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RESEARCH ARTICLE

Functional foods

Development of vacuum-dried powder and drinking yoghurt from soursop fruit (*Annona muricata* L.) and evaluation of their physico-chemical and functional properties

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Abstract: Soursop (*Annona muricata* L.) is an underutilized seasonal climacteric fruit which exhibits many therapeutic and nutritive values. The objective of the present study was to develop soursop fruit powder and soursop incorporated drinking yoghurt and to evaluate their physico-chemical and antioxidant properties. Soursop fruit powder was developed by steam blanching of matured fresh cuts followed by vacuum drying at 60 °C for 6 hours. Soursop drinking yoghurt was developed by incorporating soursop pulp (15%, 20%, and 25% w/v) followed by selecting the best fruit content (*i.e.*, 20% w/v) based on the sensory evaluation trials. Titratable acidity, pH, total soluble solids, syneresis, and milk solid non-fat of selected soursop drinking yoghurt were 0.85%, 4.5, 16.3 °Brix , 29.3%, and 13.8%, respectively. The soursop drinking yoghurt (20% w/v) with added sucralose and sugar were separately compared with normal drinking yoghurt as a control. The drinking yoghurt with added sucralose was selected as the most preferable product based on the sensory attributes of odour, appearance, texture, taste, aftertaste, and overall acceptability. A significantly ($p < 0.05$) higher antioxidant potential in terms of total polyphenolic content, total flavonoids content, ferric reducing antioxidant power, and radical scavenging activities of 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) and 2,2-diphenyl-1-picrylhydrazyl were shown in the soursop drinking yoghurt (14.13 ± 0.63 mgGAE/g, 3.39 ± 0.36 mgQE/g, 1.32 ± 0.30 mgTE/g, 30.1 ± 3.24 mgTE/g, and 25.67 ± 3.38 mgTE/g respectively) than in the vacuum-dried powder. The vacuum dried powder had a high content of protein (8.71%), crude fibre (4.28%) and ash (3.97%). Further study showed that the soursop drinking yoghurt is a potential source of functional food, while soursop fruit powder is a good source of supplementary food.

Keywords: Antioxidant potential, drinking yoghurt, functional properties, soursop fruit, vacuum-dried powder.

INTRODUCTION

Soursop (*Annona muricata* L.) is an evergreen plant growing in tropical and subtropical regions, and belongs to the family *Annonaceae*. Those seasonal climacteric soursop fruits had a high rate of respiration and ethylene production during the ripening stage, leading to high perishability and post-harvest losses (Kader, 2005; Lagunju & Sandewa, 2018).

Soursop fruit is therapeutically very popular for its outstanding antioxidant properties throughout the world (Kaur & Kapoor, 2001; Villegas, 2002; Gyesi *et al.*, 2019) and has the ability to reduce tumour size, kill off breast cancer cells, and enhance the activity of the immune system (Fidianingsih & Handayani, 2015). It is a good natural remedy for diabetics by inhibition of α-amylase and α-glucosidase activities with minimal or no adverse side effects (Agu *et al.*, 2019). Recent research reported that *Annona muricata* L. fruit extract is used for the treatment of depression (Solanki *et al*., 2020).

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Based on their remedial properties to many chronic diseases, soursop fruit can be used to generate varieties of value-added food products such as yoghurts, jams, fruit powders and beverages. Sinthiya and Poornima (2017) had developed several value added products from soursop such as fruit pulp concentrate, syrup, jam, and juice. It was reported that soursop nectar incorporated stirred yoghurt was developed by Dias and Jayasooriya (2017) and soursop incorporated set yoghurt was developed by Virgen-Ceceña *et al*. (2019). Further preparation and quality evaluation of soursop jelly was performed by Shashikala and Mahendran (2019). In most of the above studies, the functional properties or potential antioxidant properties had not been studied.

Although soursop fruits have many health promoting functional properties, consumption of fresh soursop fruit is low among the younger and adult generation, due to the time consumed for the peeling and seed removal process. Therefore there is a high potential for implementing the development of different attractive products to preserve soursop worldwide. The development of value added products like functional drinking yoghurt and vacuum-dried powder using soursop fruit may be considered as a timely solution for higher postharvest losses and its unavailability off-season.

Dehydration and drying is one of the most commonly used methods to prepare powders from fresh fruits and vegetables. Vacuum-drying is ideal for heat and oxygen-sensitive foods such as fruits and vegetables due to the advantage of removing moisture at low temperatures and minimizing the possibility of oxidation reactions (Wijewardana *et al*., 2016; Ngamwonglumlert & Devahastin, 2018).

