running tap water to remove debris and damaged portions. The leaves and petioles of pennywort were stripped from the plant and extracted with water (1:1 w/v) by grinding at room temperature for 15 min. This juice was filled into the double layer of an LDPE Stomacher pouch (Seward Limited, UK) before HPP and filled in a retort pouch (PET/nylon/aluminium/PP) (Royal Can Industries Co., Ltd, Thailand) before pasteurisation and sterilisation. The samples were subjected to pasteurisation (90 °C for 3 min), sterilisation (121 °C for 4 min) and HPP (400 MPa for 20 min at <30 °C).

2.2. Chemicals

1,2-Dichlorobenzene (99% from Sigma–Aldrich Company, Ltd, Poole, UK) was used as an internal standard (IS) for GC–MS analysis, as this compound was not found in the pennywort juice.

2.3. Headspace concentration

The sample (20 ml) was transferred to a 40 ml amber vial which was capped with a PTFE septum. 1,2-Dichlorobenzene (10 μl) was added and the vial was incubated for 5 min at 35 °C, before a 75 μm Supleco SPME fibre Carboxen/Polydimethylsiloxane (PDMS) was introduced and exposed to the sample headspace for 30 min. The SPME fibre was then inserted into the GC–MS injection port and held for 15 min with the adsorbed components being effectively desorbed. The isolation and GC–MS analysis of volatiles from each sample of pennywort juice was repeated 4 times.

2.4. GC–MS analysis

Analysis was carried out with a Hewlett–Packard 5890 series II GC connected to a 5972 series MS and a 60 m VF-5 ms capillary column (i.d. 0.25 mm, 0.25 μm film thickness, Varian). The injector port was in splitless mode, and split flow was programmed to turn on after 0.5 min. The temperature of the injector was 250 °C. The oven temperature was kept at 50 °C for 3 min and programmed to increase at 4 °C min^{-1}. The final temperature was set at 240 °C and held for 5 min.

Retention times for a series of n-alkanes (C_5–C_{25}) were determined in the study by an analysis under exactly the same conditions, and they were used to calculate the LRI values of detected compounds.

The relative concentrations of the investigated compounds were calculated by relating the area of the internal standard to the area of the compound of interest, defined as:

$$\text{Relative conc.} = \frac{[\text{Peak area of particular compound}]}{\text{Peak area of IS}} \times \text{IS conc.}$$

2.5. Statistical analysis

Mean total volatiles were analysed by ANOVA one-way with Tukey's post hoc test to assess significant differences between processing methods ($p < 0.05$).

3. Results and discussion

3.1. Flavour of fresh juice

The flavour compounds in fresh and processed pennywort juice treated by heat and high pressure treatment are shown in Tables 1 and 2. Fresh juice and high pressure processed juice contained 40 and 39 volatile components that could be identified, respectively, whereas pasteurised and sterilised juice contained 39 and 38 volatile compounds, respectively. Twenty-nine components occurred in all the fresh and processed juice samples, although in some samples only traces of some compounds were detected. Fresh juice was characterised by a high content of the oxygenated monoterpenes; linalool (335.5 ng/l), geraniol (146.9 ng/l) and β-cyclocitral (42.5 ng/l), and the sesquiterpenoid hydrocarbons β–caryophyllene (1344.0 ng/l) and humulene (1602.2 ng/l) were present at higher concentrations in the fresh juice than other volatiles. Chou (2005) and Oyedeji and Afolayan (2005) reported that the main compounds in the essential oil of pennywort were linalool, copaene, elemene, caryophyllene, cadinene, humulene, alloaromadendrene, farnesene, selinene, cuparene, caryophyllene oxide, bicyclogermacrene and myrcene. All these components except for bicyclogermacrene were detected in fresh juice in this study, and the volatile compounds included 4 alcohols, 6 aldehydes, 4 monoterpenoid hydrocarbons, 3 oxygenated monoterpenoid, 19 sesquiterpenoid hydrocarbons, 1 oxygenated sesquiterpenoid, 4 miscellaneous compounds (Table 1) as well as 19 unknown components (data not shown). Forty different varieties of *C. asiatica* have been identified throughout the world (de Pauda, Bunyapraphatsara, & Lemmens, 1999), so differences in reported volatile components are not surprising.

3.2. Effects of processing on chemical classes

There is no published information about the effect of heat and HPP treatment on volatile components of pennywort juice. The total concentration of volatile compounds in fresh juice was higher than in processed juice ($p < 0.05$) and there was a non-significant trend in the order sterilised juice > HPP juice > pasteurised juice, respectively. Reductions in the concentration of acyclic alcohols, aldehydes, oxygenated monoterpenes, sesquiterpene hydrocarbons, and oxygenated sesquiterpenes were detected after some of the processing operations, whereas ketones increased significantly (Table 2). Aldehydes and oxygenated sesquiterpenes were present at higher concentration in fresh juice than in other samples ($p < 0.05$), monoterpene hydrocarbons were also found in high concentrations but there was no significant difference between HPP and heat-treated samples ($p > 0.05$). In addition, there was no

Table 1
Concentrations of volatile compounds identified in fresh and processed pennywort juices.

Compounds (*m/z*)	Mean concentration ± sd (ng/L)[A]				Method of identification[B]	LRI[C]
	Fresh	HPP	Pasteurised	Sterilised		
Acyclic alcohols						
1-Penten-3-ol	123.5 ± 54.0^{a}	28.1 ± 21.4^{b}	nf^{b}	nf^{b}	MS + LRI	689
1-Butanol, 3-methyl-29, 37, 39, 42, 51, 53, **55**, 57, 60, 70, 77	19.7 ± 23.2^{ns}	6.2 ± 8.7	nf	nf	se	736
1-Butanol, 2-methyl-39, 41, 43, 45, 53, 55, **57**, 59, 70	43.5 ± 29.5^{a}	12.2 ± 10.4^{ab}	nf^{b}	nf^{b}	se	741
Hexanol	nf^{b}	20.1 ± 16.2^{a}	nf^{b}	nf^{b}	MS + LRI	873
Aldehydes						
Propanal, 2-methyl-29, 37, 39, 41, **43**, 45, 50, 53, 55, 57, 59, 72, 74	97.5 ± 85.6^{a}	35.2 ± 25.2^{ab}	trace^{b}	trace^{b}	se	567
Butanal, 3-methyl-29, 37, 39, 41, **44**, 50, 53, 55, 58, 71, 86	79.5 ± 38.0^{a}	26.2 ± 20.0^{ab}	nf^{b}	nf^{b}	se	659
Butanal, 2-methyl-**41**, 57, 71, 86	187.1 ± 105.1^{a}	62.5 ± 47.6^{ab}	8.1 ± 6.3^{b}	–^{b}	se	669
Hexanal	142.4 ± 68.2^{a}	8.6 ± 9.9^{b}	53.2 ± 27.8^{ab}	90.4 ± 48.9^{ab}	MS + LRI	805
2,6-Nonadienal	88.2 ± 68.6^{a}	nf^{b}	nf^{b}	nf^{b}	ms[O]	1162
2-Nonenal	72.7 ± 55.0^{a}	nf^{b}	nf^{b}	nf^{b}	MS + LRI	1168
Ketone						
2-Butanone	nf^{ns}	12.8 ± 9.0	18.5 ± 18.3	11.1 ± 9.5	MS + LRI	601
2-Nonanone	nf^{b}	nf^{b}	21.2 ± 10.7^{ab}	58.8 ± 32.6^{a}	MS + LRI	1095
2-Hexanone, 5-methyl-**43**, 53, 58, 71	nf^{b}	nf^{b}	30.9 ± 22.6^{a}	14.5 ± 12.1^{ab}	se	1095
3-Nonen-2-one	nf^{b}	16.8 ± 27.6^{b}	123.5 ± 72.5^{ab}	185.7 ± 98.2^{a}	MS + LRI	1145
Monoterpenes hydrocarbon						
α-Pinene	92.5 ± 39.1^{a}	24.7 ± 22.8^{b}	20.1 ± 11.3^{b}	31.4 ± 23.9^{b}	MS + LRI	940
β-Pinene	108.2 ± 43.5^{a}	29.1 ± 26.5^{b}	21.9 ± 16.4^{b}	36.8 ± 27.1^{b}	MS + LRI	987
Myrcene	167.2 ± 77.4^{ns}	86.8 ± 110.2	72.2 ± 61.6	160.6 ± 117.7	MS + LRI	993
α-Terpinene	nf^{ns}	nf	nf	15.3 ± 15.3	ms[N]	1025
Limonene	trace^{ns}	9.3 ± 15.8	15.3 ± 11.1	28.6 ± 18.3	MS + LRI	1037
γ-Terpinene	nf^{b}	13.0 ± 22.3 ^{ab}	65.1 ± 50.2^{ab}	131.9 ± 92.8^{a}	MS + LRI	1066
Terpinolene	nf^{b}	nf^{b}	25.5 ± 19.6^{a}	8.1 ± 7.3^{ab}	ms[F]	1094
Oxygenated monoterpenoids						
Linalool	335.5 ± 148.6^{a}	208.0 ± 177.4^{ab}	83.6 ± 48.1^{b}	82.0 ± 49.5^{b}	MS + LRI	1106
α-Terpineol	nf^{ns}	5.4 ± 7.9	–	–	MS + LRI	1210
β-Cyclocitral	42.5 ± 30.4^{a}	nf^{b}	nf^{b}	nf^{b}	MS + LRI	1235
Geraniol	146.9 ± 74.6^{a}	45.7 ± 45.0^{b}	22.9 ± 17.1^{b}	trace^{b}	MS + LRI	1258
Sesquiterpene hydrocarbons						
α-Cubebene	147.3 ± 57.5^{ns}	71.7 ± 90.2	44.7 ± 24.7	89.8 ± 56.7	ms[D]	1361
α-Ylangene	nf^{b}	nf^{b}	6.8 ± 9.0^{ab}	17.4 ± 9.7^{a}	ms[L]	1387
α-Copaene	537.6 ± 221.1^{ns}	250.6 ± 302.5	108.0 ± 54.1	207.4 ± 140.2	ms[D]	1394
β-Elemene	248.6 ± 107.5^{a}	78.3 ± 96.3^{ab}	67.9 ± 40.0^{b}	111.7 ± 66.3^{ab}	ms[E]	1405
β-Caryophyllene	1344.0 ± 1049.2^{ns}	710.3 ± 864.0	464.3 ± 252.4	750.0 ± 449.8	ms[F]	1444
β-Copaene 41, 55, 69, 91, 105, 119, 133, **161**, 204	16.6 ± 14.6^{ns}	–	15.7 ± 11.8	22.4 ± 12.1	se	1451
(E)-β-Farnesene	551.8 ± 200.5^{ns}	248.5 ± 338.9	520.8 ± 274.6	882.2 ± 562.5	ms[G]	1461
Humulene	1602.2 ± 668.7^{ns}	685.6 ± 827.7	533.7 ± 292.8	777.5 ± 425.3	ms[H]	1481
Alloaromadendrene	258.3 ± 105.0^{a}	105.8 ± 134.0^{ab}	35.0 ± 42.8^{b}	48.5 ± 41.9^{b}	ms[D]	1485
δ-Cadinene	29.7 ± 8.3^{ns}	29.0 ± 26.2	35.7 ± 38.7	34.1 ± 23.7	ms[J]	1393
γ 2-Cadinene	nf^{b}	nf^{b}	78.3 ± 40.1^{a}	88.6 ± 57.0^{a}	ms[I]	1494
γ-Curcumene	trace^{ns}	34.8 ± 44.3	39.9 ± 18.2	64.7 ± 40.3	ms[E]	1498
Germacrene D	trace^{b}	trace^{b}	93.7 ± 72.8^{ab}	186.8 ± 118.4^{a}	ms[D]	1504
Valencene	146.0 ± 102.0^{ns}	17.2 ± 21.7	68.6 ± 32.0	75.4 ± 50.5	MS + LRI	1514
β-Selinene	34.9 ± 27.3^{ns}	52.8 ± 83.2	–	–	ms[E]	1515
α-Muurolene	50.5 ± 42.4^{ns}	24.1 ± 32.2	13.9 ± 9.2	25.3 ± 27.6	ms[L]	1518
Bicyclogermacrene	nf^{b}	–^{b}	11.6 ± 12.6^{ab}	17.0 ± 9.7^{a}	ms[D]	1520
α-Selinene	130.2 ± 68.1^{a}	46.7 ± 61.5^{ab}	17.4 ± 16.7^{b}	34.2 ± 24.4^{ab}	ms[K]	1521
Cuparene	192.8 ± 81.1^{a}	65.2 ± 80.5^{ab}	nf^{b}	nf^{b}	ms[M]	1535
γ-Cadinene	96.5 ± 30.8^{ab}	nf^{b}	84.0 ± 46.2^{ab}	128.0 ± 84.0^{a}	ms[D]	1535
δ-Cadinene (armoise-Maroc)	163.1 ± 50.7^{ns}	72.3 ± 94.8	126.7 ± 67.6	209.1 ± 135.7	ms[D]	1538
Calamenene	101.7 ± 34.1^{ns}	38.0 ± 42.5	65.0 ± 34.5	89.9 ± 57.2	ms[J]	1544
Oxygenated sesquiterpene						
Caryophyllene oxide	37.7 ± 28.8^{a}	nf^{b}	nf^{b}	nf^{b}	ms[E]	1613
Miscellaneous						
Dimethyl sulfide 29, 35, 43, 45, 47, 49, 58, **62**, 64	840.9 ± 429.6^{a}	190.3 ± 159.7^{b}	187.9 ± 125.5^{b}	361.1 ± 149.9^{ab}	se	532
Tetrahydrofuran	nf^{b}	71.5 ± 58.1^{a}	trace^{b}	–^{b}	MS + LRI	633
Furan, 2-pentyl-53, **81**, 109, 138	87.9 ± 23.7^{a}	trace^{c}	13.7 ± 12.3^{bc}	34.2 ± 19.9^{b}	se	995
1-Methyl-3-isopropylbenzene 51, 57, 65, 77, 91, 103, 115, **119**, 134	138.3 ± 53.9^{ns}	54.3 ± 63.8	91.6 ± 68.1	148.3 ± 95.3	se	1033
5-Ethyl-1-formylcyclopentene 39, 63, **67,** 77, 81, 91, 95, 109, 124	64.7 ± 49.9^{a}	nf^{b}	nf^{b}	nf^{b}	se	1042

