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Storage stability of pennywort juice as affected by high pressure and thermal processing

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Abstract

The physicochemical and microbiological qualities of pressurized (500 MPa/30ºC/20 min) and pasteurized (90ºC/3 min) pennywort juices were determined during chilled storage (4ºC) for 4 months. The color parameters and pH values in both processed juices exhibited no changes upon storage. Moreover, it was found that high pressure could preserve various bioactive components including ascorbic acid, total phenolic compounds and antioxidant capacity (FRAP assay), superior than pasteurization. Asiaticoside, madecassoside and β-carotene were relatively stable on the processing and storage. The concentrations of total phenolic compounds and ascorbic acid as well as antioxidant capacity in both processed juices significantly decreased with an increasing of the storage times; however these components still retained in pressurized juice higher than pasteurized juice. The microbiological assessment displayed that standard plate count, yeasts and moulds in both processed juices were pleasingly eliminated.

Introduction

Pennywort or *Centella asiatica* (Linn.) Urban, one of the traditional Asian medicine herbal plants, is indigenous to Thailand, Malaysia, India, Srilanka and China. It contains various bioactive components such as triterpene acids, alkaloids, glycosides (i.e. asiaticoside and madecassoside), flavoniods, caroteniods, phenols and ascorbic acid (Aruoma, 2003; Jamil *et al*., 2007). Several researches have reported different pharmaceutical properties of pennywort extract including gastric ulcer healing, wound healing, anti-tumor, memory enhancing, neuroprotective, cardioprotective, hepatoprotective, immunomodulating, anti-viral and anti-inflammatory (Punturee *et al*., 2005; Jamil *et al*., 2007).

Recently, consumers are demanding high quality and convenient products with natural flavor and taste, and greatly appreciate the fresh appearance of minimally processed food (Oey *et al.*, 2008). In order to extend the shelf life of these products they are usually processed by conventional methods such as pasteurization, however this process can cause a diminution in antioxidant capacity (Dewanto *et al.*, 2002) and losses of some bioactive components (Chaikham and Apichartsrangkoon, 2012a, b). High pressure processing, an essentially non-thermal pasteurization process, has been pointed as the most novel technology to stabilize and extend the shelf life of various foods (Butz *et al.*, 2002; Apichartsrangkoon *et al*., 2012; Chaikham and Apichartsrangkoon, 2012 a, b). It is well known that high pressure processing has little effect on low molecular weight molecules when immediately evaluated after pressurization, contributing to the preservation of various vitamins and pigments in fruit and vegetable products when compared with thermal processing (Polydera *et al.*, 2004). According to Apichartsrangkoon *et al.* (2009), Chaikham and Apichartsrangkoon (2012a, b) and Barba *et al.* (2013), food qualities such as natural color, flavor, bioactive components and food nutrients are unaffected or only minimally altered on high pressure processing at 25-30ºC. Apichartsrangkoon *et al.* (2013) found that high pressure retained some volatile compounds including aldehydes (glassy) and esters (freshness/fruity) in Thai green-chili paste better than pasteurization and sterilization. In addition, pressurized and pasteurized longan juices were displayed that pressurization stabilized color parameters and some bioactive compounds, such as ascorbic acid, total phenols and antioxidant capacity (DPPH assay), better than pasteurization (Chaikham and Apichartsrangkoon, 2012a).

However, there are small reports on the effects of pressurization and its consequent chilled storage on the stability of bioactive components present in pennywort juice. Hence, the objective of this research was to study the effects of high pressure

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processing on bioactive components including asiaticoside, madecassoside, β-carotene, ascorbic acid, total phenolic compounds and antioxidant capacity (FRAP assay) as well as color parameters, pHs and microbiological qualities of pennywort juice in comparison to pasteurization. The stability of such qualities was also determined during chilled storage at 4ºC for 4 months.

