Original Article

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Three functional foods from *Garcinia mangostana* L. using low-α-mangostin aqueous extract of the pericarp: Product development, bioactive compound extractions and analyses, and sensory evaluation

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ABSTRACT

Background: As a result of the expansion of the aging society worldwide, the trend in consuming healthy foods as well as functional foods for maintaining good health and preventing chronic diseases is increasing. At present, functional foods are commercially available in the market in many forms. However, their health benefits and stabilities are not routinely confirmed. Mangosteen pericarp has been well-known for its high content of xanthones which exhibited various biological activities. Aqueous extract of mangosteen pericarp which contained a low amount of α-mangostin is abundant in polymeric flavonoids such as epicatechin. **Materials and Methods:** This study aimed to develop three functional foods containing low-α-mangostin aqueous extract of *Garcinia mangostana* L. pericarp as a functional ingredient in several applications including homemade chocolate, crunchy cornflakes, and chrysanthemum and chamomile flower tea, using processes avoiding the degradation of bioactive compounds. The bioactive compounds contained in the finished products were extracted and analyzed. **Results:** The three functional foods obtained the mean liking scores between 7 ("like moderately") and 8 ("like very much") in all major attributes and overall preference in sensory evaluation using 9-point hedonic scale method. Epicatechin, the major compound of the aqueous extract of mangosteen pericarp, was used as the biomarker in the thin-layer chromatography and high-performance liquid chromatography analyses of developed functional foods. The result showed that the percentage retention of epicatechin in three functional foods was in the range of 55.85–84.46. Similarly, due to the complexity of the food matrix, total phenolic, and total flavonoid contents of functional foods extracts were lower than anticipated. Adjustment of the extraction of the active compounds was discussed. **Conclusions:** The information obtained from this study suggested the potential uses of the aqueous extract from mangosteen pericarp in several applications as a functional ingredient.

Keywords: Functional foods, *Garcinia mangostana* L., mangosteen pericarp, product development, sensory evaluation

INTRODUCTION

Due to the progress in science and technology especially medical technology, the life span of people is longer, leading to the growing size of aging society worldwide. Thus, the trend in consuming healthy foods as well as functional foods for maintaining good health and preventing chronic diseases is increasing. Functional foods are foods which provide health benefits beyond nutrition.[1] Recently, the global revenue from functional food market was

approximately 43.27 billion USD in 2013 and was expected to reach 190 billion USD by 2015.[2] There are many forms of functional foods such as beverages and snacks. All of which are preferable for all groups of consumers. Although there are a large number of functional foods commercially available in the market, their health benefits and stabilities are not routinely confirmed.

Mangosteen (*Garcinia mangostana* L., Family Clusiaceae), the queen of fruits, is a tropical plant native to Southeast Asia. In addition to the unique and pleasant taste of the pulp, the pericarp of mangosteen has been well-known for its high content of xanthones which possessed a wide variety of biological activities such as antioxidant, antiproliferative, anti-inflammatory, neuroprotective, and hypoglycemic.[3-5] Alcoholic extract from mangosteen pericarp is known to be a rich source of xanthones, especially α-mangostin.[6] However, aqueous extract of mangosteen pericarp which contained a low amount of α-mangostin is abundant in polymeric flavonoids such as epicatechin.[7] Although the products containing mangosteen pericarp and pulp are popularly consumed as beverages, powders, capsules, and extracts, the development of the products containing mangosteen pericarp with the suitable process and the analysis of bioactive compounds contained in the finished products have not been carried out. According to a recent clinical study, the aqueous mangosteen extract showed a positive effect and safety on Alzheimer's patients at the double dose of 220 or 280 mg daily for 60 days.[8] Thus, the dose of 220 mg of the aqueous extract was set for each serving of the three functional foods to be developed. Preliminary study by our research group revealed that the aqueous mangosteen extract was able to withstand the heat treatment not higher than 100°C for up to 30 min as its biological activities and the content of the bioactive compound, epicatechin, were still retained. In this study, the functional foods containing low-α-mangostin aqueous extract of mangosteen pericarp with processes avoiding the degradation of bioactive compounds were developed and subjected to sensory evaluation. The bioactive compounds contained in the finished products were extracted and analyzed.

