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Effects of high pressure and thermal processing on phytochemical, color and microbiological qualities of herbal-plant infusion

[1*]Chaikham, P., [2]Worametrachanon, S. and [3]Apichartsrangkoon, A.

[1]*Division of Food Science and Technology, Faculty of Science and Technology, Phranakhon Si Ayutthaya Rajabhat University, Phranakhon Si Ayutthaya 13000, Thailand*
[2]*Division of Food Science and Technology, Faculty of Agro-Industry, Chiang Mai University, Chiang Mai 50100, Thailand*
[3]*Postharvest Technology Research Institute, Chiang Mai University, Chiang Mai 50200, Thailand*

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Abstract

The objective of the present study was the evaluation of the bioactive compounds, physical properties and microbiological qualities of herbal-plant infusion as affected by high pressure processing (400 or 500 MPa at 25°C for 15 or 30 min) and thermal processing (90°C for 1 - 3 min). It was found that pressurized infusions comprised of high concentrations of gallic acid, ellagic acid, total polyphenols, ascorbic acid, asiatic acid and gamma oryzanol compared to the pasteurized samples. Color parameters displayed that pressurization could conserve the natural color of the products better than pasteurization. The microbiological assessments showed that total plate counts, yeasts-moulds and fecal coliforms in both processed infusions were acceptably eliminated.

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Introduction

Currently, consumers are demanding new healthy beverages, which containing high bioactive components and antioxidant capacity. Herbal-plant infusion is a new product prepared from fresh pennywort, dried longan and purple rice extracts. Pennywort (*Centella asiatica* L.) is traditionally used as a medicinal herbs and alternative medicine for treating many kinds of diseases, since it contains several bioactive triterpenes such as asiaticoside, asiatic acid, madecassoside and madecassic acid (Hashim, 2011). Longan fruit (*Euphoria longana* Lam.) also contains significant amounts of bioactive compounds such as corilagin, ellagic acid and gallic acid (Rangkadilok *et al*., 2005; Chaikham and Apichartsrangkoon, 2012a,b). This fruit has been used in the traditional Chinese medicinal formulation, serving as an agent in relief of neural pain and swelling (Yang *et al.*, 2011). Recently, the extract from longan fruit has exhibited excellent antioxidant ability and good anti-tyrosinase and anticancer activities (Rangkadilok *et al.*, 2007; Prasad *et al*., 2009; Yang *et al.*, 2011). Moreover, several studies showed that gamma oryzanol which found in purple rice (*Oryza sativa* L.) exhibits antioxidant properties, reduction of tumor incidence, inhibition of platelet aggregation and anti-inflammatory activity as well as cholesterol-lowering effects (Rong *et al*., 1997; Gerhardt and Gallo, 1998; Wilson *et al.*, 2002; Lerma-Garcia *et al.*, 2009).

Thermal processing, i.e. pasteurization and sterilization, is widely used for self-life prolongation of food products, on the other hand this technology is not able to preserve their natural color, flavor and nutrients (Barba *et al*., 2013). Thus, non-thermal technologies such as high pressure processing are preferred to maintain the sensory attributes and nutritional values of the products (Viljanen *et al.*, 2011; Chaikham and Apichartsrangkoon, 2012a, b; Apichartsrangkoon *et al*., 2012, 2013). Chaikham and Apichartsrangkoon (2012a) pressurized and pasteurized longan juices at 300 - 500MPa at 25°C for 20 min and at 90°C for 2 min, respectively. They found that the pressurized juices were brighter and more transparent than pasteurized or untreated juices. Additionally, total phenolics and antioxidant capacity were relatively stable on pressurization but apparently reduced on pasteurization. Apichartsrangkoon *et al.* (2012) observed that pressurization (400 MPa at <30°C for 20 min) preserved several bioactive compounds, such as asiaticoside, madecassoside, β-carotene, ascorbic acid, total phenols and antioxidant capacity in pennywort juice, better than pasteurization (90°C for 3 min) or sterilization (121°C for 4 min).

This study was aimed to develop a high quality of herbal-plant infusion using a high pressure technique to minimize the loss of important bioactive

*Corresponding author.
Email: *osirismu@hotmail.com*

constituents in comparison with untreated and pasteurized infusions. Moreover, the color parameters and microbiological qualities of both processed infusions were evaluated.

Materials and Methods

Herbal-plant infusion preparation

Pennywort leaves, dried longans and purple rice were purchased from a local market in Chiang Mai, Thailand. Fresh pennyworts were extracted with drinking water at a ratio of 1:1 (w/w) then filtered by a cotton cloth. Dried longans and black rice were boiled in water at a ratio of 1:4 (w/w) for 3 min and 1:15 (w/w) for 15 min respectively, and then separately filtered by the cotton clothes. The formula of herbal-plant infusion was a mixture of pennywort, dried longan and black rice extracts at a ratio of 1:1:1.

One hundred milliliters of infusion were then packed in a laminated bag (nylon plus polyethylene) and subjected to pressure 400 or 500 MPa at 25°C for 15 or 30 min. The high pressure vessel was a 'Food Lab' model 900 high pressure rig (Stansted Fluid Power; Essex, UK). The rate of pressure increase was about 330 MPa/min. During this high pressure treatment an adiabatic increase in temperature occurs. At ambient temperature (25°C), the monitored cell temperature increased by about 7°C to 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 and Lab Supplies) at the ratio of 20:80 (v/v) (Chaikham and Apichartsrangkoon, 2012a, b). For pasteurization, 100 ml infusion was packed in a retort pouch, heated in boiling water until the inside core of the package reached 90 ± 5°C for 1, 2 and 3 min. Subsequently the processed infusions were cooled to room temperature and kept at 4°C before analysis.

Determination of gallic and ellagic acids

Gallic and ellagic acids were determined using a modified HPLC method described by Rangkadilok *et al.* (2005) and Chaikham and Apichartsrangkoon (2012a, b). One gram of freeze dried sample was mixed with 9 ml of 100% methanol (RCI Lab-Scan, Thailand) and stirred for 30 min, then centrifuged at 2,500 rpm for 15 min. The supernatant was filtered though a 0.20 μm nylon filter and the filtrate used for HPLC assay. The HPLC system (Shimadzu LC-10AD; Shimadzu) consisted of a low-pressure pump and a photodiode array detector (SPD-M20A; Shimadzu) adjusted to a λ_{max} 270 nm. Chromatographic separation was performed with a C18 column (YMC-Pack ODS-AM, 5 μm, 4.6 mm ID x 250 mm; YMC). The mobile phase was a mixture of 0.4% formic acid (solvent A) (Merck, Germany) and 100% methanol (solvent B) (RCI Lab-Scan) with a flow rate of 1.0 ml/min. The gradient system of the mobile phase commenced from 0 min (100% A) to 4 min (95% A/5% B), 10 min (70% A/30% B), 16 min (66% A/34% B), 22 min (45% A/55% B), 28 min (55% A/45% B) and 34 min (100% A), and maintained at this state to 40 min. The temperature of the column was adjusted to 25°C and the injection volume was 20 μl. Peak areas were determined and converted to the content of each component.

Determination of total polyphenols

Total polyphenols were determined using the Folin-Ciocalteu reagent (Zainol *et al*., 2003). Two milliliters of infusion were stirred with 8 ml of 100% cooled ethanol (Chemical & Lab Supplies) for 15 min and centrifuged at 2,500 rpm for 15 min. A 0.5 ml of supernatant was added to 2.5 ml of 10% Folin-Ciocalteu reagent (Sigma, Germany) and allowed to react for 5 min. Subsequently, 2 ml of saturated sodium carbonate solution (Ajax, Australia) were added to the mixture and held for 2 h at room temperature. The apparent blue complex was determined at a λ_{max} 765 nm (Spectophotometer; Perkin Elmer UV WINLAB, USA). Total polyphenols were expressed as mg gallic acid equivalent per 100 ml sample (mg GAE/100 ml).

Determination of ascorbic acid

Ascorbic acid content was determined following the method described by Chaikham and Apichartsrangkoon (2012a, b) with some modifications. One gram of freeze dried infusion was mixed with 9 ml diluted sulfuric acid (pH 2.2) (Merck), stirred for 30 min and then centrifuged at 2,500 rpm for 10 min. The supernatant was filtered though a 0.20 μm nylon membrane and the filtrate used for HPLC analysis. The isocratic system used 0.1 M acetic acid (Merck) in deionized water (RCI Lab-Scan) as a mobile phase with a flow rate of 1.5 ml/min. The temperature of the column was adjusted to 30°C and UV detection was at a λ_{max} 250 nm with an injection volume of 20 μl. The peak area was determined and converted to concentration of ascorbic acid.

Determination of asiatic acid

The analysis of asiatic acid was carried out using a modified method as described by Inamdar *et al.* (1996). One gram of freeze dried infusion sample was mixed with 9 ml methanol (Merck), stirred for 2 h

and then centrifuged at 2,500 rpm at 25°C for 10 min. The supernatant was filtered though a 0.20 µm nylon membrane and the filtrate used for HPLC analysis. The mobile phase was a mixture of acetonitrile (solvent A) (Merck) and deionized water (solvent B) (RCI Lab-Scan) with a flow rate of 1.4 ml/min. The gradient system of the mobile phase commenced from 0 min (20% A/80% B) to 30 min (55% A/45% B), 35 min (55% A/45% B) and 45 min (80% A/20% B). The temperature of the column was adjusted to 25°C and UV detection was at a λ_{max} 220 nm with an injection volume of 20 µl. The peak area of asiatic acid was determined and converted to concentration.

Determination of gamma oryzanol

Gamma oryzanol content was determined following the procedure as described by Iqbal *et al*. (2005) with some modifications. Two grams of freeze dried sample were extracted by dichloromethane (RCI Lab-Scan) in Soxhlet apparatus for 6 h. The crude oil was diluted with dichloromethane and filtered though a 0.20 µm nylon membrane. The isocratic system used methanol, acetonitrile, dichloromethane and acetic acid (50:44:3:3, v/v) as a mobile phase with a flow rate of 1.4 ml/min. The UV detection was at a λ_{max} 330 nm with an injection volume of 20 µl. The peak area of each component was determined and converted to concentration.

Color parameter measurements

A colorimeter (Minolta Chroma Meter, CR-300, Japan) was used to measure the color parameters of untreated and processed infusions. Analytical data were expressed as Hunter *L* (lightness), a^* (greenness/redness) and b^* (yellowness/blueness) parameters.

Determination of microbiological qualities

The assessments of total plate counts, yeasts, moulds and fecal coliforms in untreated and processed infusions were followed the method of U.S. Food and Drug Administration (2001).

Data 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 ($P \leq 0.05$).

Results and Discussion

Phenolic compounds

Gallic and ellagic acids, with antioxidant and chemopreventive properties, are the predominant phenolic compounds in longan fruit (Rangkadilok *et al*., 2005). The results in Table 1 indicated that gallic acid, ellagic acid and total polyphenols contents in pressurized infusions were significantly higher ($P \leq 0.05$) than those in pasteurized batches, but were lower than untreated sample. Except, infusion pressurized at 500 MPa for 15 min these phenols were relative stable. In thermally processed infusions, these phenolics apparently reduced ($P \leq 0.05$) in accordance with the increase of processing times. In this study, total polyphenolic compounds in pressurized infusions slightly decreased, which might be due to the involvement of residual polyphenol oxidase and peroxidase activities in the degradation of these phenols. Since both enzymes could be activated during high pressure processing (Talcott *et al.*, 2003). Chaikham and Apichartsrangkoon (2012a) found that polyphenol oxidase was completely inactivated in pasteurized longan juices (90°C for 2 min), whereas in pressurized batches (500 MPa at 25°C for 30 min), the residual activities were 95 - 99%. Cao *et al.* (2011) illustrated that total phenols decreased significantly in high pressure treated strawberry pulps at 400 MPa in accordance with an increasing of holding times from 15 to 25 min. Landl *et al.* (2010) elucidated that total phenolic compounds in Granny Smith apple purée significantly decreased after pressurization at 400 MPa and 20°C for 5 min, as compared to unprocessed sample. Additionally, Patras *et al*. (2009a, b) observed that total polyphenols in pressurized strawberry and carrot purées (400 - 600 MPa at 10 - 30°C for 15 min) were higher than in thermally treated samples (70°C for 2 min). In all over pressurization could preserve

Table 1. Phenolic compounds of untreated, pasteurized and pressurized herbal-plant infusions

Treatment conditions	Gallic acid (mg/100 ml)	Ellagic acid (mg/100 ml)	Total polyphenols (mg GAE/100 ml)
Untreated infusion	7.43 ± 0.10^{a}	22.60 ± 0.68^{a}	98.48 ± 6.50^{a}
Pasteurized infusion 90ºC/1 min	5.83 ± 0.17^{c}	17.50 ± 0.59^{c}	75.59 ± 1.39^{c}
Pasteurized infusion 90ºC/2 min	5.78 ± 0.15^{c}	15.98 ± 0.27^{d}	71.96 ± 2.50^{d}
Pasteurized infusion 90ºC/3 min	4.90 ± 0.18^{d}	14.62 ± 0.20^{e}	65.97 ± 2.44^{e}
Pressurized infusion 400 MPa/15 min	7.10 ± 0.10^{b}	19.18 ± 0.47^{b}	88.47 ± 2.53^{b}
Pressurized infusion 400 MPa/30 min	7.08 ± 0.22^{b}	18.99 ± 0.31^{b}	83.83 ± 4.51^{b}
Pressurized infusion 500 MPa/15 min	7.09 ± 0.12^{b}	19.02 ± 0.44^{b}	90.15 ± 3.88^{ab}
Pressurized infusion 500 MPa/30 min	7.11 ± 0.16^{b}	18.70 ± 0.62^{b}	88.10 ± 2.64^{b}

Means in the same column followed by the same letter indicate an insignificant difference ($P > 0.05$). Each data point is the average of three replications.

Table 2. Ascorbic acid, asiatic acid and gamma oryzanol contents in untreated, pasteurized and pressurized herbal-plant infusions

Treatment conditions	Ascorbic acid (mg/100 ml)	Asiatic acid (mg/100 ml)	Gamma oryzanol (mg/100 ml)
Untreated infusion	1.98 ± 0.10^{a}	1.97 ± 0.07^{a}	1.16 ± 0.14^{a}
Pasteurized infusion 90ºC/1 min	1.13 ± 0.12^{d}	1.54 ± 0.03^{c}	0.95 ± 0.25^{b}
Pasteurized infusion 90ºC/2 min	0.82 ± 0.08^{e}	1.46 ± 0.02^{d}	0.80 ± 0.06^{b}
Pasteurized infusion 90ºC/3 min	0.56 ± 0.03^{f}	1.13 ± 0.11^{e}	0.79 ± 0.10^{b}
Pressurized infusion 400 MPa/15 min	1.58 ± 0.06^{b}	1.81 ± 0.05^{b}	1.10 ± 0.09^{a}
Pressurized infusion 400 MPa/30 min	1.40 ± 0.07^{c}	1.77 ± 0.04^{b}	1.23 ± 0.15^{a}
Pressurized infusion 500 MPa/15 min	1.59 ± 0.05^{b}	1.79 ± 0.04^{b}	1.05 ± 0.08^{a}
Pressurized infusion 500 MPa/30 min	1.36 ± 0.08^{c}	1.75 ± 0.07^{b}	1.30 ± 0.21^{a}

Means in the same column followed by the same letter indicate an insignificant difference ($P > 0.05$). Each data point is the average of three replications.

polyphenol compounds in food products better than pasteurization.

Other bioactive components

Ascorbic acid is a crucial nutrient in fruits and vegetables. Table 2 illustrates that ascorbic acid contents significantly decreased ($P \leq 0.05$) by 42 - 72% and 19 - 31% in pasteurized and pressurized infusions respectively, as compared to untreated infusion. The amounts of ascorbic acid in both processed infusions apparently reduced ($P \leq 0.05$) according to the increase of process times. In overall pressurization could conserve ascorbic acid better than pasteurization, since this vitamin was relatively sensitive to heat. Chaikham and Apichartsrangkoon (2012a) revealed that ascorbic acid contents were significant lowest in pasteurized longan juices compared to fresh and pressurized juices. Keenan *et al.* (2012) pressurized (450 - 600 MPa at 20°C for 5 - 10) and pasteurized (70°C for >10 min) fruit smoothies, and observed that ascorbic acid contents in pressurized samples had the highest retention and were close to fresh samples. In addition, Patras *et al.* (2009b) illustrated that 94% and 54% of ascorbic acid were retained in pressurized (600 MPa at 20°C for 15 min) and pasteurized (70°C for 2 min) tomato purées, respectively. In general, ascorbic acid is easily destroyed at high temperatures. Gil-Izquierdo *et al.* (2002) found that ascorbic acid in orange juice decreased from 150.1 to 143.7 mg/L after pasteurization at 95°C for 30 sec.

Asiatic acid, one of the main triterpene compounds in pennywort, exerts neuroprotective effects on cultures cortical cells by potentiation of the cellular oxidative defense mechanism (Jamil *et al*., 2007). Asiatic acid contents in herbal-plant infusion after high pressure and thermal processes are presented in Table 2. In general the amounts of this triterpene followed similar trended to those seen gallic and ellagic acids, which means for the processed overall displayed pasteurized infusions were lower ($P \leq 0.05$) than untreated and pressurized infusions in order. The degradation of asiatic acid in pennywort products was reported by several researchers. For instance, Sangkam *et al.* (2010) and Thonabut *et al.* (2010) reported that asiatic acid in pennywort jellies and leaves significantly reduced after dehydrated by a vacuum infrared dryer at temperatures of 40, 50 and 60°C. Besides asiatic acid, Apichartsrangkoon *et al.* (2012) revealed that asiaticoside, madecassoside and β-carotene in pennywort juice remained unchanged after pressurization at 400 MPa and <30°C for 20 min and pasteurization at 90°C for 3 min.

Gamma oryzanol contents in herbal-plant infusion remained virtually unaltered ($P > 0.05$) after pressurization, while loss of this compound in pasteurized infusions was noticeably observed ($P \leq 0.05$), as compared to untreated sample (Table 2). Finocchiaro *et al*. (2007) observed an 8% loss of gamma oryzanol in cooked brown rice, compared with that of raw brown rice. Similarly, de Simone Carlos Iglesias Pascual *et al*. (2013) found that an average gamma oryzanol content of 27 brown rice samples was lost by 20% after parboiling. In contrast, Khatoon and Gopalakrishna (2004) reported that gamma oryzanol was not changed after parboiling.

Table 3. Color parameters of untreated, pasteurized and pressurized herbal-plant infusions

Treatment conditions	*L*	*a**	*b**
Untreated infusion	31.91 ± 0.45^{a}	-0.06 ± 0.01^{b}	3.44 ± 0.05^{c}
Pasteurized infusion 90ºC/1 min	28.91 ± 0.22^{b}	1.98 ± 0.05^{a}	4.08 ± 0.03^{b}
Pasteurized infusion 90ºC/2 min	27.28 ± 0.17^{c}	2.00 ± 0.02^{a}	4.06 ± 0.04^{b}
Pasteurized infusion 90ºC/3 min	25.97 ± 0.14^{d}	2.04 ± 0.03^{a}	4.19 ± 0.02^{a}
Pressurized infusion 400 MPa/15 min	32.43 ± 0.30^{a}	-0.04 ± 0.02^{b}	3.78 ± 0.04^{c}
Pressurized infusion 400 MPa/30 min	32.57 ± 0.28^{a}	-0.10 ± 0.03^{c}	3.86 ± 0.06^{c}
Pressurized infusion 500 MPa/15 min	32.40 ± 0.20^{a}	-0.13 ± 0.04^{c}	3.60 ± 0.10^{d}
Pressurized infusion 500 MPa/30 min	32.81 ± 0.31^{a}	-0.16 ± 0.05^{c}	3.74 ± 0.08^{cd}

Means in the same column followed by the same letter indicate an insignificant difference ($P > 0.05$). Each data point is the average of three replications.

Table 4. Microbiological qualities of untreated, pasteurized and pressurized herbal-plant infusions

Treatment conditions	Total plate counts (log CFU/ml)	Yeasts and moulds (log CFU/ml)	Fecal coliforms (log CFU/ml)
Untreated infusion	4.37 ± 0.83^{a}	2.56 ± 0.41^{a}	1.64 ± 0.32^{a}
Pasteurized infusion 90ºC/1 min	<10^{b}	nd^{b}	nd^{b}
Pasteurized infusion 90ºC/2 min	nd^{c}	nd^{b}	nd^{b}
Pasteurized infusion 90ºC/3 min	nd^{c}	nd^{b}	nd^{b}
Pressurized infusion 400 MPa/15 min	<10^{b}	nd^{b}	nd^{b}
Pressurized infusion 400 MPa/30 min	nd^{c}	nd^{b}	nd^{b}
Pressurized infusion 500 MPa/15 min	nd^{c}	nd^{b}	nd^{b}
Pressurized infusion 500 MPa/30 min	nd^{c}	nd^{b}	nd^{b}

Means in the same column followed by the same letter indicate an insignificant difference ($P > 0.05$). nd = not detected. Each data point is the average of three replications.

Instrument color parameters

The color parameters of food products are an important aspect for consumer satisfactoriness. As shown in Table 3, the *L* parameters (lightness) of pressurized infusions were equivalent ($P > 0.05$) to the untreated sample, and were higher than pasteurized infusions. The brightness of pasteurized infusions significantly deceased ($P \leq 0.05$) with an increasing the process times. The a^* parameters (redness) displayed significantly higher values ($P \leq 0.05$) in untreated and pressurized infusions than that in pasteurized infusions. These results indicate that high pressure can be preserved the greenness of this product. The b^* (yellowness) of herbal-plant infusion significantly increased ($P \leq 0.05$) after pressurization and pasteurization, however the *L* of pressurized samples still had the lower values ($P \leq 0.05$) than thermally treated samples. In overall high pressure processing gave rise to better *L*, a^* and b^* parameters. This indicates that pressurization

improved or preserved the natural color of the products better than thermally treatment. Several researchers reported the effect of high pressure and thermal processing on color parameters of various products. For instance, Barba *et al*. (2010) found *L* values of pressurized vegetable beverages processed at 100 - 400 MPa and 30°C for 2 - 9 min similar to those of unprocessed samples. Similarly, Keenan *et al.* (2012) with pressurized fruit smoothies at 450 MPa and 20°C for 5 min. Chaimoon *et al.* (2009) elucidated that the a^* parameters of pressurized longan pulps in syrup at 500 MPa and 30 - 40°C for 40 min were lower than those unprocessed and pasteurized samples. In this study, amongst pasteurized infusions the loss of brightness or the increases of redness and yellowness could be associated with the increase of Maillard condensation, caramelization and pigment destruction (Ibarz *et al.*, 2000).

Microbiological qualities

The total plate counts exhibited that aerobic mesophilic microorganisms in all processed herbal-plant infusions were satisfactorily eliminated ($P \leq 0.05$), except those in pasteurized infusion at 90°C for 1 min and in pressurized fusion at 400 MPa for 15 min were less than 10 CFU/ml. Moreover, other bacteria including yeasts, moulds and fecal coliforms in all the processed infusions were completely eradicated (Table 4). Landl *et al*. (2010) pressurized (400 and 600 MPa at 20°C for 5 min) and pasteurized (75°C for 10 min) acidified Granny Smith apple purées, they found that total aerobic mesophilic microorganisms and yeasts-moulds were below the detection limit (<50 CFU/g). In addition, Lavinas *et al*. (2008) pressurized cashew apple juice at 350 MPa for 7 min or 400 MPa for 3 min and observed that aerobic mesophilic bacteria decreased from a range of 4.6 to 5.9 log CFU/ml to undetectable levels (<10 CFU/ml). They also reported that pressure treatments were efficient in inactivating yeasts, filamentous fungi and *Escherichia coli.* Identical observations were displayed by Krebbers *et al*. (2003) with tomato purée processed by pressure 700 MPa at 20°C for 2 min.

Conclusion

The effects of high pressure and thermal processing on the bioactive compounds and qualities of herbal-plant infusion were determined. The result illustrated that pressurized infusions comprised of high amounts of gallic acid, ellagic acid, total polyphenols, ascorbic acid, asiatic acid and gamma oryzanol compared to the pasteurized samples. Color parameters displayed that pressurization could improve or conserve the natural color of the products greater than pasteurization. The microbiological assessments showed that total plate counts, yeasts-moulds and fecal coliforms in both processed infusions were agreeably eliminated.

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