Materials and Methods

Raw materials and preparation

Pennywort was harvested, from high land of Chiang Mai, Thailand. Its leaves were washed and extracted with drinking water at a ratio of 1:1 (w/v); subsequently the solids were separated by centrifuging at 1,000 rpm through sterile filter cloth. The filtrate was adjusted to a total soluble solid of 12°Brix with sucrose for standardization. One hundred milliliters of pennywort extract were then packed in a laminated bag (nylon plus polyethylene) and subjected to a pressure of 500 MPa at 30ºC for 20 min. The high pressure vessel was a 'Food Lab' model 900 high pressure rig (Stansted Fluid Power; Stansted, UK). The rate of pressure increase was about 330 MPa/min. During this high pressure treatment an adiabatic increase in temperature occured. At ambient temperature (25ºC), the temperature of monitored cell increased by about 7ºC upto 500 MPa but decreased to the set equilibrium value in less than 2 min. The pressure transmitting medium was a mixture of castor oil (Chemical & Lab Supplies, Thailand) and 98% ethanol (Chemical & Lab Supplies) at a ratio of 20:80 (v/v) (Chaikham and Apichartsrangkoon, 2012a). For pasteurization, 150 ml of extract was packed in a retort pouch, heated in boiling water until the core temperature of the package reached 90ºC for 3 min. Subsequently, the processed juice was cooled to room temperature and kept at 4ºC for 4 months. The physicochemical and microbiological qualities were evaluated at every 15 days intervals during storage.

Determination of proximate compositions

Proximate compositions of pennywort leaves including moisture content, ash content, crude protein, fat, crude fiber and carbohydrate were determined according to AOAC method (AOAC, 2005). In brief, 100 g of sample were extracted using a fruit extractor (Blender PANASONIC MJ-68M, Thailand). Total soluble solids were assessed using a Pocket Refractometer (N-10E, Atago, Japan).

Color parameter and pH measurements

A colorimeter, model Color Quest XE (HunterLab, Reston, VA) was used to measure the color of fresh and processed pennywort juices. Color parameters, a^* and b^* were used to calculate chroma [$C^* = [(a^*)^2 + (b^*)^2]^{1/2}$] and hue angle [$H° = \arctan (b^*/a^*)$].

The pH values were measured using a pH meter (Sartorius PB-20, Germany) according to the procedure as described by Chaikham and Apichartsrangkoon (2012a).

Determination of glycosides

Sample extraction for the analysis of glycosides was carried out using a modified method described by Inamdar *et al.* (1996). One milliliter of pennywort juice was mixed with 1 ml of 90% methanol (Lab-Scan, Thailand) and stirred for 2 h at room temperature. The solution was filtered through a 0.20 μm filter (Minisart, Sartorius, USA) and the clear filtrate was used for HPLC assay. The Agilent 1050 HPLC system (Agilent Technologies, USA) consisted of a pressure flow pump and a UV spectrophotometric detector. Chromatographic separation was performed with YMCS5 ODSAM 5 μm, 4.6 mm ID × 250 mm column with Waters S5ODS2 guard column compartment. C18 Column with deionized water (Lab-Scan) and acetonitrile (Fisher Scientific, UK) as the mobile phase with a λ_{max} of 220 nm was used. The flow rate of the mobile phase was 1.4 ml/min and the gradient was water 80% decreased within 30 min to 45%, maintained for 5 min and increased to 80% within 10 min. A constant volume (20 μl) of each sample was injected into the column. The peak areas of each component were determined, and the concentrations were conversed from the standard curves.

Determination of β-carotene

Ten milliliters of the extracted juice were mixed with 20 ml of cold acetone (Fisher Scientific). Twenty milliliters of petroleum ether (40–60ºC) (Fisher Scientific) were then added, and the organic layer was separated and washed with 40 ml phosphate buffer (pH 7; Lab-Scan). The separated organic layer was stirred overnight with 20 ml of 10% potassium hydroxide (Fisher Scientific) in methanol and washed successively with 40 ml distill water, 40 ml phosphate buffer, 40 ml saturated sodium chloride (Chemical & Lab Supplies) and dried with sodium sulfate (Fisher Scientific). The solvent was evaporated and the residue was dissolved in 5 ml methanol. The solution was filtered through a 0.20 μm filter (Minisart, Sartorius) and the clear filtrate was used for HPLC assay. β-Carotene concentration was determined using the method described by Lefsrud *et al*. (2007). A HPLC unit model 1200 with photo diode array detection (Agilent Technologies) was used. Samples were analyzed using a Water spherisorb S5 ODS2 4.6

mm ID × 250 mm column with Waters S5ODS2 guard column compartment. The column was maintained at 16°C using a thermostatic column compartment. Eluents were A: 75% acetronitrile, 20% methanol, 5% hexane, 0.05% BHT and 0.013% triethylamine and B: 50% acetronitrile, 25% tetrahydrofuran, 25% hexane and 0.013% triethylamine. The flow rate of the eluents was 0.7ml/min and the gradient was 100% eluent A for 30 min; a change to 50% A and 50% B over the next 2 min; a change to 100% B over the next 2 min; followed by a change to 50% A and 50% B for the next 2 min. The eluent was instantly returned to 100% A for 10 min before the next injection. Components eluted from a 20 µl injection were detected at a λ_{max} of 452 nm.

Determination of ascorbic acid

One milliliter of juice sample was extracted with 2 ml of 80% methanol (Lab-Scan), 19.9% distill water (Lab-Scan) and 0.1% hydrochloric acid (Merck, Germany) and then stirred for 2 h. Subsequently, the mixture was diluted with 2 ml of 5% meta-phosphoric acid (Sigma-Aldrich, USA) and filtered through a 0.20 µm filter (Minisart, Sartorius). The clear filtrate was used for HPLC assay. Ascorbic acid content was determined using the method described by Rodriguez-Comesana *et al.* (2002). HPLC instrument used was the same condition as above, using acetic acid (Merck) in deionized water (0.1 M) as the mobile phase with a λ_{max} of 250 nm. The flow rate of the mobile phase was 1.5 ml/min and at single run isocratic mode at room temperature with 20 µl injection volume. L-ascorbic acid (Sigma-Aldrich) was dissolved in sulfuric acid (pH 2.2; Merck), to obtain concentrations of 5-500 µg/ml from the calibration curve.

Determination of total phenol compounds

The total phenolic contents were determined using the modified Folin-Ciocalteu assay described by Zainol *et al.* (2003). An aliquot of 1 ml of the extract was added to 10 ml of deionized water and mixed with 2 ml of Folin-Ciocalteu phenol reagent (Fisher Scientific). The mixture was then allowed to react for 5 min and 2 ml of the saturated sodium carbonate solution (Sigma) was added to the mixture. The blue complex was then determined at a λ_{max} of 680 nm with gallic acid (Fluka, Switzerland) as a standard. The total phenolics content of the extract was expressed as milligram gallic acid equivalents per 100 ml of sample (mg GAE/100 ml).

Ferric-reducing antioxidant power (FRAP) assays

The ability to reduce ferric ions was measured using a method described by Benzie and Stain (1996) with some modifications. An aliquot (l ml) of extract juice was added to 10 ml deionized water and 3 ml of FRAP reagent (10:1:1 of 300 mM sodium acetate buffer at pH 3.6, 10 mM 2,4,6-tripyridyls-triazine solution and 20 mM $FeCl_3.6H_2O$ solution) and the mixture was incubated in a water bath at 37°C for 30 min. Absorbance was measured at a λ_{max} of 593 nm. The antioxidant capacity based on the ability to reduce ferric ions of the extract was expressed as µmol Fe (II) per liter of the sample (µM $FeSO_4$). The measurement of FRAP was performed in disposable cuvettes using a UV–Visible spectrophotometer model Lambda Bio-20 (Perkin Elmer, USA).

Microbiological assessments

The assessments of standard plate count, yeasts and moulds in fresh and processed pennywort juices followed the method of the US Food & Drug Administration (BAM, 2001).

Statistical analysis

All data were the means of triplicate determinations with individual duplication (n = 6). Analysis of variance (ANOVA) was carried out using SPSS Version 15.0, and determination of significant differences among treatment means was done by Duncan's multiple range tests ($P < 0.05$).

Results and Discussion

Proximate compositions

The proximate compositions of edible portion (100 g) of pennywort leaves are shown in Table 1. Several researchers also reported the compositions of this plant. For instance, Tee *et al.* (1997) reported that the analysis of pennywort leaves in Malaysia gave the following values: 88% moisture, 2% crude protein, 0.2% fat, 1.6% fiber, 6.7% carbohydrate, 1.8% ash and energy 37 kcal/100 g. Furthermore, it also contained various minerals and vitamins including 391 mg/100 g potassium, 171 mg/100 g calcium, 32 mg/100 g phosphorus, 21 mg/100 g sodium and 5.6 mg/100 g iron as well as 2.65 mg/100 g carotene, 0.44 mg/100 g retinol equivalents, 48.5 mg/100 g ascorbic acid, 0.19 mg/100 g vitamin B2, 0.1 mg/100 g niacin and 0.09 mg/100 g vitamin B1. Similar results were reported by Jamil *et al*. (2007) with Indian pennywort.

Color parameters

The chroma and hue angle parameters of pasteurized and pressurized pennywort juices are shown in Table 2. The chroma of pressurized juice was significant higher ($P < 0.05$) than pasteurized and fresh juices in order, while fresh juice showed highest in hue angle than pressurized and pasteurized juice. Upon storage at 4°C for 4 months, both processed

Table 1. Proximate compositions of fresh pennywort

Proximate compositions	g/100 g
Moisture content	90.92±0.03
Ash content	1.44±0.06
Crude protein	1.80±0.05
Fat	0.48±0.04
Crude fiber	1.21±0.05
Carbohydrate	4.15±0.11
Total soluble solids (°Brix)	5.27±0.21

Each data point is the average of triplication.

Table 2. Color parameters and pH values of fresh and processed pennywort juices kept at 4ºC for 4 months

Storage times (months)	Fresh juice	Pressurized juice	Pasteurized juice
*Chroma, C**			
Initial state	5.84±0.02^{C}	11.06±0.05^{A,a}	7.02±0.03^{B,a}
1		11.05±0.07^{a}	7.02±0.05^{a}
2		11.04±0.04^{a}	7.00±0.04^{a}
3		11.01±0.05^{a}	6.65±0.08^{a}
4		11.02±0.03^{a}	6.68±0.03^{a}
Hue angel, H°			
Initial state	102.91±0.38^{A}	99.39±0.20^{B,a}	79.26±0.17^{C,a}
1		99.36±0.23^{a}	79.19±0.56^{a}
2		99.20±0.41^{a}	79.27±0.33^{a}
3		99.30±0.15^{a}	79.20±0.44^{a}
4		98.95±0.44^{a}	79.17±0.06^{a}
pH value			
Initial state	5.62±0.05^{A}	5.56±0.02^{A,a}	5.50±0.02^{B,a}
1		5.56±0.02^{a}	5.51±0.01^{a}
2		5.53±0.05^{a}	5.50±0.01^{a}
3		5.54±0.02^{a}	5.47±0.03^{a}
4		5.53±0.01^{a}	5.48±0.02^{a}

Means with same letters within a column of each quality are significantly different (P > 0.05). Means with the same capital letters within a row of initial storage are significantly different (P > 0.05) in comparison with fresh juice. Data are expressed as means ± standard deviation (n = 6).

pennywort juices exhibited no changes (P > 0.05) in chroma and hue angle. Ahmed *et al.* (2005) observed that the color parameters including chroma and hue angle of processed mango pulps remained constant during storage indicating the pigment stability, while Choi *et al*. (2002) found that the hue angle increased in blood orange juice and chroma decreased during refrigerated storage for 7 weeks.

pH values

The pH of pasteurized pennywort juice significantly changed (P < 0.05) compared to fresh and pressurized juices (Table 2). This might be due to thermal acceleration of hydrogen ion concentration by food decomposition processes, such as hydrolysis (Queiroz *et al*., 2010). Chaikham and Apichartsrangkoon (2012a) found that pH and total acidity of pasteurized juices significantly decreased as compared to fresh and pressurized juices. During 4 months of storage, these processed juices exhibited non-significant changes (P > 0.5) in pH value. This result is in accord with Esteve *et al.* (2005)'s report, which kept orange juice at 4 and 10ºC for 6 weeks and found that the variations in pH observed in the juices did not become statistically significant.

Asiaticoside, madecassoside and β-carotene

Asiaticoside and madecassoside have been reported to possess wound healing, anti-ulcer, antioxidant, anti-inflammatory activities (Shukla *et al*., 1999; Guo *et al.*, 2004), cardioprotective (Bian *et al*., 2008; Cao *et al*., 2010) and neuroprotective effects (Shinomol and Muralidhara, 2008; Dhanasekaran *et al*., 2009; Haleagrahara and Ponnusamy, 2010), while the nutrition of β-carotene also has a significant role in the prevention of many chronic diseases such as cardiovascular diseases, cancers and degenerative brain diseases (Voutilainen *et al*., 2006).

Table 3. Main glycosides and β-carotene concentrations of fresh and processed pennywort juices kept at 4°C for 4 months

Storage times (months)	Fresh juice	Pressurized juice	Pasteurized juice
Asiaticoside (mg/100 ml)			
Initial state	4.49±1.86^{A}	4.30±1.95^{A,a}	3.55±0.75^{A,a}
1		4.03±1.86^{a}	3.28±1.94^{a}
2		3.91±0.96^{a}	3.33±1.05^{a}
3		4.18±2.04^{a}	3.25±1.97^{a}
4		3.99±1.56^{a}	3.35±2.17^{a}
Madecassoside (mg/100 ml)			
Initial state	3.80±0.41^{A}	3.77±1.19^{A,a}	3.32±1.29^{A,a}
1		3.62±1.46^{a}	3.28±1.56^{a}
2		3.70±0.73^{a}	3.30±0.48^{a}
3		3.55±1.50^{a}	3.31±1.46^{a}
4		3.55±1.66^{a}	3.26±0.88^{a}
β-Carotene (mg/100 ml)			
Initial state	2.50±0.65^{A}	2.52±0.70^{A,a}	2.48±0.59^{A,a}
1		2.32±0.25^{a}	2.43±0.68^{a}
2		2.23±0.32^{a}	2.23±0.42^{a}
3		2.10±0.21^{a}	1.97±0.31^{a}
4		2.04±0.11^{b}	1.96±0.19^{b}

Means with the same letters within a column of each quality are none significantly different (P > 0.05). Means with the same capital letters within a row of initial storage are none significantly different (P > 0.05) in comparison with fresh juice. Data are expressed as means ± standard deviation (n = 6).

In this study, we found that asiaticoside, madecassoside and β-carotene were relatively stable (P > 0.05) on pressurization and pasteurization in comparison to fresh sample (Table 3). Asiaticoside and madecassoside are more stable than phenolic compounds (Apichartsrangkoon *et al*., 2012), while β-Carotene is also known to be stable during heat treatment (Elkins, 1979). The amounts of asiaticoside and madecassoside still remained during storage with no significant losses (P > 0.05) (Table 3). This finding is in agreement with Kim *et al.* (2001)'s study, which reported that glycosides such as asiaticoside was stable in micellar formulations with no significant changes during storage for 60 days at room temperature.

β-Carotene showed small degradation percentages around 20% after 4 months of storage when compared to the freshly processed juices of day 0 (Table 3), indicating that this component was less susceptible to oxidation during storage (Jacobo-Velázquez and Hernández-Brenes, 2012). Pérez-Gálvez and Mínguez-Mosquera (2001) revealed that the stability of some carotenoids such as lutein and β-carotene was related to the chemical structure of the carotenoids and was also affected by the nature and concentration of other oxidizing molecules in the

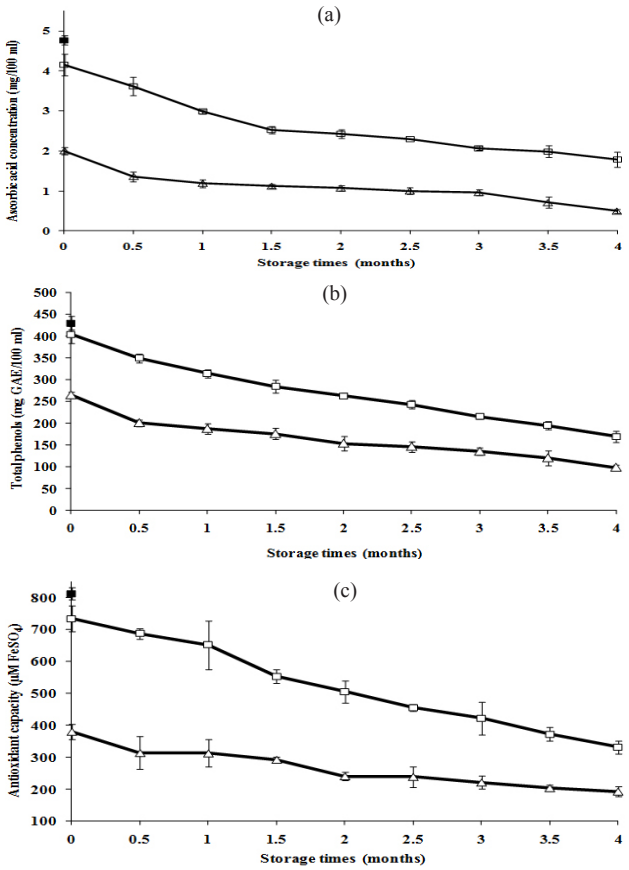

Figure 1 Phytochemical properties of fresh pennywort juice (■), pressurized pennywort juice (□) and pasteurized pennywort juice (△) kept at 4°C for 4 months; (a) ascorbic acid, (b) total phenols and (c) antioxidant capacity (FRAP assay). Each data point is the average of triplication.

product.

Ascorbic acid

Ascorbic acid constitutes the most important antioxidant compound reacting instantaneously with free radicals. As shown in Figure 1(a), an amount of ascorbic acid significantly reduced ($P < 0.05$) on pasteurization and pressurization when compared with fresh juice, in particular loss in pasteurized juice. However, it must be noted that in the present study high pressure processing was much more effective than thermal processing in retaining ascorbic acid levels with over 75%, while in pasteurized juice retained around 32%. Ascorbic acid is easily destroyed by oxidation at high temperatures. 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; Wolbang *et al.*, 2008). Gil-Izquierdo *et al.* (2002) pasteurized orange juice at 95°C for 30 sec and found that ascorbic acid decreased from 150.1 to 143.7 mg/L. Similar result was observed by Chaikham and Apichartsrangkoon (2012a, b) with pressurized (500 MPa/25°C/20 min) and pasteurized (90°C/2 min) longan juices.

In this study, it was seen that high pressure processing could not completely preserve ascorbic acid in pennywort juice. Nuñez-Mancilla *et al.* (2012) observed that ascorbic acid in strawberry (*Fragaria vesca*) pressurized at 100 and 300 MPa for 10 min were significantly lower than in fresh samples, remaining around 96%. Hsu *et al.* (2008) found that ascorbic acid content in pressurized tomato juices (300, 400 and 500 MPa/25°C/10 min) significantly decreased when compared to untreated juice. Patras *et al.* (2009) pressurized strawberries purées at 400-600 MPa and 10-30°C for 15 min, and found that pressurization could be preserved ascorbic acid around 94% in the samples. Similar result was seen by Barba *et al.* (2013) and Sanchez-Moreno *et al.* (2003) with pressurized blueberry juice and orange juice, respectively. Chaikham and Apichartsrangkoon (2012a) stated that ascorbic acid reduction could be induced by enzyme activation during pressurization. Nagy (1980) and Talcott *et al.* (2003) suggested two main reasons for ascorbic acid reduction: oxidative reactions by enzymes such as ascorbic acid oxidase, cytochrome oxidase, polyphenoloxidase and peroxidase found in fruits and vegetables; and aerobic and anaerobic non-enzymatic reactions. High pressure affects the secondary, tertiary and quaternary structures of proteins; such conformational changes can enhance enzyme activity by uncovering active sites and consequently facilitate catalytic conversion.

Ascorbic acid was increasingly lost ($P < 0.05$) during the storage for 4 months at 4°C. However, the total amount of ascorbic acid retained in pressurized juice was still higher than pasteurized products in every respect, suggesting that ascorbic acid, a heat-sensitive component tends to decline by heat than pressure. Esteve *et al.* (2005) found that the ascorbic acid concentration of pasteurized orange juices (77°C/20 sec) significantly decreased during chilled storage at 4 and 10°C. Choi *et al.* (2002) also observed that during storage at 4.5°C for 7 weeks, ascorbic acid in pasteurized blood orange (*Citrus sinensis*) juice (90°C/90 sec) apparently diminished with an increase the storage time; more than 50% was lost within 3 weeks of storage and completely degraded after 5 weeks of storage. Besides pasteurized products, Cao *et al.* (2012) displayed that ascorbic acid in pressurized cloudy and clear strawberry juices (600 MPa/43°C/4 min) kept at 4 and 25°C gradually reduced with an increasing the storage time. Decreases of 48.91% and 89.21% for the ascorbic acid in clear juices and of 39.61% and 89.71% in cloudy juices were seen after 6 months of storage at 4 and 25°C,

respectively. Similar result was reported by Chaikham and Apichartsrangkoon (2012b) with pressurized longan juice (500 MPa/25°C/4 min) stored at 4°C for 4 weeks.