[A] Approximate quantities (ng) in headspace from 20 ml of sample were estimated by comparison with 100 ng of 1,2-dichlorobenzene internal standard, mean values of 3–4 replicates analyses are given; compounds identified below 2 ng are reported as trace; –, less than 0.5 ng; nf, not found.

[B] MS + LRI, mass spectrum and LRI agree with those of authentic compound; ms, mass spectrum agree with spectrum in mass spectral database or with those reported in previous studies as listed below; se, tentative identification from structure elucidation of mass spectrum.

[C] LRI; Liner retention indices on a VF-5MS column.

[D] Kondjoyan and Berdague (1996).

[E] Noueira, Bittrich, Shepherd, Lopes, and Marsaioli (2001).

Table 1 (*continued*)
[F] Quorn® (2009).
[G] Priestap, van Baren, Lira, Coussio, and Bandoni (2003).
[H] Flavornet (2004).
[I] Kilic, Hafizoglu, Kollmannsberger, and Nitz (2004).
[J] Vichi et al. (2007).
[K] Adams (1995).
[L] Baranauskinene, Venshutonis, and Demyttenaere (2003).
[M] Sacchetti et al. (2005).
[N] Flamini, Cioni, Morelli, Macchia, and Ceccarini (2002).
[O] Skaltsa, Demetzos, Lazari, and Sokoric (2003).

Table 2
Concentrations (ng/L) of volatile product groups.

Compound groups	Total concentration ± sd (ng/L)			
	Fresh	HPP	Pasteurised	Sterilised
Acyclic alcohols	186.8 ± 54.4^{a}	66.7 ± 9.5^{ab}	nf^{b}	nf^{b}
Aldehydes	665.6 ± 44.7^{a}	132.6 ± 24.4^{b}	61.4 ± 21.3^{b}	90.4 ± 36.9^{b}
Ketones	nf^{b}	29.5 ± 8.7^{b}	194.1 ± 50.3^{ab}	270.1 ± 81.7^{a}
Monoterpene hydrocarbons	367.9 ± 69.4^{ns}	162.8 ± 30.2	219.9 ± 26.8	412.8 ± 61.0
Oxygenated monoterpene	524.9 ± 149.5^{a}	259.1 ± 97.6^{ab}	106.4 ± 39.5^{b}	82.0 ± 41.0^{b}
Sesquiterpene hydrocarbons	5651.9 ± 424.9^{a}	2530.8 ± 201.1^{b}	2431.6 ± 165.2^{b}	3859.8 ± 263.6^{ab}
Oxygenated sesquiterpene	37.7 ± 28.8^{a}	nf^{b}	nf^{b}	nf^{b}
Miscellaneous	1634.2 ± 168.6^{a}	435.8 ± 41.3^{b}	550.6 ± 42.4^{b}	1168.6 ± 77.5^{ab}
Total	9069.±1898.3^{a}	3617.2 ± 851.5^{b}	3564.0 ± 821.9^{b}	5883.7 ± 1319.9^{ab}

Total concentration with different letters within a row are significantly different ($p < 0.05$) (Tukey's), non-significantly different (ns), compounds identified below 0.5 ng/L are reported as not found (nf).

significant difference between fresh and HPP juice ($p > 0.05$) in the total content of alcohols, ketones and oxygenated monoterpenoids. Moreover, there was no significant difference between HPP and processed juice ($p > 0.05$) in the content of acyclic alcohols, aldehydes, oxygenated monoterpenes, sesquiterpene hydrocarbons, oxygenated sesquiterpenes, miscellaneous compounds and total volatiles as shown in Table 2. However, HPP caused more flavour volatiles in the acyclic alcohols class to be retained, with a trend to greater retention of aldehydes and oxygenated monoterpenoids ($p > 0.05$) than pasteurisation and sterilisation.

3.3. Effects of processing on non-terpenoid compounds

The fresh pennywort juice contained aldehydes including hexanal and 2-nonenal that were presumably formed by enzymatic oxidation in the plant post-harvest and during preparation of the juice. (*E*)-2-Nonenal and hexanal are biosynthesised in plant tissues by lipoxygenase-mediated lipid oxidation of polyunsaturated fatty acids (Hatanaka, 1996; Vichi, Guadayol, Caixach, Lopez-Tamames, & Buxaderas, 2007), although they may also be formed by autoxidation. Processing of the juice reduced the concentration of these components, but hexanal was found in sterilised samples in higher concentrations than in pasteurised and HPP juice, so some formation of this compound by autoxidation is suspected under sterilisation conditions. Other aldehydes including 2-methyl-propanal, 3-methyl-butanal, 2-methyl-butanal, 2,6-nonadienal and 2-nonenal had decreased or disappeared after thermal treatment. Aldehydes are known to degrade readily by chemical reactions including acetal formation which occurs in the presence of alcohols under acid conditions (Jerry, 1992).

Ketones including 2-butanone, 2-nonanone, 5-methyl-2-hexanone and 3-nonen-2-one were present after processing but not in the fresh juice, with concentrations of 2-nonanone and 3-nonen-2-one significantly higher in sterilised than in high pressure processed juice indicating these components were formed due to the high temperatures employed in sterilisation.

Acyclic alcohols including 1-penten-3-ol, 3-methyl-1-butanol, 2-methyl-1-butanol and hexanol were found in high abundance in fresh and HPP juice. These compounds might be formed during sample preparation because no action was taken to inactivate enzymes prior to extraction in this study. Hexanol was detected in the HPP juices. Stone, Hall, and Kazeniac (1975) reported that alcohols (hexanol) are formed in plant tissues by alcohol oxidoreductase activity on C_6 aldehydes (hexanal).

3.4. Effects of processing on terpenoid compounds

The class of oxygenated monoterpenes was reduced in concentration by processing (Table 2). β-Cyclocitral and caryophyllene oxide were found in fresh juice but not in processed juice. Since these components were amongst the less volatile of the volatile fraction, it is probable that these components were lost by chemical transformation. It was clear that these volatile components were retained better by HPP than by pasteurisation or sterilisation.

Linalool was the major component of the oxygenated monoterpene class present in fresh juice, but it was found to be reduced ($p < 0.05$) by the thermal processing of pennywort juice. Sterilisation and pasteurisation caused big drops in the concentration of linalool. α-Terpineol was not detected in fresh juice but it was formed in low concentrations in high pressure processed juice. This component is considered to be desirable in many fruits, whereas in others it is perceived as an off-flavour (terpentine-like) (Dung, Moi, Nam, Cu, & Leclercq, 1995). It is known to be formed in citrus juices from limonene and linalool by acid-catalysed reactions (Haleva-Toledo, Naim, Zehavi, & Rouseff, 1999). Rui, Xiaoyan, Taixiang, and Guanjian (2007) reported isomerisation of linalool and dehydration to monoterpenes shown in Fig. 1. Geraniol was not detected in sterilized pennywort juice, but was present in the fresh juice and in samples processed by other methods. This is also susceptible to dehydration under acid conditions (Jerry, 1992).

In this study, terpinolene and α-terpinene were found in heat-treated samples but myrcene, α-pinene and β-pinene were found in all samples. In contrast, limonene was present only at trace levels in fresh juice, which is in agreement with the findings of Chou (2005), although Ali (2007) did detect D-limonene in pennywort juice. Our studies show that γ-terpinene, which contributes bitter

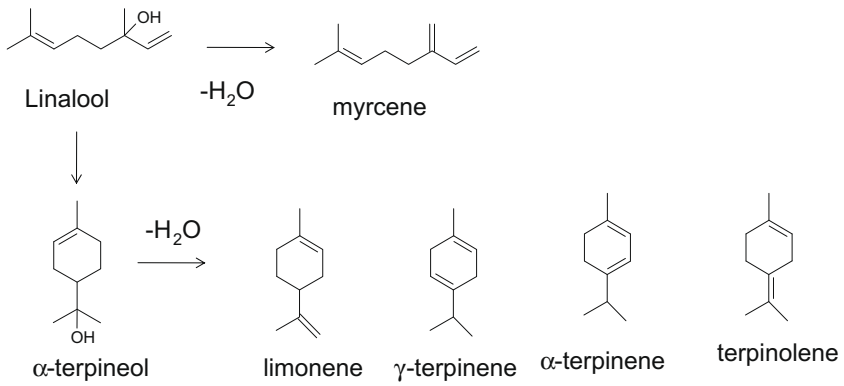

Fig. 1. Isomerisation of linalool and dehydration to monoterpenes (Rui et al., 2007).

flavours, was found in higher concentrations in processed samples. Myrcene, which was described as contributing pine odour to *Piatacia lenticus* (Seo & Beak, 2005) was present at a high concentration in all samples. β-Pinene is a compound with a plastic and pine-like aroma present in *Piper nigrum*, *Pistacia lenticus, Argyranthemum adauctum*, *Sideritis bigerana* (Zheng, Kim, Kim, & Lee, 2005), water dropwort (Seo & Beak, 2005), carrot (Kreutzmann, Thybo, Edelenbos, & Christensen, 2008) and it is also important for the overall aroma of omija leaves (Zheng et al., 2005). It was found at higher concentrations in fresh pennywort juice than in processed samples ($p < 0.05$).

The sesquiterpene class, including β-caryophyllene, humulene, *E*-β-farnesene, α-copaene, alloaromadendrene and β-elemene was the major class of volatiles present in pennywort juice. β-Caryophyllene and (*Z, E*)-β-farnesene contributed to the woody note in water dropwort (Seo & Beak, 2005). All samples contained germacrene D but only trace levels were present in fresh and HPP juice. It has been reported as the predominant sesquiterpene in various essential oils of *Pinus canariensis, P. pauce* and *P. pinaster* and as the major volatile compound of omija leaves (Zheng et al., 2005). β-Elemene was found at a higher concentration in fresh pennywort juice than in other samples ($p < 0.05$). It has also been reported in omija fruits, *Murraya koenigii, Stachys swainsonii* spp *melangavica* and it was identified as the main component of basil oil and omija leaves (Zheng et al., 2005).

The oxygenated sesquiterpene, caryophyllene oxide was only detected in the fresh juice. Chou (2005) and Ali (2007) also detected caryophyllene oxide in pennywort.

Temperature and pressure are important parameters in determining the juice aroma which may be modified by thermal and high pressure-induced reactions. Some of the compounds detected in the processed juice including calamenene have not been reported previously in fresh pennywort juice. However, the effects of processing on volatile compounds in pennywort juice have not been reported previously.

3.5. Benefits of HPP processing compared to thermal processing methods

The main justification for HPP of foods is that more valuable constituents, for example, aroma compounds and vitamins (Gotz & Weisser, 2002), are retained than in thermally processed foods. In this study, many compounds were conserved better by HPP treatment including linalool, geraniol, α-copaene, alloaromadendrene, β-selinene, α-selinene and cuparene. Some compounds were present in fresh juice but were lost during HPP including 2,6-nonadienal, 2-nonenal, β-cyclocitral, γ-cadinene, caryophyllene oxide and 4 unknown compounds (data not shown). In contrast, some of the identified compounds were not present in fresh juice but were found in HPP juice including γ-terpinene, 2-butanone, 3-nonen-2-one, α-terpineol, tetrahydrofuran and 3 unknown compounds. These findings indicate that HPP can induce chemical changes which generate new compounds from components of the original juice.

Several compounds were detected in heat-treated juice but were not found in fresh and HPP juice, including α-terpinene and α-ylangene. Some compounds were only detected in processed juice including hexanol, α-terpinene, γ-terpinene, terpinolene, ketones, α-terpineol, α-ylangene, bicyclogermacrene, γ-cadinene, tetrahydrofuran and 13 unknown compounds. However, some compounds which have been previously reported as components of fresh pennywort juice including bicyclogermacrene, which was present at 11.2% of the essential oil isolated by Oyedeji & Afolayan, 2005, were not detected in the current study.

Hendrickx, Ludikhuyze, Van den Broeck, and Weemaes (1998) reported that high pressure process-induced enzyme activation and inactivation are relevant to food quality; enzyme activation can arise from pressure-induced decompartmentalisation (Butz, Koller, Tauscher, & Wolf, 1994 and Gomes & Ledward, 1996). However, the aldehydes e.g. hexanal, which may be formed by enzymatic processes were at low concentrations after high pressure processing, so there is no evidence for this phenomenon in this study.

Yu and Chiang (1986) reported that pasteurisation (75 °C for 40 s) of passion fruit juice caused about 45% loss of flavour compounds based on total volatiles. For this study, there was a significant reduction in volatiles between fresh and high pressure and pasteurised samples ($p < 0.05$) and a non-significant trend for a reduction in total volatiles between fresh and sterilized juice ($p > 0.05$) (Table 2). Carelli, Crapiste, and Lozano (1991) considered the effect of temperature on the release of aroma compounds in apple juice, and it was found that raising the temperature from 25 to 65 °C caused an increase in interactions between aroma compounds (pentyl acetate, hexanal, hexanol) and macromolecules in apple juice (fructose). Jouquand, Ducruet, and Giampaoli (2004) also reported that aroma compounds bind to macromolecules by hydrophobic interactions and this effect was enhanced by heat treatment, which caused the lower release of aroma compounds in pasteurised orange juice. A decrease in juice flavour on processing may be caused by these interactions or by evaporation or thermal degradation (Su & Wiley, 1998).

γ 2-Cadinene was not detected in the fresh juice and in the high pressure processed samples but was present in the thermally

ftreated samples. However, γ-cadinene was present in the fresh juice but may have isomerised to the 2-isomer on heating.

In conclusion, it appears that several chemical changes occurred as well as loss of volatiles during the heating processes of pasteurisation and sterilisation. Our data suggests that thermal and HPP treatment can cause chemical changes in some plant components e.g. by dehydration or isomerisation reactions. Although the total volatile concentration in sterilised juice was higher than in juice processed by other methods ($p > 0.05$), some volatile components that were not present in the fresh juice were formed at high levels in the sterilised juice e.g. γ-terpinene, ketones, γ 2-cadinene and germacrene D. This indicates that high pressure treatment could maintain the flavour better than pasteurisation and sterilisation. The detection of ketones is a good marker that processing has been applied to the juice.

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