MATERIALS AND METHODS

Preparation of α-Mangostin-free Aqueous Mangosteen Extract and Isolated Epicatechin

Low-α-mangostin aqueous mangosteen extract in powder form was prepared from the pericarp of mangosteen as described by Tunrungruangtavee.[9,10] Briefly, small mangosteen pericarp pieces were dried and ground. The dried powder was then macerated with ethanol and concentrated using a rotary evaporator at 45°C. The crude ethanolic extract was then partitioned with ethyl acetate and distilled water. The crude water extract was dissolved in distilled water at 55–60°C. The concentrated crude water extract was collected and centrifuged at 6000 rpm for 15 min. The supernatant was spray-dried at 180°C into powder and stored at 4°C. Isolated epicatechin, a marker compound, was prepared and stored at 4°C as described in the studies as well.

Identification and Determination of the Active Compounds

Thin-layer chromatography (TLC) analysis

The aqueous mangosteen extract was subjected to TLC analysis using silica gel 60 F254 aluminum plate as the stationary phase and EtOAc-hexane-EtOH-formic acid (10:6.0:0.7:0.25) as developing solvent. After development, the plate was dried in the fume hood. TLC chromatogram was observed under UV light at the wavelengths of 254 and 366 nm and compared with that of the marker compound (Isolated epicatechin).

High-performance liquid chromatography (HPLC) analysis

The chromatographic analyses were performed using a system of HPLC Shimadzu CLASS-VP V6.14 SP2 model. The separation of chemical constituents was carried out, using C18 column, Hypersil BDS-C18, with guard column. The mobile phase consisted of 0.1% (v/v) formic acid in water and methanol using a gradient program at 254 nm, in the run time of 65 min.[11]

Determination of total phenolic contents

Total phenolic contents of the samples were assessed using Folin–Ciocalteu's method. The method was slightly modified from Vongsak *et al*.[12,13] The sample, at 150 mg/ml concentration, was prepared by diluting in water. 20 μl of the sample was added into 96-well microtiter plate followed by 50 μl of 10% Folin–Ciocalteu reagent and then allowed to react for 3 min. 80 μl of 7.5% w/v of Na2 CO3 was added. After incubating in the dark for 2 h, the absorbance was measured at 765 nm. The assay was carried out in triplicate. The phenolic content was expressed as gallic acid equivalent (GAE) in mg/g of extract, using a standard curve generated from standard gallic acid.

Determination of total flavonoid contents

Total flavonoid contents were determined by the aluminum microliters chloride colorimetric test modified from Herald *et al*.[14] 160 μl of sterile water was added into the Eppendorf followed by 40 μl of sample (1 mg/ml) and 15 μl of 5% $NaNO_2$. After 5 min, 24 μl of 10% $AlCl_3.6H_2O$ was added. The mixture was allowed to react for 6 min. Finally, 80 μl of 1 M NaOH microliters and 80 μl of sterile water were added and mixed well. Then, 250 μl of the reaction mixture was transferred to the 96-well microtiter plate. The plate was shaken for 30 s and the absorbance was read at 510 nm. The assay was performed in triplicate. The total flavonoid content was expressed as quercetin equivalent (QE) in mg/g of extract.

Functional Foods Development

Three functional foods, containing aqueous mangosteen extract, were developed. The dose of the extract in each product was set at 220 mg per serving. The heat treatment applied in the processing of functional foods was not higher than 100°C for up to 30 min to avoid the degradation of bioactive compounds.

Preparation of homemade chocolate containing aqueous mangosteen extract

Three parts of the products were prepared: (1) Coating chocolate part (23.3%w/w of the formula), the ingredients were, in %w/w, chocolate bar (Cacao Barry®-58%cocoa) (11.65) and chocolate compound (Tulip®) (11.65); (2) body chocolate part (70.0%w/w of the formula), the ingredients were, in %w/w, chocolate (Cacao Barry®-58%cocoa) (46.67) and chocolate compound (Tulip®) (23.33). All the ingredients of the two parts were separately melted in two separate saucepans over low heat; and (3) yogurt filling part (6.70%w/w of the formula), the ingredients were, in %w/w, yogurt (Dutch Mill®) (4.06), water (2.03), aqueous mangosteen extract (0.45), gelatin (McGarrett®) (0.12), and strawberry flavor (Winner®) (0.04). Gelatin was dissolved in water, at 60°C, and then combined with yogurt and strawberry flavor. The mixture was then pasteurized at 72°C, for 15 s. The oval mold, $\pi/4 \times 2.5 \times 3 \times 1.7$ cm^3, was selected for the product. One serving (10.0 ± 0.1 g) was set to comprise 5 pieces of the product and contain 220 mg of aqueous mangosteen extract. The melted coating chocolate was poured into the mold to cover the base and the side of the mold, and cooled to 10°C for 5 min. The melted body chocolate was poured into the mold to fill approximately 1/5 of the height and cooled to 10°C for 5 min. Yogurt filling was added and cooled to 10°C for 5 min. The remaining body chocolate was poured into the mold to fill approximately 4/5 of the height and then cooled to 10°C for 10 min. The remaining coating chocolate was poured into the mold and then cooled to <25°C. The finished homemade chocolate pieces were weighed. The average weight was calculated from 20 pieces of the chocolate products.

Preparation of crunchy cornflakes containing aqueous mangosteen extract

The ingredients of crunchy cornflakes were, in %w/w, cornflakes (Kellogg's Cornflakes) (56.6), white chocolate compound (Tulip®) (25.8), brown sugar (6.5), sliced almond (12.9), water (2.5), and aqueous mangosteen extract (0.7). The product was set to be 30 g per serving and contain 220 mg of the aqueous mangosteen extract. Almond flakes were spread onto a metal tray and then put into a 100°C oven for 30 min or until slightly golden. The white chocolate, sugar, water, and the aqueous mangosteen extract were heated in a saucepan, using low heat, until sugar was dissolved and the mixture was frothy. Cornflakes were spread onto a tray. The mixture was poured over the cornflakes until all the cornflakes were coated. The coated cornflakes were heated at 100°C in an oven for 15 min or until crispy and allowed to cool and harden. The coated cornflakes were stored in an airtight container.

Preparation of chrysanthemum and chamomile flower tea containing aqueous mangosteen extract

The ingredients of chrysanthemum and chamomile flower tea were, in chamomile flower %w/w, dried chrysanthemum flower (56.39), dried chamomile flower (18.80), maltodextrin (16.54), and aqueous mangosteen extract (8.27). Both chrysanthemum and chamomile flowers were heated at 60°C for 30 min in a hot air oven and allowed to cool before weighing and mixing. The aqueous mangosteen extract was blended with maltodextrin in the ratio of 1:2. The ingredients were then mixed together and packed in a paper sachet and sealed. The product was set to be 2.66 g per serving and contain 220 mg of the aqueous mangosteen extract.

Analysis of Functional Foods

Extraction of bioactive compounds from functional foods

About 250 g of homemade chocolate with and without the aqueous extract was weighed. Both yogurt fillings with and without aqueous mangosteen extract were separated from the chocolate and weighed. They were extracted with 50 ml of methanol by maceration in a shaker at 65°C for 45 min and frozen in a freezer at 0°C for 30 min. Each of the macerate was centrifuged, filtered and the marc was re-extracted twice. Then, the extracts were combined and evaporated using a rotary evaporator. The extracts (2.31 g) were stored at −20°C until further analysis.

About 156 g and 15 mg of crunchy cornflakes with and without the aqueous extract were weighed. Both caramel coatings of crunchy cornflakes with and without aqueous mangosteen extract were grounded into coarse powder and extracted with 50 ml of methanol by maceration in a shaker at 65°C for 45 min. Each of the macerate was centrifuged, filtered and the marc was re-extracted twice. Then, the extracts were combined and evaporated to dryness using a rotary evaporator and weighed. The extracts (2.77 g) were stored at −20°C until further analysis.

13 g and 30 mg of flower tea with and without aqueous mangosteen extract were weighed and extracted with 150 ml of sterile water by maceration in a shaker at 65°C for 45 min. Each of the macerate was centrifuged, filtered and the combined, lyophilized marc was re-extracted twice. Then, the extracts were combined, lyophilized, and weighed. The extracts (1.39 g) were stored at -20°C until further analysis.

TLC analysis

About 10 μl of each of the eight sample solutions were applied on the TLC plate and were subjected to TLC analysis as described.

HPLC analysis microliters

Functional foods extracts from the three functional foods were analyzed using HPLC in the same manner as explained.

Determination of total phenolic and total flavonoid contents

Functional foods extracts from the three functional foods were analyzed in the same manner as previously described.

Sensory Evaluation

Fifty-four panelists, aged ≥40 years, were recruited for the sensory evaluation test. The sensory panel comprised 27 females and 27 males. Three functional foods formulations were prepared. The products were packed in boxes before being sent to the panelists for a home use test. Each panelist was asked to fill a questionnaire regarding the attributes of the products, appearance, flavor, and overall preference of the samples, using 9-point hedonic scale method. The experiment

was approved by the Ethical Review Committee for Human Research, Mahidol University (COA.No. MU-DT/PY-IRB 2016/014.0303).

Statistical Analysis

All quantitative data were reported as mean ± standard error of the mean. Statistical analyses were carried out using SPSS version 11.5. Multiple comparisons of more than two groups ($n = 3$ for each group) of variables were performed using two-way analysis of variance with Scheffe *post hoc* test. Statistical significance was considered when $P < 0.05$.

RESULTS AND DISCUSSION

Low-α-Mangostin Aqueous Mangosteen Extract and Isolated Epicatechin

The aqueous mangosteen extract powder obtained was reddish brown in color. Initially, the preparation process was intended for the extraction of α-mangostin using ethyl acetate. The aqueous mangosteen extract, the byproduct of the initial process, containing other active compounds was shown to exhibit antioxidant activity and other activities.[7] The powder was soluble (1 mg/30 μl) in water up to 120°C. In the previous studies, the aqueous mangosteen extract showed no toxicity, both acute and subchronic toxicities.[3,15,16] Isolated epicatechin was also obtained and was used as a marker compound in the analytical step.

Identification and Determination of the Active Compounds in the Aqueous Mangosteen Extract

TLC analysis

TLC analysis revealed the presence of the major compound at the same Rf as epicatechin in the aqueous mangosteen extract as shown in Figure 1.

HPLC analysis

HPLC analysis was performed to compare the pattern of HPLC chromatograms of the aqueous mangosteen extract and the isolated epicatechin. The aqueous mangosteen extract contained epicatechin as major chemical constituent which appeared at the same retention time as the isolated epicatechin (46 min) [Figure 2]. 1 g of the aqueous mangosteen extract contained 35.06 ± 0.79 mg of epicatechin.

Determination of total phenolic and total flavonoid contents

The total phenolic and total flavonoid contents of the aqueous mangosteen extract were 593.29 ± 4.34 mg GAE/g of the extract and 1446.44 ± 2.83 mg QE/g of the extract, respectively. The result of total phenolic content from our study was in accordance with previous reports.[17,18]

Functional Foods Development

Three functional foods containing aqueous mangosteen extract have been developed. Homemade chocolate piece weighed 9.99 ± 0.05 g per piece (average weight of 20 pieces). The color was glossy dark brown and the flavor was sweet and mild chocolate, with sour and strawberry flavor [Figure 3a]. Crunchy cornflakes were golden brown in color, and crispy. The serving size was 30 g [Figure 3b]. Chrysanthemum and chamomile flower tea, the dry mix, was yellow-green in color [Figure 3c]. When the dry mix was dissolved in 150 ml of water, the color of tea was yellow, with a fragrant aroma and mild chrysanthemum flavor. The serving size was 2.66 g.

This study aimed to develop functional foods which included the aqueous extract of *G. mangostana* L. in several applications as a functional ingredient. The extract was used in three applications, filling, coating, and dry mix form. Filling was a suitable matrix to apply at room temperature in the last step of preparation for products requiring high temperature. Coating is used as a matrix for the dry,

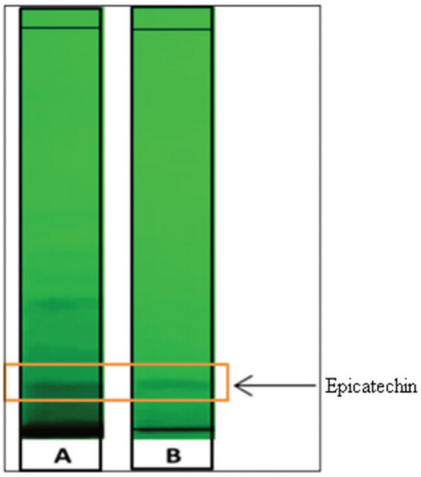

Figure 1: Thin-layer chromatography chromatograms of the aqueous mangosteen extract (A) and the isolated epicatechin (B) using silica gel aluminum sheet 60 F_{254} as an absorbent, EtOAc-hexane-EtOH-formic acid (10:6.0:0.7:0.25) as developing solvent, and observed under UV 254 nm

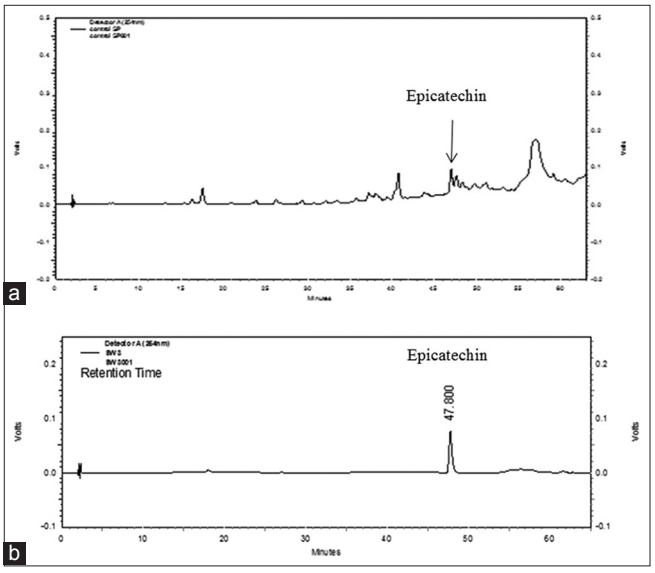

Figure 2: High-performance liquid chromatography chromatograms of the aqueous mangosteen extract (a) and isolated epicatechin (b)

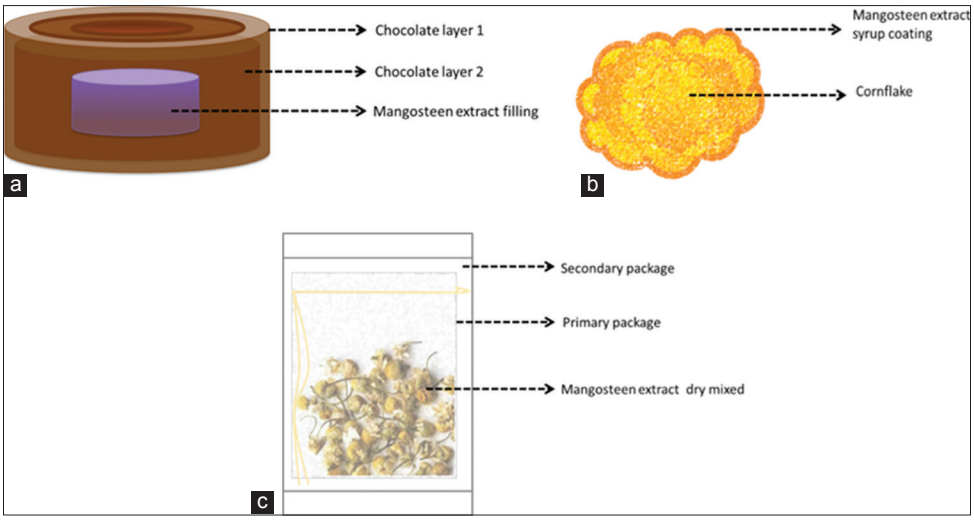

Figure 3: Functional foods containing aqueous mangosteen extract; homemade chocolate (a), crunchy cornflakes coated with white chocolate (b), and chrysanthemum and chamomile flower tea (c)

crispy products. Dry mix was the general use of powder ingredients in foods. Therefore, homemade chocolate, crunchy cornflakes, and chrysanthemum and chamomile flower tea were developed, with the inclusion of the aqueous mangosteen extract in the form of "filling," "coating," and "dry mix," respectively.