Total phenols and antioxidant capacity

Losses of total phenols and antioxidant capacity (FRAP assay) in pasteurized pennywort juice were significantly higher ($P < 0.05$) than those in pressurized juice in comparison to fresh juice, as shown in Figures 1(b) and 1(c). Phenolic compounds appeared to be more sensitive to thermal processing. Keenan *et al.* (2010) found that total phenolic compounds in pasteurized smoothies (70°C/>10 min) were lower than fresh or pressurized smoothies at 450 MPa and 37.5°C for a holding time of 1, 3 or 5 min. Moreover, Patras *et al.* (2009) reported that pressurized strawberry purées treated at 400 MPa had significantly lower antioxidant capacities when compared to unprocessed samples.

In this study, total phenols and antioxidant capacity of both processed pennywort juices apparently diminished ($P < 0.05$) throughout the storage period. Keenan *et al.* (2010) reported that chill storage at 4°C for 30 days resulted in a significant reduction in total antioxidant activity (DPPH and FRAP assays) and total phenols for all pressurized smoothies (450 MPa/37.5°C/1-3 min). In general, both processed juices exhibited expected degradation during storage. During storage, oxygen can diffuse into the juices from the headspace present in the package. The formation of these free radical species will oxidize the antioxidant compounds, degrading the antioxidant activity and reducing their quality attributes over time (Zerdin *et al*., 2003). In addition, Oey *et al*. (2008) stated that high pressure processing minimally affects qualities of fruit products, which they will change during storage due to coexisting chemical reactions such as oxidation and biochemical reactions when endogenous enzymes such as polyphenol oxidase and peroxidase are incompletely inactivated. Chaikham and Apichartsrangkoon (2012a) found that polyphenol oxidase was completely inactivated in pasteurized longan juices, whereas in pressurized juices at 300 and 500 MPa, the activities were more than 100% and 95-99%, respectively.

Microbiological quality

The standard plate count, yeasts and moulds in Table 4 indicated that general microbes in processed pennywort juices were satisfactorily eliminated ($P < 0.05$). The microbiological qualities of the juices during storage at 4ºC for 4 months showed pleasing results. Yeasts, moulds, *Staphylococcus aureus*, *Clostridium perfingens* and *Escherichia coli* were not detected throughout the keeping period (data not shown). These qualities of both processed juices were in accord with Thai industrial standard (2003) for processed pennywort juice.

Table 4. General microbiological qualities of fresh and processed pennywort juices kept at 4°C for 4 months

Storage times (months)	Fresh juice	Pressurized juice	Pasteurized juice
Standard plate count (CFU/ml)			
Initial state	10,786±635^{A}	nd^{B,d}	nd^{B,c}
1		nd^{d}	nd^{c}
2		21±8^{c}	nd^{c}
3		152±29^{b}	98±14^{b}
4		225±35^{a}	182±37^{a}
Yeasts & moulds (CFU/ml)			
Initial state	142±45^{A}	nd^{B,a}	nd^{B,a}
1		nd^{a}	nd^{a}
2		nd^{a}	nd^{a}
3		nd^{a}	nd^{a}
4		nd^{a}	nd^{a}

Means with the same letters within a column of each quality are none significantly different ($P > 0.05$). Means with the same capital letters within a row of initial storage are none significantly different ($P > 0.05$) in comparison with fresh juice. nd = not detected. Data are expressed as means ± standard deviation (n = 6).

Conclusion

In this study it was found that pressurization could preserve various bioactive components including ascorbic acid, total phenolic compounds and antioxidant capacity (FRAP assay), superior than pasteurization. Asiaticoside, madecassoside and β-carotene were relatively stable on the processing and storage. The concentrations of total phenolic compounds and ascorbic acid as well as antioxidant capacity in the juice apparently diminished with an increasing the storage times; however these components still retained in pressurized juice higher than pasteurized juice. The microbiological assessment showed that standard plate count, yeasts and moulds in both processed juices were satisfactorily eliminated.

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