Drinking yoghurts are one of the fastest growing fermented products in the functional food market, because of their multi-nutrient content and health-promoting properties (Sun-Waterhouse *et al.*, 2012). The fermentation process is carried out by various characteristic bacterial cultures such as *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. It has been showed that the incorporation of fresh or processed fruits (e.g., chokeberry, salal berry, water melon, cocoa) into regular drinking yoghurts enhanced the nutritional, functional, and antioxidant profile and sensory properties (Gonzalez *et al*., 2011; Sengupta *et al.*, 2014; Nguyen & Hwang, 2016; Raikos *et al.,* 2019).

The objective of the present research study was to evaluate the feasibility of incorporating soursop fruit into regular drinking yoghurt and developing soursop powder by vacuum drying and evaluation of physico-chemical characteristics, antioxidants, and sensory properties of those products.

MATERIALS AND METHODS

Materials

Raw materials

Ripened soursop (*Annona muricata L.*) fruits were obtained from the local market at Malabe, Sri Lanka. Ultra Heat Treated (UHT) full cream fresh milk was purchased from a local supermarket in Malabe, and yoghurt cultures were obtained from Morison PLC, Colombo.

Chemicals and reagents

2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulphonic acid), diammonium salt (ABTS), 1,1-diphenyl-2-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), gallic acid, 2,4,6-tripyridyl-s-triazine (TPTZ), potassium persulphate, ferric chloride, quercetin and Folin-Ciocalteu phenol reagent were purchased from Sigma-Aldrich, USA. All the other chemicals used were of analytical grade.

Preparation of vacuum-dried soursop powder

Soursop fruit powder was prepared by steam blanching followed by a vacuum drying technique as described by Bourdoux *et al*. (2016).

The edible portion of soursop fruit was cut into thin slices (2 cm x 2 cm) and dipped in 1% sodium meta bisulphite (SMS) solution. The pieces were steam blanched for 1 min and then were vacuum dried at 60 ^{o}C for 6 h using dehydrator (American Harvest, USA). The dried slices were ground using a pin mill (Munson cim-18-S316, Shanghai, China) to pass through 0.5 mm sieve size. Powdered samples were stored at 15 ^{o}C in airtight containers until assaying.

The recovery percentage of vacuum-dried powder was calculated using following equation.

$$\text{Vacuum-dried recovery percentage} = \frac{\text{Weight of vacuum-dried powder}}{\text{Weight of edible portion}} \times 100$$

Preparation of soursop drinking yoghurt

The edible portion of the soursop fruit was obtained and ground well using a wet grinder (Prestige Deluxe LS 304, Bangalore, India). Three different formulae of drinking yoghurt were developed using the fruit content of soursop: 15%, 20%, and 25% (w/v) and other ingredients, *i.e.*, milk, sugar, and yoghurt culture. Ingredients required for each formula were put into a separate container and homogenized using a laboratory homogenizer (Pro 250 Scientific, Oxford, USA) at 3000 rpm for 10 min. The homogenized samples were pasteurized at 85°C for 15 min. The pasteurized samples were allowed to cool up to 42°C and the yoghurt culture was added to each sample. Samples were then incubated in an incubator (Memmert c1810, Munich, Germany) at 44°C for 3 h and 30 min until the samples reached a pH of 4.6. The incubated yoghurt samples were separately homogenized to break down the coagulum. The resulting drinking yoghurts were filled into previously autoclaved glass bottles (at 121°C for 20 min under the pressure of 15 psi) under a laminar air flow cabinet and hermetically sealed with lug cap. Bottled soursop drinking yoghurts were labelled and stored at 4°C in a refrigerator until use. After selecting the best soursop fruit content in drinking yoghurt using a sensory panel, the second trial was carried out to replace the sugar content in soursop drinking yoghurts by adding sucralose.

Sensory evaluation

Soursop incorporated drinking yoghurts with 3 different soursop fruit contents, 15%, 20% and 25% (w/v), were subjected to a sensory evaluation trial to select the most preferable soursop level. Thereafter, the sugar content of the drinking yoghurt with the most preferable level of soursop was replaced by adding 200 mg/kg of sucralose (*i.e.*, less than the maximum level of sucralose addition to milk based preparations, 400 mg/kg) according to Food (Sweeteners) Regulations published by Ministry of Health and Indigenous Medicine (2015). The sucralose and sugar-sweetened yoghurts were separately compared, with normal drinking yoghurt as the control, in another sensory evaluation trial. Both sensory evaluation trials were conducted by using twelve trained panellists and the samples were evaluated in terms of colour, odour, appearance, texture, taste, aftertaste, and overall acceptability, using the nine point hedonic scale.

Determination of physico-chemical properties of drinking yoghurt

Determination of total soluble solids (TSS) content

The total soluble solids of drinking yoghurt samples was measured using a refractometer at room temperature (27°C) (Atago automatic1620, