CHAPTER 4
RESULTS AND DISCUSSION

4.1 Analysis of the composition of raw materials

Pennywort samples were purcharsed from local markets and Chiang Mai farm in 2007. The fresh pennywort from local market contained the content of some bioactive compounds including madecassoside, asiaticoside, madecassic acid, asiatic acid and ascorbic acid at 1.63-7.60, 2.94-8.98, 0.07-0.58, 0.34-2.66 and 1.38-2.59 mg/100 g. The content of bioactive compounds from local market was lower than that from Chiang Mai farm. In this study, the fresh pennywort from Chiang Mai farm was used for all experiments.

4.1.1 Physical properties of raw materials

The yield of pennywort leaves and petioles by stripping was 27.3±3.8% (w/w) from the whole plant and the yield of juice was 46.7±4.6% (w/v) from the leaves and petioles to the juice extract by grinding.

Colour is one of the main characteristics of food and these important quality parameters affects consumer behavior but the green colours, due to chlorophyll in pennywort juices is unstable and easily degrade leading to a brown colour. Hue angles, the qualitative attributes of colour, are defined as 0/360˚ for magenta, 90˚ for yellow, 180˚ for green and 270˚ for blue (Rein and Heinonen, 2004). Chroma is the quantitative attribute of colourfulness, and its values are matched with vivid colours. The chroma values show the distance away from the grey tone (i.e., degree of colour saturation) on a scale from 0-100. The colour parameters comprising chroma and hue angle of the fresh extract prepared with water (1:1 w/v) were 5.84±0.02 and 102.91˚±0.38, respectively, suggesting that this juice had a yellow-green colour. For comparison with other juices, the hue value was 45.11-55.72, 89.64˚ and the chroma was 9.92-13.00, 6.40 for samples of guava juices (Phongphisutthinant, 2006; Thongsroy, 2003). In addition, fresh Indian mulberry juice had hue value was 69.50-

87.56˚ and chroma was 2.46-6.36 (Srimuang, 2003). The fresh pennywort juice was brighter and greenish with higher chroma values and hue angle than guava juice and Indian mulberry juice.

4.1.2 Chemical properties of raw materials

The chemical properties of fresh pennywort including proximate and pesticide residues were determined and the result is shown **Table 4.1.** The biological compounds of fresh pennywort and juice are shown in **Table 4.2**.

Table 4.1 Proximate compositions of fresh pennywort

Proximate compositions	**g/100 g**
Moisture content (%)	90.92±0.02
Ash content	1.44±0.06
Crude protein	1.80±0.01
Fat (Extraction)	0.48±0.04
Crude fibre	1.21±0.04
Carbohydrate by difference	4.15±0.11
Total soluble solids (%)	5.27±0.21
Organochlorine	Not detected
Organophosphate	Not detected

The proximate compositions of edible portion (100 g) of pennywort were reported as moisture 69%, crude protein 2 g, fat 2 g, crude fibre 0.7 g, carbohydrate 1.7 g and energy 23 kcal (Sheela *et al*., 2004). Mudzwiri (2007) reported 87.78% moisture, 3.15 g crude protein, 2.72 g fat, 1.92 g crude fibre, 2.54 g ash, 3.81 g carbohydrate and 219.01 kJ energy. Ali (2008) also reported the composition as 88% moisture, 2 g crude protein, 0.2 g fat, 1.6 g crude fibre and 6.7 g carbohydrate. In addition, Tee *et al*. (1997) reported that the proximate composition of fresh pennywort in Malaysia was 87.7% moisture, 2 g crude protein, 0.2 g fat, 1.6 g fibre, 6.7 g carbohydrate, 1.8 g ash and 37 kcal energy. It also contained minerals including potassium, calcium, phosphorus, sodium and iron at concentrations of 391, 171, 32,

21 and 5.6 mg/100 g, respectively (Tee *et al*., 1988). Similarly, it contained vitamins such as carotene, retinol equivalents, ascorbic acid, vitamin B_2, niacin and vitamin B_1 were 2.649, 0.442, 48.5, 0.19, 0.1 and 0.09 mg/100 g (Tee *et al*., 1988).

The antioxidant activity and the content of active compounds and pigments of fresh pennywort juices are summarized in **Table 4.2**. The 4 major bioactive components of pennywort juice observed at 220 nm were the terpene acids and the glycosides which were well separated due to the large difference in polarity of the terpene acids and the glycosides. The results in this study demonstrated that the pennywort juice containing a high amount of active compounds or vitamins also possessed a high level of antioxidant activity. These results are in agreement with those in previously published literature by Kormin (2005).

Analysis of the fresh pennywort juice showed that madecassoside, asiaticoside, madecassic acid and asiatic acid were present at concentrations of 3.80, 4.49, 1.66 and 2.69 mg/100 ml juice, respectively. Asiaticoside was markedly higher in this sample than in the sample analyzed by Ali (2008) who reported that the concentrations of these compounds were 7.7-9.1, 1.1-1.3, 5.4-7.1 and 4.2-4.6 mg/100 g (fw), respectively. Kormin (2005) extracted pennywort with a 1:5 plant: water ratio and analysis of the extract indicated that the original pennywort contained 15.60, 19.66, 12.80 and 12.25 mg/100 ml, respectively. Also, fresh drink contained 3.12, 3.91, 2.56 and 2.45 mg/100 ml, respectively. Sribusarakum (1997) reported the presence of asiatic acid, mixture of madecassic acid and terminolic acid, asiaticoside and mixture of asiaticoside A and B based on dried sample extract at concentrations of 6.0, 7.0, 21.0 and 30.0 mg/100 g, respectively, with relative amounts of 9.38, 10.94, 32.81 and 46.88%, respectively. Mahapunt and Chaicharoentharwekit (1987) reported that the yield of asiaticoside in leaves and petioles from pennywort grown in Thailand were 1.07 and 0.16%, respectively, and the yield was only 0.07-0.12% in pennywort leaves from Madagascar (Bontems, 1941) and the yield was up to 1.7 % in pennywort root from India (Jain and Agrawal, 2008). Castellani *et al*. (1981) reported that the concentrations of madecassoside, asiatic acid and madecassic acid in the entire plant from Madagascar were 0.0051, 0.07 and 0.079% but Sing and Rastogi (1969) reported that the concentration of asiatic acid in the whole plant from India was 0.009%. Aziz *et al*. (2007) reported that asiaticoside and madecassoside

concentrations in the leaves of glasshouse-grown and tissue culture-derived pennywort were asiaticoside 0.79 and 1.15% (dw) and madecassoside 0.97 and 1.65% (dw), respectively. The yield of madecassoside, asiaticoside, madecassic acid and asiatic acid in dried pennywort leaves grown in Madagascar were 1.27-1.7, 1.63-2.0, 0.72-0.95 and 0.72-.98%; 2.38-5.89, 2.67-6.42, 0.12-1.97 and 0.09-1.89%; 2.76-3.62, 3.13-3.68, 0.11-0.59 and 0.05-0.64%, respectively (Rafamantanana *et al*., 2009; Randriamampionona *et al*., 2007; Jacinda *et al*., 2008). A relatively wide range for the content of asiaticoside and asiatic acid in dried pennywort was reported by Fernandez *et al*. (1997), Akerlof (1932) and Kim *et al*. (2009), who reported that the asiaticoside content was 100-300, 52-1,770 and 730-1,000 mg/100 g, respectively. The content of asiatic acid was found to be 200-1,000 and 700-780 mg/100 g (Akerlof, 1932; Kim *et al*., 2009).

Table 4.2 Bioactive compounds and antioxidant properties of fresh pennywort and fresh juice

Compounds/properties	**Fresh pennywort (mg/100 g)**	**Fresh juice (1:1) (mg/100 ml)**
Madecassoside	7.60-12.67	3.80 ± 0.41
Asiaticoside	8.98-21.08	4.49 ± 1.86
Madecassic acid	0.58-3.32	1.66 ± 1.12
Asiatic acid	2.66-5.38	2.69 ± 1.81
Ascorbic acid	1.38-9.54	4.77 ± 0.42
β-Carotene	3.04-5.00	2.50 ± 0.65
Chlorophylls *a*	-	0.33±0.18
Chlorophylls *b*	-	0.15±0.07
Total phenolic content	1,977± 97.32*	988.54±45.16
FRAP (μM $FeSO_4$)	1,827±37.22*	913.51±18.61

*calculated from fresh juice

Data in this study showed that fresh pennywort also contained significant concentrations of ascorbic acid and *β*-carotene. Tee *et al*. (1988) reported the concentrations of 2.65 and 48.5 mg/100 g for carotene and. ascorbic acid,

respectively. However, Ali (2008) reported that the concentration of carotenoids (2.7 mg/100 g) and ascorbic acid (29 mg/100 g) were low compared to previous reports due to dried samples being used (oven dried at 40˚C, overnight). Some of the compounds would have degraded during the drying process. This affected the levels of ascorbic acid which contributed 0.9-5.5% to the total antioxidant activity. The amount of ascorbic acid in fresh juice is significantly lower than the concentrations determined for guava juice (80.1 mg/100 ml), passion fruit (39.1 mg/100 ml) and lemon juice (10.5 mg/100 ml) but similar to that of *G. schomburgkiana* juice (4.6 mg/100 ml) (Suntornsuk *et al*., 2002). Also, Kormin (2005) reported that fresh pennywort juice (1:5 extract) contained ascorbic acid 4.23 mg/100 ml. In addition, Mudzwiri (2007) reported that the content of phytic acid, a natural organic compound, was 37 mg/100 ml and that of oxalic acid was 127.03 mg/ml. Ascorbic acid and total vitamin C in fresh tomato juice was reported as 18.6 and 21.0 mg/100 ml, respectively (Hsu *et al*., 2008) and the content of ascorbic acid in various cultivars of orange juice was in the range 25-59 mg/100 ml (Niu *et al*., 2008). The total carotenoid and lycopene contents in fresh tomatoes were 212.8 and 145.6 mg/g, respectively (Hsu *et al*., 2008) and the concentration of total carotenoids in blood orange juice was 0.286 mg/100 ml (Choi *et al*., 2002).

Fruits and vegetables especially citrus fruits are sources of ascorbic acid. Ascorbic acid is considered an indicator of the nutritional quality of processed foods. Because of the instability of ascorbic acid and its nutritional importance, its content guarantees the presence of other nutrients (Bottcher, 1993; Williams *et al*., 1995; Canet, 1996). Ascorbic acid is a major contributor to the antioxidant activity of oranges (66%), Florida oranges (100%), grapefruit (89%) (Gardner *et al*., 2000) and orange juice (87%) (Miller *et al*., 1997). The content of ascorbic acid present in fresh orange juice was 42.46-73.45 mg/100 ml (Esteve *et al*., 2005), which is higher than the content in pennywort juice. Majchrzak *et al*. (2004) reported that addition of lemon, which contains ascorbic acid, to a tea drink can positively influence the antioxidant potential. The total antioxidant capacity of green tea was increased by the addition of ascorbic acid up to 30 mg/100 ml of tea extract.

Chlorophyll contributes to the attractive fresh greenish appearance of juice. In general, the amount of chlorophyll *a* (blue-green) and chlorophyll *b* (yellow-green) is

3:1. Then *et al*. (1998) reported the total chlorophyll content in fennel (10-26 mg/g) and parsley (12-17 mg/g). Also, some algae contained chlorophyll *a* and chlorophyll *b* in the concentration ranges 49-60.7 and 21.3-24.1 μg/g (fw), respectively (Dere *et al*., 1998). The results (**Table 4.2**) showed that the chlorophyll concentration of the raw pennywort was lower than that in several literature reports. Yusof *et al*. (2000) also found similar results. The chlorophyll content of juice extracted from stored canes decreased within 3 days of storage. The juices of canes stored at 27˚C showed a faster rate of chlorophyll degradation than that stored at 10˚C. It is known that chlorophyll degrades readily to pheophytin at acidic pH, and this was probably the main cause of the colour change. The presence of oxygen, light and internal enzymes may also contribute to the loss of chlorophyll, resulting in the colour of the juice changing from greenish to yellowish. The decrease in chlorophyll content due to enzymics oxidation resulted in the juice turning slightly yellowish in colour (less green) immediately after extraction. Thus, extraction may reduce the colour of fresh pennywort juice by degreening.

The total antioxidant capacity of the fresh pennywort, determined by the FRAP assay, was 1,827 μM $FeSO_4$/kg, which is higher than the values reported in the literature. Ali and Crozier (2007) and Ali (2008) reported FRAP values of 300 and 400 μmol $FeSO_4$/L for pennywort, respectively. In contrast, it was reported that this plant possessed high antioxidant activity by Vimala *et al*. (2003) and Hussin *et al*. (2007). In this study, FRAP in fresh juice was 913.51 μM $FeSO_4$/L which is similar to the value of Kormin (2005), who reported the value of 860 μM $FeSO_4$/L.

The total phenolics content (TPC) of pennywort was found to be 988.54 mg/100 ml, which is much lower than the values of 1,470.14 mg/100 ml of gallic acid equivalents reported by Kormin (2005). Ali (2008) reported that the TPC of pennywort was 3.2-3.5 mg/g (fw). The TPC of other fruits were reported as 0.5 g/L in strawberry juice; 0.4 g/L in raspberry juice, 1.3 g/L in lingonberry and 0.8 g/L in cranberry (Rein and Heinonen, 2004). TPC values are influenced by various factors, including different varieties used, different extraction methods, and also differences in harvesting season, exposure to sunlight and storage (Harborne and Williams, 2000; Robards, 2003). During extraction, phenolic compounds are usually sensitive to acidic solution and high temperature (Kormin, 2005). Zainol *et al*. (2003) suggested that

phenolic compounds are responsible for the antioxidant capacity of this plant. Pennywort is a very popular vegetable and it was reported to have high antioxidant activity (Vimala *et al*., 2003; Hussin *et al*., 2007) and increase the levels of antioxidant enzymes (Jayashree *et al*., 2003). Ali (2008) reported that pennywort contained relatively low antioxidant capacity compared with some other vegetables, with the order of activity being *Anacardium occidentale* > *Pluchea indica* > *C. asiatica* > *Premna cordifolia* and *Colubrina asiatica*. Wong *et al*. (2006) concluded that the phenols were partly responsible for the antioxidant capacity of a range of plants including pennywort. Zainol *et al*. (2003) and Fezah *et al*. (2000) suggested that phenolic compounds are the major contributors to the antioxidant capacity of pennywort. Ascorbic acid also contributes to the antioxidant capacity (Gardner *et al*., 2000). However, flavonoids have much stronger antioxidant activities against peroxy radicals than ascorbic acid (Cao *et al*., 1996). The antioxidant mechanism of phenolic antioxidants is associated with their ability to donate hydrogen atoms to free radicals. Phenolic compounds are proper antioxidants or hydroperoxide stabilizers because they are able to inactivate the lipid free radicals as well as prevent the decomposition of hydroperoxides to free radicals (Pokorny, 2001).

Phenolic compounds containing aromatic rings are found naturally in plants, they provide much of the flavour, colour and texture. Also, they are observed to be responsible for some taste attributes, including bitterness and astringency (Lea and Arnold, 1978). Thus, the addition of sugar to pennywort juice helps to improve sensory properties in making the drink more palatable due to reduction in the bitter taste of this juice.

Traditionally, pennywort juice was prepared by blending the leaves with water then filtered and mixed with hot syrup. Sugar is generally added in processed food partially to increase the product stability by lowering the a_w and increased the concentration of products. In this study, the fresh juice without sugar added was used in order to compare the quality of juice with sugar added. The range of total soluble solids used in this study was based on consumer acceptance as previously reported by Kormin (2005), who mixed sugar at 11% (w/v) in fresh pennywort juice. In addition, Kormin (2005) reported that the pennywort juice was prepared based on formulation that was developed by Malaysian Agricultural Research and Development Institute

(MARDI). The amount of sugar added to fruit and vegetable juices was mainly based on sensory test or consumer acceptance. The total soluble solids in the beverage are widely varied from 5-15% (Lea, 1991; Henrix, 1995).

In this study, the physico-chemical analysis of the leaves and petioles extracted with water (1:1 w/v) with sugar added (10%, w/v) and without sugar added was performed. The result for pH, total soluble solid, viscosity and colour including chroma and hue angle of samples are shown in **Table 4.3**.

The pH value and chroma (colour saturation) of juice without added sugar were higher than in juice with added sugar, although the difference in the pH values was not statistically significant ($p>0.05$).

Total soluble solids (TSS) and acidity (or pH) are important factors in determining the eating quality of fruits and vegetables. Pennywort juice is regarded as one of many healthy products which contain natural compounds and it is recommended that only sugar should be added to this juice in accordance with the Thai Food Regulation-Standard (2003). The pH of the juice with added sugar was slightly lower than the value before sugar addition, and this could be due to a reduction in the free water content in the sample by sugar. The total soluble solids content of this juice was 2.53% (control juice) and 12.17% (sugar added juice), and these values are similar to commercial fruit and vegetable juice. For example, the TSS of commercial strawberry and raspberry juices fortified with sugar was 10% for strawberry juice and 11% for raspberry juice. Sugar was not added to lingonberry juices, cranberry juices, pomegranate juice, pear juice and orange juice which contain TSS 5, 3, 11, 15.2 and 8.7-10.8%, respectively (Rein and Heinonen, 2004; Magerramov *et al*., 2007; Niu *et al*., 2008). Kormin (2005) reported that the pH and TSS content of fresh pennywort juice prepared from the plant with a concentration of 1:5 dilution was 5.93 and 1%, respectively. Chaiwanichsiri *et al*. (2000) also reported that the pH and TSS content of fresh pennywort juice (100%) was 5.56 and 4.10%, respectively. The pH of this juice is higher than that of apple juice, orange juice and grape juice (2.8-3.8) (Lea, 1991; Henrix and Redd, 1995), berry juices (2.5-3.5) (Rein and Heinonen, 2004), pear juice (4.15) (Magerramov *et al*., 2007), and 7 cultivars of orange juice had pH values in the range 3.81-4.31 (Niu *et al*., 2008). Fruit juices have a lower pH because of the presence of organic acids such as malic acid. This study

showed that the pennywort juice prepared was markedly different in pH from fruit juices from previously studied samples. Differences from the values reported by Kormin (2005) may due to the concentration of the juice, or effect of various variables including soil, climatic or environmental factors.

Table 4.3 Physico-chemical properties of fresh pennywort juice with and without added sugar

Properties	Juice with sugar added	Juice without sugar added
pH	5.57±0.08 [ns]	5.62±0.09
Total soluble solids (%)	12.17±0.06[a]	2.53±0.55[b]
Chroma, saturation	5.63±0.03 [b]	5.84±0.02 [a]
Hue angle, ˚Hue	101.00±0.33 [b]	102.91±0.38 [a]
Viscosity (cP) at 27 ˚C	1.82±0.05 [a]	1.71±0.04 [b]

Means with different letters within a row of each component are significantly different ($p \leq 0.05$) (Tukey's) and non significantly different (ns)

The colour is often used as an indicator of the natural transformation of a fresh food or of changes that occur during its processing or storage (Calvo and Duran, 1997). The CIELAB system enables an approach to the changes of juice colours where the changes in hue angle, chromatic saturation and overall lightness (L^*) are taken into account (Rein and Heinonen, 2004). The perceptible and overall colour impression of the berry juices depends on the relative amount of red and yellow colour, which is expressed as an angle of hue. Since the original colour of pennywort juice was dark green, the L^* value was not suitable for indicating the colour changes (Kormin, 2005). The changes in green colour of pennywort juice were expressed as chroma and hue angle (Jannok, 2007). In this study, the chroma and hue angle of juice with added sugar was higher than that of the juice without added sugar ($p \leq 0.05$). Sugar addition to the juice reduced the colour saturation and the hue angle showed a shift from a green colour to a colour with decreasing greenish tones. The chroma of fresh pennywort juice was 5.63-5.84 and the hue angle was 101.00-102.91. The colours of fresh pennywort gradually changed toward more dark-greenish tones immediately after juice extraction. These values can be compared with the colour of

other research, eg. the chroma and hue angle of 5.64 and 59.88, respectively, in fresh pennywort juice from Malaysia (Kormin, 2005).

The viscosity of food products can not be predicted theoretically, due to complicated physical and chemical interactions. Fruit and vegetable juices vary greatly in their viscometric behavior. There is a very strong effect of temperature and concentration on the viscosity of fruit and vegetable juices (Magerramov *et al*., 2007).

The changes in pennywort juice viscosity were similar to the changes in TSS. The viscosity of pennywort juice with added sugar was higher than that of the juice without added sugar ($p \leq 0.05$), as shown in **Table 4.3**. Pennywort juices consist of an aqueous dispersing phase, in which sugars, acids, soluble pectins, proteins, etc., are dissolved and a dispersed phase made up of particles of different sizes which come from the tissues of the plant. The viscosity of fruit and vegetable juices changes with content of soluble and suspended solids. Pectin and sugar concentrations are the main variables causing changes of viscosity (Rouse *et al*., 1974). Hsu *et al*. (2008) reported that the consistency of tomato products is strongly affected by pectins. Controlling the breakdown or retention of the pectins, and the enzymes that lead to changes in the pectins, is thus of importance during processing (Hayes *et al*., 1998). The degradation of the pectin chains reduces the viscosity of the juice. The viscosity of others fruit and vegetable juices can be compared, for example, 2.03 cP for sugarcane juice (16%, pH 5.71) (Yusof *et al*., 2000) and 1.09 cP for guava juice (Thongsroy, 2003). Magerramov *et al*. (2007) reported that the viscosity at 0.1 MPa of pomegranate juice (11%) and pear juice (15.2%) was 1.642 and 1.975 cP (at 20-21˚C), respectively. The viscosity values of fruit and vegetable juices previously reported are systematically lower than the present results, in which pennywort juice with added sugar (12.17%) and without added sugar (2.53%) had viscosities of 1.82 and 1.71 cP (at 24-28˚C), respectively.

The behavior of the concentration dependence of the viscosity of juices depends on temperature. The large deviations between various viscosities of juices are probably due to the effect of chemical composition of the juices. For example, it is well known that the viscosity of juices is significantly affected by pectin and sugar concentration (Rouse *et al*., 1974). However, the viscosity of juices considerably decreases with increasing temperature.

4.2 Optimization of different processes based on microbiological quality

The original extracted juice was used as a control. Ten percent sucrose was added to the juice. The juice samples were subjected to 3 processing treatments including HPP (400 and 600 MPa) at room temperature (<30˚C) for 20 and 40 min, pasteurization by heating at 90˚C for holding times of 3, 5 and 7 min and sterilization at 121˚C for 4 and 6 min. The finished products were assessed for microorganisms including total plate count (total aerobe-microorganisms), *S. aureus*, *C. perfringens*, *E. coli*, yeasts and moulds.

Based on microbiological quality, the values for total plate count as well as yeasts and moulds count in the fresh juice were 580 and 132 cfu/ml, respectively and *S. aureus*, *C. perfringens* and *E. coli* were not present in all processed samples. In addition, the total plate and yeasts and moulds counts were <30 cfu/ml and *E. coli* (MPN method) was <2.2/100 ml in all samples. It is known that asiaticoside retains the most profound effect on antibacterial and fungicidal activity against pathogens and fungi (Hausen, 1993).

The content of microorganisms in each sample must be less than those recommended in the Thai Food Regulation-Standard (2003). According to this standard, the content of total plate and yeasts and moulds counts should be less than 1 x 10^4 and 1 x 10^2 cfu/ml, respectively. *S. aureus* and *C. perfringens* must not present in 1 ml sample, also *E. coli* (MPN method) must be less than 2.2/100 ml of sample.

Sancho *et al*. (1999) reported that the initial total mesophilic aerobic flora of strawberry *coulis* was 3.29 log cfu/ml and it was reduced to 2.35 log cfu/ml after a treatment at 400 MPa for 30 min at 20˚C. This data proved the efficiency of HPP to inactivate bacteria on fruits and vegetables, a pressurization of 400 MPa for 15 min at 30˚C is usually sufficient (Butz and Tauscher, 1997). Total viable counts of tomato juices treated by 300 and 400 MPa for 10 min at 25˚C were decreased to 0.9 and 1.5 log cfu/ml, respectively (Hsu *et al*., 2008). Mild HPP (500 MPa for 3 min at 25˚C) resulted in a stable refrigerated tomato juice product (Porretta *et al*., 1995), as vegetative microorganisms were inactivated and outgrowth of remaining *Bacillus* spores was prevented by the low pH (pH 4.0-5.0). A HPP treatment (700 MPa for 30 s at 90˚C) reduced *B. stearothermophilus* spore contamination level in inoculated

meatballs in tomato puree by 4.5 log cfu/ml. Treatment at 700 MPa for 2 min at 20˚C resulted in inactivation of natural flora to a level below the detection limit but 500 MPa for 2 min at 20˚C decreased natural flora in tomato puree by only 0.7 log cfu/ml (Krebbers *et al*., 2003). HPP treatments (300-500 MPa) at 25˚C for 10 min could be useful in processing tomato juice in considering the inactivation of the enzymes and maintenance or improvement of viscosity, colour, extractable carotenoids and radical-scavenging capacity (Hsu *et al*., 2008).

In order to reduce microorganisms to safe levels and to extend the shelf-life of this juice, pasteurization or sterilization is required. Thermal treatment is one of the most important methods of food preservation (Lund, 1975). However, quality losses in terms of flavour, colour, sensory and nutritive qualities are shown in section 4.3.

Suitable processing conditions for HPP were 400 MPa for 20 min at room temperature. Also, heat treatments including pasteurization (90˚C) and sterilization (121˚C) were applied for 3 and 4 min, respectively. Optimum conditions for each processing technique were selected for use in section 4.3.

4.3 Optimization of different processes based on chemical quality

The optimum conditions of each processing operation from the experiments described in section 4.2 were selected, and the chemical properties of the samples after each processing were analyzed. The treated juice samples were analyzed for triterpenes, active compounds including asiaticoside, madecassoside, chlorophyll content, ascorbic acid, total phenolic compounds (TPC), ferric reducing antioxidant potential (FRAP) assay, pH and total soluble solids. Also, the *β*-carotene content was determined since it occurred with chlorophyll in the sample. Chemical, physical and biological changes of food influence organoleptic properties, nutritional value, safety and health benefits of the juice (Kormin, 2005). The antioxidant activity and the content of active compounds of processed pennywort juices are summarized in **Table 4.4**. These results are in agreement with those in previously published literature by Kormin (2005). The juice composition and properties after each processing operation showed small changes in madecassoside, asiaticoside, ascorbic acid, chlorophyll, *β*-carotene and pH.

4.3.1 Physico-chemical properties of the processed juice

The pH, TSS and viscosity of HPP juice were higher than after thermal treatment. The pH of juice with added sugar was lower than that without added sugar. Kormin (2005) indicated that the moisture content of fresh samples was significantly higher than heat-treated samples due to water evaporation which occurred during heating, and the free moisture was also reduced by sugar addition. However, the pH of HPP juice was not different from fresh juice with added sugar (5.57±0.08) and without added sugar (5.62±0.09). Jannok (2007) also reported that the pH of HPP pennywort juice was 6.00±0.10, which was not significantly different from that of the fresh juice.

Table 4.4 Physico-chemical properties of processed juice

Compounds/ properties	Concentration (mg/100 ml)					
	HPP (400 MPa, 20 min)		Pasteurization (90˚C, 3 min)		Sterilization (121˚C, 4 min)	
	sugar added	Without sugar	sugar added	Without sugar	sugar added	Without sugar
Madecassoside	3.77±1.19 ns	3.70±1.08	3.32±1.29	3.20±0.33	3.07±1.33	2.87±1.02
Asiaticoside	4.30±1.95 ns	4.28±0.66	3.55±0.75	3.57±1.36	3.20±1.00	3.16±1.45
Ascorbic acid	4.16±0.27 a	4.03±0.63 a	2.00±0.09 b	1.70±0.09 b	0.85±0.04 c	0.84±0.02 c
Chlorophyll a	0.28±0.08 ns	0.21±0.06	nd	nd	nd	nd
Chlorophyll b	0.11±0.05 ns	0.08±0.01	nd	nd	nd	nd
β-Carotene	2.52±0.70 ns	2.51±0.79	2.48±0.59	2.39±0.64	2.32±0.03	2.26±0.52
TPC	404.23±20.43 a	385.70±16.25 a	265.27±7.06 b	235.75±20.16 b	170.63±31.21 c	158.13±8.60 c
FRAP (μM FeSO4/L)	734.87±80.67 a	462.58±64.79 b	381.00±23.64 bc	366.62±23.57 bc	345.00±6.00 c	333.33±32.01 c
pH	5.56±0.17 ns	5.61±0.24	5.54±0.03	5.57±0.20	5.53±0.08	5.56±0.14
TSS (%)	12.13±0.12 a	2.47±0.42 b	11.87±0.23 a	2.50±0.52 b	11.77±0.38 a	2.47±0.12 b

Means followed by different letters in same row indicate significant differences at p≤0.05 (Tukey's), non significantly different (ns) and not detected (nd).

Processing of juice by HPP, pasteurization and sterilization caused a change in the pH of juice with added sugar from that of the fresh juice (5.57) to 5.56, 5.54 and 5.53, respectively. Also, the pH of juice without added sugar decreased from that of the fresh juice (5.62) to the values of 5.61, 5.57 and 5.56, respectively. Phunchaisri and Apichartsrangkoon (2005) reported that the pH of fresh lychee was 4.72 and it decreased to 4.60 after HPP treatment. The pH of lychee in syrup was 4.22 and up to 4.25 after HPP and decreased to 4.12 after canning (boiled for 18 min). The lychee processed in syrup unexpectedly had a lower pH and higher TSS than fresh lychee. The TSS of fresh lychee was 11.2% and it increased to 16.3 after HPP. For lychee in

syrup, the TSS was 22.6% and also increased to 22.8 and 23.9% after HPP and canning, respectively. The reduction in pH on pasteurization and sterilization indicates that amino groups on proteins and amino acids were removed by reaction with reactive molecules such as aldehydes on heating. The observation that the ascorbic acid and TPC content fell strongly during these processing operations makes it probable that oxidation products of ascorbic acid and phenols with carbonyl groups in their structure were formed during processing, and they reacted with amino groups to cause a reduction in pH. It is known that oxidation products of ascorbate react one hundred times faster with amine groups than glucose does (Lee *et al*., 1998).

4.3.2 Phytochemical compositions of the processed juice

The present study showed the effects of HPP and heat treatment during processing of pennywort juice on phytochemicals composition, with changes evident particularly in the content of madecassoside and asiaticoside. The effects of heat treatment on triterpene glycosides in pennywort have been investigated by Kormin (2005). However, the effects of HPP treatment on active constituents in this plant have not been reported.

Changes of triterpenes glycosides

The madecassoside content in fresh juice was 3.80 mg/100 ml which was higher than the content after HPP (3.70-3.77 mg/100 ml), pasteurization (3.20-3.32 mg/100 ml) and sterilization (2.87-3.07 mg/100 ml). Kormin (2005) reported that the contents of madecassoside in fresh drink samples were 12.2-22.1% which was higher than those in the corresponding heat-treated samples. The level of this component decreased from 3.12 mg/100 ml in fresh juice to 2.70 mg/100 ml (65°C, 15 min) and 2.43 mg/100 ml (80°C, 5 min) in pasteurization juices. However, it slightly increased when the temperature was increased to 100°C for 10 min (2.74 mg/100 ml). Kormin (2005) assumed that the target constituent was extracted and dissolved easily at high temperatures during juice processes involving heating.

The asiaticoside content in fresh juice was 4.49 mg/100 ml, which was higher than the content after HPP (4.28-4.30 mg/100 ml), pasteurization (3.55-3.57 mg/100 ml) and sterilization (3.16-3.20 mg/100 ml). In contrast, Kormin (2005) showed that the asiaticoside content in the fresh drink (3.92 mg/100 ml) was significantly lower

than in samples processed at 65°C (4.32 mg/100 ml). However, it was significantly higher than in the other samples. The concentration of asiaticoside was reduced to 8-22.5% of the initial value when exposed to high temperatures up to 80°C (3.61 mg/100 ml) and 100°C (3.03 mg/100 ml). Kormin (2005) concluded that heat treatment at moderate temperature (65°C) is likely to increase the ability of water (as a medium) to dissolve the asiaticoside. An increase in heating temperature decrease the concentrations of madecassoside (65-80°C) and asiaticoside (80-100°C) in the ranges studied.

Heat treatment may reduce the saponin concentration (Court *et al*., 1996). Vongsangnak *et al*. (2003) also reported that the maximum saponin yield was achieved when the extract temperature was 50°C.

The variations of triterpene glycoside content in different samples occurs due to various factors including species, geographical source, cultivation, harvest, storage as well as method used to prepare the plant for analysis (Kormin, 2005) as well as the extraction and HPLC analytical methods.

Changes of ascorbic acid

Ascorbic acid present in processed food is considered as an indicator for the product quality due to its relative instability to heat, oxygen and light (Birch *et al*., 1974). It is highly sensitive to degradation at a rate that depends on temperature, salt, sugar concentration, pH, oxygen, enzymes, metal catalyzts and its initial concentration (Tannenbaum *et al*., 1985). It also degraded by active oxygen and by reactions initiated by transition metals. It removes oxygen in systems where oxygen is present in limited amounts and oxidized to dehydroascorbic acid (Jadhav *et al*., 1996). Furthermore, Van den Broeck *et al*. (1998) concluded that the sensitivity of ascorbic acid to heat depends on the type of product. The ascorbic acid content after HPP and heat treatment was lower than that of the fresh juice. The concentration of ascorbic acid was reduced significantly after processing. Fresh juice contained the highest amount of ascorbic acid (4.77 mg/100 ml) followed by HPP (4.03-4.16 mg/100 ml), pasteurization (1.70-2.00 mg/100 ml) and sterilization (0.84-0.85 mg/100 ml). Losses of ascorbic acid in processed pennywort juice were also observed by Kormin (2005), with losses in a commercial sample due to high temperature pasteurization (90°C, 1 min), low temperature pasteurization (80°C, 5 min) and sterilization (100°C, 10 min)

being 33.2, 16.6 and 66.8%, respectively. Somsub *et al*. (2008) reported losses of ascorbic acid of 49.2% during a blanching process. Mahanom *et al*. (1999) reported that the loss of ascorbic acid in dried tea at 50°C for 9 hours and 70°C for 5 hours was 75.60 and 34.19%, respectively. In addition, the concentration of ascorbic acid in tomato puree and oil samples was reduced to 46 and 55%, respectively of the initial value after heat treatment at 95°C for 30 min (Nicoli *et al*., 1997). However, low temperature treatments including, freeze-drying of guava juice and myrobolan juice also caused a reduction in the concentration of ascorbic acid to 41.4 and 20.4% of the initial value, respectively (Suntornsuk *et al*., 2002). Lea (1992) also reported that ascorbic acid in fresh apples is rapidly lost during processing into juice. The loss of ascorbic acid may be attributed to oxidation at the temperature applied (Yang and Atallah, 1985). Ascorbic acid is easily destroyed by oxidation at high temperatures and during processing and also during storage. The effect of temperature on ascorbic acid content is more than the effect of heating duration. Since ascorbic acid is soluble in water, it is readily lost via raw material preparation but the most significant losses result from chemical degradation (Tannebaum, 1985). Conversion of ascorbic acid to diketoglutanic acid due to reaction with air, light and metal ions may also contribute to the losses encountered (Harris, 1975; Addo, 1981). However, ascorbic acid is well known as an antioxidant nutrient and *in vitro*, protects some flavonoids against oxidative degradation during processing and storage in elderberry juice (Kaack and Austed, 1998). There is extensive literature about the stability of ascorbic acid in juices. Ascorbic acid of canned tomato juice was decreased by treatment at 100˚C for 10 min (Youssef and Rahman, 1982). The ascorbic acid and total vitamin C contents decreased to 38 and 42% compared with fresh tomato juice (Hsu *et al*., 2008). Although Sanchez-Monreno *et al*. (2006) revealed that ascorbic acid and total vitamin C contents of tomato puree treated by pasteurization at 90˚C for 1 min declined only 34 and 26%. Sancho *et al*. (1999) have also measured ascorbic acid concentrations in Strawberry *coulis* after treated with pasteurization (72˚C for 20 s), and obtained a 91.52% ascorbate retention. These non-significant losses indicate that ascorbic acid is conserved in the pasteurization process under these conditions. There was, thus, no significant effect on the destruction of the ascorbic acid of the strawberry *coulis*. On the other hand, a sterilization process (120˚C for 20 min) had a more noticeable effect

on ascorbic acid concentrations in strawberry *coulis*. Only 67.1% of the initial ascorbic acid was conserved after sterilization.

In this study the effect of temperature and time used for the heat treatments, pasteurization (90˚C for 3 min) as well as sterilization (121˚C for 4 min), on ascorbic acid content were greater at higher temperature and longer time, which is in accordance with the evidence supporting ascorbic acid destruction in tomato products with thermal treatments (Hayes *et al*., 1998; Nicoli *et al*., 1997).

Methods of juice processing with minimal heating such as HPP are necessary to preserve as much ascorbic acid as possible. The ascorbic acid content in Broccoli juice was reduced from the value for fresh juice of 41.2 mg/100 g to the values of 38.2, 40.8, 41.9, 40.1, 36.4, 34.5, 36.6, 36.3, 31.4, 30.5, 31.6, 33.8, 28.8, 25.2, 28.0 and 29.9 mg/100 g, respectively after HPP treatments at 350, 400, 450 and 500 MPa for 5 min; 350, 400, 450 and 500 MPa for 10 min; 350, 400, 450 and 500 MPa for 15 min; 350, 400, 450 and 500 MPa for 20 min (Houlka *et al*., 2006). Pressure alone was not found to significantly change the ascorbic acid concentration of orange juice but at temperatures above 60˚C during HPP processes, ascorbic acid degradation was observed (Taoukis *et al*., 1998; Van den Broeck *et al*., 1998). According to reported by Hayashi (1989) and Kimura *et al*. (1994), HPP had less effect on the tested hydrosoluble vitamins, and thus it helps to preserve the nutritional quality of food products. Wolbang *et al.* (2008) reported that the ascorbic acid content was significantly affected by melon cultivar and HPP (600 MPa for 10 min), and the various cultivars responded differently to processing. Cultivar is importance and may also be a contributing factor in ascorbic acid retention. In this study, it was found that HPP could not completely preserve ascorbic acid in pennywort juice and losses of ascorbic acid occurred during HPP at 400 MPa for 20 min at 30˚C. In addition, Hsu *et al.* (2008) also found losses in the ascorbic acid content in the HPP (300, 400 and 500 MPa) tomato juice, which could be due to the long time treatment (10 min) and mild temperature (25˚C). Several studies of orange juice and tomato puree showed that the loss of ascorbic acid after HPP is dependent mainly on temperature and time (Sanchez-Moreno *et al*., 2003, 2006). Hsu *et al*. (2008) reported that the pressure levels of 300-500 MPa caused the same effect on degradation of ascorbic acid in tomato juice. HPP treatment (at 300, 400 and 500 MPa for 10 min at 25˚C) of tomato

juices also caused a decrease in ascorbic acid and total vitamin C of about 30%, with no significant difference between different samples (Hsu *et al*., 2008). For a strawberry *coulis*, which has a high content of ascorbic acid, HPP had no significant effect on its loss. In strawberry *coulis*, HPP treatment at 400 MPa for 30 min at 20˚C was responsible for 88.68% retention. In a strawberry nectar (50% juice, 49.5% sucrose syrup, 0.2% ascorbic acid and 0.3% citric acid), after HPP under the same conditions as applied to the strawberry *coulis*, the measured quantity of ascorbic acid was 1,129 ppm for an initial concentration of 1,100 ppm (Sancho *et al*., 1999). The concentration of ascorbic acid in citrus juice did not indicate any change after HPP. The concentration for fresh citrus juice was 27.2 mg/100 g and after treatment at 500 MPa for 10 min at 20˚C was reduced to 27.1 mg/100 g (Ogawa *et al*., 1992).

According to the Association of the Industry of Juices and Nectars from Fruits and Vegetables of the European Union, the ascorbic acid content of orange juice has to be more than 20 mg/100 ml at the expiry date for the product. This ascorbic acid content was used to estimate the end of the shelf life of orange juice. The shelf life of orange juice was estimated, as the time period in which there is a 50% loss since the initial ascorbic acid concentration of the orange juice was about 40 mg/100 ml (Polydera *et al*., 2003). Ascorbic acid concentration was found to always be above the minimum values recommended for processed orange juice (40 mg/100 ml) (Esteve *et al*., 2005), but there is no recommended value for processed pennywort juice according to Thai Food Regulation-Standard (2003).

Changes of pigments

Changes in food colour can be associated with its previous heat treatment history. Various reactions such as pigment destruction (carotenoids and chlorophylls) and non-enzymatic browning (Maillard) reactions can occur during heating of fruits and vegetables and therefore affect its colour (Reyes and Luh, 1960; Abets and Wrolstad, 1979; Resnik and Chirife, 1979; Cornwell and Wrolstad, 1981). The retention of total colour can be used as a quality indicator to evaluate the extent of deterioration due to thermal processing (Shin and Bhowmik, 1995).

Chlorophyll is unstable at elevated temperatures and change to brown colour. This colour change is due to chlorophyll conversion to pheophytin and it is favoured by acid conditions (Potter, 1986; Francis, 1985). The rate of brown pigment formation

is increased at higher heating temperatures and longer heating times (Labuza and Baisier, 1992), and Roig *et al*. (1999) reported that this change is accompanied by ascorbic acid loss. Browning in this product is undesirable due to consumers perceiving less desirable sensory characteristics including appearance and aroma (Kormin, 2005).

The chlorophyll concentrations in fresh pennywort and in the juice were lower than those in several literature reports. The fresh juice was deep green in colour, and a small concentration of chlorophyll *a* and *b* was still present in HPP samples with added sugar (0.28 and 0.11 mg/100 ml, respectively) and without added sugar (0.21 and 0.08 mg/100 ml, respectively). The errors in the determination of chlorophyll content are due to juice extraction, freezing before transfer to another laboratory, thawing, juice preparation and extraction for analysis. The stability of the pigment depends on pH, temperature, storage time etc. The mild acid pH of the juice would lead to considerable degradation of chlorophyll to pheophytin. Thus, the main focus on pigment analysis was switched from chlorophyll to *β*-carotene in the next experiment.

β-Carotene is known to be relatively stable during heat treatment (Elkin, 1979; Miki and Akatsu, 1971). Lycopene in tomato is relative resistant to degradation, whereas other antioxidants (ascorbic acid, tocopherol and *β*-carotene) decrease as a function of thermal processing (Abushita *et al*., 2000). In this study, *β*-carotene was present at concentrations of 2.39-2.48 and 2.26-2.32 mg/100 ml in the pasteurized and sterilized juice samples. After thermal processing (98˚C for 15 min) the total carotenoid and lycopene contents of tomato juices were decreased by about 1%, which was insignificant (Hsu *et al*., 2008). Miki and Akatsu (1970) demonstrated that heating tomato juice at 90, 100 and 130˚C for 7 min resulted in a 1.1, 1.7 and 17.1%, respectively, decrease in lycopene content. Increases in lycopene concentration in tomato puree have been found at temperatures of 90˚C for 110 min and 110˚C for 1.1 min but not at 120˚C for 0.1 min (Anese *et al*., 2002). Hsu (2008) showed that the temperatures 60˚C and 92˚C for 2 min applied to tomato juice did not affect lycopene extractability or degradation probably due to insufficient treatment temperature and time.

HPP pennywort juice contained a concentration of *β*-carotene 2.51-2.52 mg/100 ml, which was similar to that of fresh juice (2.50 mg/100 ml). HPP melon samples had higher levels of *β*-carotene compared to fresh samples, which is also consistent with the findings for orange juice. De Ancos *et al*. (2002) reported that the increase of carotene in orange juice was thought to be due to enhanced extraction due to the denaturation of the protein-carotenoid complex (Sanchez-Moreno *et al*., 2003). Hsu *et al*. (2008) reported that after HPP at 300 MPa for 10 min at 25˚C, total carotenoids and lycopene contents increased by up to 62 and 60%, respectively. Hsu (2008) also reported that after HPP above 300 MPa for 10 min at 4 and 25˚C, total carotenoid and lycopene contents of tomato juice significantly increased up to 62 and 56%, respectively, as compared with control. However, HPP below 200 MPa at 4 and 25˚C might only slightly modify the structure of proteins which are bound to carotenoids but these conditions could not induce the pigments extraction. Total carotenoid and lycopene contents of the tomato juices processed by HPP at 50˚C were much lower than those processed at either 4 or 25˚C, probably due to the pressure preventing protein thermal denaturation at denaturing temperatures (Heremans and Smeller, 1998). It has been reported that HPP (500 MPa for 2 min at 20˚C) increased the lycopene content of tomato puree, compared with the raw puree (Krebbers *et al*., 2003). In addition, Sanchez-Moreno *et al*. (2006) have shown that increases in carotenoid and lycopene occur as a result of HPP (400 MPa for 15 min at 25˚C) of tomato puree. The enhanced extraction of carotenoid and lycopene by HPP is attributed to an effect on the membranes in vegetable cells (Shi and Le Maguer, 2000). Carotenoids are tightly bound to macromolecules, in particular to protein and membrane lipids, and HPP is known to affect macromolecular structures such as proteins and carbohydrate polymers (Gartner *et al*., 1997).

β-Carotene, madecassoside and asiaticoside were relatively stable on processing with no significant losses occurring. There was a trend for losses of *β*-carotene, madecassoside and asiaticoside to be greater in samples heat treated by pasteurization and sterilization but these did not reach statistical significance. Madecassoside and asiaticoside are triterpene alcohols and therefore are more stable than phenolic compounds.

Changes of total phenolics

This juice contained high concentrations of phenolic compounds as compared to orange juice (75.5 mg/100 ml), pineapple juice (35.8 mg/100 ml) and vegetable juice (29.3 mg/100 ml) (Gardner *et al*., 2000). However, the concentrations in HPP and heat-treated pennywort juice were lower than the values for tea beverages according to several reports.

HPP and heat treatment applied during juice processing causes a reduction in the content of total phenolics and the FRAP values. After treatment, the total phenolic content was decreased from a value of 988.54 mg/100 ml for fresh juice to 385.7-404.23 mg/100 ml for HPP juice (400 MPa for 20 min), 235.75-265.27 mg/100 ml for pasteurized juice (90˚C for 3 min) and 158.13-170.63 mg/100 ml for sterilized juice (121˚C for 4 min). The FRAP value decreased from fresh to HPP, pasteurized and sterilized juice, from 913.51 µM FeSO4/L to 462.58-734.87, 366.62-381.00 and 333.33-345.00 µM FeSO4/L, respectively. Kormin (2005) reported that about 50% of the total phenolic content was retained in this juice after canning (100˚C for 10 min) and the loss after treatment at 65˚C for 15 min and 80˚C for 5 min was 45 and 49%, respectively. In agreement with the previous reports, the phenolic compounds in pennywort juice were significantly lost during heat processing. Fezah *et al*. (2000) reported that the air-dried treatment at room temperature of pennywort leaf significantly reduced the pyrogallol content. The total phenolics in pennywort leaf and root were reduced by 52 and 50%, respectively. Gil-Izquierdo *et al*. (2002) reported that pasteurization led to degradation of several phenolic components in orange pulp.

Since the unstable compounds may be present in this juice as major components, the content of total phenolics can be significantly reduced after high pressure and thermal treatment, Zainol *et al*. (2003) and Fezah *et al*. (2000) reported that pennywort leaf extract contained total phenolics at concentrations of 3.23-11.70 and 23.00%, respectively. The phenolic content of pennywort juice was significantly lower than that in the raw plant due to the dilution process (1:1, extracted with water) and the reduction of this compound by chemical reaction during juice processing. As previously observed the processing treatment of pennywort juice at the high temperatures potentially caused thermal decomposition of some phenolic antioxidants (Kormin, 2005). However, juice extraction leaves behind some phenolic compounds

in the residue. Skrede and Wrolstad (2002) reported that the loss of polyphenolic compounds occurred during the processing of single strength juice. The pasteurization of blueberries allowed the recovery of 32% of the anthocyanins, whereas 18% remained in the press-cake residue after pulp pressing. Koo and Suhaila (2001) reported that at high temperatures certain phenolics decompose or combine with other plant components. Moreover, some phenolics are excellent substrates for polyphenol oxidase (PPO) which leads to enzymatic browning (Kormin, 2005).

Velioglu *et al*. (1998) reported that phenolic compounds are responsible for most of the antioxidant activity in selected vegetables, fruits and grains. Nicoli *et al*. (1999) also reported that the antioxidant effectiveness of most plant materials is reported to be mostly due to phenolic compounds. The antioxidant potential of phenolic compounds depends on the stability of the radical formed from the compound after hydrogen abstraction by a radical. The mechanism of protection from oxidative insults of each compound is very specific (Pokorny *et al*., 2001).

The high pressure and thermal processing operations studied caused a decrease in the antioxidant potential of the juice. The FRAP value of the fresh juice was 860 μM $FeSO_4$/L whereas juice processed at 65˚C for 15 min, 80˚C for 5 min and 100˚C for 10 min had FRAP value in the range of 404-740 μM $FeSO_4$/L (Kormin, 2005). Gardner *et al*. (2000) reported that the ability of vegetable and orange juice to reduce Fe (III) gave FRAP values of 1.2 and 6 mM, respectively. Tsai *et al*. (2002) reported the FRAP activity of rosella extract and green tea was 2 mM and 8 mM, respectively. Kormin (2005) reported that the antioxidant capacity after heat treatment was reduced by 14-53.59% when assessed by the FRAP assay. The polyphenols in pennywort juice can be destroyed or transformed into other phytochemicals during heat treatment and processing. Transformations of the structure, phenolic compound oxidation during processing and reaction of phenolic antioxidants with other components (Kikuzaki and Nakatani, 1993; Nicoli *et al*., 1999) may explain the reduction in antioxidant capacity value in pennywort juice. The high capacity of antioxidants in processed juice is probably partly due to development of new components, having antioxidant properties. Food processing can promote polymerization of phenols to form brown coloured macromolecular products, which are expected to possess about the same antioxidant activity as the original phenols (Mazocco *et al*., 1999). Wang *et al*. (1996)

also reported that heat-processed tomato juice and grape juice had higher antioxidant activity than the fresh juice.

However, the processing of pennywort juice in this study was applied to juice sealed in pouches with little air in the headspace before it was subjected to HPP, pasteurization and sterilization, but the oxidative degradation of active biological compounds was not inhibited under these conditions.

4.3.3 The effect of sugar addition

The total soluble solids (TSS) in food products can be increased by evaporation of moisture and by sugar addition. There was a marked increase in the TSS contents and concomitant decrease in water activity (a_w) in the samples, indicating some loss of water during processing and/or solubilisation of previously insoluble material. Wrolstad *et al*. (1990) suggested that a high sugar concentration increased the stability of some phenolic compounds by lowering the a_w of the food. In this study, the concentration of TSS was increased from 2.53 to 12.17%. The FRAP value of the juice with added sugar after HPP and heat treatment was higher than that for the juice without added sugar. Kormin (2005) also investigated the dependence of the antioxidant activity of this juice on TSS, with high absorbance values in the FRAP assay being observed for juice with TSS 15% (0.057) followed by 10% (0.050), 5% (0.044) and the control sample (0.032). The antioxidant activity was gradually increased at 5 and 10% when assessed by the antioxidant activity in a linoleic acid system (FTC assay), but it declined at TSS 15%. Takeoka *et al*. (2001) reported that the antioxidant capacity and lycopene content increased up to a TSS level of 25-30% in tomatoes. The increase in TSS through the addition of sugar also reduced a_w in pennywort juice (Kormin, 2005). The rate of chemical reactions, including lipid oxidation and ascorbic acid degradation, increases as water is added up to a higher a_w value with maximum rates occurring in the range of intermediate moisture foods (a_w 0.7-0.9) (Fennema, 1985). The presence of free water may accelerate oxidation by increasing the solubility of oxygen and by causing macromolecules to swell, thereby exposing the molecules to more reactions.

Phunchaisri and Apichartsrangkoon (2005) reported that the PPO in non-syrup lychee was decreased in activity after HPP (200-600 MPa for 10-20 min at 20, 40 and

60˚C). When processed in syrup (22.6%), the PPO activity was decreased after HPP (200 MPa for 10 min at 40˚C, 200-600 MPa for 10-20 min at 60˚C and 600 MPa for 20 min at 20-40˚C). These results are undoubtedly due to the baroprotective effect of the syrup according to Seyderhelm *et al*. (1996), who reported that the pectinesterase inactivation in orange juice containing 30% sucrose (60˚Brix) was much lower than in a sample containing 11% sucrose.

The next study comprised experiments designed to determine the change in quality of pennywort juice during storage. Juice was processed to produce the finished products using conditions based on the results from section 4.2.

The juice was kept at 4˚C for products treated by HPP and pasteurization as well as stored at 40˚C for product treated by sterilization. The stability of the sterilized sample towards microbial decay allowed the storage to be studied at a higher temperature, which represents more demanding conditions for chemical deterioration. All samples were kept for 4 months and/or until the products degraded due to the content of microorganisms exceeding the Thai Food Regulation-Standard (2003). The physical properties of the products were determined at 1 month intervals, the chemical properties at 15 day intervals and the microbiological quality at 1 week intervals during the storage time.

4.4 The effects of storage time on the physico-chemical quality

The changes in physical properties and chemical compositions as a consequence of HPP and heat treatment were determined.

4.4.1 Change in physical properties

The visual colour changes defined in term of chroma and hue angle are shown in **Tables 4.5-4.6** and viscosity in **Table 4.7** but turbidity was not determined due to the fact that few microorganisms were detected in all processed samples.

During storage at 4˚C for HPP and pasteurized juices and 40˚C for sterilized juice, gradual degradation of the green colour was observed in juice samples (**Tables 4.5-4.6**).

Table 4.5 Chroma values of processed pennywort juice

Shelf life (months)	Chroma (C)					
	HPP + sugar NS	HPP NS	Pasteurized + sugar NS	Pasteurized NS	Sterilized + sugar NS	Sterilized NS
0.0	11.06±0.05 b	12.99±0.04 a	7.02±0.03 c	6.54±0.06 d	6.07±0.23 e	5.99±0.44 e
1.0	11.05±0.07 b	13.00±0.06 a	7.02±0.05 c	6.56±0.05 d	6.07±0.34 e	5.98±0.75 e
2.0	11.05±0.04 b	12.98±0.07 a	7.02±0.04 c	6.55±0.10 d	6.05±0.18 e	5.97±0.82 e
3.0	11.04±0.05 b	12.99±0.05 a	7.02±0.08 c	6.54±0.08 d	6.08±0.22 e	5.98±0.18 e
4.0	11.04±0.03 b	12.97±0.08 a	7.02±0.03 c	6.55±0.05 d	6.04±0.10 e	5.97±0.25 e

Means with different small letters within a row of each component are significantly different ($p \leq 0.05$) (Tukey's) and non significantly different (ns)

Means with different capital letters within a column of each component are significantly different ($p \leq 0.05$) (Tukey's) and non significantly different (NS)

Table 4.6 Hue angle values of processed pennywort juice

Shelf life (months)	Hue angle (h˚)					
	HPP + sugar NS	HPP NS	Pasteurized + Sugar NS	Pasteurized NS	Sterilized+ sugar	Sterilized
0.0	99.39±0.11 a	97.78±0.26 a	79.26±0.17 b	78.18±0.81 b	73.07±0.35 cA	72.94±0.19 cA
1.0	99.36±0.23 a	97.78±0.18 a	79.19±0.56 b	78.20±0.43 b	73.01 ±0.26 cA	72.88±0.41 cA
2.0	99.39±0.41 a	97.78±0.23 a	79.27±0.33 b	78.20±0.72 b	72.94±0.38 cA	72.05±0.28 cAB
3.0	99.39±0.15 a	97.78±0.12 a	79.19±0.44 b	78.20±0.29 b	72.80±0.48 cA	71.37±0.32 cB
4.0	99.36±0.44 a	97.78±0.16 a	79.19±0.06 b	78.20±0.40 b	71.37±0.40 cB	71.11±0.16 cB

Means with different small letters within a row of each component are significantly different ($p \leq 0.05$) (Tukey's) and non significantly different (ns)

Means with different capital letters within a column of each component are significantly different ($p \leq 0.05$) (Tukey's) and non significantly different (NS)

The hue angle of HPP samples was higher than 90, indicating that they all had a deep-green colour. High storage temperatures have been studied in order to observe clearly the effects of temperature on certain factors including browning, loss of biological components, etc. Since original colour of the pennywort juice was dark green, the *L** value was not suitable for indicating the colour changes (Kormin, 2005). The formation of browning was monitored by CIE values including chroma and hue angle. The chroma values of HPP, pasteurized and sterilized juices were 11.04-13.00, 6.54-7.02 and 5.97-6.08, respectively. Kormin (2005) reported that the chroma values of heated juices (65˚C for 15 min, 80˚C for 5 min and 100˚C for 10 min) were 7.21, 6.38 and 7.08, respectively. However, Jannok (2007) reported that the chroma of

pasteurized juice (90˚C for 15 s) and same HPP (400 MPa for 20 min at <30˚C) treated juice (1:4 dilution) were 63.61 and 78.44, respectively, which were higher than the values found in this study. The chroma slightly decreased ($p>0.05$) during 4 months of the storage, which could be due to the sedimentation of brown compounds. For the hue angles, HPP, pasteurized and sterilized juices were 97.78-99.39, 78.18-79.27 and 71.11-73.07, respectively. These values can be compared with the colour from other research; for example, the hue angle of HPP treated juice and pasteurized juices were 94.20 and 81.36, respectively (Jannok, 2007). Kormin (2005) reported that the hue angles of heated juice (65˚C for 15 min, 80˚C for 5 min and 100˚C for 10 min) were 72.50, 70.88 and 67.85, respectively, which were lower than those found in this study.

The chroma and hue angle showed no changes during 4 months except for sterilized pennywort juice which had a change in hue angle within 3 months. The HPP (600 MPa for 40 min at 50˚C) and pasteurization treated juice stored at 4˚C exhibited no change in chroma and hue angle but changes were detected when stored at room temperature during 4 weeks of storage (Jannok, 2007).

For other food samples, orange juice pressurized at 800 MPa for 1 min at 25˚C showed that the colour value was stable during storage at 4, 15 and 26˚C. The colour was not changed over time, except in the samples stored at 37˚C (Nienaber and Shellhammer, 2001). Rein and Heinonen (2004) concluded that for the copigmentation compounds, phenolic acid enrichment improved and stabilized the colour of the berry juices during 103 days of storage. The changes in hue angle and chroma were observed in untreated strawberry, raspberry, lingonberries and cranberries juice, the hue angle of which increased from 59.9, 33.4, 38.2 and 33.4 to 71.1, 62.5, 48.2 and 48.5 but the chroma was changed from 11.7, 21.3, 39.5 and 37.8 to 11.8, 10.4, 22.4 and 14.5, respectively during storage for 103 days. Choi *et al*. (2002) reported that increasing in hue angle and decreasing in chroma were the characteristic changes in blood orange juice colour during refrigerated storage for 7 weeks. Esteve *et al*. (2005) concluded that the colour values were always higher in the orange juice samples stored at 4˚C than in those stored at 10˚C.

After guava juice processing, Phongphisutthinant (2006) reported that the green colour (-a^*) of guava juice was decreased and the yellow colour (+b^*) was

increased in order of heating time (90˚C) for 30, 60, 90 and 120 s, respectively. The hue angle was decreased and the chroma was increased with increasing holding time (5-20 min) during HPP at 500 MPa and 30˚C. During storage at 4˚C for 28 days, HPP treatment of guava juice caused a decrease in the L^*, a^* and b^* values but not significantly affected when compared with fresh juice. However, the heated sample at 90˚C for 120 s results in significant changes of L^*, a^* and b^* values ($p \leq 0.05$).

The colour of the fresh berry juices was not stable during storage. The red colour of strawberry juice was imperceptible after 36 days. Raspberry juice colour was not measurable in the red region after 51 days. The colour of lingonberries and cranberry juices was maintained in the red region of the spectrum throughout storage, but the colour intensity was only 33% of the original for lingonberries juice and 19% for cranberry juice in the end of storage. Thus, copigmentation of berry juices occurs and the colour is enhanced by phenolic acids and commercial colour enhancers for colour stability during storage (Rein and Heinonen, 2004). The colour of tomato juice degraded more rapidly with increasing temperature (Goodman *et al*., 2002). Ahmed *et al*. (2005) observed that the colour parameters such as a^*, b^*, chroma and hue angle of mango pulps remained constant after HPP, indicating pigment stability. Palou *et al*. (2000) reported that the hue angle of HPP (689 MPa at 21˚C) treated guacamole sauce was increased when HPP holding time increased. HPP did not affect the colour of guacamole. The colour of guacamole right after HPP was not significantly different from control sample (Palou *et al*., 2000; Lopez-Malo *et al*., 1999; Palou *et al*., 1999). However, during storage at 5, 15 and 25˚C, changes in the hue angle and a^* value occurred, and the green contribution to the colour gradually decreased and was lost in less than 5 days for guacamole stored at 15 or 25˚C. Only HPP guacamole stored at 5˚C retained the green hue for more than 10 days. Storage temperature drastically affected the rate of hue angle decrease. Changes in hue angle and chroma were correlated with the a^* value, indicating that severe browning of guacamole was observed when the a^* value was positive (redness) (Palou *et al*., 2000). Hsu *et al*. (2008) concluded that the colour of tomato juice treated by HPP above 400 MPa (at 25˚C for 10 min) was appreciated more than that of the control and that of samples treated by thermal treatments (98˚C for 15 min). The colour declined and was not significantly different from the control after storage for 21-28 days. However, the

colour was slightly lower than the control probably due to the isomerization of lycopene. Isomerization converts all-*trans* isomers to *cis*-isomers during HPP (Qiu *et al*., 2006). HPP treatment of lychees at 200-600 MPa for 20 min (at 20 and 60˚C) caused a decrease in the *a** value indicating that HPP may be an effective means of minimizing the pink discolouration, but the *b** value in this sample had changed a little (Phunchaisri and Apichartsrangkoon, 2005). Products with high pH such as melon (pH 7.0), may require increased temperatures in the HPP treatment (600 MPa for 10 min) to inactive enzymes and retain colour.

The cultivar effects on the colour change

Wolbang *et al*. (2008) found that HPP (600 MPa for 10 min) resulted in significant reductions of colour parameters (based on *L**, *a** and *b**) in all cultivars of melon. Although both cultivar and HPP had an effect on melon colour, there were no significant interactions between cultivar and HPP contributing to variation in the colour. The changes in colour due to HPP were not influenced by differences in the colour of the raw material.

The packaging effects on the colour change

The colour of orange juice stored in laminated flexible pouches indicated that the chroma slightly changed with storage time but this change did not correlate with processing method and storage temperature (0-15˚C) (Polydera *et al*., 2003). Colour parameters for orange juice, packed in monolayer PET bottles with and without dissolved oxygen, stored at 4 and 25˚C did not present significant variations until 60 days of storage for both temperatures. After this time, values of *L** and hue angle started to decrease. However, the chroma values showed an increasing trend. At the end of storage for 180 days, only juice stored at 25˚C showed a significant change ($p \leq 0.05$) in the orange colour (Ros-Chumillas *et al*., 2007).

Effects of non-enzymatic reactions on the colour degradation

Browning and discolouration due to thermal treatments occur due to many reactions including non-enzymatic browning (Maillard) reactions, caramellisation, the ascorbic acid browning process (Cornwall and Woodstad, 1981) and the destruction of pigments (Beveridge *et al*., 1986). Hsu (2008) reported that colour degradation of tomato juice occurred due to thermal treatments (60 and 92˚C for 2 min), and this was caused by non-enzymatic reactions. Kormin (2005) also reported that heat treatments

significantly increased brown colour development in pennywort juice but it is not clear which reactions are involved to enhance non-enzymatic browning. The rate of non-enzymatic browning depends on a_w, pH, temperature and chemical composition of the food system (Whristler and Daniel, 1985; Potter, 1986). A longer heating time and complex reactions between components during the initial stages of browning may be associated with the increase of colour (Labuza and Baisier, 1992). Marchese (1995) reported the impact of pasteurization time and temperature on blood orange juice discolouration, and suggested that mild pasteurization (less than 80˚C) minimized the degradation of anthocyanins. Those discrepancies might be due to the different colour contributors, colour degradations and various processing conditions including pH, time and temperature (Rodrigo *et al*., 2007a).

Ascorbic acid is a reducing compound and can be easily oxidized, which might reduce browning through preventing and/or reversing the oxidation of *o*-diphenols to *o*-quinones. In addition, Poei-Langston and Wrolstad (1981) concluded that the addition of ascorbic acid resulted in the loss of pigment stability. However, browning formation is often attributed to reactions of *L*-ascorbic acid (Clegg, 1966; Roig *et al*., 1999).

The natural antioxidant concentration is significantly reduced as a result of heat treatment, but the overall antioxidant properties of the products may be maintained by the Maillard browning reaction (Nicoli *et al*., 1997). Maillard browning involves reactions of carbohydrates with amino compounds; particularly reducing sugar and amino acids, and the reaction depends on water content and it increases with increasing heating temperature and pH (Cheftel *et al*., 1985). Non-enzymatic browning in food products has a positive and negative impacts but it has a negative impact in fruit juice due to changes in sensory properties including colour and aroma (Carabasa-Giribet and Ibarz-Ribas, 2000). However, Maillard browning reactions are reported to cause the formation of compounds with antioxidant capacity (Manzocco *et al*., 2000). Formation of Maillard reaction products during prolonged heating and storage times generally produced products that exhibited strong antioxidant properties (Eichner, 1981; Nicoli *et al*., 1999). For example, carrot, cauliflower and zucchini juices were heated at 102˚C for 10 min showed higher antioxidant capacity and also increased with an increase of heating temperature and time (Gazzani *et al*., 1998).

Enzymatic reaction effects on colour degradation

The colour deterioration in pasteurized fruit juice is due to enzymatic browning of polyphenols, catalyzed by PPO in the presence of dissolved oxygen (Pokorny, 2001). Browning is mostly the result of the activity of PPO acting on phenolic compounds to produce dark coloured polymers when pennywort juice is extracted. Flavour and colour deterioration of HPP foods during storage were attributed to residual enzyme activity by Horie *et al*. (1991) and Cano *et al*. (1997). HPP has been found to induce discolouration in mushrooms and onions due to enzyme (PPO) activity, responsible for browning (Butz *et al*., 1994). After HPP (689 MPa for 20 min at 21˚C) applied to avocado products like guacamole sauce, PPO residual activity was 22% demonstrating the baroresistance of PPO (Palou *et al*., 2000). Lopez-Malo *et al*. (1999) reported that the storage time to loose the green colour of avocado puree depended on storage temperature, initial pH and residual PPO activity. Longer storage time for the avocado puree was obtained with treatments that combined a residual PPO activity below 45% with a storage temperature of 5˚C. An acceptable colour of the avocado puree was maintained during refrigerated storage for a longer time when HPP was applied at 517 or 689 MPa and the pH of the puree was 4.1 or 3.9. Residual PPO activities of 86 and 63% in guava puree after HPP (400 and 600 MPa for 15 min at 25˚C) was reported by Yen and Lin (1996). During storage at 4˚C, the lightness and greenness of the HPP guava puree decreased continuously and the authors concluded that during storage at 4˚C, guava puree processed at 400 and 600 MPa at 25˚C for 15 min maintained acceptable quality for 20 and 40 days, respectively. Enzymatic reactions may be enhanced or inhibited by HPP, depending on whether the volume change associated with the reaction is positive or negative. Pressure induced changes in the catalytic rate may be due to changes in the enzyme-substrate interaction, change in the reaction mechanism, the effect on a particular rate-limiting step and the overall catalytic rate (Ludikhuyze and Hendrickx, 2001).

High temperatures during processing inactivated PPO, which confirmed the observation of Vamos-Vigyazo (1981) who reported that PPO enzymes are destroyed at 80˚C because they are relatively heat labile. However, the colour change of both HPP and thermally treated orange juice after storage at different temperatures and

times was found to correlate linearly with the corresponding ascorbic acid loss (Polydera *et al*., 2005). Ascorbic acid can act as an inhibitor of PPO activity due to a reduction of the rate of reaction at the lower pH (Lindsay, 1985).

However, the results suggested that degreening of pennywort juice would be related much more with chlorophyll degradation than with browning reactions. Chlorophyll degradation occurs by several mechanisms including the effects of several enzymes including chlorophyllase and pheophorbide *a* oxygenase (Takamiya *et al*., 2000).

HPP improved the viscosity and colour properties of tomato juice in comparison with conventional heat-processed samples (Porretta *et al*., 1995). Like flavour components, pigments that contribute to fruit and vegetable colour are generally considered to be unaffected by HPP (Ahmed and Ramaswamy, 2006). However, Krebbers *et al*., (2002a) have noted significant differences between the colour of HPP products and the colour of fresh green beans and conventional thermally preserved products.

The difference between this study and previous reports may be attributed to differences in operating parameters and the consequent inconsistent inactivation of enzymes that play a major role in colour changes of fruit and vegetable products during processing and storage (Ahmed and Ramaswamy, 2006).

The viscosity of the juices is an important physical characteristic because it affects the manufacturing process. There were slight increases ($p>0.05$) in viscosity of all pennywort juices as shown in **Table 4.7**. The viscosity was increased with increasing of the total plate count. The increase in viscosity of juice stored at low temperatures might be due to the formation of dextran in gummy substances produced by bacteria such as *Leuconostoc mesenteroides* (Lotha *et al*., 1994; Yusof *et al*., 2000). Lotha *et al*. (1994) reported that the viscosity of mandarin juices increased during refrigerated storage and decreased when stored at ambient temperature. There were significant differences ($p\leq0.05$) in the viscosity of sugarcane juice (16%, pH 5.71) which increased from 2.03 to 2.63 cP after 15 days of storage at 5˚C and decreased from 2.03 to 1.30 cP after 3 days of storage at 27˚C (Yusof *et al*., 2000).

Table 4.7 Viscosity of processed pennywort juice

Shelf life (months)	Viscosity (cP) (25-27˚C)					
	HPP+sugar^{NS}	HPP^{NS}	Pasteurized+ sugar^{NS}	Pasteurized^{NS}	Sterilized+ sugar^{NS}	Sterilized^{NS}
0.0	1.81±0.11 ^{a}	1.74±0.03 ^{b}	1.81±0.06 ^{a}	1.73±0.04 ^{b}	1.79±0.08 ^{a}	1.70±0.04 ^{b}
1.0	1.82±0.06 ^{a}	1.74±0.10 ^{b}	1.81±0.08 ^{a}	1.72±0.06 ^{b}	1.79±0.05 ^{a}	1.75±0.03 ^{b}
2.0	1.82±0.13 ^{a}	1.75±0.07 ^{b}	1.82±0.14 ^{a}	1.73±0.11 ^{b}	1.80±0.10 ^{a}	1.75±0.12 ^{b}
3.0	1.84±0.20 ^{a}	1.76±0.06 ^{b}	1.82±0.06 ^{a}	1.73±0.08 ^{b}	1.82±0.03 ^{a}	1.72±0.06 ^{b}
4.0	1.86±0.15 ^{a}	1.76±0.12 ^{b}	1.82±0.10 ^{a}	1.74±0.03 ^{b}	1.85±0.21 ^{a}	1.75±0.14 ^{b}

Means with different small letters within a row of each component are significantly different ($p \leq 0.05$) (Tukey's) and non significantly different (ns)

Means with different capital letters within a column of each component are significantly different ($p \leq 0.05$) (Tukey's) and non significantly different (NS)

Consistency of products refers to their viscosity and the ability of their solid portion to remain in suspension throughout the shelf life of the product. During 6 weeks of storage at 4˚C, there were no significant changes in the viscosity of fresh orange juices but significant reductions were observed after 6 weeks of storage. At 10˚C there was a decrease in viscosity in the juices towards the end of the storage period. This may be connected with the on-set of spoilage or the enzymatic reaction (Esteve *et al*., 2005). The consistency index of thermally treated (80˚C for 30 s) orange juice was not found to significantly change during storage (at 15˚C), leading to an almost constant apparent viscosity (Polydera *et al*., 2003). Hsu (2008) reported that thermally processed (60 and 92˚C for 2 min) tomato juice had a significantly higher viscosity (1.547 and 1.986 cP) than the control. Hsu *et al*. (2008) also reported that the tomato juice treated by thermal processing at a higher temperature (98˚C for 15 min) had a significantly lower viscosity (1.624 cP) than the control (1.875 cP). Fito *et al*. (1983) and Goodman *et al*. (2002) reported that when using a lower temperature processing method, the temperatures are high enough for pectolytic enzyme inactivation, and this leads to a concentrate of greater viscosity.

For orange juice, with HP treatment at high pressure (500 MPa for 5 min at 35˚C), the consistency index increased with storage time. Higher apparent viscosity values were determined for HPP orange juice treated at higher pressure compared to thermally treat immediately after processing and at each storage day. Similar results were obtained for all storage temperatures (0, 5, 10 and 15˚C) (Polydera *et al*., 2003).

Hsu (2008) reported that the viscosity of tomato juice increased linearly with pressure levels elevated from 100 to 500 MPa at various temperatures (4, 25 and 50˚C), but a reduction in viscosity occurred with the pressures at 100 and 200 MPa in comparison with fresh tomato juice. HPP 300 MPa at various temperatures resulted in retention of the viscosity. The change in viscosity may partly be caused by heat-degradation or enzymatic-degradation of pectins (Thakur *et al*., 1996). HPP at 400-500 MPa could increase the viscosity by up to 20%. The results were probably due to enzyme inactivation, compacting effects, or protein–tissue coagulation (Porretta *et al*., 1995; Krebbers *et al.*, 2003). Hsu *et al*. (2008) also reported that the viscosity of the tomato juice treated by HPP 300 MPa for 10 min at 25˚C was 1.856 cP, which was a lower viscosity than the control (1.875 cP). Moreover, the viscosity value of the tomato juice treated by HPP at 500 MPa increased by about 20% (2.255 cP), and application of HPP at 400 MPa also caused an increase of 8% (2.020 cP). The results were probably due to enzyme inactivation, compacting effects, or protein-tissue coagulation (Porretta *et al*., 1995; Krebbers *et al*., 2003). Some researchers have reported contradictory results that the highest loss in consistency of the tomato homogenate after combined pressure–temperature treatment was found at 300 MPa at temperatures (30-70˚C) for 15 min compared with those at pressure levels of 100-500 MPa (Verlent *et al*., 2006). Moreover, high pressure sterilization (700 MPa at 80 or 90˚C) of tomato puree brought about a considerable viscosity reduction maybe due to the relative long preheating time applied to reach the target temperature (80 or 90˚C) for pressurization (Krebbers *et al*., 2003). The study of the effects of HPP on loss of viscosity of tomato juice also showed that pressure levels less than 200 MPa could not inactivate enzymes (Hsu, 2008).

High-pressure treatments at ambient temperature resulted in a more jelly-like, homogenous structure of the tomato puree due to protein-tissue coagulation and compacting compared to thermal treatments (Krebbers *et al*., 2003; Porretta *et al*., 1995; Verlent *et al*., 2006). In addition, HPP could improve the viscosity by enzyme inactivation and enhancement of water binding by pectins (Fernandez Garcia *et al*., 2001b; Krebbers *et al*., 2003). The increase in viscosity due to pressure was attributed to an increase in the linearity of cell walls and volumes of particles due to rupture of the cellular envelope (Hayes *et al*., 1998). The results in the loss of viscosity of

tomato juices during storage showed that HPP at 300 and 400 MPa could not inactivate enzymes which degraded pectins (Thakur *et al*., 1996). Rovere *et al*. (1997) also showed a decrease in syneresis, positively correlated with pressure, temperature and treatment time. The results showed that the HPP at 500 MPa would be the optimal pressure level to produce a tomato juice with improved and stable consistency.

The viscosity of tomato juices processed by thermal treatment (98˚C for 15 min) and HPP (500 MPa for 10 min at 25˚C) remained higher than the control even after storage at 4˚C for 28 days, which was attributed to complete enzyme inactivation. However, the viscosity value of the juice processed by HPP at 300 and 400 MPa significantly decreased with elongation of the treatment time (Hsu *et al*., 2008)**.** The lower temperature (60˚C) reduces the amount of thermal abuse of the product, giving a greater retention of colour and flavour components and reducing production of undesirable compounds. The lower temperature also does not entirely inactivate the enzymes and allows these enzymes to break down some of the pectins reducing the viscosity of the tomato juice (Luh and Daouf, 1971). A heat-labile enzyme in tomato at ambient pressure, was dramatically stabilized against thermal denaturation at pressures up to 500-600 MPa, and was completely inactivated at 800 MPa for 70˚C more than 20 min (Crelier *et al*., 2001).

4.4.2 Changes in physico-chemical properties

Changes of pH value

Processed pennywort juice exhibited small non-significant changes in pH value during 4 months of storage as shown in **Table 4.8** ($p>0.05$). Jannok (2007) reported that the pH of HPP (600 MPa for 40 min at 50˚C) pennywort juice (1:1, pennywort: water) was 6.00±0.10, which was not significantly different from fresh juice. The pH value was decreased with increasing of the total plate count. The small pH changes can be explained by the formation of small amounts of free acids (Kaanane *et al*., 1988) which may be due to the production of acids during microbial growth (Bhupinder *et al*., 1991). The HPP juice with and without added sugar contained a total plate count of >30 cfu/ml after 9 and 7 weeks of storage, respectively. Yusof *et al*. (2000) also reported a similar effect, when they found a decrease in the pH value of sugarcane during storage perhaps due to the breakdown of

sucrose to simple sugars and acids. Bhupinder *et al.* (1991) also found that the acidity of juice increased during storage, and the increase in acidity caused a concomitant decrease in pH value. High acid and low pH could be due to acetic acid and lactic acid production. However, Kormin (2005) reported that after heat processing of pennywort juice containing 0.12% citric acid added, the pH of juice pasteurized at 80˚C, 5 min (3.79) was higher than that of juice pasteurized at 65˚C, 15 min (3.72) and also higher than that of canned juice (3.72) (heated at 80˚C, 5 min and boiled at 100˚C, 10 min). Esteve *et al*. (2005) reported that during the 6 weeks of orange juice storage at 4 and 10˚C, the variations in pH observed in the juices did not become statistically significant.

Table 4.8 pH values of processed pennywort juice

Shelf life (months)	pH values					
	HPP + sugar ^{NS}	HPP ^{NS}	Pasteurized + sugar ^{NS}	Pasteurized ^{NS}	Sterilized + sugar ^{NS}	Sterilized ^{NS}
0.0 ^{ns}	5.56±0.17	5.61±0.24	5.54±0.03	5.57±0.20	5.53±0.08	5.56±0.14
0.5 ^{ns}	5.55±0.09	5.60±0.30	5.55±0.14	5.58±0.21	5.53±0.09	5.56±0.20
1.0 ^{ns}	5.56±0.09	5.57±0.21	5.53±0.10	5.58±0.44	5.53±0.12	5.54±0.12
1.5 ^{ns}	5.53±0.17	5.60±0.30	5.55±0.04	5.50±0.11	5.49±0.10	5.54±0.07
2.0 ^{ns}	5.53±0.15	5.59±0.36	5.55±0.16	5.56±0.14	5.53±0.12	5.52±0.11
2.5 ^{ns}	5.55±0.30	5.59±0.35	5.52±0.14	5.50±0.27	5.47±0.19	5.52±0.10
3.0 ^{ns}	5.54±0.19	5.56±0.15	5.51±0.13	5.55±0.06	5.44±0.24	5.54±0.20
3.5 ^{ns}	5.54±0.35	5.55±0.14	5.53±0.14	5.56±0.22	5.51±0.11	5.53±0.23
4.0 ^{ns}	5.53±0.11	5.56±0.11	5.54±0.06	5.53±0.07	5.51±0.08	5.52±0.11

Means with different small letters within a row of each component are significantly different ($p \leq 0.05$) (Tukey's) and non significantly different (ns)

Means with different capital letters within a column of each component are significantly different ($p \leq 0.05$) (Tukey's) and non significantly different (NS)

Changes of total soluble solids

Processed pennywort juice exhibited little change in TSS during 4 months of storage. The TSS of HPP was higher than that of pasteurized and sterilized juice ($p>0.05$) as shown in **Table 4.9**. With 11% sugar added after pasteurization at 80˚C, 5 min (TSS 11.8%), the TSS was higher than that of juice pasteurized at 65˚C, 15 min and that of canned juice (heated at 80˚C, 5 min and boiled at 100˚C, 10 min) (TSS 11.2%) (Kormin, 2005). The TSS content of some treatments of pennywort juice was slightly increased after 0.5 months of storage ($p>0.05$) but this value decreased

slightly by the end of the storage period ($p>0.05$). Yusof *et al*. (2000) also found similar results in the storage of a sugarcane sample, and the TSS increase was perhaps due to the breakdown of oligomers, such as sucrose, into simple sugars and acids.

Table 4.9 Total soluble solids content of processed pennywort juice

Shelf life (months)	Total soluble solids (%)					
	HPP + sugar ^{NS}	HPP ^{NS}	Pasteurized + sugar ^{NS}	Pasteurized ^{NS}	Sterilized + sugar ^{NS}	Sterilized ^{NS}
0.0	12.13±0.12^{a}	2.47±0.42^{b}	11.87±0.23^{a}	2.50±0.52^{b}	11.77±0.38^{a}	2.47±0.12^{b}
0.5	12.13±0.12^{a}	2.50±0.44^{b}	11.93±0.46^{a}	2.50±0.44^{b}	11.80±0.20^{a}	2.33±0.50^{b}
1.0	12.13±0.12^{a}	2.47±0.42^{b}	11.93±0.50^{a}	2.50±0.52^{b}	11.53±0.23^{a}	2.27±0.42^{b}
1.5	12.13±0.31^{a}	2.50±0.44^{b}	11.93±0.12^{a}	2.50±0.52^{b}	11.80±0.35^{a}	2.27±0.23^{b}
2.0	12.07±0.12^{a}	2.40±0.35^{b}	11.93±0.31^{a}	2.50±0.44^{b}	11.80±0.20^{a}	2.33±0.50^{b}
2.5	12.07±0.12^{a}	2.47±0.49^{b}	11.93±0.31^{a}	2.50±0.61^{b}	11.70±0.44^{a}	2.27±0.42^{b}
3.0	12.13±0.12^{a}	2.43±0.47^{b}	11.93±0.31^{a}	2.47±0.42^{b}	11.80±0.35^{a}	2.20±0.53^{b}
3.5	12.07±0.12^{a}	2.43±0.55^{b}	11.67±0.46^{a}	2.47±0.51^{b}	11.73±0.23^{a}	2.33±0.31^{b}
4.0	12.07±0.12^{a}	2.43±0.55^{b}	11.93±0.31^{a}	2.47±0.58^{b}	11.73±0.42^{a}	2.37±0.49^{b}

Means with different small letters within a row of each component are significantly different ($p\leq0.05$) (Tukey's) and non significantly different (ns)

Means with different capital letters within a column of each component are significantly different ($p\leq0.05$) (Tukey's) and non significantly different (NS)

There were significant cultivar differences in TSS, but neither packaging for HPP nor the HPP process itself had any impact on TSS levels. Cultivar was not an important influence on the response of melons to HPP in respect to important organoleptic parameters (Wolbang *et al*., 2008). In addition, Yusof *et al*. (2000) reported that the decrease in sucrose content of sugarcane, stored at 5 and 27˚C was due to the conversion of sucrose to reducing sugars as shown by the increase in fructose and glucose contents after storage. The reducing sugars increased slightly towards the end of storage, as reported by Ewaidah (1992). The drop in sucrose that occurred in cane juice samples stored at 27˚C was higher than that of sample stored at 5˚C, perhaps caused by microbes that utilized the sugars and juice spoilage (Yusof *et al*., 2000).

4.4.3 Changes of phytochemical composition

Heat treatment caused degradation of some chemical components. After processing, madecassoside and asiaticoside content was slightly decreased from that of the fresh sample and did not change ($p>0.5$) during storage in all samples as shown in **Tables 4.10-4.11**. Madecassoside and asiaticoside were relatively stable during storage with no significant changes during 4 months storage at 4 and 40˚C.

Madecassoside contents after HPP, pasteurization and sterilization were 3.70-3.77, 3.20-3.32 and 2.87-3.07 mg/100 ml, respectively. Kormin (2005) reported that the concentrations of this component in a commercial sample: without heat treatment; with pasteurization 90˚C for 1 min; with pasteurization, 80˚C for 5 min and with sterilization, 100˚C for 10 min were 2.93, 2.54, 2.43 and 2.74 mg/100 ml, respectively.

Table 4.10 Madecassoside contents of processed pennywort juice

Shelf life (months)	Madecassoside contents (mg/100 ml)					
	HPP + sugar^{NS}	HPP^{NS}	Pasteurized + sugar^{NS}	Pasteurized^{NS}	Sterilized + sugar^{NS}	Sterilized^{NS}
0.0^{ns}	3.77±1.19	3.70±1.08	3.32±1.29	3.20±0.33	3.07±1.33	2.87±1.02
0.5^{ns}	3.71±0.27	3.71±2.02	3.31±1.50	3.23±1.77	3.04±0.91	2.85±1.84
1.0^{ns}	3.62±1.46	3.58±0.89	3.28±1.56	3.14±1.17	3.02±1.34	2.78±1.29
1.5^{ns}	3.74±2.11	3.60±1.24	3.27±1.08	3.18±1.25	2.91±1.35	2.76±0.59
2.0^{ns}	3.70±0.73	3.54±0.71	3.30±0.48	3.16±0.83	2.97±0.41	2.74±0.69
2.5^{ns}	3.65±0.55	3.57±1.72	3.22±1.29	3.03±0.65	2.97±1.15	2.78±1.32
3.0^{ns}	3.55±1.50	3.54±0.83	3.31±1.46	3.13±0.53	2.94±0.58	2.75±1.67
3.5^{ns}	3.56±1.09	3.51±1.19	3.30±1.55	3.04±0.62	2.93±0.34	2.81±0.11
4.0^{ns}	3.55±1.66	3.52±1.00	3.26±0.88	3.14±1.38	2.92±0.81	2.83±1.65

Means with different small letters within a row of each component are significantly different ($p\leq0.05$) (Tukey's) and non significantly different (ns)

Means with different capital letters within a column of each component are significantly different ($p\leq0.05$) (Tukey's) and non significantly different (NS)

Asiaticoside contents in this juice after HPP, pasteurization and sterilization were 4.28-4.30, 3.55-3.57 and 3.16-3.20 mg/100 ml, respectively. The samples without heat treatment, after pasteurization 90˚C for 1 min, after pasteurization 80˚C for 5 min and after sterilization 100˚C for 10 min had asiaticoside contents of 2.60, 3.10, 3.61 and 3.03 mg/100 ml, respectively (Kormin, 2005). This is consistent with the findings of Kim *et al*. (2001) who reported that asiaticoside was stable in micellar

formulations with no significant changes during storage for 60 days at room temperature. Similarly, a stock solution of asiaticoside was found to be stable under refrigeration conditions, with 99.2% remaining after storage for 90 days (Qi *et al*., 2000). However, saponin, a triterpene glycoside in *Panax notoginseng* was significantly degraded upon prolonged steaming (Lau *et al*., 2003). Pokorny (2001) concluded that food processing in some cases increased resistance to oxidation of glycosides and transformation to active compounds (such as aglycones) by reducing oxygen access and thereby reducing formation of new compounds.

Table 4.11 Asiaticoside contents of processed pennywort juice

Shelf life (months)	Asiaticoside contents (mg/100 ml)					
	HPP + sugar ^{NS}	HPP ^{NS}	Pasteurized + sugar ^{NS}	Pasteurized ^{NS}	Sterilized + sugar ^{NS}	Sterilized ^{NS}
0.0 ^{ns}	4.30±1.95	4.28±0.66	3.55±0.75	3.57±1.36	3.20±1.00	3.16±1.45
0.5 ^{ns}	4.19±1.38	4.03±0.97	3.29±1.94	3.36±0.67	3.17±1.67	3.14±1.19
1.0 ^{ns}	4.03±1.86	3.82±1.60	3.28±1.94	3.16±0.80	3.18±1.17	3.08±0.64
1.5 ^{ns}	4.04±1.78	3.85±2.31	3.19±1.91	3.36±0.92	3.09±0.82	3.00±1.47
2.0 ^{ns}	3.91±0.96	3.80±1.23	3.33±1.05	3.27±1.02	3.06±1.26	3.10±1.15
2.5 ^{ns}	4.07±1.86	3.84±1.28	3.35±1.92	3.17±1.01	3.11±1.27	3.01±0.80
3.0 ^{ns}	4.18±2.04	3.83±0.74	3.25±1.97	3.16±1.26	3.11±1.55	3.02±1.38
3.5 ^{ns}	4.08±2.02	3.73±0.90	3.37±0.24	3.41±1.05	2.95±0.66	2.92±1.52
4.0 ^{ns}	3.99±1.56	3.84±1.28	3.35±2.17	3.24±0.82	3.03±0.98	2.94±1.16

Means with different small letters within a row of each component are significantly different ($p \leq 0.05$) (Tukey's) and non significantly different (ns)

Means with different capital letters within a column of each component are significantly different ($p \leq 0.05$) (Tukey's) and non significantly different (NS)

Ascorbic acid, which is labile, is easily destroyed during processing and storage (Davey *et al*., 2000). During storage of the juice ascorbic acid is degraded, following 2 parallel pathways, aerobically and anaerobically, with rates depending on storage conditions, packaging and the processing method (Gregory, 1996; Kenawi *et al*., 1994; Kennedy *et al*., 1992; Sadler *et al*., 1997; Tawfik and Huyghebaert, 1998). In this study, the ascorbic acid content in HPP treated pennywort juice was higher than that of pasteurized and sterilized juice ($p \leq 0.05$) as shown in **Table 4.12**.

Table 4.12 Ascorbic acid contents of processed pennywort juice

Shelf life (months)	Ascorbic acid contents (mg/100 ml)					
	HPP+sugar	HPP	Pasteurized+ sugar	Pasteurized	Sterilized+ sugar	Sterilized
0.0	4.16±0.27^{aA}	4.03±0.63^{aA}	2.00±0.09^{bA}	1.70±0.09^{bA}	0.85±0.04^{cA}	0.84±0.02^{cA}
0.5	3.62±0.53^{aB}	3.41±0.03^{aB}	1.36±0.12^{bB}	1.29±0.08^{bcB}	0.84±0.01^{cA}	0.80±0.01^{cA}
1.0	2.60±0.05^{aC}	2.47±0.04^{bC}	1.19±0.02^{cBC}	1.16±0.01^{cBC}	0.81±0.03^{dAB}	0.78±0.01^{dA}
1.5	2.53±0.07^{aCD}	2.33±0.03^{bC}	1.12±0.02^{cC}	1.07±0.02^{cC}	0.79±0.01^{dAB}	0.77±0.04^{dA}
2.0	2.43±0.02^{aCD}	2.13±0.09^{bCD}	1.07±0.02^{cC}	1.03±0.02^{cCD}	0.78±0.03^{dAB}	0.76±0.02^{dAB}
2.5	2.30±0.07^{aCD}	1.92±0.18^{bCD}	1.00±0.01^{cCD}	0.98±0.01^{cCD}	0.75±0.04^{dAB}	0.74±0.01^{dAB}
3.0	2.07±0.05^{aD}	1.68±0.04^{bD}	0.96±0.03^{cCD}	0.94±0.02^{cD}	0.73±0.05^{dAB}	0.71±0.04^{dAB}
3.5	1.99±0.05^{aD}	1.63±0.04^{bD}	0.91±0.01^{cD}	0.90±0.02^{cD}	0.72±0.05^{dB}	0.70±0.03^{dB}
4.0	1.79±0.19^{aD}	1.50±0.07^{bD}	0.90±0.01^{cD}	0.87±0.02^{cdD}	0.70±0.04^{dB}	0.69±0.05^{dB}

Means with different small letters within a row of each component are significantly different ($p \leq 0.05$) (Tukey's) and non significantly different (ns)

Means with different capital letters within a column of each component are significantly different ($p \leq 0.05$) (Tukey's) and non significantly different (NS)

Ascorbic acid was lost during processing, and losses continued during the storage of processed juices for 4 months at 4˚C. A literature report describes that there were some losses just after HPP for tomato juices compared to control, at the end of the shorter period of 28 days at 4˚C there were no significant losses of ascorbic acid and total vitamin C (Hsu *et al*., 2008).

Losses of ascorbic acid during storage at 4˚C of high pressure processed pennywort juice were greater than those of pasteurized juices and these were greater than in sterilized juices stored at 40˚C. This suggests that microbial activity was the cause of these losses with HPP being unable to inactivate all microorganisms. A small reduction in pH of all samples was detected during 4 months storage with falls of 5.61-5.53, 5.57-5.53 and 5.56-5.51 for the high pressure processed, pasteurized, and sterilized juices, respectively. However, Gil-Izquierdo *et al*. (2002) who investigated effects of processing on *L*-ascorbic acid in orange juice reported that the concentration increased from 126.8 to 135.3 mg/L after pasteurization at 75˚C but decreased from 150.1 to 143.7 mg/L after pasteurization at 95˚C for 30 s.

Ascorbate content of untreated and pressurized (400 MPa for 30 min, 20˚C) strawberry *coulis* decreased with time. Samples were stored at 4˚C and without light. The initial concentration of ascorbic acid was 33.13 mg/100 g, and this was reduced

to 29.38 mg/100 g (88.68%) after HPP. After 28 days of storage, it was 26 mg/100 g (78.48%) for untreated strawberry *coulis* and 22.8 mg/100 g (68.82%) for pressurized *coulis*, which were acceptable during the 1 month storage period. Oxidation is increased in the presence of light, air and heat, so it is important to preserve the samples in darkness at 4˚C (Sancho *et al*., 1999). Rovere *et al*. (1996) found that the content of ascorbic acid of strawberry nectar remained unchanged during HPP but decreased during storage, reaching 75% of the original content after 60 days at 3˚C. During storage for 28 days at 4˚C, analysis of tomato juices treated by HPP at 300, 400 and 500 MPa for 10 min at 25˚C showed that ascorbic acid and total vitamin C contents almost remained constant. Furthermore, the HPP at 500 MPa of tomato juice showed significantly greater ascorbic acid content during storage from 7-21 days and in total vitamin C content during storage at 7 days than the juice treated by HPP at 300 MPa. However, at the end of the storage for 28 days, there were no significant differences of ascorbic acid and total vitamin C contents between all the HPP tomato juices (Hsu *et al*., 2008). Ascorbic acid loss was slower in the case of HPP treated (500 MPa for 5 min, 35˚C) orange juice than in the juice HPP treated at a lower pressure. The retention of ascorbic acid after storage of high pressurised orange juice for 1 month at 5˚C was 70 and 79% in bottles and pouch, respectively. In contrast, thermal treatment (80˚C for 30 s) led to retentions of 57 and 77% ascorbic acid in plastic bottles and flexible pouches, respectively (Polydera *et al*., 2003). The ascorbic acid content of untreated orange juice did not vary significantly during 18 days of storage, but declined between 18-22 days. The profile of diminution of the content in ascorbic acid during storage was similar in samples processed by HPP to that of the untreated sample (Lamballerie-Anton *et al*., 1997). The degradation of ascorbic acid and total vitamin C after thermal processing (98˚C for 15 min) of tomato juice was about 18% during 14 days of storage. This was, probably due to the presence of residual oxygen in the juice (Lin and Chen, 2005), and the content did not significantly decline during further storage (Hsu *et al*., 2008).

Nienaber and Shellhammer (2001) reported that more than 80% of ascorbic acid was retained after 3 months at 4˚C and 2 months at 15˚C storage of orange juice pasteurized at 800 MPa for 1 min at 25˚C. At the beginning of the storage period, a drop in ascorbic acid concentration occurred at all temperatures (4, 15, 26 and 37˚C).

This may be contributed by the fact that the juice was not deaerated and therefore, a portion of the ascorbic acid reacted with dissolved oxygen. During storage, ascorbic acid concentration gradually decreased at all temperatures. This could be a consequence of oxygen diffusion through the PET bottle.

The content of ascorbic acid depends on the holding time during HPP, but it is independent of the pressure level. The concentration of ascorbic acid in HPP apple-broccoli juice was lower than that in juice preserved by freezing. This effect can be caused by a decay of ascorbic acid during juice preparation and HPP treatment. The content of ascorbic acid decreased during storage. Higher rates of decay in HPP juice can be caused by the storage of this juice chilled in PET bottles that are a less effective barrier for oxygen for long-term storage than a glass bottle. It is better to use higher pressure and shorter holding times for preservation of ascorbic acid than lower pressures and longer times (Houlka *et al*., 2006).

The shelf life of orange juice (based on ascorbiv acid loss) was increased by HPP (500 MPa for 5 min at 35˚C) treatment compared to pasteurized (80˚C for 30 s) juices when stored in polypropylene (PP) bottles, with the shelf life increased from 18 to 20 days at 15˚C, 34 to 50 days at 5˚C and 49 to 81 days at 0˚C. When laminated flexible pouches were used, the shelf life increased from 50 to 62 days at 15˚C, 62 to 90 days at 5˚C and 69 to 109 days at 0˚C. However, the shelf life based on ascorbic acid degradation was shorter compared to the shelf life based on sensory evaluation. No microbial growth was observed during storage of either high pressure or heat treated orange juice until the end of its shelf life (Polydera *et al*., 2003).

The level of vitamins in HPP treated juice was reported to be fully maintained in one study (Donsi *et al*., 1996). The conclusion was drawn that, it is possible to stabilize fruit juices or jams over a long time after HPP (300 MPa for 30 min at 20˚C) without modification of colour, flavour and vitamins (Cheftel, 1991).

Choi *et al*. (2002) reported that during storage of orange juice at 4.5˚C, ascorbic acid decreased gradually with storage time; more than 50% was lost within 3 weeks of storage and completely degraded after 5 weeks of storage. The rate of ascorbic acid decomposition in the control juice was greater than 1.5-2% per day, which is previously reported as the decomposition rate of ascorbic acid in single-strength orange juice at refrigerated conditions (Shaw and Moshonas, 1991; Squires

and Hanna, 1979). Esteve *et al*. (2005) reported that the ascorbic acid content of the mild heat treated (77˚C for 20 s) orange juices decreased faster during storage at 10˚C than at 4˚C. Storage stability of orange juice for a period longer than 42 days at 4˚C and 35-42 days at 10˚C would be recommended.

Significant losses of ascorbic acid in processed foods are associated with chemical degradation, usually associated with non enzymatic browning reactions, as well as aerobic oxidation which produces *L*-dehydroascorbic acid (Davidek *et al*., 1990). This molecule undergoes hydrolysis by alkaline nucleophilic reagents, finally yielding a wide variety of chemical products (reductones in alkaline solutions and furan derivatives in neutral and acid solutions). The HPP suppresses browning reactions (Tamakoa *et al*., 1991). However, Sancho *et al*. (1999) proposed that this is caused by the previously cited aerobic oxidation.

Table 4.13 *β*-Carotene contents of processed pennywort juice

Shelf life (months)	*β*-carotene contents (mg/100 ml)					
	HPP + sugar ^{NS}	HPP ^{NS}	Pasteurized + sugar ^{NS}	Pasteurized ^{NS}	Sterilized + sugar	Sterilized
0.0 ^{ns}	2.52±0.70	2.51±0.79	2.48±0.59	2.39±0.64	2.32±0.03 ^{A}	2.26±0.26 ^{A}
0.5 ^{ns}	2.43±0.78	2.42±0.74	2.46±0.78	2.27±0.56	2.03±0.11 ^{AB}	2.11±0.08 ^{AB}
1.0 ^{ns}	2.32±0.25	2.35±0.21	2.43±0.68	2.25±0.69	2.03±0.13 ^{AB}	1.87±0.04 ^{AB}
1.5 ^{ns}	2.23±0.29	2.25±0.13	2.43±0.85	2.07±0.14	1.87±0.10 ^{AB}	1.80±0.05 ^{AB}
2.0 ^{ns}	2.23±0.32	2.16±0.28	2.23±0.42	2.00±0.18	1.75±0.03 ^{B}	1.80±0.09 ^{AB}
2.5	2.21±0.31 ^{a}	2.17±0.37 ^{a}	2.03±0.41 ^{ab}	1.98±0.15 ^{ab}	1.79±0.07 ^{abB}	1.52±0.07 ^{bB}
3.0 ^{ns}	2.10±0.21	1.91±0.60	1.97±0.31	1.98±0.65	1.77±0.04 ^{B}	1.68±0.24 ^{AB}
3.5	2.09±0.18 ^{a}	1.90±0.33 ^{a}	2.01±0.44 ^{a}	2.03±0.51 ^{a}	1.84±0.20 ^{abB}	1.58±0.09 ^{bB}
4.0	2.04±0.11 ^{a}	1.86±0.62 ^{ab}	1.96±0.19 ^{a}	1.89±0.21 ^{a}	1.55±0.06 ^{bB}	1.50±0.11 ^{bB}

Means with different small letters within a row of each component are significantly different ($p \leq 0.05$) (Tukey's) and non significantly different (ns)

Means with different capital letters within a column of each component are significantly different ($p \leq 0.05$) (Tukey's) and non significantly different (NS)

β-Carotene content of juice after HPP, pasteurization and sterilization was found to be 2.51-2.52, 2.39-2.48 and 2.26-2.32 mg/100 ml, respectively. There was a trend for losses of it to be greater in sterilized juice during 2.5 months of storage. It was found that *β*-carotene in sterilized juice significantly decreased ($p \leq 0.05$) during storage but it did not change in pasteurized and high pressure processed juices (**Table 4.13**). It has been reported that the extract of this plant contained a relative high

amount of total carotenes (Chanwitheesuk *et al*., 2005). *β*-Carotene in high pressure processed juice was higher than in the fresh juice but the difference was not significant ($p>0.05$). Spanos *et al*. (1990) reported that a high temperature during processing of apple juice produced up to a 5-fold increase in the concentration of phloretin glucosides compared to that obtained in pressed juice without temperature elevation. In addition, the anthocyanin concentration of pasteurized (80˚C for 1 min) blood orange juice was higher than that in non-thermally treated juice (Scalzo *et al*. 2004). On the other hand, the loss of anthocyanins was increased by 2-fold after 2 months storage when the storage temperature was increased from 10 to 23˚C (Cabrita *et al*. 2000). Mohanom *et al*. (1999) also reported that the carotenoid content of 8 medicinal plants was reduced by 27 and 20% after oven drying at 50˚C for 9 hr and 70˚C for 5 hr.

The total carotenoid content in the juice fluctuated but appeared to gradually decrease as a function of storage time. The initial concentration of total carotenoids in the juice samples was 0.286 mg/100 ml and it decreased to 0.267 mg/100 ml (a 6.6% decrease) after 7 weeks. Losses of carotenoids in blood orange juice are limited compared to anthocyanin pigments under the same condition, which is probably due to the natural stability of carotenoids and to the stabilizing effect of ascorbic acid on carotenoid (Choi *et al*., 2002). Oxidation is the major cause of carotenoids loss, and it depends on the carotenoids involved. Oxidation is stimulated by light, heat, metals, enzymes, and peroxides, and it is inhibited by antioxidants including tocopherols and ascorbic acid (Sanchez-Moreno *et al*., 2003). Hsu *et al*. (2008) reported that during storage for 14 days, total carotenoids and lycopene of the thermally processed (98˚C for 15 min) tomato juice rapidly degraded by 16 and 12% compared with fresh juice, but there was no further degradation during storage from 14-28 days. On the other hand, the carotenoid content of HPP (300, 400 and 500 MPa for 10 min at 25˚C) treated tomato juice was slightly reduced compared with fresh juice, but it did not significantly decrease during storage for 28 days.

Oxidation could be prevented by HPP due to the inactivation of peroxidase and the maintenance of ascorbic acid content. The enzyme, peroxidase in orange juice was inactivated under HPP 300 MPa at 20˚C, and HPP may lead to a release of other antioxidants found in tomatoes (some carotenoids, ascorbic acid and phenolic

components) (Cano *et al*., 1997; Qiu *et al*., 2006). Lin and Chen (2005) suggested that isomerization and oxidative degradation are major pathways for loss of lycopene and other carotenoids during storage of thermally processed tomato juice.

The total carotenoid and lycopene contents of thermally processed tomato juice were reduced during storage at low temperature and without light probably due to the presence of residual oxygen in the juice (Lin and Chen, 2005).

Storage of concentrates of apple juice for 9 months resulted in 50-60% loss of quercetin and phloretin derivatives (Spanos *et al*., 1990). However, the heat treatment did not cause a significant change to the content and antioxidant activity of lycopene and *β*-carotene, which were very heat stable even after prolonged heat treatments (Elkin, 1979; Miki and Akatsu, 1971). The carotene content of apple-broccoli juice was very low (0.42-0.46 mg/100 g) and there were no changes during storage (34 days) (Houlka *et al*., 2006).

4.4.4 Changes of antioxidant properties

An antioxidant is an oxidation inhibitor (Pokorny *et al*., 2001), and it can be defined as a compound that inhibits or delays the oxidation of other molecules through inhibiting the initiation or propagation of oxidizing chain reactions. It is well established that lipid peroxidation is set in motion as a consequence of free radical formation in cells and tissues. Antioxidants reduce oxidation of lipids, natural pigments and other active chemicals (Anese and Nicoli, 2001). They are used to prolong the shelf life as well as maintain the nutritional quality of lipid-containing processed foods.

The antioxidant capacities of pennywort grown in farms in Chiang Mai, Thailand measured with the Folin-Ciocalteu and FRAP assays varied depending on the processing conditions and the presence of added sugar as shown in **Tables 4.14-4.15**. The extracts that contained a high concentration of polyphenols also exhibited a high antioxidant capacity. The Folin-Ciocalteu phenol reagent is used to obtain a crude estimate of the concentration of phenolic compounds present. The ability of the extracts to reduce ferric ions was determined using the FRAP assay. There was a wide range of TPC and FRAP values concentrations in the processed pennywort juice. The TPC value varied from 55.53-404.23 mg/100 ml and the FRAP value varied from

126.00-734.87 μM $FeSO_4$/L. The antioxidant properties were strongest in the order of high pressure processed juice > pasteurized juice >sterilized juice.

Table 4.14 Total phenolics content of processed pennywort juice by Folin Ciocalteu method without correction for ascorbic acid content

Shelf life (months)	Total phenolics content(mg/100 ml)					
	HPP + sugar	HPP	Pasteurized + sugar	Pasteurized	Sterilized + sugar	Sterilized
0.0	404.23±20.43^{aA}	385.70±16.25^{aA}	265.27±7.06^{bA}	235.75±20.16^{bA}	170.63±31.21^{cA}	158.13±8.60^{cA}
0.5	350.07±10.30^{aB}	343.47±7.77^{aB}	201.39±7.66^{bB}	207.24±3.35^{bB}	162.98±20.33^{cAB}	147.63±3.38^{cA}
1.0	314.73±9.41^{aC}	309.10±6.75^{aC}	187.68±2.81^{bBC}	187.09±10.45^{bB}	134.53±13.57^{cB}	132.41±1.82^{cAB}
1.5	285.10±14.75^{aCD}	264.10±5.33^{aD}	176.17±12.60^{bC}	142.20±4.01^{bcC}	126.72±5.60^{cB}	123.00±4.56^{cB}
2.0	263.53±7.24^{aD}	236.73±16.58^{aE}	154.03±5.99^{bD}	120.47±19.15^{cC}	113.53±1.23^{cBC}	100.22±5.65^{cC}
2.5	243.42±9.21^{aDE}	209.63±7.99^{bF}	146.60±2.01^{cD}	94.47±1.89^{dD}	102.77±3.65^{dBC}	96.20±1.90^{dC}
3.0	215.63±5.97^{aE}	183.07±5.47^{bFG}	135.53±3.76^{cDE}	93.17±1.86^{deD}	96.53±3.46^{dC}	84.20±2.86^{eCD}
3.5	195.47±9.69^{aEF}	167.37±2.60^{bG}	120.57±7.10^{cE}	85.93±1.55^{dD}	78.80±5.54^{dCD}	73.76±13.12^{dD}
4.0	170.07±13.00^{aF}	138.73±4.80^{bH}	98.80±5.46^{cF}	79.03±3.26^{dD}	59.60±5.06^{eD}	55.53±5.39^{eE}

Means with different small letters within a row of each component are significantly different ($p \leq 0.05$) (Tukey's) and non significantly different (ns)

Means with different capital letters within a column of each component are significantly different ($p \leq 0.05$) (Tukey's) and non significantly different (NS)

Kormin (2005) also reported that the phenolic compounds in pennywort drink declined with increasing heat processing temperature. The juices with 10% sucrose addition maintained antioxidant properties better than the juice without added sugar. After processing, the TPC of the juice treated by HPP, pasteurization and sterilization was 385.7-404.23, 235.75-265.27 and 158.13-170.63 mg/100 ml, respectively. Similar trends were reported by Kormin (2005) for a commercial sample; where the TPC value without heat treatment and with pasteurization 90˚C, 1 min; pasteurization 80˚C, 5 min; and sterilization 100˚C, 10 min were 1140.24, 797.53, 805.54 and 730.27 mg/100 ml, respectively. The trend in the FRAP values was similar to that of the TPC. The FRAP values of this juice after HPP, pasteurization and sterilization were 462.58-734.87, 366.62-381.00 and 333.33-345.00 μM $FeSO_4$/L, respectively. Kormin (2005) reported that the FRAP values of a sample of a commercial juice without heat treatment and pasteurized (90˚C, 1 min), pasteurized (80˚C, 5 min) and sterilized (100˚C, 10 min) were 620, 370, 560 and 370 μM $FeSO_4$/L, respectively. Zainol *et al*. (2003) reported that the antioxidant capacity of pennywort extracts

correlated with the total phenolics content. This suggests that the phenolic compounds are the major contributors to the antioxidant capacity of pennywort. However, the content of phenolic compounds and the FRAP values were reduced when the juice was stored for a long time. The antioxidant capacity fell during the storage period from 0 to 4 months. High pressure processed and pasteurized juices had changes in TPC within 0.5 month and sterilized juices had changes within 1-1.5 months. The FRAP values of HPP juices had changes within 1-1.5 months, whereas the values for pasteurized juices and sterilized juices had changes within 1.5 months.

Several papers have reported that most food processing operations significantly reduced the phenolic compounds concentration. After blanching of peas and spinach (97˚C for 85-90 s) and boiled peas at 100˚C for 8 min, the antioxidant capacity was reduced by 50, 20 and 34%, respectively but it remained constant and stable during frozen storage after blanching (Hunter and Flatcher, 2002). In addition, onions and asparagus boiled for 60 min suffered flavonol losses of 20.6 and 43.9%, respectively (Makris and Rossiter, 2001).

Heat treatment of pennywort juice led to considerable loss of ascorbic acid, TPC and antioxidant capacity. HPP caused losses of 16% ascorbic acid, 61% TPC and a 49% reduction in FRAP value. This compared with pasteurization where the losses were 64, 76 and 64%, respectively, and sterilization where the losses were 82, 84 and 64%, respectively. Choi *et al*. (2006) reported that the phenolic contents and antioxidant capacity of shitakae mushroom extracts increased as the heating temperature and time increased. For example, the free phenolics content in the extract heated at 121˚C for 30 min was increased by 1.9-fold compared to that in the extract from the raw sample. In addition, Jeong *et al*. (2004) also reported that the antioxidant capacity of citrus peel extracts increased as the heating temperature increased. In contrast, the reducing power of the extracts of purple yam decreased with increasing heating time. Moreover, the anthocyanin and total phenolics content of the extracts of purple yam decreased with increasing extraction temperature and time (Yin and Wang, 2003). The total phenolics in roselle remained at 85% of the initial value after drying at 75˚C and storage for 15 weeks at 40˚C (Tsai *et al*., 2002).

Kormin (2005) also reported that the phenolic content of pennywort juice was reduced to an extent that increased with increasing heat processing temperature.

Kormin (2005) reported that losses of phenolics from a commercial sample were 29, 30 and 36% following pasteurization at 80˚C for 5 min; pasteurization at 90˚C for 1 min, and sterilization at 100˚C for 10 min respectively. Kormin (2005) reported that loss of antioxidant capacity due to these processing conditions was 10-40%. Zainol *et al*. (2003) reported that the antioxidant capacity of pennywort extracts correlated with the total phenolic content. This is consistent with the phenolic components being major contributors to the antioxidant capacity of pennywort.

Table 4.15 Ferric reducing antioxidant potential (FRAP) values of processed pennywort juice

Shelf life (months)	FRAP values (μM $FeSO_4$/L)					
	HPP + sugar	HPP	Pasteurized + sugar	Pasteurized	Sterilized + sugar	Sterilized
0.0	734.87±80.67^{aA}	462.58±64.79^{bA}	381.0±23.64^{bcA}	366.62±23.57^{bcA}	345.0±6.00^{cA}	333.33±32.01^{cA}
0.5	688.34±16.78^{aAB}	405.0±39.59^{bAB}	314.33±51.33^{cAB}	305.86±6.79^{cAB}	288.33±49.52^{cAB}	290.0±26.46^{cAB}
1.0	652.0±77.27^{aAB}	353.24±35.67^{bB}	313.0±72.96^{bAB}	305.67±50.82^{bAB}	252.67±12.74^{bAB}	272.67±41.74^{bAB}
1.5	554.0±21.93^{aB}	340.0±19.16^{bB}	293.0±7.94^{bcB}	284.75±9.21^{bcB}	241.0±49.51^{cB}	212.0±83.29^{cB}
2.0	506.0±34.22^{aBC}	296.67±11.06^{bBC}	241.06±13.58^{bBC}	237.83±63.22^{bBC}	222.67±36.18^{bBC}	212.67±5.69^{bB}
2.5	455.67±10.60^{aBC}	270.67±12.66^{bBC}	239.0±31.61^{bBC}	203.0±14.18^{bC}	206.33±48.85^{bBC}	198.67±59.21^{bB}
3.0	422.33±52.20^{aC}	242.0±48.59^{bC}	221.33±20.65^{bBC}	200.0±22.65^{bC}	180.0±36.17^{bBC}	175.33±62.12^{bB}
3.5	373.0±22.00^{aC}	234.33±28.22^{bC}	204.03±9.80^{bC}	183.80±27.53^{bC}	175.0±60.75^{bBC}	160.0±44.91^{bB}
4.0	331.67±20.26^{aC}	223.67±26.56^{bC}	193.0±15.62^{bcC}	159.0±12.17^{bcC}	138.0±46.86^{cC}	126.0±28.69^{cB}

Means with different small letters within a row of each component are significantly different ($p \leq 0.05$) (Tukey's) and non significantly different (ns)

Means with different capital letters within a column of each component are significantly different ($p \leq 0.05$) (Tukey's) and non significantly different (NS)

The antioxidant capacity of pennywort juice fell during storage for 4 months at 4 and 40˚C. The total phenolics content fell in parallel with the FRAP value. The Folin-Ciocalteu phenol reagent is used to obtain a crude estimate of the concentration of phenolic compounds present. The relative rate of reduction of FRAP values was highest for the sterilized sample at 1.5 months storage. After 4 months storage, the FRAP values were similar for samples with high pressure processing, pasteurization, and sterilization with reductions of 64, 66 and 65% from the value at the start of storage, despite the sterilized sample being stored at 40˚C, with the other samples being stored at 4˚C. The phenolic content was reduced by 50.5, 55.5 and 62.5% for the HPP, pasteurized and sterilized samples after storage for 4 months, so it is likely

that the increasing loss of ascorbic acid in the high pressure processed and pasteurized samples offset the reducing loss of phenolic components in these samples. The contribution of ascorbic acid to the antioxidant capacity of the juice was a relatively proportional to FRAP during the 4 months storage period, which indicates that the rate of loss of ascorbic acid was similar to the rate of loss of phenolic components with antioxidant activity during storage. Jannok (2007) reported that the total phenolic content of HPP (600 MPa for 40 min at 50˚C) and pasteurized (90˚C for 15 s) pennywort juice was reduced by 48.48 and 19.80%, respectively, when stored at room temperature for 4 weeks, and by 6.54 and 4.75%, respectively, when stored at 4˚C for 4 weeks.

Table 4.16 Contributions of ascorbic acid to antioxidant capacity of the processed pennywort juice

Shelf life (months)	Contributions of ascorbic acid to antioxidant capacity (%)					
	HPP + sugar	HPP	Pasteurized + sugar	pasteurized	Sterilized + sugar	sterilized
0.0	8.56 ± 0.56^{bA}	12.74 ± 2.00^{aA}	7.63 ± 0.37^{bA}	6.66 ± 0.37^{bA}	3.53 ± 0.17^{cD}	3.56 ± 0.11^{cD}
0.5	7.92 ± 1.17^{bAB}	12.17 ± 0.10^{aA}	6.10 ± 0.51^{cB}	5.93 ± 0.37^{cB}	3.84 ± 0.42^{dCD}	3.85 ± 0.06^{dD}
1.0	5.99 ± 0.12^{bC}	9.95 ± 0.15^{aB}	5.37 ± 0.07^{bC}	5.33 ± 0.06^{bC}	4.38 ± 0.14^{cC}	3.93 ± 0.06^{cD}
1.5	6.71 ± 0.07^{bBC}	9.76 ± 0.12^{aB}	5.34 ± 0.08^{cC}	5.23 ± 0.09^{cC}	4.46 ± 0.06^{dC}	4.84 ± 0.23^{dC}
2.0	7.07 ± 0.07^{bBC}	10.02 ± 0.41^{aB}	6.06 ± 0.12^{cB}	5.88 ± 0.12^{cB}	4.70 ± 0.18^{dBC}	4.77 ± 0.15^{dC}
2.5	7.38 ± 0.22^{bB}	9.78 ± 0.94^{aB}	5.68 ± 0.06^{cdBC}	6.34 ± 0.09^{cAB}	4.79 ± 0.29^{dBC}	4.91 ± 0.10^{dC}
3.0	7.11 ± 0.18^{bBC}	9.46 ± 0.21^{aB}	5.82 ± 0.16^{cBC}	6.20 ± 0.13^{cB}	5.30 ± 0.37^{dB}	5.23 ± 0.28^{dBC}
3.5	7.67 ± 0.19^{bAB}	9.44 ± 0.22^{aB}	$5.88\pm0.0cd^{bBC}$	6.31 ± 0.14^{cAB}	5.29 ± 0.39^{dB}	5.51 ± 0.20^{dB}
4.0	7.66 ± 0.79^{bAB}	8.99 ± 0.39^{aB}	6.12 ± 0.07^{cB}	6.91 ± 0.15^{bcB}	6.16 ± 0.40^{cA}	6.50 ± 0.44^{cA}

Means with different small letters within a row of each quality are significantly different ($p\leq0.05$) (Tukey's) and non significantly different (ns)

Means with different capital letters within a column of each component are significantly different ($p\leq0.05$) (Tukey's) and non significantly different (NS)

Wong *et al*. (2006) concluded that the phenolics were partly responsible for the antioxidant capacity of a range of plants including pennywort. Ascorbic acid also contributes to the antioxidant capacity. After HPP, pasteurization and sterilization, the ascorbic acid was found to contribute 12.74, 6.66 and 3.56% of the antioxidant capacity of the juice without added sugar, and 8.56, 7.63 and 3.53%, respectively of the antioxidant capacity of the juice with added sugar (**Table 4.16**).