Homemade chocolate

The aqueous mangosteen extract was added to the yogurt filling because this part was the least-complex matrix among the three parts of the product and required the shortest mixing time. The extract was added last in the yogurt mixture. The purpose was to maintain the stability of the extract and facilitate subsequent extraction process before the analysis of the active compounds. Coating chocolate and body chocolate were prepared separately due to the difference in fat contents. The chocolate compound in coating chocolate contained 34% of palm oil, and 18.2% of cocoa, and the chocolate bar in body chocolate contained 38% of cocoa butter, and 58% of cocoa. Both chocolate mixtures required different melting processes. In coating chocolate, the ratio of chocolate bar and chocolate compound was 1:1 to avoid melting at room temperature in the rotation step. As for body chocolate, the ratio of chocolate bar and chocolate compound was 2:1 to provide prompt melting in the mouth, thus resulting in smooth texture and sweet flavor. Chocolate compound contained palm oil, leading to high-temperature melting. Chocolate bar contained chocolate mass, leading to easier melting, and smooth-texture mouthfeel. For the chocolate tempering method, the temperature of 55°C was maintained for the melted chocolate to minimize chocolate fat crystallization.

Crunchy cornflakes

The aqueous mangosteen extract was added to the coating syrup. The aqueous mangosteen extract powder was dissolved in water before being added into the syrup. To maintain the stability of the aqueous mangosteen extract, coating syrup was exposed to heat not higher than 100°C. In addition, temperature control was also essential in the oven drying steps to obtain the crispy product; cornflakes had to be heated at 100°C before and after the coating.[19] The finished product was allowed to cool to room temperature before being filled into a container to maintain the crispiness. Silica gel sachet was placed in the bottom of the container to absorb the moisture until the next step.

Chrysanthemum and chamomile flower tea

Chrysanthemum and chamomile flower tea containing the aqueous mangosteen extract was developed to show the solubility of the extract to be used as a dry mix. Chrysanthemum and chamomile flower was heated to reduce the moisture content. The aqueous mangosteen extract was blended with maltodextrin in the ratio of 1:2 to avoid caking and to increase the bulk of the mix. The developed tea could be brewed with hot water, and there was no lump of mangosteen extract mixture in the bottom of the glass. The color of the drink containing the aqueous mangosteen extract was slightly brown when compared with control, but the flavor difference could not be detected. Primary packaging is paper sachet; the secondary packaging is PE bag for moisture control.

Extraction and Analysis of Bioactive Compounds in Functional Foods

Extraction of bioactive compounds from functional foods

The results of the extraction of bioactive compounds from the three functional foods were as follows. Caramel coating and flower tea extracts were in dried form, but the extract from yogurt filling was in semisolid form. Dried weights of the extracts from yogurt filling: Control = 12.26 g, test = 14.46 g; caramel coating: Control = 2.84 g, test = 3.15 g; and flower tea: Control = 18.36 g, Test = 18.15 g. Since each of the products contained different ingredients; therefore, the extraction methods were adjusted accordingly. The extraction of caramel coating with methanol and the extraction of flower tea with water was carried out without any problems. However, during the extraction process of the matrix of yogurt filling, the brown-colored gel separated from yogurt filling supernatant. Yogurt

filling contained gelatin, a soluble fiber, which could bind with phenolic compounds.[20] Thus, some adjustment was applied. The maceration with methanol under low chilling temperature was used to accelerate the soluble fiber precipitation from yogurt filling before being centrifuged and filtered.

TLC analysis

The TLC chromatograms of eight extracts from functional foods compared with aqueous mangosteen extract and the isolated epicatechin were shown in Figure 4. High-intensity bands of epicatechin in all products were observed under UV 254 nm with R_f value of 0.133.

