Biofunctional stirred yoghurt production using Mangosteen (*Garcinia mangostana* L.) peel powder extract

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ABSTRACT

Mangosteen (*Garcinia mangostana* L.) peel powder (MPP) extract was incorporated to develop stirred yoghurt rich in functional properties. Mangosteen peels are seasonal agricultural waste, rich in phenolic compounds and antioxidant activity. Ethanol (80%) with MPP (10:1) extract was prepared. Stirred yoghurt was produced using pasteurized cow-milk (3.25% fat, 23% total solids) incorporating 1.0, 1.5 and 2.0 mL of MPP extract/kg of yoghurt mixture as treatments. Sensory evaluation in a nine-point hedonic scale was conducted to determine the best level of MPP extract that suit the best sensory attributes in stirred yoghurt. The control was prepared without MPP extract. Total phenolic content (TPC, measured as mg GAE/g) and antioxidant activity of MPP extract and the treatments were determined using the standard procedures. pH profile during storage of yoghurt, physiochemical analysis, and microbiological parameters was determined. Data were analyzed by Kruskal-Wallis test, nonparametric one-way ANOVA test at $p<0.05$. Among all treatments, MPP extract-treated (1.5 mL/kg) yoghurt was selected as the best. TPC of MPP treated sample and control showed 3.88 ± 0.14 and 2.5 ± 0.04 respectively, where treated sample showed the highest TPC value. MPP extract of 1.5 mL/kg of yoghurt mixture was insufficient to provide a substantial antioxidant activity in the yoghurt whereas, it was sufficient to ensure the organoleptic acceptability and microbiological safety of stirred yoghurt. pH reduction was significantly higher ($p<0.05$) in the best treatment compared to the control samples. Even though the acceptability of the sample was lower than the control due to the presence of noticeable aftertaste, the best-selected yoghurt with MPP extract 1.5 mL/kg possessed 26 days of shelf life compared to the control (24 days) at 4 ± 1 °C without preservatives.

Keywords: Mangosteen peel powder extract, stirred yoghurt, Total phenolic content.

INTRODUCTION

Since the beginning, humans generate waste through various means. With the progress of population, industrialization and urbanization, the waste generated became more complex. Processing of fruits produces mainly two types of wastes, solid waste of peel/skin and seeds and liquid waste of juice and wash water. The discarded portion can be very high (e.g., mango 30-50%, banana 20%, pineapple 40-50% and orange 30-50%) (Happi Emaga et al., 2008). Therefore, there is often a serious waste disposal problem. There are several possibilities for the use of some types of solid fruit wastes. The present study focused on the utilization of mangosteen peel for the production of functional stirred yoghurt.

Mangosteen belongs to the family Guttiferae, and the botanical name is *Garcinia mangostana* L. It is native to Asia (Ovalle-Magallanes et al., 2017Mangosteen peel contain various kinds of bioactive compounds which act as therapeutic agents, functional food additives as well as phenolic acids, tannins and xanthones (Pothitirat et al., 2009; Zadernowski et al., 2009; Zarena and Sankar, 2009) It was found that mangosteen peel contains protocatechuic acid a, cyanidin- 3-sophoroside b, cyanidin-3-glucoside c, pelargonidin-3- glucoside d, procyanidin A-2 e, procyanidin B-2 f, (−)-epicatechin g, garcimangosxanthone A h, garcimangosxanthone B i and garcimangosxanthone C (Zadernowski et al., 2009). Therefore, the present study was carried out to determine the suitability of utilizing this valuable mangosteen fruit peel waste for the production of functional stirred yoghurt.

METHODOLOGY

Raw materials: Fresh ripe peels of Mangosteen collected from the fruit stalls in Kandy, Sri Lanka.

Chemicals: Ethanol, Petroleum Ether, DPPH (2,2 Diphenyl-1-picrylhydrazyl), Folin-Ciocalteu Reagent, Sulphuric Acid 90-91% (Density - 1.815 g/cm^3), Amyl Alcohol (Sigma-Aldrich UNI 170), Sodium Hydroxide Pellets.

Preparation of MPP extract: Ripen Mangosteen peels were cleaned, cut, dried at 50 °C in a drier for around 18-24 h to a moisture content less than 10%. The dried peels were ground into powder using a grinder and sieved through a 150 mm sieve. The powdered sample was kept in air-tight containers protected from light in a freezer until used for the experiments. Mangosteen peel powder extract was prepared according to the method given by Singh et al. (2013). Cleaned centrifuge tubes were taken, and 10 mL of 80% ethanol was added. One gram of MPP was added to the centrifuge tubes and mixed well until all powder was dissolved in ethanol. Eighteen centrifuge tubes were filled and kept for 2 minutes. Moreover, the tubes were centrifuged at 4000 rpm for 10 min. The supernatant was separated us-

ing a micropipette and was concentrated using a water bath maintained at 60 °C for 3-4 hrs. The extract was stored at -18 °C before analysis.

Manufacture of stirred yoghurt fortified with MPPE: Yoghurt mixture was standardized using Pasteurized fresh milk [3.5% fat and 8.5% Solid Non-fat (SNF)], full-cream milk powder (26.5% fat and 8.5% SNF) and skim milk powder (96% SNF). The yoghurt mix was pasteurized at 105 °C for 1 minute, cooled to 40 °C, inoculated with 2% starter culture and 1.5 mL (based on a sensory evaluation) of MPPE was added finally. Then the yoghurt mix was incubated at 42 °C until pH reached approximately 4.6 and stored overnight at 4 °C. The coagulum was broken by gentle stirring using a hand blender. The resulting stirred yoghurt preparation was transferred into sterile polystyrene containers, covered with lids and stored at refrigeration temperature (7-8 °C) for 21 days. The stirred yoghurts prepared without MPPE was taken as the control.

Total phenolic content: It was determined using spectrophotometry, equivalent to gallic acid standard and the method described by Huang, Boxin and Prior, 2005 with slight modifications. The concentration series was briefly prepared using a gallic acid standard solution to 10, 20, 30, and 70 ppm. For every 1 mL from this series, 5 mL of 10% of Folin-Ciocalteu reagent (water) and 4 mL of Na_2CO_3 (7.5% w/v) were added and allowed for 1 hour in room temperature, and absorbance was measured at 765 nm. The same procedure was followed to twenty times dilute 1 mL from MPP extract and 1 mL from yoghurt samples extracts. Results were expressed as gallic acid equivalents (GAE) in the mg/g sample.

DPPH radical scavenging assay: The free radical scavenging activity was measured using 2, 2-diphenyl-1 –picryl-hydrazyl (DPPH) assay proposed by Blois in 1958. The reaction mixture (1.5 mL) consists of 1.0 mL of DPPH in methanol (0.1 mM), 50 μl of the extract and 450 μl of Tris HCl. It was incubated for 10 minutes in the dark, and then the absorbance was measured at 517 nm. Methanol was used as the control, and its absorbance was also measured. The percentage of inhibition was calculated using the following formula;
Inhibition (%) $= (A_0 – A_1/A_0) \times 100$
where A_0 is the absorbance of the control and A_1 is the absorbance of the test sample.

Sensory evaluation: Sensory evaluation was carried out to select the best concentration of MPPE incorporated into the yoghurt. Stirred yoghurt was incorporated with MPPE at the rate of 1, 1.5, and 2 mL/kg of yoghurt mix. Yoghurts were marked with a 3-digit code and followed by a sensory evaluation using 30 untrained panels of judges from the yoghurt factory. The panellists were asked to rate each sensory attribute (appearance, after taste aroma, flavour and overall acceptability) using a 'nine-point hedonic scale'.

Chemical analysis and microbial analysis: Proximate analysis (moisture content, fat, total solids content and crude protein) and evaluation of shelf life based on physiochemical (pH, titratable acidity), microbiological (coliform, yeast and mold count) (AOAC, 1999).

Statistical analysis: Data analysis was done using Statistix ver.10 software package; the mean comparison was made using Duncan's new multiple range test (significance level at $p<0.05$). Results were expressed as mean±standard deviation. For sensory data, results were analyzed by using Kruskal- Wallis nonparametric one-way ANOVA test.

RESULTS AND DISCUSSIONS

Sensory evaluation: Sensory evaluation was conducted to find out the most suitable level of MPPE that can be incorporated into the yoghurt—according to the sensory evaluation results, aroma, flavour and after taste had no significant difference between the samples. However, appearance was observed to be significantly ($p<0.05$) different with the changing of the concentration of MPPE in the yoghurt mix. It was observed that the appearance was highest in the sample containing 1.5 mL of MPPE per kg of yoghurt mix. However, further increase of MPPE decreased the appearance score of the yoghurt. This might be due to the dark brown colour of the MPPE, which imparts a bad appearance to the final product even though, the added amounts are small. The overall acceptability score of the yoghurt was observed to be highest at 1.5 mL of MPPE/kg of yoghurt mix. With the increase of the MPPE level in the yoghurt mix, it was observed that the overall acceptability score was decreased significantly ($p<0.05$). Based on the results of the sensory evaluation, 1.5 mL MPPE/kg of yoghurt mix was selected for further experiments.

Proximate composition: Proximate compositions of MPP were moisture 8.61±0.18%, dry matter 91.39±0.18%, Crude protein 5.68±0.047%, Fat 2.41±0.34%, Ash 4.19±0.17%. The water content of MPP was observed to be 8.6%, and therefore, this material is dry enough to protect from fungus and other contaminations. It was better to keep moisture content less than 10% to have a better storage life of a food product. This powder can be stored in the freezer without any contamination. According to (Tjahjani et al., 2014), proximate composition results are numerically the same. Proximate composition of MPPE incorporated (1.5 mL/kg yoghurt mix) and control yoghurt were Fat 3.24±0.04%, 3.23±0.02%, Protein 5.12±0.05%, 5.32±0.04%, Moisture 76.57±0.56%, 78.17±0.04%, Total solid 23.42±0.05%, 21.84±0.04% comparatively (Table 1). According to the results, fat and protein contents did not show any difference between selected and control yoghurt samples, and moisture content was comparatively low in MPPE incorporated yoghurt samples because of the bitter and sticky substance of extract.

Total phenolic content and antioxidant activity: Ad-

Table 1: Proximate composition of MPPE incorporated (1.5 mL/kg yoghurt mix) and control yoghurt

Component	Selected sample (%)	Control (%)
Fat	3.24±0.04	3.23±0.02
Protein	5.12±0.05	5.32±0.04
Moisture	76.57±0.56	78.17±0.04
Total solids	23.42±0.05	21.84±0.04

Table 2: Total Phenolic Content and Antioxidant activity of MPPE and yoghurt samples

Sample	TPC (mg/GAE)	Antioxidant activity (% of scavenging radicals)
MPPE	115.10±0.05	57.81±6.56
Selected yoghurt	3.88±0.14	Not detected
Control yoghurt	2.5±0.04	Not detected

ND:

Table 3: The mean rank scores of sensory attributes in the control and selected treatment (1.5 mL MPP extract per kg yoghurt mixture)

Sample	Appearance	Aroma	Flavor	After taste	Overall
Control	7.14 ± 0.85^{a}	6.90 ± 0.83^{a}	7.14 ± 0.91^{a}	5.67 ± 0.86^{a}	7.10 ± 0.89^{a}
Treatment	6.90 ± 0.94^{a}	6.71 ± 0.96^{a}	6.67 ± 0.91^{a}	7.10 ± 1.04^{b}	6.62 ± 0.92^{a}

^{a,b}Mean±standard deviation (n=3) different letter in each column denote significant ($p<0.05$) difference from each other

dition of 1.5 mL of MPPE to 1 kg of yoghurt mix, the TPC in water extract of stirred yoghurt was observed to be 3.88 ± 0.14 mg GAE per g compared to 2.5 ± 0.04 mg GAE per g for the control yoghurt. The TPC value observed in control yoghurt can be due to non-specific reactions of Folin Cio-calteu reagent with milk components (Everette et al., 2010). Deviations of TPC can be happened due to storage of the product at 4 °C for three weeks. Deviations of TPC can be happened due to storage of the product at 4°Cfor 3 weeks, but according to Rita et al., 2018, no changes in polyphenol content was observed in the yoghurt. Antioxidant activity was determined as a percentage of scavenging radicals; for MPPE, it was observed to be 57.81±6.56% (Table 1). However, a sufficient amount of antioxidant activity was not detected (Table 2). Due to the limited amount (1.5mL) of MPPE added and the delay (3 weeks after sample preparation) of conducting the DPPH assay, it might affect the antioxidant activity. Various workers have reported the positive correlation between total phenolic contents and free-radical scavenging activity of the fruit polyphenolic extracts (Skrede et al., 2004; Caillet et al., 2006).

Physiochemical properties (pH): MPP extract incorporated yoghurt showed 4.34 of pH value on its first day of storage and observed a reduction of that value to 4.11 on the final day of storage period (Figure 1). This might be due to the changes in lactic acid production of microorganisms during storage. The changes in pH value in control yoghurt during the storage period showed the same trend, but the values were always beyond the experimented sample.

