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Comparison of bioactive components in fresh, pressurized, pasteurized and sterilized pennywort (*Centella asiatica* L.) juices

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The biologically active constituents of pennywort juice were analyzed by HPLC. The juice extract contained the bioactive glycosides, including asiaticoside and madecassoside. Antioxidant properties of juices were determined in terms of ferric-reducing antioxidant power assay, total polyphenol, β -carotene and ascorbic acid contents. After processing, asiaticoside, madecassoside and β -carotene in the extracted juice were relatively stable with no significant losses occurring. Pressurization could significantly retain ascorbic acid, polyphenols and antioxidant capacity than those pasteurization or sterilization. For storage assessment, asiaticoside in the processed juices was relatively stable during 4 months storage. Losses of ascorbic acid in the pressurized juice during storage were greater than in pasteurized and sterilized juice. However, the total amount of ascorbic acid retained in pressurized juice was still higher than those thermal-treated products.

Keywords: high pressure processing; pasteurization; sterilization; pennywort juice; bioactive compounds

1. Introduction

Pennywort (*Centella asiatica*) is one of the local Asian herbs that is claimed to possess various physiological effects as wound and ulcer healing which acts by promoting fibroblast proliferation and collagen synthesis [1]. In Chinese Pharmacopoeia, it is used as an antipyretic, diuretic and antidote in the treatment of icterus, heat stroke, diarrhea, ulcerations, eczema and traumatic diseases [2]. In Thai traditional recipes, it is regularly used as a vegetable and tonic [3]. The active principles of pennywort include asiatic acid, asiaticoside, madecassic acid and madecassoside which are pentacyclic triterpenes, found to reduce chronic venous insufficiency [4] and have wound-healing properties. The extracts of pennywort also contain vitamin E, ascorbic acid, β -carotene, flavonoids and other polyphenols [5].

To preserves all these phytochemical constituents, ultra-high pressure, a nonthermal processing, is preferred to be superior to the conventional thermal process. Jannok et al. [6] pressurized pennywort juice 400 and 600 MPa/30 min and 50°C/20 min and found that residual polyphenol and

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chlorophyll were higher than those samples heated at 90°C/15 s. In addition, Chaimoon et al. [7] pressurized longan in syrup (23°Brix) with pressure 400 and 500 MPa/30 min and 40°C/20 min and found that the residual ascorbic acid in the products was in the range of 74–79% in comparison to 46% pasteurized sample (93°C/3 min). Panyada et al. [8] also reported identical effects of pressure on carotenoid contents of carrot juice. This research is proposed to develop a high-quality pennywort juice using an ultra-high pressure technique to minimize the loss of essential bioactive compounds in comparison with fresh juice as well as other pasteurized and sterilized juices.

2. Materials and methods

2.1. Raw material and preparation

Pennywort with commercial maturity (2–4 months) was freshly harvested, from high land, Chiangmai, Thailand. Its leaves and petioles were extracted with water 1:1 w/v, using the method described by Jannok et al. [6]. The 100 mL extract was filled into a laminated pouch before pressurization. For pasteurization and sterilization, 150 mL extracts were filled into a retort pouch before the treatment conditions. The packed samples were subjected to either high pressure (400 MPa/20 min at <30°C) in a high pressure rig (Food Lab; Stansted Fluid Power, Essex, UK.) or pasteurization (90°C/3 min) or sterilization (121°C/4 min) in a spray retort (Saha Thai Factory, Model RT-6/FT). For shelf-life predication, pressurized and pasteurized juices were kept at 4°C as well as sterilized juice was kept at 40°C (modification of accelerated condition for room temperature). The products were determined for chemical quality at every 15 days intervals throughout the storage period of 4 months.

2.2. Biochemical analysis

2.2.1. Analysis of asiaticoside, madecassoside, asiatic acid and madecassic acid

Sample preparation for the analysis of asiaticoside and madecassoside asiatic acid and madecassic acid was carried out using a modified method described by Inamdar et al. [9]. One milliliter of pennywort juice was mixed with 1 mL of 90% methanol and stirred for 2 h at room temperature. The solution was filtered through a 0.20 µm filter (Minisart, Sartorius) and the clear filtrate was used for HPLC assay. The Agilent 1050 HPLC system consisted of a pressure flow pump and a UV spectrophotometric detector. Chromatographic separation was performed with YMC S5 ODS-AM 5 µm, 4.6 mm ID × 250 mm column with Waters S5ODS2 guard column compartment. C18 Column with water–acetonitrile as the mobile phase with a $\lambda_{\max}$ 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 determined from the curves. The concentrations of standard components in the juice were determined by a standard addition method. Pennywort juice samples were prepared by the addition of the stock solution of standard components to obtain a concentration of 10–400 µg/mL juice.

2.2.2. Analysis of ascorbic acid

One milliliter of juice sample was mixed with 2 mL of 80% methanol, 19.9% water, 0.1% hydrochloric acid and stirred for 2 h. It was then diluted with 2 mL of 5% meta-phosphoric acid. The solution was filtered through a 0.20 µm filter (Minisart, Sartorius) and the clear filtrate

was used for HPLC assay. Ascorbic acid content was determined using the method described by Rodriguez-Comesana et al. [10]. HPLC instrument used was the same condition as above, using acetic acid in water (0.1 M) as the mobile phase with a $\lambda_{\max}$ 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 μ L injection volume. L-ascorbic acid was dissolved in sulfuric acid, pH 2.2, to obtain concentrations of 5–500 μ g/mL from the calibration curve.

2.2.3. Analysis of β -carotene

Ten milliliters of the extracted juice were mixed with 20 mL of cold acetone. Petroleum ether (40–60°C) (20 mL) was then added, and the organic layer was separated and washed with 40 mL phosphate buffer, pH 7. The separated organic layer was stirred overnight with 20 mL of 10% potassium hydroxide in methanol and washed successively with 40 mL water, 40 mL phosphate buffer, 40 mL saturated sodium chloride and dried with sodium sulfate. The solvent was evaporated and the residue was dissolved in 5 mL methanol. The solution was filtered through a 0.20 μ m filter (Minisart, Sartorius) and the clear filtrate was used for HPLC assay.

β -Carotene content was determined using the method described by Lefsrud et al. [11]. A HPLC unit with photo diode array detection (model 1200, Agilent Technologies) was used. Samples were analyzed using a Waters spherisorb S5 ODS2 4.6 mm ID $\times$ 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% acetonitrile, 20% methanol, 5% hexane, 0.05% BHT and 0.013% triethylamine and B: 50% acetonitrile, 25% tetrahydrofuran, 25% hexane and 0.013% triethylamine. The flow rate of the eluents was 0.7 mL/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 μ L injection were detected at $\lambda_{\max}$ 452 nm.

2.2.4. Total polyphenol determination

The total phenolic content was determined using the modified Folin-Ciocalteu assay described by Zainol et al. [12]. 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. The mixture was then allowed to react for 5 min and 2 mL of the saturated sodium carbonate solution was added to the mixture. The blue complex was then determined at $\lambda_{\max}$ 680 nm with gallic acid as a standard. The total phenolics content of the extract was expressed as milligram gallic acid equivalents per 100 mL of sample.

2.2.5. Ferric-reducing antioxidant power (FRAP) assays

The ability to reduce ferric ions was measured using a method described by Benzie and Stain [13], with some modifications. An aliquot (1 mL) of extract juice was added to 10 mL 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-tripyridyl-s-triazine solution and 20 mM $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ solution) and the mixture was incubated in a water bath at 37°C for 30 min. Absorbance was measured at $\lambda_{\max}$ 593 nm. The antioxidant capacity based on the ability to reduce ferric ions of the extract was expressed as μ mol Fe (II) per liter of the sample. The measurements of total phenol and FRAP were performed in disposable cuvettes using a UV-Visible spectrophotometer model Lambda Bio-20 Perkin Elmer (USA).

2.2.6. Statistical analysis

One-way ANOVA with Tukey's *post hoc* test was used to identify significant difference between samples. Results with $p \leq 0.05$ were considered to be statistically significant. All data were performed in triplicate.

3. Results and discussion

Comparison of bioactive components and antioxidant activity of fresh and processed pennywort juices

Bioactive components and the antioxidant activity of fresh and processed pennywort juices are summarized in Table 1. The major compounds of extracted pennywort juice were the glycosides (asiaticoside and madecassoside) which were well separated due to the large difference in polarity of the glycosides. However, the results in this study demonstrate that the pennywort juice which contained high amount of active compounds or vitamin also possessed high level of antioxidant activity. In addition, fresh pennywort also contained significant concentrations of total phenols, ascorbic acid and β -carotene. The total antioxidant capacity of the fresh pennywort juice, determined by FRAP assay, was $913.51 \mu\text{mol FeSO}_4/\text{L}$ and was comparable to the value obtained by Kormin [14], who found $860 \mu\text{M FeSO}_4/\text{L}$. All these quantities were higher than the values reported by Ali [15] ($300 \mu\text{mol FeSO}_4/\text{L}$).

After processing, asiaticoside, madecassoside and β -carotene were relatively stable with no significant losses occurring ($p > 0.05$), whereas asiatic acid and madecassic acid were markedly lost in processing. Madecassoside and asiaticoside are triterpene alcohols and therefore are more stable than phenolic compounds. β -Carotene is also known to be stable during heat treatment [16]. Pressurization could significantly retain ascorbic acid, total phenol and antioxidant capacity than those pasteurization or sterilization ($p \leq 0.05$). High pressure led to loss of ascorbic acid, total phenols and antioxidant capacity for 15.51%, 60.98% and 49.36% respectively, while pasteurization caused losses 64.36%, 76.15% and 59.87% respectively, as well as sterilization the losses were greater at 82.39%, 84% and 63.51%, respectively. However, the reduction of ascorbic acid and total phenolic contents during these processing makes it probable that oxidation products of ascorbic acid and phenols with carbonyl groups in their structures were formed during processing, and they reacted with amino groups. It is known that oxidation products of ascorbate react one hundred times faster with amine groups than glucose does [17]. Total antioxidant activity,

Table 1. Bioactive components and antioxidant activity of fresh and processed pennywort juices.

Compounds or property	Processed pennywort juices			
	Fresh juice	Pressurized	Pasteurized	Sterilized
Asiaticoside ^a (mg/100 mL)	4.49 ± 1.86	4.28 ± 0.66	3.57 ± 1.36	3.16 ± 1.45
Madecassoside ^a (mg/100 mL)	3.80 ± 0.41	3.70 ± 1.08	3.20 ± 0.33	2.87 ± 1.02
Asiatic acid (mg/100 mL)	2.69 ± 1.81	nd	nd	nd
Madecassic acid (mg/100 mL)	1.66 ± 1.12	nd	nd	nd
Ascorbic acid (mg/100 mL)	$4.77 \pm 0.42\text{a}$	$4.03 \pm 0.63 \text{ a}$	$1.70 \pm 0.10 \text{ b}$	$0.84 \pm 0.03 \text{ b}$
β -Carotene ^a (mg/100 mL)	2.50 ± 0.65	2.51 ± 0.79	2.39 ± 0.64	2.26 ± 0.52
Total phenol content (mg/100 mL)	$988.54 \pm 45.16 \text{ a}$	$385.70 \pm 16.25 \text{ b}$	$235.75 \pm 20.16 \text{ c}$	$158.13 \pm 8.60 \text{ d}$
FRAP ($\mu\text{M FeSO}_4/\text{L}$)	$913.51 \pm 18.61 \text{ a}$	$462.58 \pm 64.79 \text{ b}$	$366.62 \pm 23.56 \text{ c}$	$333.33 \pm 32.01 \text{ c}$

Note: Means with different letters within a row of each quality are significantly different ($p \leq 0.05$) (Tukey's). Values are mean $\pm$ SD ($n = 3$).

^aNon-significant difference, nd : not detected.

Table 2. Storage stability of asiaticoside contents in processed pennywort juices (mg/100 mL).

Storage (months)	Processed pennywort juices		
	Pressurized*	Pasteurized ^a	Sterilized ^a
0.0	4.28 ± 0.66	3.57 ± 1.36	3.16 ± 1.45
0.5	4.03 ± 0.97	3.36 ± 0.67	3.14 ± 1.19
1.0	3.82 ± 1.60	3.16 ± 0.80	3.08 ± 0.64
1.5	3.85 ± 2.31	3.36 ± 0.92	3.00 ± 1.47
2.0	3.80 ± 1.23	3.27 ± 1.02	3.10 ± 1.15
2.5	3.84 ± 1.28	3.17 ± 1.01	3.01 ± 0.80
3.0	3.83 ± 0.74	3.16 ± 1.26	3.02 ± 1.38
3.5	3.73 ± 0.90	3.41 ± 1.05	2.92 ± 1.52
4.0	3.84 ± 1.28	3.24 ± 0.82	2.94 ± 1.16

*Non-significant difference ($p > 0.05$) (Tukey's). Values are mean ± SD ($n = 3$).

Table 3. Storage stability of ascorbic acid contents in processed pennywort juices (mg/100 mL).

Storage (months)	Processed pennywort juices		
	Pressurized	Pasteurized	Sterilized
0.0	4.03 ± 0.63 a	1.70 ± 0.09 a	0.84 ± 0.02 a
0.5	3.41 ± 0.03 b	1.29 ± 0.08 b	0.80 ± 0.01 a
1.0	2.47 ± 0.04 c	1.16 ± 0.01 bc	0.78 ± 0.01 a
1.5	2.33 ± 0.03 c	1.07 ± 0.02 c	0.77 ± 0.04 a
2.0	2.13 ± 0.09 cd	1.03 ± 0.02 cd	0.76 ± 0.02 ab
2.5	1.92 ± 0.18 cd	0.98 ± 0.01 cd	0.74 ± 0.01 ab
3.0	1.68 ± 0.04 d	0.94 ± 0.02 d	0.71 ± 0.04 ab
3.5	1.63 ± 0.04 d	0.90 ± 0.02 d	0.70 ± 0.03 b
4.0	1.50 ± 0.07 d	0.87 ± 0.02 d	0.69 ± 0.05 b

Means with different letters within a column of each component are significantly different ($P \leq 0.05$) (Tukey's). Values are mean ± S.D ($n = 3$)

polyphenols, ascorbic acid and anthocyanin content were better retained in pressurized (400–600 MPa/20°C/15 min) strawberry and blackberry purees compared to heated (70°C/20 min) sample [18], whereas phenolic compounds of tomato and carrot puree were unaffected by both treatment conditions [19].

Stability of asiaticoside and ascorbic acid on storage

Asiaticoside in the processed juices similar to madecassoside was relatively stable during storage with no significant changes for a period of 4 months at 4°C and at 40°C for pressurized, pasteurized and sterilized samples, respectively (Table 2). This is consistent with the findings of Kim et al. [20], who reported that asiaticoside was stable in micellar formulations with no significant changes during storage for 60 days at room temperature. Significant losses of ascorbic acid (Table 3) in pressurized juice during storage at 4°C ($p \leq 0.05$) were greater than in pasteurized juice stored at 4°C and in sterilized juice at 40°C. However, the total amount of ascorbic acid retained in pressurized juice was still higher than those thermal-treated products in every respect, suggesting that ascorbic acid, a heat-sensitive component tends to deteriorate by heat than pressure. Polydera et al. [21] work also supported the total antioxidant activity of pressurized (600 MPa/40°C/4 min) and pasteurized (80°C/60 s) Navel orange juice decreased during storage mainly due to ascorbic acid loss. Vega-Galvez [22] demonstrated that after 35 days of storage, pressurized aloe vera gel by low pressure (300 MPa/3 min) retained better ascorbic acid and total phenolic compounds than by higher pressure (500 MPa/3 min), whereas 2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity was well maintained for those pressurization at only high pressure level.

4. Conclusion

After processing, asiaticoside, madecassoside and β -carotene were relatively stable with no significant losses occurring, whereas asiatic acid and madecassic acid were markedly lost during processing. Pressurization could significantly retain ascorbic acid, total phenols and antioxidant capacity than those pasteurization or sterilization. Asiaticoside in processed juice was relatively stable for 4 months storage. Losses of ascorbic acid in pressurized juice during storage were greater than those in pasteurized and in sterilized juice. However, the total amount of ascorbic acid retained in pressurized juice was still higher than those thermal-treated products.

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