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Some factors affecting the bioactive substances of lactogenic tea

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PAPER

Abstract

This study aims to perform the phytochemical screening composition for galactagogue herbs and determining the total phenolic (TPC) and flavonoid contents (TFC). Terpenes, saponins, tannins, alkaloids, phenolics, and flavonoids were found in water extracts of banana inflorescence *(Musa sapientum* L), white holy basil leaves (*Ocimum sanctum* L.), rhizomes of Thai ginger (*Zingiber officinale* Roscoe), and powdered lactogenic product. The results show that the TPC were greater than the TFC for all fresh parts of banana inflorescences. Following the M1 method, fresh banana floret gave the greatest TPC, superior to those with M2 or M3, while the TFC with M1 and M2 methods applied to banana floret was significantly greater than that of banana bracts or banana cores. After harvest and dehydration of the banana inflorescence, TPC and TFC after one-day storage were higher than those at five-days of storage. Meanwhile, TPC and TFC on both fresh and dehydrated white holy basil tended to decrease due to heat intolerance, deterioration, and brewing. In contrast, TPC and TFC of Thai ginger increased on dehydration. The maceration with press-shear force interaction (PAI) pretreatment, and with PAI combined with moist heat, strongly increase the bioactive contents. SEM imaging demonstrated that PAI with moist heat tended to enlarge the micro-pore volume and simultaneously increased pores, resulting in more adsorption of these compounds.

***Keywords*:** breastfeeding, galactagogue herbal tea, herbal tea brew, lactogenic product, particle size distribution, total phenolic and flavonoid content

Introduction

Both World Health Organization, the American Academy of Pediatrics and the European Society for Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) strongly recommended the breast milk as the best and exclusive natural food serving as the nutritional source for infants (Embleton *et al.*, 2023; Kong *et al.*, 2021), reinforcing the immune system and development on their digestive system. Human milk is a major food to feed infants from newborns to six months of life, and is supplemented with soft foods up to two years of age (Perrella *et al.*, 2021). Human milk is slightly acidic and usually contains mainly water with approximately 1% protein, 5% fat, 7% carbohydrates, and 0.2% minerals, vitamins and various types of bacteria probiotics (Perrella *et al.*, 2021; Luz *et al.*, 2021). Being a source of high nutritional energy, polyunsaturated fatty acids in breast milk, also function in the transport of fat-soluble vitamins (Perrella *et al.*, 2021). PUFAs in breast milk include linoleic (LA-18:2 *n*-6), arachidonic (ARA-20:4 *n*-6), alpha-linolenic (ALA-18:3 *n*-3), eicosapentaenoic (EPA-20:5 *n*-3) and docosahexaenoic (DHA-22:6 *n*-3) fatty acids. The fatty acid content in breast milk according to dietary intake of the mother, and may affect the n-6 and n-3 levels in the blood stream of children (Perrella *et al.*, 2021). Otherwise, breast milk also contains antioxidant compounds including superoxide dismutase, glutathione peroxidase, catalase, vitamin E, vitamin C, and beta-carotene, flighting against the reactive oxygen species (ROS) in a newborn (Sinkiewicz-Darol *et al.*, 2022). Breastfeeding could help the mother's health lowering the risk of postpartum bleeding, and of ovarian and breast cancers, or cardiovascular disease (Amorim *et al.*, 2023). Both the mature breast milk (MBM) and breast colostrum (BC) contain immune and growth factors including casein, serum, albumin, *a*-lactabumin, *b*-lactoglobulins, immunoglobulins and lactoferrin, which can prevent infants against allergies and gastrointestinal infections. The beneficial breast milk and breast colostrum can also enhance their neuronal evolution and help to stimulate the innate immune system and gut function, which it induces the development of a healthy gut microbiome in newborns (Perrella *et al.*, 2021). Immune cells, antibodies in the adaptive immune system include immunoglobulins A, G and M and enzymatic substances in the innate immune system include lysozyme (LZ), lactoferrin (LF) and lactoperoxidase (LPOS) are provided in breast colostrum (Li *et al.*, 2022). Among LZ, LF and LPOS, all enzymes also exhibit antimicrobial properties (El-Loly, 2022). Breast milk can contain 600 types of microbes belonging the genus *Lactobacillus*, which exhibit antimicrobial activity (Luz *et al.*, 2021). The nutritional requirements of an infant, affecting each span of growth and developmental stages, are such that breast milk is considered optimal food for the infant and may alter each infant during breastfeeding (Li, Zheng *et al.*, 2020). During the breastfeeding period, the released antibodies, oxytocin and prolactin hormones will strengthen the bond between mother and infant. Various factors including circadian rhythm, diet, physical condition, environment, and lactation period can affect the nutrient contents (Karcz and Królak-Olejnik, 2020; Martín-Carrasco *et al.*, 2023). The predominant problem in the mother's lactation period is too low volume of milk production and its inconsistent flow. The report of Australian National Breastfeeding Strategy 2010-2015 indicated at least 75% of new postpartum mothers only partially breastfeeding during the early postbirth months. The study reported that insufficient or decreased production of breast milk was mainly caused by some diseases on structural abnormalities in the mother and in her infant, such as inverted nipples, oral clefts, infection, maternal pain and poor latching. The psychological disorders of mothers constitute an important reason that can inhibit lactation (Chrzan-Detkoś, Walczak-Kozlowska and Pietkiewicz, 2021). As medication in ablactation period, there are various galactagogue synthetic drugs including the dopamine antagonists, such as domperidone (motilium®) (Budiono *et al.*, 2023) and the antiemetics metoclopramide (Samal *et al.*, 2021), chlorpromazine (Lawrence, *et al.*, 2022) and antipsychotics sulpiride (Cheng *et al.*, 2020). Among predominant hormone analogues, they have also influenced galactagogue activity including prolactin (PRL), somatotropine, cortisol, insulin, leptin, estrogen, progesterone, and medroxyprogesterone, oxytocin, recombinant bovine somatotropin (rBST) and thyrotropin releasing hormone (TRH) (Kim, 2020). Domperidone has affected milk production, contributing to milk breastfeeding, calories consumption, regularity of pumping, drainage of milk from the breast, and psychiatric history (McBride *et al.*, 2023). Regarding limitations, treatments with these drugs have been prescribed under the supervision of a doctor. However, adverse side effects were noted, such as dry mouth, exhaustion, diarrhea (McBride *et al.*, 2023), headaches, constipation, and depression, reviewed by Medicines and Healthcare Products Regulatory Agency (MHRA) of the United Kingdom. Finally, the Food and Drug Administration of the United States has not approved domperidone for improving milk production (No authors listed, 2023).

Regarding the advantages of galactagogues properties in medicinal herbs and food diets, the mothers can apply self-help with these. The dietary choice of drinking sufficient water can significantly increase breast milk production. Special hot foods and herbal drinks, including fenugreek seeds (*Trigonella foenum-graecum)*, *Zingiber officinalis* (ginger) and *Piper nigrum* L. have biological galactagogue activity, which could improve human milk and support breastfeeding. Polyphenols in human milk exert certain beneficial promotion of breastfeeding, pregnancy, and postpartum health (Miranda *et al.*, 2022). Moreover, polyphenols have affected the infant gut microbiota, resulting in the improvement of immunity and the prevention of the chronic diseases in adulthood (Cortes-MacÍas *et al.*, 2021). In Thai traditional medicine, the banana inflorescences from *Musa sapientum* L. are considered an alternative source of added-value beverage for stimulating breast milk production (Yimyam, Suppansan and Jankajonchai, 2023). They are always an agricultural by-product and are often consumed in many Asian countries, such as Thailand, Malaysia, Indonesia, India, Sri Lanka, and Myanmar, but they become agricultural waste in the industry (Liang *et al.*, 2023). The study reported proanthocyanidins in the banana inflorescence extract can inhibit dihydrotessterone (DHT) production and proliferation of prostate cell. Furthermore, high amounts of fiber, iron, potassium, calcium, and magnesium in its extract can decrease blood sugar and increase hemoglobin. It can also decrease menstruation and muscle cramps (Liang *et al.*, 2023). The secondary metabolites found in banana inflorescence extract are polyphenols, triterpenes, and sterols (Panyayong and Srikaeo, 2023). These pharmacological activities exhibit potential antioxidant activity (Panyayong and Srikaeo, 2022), anti-hyperglycemic, anti-inflammatory, and anti-cholesterolemic effect. In addition, the high-quality amino acids have the beneficial function on maternal health and infant development in the hormone synthesis (Panyayong and Srikaeo, 2022; 2023). According to its unsaturated fatty acids, it contains oleic, linoleic and a-linolenic acids accounting for greater than 60% of total fatty acids, which linoleic acid contains in high concentration. Thus, the beneficial unsaturated fatty acids in banana inflorescence can reduce the risk of cardiovascular diseases (Lau *et al.*, 2020).

Recently, natural galactagogue herbs have been developed into powder, packed in sachet, and serving as brewing tea to enhance postpartum mothers. These cases comprise many types of galactagogue herb mixes for brewing tea. This product has been launched into the market. The consumer acceptance of product quality and other attributes has been quite tremendous. This lactogenic products are continue to develop in releasing more biological compounds by using natural press-shear assisted process (PAI) with eggshell powder as an adjuvant on pulverizing natural galactagogue herbs. This technology had been patented.

The purpose of this study was to assess the contents of bioactive galactagogue compounds in lactogenic products, which used without and with PAI pretreatment and plus moisture heated. The compositions of lactogenic products from banana flower, Thai ginger, and Thai white holy basil, were investigated by phytochemical screening and examined for total phenolic (TPC) and flavonoid contents (TFC) based on dry weight. The compositions, especially in phenolics and flavonoids from the dehydration in a hot air oven at a specific temperature were monitored and compared to the fresh herbs. The polyphenols were identified, and their quantities were determined using various brewing methods. In addition, particle size distribution and morphology of the galactagogue microparticles were evaluated.

Materials and Methods

Materials

The three lactogenic products were obtained from an entrepreneur. Briefly, the product consisted of banana inflorescences, ginger, basil and secret herbs before taking to dry and crushed with eggshell as press-shear assisted (PAI) and further with most-heated. The lactogenic product was crushed by using without PAI pretreatment as a control. Banana inflorescences (*Musa sapientum* L.) were collected during flowering period in May of 2021. Both the banana inflorescences and the Thai white holy basil (*Ocimum sanctum*) were provided by an organic orchardist, while the Thai ginger (*Zingiber officinale* Roscoe) rhizome was acquired from a local market. All other chemicals and reagents were of analytical grade.

Sample preparation and acquisition

In this study, three major ingredients such as banana inflorescence, Thai ginger, and white holy basil were used. Briefly, banana inflorescence was separated to bract, floret and core, while white holy basil leaf was detached, and ginger rhizome was peeled out before drying in hot air oven at 60±2°C until moisture content was less than 8% for further analyses. The three obtained lactogenic products included treatment with PAI, combined PAI and moist heating, and control (traditional one without any treatment). All samples were extracted with distilled water, in hot extraction at 90°C and maceration overnight.

Hot extraction and maceration procedure

Ten grams of samples were accurately weighed and mixed with 200 mL of distilled water. The blend was placed in a water bath at 90°C for 4 h (hot extraction), always keeping the volume at 200 mL and swirling intermittently. For maceration procedure, the aqueous solution was gently stirred on a shaker at room temperature overnight. For hot extraction, the solution was left to cool down to room temperature. After that, the aqueous suspension was centrifuged at 1,600 g for 15 min to separate solids from supernatant. The supernatant was evaporated in a water bath at 60°C overnight. The crude extract was dried in a hot air oven at 60°C for 3 h to obtain the final sample and kept in a dark bottle until use.

Phytochemical screening

Several secondary metabolites have been tested for their presence, and alkaloids (Dragendroff's test), steroids (Salkowski test), terpenoids (Modified Salkowski test), saponins (foam test) (Parekh and Chanda, 2007; Ukoha *et al.*, 2011; Yadav *et al.*, 2014), tannins ($FeCl_3$ test) (Kumar *et al.*, 2007; Parekh and Chanda, 2007; Yadav *et al.*, 2014) were identified using different assays (Atraqchi and Hamed, 2014; Odeja *et al.*, 2014). The presence of total phenol and flavonoid in the extracts was tested by using the Folin Ciocalteu's reagent and the aluminum nitrate method, which can serve both qualitative and quantitative analysis.

Brewing procedures of galactagogue herbs and lactogenic products

Separated fresh banana inflorescences into banana bracts, banana cores and banana florets were made before taken in different three brewing methods. M1 brewing method, the sample was immersed similarly as common tea into hot water at 90°C for 20 min. M2 brewing method, the sample was boiled in water for 20 min, following the entrepreneur's method, subsequently the temperature was decreased to 80°C and held by heating for 20 min. While the last brewing method, M3, the sample was boiled at 110°C for 20 min. All brewing methods, the volume of product was controlled at 200 mL and swirled intermittently. Total phenolic and flavonoid contents were investigated and calculated as the basis of one g solid content (dry weight).

Determination of total polyphenol content

Total phenolic content (TPC) was determined by the Folin-Ciocalteu method, as described by Yeo and Shahidi (2017). Briefly, 2.5 mL of the diluted Folin Ciocalteu's phenol reagent (the ratio between Folin Ciocalteu's reagent to distilled water was 1:10 v/v) was mixed with 2 mL of aqueous Na_2CO_3 (Na_2CO_3 7.5 g dissolved in distilled water 100 g). 0.5 mL of the diluted extract was then added to the solution. The mixture was allowed to stand in the dark at room temperature for 30 min. Gallic acid was used as the standard at concentrations of 1-100 μg mL^{-1}. A microplate reader was used to measure the absorbance at l760 nm (Gen5, Biotek PowerWave XS, Marshall Scientific, United States). The content of phenolics was expressed in mg gallic acid equivalents (GAE) g^{-1} of dry matter (DM) (mg GAE g^{-1} DM).

Determination of total flavonoid content

The total flavonoid content was measured using the aluminum nitrate method, as described by Chandra *et al.* (2014) and Bajalan *et al.* (2017). Briefly, 0.1 mL of the aqueous extract was dissolved in 1.8 mL of distilled water. 0.1 mL of aqueous 5%$NaNO_2$ was added into the solution. The mixture was then allowed to stand for 5 min. After that, 0.1 mL of aqueous 10%$Al(NO_3)_3$ and 0.4 mL of 1 M NaOH were added to the mixture. The mixture was allowed to incubate in the dark for 15 min. The absorbance was read at l510 nm (Gen5, Biotek PowerWave XS, Marshall Scientific, United States). The results were reported in mg of quercetin equivalents (QE) g^{-1} of dry matter (DM).

The analysis of particle size distributions

The three lactogenic powder products (a control without PAI pretreatment, that with PAI, and that with PAI combined with moist heating) were passed through a standard No. 60 mesh sieve. The particle size distributions of the lactogenic products were analyzed when suspended in water, by using the LS Particle Size Analyzer (Analysette 22 MicroTec plus, FRITSCH, Germany). The speed for stirring was set at 500 rpm. For correct measurement, the flocculated solids were broken down by sonication. Then, the control software calculated the average particle size and particle size distribution based on the Fraunhofer theory (Xie *et al.*, 2018).

Zeta potential analysis

The dispersions of lactogenic products were characterized using a Zetasizer 5000 (Malvern, UK) to determine their mean droplet size, polydispersity index and zeta potential. The products were diluted in distilled water (1:25) and measurements were repeated ten times. Results were expressed as mean±standard deviation.

Microstructure analysis

The microstructures of the three lactogenic powder products were observed using scanning electron microscopy (SEM, Quanta 450, FEI, Columbus, USA). The powder sample was fixed on an aluminum stub after dispersing it on double-sided conductive adhesive tape, and sputter-coating with gold for imaging. The acceleration voltage was set as 10 kV and the magnifications were x1.5K and x5K.

Statistical analysis

All measurements were done in quintuplets and are reported as mean±standard deviation. The experimental data were analyzed by using statistical software. Analysis of variance (ANOVA) was followed by Duncan's test to determine the effects of main parameters and to compare differences between means for statistical significance at $p<0.05$ level.

Results and Discussion

Phytochemical screening

Phytochemical screening is useful for assessing biologically active components in products for traditional medicine. Such phytochemical screening is a preliminary step to investigation of the secondary metabolites, derived from the primary metabolites, also contributing pharmacological effects in therapeutic treatment. This study aimed to screen the secondary metabolites crucial in herbal medications, such as alkaloids, glycosides, saponins, terpenes, tannins, flavonoids, phenolics, etc. Both fresh and dehydrated of main natural galactagogue herbs (i.e. banana inflorescences: banana bracts, banana cores and banana florets; Thai white holy basil leaves; and Thai ginger rhizomes) and three lactogenic products (mixed galactagogue herbal tea without PAI pretreatment; that with PAI pretreatment; and that with PAI combined with moist heating) were analyzed for the presence of phytochemical contents of terpenoids, saponins, tannins, alkaloids, phenolics and flavonoids, as summarized in Table 1.

The phytochemical screening of crude water extract from hot extraction and maceration methods of banana inflorescence (*Musa sapientum* L. in the family Musaceae) revealed the presence of terpenoids, saponins, tannins, alkaloids, etc. The result indicated that phytochemicals contained in the sample depended on types, part, and extraction methods. Generally, maceration method seemed to provide greater phytochemicals content when compared to hot water extraction with less time extraction providing at high temperature. Due to business cancelation, therefore the deep information could not be announced to indicate the PAI and moist heating condition. Interestingly, the sample with PAI which was a shear force treatment provided more phytochemicals particularly when moist heated was combined. The result indicated that application of PAI and moist heated should facilitate extraction process and/or protect active compound from high temperature.

As known that secondary metabolites have pharmacological effects on animals' health, such as antioxidant, antibacterial, anti-inflammatory, anti-diabetic and antibiotic, etc (Tabares, Jaramillo and Ruiz-Cortés, 2014). Phytochemicals containing in both raw materials and lactogenic products in this present work were in accordance with other authors studied, for instance, in traditional Indian medicine, ginger demonstrated that alkaloids, saponins and tannins, which provided various pharmaceutical or medicinal activities, such as antibacterial, antiviral, anti-hypertension, nephroprotective, angiotensin and serotonin receptor blocking activities (Jaiswal and Solanum, 2012). Therefore, both major raw material and lactogenic products could be potential candidates for postpartum mothers due to phytochemicals having pharmacological effects.

Bioactive contents on both fresh and dehydrated of each type of major ingredients

The results revealed that the phenolic contents were greater than the flavonoid contents across all three methods of tea brewing. According to Figure 1, the total phenolic content (TPC) for M1 of fresh banana floret (0.43±0.005 mg mL^{-1} g^{-1} dry weight) was greater than those for M2 (0.27±0.01 mg mL^{-1} g^{-1} dry weight) and M3 (0.38±0.01 mg mL^{-1} g^{-1} dry weight). However, M3 provided the total phenolic content higher than M2 even higher temperature. This may be explained by law of synthesis is bigger than deterioration due to chemical reaction rate, releasing power and product regeneration. As known that higher temperature can lead to open cell tissue easily because of structure weakening. In addition, M1 and M2 yielded about 3 times flavonoid content in banana floret (0.28±0.01 mg mL^{-1} g^{-1} dry weight and 0.26±0.004 mg mL^{-1} g^{-1} dry weight) higher than of banana bracts and core (0.07±0.01 – 0.07±0.004 mg mL^{-1} g^{-1} dry weight and 0.08±0.002-0.11±0.005 mg mL^{-1} g^{-1} dry weight, respectively). It was noticed that banana floret was easily to turn brown and provide more bitter taste when compared with other parts (data not show).

The post-harvest of banana inflorescences

After harvesting the banana inflorescences, their storage period influenced the bioactive contents as well. In our experiments. The effects of duration of storage were studied for 1 and 5 days. Total phenolic and flavonoid contents with alternative tea brewing methods were then determined after dehydration the sample at 60°C until the moisture content was below 7.8%. The results revealed that total phenolic and flavonoid contents in banana florets were above those in banana bracts and banana cores. Apart from that, obviously the contents of phenolics and flavonoids were higher when stored for one day than when stored for five days. It was concluded that storage time could reduce bioactive contents in banana inflorescence, therefore the more storage time the less bioactive value retained. Also, the results demonstrated that the bioactive compounds with M1 and M3 tea brew methods were higher than with M2, as shown in Figure 2.

Flavonoid content of 1-1-banana floret (TFC = 0.47±0.01 mg mL^{-1} g^{-1} dry weight), 1-3-banana floret (TFC = 0.52±0.01 mg mL^{-1} g^{-1} dry weight), and 2-1-banana floret (TFC = 0.47±0.01 mg mL^{-1} g^{-1} dry weight) had significantly greater total flavonoid contents than the others ($p<0.05$). The brewing method had a bigger relationship with the obtained contents of polyphenols (phenolic and flavonoids) when compared with storage time. Stored banana inflorescence longer 5 days can retain flavonoids content, however, the yield was lower about 30-40% (data do not show) due to senescence, then many bracts and floret were dropped. Therefore, freshness of this raw material is important and needs to be used fastest for better yield obtaining. From the result, (Fig 2), it pointed out that using higher temperature (M3) may not show negative effect for both total phenolic and flavonoids, which is good for drinking product by thermal processing further. Generally, the phenolic and flavonoid compositions in banana inflorescence depended on the freshness or storage time, part of use and extraction method. In addition, temperature (60°C) of dehydration, and other exposure to heating along with

Table 1. A comprehensive phytochemical screening of aqueous extracts of galactagogue herbal compositions, and aqueous extracts of lactogenic products.

Scientific name (Family)	Common name	Part / form of detection	Terpenoids	Saponins	Tannins	Alkaloids
Musa sapientum L. (Musaceae) Hot extraction	Kluai Nam Wa	banana inflorescence / powder	+++	++	++	+++
Musa sapientum L. (Musaceae) Maceration	Kluai Nam Wa	banana inflorescence / powder	+++	+++	++	++
Banana bract, hot extraction		bract / fresh, powder	-	+	+	-
Banana core, hot extraction		core / fresh, powder	-	-	+	-
Banana floret, hot extraction		floret / fresh, powder	++	+	++	++
Banana bract, maceration		bract / fresh, powder	-	-	++	++
Banana core, maceration		core / fresh, powder	-	-	+	+
Banana floret, maceration		floret / fresh, powder	+	+	++	++
Banana bract, hot extraction		bract / dehydrated, powder	++	-	-	-
Banana core, hot extraction		core / dehydrated, powder	+	-	-	-
Banana floret, hot extraction		floret / dehydrated, powder	++	+	+++	+++
Banana bract, maceration		bract / dehydrated, powder	+	-	-	-
Banana core, maceration		core / dehydrated, powder	+	-	-	-
Banana floret, maceration		floret / dehydrated, powder	++	+	+++	+++
Ocimum sanctum L. (Labiatae)	Holy basil, Ka-Pow-Kaow	Leave				
Thai white holy basil, hot extraction		Leaves / fresh, powder	-	-	-	-
Thai white holy basil, hot extraction		Leaves / dehydrated, powder	-	-	-	++
Thai white holy basil, maceration		Leaves / fresh, powder	-	+++	-	-
Thai white holy basil, maceration		Leaves / dehydrated, powder	++	+++	-	-
Zingiber officinale Roscoe. (Zingiberaceae)		Rhizome				
Thai ginger, M1 tea brew		Rhizome / fresh, powder	-	-	-	+++
Thai ginger, M1 tea brew		Rhizome / dehydrated, powder	+++	-	++	+++
Thai ginger, M2 tea brew		Rhizome / fresh, powder	-	+++	-	+++
Thai ginger, M2 tea brew		Rhizome / dehydrated, powder	++	+++	+	+++
Thai ginger, M3 tea brew		Rhizome / fresh, powder	-	-	-	-

(*continues*)

Table 1. Continued.

Scientific name (Family)	Common name	Part / form of detection	Terpenoids	Saponins	Tannins	Alkaloids
Thai ginger, M3 tea brew		Rhizome / dehydrated, powder	+++	+	+++	++
Lactogenic products						
Mixed herbs with PAI treatment, hot extraction		powder	++	-	+++	+++
Mixed herbs with PAI treatment, maceration		powder	++	-	+++	+++
Mixed herbs without PAI treatment, hot extraction		powder	++	-	+++	+++
Mixed herbs without PAI treatment, maceration		powder	++	++	+++	+++
Mixed herbs with PAI treatment combined moist heating, hot extraction		powder	+++	++	+++	+++
Mixed herbs with PAI treatment combined moist heating, maceration		powder	+++	+++	+++	+++

(+) = fairly presence, (++) = moderately presence, (+++) = very presence, (-) = absence.

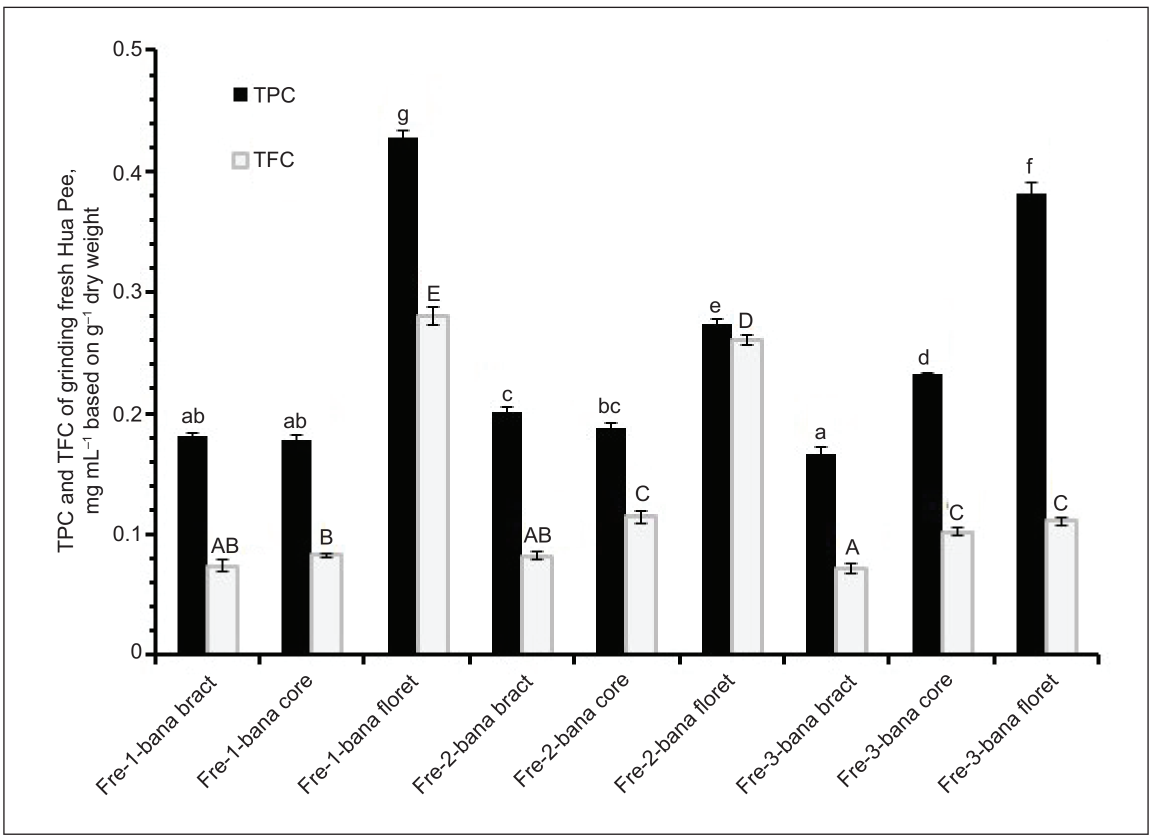

Figure 1. Total phenolic (TPC) and flavonoid contents (TFC) in different parts (banana bract, core, and banana floret) of freshly ground banana inflorescence (*Musa sapientum* L.). Fre- represents "Fresh", middle letters ("1", "2" and "3") represent the brewing method (M1, M2 or M3). Different letters above bars indicate statistically significant difference (p<0.05) according to Duncan's (D) multiple comparison test.

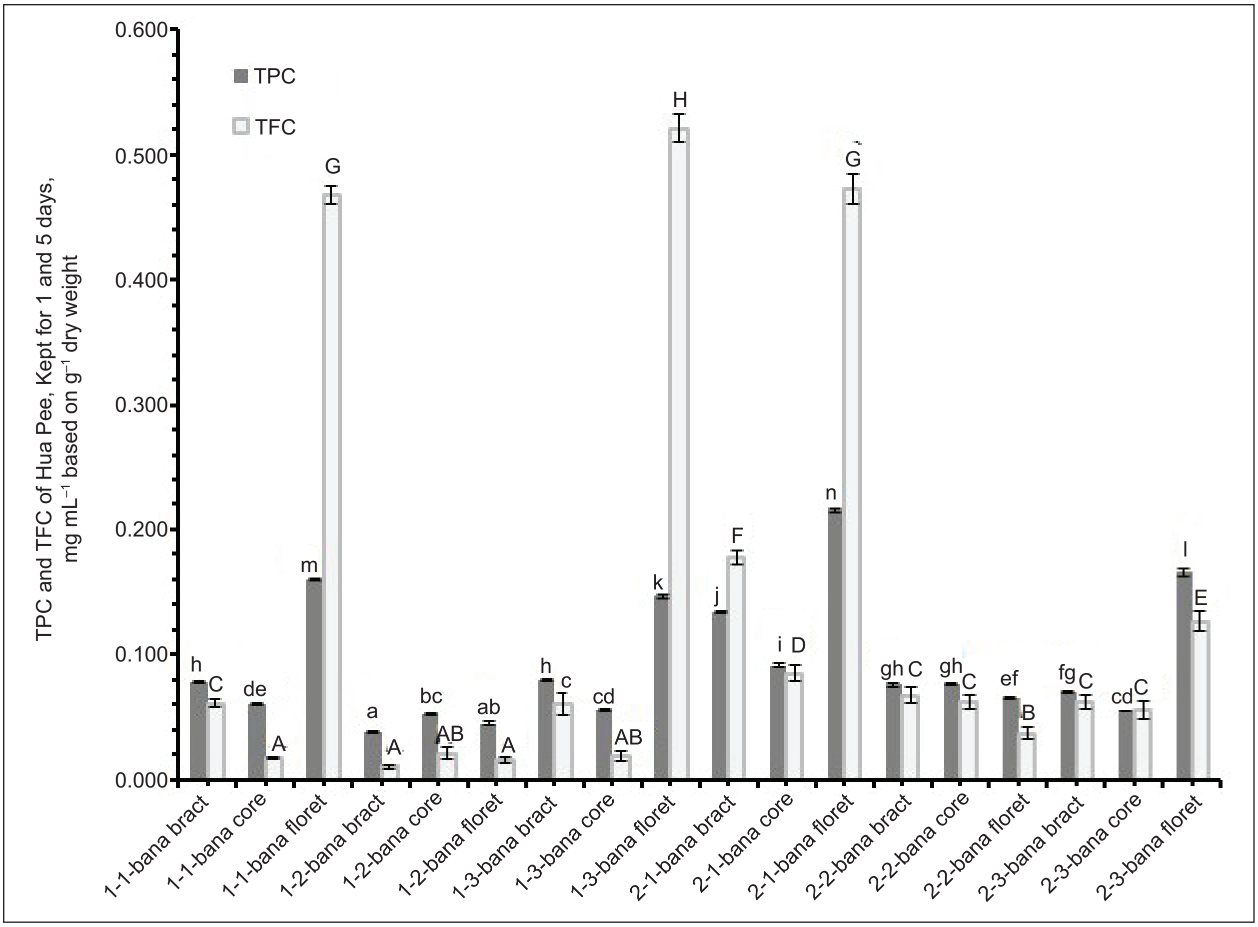

Figure 2. Total phenolic (TPC) and flavonoid (TFC) contents of different parts of ground banana inflorescences, stored for 1 and 5 days before test. In labeling of the samples, the first index, "1" represents storage for 1 day and "2" represents storage for 5 days. The middle index, "-1-", "-2-", or"-3-" represents the brewing method, M1, M2 or M3. The last one showe the part of banana inflorescences used. Different letters above bars indicate statistically significant difference (p<0.05) according to Duncan's (D) multiple comparison test.

the method of herbal tea brewing (M1, M2 and M3) were important factors affecting these compositions.

Bioactive contents on both fresh and dehydrated Thai white holy basil (*Ocimum sanctum* L.)

In the postharvest method, the Thai white holy basil was dehydrated at 60°C until the moisture content no longer exceeded 7%. Both fresh and dry Thai white holy basil contained phenolic and flavonoid substances. According to Figure 3, the dehydration caused a significant ($P<0.05$) reduction of lower both total phenolic and flavonoids. It pointed out that active compounds in this material could be removed by evaporation of water and essential oil. Interestingly, the total flavonoid content of white holy basil was bigger than total phenolic content. Brewing methods of the fresh leaves provided different content of both TPC and TFC but not in dried one. M1 (TFC = 66.72±1.50 mg mL^{-1} g^{-1} dry weight) gave the highest of total flavonoids followed by M2 (TFC = 45.62±7.88 mg mL^{-1} g^{-1} dry weight) and M3 (TFC = 27.96±2.63 mg mL^{-1} g^{-1} dry weight) statistically ($P<0.05$). In the same way that total phenolic contents for the M2 case (TPC = 8.03±0.28 mg mL^{-1} g^{-1} dry weight) had content exceeding those with M1 (TPC = 3.74±0.61 mg mL^{-1} g^{-1} dry weight) or M3 (TPC = 3.29±0.38 mg mL^{-1} g^{-1} dry weight) with significant difference ($P<0.05$). In the results overall, these bioactive contents in both fresh and dehydrated Thai white holy basil tended to decrease. Likely these substances are heat intolerant, degrading easily during dehydration and brewing. Therefore, the dehydration and brewing for better bioactive compounds retaining may need to concern and modify.

Bioactive contents on both fresh and dehydrated Thai ginger (*Zingiber officinale* Roscoe)

As shown in Figure 4, the results showed that dehydrated ginger with M1 brewing method had the highest phenolic

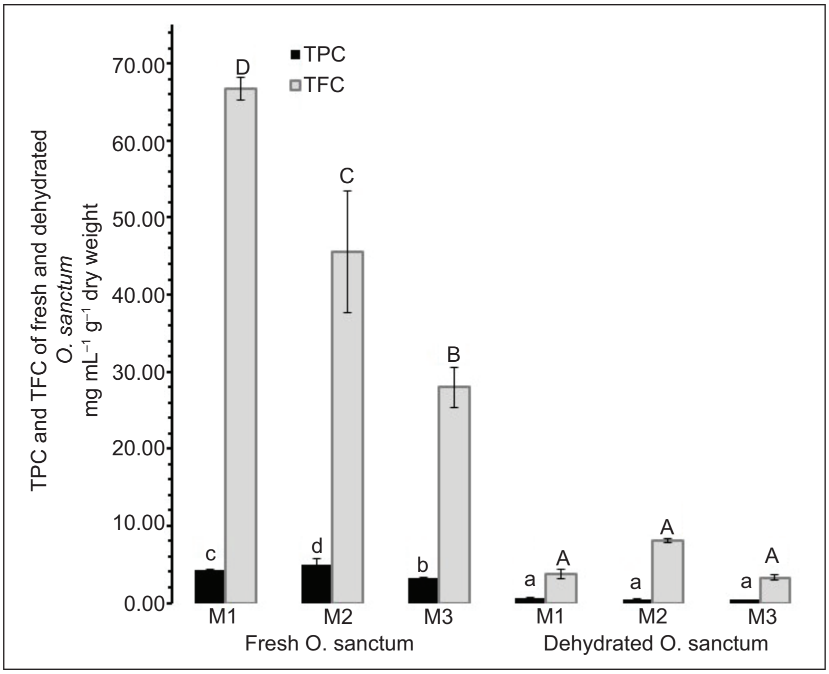

Figure 3. Total phenolic and flavonoid contents in both fresh and dehydrated Thai white holy basil (*O. sanctum*), when brewed with 3 alternative methods (M1, M2 and M3). Different letters above bars indicate statistically significant difference (p<0.05) according to Duncan's (D) multiple comparison test.

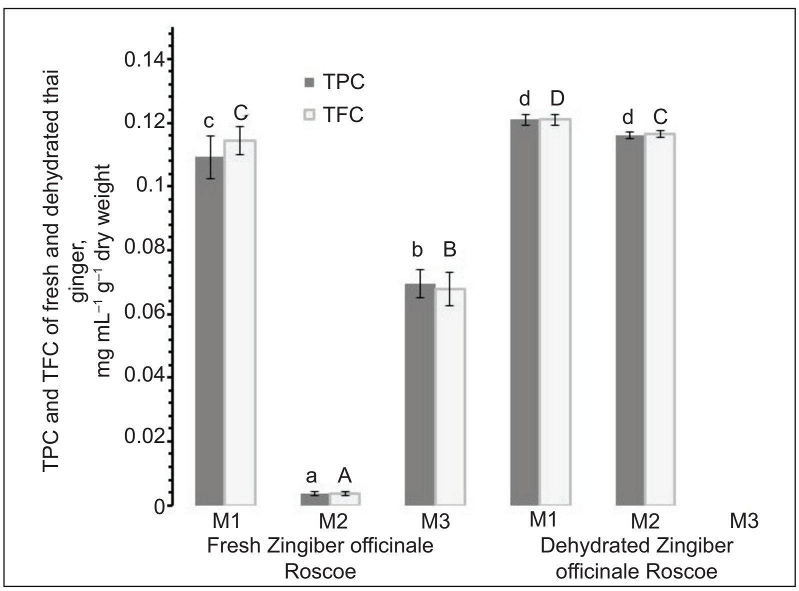

Figure 4. Total phenolic and flavonoid contents in both fresh and dehydrated Thai ginger (*Zingiber officinale* Roscoe), when brewed with alternative brewing methods (M1, M2 and M3). Different letters above bars indicate statistically significant difference (p<0.05) according to Duncan's (D) multiple comparison test.

and flavonoid contents superior to those for fresh with M1, M2 or M3. The phenolic and flavonoid contents with M3 brewing method indicated by no detectable level (P<0.05). This might be caused by thermal intolerant bioactive compounds (TPC = 0.069±0.004 mg mL^{-1} g^{-1} dry weight, TFC = 0.068±0.005 mg mL^{-1} g^{-1} dry weight). The anhydrous bioactive compounds in dehydrated ginger could not be maintained at the high temperature of the M3 brewing tea procedure (110°C, 20 min). However, it was concluded that dehydration influenced the phenolic and flavonoid contents, as in *Ocimum sanctum* L. they decreased while in *Zingiber officinale* Roscoe they increased. Overall, it was revealed that using a high temperature beyond 100°C in brewing more strongly impacted these bioactive contents than traditional steam brewing.

Interesting, on dehydration, both TPC and TFC in ginger sample did not reduce dissimilar as finding those in white holy basil. There was a study that demonstrated the dehydration methods significantly influenced total soluble phenolic and flavonoid contents of dried holy basil leaves. Total soluble phenolic content was decreased when leaves dried at 45°C, comparing to fresh holy basil phenolic and flavonoid contents being 2,927.00 and 3,091.98 mg 100 g^{-1} dry weight (Rudrakshi, Surekha and Preetinder, 2018). Zafer and Filiz (2009) reported that oleuropein in olive leaves could be hydrolyzed to hydroxytyrosol, in addition thermal drying treatment induced deterioration of the phenolic. Moreover, enzymatic reaction and free water content also affected the phenolic. Korus (2011) reported that among flavonoids, kaempferol and quercetin contents of kale dried with hot air drying at 55°C for 5.5 h had a reduction by 51-73%, compared to their fresh one.

Bioactive contents of lactogenic products with PAI pretreatment with and without moist heated treatment

Lactogenic products composed of whole banana inflorescence, Thai ginger and white Thai holy basil and other herbs and prepared with PAI pretreatment, and PAI combined with moist heating, as well as without PAI pretreatment (control) provided from BD Care and packed in sachet. According to Figure 5, using hot extraction, lactogenic herbal product without PAI process had significantly greater phenolic and flavonoid contents (TPC = 18.73±0.17 μg mL^{-1} g^{-1} product, TFC = 10.37±0.17 μg mL^{-1} g^{-1} product) than one with PAI process (TPC = 9.21±0.26 μg mL^{-1} g^{-1} product, TFC = 1.15±0.37 μg mL^{-1} g^{-1} product). The reduction of particle size caused by shear forces in the raw material could increase specific surface and access more contents, however, this process also exposed the bioactive compounds to thermal degradation.

In addition, crushed eggshell was a porous powder, then some phenolic and flavonoid substances might be absorbed by the pores. Therefore, reductions of TPC and TFC were noticed in the product treated with PAI. However, the phenolic and flavonoid contents were significantly enhanced when the moist heat was applied. This might indicate that the moist heating affected the pore sizes in the bioactive particles, affecting binding of the flavonoid's molecules. Furthermore, the results showed that the flavonoid content (TFC = 209.58±1.17 μg mL^{-1} g^{-1} product) was dramatically increased ($P<0.05$). This suggests that using crushed eggshell as the press-shear force was a potential bioactive absorber, which is an advantage in further research work while moist heated treatment helped for bioactive compounds releasing during extraction (see more