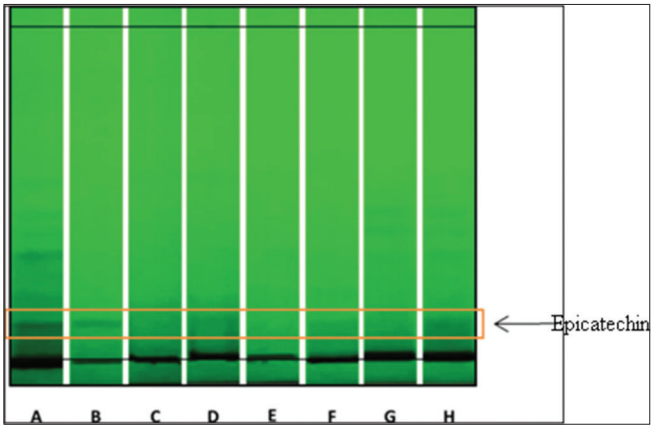

Figure 4: Thin-layer chromatography chromatograms of A = aqueous mangosteen extract, B = epicatechin, C = crunchy cornflakes (Control), D = crunchy cornflakes (Test), E = homemade chocolate (Control), F = homemade chocolate (Test), G = chrysanthemum and chamomile flower tea (Control), H = chrysanthemum and chamomile flower tea (Test) using silica gel aluminum sheet 60 F_{254} as an absorbent; EtOAc-hexane-EtOH-formic acid (10:6.0:0.7:0.25) as developing solvent and observed under UV 254 nm

HPLC analysis

From HPLC analysis, the active compounds of the three functional foods were similar as shown in Figure 5. The content of the active marker, epicatechin, in each sample was calculated from the linear regression equation. The percentage retention of epicatechin in yogurt filling (homemade chocolate), caramel coating (crunchy cornflakes), and chrysanthemum and chamomile flower tea was 55.85, 79.61, and 84.46, respectively. The retention of epicatechin varied in three functional foods. This might be the result of the difference in the ingredients used in each functional food. As gelatin which is a soluble fiber used in the preparation of yogurt filling was reported to possibly bind with phenolic compounds,[20] the lowest retention of epicatechin was found in yogurt filling.

Determination of total phenolic and total flavonoid contents

Total phenolic and total flavonoid contents of the three functional foods were shown in Table 1. From the result, total phenolic and total flavonoid contents of the three functional foods were lower than anticipated. The interactions between phenolic or flavonoid compounds and the matrices of functional foods may be the cause of these differences. On the other hand, other ingredients in the flower tea also contributed to the phenolic and flavonoid contents, thus increasing the total values of the whole product.