Table 4.17 Phenolic contents of processed pennywort juice (subtracted from ascorbic acid content)

Shelf life (months)	Phenolic contents (mg/100 ml, subtracted from ascorbic acid content)					
	HPP + sugar	HPP	Pasteurized + sugar	Pasteurized	Sterilized + sugar	Sterilized
0.0	225.13±11.57^{aA}	214.77±9.20^{aA}	148.58±4.51^{bA}	132.16±3.26^{bA}	96.13±17.68^{cA}	89.06±4.87^{cA}
0.5	195.00±5.83^{aB}	191.47±4.40^{aB}	113.04±4.34^{bB}	116.43±3.26^{bB}	91.81±11.52^{cAB}	83.15±1.92^{cA}
1.0	176.00±5.33^{aC}	172.94±3.82^{aC}	105.45±1.59^{bBC}	105.14±3.26^{bB}	75.73±7.69^{cB}	74.56±1.03^{cB}
1.5	159.29±8.35^{aD}	147.59±3.02^{bD}	99.00±7.13^{cC}	79.81±3.26^{dC}	71.32±3.17^{dBC}	69.23±2.58^{dB}
2.0	147.18±4.10^{aDE}	132.30±9.39^{bDE}	86.51±3.39^{cD}	67.54±3.26^{dCD}	63.86±0.70^{dBC}	56.34±3.20^{eC}
2.5	135.91±5.21^{aE}	117.15±4.53^{bE}	82.37±1.14^{cD}	52.87±3.26^{dD}	57.80±2.07^{dC}	54.08±1.08^{dC}
3.0	120.40±3.38^{aF}	102.34±3.10^{bF}	76.14±2.13^{cDE}	52.16±3.26^{dD}	54.28±1.96^{dC}	47.31±1.62^{eCD}
3.5	109.06±5.49^{aFG}	93.50±1.47^{bF}	67.72±4.02^{cE}	48.11±3.26^{dD}	44.25±3.14^{dCD}	41.41±7.43^{dD}
4.0	94.87±7.36^{aG}	77.41±2.72^{bG}	55.40±3.09^{cF}	44.23±3.26^{dD}	33.40±2.87^{eD}	31.10±3.05^{eE}

Means with different small letters within a row of each quality are significantly different ($p \leq 0.05$) (Tukey's) and non significantly different (ns)

Means with different capital letters within a column of each component are significantly different ($p \leq 0.05$) (Tukey's) and non significantly different (NS)

After HPP, pasteurization and sterilization, the phenolics content subtracted from ascorbic acid was found to be 214.77, 132.16 and 89.06 mg/100 ml, respectively in the juices without added sugar, and 225.13, 148.58 and 96.13 mg/100 ml, respectively, in the juices with added sugar (**Table 4.17**).

FRAP due to phenolic compounds of HPP, pasteurized and sterilized pennywort juice was 87.11, 93.34 and 96.42%, respectively, in the juices without sugar added, and 91.38, 92.42 and 96.48%, respectively, in the juices with added sugar (**Table 4.18**). There was a FRAP reducing trend in the order of sterilized juice > pasteurized juice > HPP juice.

The antioxidant activity of phenolic compounds depends on their chemical reactivity towards peroxy radicals and other active species. The activity also changes due to many other factors including antioxidant concentration, substrate type, physical state of the system and the number of microcomponents acting as pro-oxidants or synergists (Yanishlieva-Maslarova, 2001). Gazzani *et al*. (1998) suggested that pro-oxidant activity, due to peroxidases, may be reduced by inactivatation at high temperature. For example, the antioxidant capacity of carrot juice at 25˚C for 10 min was 24% and it increased to 75% after heating at 102˚C for 30 min. Wang *et al*.

(1996) have also observed that heated tomato and grape juice had much higher antioxidant activity than fresh juices.

Table 4.18 Ferric reducing antioxidant potential values due to phenolic compounds of processed pennywort juice

Shelf life (months)	FRAP values (%, due to phenolic compounds)					
	HPP + sugar	HPP	Pasteurized + sugar	Pasteurized^{NS}	Sterilized + sugar	Sterilized
0.0	91.38±0.88^{bC}	87.11±1.61^{cB}	92.42±0.41^{bB}	93.34±0.39^{b}	96.48±0.05^{aA}	96.42±0.31^{aA}
0.5	92.08±0.18^{cBC}	87.76±1.07^{dAB}	93.83±0.92^{bAB}	94.07±0.12^{b}	95.89±0.67^{aAB}	96.14±0.30^{aA}
1.0	93.96±0.70^{bA}	89.99±0.86^{cA}	94.50±1.03^{bA}	94.58±0.86^{b}	95.62±0.19^{aAB}	96.03±0.50^{aA}
1.5	93.23±0.25^{bAB}	90.23±0.48^{cA}	94.68±0.13^{aA}	94.77±0.15^{a}	95.46±0.86^{aAB}	94.85±1.42^{aAB}
2.0	92.91±0.43^{bAB}	89.98±0.33^{cA}	93.96±0.28^{bA}	93.90±1.58^{b}	95.24±0.70^{aAB}	95.22±0.11^{aAB}
2.5	92.62±0.16^{cB}	90.21±0.40^{dA}	94.28±0.66^{abA}	93.64±0.36^{b}	95.07±1.09^{aAB}	94.86±1.41^{aAB}
3.0	92.84±0.77^{cAB}	90.38±1.50^{dA}	94.17±0.44^{abA}	93.76±0.59^{b}	94.64±0.90^{aAB}	94.52±1.34^{aAB}
3.5	92.32±0.41^{cBC}	90.50±1.02^{dA}	94.11±0.24^{abA}	93.61±0.82^{b}	94.36±1.87^{aAB}	94.30±1.23^{aAB}
4.0	92.33±0.42^{bBC}	90.93±0.96^{cA}	93.88±0.42^{aAB}	93.08±0.43^{ab}	93.60±1.45^{aB}	93.37±1.03^{aB}

Means with different small letters within a row of each quality are significantly different ($p \leq 0.05$) (Tukey's) and non significantly different (ns)

Means with different capital letters within a column of each component are significantly different ($p \leq 0.05$) (Tukey's) and non significantly different (NS)

The antioxidant capacity of polyphenol-containing food can be improved depending on the processing conditions including a_w, pH, temperature, time and oxygen content (Nicoli *et al*., 1999; Kikugava *et al*., 1990). With process temperatures below 50˚C, HPP does not cause browning of the juice during processing (Nienaber and Shellhammer, 2001).

The bioactive compounds related to the juice processing

Some of the compounds would have degraded during the processing. Heat treatment may reduce the saponin concentration (Court *et al*., (1996). The juice properties after processing showed changes of madecassoside, asiaticoside, ascorbic acid, *β*-carotene and total phenolic contents. Madecassoside, asiaticoside and *β*-carotene were relatively stable on processing with no significant losses occurring. There was a trend for losses of *β*-carotene, madecassoside and asiaticoside to be greater in samples heat treated by pasteurization and sterilization but did not reach statistical significance. The madecassoside and asiaticoside contents in fresh juice

were higher than the content after HPP, pasteurization and sterilization. Madecassoside and asiaticoside are triterpene alcohols and therefore, are more stable than phenolic compounds. β-Carotene is known to be relatively stable during heat treatment (Elkin, 1979; Miki and Akatsu, 1971). HPP pennywort juice contained a concentration of β-carotene was similar to that of fresh juice, which was present at higher concentration than pasteurized and sterilized juices, respectively.

Ascorbic acid is considered as an indicator of the nutritional quality of processed foods. Because of its content guarantees the presence of other nutrients and its relative instability to heat. It is highly sensitive to degradation at a rate that depends on temperature, sugar concentration, pH and its initial concentration. The sensitivity of ascorbic acid to heat depends on the type of product. In this study, the effect of temperature and time used for the heat treatments, pasteurization as well as sterilization, on ascorbic acid destruction was greater for higher temperature and longer time. Methods of juice processing with minimal heating such as HPP are necessary to preserve ascorbic acid. HPP had less effect on the hydrosoluble vitamins, and thus it helps to preserve the nutritional quality of food products. The ascorbic acid content after HPP and heat treatment was lower than that of the fresh pennywort juice. The concentration of ascorbic acid was reduced significantly after processing. Fresh juice contained the highest amount of ascorbic acid followed by HPP, pasteurization and sterilization, respectively. In this study, it was found that HPP could not completely preserve ascorbic acid in pennywort juice and losses of ascorbic acid occurred during HPP at 400 MPa for 20 min at <30˚C. Several studies showed that the loss of ascorbic acid after HPP is dependent mainly on temperature and time. The loss of ascorbic acid may be attributed to oxidation at the temperature applied (Yang and Atallah, 1985). Ascorbic acid is easily destroyed by oxidation at high temperatures and during processing and also during storage. The effect of temperature on ascorbic acid content is more than the effect of heating duration.

This juice contained high concentrations of phenolic compounds. The phenolic compounds are responsible for the antioxidant capacity of this plant. Total phenolic contents are influenced by various factors including different processing used. During processing, phenolic compounds are usually sensitive to acidic solution and high temperature.

HPP and heat treatment applied during juice processing causes a reduction in the content of total phenolics and the FRAP values. After treatment, the total phenolic content and FRAP value were decreased. The phenolic compounds in pennywort juice were significantly lost during heat processing. Since the content of total phenolics was significantly reduced after HPP and thermal treatment, the unstable compounds may be present in this juice as major components. The processing treatment of pennywort juice at the high temperatures potentially caused thermal decomposition of some phenolic antioxidants may be due to high temperature breakdown of phenolics or combination with other plant components. The phenolics in pennywort juice can be destroyed by oxidation or the structure transformed into other phytochemicals during processing.

The bioactive compounds related to antioxidant activity

Ascorbic acid is a major contributor to the antioxidant activity of pennywort. It is well known as an antioxidant nutrient and protects some flavonoids against oxidative degradation during processing and storage. Ascorbic acid contents in the HPP, pasteurized and sterilized juices contributed 5.99-12.74, 5.23-7.63 and 3.53-6.50% the total antioxidant activity, as shown in **Table 4.16**. Ascorbic acid were degraded by active oxygen and initiated by transition metals. It removes oxygen in systems where oxygen is present in limited amounts and gets oxidized to dehydroascorbic acid (Jadhav *et al*., 1996). However, ascorbic acid has much weaker antioxidant activity than flavonoids.

The phenolics were partly responsible for the antioxidant capacity. The phenolic compounds are the major contributors to the antioxidant capacity of pennywort. Phenolic compounds are proper antioxidants because they are able to inactivate the lipid free radicals as well as prevent the decomposition of hydroperoxides to free radicals. The total antioxidant capacity of the pennywort determined by the FRAP assay and total phenolics content. This juice contained high concentrations of phenolic compounds. The polyphenols in pennywort juice can be destroyed or transformed into other phytochemicals which explains the reduction in antioxidant capacity value in pennywort juice.

4.5 The effects of storage time on the microbial quality

Effects of storage on the microbiological properties of pennywort juices during storage for 4 months at 4˚C for pressurized and pasteurized juices, and at 40˚C for sterilized juice showed satisfactory results. *S. aureus* and *C. perfingens* were not detected in all samples during storage and *E. coli* by MPN method was less than 2.2 per 100 ml and also yeasts and moulds count was less than 30 cfu/ml. The total plate count of HPP juice increased in the order of 35, 41 62, 80, 97, 119, 142 and 187 cfu/ml during 9-16 weeks of storage, respectively. Also, the HPP juice with added sugar had total plate count in the order of 31, 45, 52, 64, 85, 112, 155, 178, 221 and 258 cfu/ml during the 7-16 weeks of storage, respectively. Addition of sucrose may not have retarded the growth of organisms. In contrast, high sugar content was reported to contribute to faster multiplication of bacteria (Yusof *et al*., 2000). *S. aureus* can grow in a temperature range of 15-45˚C. Oyedeji and Afolayan (2005) reported that pennywort oil exhibited a broad spectrum of antibacterial activities against *S. aureus* and *E. coli*. However, the microbial growth found in this work was not affected by the antibacterial property of pennywort due to very low concentration of extracted oil in the juice.

The effects of HPP on microorganisms in other plants have been widely reported, and the microbiological stability of orange juice was easily achieved by HPP since yeasts, moulds and lactic acid bacteria remained low during storage at 4˚C and 37˚C over a period of more than 2 months after HPP (Nienaber and Shellhammer, 2001). HPP at 500 MPa for 10 min was capable of inactivating more than 5 log decades of microorganisms in apple-broccoli juice. This juice was free of coliforms, yeasts, moulds and *Salmonella* during 30 days of storage at 5-8˚C. HPP juice (500 MPa for 10 min) was found to have a total plate count 920 cfu/ml, coliforms were 920 cfu/ml and yeasts and moulds were less than100 cfu/ml, when stored for 30 days. Treatment with pressures higher than 300 MPa for 10 min totally inactivated microorganisms present at an initial concentration higher than 10^5 cfu/ml. On the other hand, pressures of 250 MPa and lower were not able to totally destroy the microorganisms. *E. coli* can be inactivated by using pressures of 400 MPa when the initial concentration is of 10^6 cfu/ml. The USDA-FDA requires orange juice to

demonstrate the effect when the initial concentration is 10^5 cfu/ml (Houska *et al*., 2006).

HPP at 700 MPa for 2 min at 20˚C resulted in inactivation of natural flora in tomato puree even after storage at 4˚C for 8 weeks (Krebbers *et al*., 2003). Sancho *et al*. (1999) reported that the total mesophilic aerobic flora were nearly the same for the pressurized (400 MPa for 30 min at 20˚C) *coulis* after 28 days of storage at 4˚C and the untreated *coulis* after 7 days of storage. Thus, the shelf life of *coulis* is increased by HPP. HPP orange juice had a shelf life at least 2 months at 8˚C, as proved by microbiological analysis (Donsi *et al*., 1996). After storage at 4˚C for 28 days, total viable counts of microornisms in tomato juices, treated by HPP at 300 and 400 MPa for 10 min at 25˚C, were slightly elevated from 3.2 and 2.6 to 4.6 and 3.1 log cfu/ml, respectively. Hsu *et al*. (2008) suggested that the different effects of HPP on inactivation of microorganisms were mainly attributed to the various tomato-based products. HPP at 400 MPa and above is sufficient to produce microbial stable tomato juices for storage at least for 28 days at 4˚C. HPP (689 MPa at 21˚C) guacamole (avocado products) was microbiologically stable for 30 days at 5, 15, and 25˚C, whereas guacamole control stored at 5˚C spoiled within the first 5 days (Palou *et al*., 2000).

4.6 Flavour profile pennywort juice

Aroma has long been known to be one of the most important quality attributes of both fresh and processed juices. Volatile compounds are especially important to the flavour of juice, with contributions from alcohols, aldehydes, esters, ketones and hydrocarbons (Nisperos-Carriedo and Shaw, 1990). One of the factors contributing to the popularity of juice is the combined quality and quantity of these highly aromatic compounds (Shaw, 1994).

This initial work has demonstrated that the use of HPP in pennywort juice may be effective on flavour compared with fresh juice and thermal treatment. The flavour volatiles in fresh and processed juice without added sugar were found as shown in **Tables 4.19-4.20** and the flavour volatiles in sugar added processed juice was as shown in **Tables 4.21-4.22**.

There are several literature reports on the effects of HPP on flavour, for example, Nienaber and Shellhammer (2001) reported that HPP did not affect the fresh citrus flavour, and the volatile components of canned tomato juice decreased by treatment at 100˚C for 10 min (Sieso and Crouzet, 1977). Thus, HPP may not affect the flavour of fresh pennywort juice, while thermal treatment would affect the flavour of fresh juice.

4.6.1 The flavour profiles of fresh and processed juices without added sugar

The flavour compounds in fresh and processed pennywort juices treated by heat and high pressure treatment are shown in **Table 4.19** and **Table 4.20**. A total of 72 volatile components were found. Fresh and high pressure processed juices contained 48 and 49 volatiles, respectively, whereas pasteurized and sterilized juice contained 55 volatiles compounds.

The total concentration of volatile compounds in fresh juice was higher than in processed juice ($p \leq 0.05$) and there was a non-significant trend in the order of sterilized juice > HPP juice > pasteurized juice, respectively. However, HPP caused more flavour volatiles in the acyclic alcohol class to be retained, with a trend to increased retention of aldehydes and oxygenated monoterpenoids ($p > 0.05$) compared to pasteurization and sterilization. Although the total volatile concentration in sterilized juice was higher than that in the juice processed ($p > 0.05$) by other methods. Some volatile components that were not present in the fresh juice were formed at high levels in the sterilized juice, e.g. γ-terpinene, ketones, γ2-cadinene and germacrene *D*. This indicates that HPP could maintain the original flavour better than pasteurization and sterilization.

Fresh juice was characterized 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 sesquiterpenoid hydrocarbons β-caryophyllene (1344.0 ng/L) and humulene (1602.2 ng/L) were present at higher concentrations than other volatiles in the fresh juice.

Table 4.19 Approximate quantities of volatile compounds identified in fresh and processed pennywort juices without added sugar

Compounds (m/z)	Mean concentration±sd (ng/L) [A]				Method of identification[B]	LRI[C]
	Fresh	HPP	Pasteurization	Sterilization		
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

Table 4.19 (continue)

Compounds (m/z)	Mean concentration±sd (ng/L) [A]				Method of identification[B]	LRI[C]
	Fresh	HPP	Pasteurization	Sterilization		
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-isopropyl benzene 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-formylcyclo pentene 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 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 *et al*. (2001)

[F] Quorn®

[G] Priestap *et al*. (2003)

[H] Flavornet

[I] Kilic *et al*. (2004)

[J] Vichi *et al*. (2007b)

[K] Adams (1995)

[L] Baranauskinene *et al*. (2003)

[M] Sacchetti *et al*. (2005)

[N] Flamini *et al.* (2002)

[O] Skaltsa *et al*. (2003)

It was clear that these volatile components were retained better by HPP than by pasteurization or sterilization. For example 62% of the original concentration of linalool remained in the juice after HPP compared with 25% after pasteurization and 24% after sterilization. Pennywort juice flavour components may be lost or transformed during heating processes. Moreover, pentanal and hexanal are formed by autooxidation and enzymatic oxidation (Hashizume, 2007).

Some other lipid oxidation products including 2-nonenal and 2,6-nonadienal has been used to indicate oxidized flavour in reduced-fat cheeses (Suriyaphan *et al*.,

1999). (*E*)-2-Nonenal is biosynthesized in green leaves via lipoxygenase-mediated lipid oxidation (Hatanaka, 1996). 2,6-Nonadienal (aliphatic aldehyde) contributes a strong cucumber-like aroma and it is known as the unique aroma of violet leaf, cucumber, muskmelon (Hatanaka, 1996; Schieberle *et al*., 1990) and also omija leaves (Zheng *et al*., 2005). 2,6-Nonadienal and 2-nonenal were found in fresh juice but not found in processed juice. 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.

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 juices. These compounds might be formed during sample preparation because no action was taken to inactivate enzymes prior to extraction in this study.

Some aldehydes including 2-methyl-propanal, 3-methyl-butanal, 2-methyl-butanal, hexanal, 2,6-nonadienal and 2-nonenal decreased or disappeared after thermal treatment, whereas the known lipid oxidation product, hexanal was found at higher concentrations in sterilized samples than in pasteurized and HPP juices. Aldehydes are known to degrade readily by chemical reactions including acetal formation which occurs in the presence of alcohols under acid conditions (Jerry, 1992).

α-Terpineol (an oxygenated monoterpene) is considered to be desirable in many fruits, whereas in others it is perceived as an off-flavour (terpentine-like) (Dung *et al*., 1995). It is known to be formed in citrus juices from limonene and linalool by acid-catalyzed reactions (Haleva-Toledom *et al*., 1999). Rui *et al*. (2007) reported isomerisation of linalool and dehydration to monoterpenes as shown in **Figure 4.11**. In this study, α-terpineol was found only in high pressure processed samples. Linalool was found to be reduced ($p \leq 0.05$) by the thermal processing of pennywort juice. Sterilization and pasteurization caused big drops in the concentration of linalool. 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).