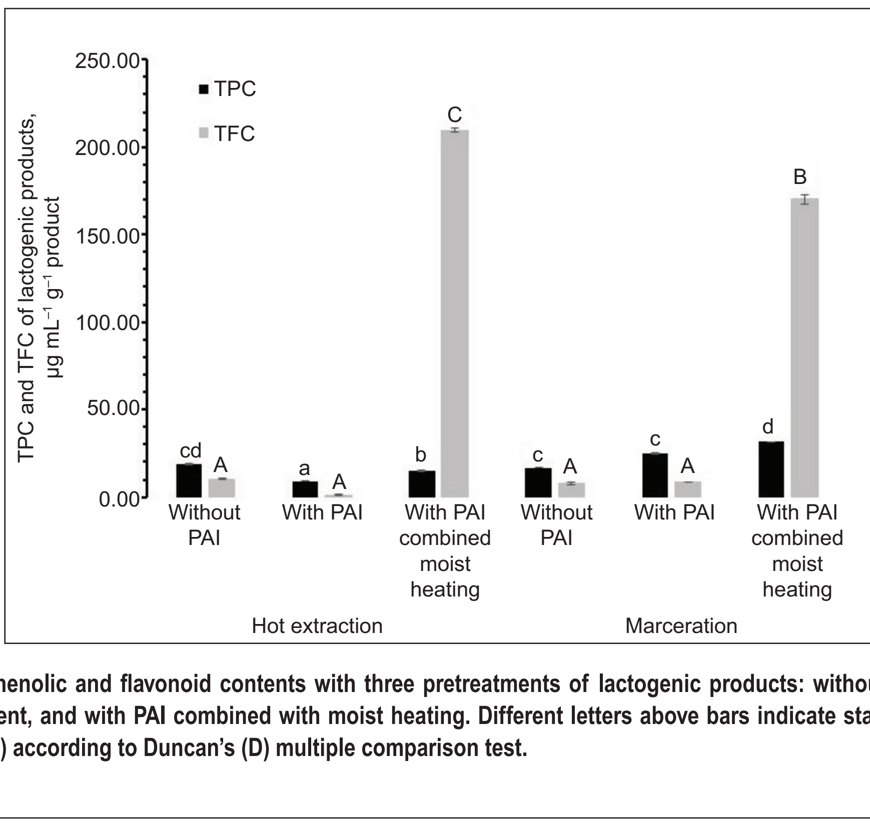

Figure 5. Total phenolic and flavonoid contents with three pretreatments of lactogenic products: without PAI pretreatment, with PAI pretreatment, and with PAI combined with moist heating. Different letters above bars indicate statistically significant difference (p < 0.05) according to Duncan's (D) multiple comparison test.

explanation in SEM part). Moreover, it was noticed that using maceration significantly gave the phenolic content higher when compared with hot extraction.

The phenolic acid and flavonoid compounds in lactogenic products with PAI pretreatment were identified by ESI mode of LC/Q-TOF/MS (details not shown). Phenolic profile presented both hydroxycinnamic acid derivatives and some flavone derivatives, such as caffeic acid, a natural and safe phenolic acid, which exerts a strong antioxidant and anti-inflammatory activities (Zhang *et al.*, 2018). Quinic acid could perform esterification with other phenolic acids, such as caffeic, *p*-coumaric, and ferulic acids, to chlorogenic acid as a product exhibiting antioxidant, antibacterial, anti-inflammatory, hepatoprotective, cardioprotective, neuroprotective and anti-obesity activities (Naveed *et al.*, 2018). Baicalin (7-glucuronic acid 5, 6-dihydroxyflavone), a natural flavone glycoside, exhibited various pharmacological activities, such as antioxidant, anti-inflammatory, anticancer, hepatoprotective, antibacterial, antiviral (Srinivas, 2010), antifungal (Yang *et al.*, 2014), cardioprotective (Huang *et al.*, 2005), photoprotective (Zhang *et al.*, 2014), antiabortive (Wang, Zhao and Zhong, 2014), anxiolytic (Wei, Yang and Wu, 2006) and immunosuppressive activities (Yang, Yang and Li, 2012). Moreover, it protects the human body against several diseases, such as pancreatitis, obesity, diabetes (Srinivas, 2010), rheumatoid arthritis (Yang, Yang and Zou, 2013), asthma (Wu *et al.*, 2012), androgenetic alopecia (Yeo *et al.*, 2014), Parkinson (Guo, Chen and Xiong, 2014) and Alzheimer (TarragÓ, *et al.*, 2008). Kaempferol, luteolin and apigenin, three types of flavone subclasses of flavonoids, also exert various pharmacological activities and possesses antioxidant, anti-inflammatory, anti-tumor and anticancer properties. In addition, kaempferol (Kaempferol 3-xylosyl-(1->2)-rhamnoside and kaempferol 3-rhamnoside-(1->2)-rhamnoside), natural and active flavonoids, could alleviate the lipopolysaccharide produced by inflammatory mediators, such as thymic stromal lymphopoietin, IL-1*b*, TNF-a, IL-6 and nitric oxide, from differentiated macrophage-like cells (Nam *et al.*, 2017). Luteolin could ameliorate chronic cerebral hypoperfusion induced cognitive dysfunction in an Alzheimer patient (Fu *et al.*, 2014). Apigenin, a natural flavone, can serve with potential chemo preventive and therapeutic effects. It could inhibit lipid peroxidation, protecting BNLCL2 cells against oxidative damage without conversion into cancer cells (Chan *et al.*, 2012).

The characteristics of fine microparticles on powered lactogenic products

Organic Thai Herbs of Community Enterprise has utilized thermal treatment to ensure microbial safety of the herb ingredients and storage stability of nutritional attributes in a quality of product. Water activity (a_w) is an index related to the food safety of lactogenic products to