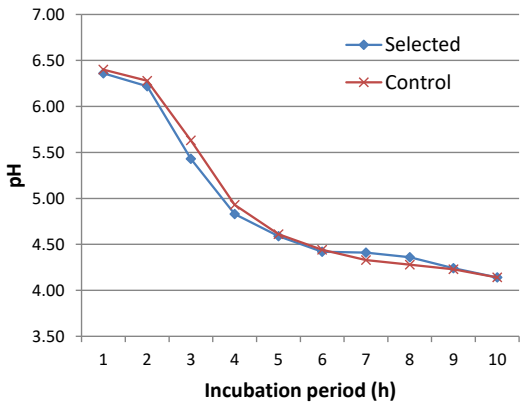

Figure 1: Changes of pH of yoghurt during the incubation

Physiochemical properties (Titratable acidity): Titratable acidity on the first day of the storage period was approximately 0.7%, and it showed a gradual increase up to 1.06% on the $14^{t}h$ day. Then the value was stagnated until the third week (Figure 2). This might be due to the changes in the lactic acid production of microorganisms. Moreover, the other reason for this condition can be that the phenolic extract act as a

prebiotic for lactic acid-producing microorganisms.

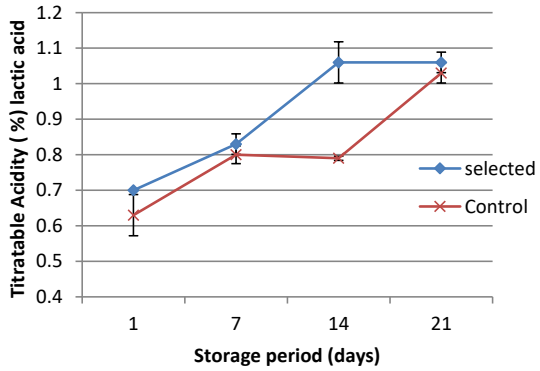

Figure 2: Changes of titratable acidity of yoghurts during the storage

Physiochemical properties (Whey syneresis): The percentage of whey syneresis of the yoghurt incorporated with 1.5 mL of MPPE/kg yoghurt mix were increased during the storage period. On the first day of storage, it showed a value of 5.8%, and at the end of the period, it increased to 8.77% (Figure 3).

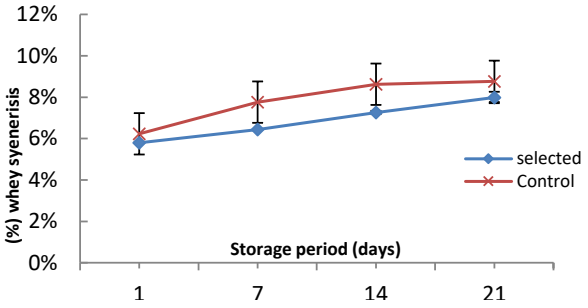

Figure 3: Whey syneresis of yoghurt samples during the storage period

This might be due to the presence of yellow, gummy, bitter latex substance of the MPP extract, which might have the ability to absorb water which imparts less whey syneresis to the MPP extract incorporated yoghurt. This compound was present in the inner bark of fruit, and it was dissolved in ethanol. It was precipitated during the storage.

Physiochemical properties (Acceptability test): The mean sensory scores obtain for control and the yoghurt incorporated with 1.5 mL of MPPE/kg yoghurt mix during the storage period of 21 days at 4±1 °C were appearance, aroma, flavour and overall acceptability had no significant difference between the samples. However, after taste was observed to be significantly different between the samples [Chi-squared value = 0.0001, *p*-value = 0.0000). The after taste of the MPPE incorporated yoghurt may be due to bitter latex substance of the extract. The control sample obtained the highest mean score for the acceptability test.

Microbiological quality (Coliform): Coliforms were not detected during the storage period. This indicated the adequate hygienic measures practiced during the production process of yoghurts.

Microbiological quality (Yeast and mold): Control and the selected yoghurt (1.5 mL/kg) did not exceed the maximum permissible limit for coliforms of 10 cfu/g during 30 days of storage period. However, control and selected yoghurt exceeded the allowable mould limit on 26^th and 28^th days, respectively. According to microbiological results, the MPP extract added yoghurt had the highest self-life of 26 days without added preservatives compared to control samples which had 25 days. Increased self-life of MPP extracts incorporated yoghurt samples might be due to the antifungal and antibacterial properties of MPP extract.

CONCLUSION

Mangosteen peel powder (MPP) extract is rich in total phenolic content of 115.10±0.05 mg GAE/g and antioxidant activity (RSA %) 57.81±6.56 %. The organoleptically acceptable level of MPP extract was 1.5 mL/kg yoghurt mixture. Bitter after the taste of yoghurt reduces consumer acceptability and masks the sugar flavour and taste of yoghurt. MPP extract 1.5 mL/kg of yoghurt mixture was insufficient to provide a substantial antioxidant activity in the yoghurt. The shelf life of MPP extract incorporated, and the control yoghurts were 26 and 24 days without any preservatives, respectively

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