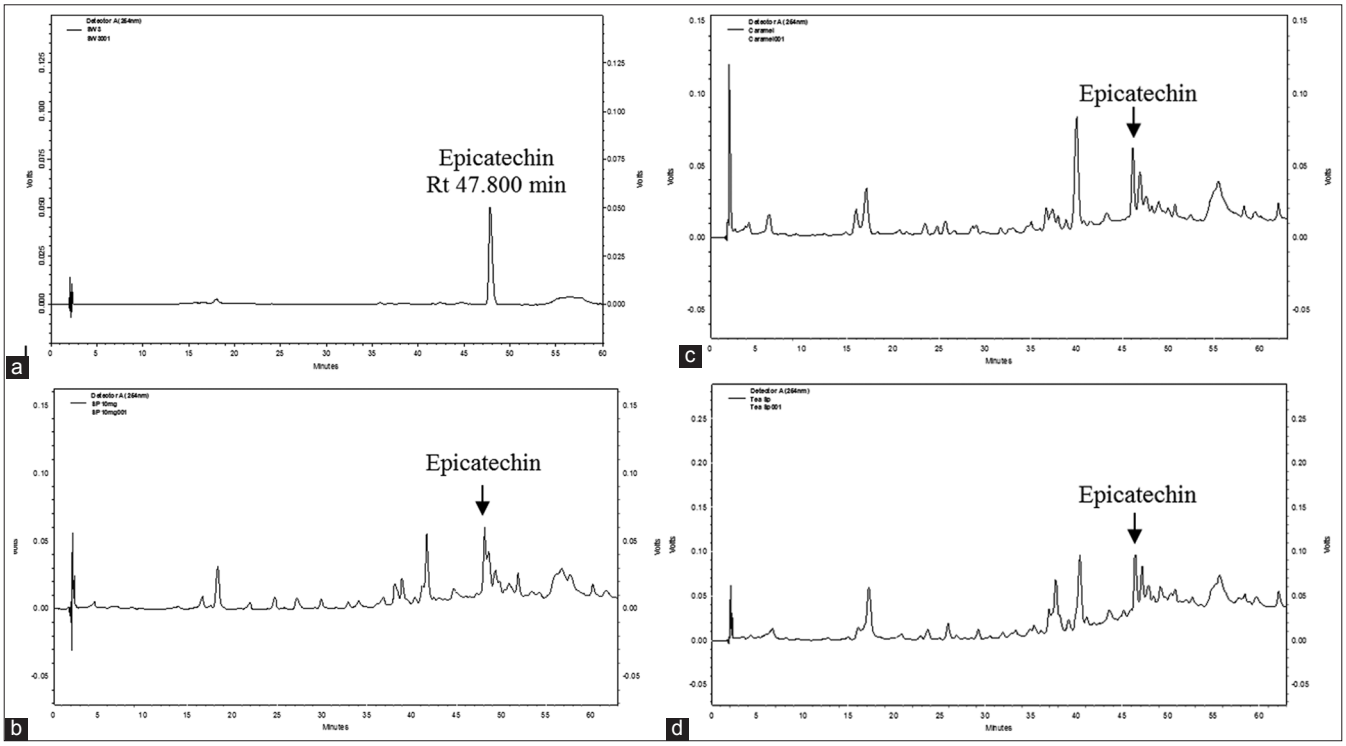

Figure 5: High-performance liquid chromatography (HPLC) chromatograms of the extracts; isolated epicatechin (marker compound) (a), homemade chocolate (b), crunchy cornflakes (c), and chrysanthemum and chamomile flower tea (d)

Table 1: Total phenolic and total flavonoid contents of the three functional foods

Extract	Total phenolic content (mg GAE/g of functional food extract)		Total flavonoid content (mg QE/g of functional food extract)	
	Control*	Test	Control*	Test
Homemade chocolate	ND	41.50±0.42	0.93±0.23	310.66±1.15
Crunchy cornflakes	ND	95.14±0.68	9.86±0.46	124.88±1.38
Chrysanthemum and chamomile flower tea	47.34±0.09	184.16±0.42	262.22±2.34	1047.33±1.76

*Control=The functional food without the aqueous mangosteen extract. Values were presented as mean±SD of triplicate measurements. ND: Not detected

Table 2: Sensory evaluation of the three functional foods using 9-point hedonic scale method

Sample	Attributes			
	Appearance	Aroma	Flavor	Overall preference
Crunchy cornflakes	7.52	7.56	7.80	7.74
Homemade chocolate	7.85	7.50	7.67	7.74
Chrysanthemum and chamomile flower tea	7.54	7.35	7.22	7.43

1=Dislike extremely, 9=Like extremely; Values were presented as means from 54 panelists (aged >40 years old, 27 males and 27 females)

Sensory Evaluation

Functional foods which were sent to 70 panelists (aged over 40 years old, 27 females and 27 males) for a home use test, 54 questionnaires were retrieved. The sensory panel for the preference test consisted of people aged over 40 years old. All of the samples obtained the mean liking scores between 7 ("like moderately") and 8 ("like very much") in all major attributes (appearance, aroma, and flavor) and overall preference [Table 2]. The mean overall preference scores of the three functional foods were not significantly different ($P > 0.05$).

A slight interference from the brown color of the aqueous mangosteen pericarp extract was observed in crunchy cornflakes. However, both the flavor and color of the extract did not affect the acceptance of the products.

CONCLUSIONS

Three functional foods containing low-α-mangostin aqueous extract of mangosteen pericarp were successfully developed. The extract was able to be used in three applications, filling, coating, and dry mix form. The results from TLC and HPLC analyses and the determination of total phenolic and total flavonoid contents confirmed the retention of the bioactive compound, epicatechin, in the developed functional foods. The information obtained from this study suggested the potential uses of the aqueous extract from mangosteen pericarp in several applications as a functional ingredient.

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