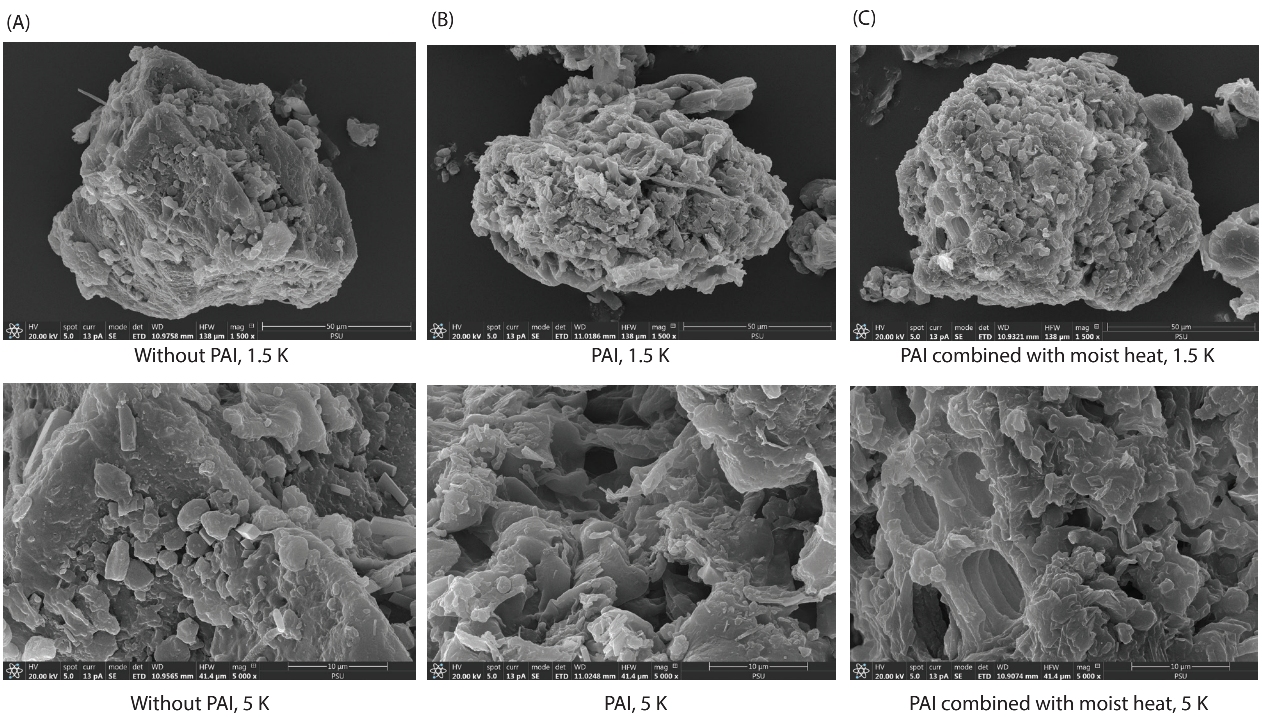

Figure 6. SEM micrograph of lactogenic products; A = lactogenic product without PAI pretreatment, B = lactogenic product with PAI pretreatment and C = lactogenic product with PAI combined with moist heat. The magnifications are 1.5 K (top row) and 5 K (bottom row).

spoilage by pathogenic bacteria, commonly *Salmonella* spp., leading to foodborne illness (Olaimat *et al.*, 2020). The growth of bacteria requires $a_w > 0.8$, for yeasts and molds $a_w > 0.6$. The lower a_w, the better the stability of a food product. The measured a_w of lactogenic product without PAI and with PAI treatment was 0.60 and 0.56, respectively, as shown in Table 2. The pH levels of lactogenic products with and without PAI pretreatment were weakly acidic due to constituting various organic acids including succinic acid, pantothenic acid, caffeic acid, quinic acid, and benzoic acid (data from LC-TOFMS but do not shown) while the color of that with PAI pretreatment was darker, more red and yellow than that without PAI. According to an approximate pH 5.5 of the case with PAI pretreatment, the magnitude of zeta potential (ZP) of suspended solution exhibited a lesser negative charge (-23.89±1.47 mV) on the surfaces of microparticles, indicating that these began to attract each other. This tended to increase both the effective diameter (1,580.25±195.31 nm) and $d_{50\%}$ (196.7±11.52 µm), compared to those without PAI pretreatment (1,572.90±245.97 nm and 184.1±7.08 µm). The lactogenic beverage without PAI pretreatment possessed a significantly lower polydispersity index (PDI) (0.30±0.04) than that with PAI pretreatment (0.50±0.02), suggesting that the suspended microparticles had more concentrated distribution. Therefore, the suspended microparticles tended to aggregate due to van der Waals interactions, after agglutination would occur, and finally, the flocculation might occur. Otherwise, the microparticle stability was considered to decrease due to attractive energy. Finally, this beverage could be allowed to settle for possible obtaining a clear solution. Furthermore, the moist heat pretreatment helped generate more porosity and the reduction of particles into a monodisperse size distribution appeared ($d_{50\%}$= 137.64±9.67, PDI = 0.09±0.01). This behavior facilitated surface attraction, resulting in an effective diameter increase (4,336.52±974.61 nm).

Morphological observation of the lactogenic products

The Scanning Electron Microscope micrographs revealed micro-morphological structures of powdered lactogenic products and obviously exhibited the grain on that without PAI pretreatment, having irregular surfaces at magnifications of 1.5 and 5 K. When press shear forces were applied with eggshell powder onto these particles, dense spherical particles appeared with a lesser number of cavities. The De Broucker mean particle size (D[4,3]) with PAI pre-treatment gave a smaller mean diameter (268.78±12.77 µm) than without PAI pretreatment (270.70±6.78 µm). Furthermore, when the moist heating was used in pretreatment, spherical microparticles with comparatively large pore sizes and an increased porosity emerged, as shown in Figure 6. The effective porosity

Table 2. Physical properties of three types of lactogenic products (without PAI pretreatment, with PAI pretreatment and with PAI combined with moist heating) including a_w, pH, color coordinates, particle size characteristics and Zeta potential of fine particles.

Lactogenic product	a_w	pH	Color measurement			Particle size analysis			Zeta potential analysis		
			L*	a*	b*	d_{50}% (µm)	Span, (d90-d10)/d50	D[4,3], (µm)	Zeta Potentail (mV)	Polydispersity index	Effective Diameter (nm)
Without PAI pretreatment	0.60±0.00	5.44±0.01	57.33±0.02	7.54±0.02	42.99±0.03	184.1±7.08[b]	3.48±0.09	270.70±6.78	−29.29±2.67[b]	0.30±0.04[b]	1,572.90±245.97[b]
With PAI pretreatment	0.56±0.00	5.54±0.01	56.93±0.01	8.48±0.01	43.48±0.06	196.7±11.52[c]	3.21±0.14	268.78±12.77	−23.89±1.47[a]	0.50±0.02[c]	1,580.25±195.31[c]
With PAI combined with moist heating	-	-	-	-	-	137.64±9.67[a]	3.40±0.12	196.2±14.32	−23.96±1.23[a]	0.09±0.01[a]	4,336.52±974.61[a]

The data on a_w, pH and color coordinates are given as mean±S.D. from triplicate were measurements.
The data on particle size and zeta potential represent ten replicates.
Different superscripts in the same column indicate statistically significantly differences ($p<0.05$) in $d_{50\%}$, Zeta Potential, Polydispersity index or Effective Diameter.

under moist heating pretreatment might increase adsorption and permeation of bioactive compounds into the structure of microparticles. It was concluded that pressing with shear force in PAI technology facilitated the formation of pores along with particle sizes reduction ($d_{50\%}$ = 137.64±9.67 μm), more concentrated effective diameter (4,336.52±974.61 nm) and monodisperse distribution (PDI = 0.09±0.01). Using PAI with moist heating could provide orderly porosity then facilitate to easily extraction.

This result highlighted a significant data whether how PAI and moist heated function that no publications have been addressed. In addition, using moist heated treatment could significantly improve and completely passed Thai FDA standard for microbiological quality (data do not show).

Conclusions

The quality of mixed galactagogue herbal tea was assessed in terms of extractable bioactive substances. The utilization of the press-shear force interaction technology (PAI) in a pretreatment tended to improve the extraction of bioactive substances, which could adsorb in the pores of spherical microparticles of the lactogenic products. Important determinants of quality included freshness, part of use, storage duration of the galactagogue herbs, dehydration step, and brewing method: all of these influenced the bioactive contents. Using PAI and moist heated treatment was an appropriate technique for the galactagogue product.

Credit authorship contribution statement

Chanisara Kluabpet: Investigation. Chaninat Anantapan: Investigation. Vatcharee Seechamnanturakit: Methodology, Investigation, Data curation, Resource, Writing – review & editing, Visualization, Supervision. Sunisa Siripongvutikorn: Conceptualization, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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