Limonene has been reported as the major flavour compound in various citrus fruits and in water dropwort (Seo and Beak, 2005). 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 (2008) did detect *D*-limonene in pennywort juice. These studies show that *γ*-terpenene, which contributes bitter flavours, was found in higher concentration in processed samples. Myrcene, which was described as contributing pine odour to *Piatacia lenticus* (Seo and 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 *et al*., 2005), water dropwort (Seo and Beak, 2005), carrot (Kreutzmann *et al*., 2008) and it is also important for the overall aroma of omija leaves (Zheng *et al*., 2005). It was found at higher concentration in fresh pennywort juice than in processed samples ($p \leq 0.05$).

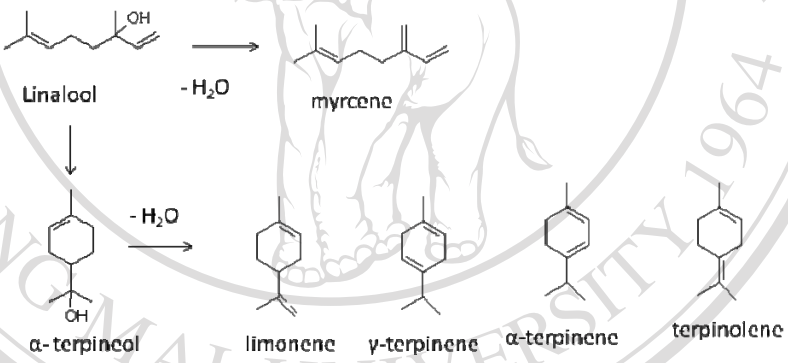

Figure 4.1 Isomerisation of linalool and dehydration to monoterpenes
Source: Rui *et al*. (2007)

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 and 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 ($p \leq 0.05$). It has also been reported in omija fruits, *Murraya koenigii, Stachys*

swainsonii spp. *melangavica* and was identified as the main component of basil oil and omija leaves (Zheng *et al*., 2005). Caryophyllene oxide was only detected in the fresh juice. Chou (2005) and Ali (2008) 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 pennywort juice. However, previous reports only describe the composition of unprocessed juice, and the effects of high pressure processing on volatile compounds in pennywort juice have not been reported previously.

The main justification for HPP of foods is that more valuable constituents, for example, aroma compounds and vitamins (Gotz and 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 some unknown compounds. 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 some 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, terpiolene, ketones, *α*-terpineol, *α*-ylangene, bicyclogermacrene, *γ*-cadinene, tetrahydrofuran and others unknown compounds. However, some compounds which have been previously reported as components of fresh pennywort juice, including bicyclogermacrene were not detected in the study of Oyedeji and Afolayan (2005).

Hexanal is normally formed in plant tissue by lipoxygenase action on polyunsaturated fatty acids (Vichi *et al*., 2007a). Su and Wiley (1998) reported that a change in the apple juice flavour profile was caused by enzyme activation at elevated

temperature (57.2˚C) which tended to increase flavour compounds including hexanal (grass-like) and trans-2-hexanal, whereas a higher temperature which simulated pasteurization decreased all flavour compounds except propyl butyrate. Yu and Chiang (1986) reported that pasteurization (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 no significant difference in total volatiles between fresh and sterilized juice ($p>0.05$) but a significant difference between fresh and other samples ($p\leq0.05$). Carelli *et al*. (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 *et al*. (2004) also reported that a hydrophobic effect, which may contribute to the interaction of aroma compounds with macromolecules, is enhanced during heat treatment and gives rise to the lower release of aroma compounds in pasteurized orange juice. This increase in concentrations of juice flavour components as a consequence of processing may due to heat activation of flavour precursors or the release of flavour compounds bound to cell membranes. Decrease in juice flavour is caused by evaporation or by thermal degradation (Su and Wiley, 1998).

Hexanol was detected in the HPP juices. Stone *et al*. (1975) reported that alcohols (hexanol) are created by alcohol oxidoreductase activity on C_6 aldehydes (hexanal). Hendrickx *et al.* (1998) also reported that HPP-induced enzyme activation and inactivation are relevant to food quality; enzyme activation can arise from pressure-induced decompartmentalization (Butz *et al*., 1994; Gomes and Ledward, 1996).

*γ*2-Cadinene was not detected in the fresh juice and in the high pressure processed samples but was present in the thermally treated samples. However, *γ*-cadinene may have isomerised to the 2-isomer on heating.

It appears that several chemical changes occurred as well as loss of volatiles during the heating processes of pasteurization and sterilization. These data suggests that thermal and HPP treatments can change chemical structures of some plant compounds or induce the biosynthesis of new compounds by enzyme activation or inactivation.

There is no published information about the effect of heat and HPP treatments on volatile components of pennywort juice. 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 this study. The volatile compounds included 4 alcohols, 6 aldehydes, 4 ketones, 7 monoterpenoid hydrocarbons, 4 oxygenated monoterpenoid, 22 sesquiterpenoid hydrocarbons, 1 oxygenated sesquiterpenoid, 5 miscellaneous and some unknown components. Forty different varieties of pennywort have been identified throughout the world (de Padua *et al*., 1999), so differences in reported volatile components are not surprising.

Table 4.20 Concentrations of volatile product groups of fresh and juice without sugar added

Compound groups	**Total concentration±sd (ng/L)**			
	Fresh	**HPP**	**Pasteurization**	**Sterilization**
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 ($p \leq 0.05$) (Tukey's), non significantly different (ns), compounds identified below 0.5 ng are reported as not found (nf)

Aldehydes and oxygenated sesquiterpenes were present at higher concentration in fresh juice than in other samples ($p \leq 0.05$), 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 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 juices ($p > 0.05$) in the content of acyclic

alcohols, aldehydes, oxygenated monoterpenes, sesquiterpene hydrocarbons, oxygenated sesquiterpenes, miscellaneous compounds and total volatiles, as shown in **Table 4.20**.

4.6.2 The flavour profiles of fresh and processed juice with added sugar

The flavour compounds in pennywort juice containing added sugar and treated by HPP and thermal processes are shown in **Table 4.21** and **Table 4.22**. Juice with added sugar had more flavour concentration than juice without added sugar. High pressure treated without added sugar juice contained 49 volatiles, whereas HPP with sugar added contained 56 volatiles. Pasteurized juice and sterilized juice without sugar contained 55 volatiles, whereas pasteurized juice with added sugar contained 60 volatiles and sterilized juice with added sugar contained 74 volatiles. This means that sugar brings about increasing volatile components through the interaction with other compounds in the food matrix. However, HPP caused more flavour volatiles from the acyclic alcohols to be retained ($p \leq 0.05$), with a trend to increase retention of aldehydes and oxygenated monoterpenoids ($p > 0.05$), as compared to pasteurization and sterilization. This indicates that the high pressure treatment maintained the flavour better than sterilization and pasteurization.

HPP juice with added sugar was characterized by a high content of the sesquiterpenoid hydrocarbons β-caryophyllene (1704.5 ng/L) and humulene (1544.0 ng/L) which were present at higher concentrations than other volatiles. It was clear that these volatile components were retained better by HPP than by pasteurization or sterilization. For example, linalool remained in the juice after HPP at 257.3 ng/L compared with concentrations after pasteurization (69.5 ng/L) and sterilization (86.0 ng/L). Pennywort juice flavour components may be lost or transformed during heating. Moreover, pentanal and hexanal might be formed by auto-oxidation and enzymatic oxidation (Hashizume, 2007).

Table 4.21 Approximate quantities of volatile compounds identified in fresh and processed pennywort juices with added sugar

Compounds (m/z)	Mean concentration±sd (ng/L) [A]				Method of identification[B]	LRI[C]
	Fresh	HPP	Pasteurization	Sterilization		
Acylic alcohols						
1-Penten-3-ol	123.5±54.0 [a]	83±86.6 [ab]	nf [b]	nf [b]	MS+LRI	689
2-Penten-1-ol 29, 32, 38, 41, 44, 50, 53, **57**, 71, 83	nf [b]	33.7±19.2 [a]	nf [b]	nf [b]	se	773
Hexanol	nf [b]	27.1±19.1 [a]	nf [b]	nf [b]	MS+LRI	873
Aldehydes						
Butanal	nf [ns]	26.5±31.6	13.5±15.7	7.5±5.3	MS+LRI	579
Propanal, 2-methyl 29, 37, 39, 41, **43,** 45, 50, 53, 55, 57, 59, 72, 74	97.5±85.6 [ns]	64.5±74.2	trace	trace	se	659
Butanal, 3-methyl 29, 37, 39, 41, **44**, 50, 53, 55, 58, 71, 86	79.5±38.0 [a]	58.8±50.7 [ab]	nf [b]	nf [b]	se	659
Butanal, 2-methyl **41**, 57, 71, 86	187.1±105.1 [a]	109.5±86.9 [ab]	trace [b]	- [b]	se	669
Pentanal	nf [b]	51.7±86.931.3 [a]	nf [b]	nf [b]		706
Hexanal	142.4±68.2 [ns]	52.8±41.1	122.7±116.6	201.7±63.3	MS+LRI	805
Heptanal	nf [b]	nf [b]	nf [b]	18.7±14.1 [a]		907
2, 6-Nonadienal	88.2±68.6 [a]	nf [b]	nf [b]	nf [b]	ms[D]	1162
2-Nonenal	72.7±55.0 [a]	nf [b]	nf [b]	nf [b]	MS+LRI	1168
Ketones						
2-Butanone	nf [ns]	38.1±62.7	29.3±25.8	13.9±7.8	MS+LRI	601
2-Nonanone	nf[b]	nf [b]	59.1±57.4 [ab]	72.1±33.7 [a]	MS+LRI	1095
3-Nonen-2-one	nf[b]	37.6±23.6 [ab]	200.8±202.4 [ab]	307.4±201.1 [a]	MS+LRI	1145
5-Nonen-2-one	nf[b]	nf[b]	nf[b]	33.4±34.3 [a]		1094
Monoterpene hydrocarbons						
α-Pinene	92.5±39.1 [ns]	43.7±31.5	43.4±56.9	34.6±16.4	MS+LRI	940
β-Pinene	108.2±43.5 [a]	51.3±33.4 [ab]	35.4±42.7 [b]	35.9±15.3 [b]	MS+LRI	987
Myrcene	167.2±77.4 [ns]	171.8±116.7	170.7±227.1	229.1±108.2	MS+LRI	993
α-Terpinene	nf[b]	nf[b]	nf [b]	16.3±10.9 [a]	ms[E]	1025
Limonene	trace [ns]	23.4±27.2	29.1±41.4	39.4±17.2	MS+LRI	1037
γ- Terpinene	nf[ns]	41.9±27.0	123.8±163.5	160.4±73.7	MS+LRI	1066
Terpinolene	nf[ns]	nf	trace	trace	ms[F]	1094
Oxygenated monoterpenes						
Linalool	335.5±148.6 [a]	257.3±107.2 [ab]	69.5±58.4 [b]	86.0±26.5 [b]	MS+LRI	1106
α-Terpineol	nf [ns]	trace	-	-	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]	63.3±24.4 [ab]	20.1±26.6 [b]	trace [b]	MS+LRI	1258
Sesquiterpene hydrocarbons						
α-Cubebene	147.3±57.5 [ns]	179.2±118.2	102.8±121.3	140.8±71.3	ms[G]	1361
α-Ylangene	nf [b]	nf [b]	trace [b]	26.9±19.4 [a]	ms[L]	1387
α-Copaene	537.6±221.1 [ns]	712.3±473.6	274.6±310.3	421.5±214.7	ms[G]	1394
β-Elemene	248.6±107.5 [ns]	176.4±89.0	167.0±170.7	239.8±119.3	ms[I]	1405
β-Copaene 41, 55, 69, 91, 105, 119, 133,**161**, 204	16.6±14.6 [ns]	46.2±50.6	35.6±36.6	56.1±32.6	se	1451
β-Caryophyllene	1344.0±1049.2 [ns]	1704.5±1084.0	946.3±100.9	1476.8±717.3	ms[F]	1444
(E)-*β*-farnesene	551.8±200.5 [ns]	571.7±383.0	1117.5±1198.6	1872.1±880	ms[J]	1461
Humulene	1602.2±668.7 [ns]	1544.0±956.7	929.9±933.2	1451.1±670.8	ms[H]	1481
Alloaromadendrene	258.3±105.0 [ns]	268.3±168.9	172.1±174.1	299.3±140.3	ms[G]	1485
δ-Cadinene	29.7±8.3 [ns]	54.4±33.0	41.3±50.9	27.7±35.4	ms[k]	1393
γ2-Cadinene	nf [b]	123.6±95.1 [a]	111.1±133.3 [a]	91.3±75.0 [ab]	ms[k]	1494
γ-Curcumene	trace [b]	108.5±57.9 [ab]	80.2±80.3 [ab]	152.6±86.8 [a]	ms[I]	1498
Germacreane *D*	trace [b]	53.3±28.3 [ab]	238.1±250.6 [ab]	381.1±195.9 [a]	ms[G]	1504
Valencene	146.0±102.0 [ab]	68.3±46.2 [b]	107.4±104.0 [ab]	244.8±113.7 [a]	MS+LRI	1514
β-Selinene	34.9±27.3 [ns]	70.0±103.3	-	-	ms[I]	1515
α-Muurolene	50.5±42.4 [ns]	44.3±20.3	25.4±31.7	30.7±26.1	ms[L]	1518

Table 4.21 (continue)

Compounds (m/z)	Mean concentration±sd (ng/L) [A]				Method of identification[B]	LRI[C]
	Fresh	HPP	Pasteurization	Sterilization		
Bicyclogermacrene	nf [ns]	-	50.7±56.5	33.6±19.6	ms[G]	1520
α-Selinene	130.2±68.1 [ns]	80.7±81.7	29.7±35.6	88.2±41.4	ms[M]	1521
Cuparene	192.8±81.1 [a]	169.5±102.9 [a]	nf [b]	nf [b]	ms[N]	1535
γ-Cadinene	96.5±30.8 [ab]	nf [b]	165.2±210.2 [ab]	228.4±106.9 [a]	ms[G]	1535
δ-Cadinene (armoise-Maroc)	161.3±50.7 [ns]	192.1±132.9	255.9±303.9	378.6±174.8	ms[G]	1538
Calamenene	101.7±34.1 [ns]	104.4±71.3	79.0±81.1	117.1±56.8	ms[O]	1544
α-Cadinene	nf [b]	nf [b]	59.7±74.9 [a]	92.8±45.4 [a]	ms[P]	1559
Oxygenated sesquiterpenes						
Caryophyllene oxide	37.7±28.8 [a]	nf [b]	nf [b]	nf [b]	ms[I]	1613
Miscellaneous						
Dimethyl sulfide 29, 35, 43, 45, 47, 49, 58, **62**, 64	840.9±429.6 [a]	283.4±218.6 [b]	274.3±199.3 [b]	341.1±140.6 [b]	se	532
Tetrahydrofuran	nf [b]	123.3±113.4 [a]	trace [b]	trace [b]	MS+LRI	633
Furan, 2-pentyl- 53, **81**, 109, 138	87.9±23.7 [ns]	80.5±67.6	32.0±42.2	58.0±29.1	se	995
1-Methyl-3-isopropylbenzene 51, 57, 65, 77, 91, 103, 115, **119**, 134	138.3±53.9 [ns]	114.3±82.6	166.1±202.8	194.3±88.3	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 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 A m/z is defined as mass per unit ion

[D] Skaltsa *et al*. (2003)

[E] Flamini *et al*. (2002)

[F] Quorn®

[G] Kondjoyan *et al*. (1996)

[H] Flavornet

[I] Noueira *et al*. (2001)

[J] Priestap *et al*. (2003)

[K] Kilic *et al*. (2004)

[L] Baranauskinene *et al*. (2003)

[M] Adams (1995)

[N] Sacchetti *et al*. (2005)

[O] Vichi *et al*. (2007b)

[P] Santos *et al*. (2001)

Acyclic alcohols were lost after heat treatment. Alcohols were found in high abundance in HPP. These compounds might be formed during sample preparation because no action was taken to inactivate enzymes prior to extraction in this study. Some aldehydes had decreased in concentration or disappeared after the thermal treatment, whereas hexanal and heptanal were found in sterilized samples at higher

concentrations than in pasteurized and HPP juices, which suggest formation by auto-oxidation.

In this study, α-terpineol was found in HPP treated samples and α-terpinene was detected only in sterilized juice. Linalool was found to be reduced by the heat treatment. Sterilization and pasteurization caused big drops in the concentration of linalool, which is also susceptible to dehydration under acidic conditions. In this study, terpinolene and α-terpinene were found in thermally samples but myrcene was found at high concentration in all samples. These studies show that γ-terpenene, which contributes bitter flavours, was found in higher concentrations in thermally samples as well.

The sesquiterpene class, including β-caryophyllene and humulene, was the major class of volatiles present in pennywort juice. Sesquiterpenes were found at high concentrations in all samples ($p>0.05$) but α-ylangene were found in thermally treated samples as well as found in sample without added sugar. In addition, α-cadinene was detected in only thermally treated sample with added sugar ($p\leq0.05$), but was not found in all samples without sugar addition and also in fresh juice.

However, previous reports (section 4.6.1) only describe the composition of fresh juice, and the effects of HPP on volatile compounds in pennywort juice without sugar addition. In this study, many compounds, including β-pinene, linalool, β-caryophyllene, humulene, geraniol, α-copaene, α-muurolene and cuparene were conserved better by HPP treatment. Several compounds, including α-terpinene, terpinolene, α-ylangene, γ-cadinene, α-cadinene and some unknown compounds were detected in heat-treated juice but were not found in HPP juice. It appears that several chemical changes occurred as well as loss of volatiles during the heating processes of pasteurization and sterilization. These data suggest that pennywort juice with sugar addition prior to treatments retains a larger quantity of flavour components than those juices with no sugar. This might be due primarily to the protective effect of sugar. However, Phunchaisri and Apichartsrangkoon (2005) treated fresh lychee and lychee in syrup with a combination of pressure (600 MPa) and temperature (60˚C) and found that this combined treatment caused extensive inactivation of enzyme POD and PPO in fresh lychee, whereas the effects were less for those processed in syrup due to baroprotection by the syrup.

In the study of juice with sugar addition, volatile compounds included 3 alcohols, 7 aldehydes, 4 ketones, 7 monoterpenoid hydrocarbons, 3 oxygenated monoterpenoids, 23 sesquiterpenoid hydrocarbons, 4 miscellaneous and some unknown componds. Acyclic alcohols were found only in fresh and HPP juices. However, there was no significant difference between HPP and heat-treated juice ($p>0.05$) in the content of aldehydes, monoterpene hydrocarbons, oxygenated monoterpenoids, sesquiterpene hydrocarbons, and total volatiles, as shown in **Table 4.22**.

Table 4.22 Concentrations of volatile product groups of fresh and juice with added sugar

Compound groups	**Total concentration±sd (ng/L)**			
	Fresh	**HPP**	**Pasteurization**	**Sterilization**
Acyclic alcohols	186.8±54.4 ^{a}	143.8±34.0 ^{a}	nf ^{b}	nf ^{b}
Aldehydes	665.6±44.7 ^{ns}	363.7±33.8	136.3±45.8	227.9±74.9
Ketones	nf ^{b}	75.7±20.7 ^{b}	289.2±83.6 ^{ab}	426.9±127.1 ^{a}
Monoterpene hydrocarbons	367.9±69.4 ^{ns}	332.1±58.6	402.4±64.9	515.5±86.1
Oxygenated monoterpenoids	524.9±149.5 ^{ns}	320.6±137.2	89.6±34.9	86.0±60.8
Sesquiterpene hydrocarbons	5651.9±424.9 ^{ns}	6272.0±447.9	4989.8±313.1	7851.3±512.0
Oxygenated Sesquiterpene	37.7±28.8 ^{a}	nf ^{b}	nf ^{b}	nf ^{b}
Miscellaneous	1634.2±168.6 ^{a}	601.4±64.7 ^{b}	601.1±63.8 ^{b}	882.2±78.0 ^{ab}
Total	9069.±1898 ^{ns}	8109.3±2261	6508.4±1801	9989.9±2448

Total concentration with different letters within a row are significantly different ($p\leq0.05$) (Tukey's), non significantly different (ns), compounds identified below 0.5 ng are reported as not found (nf).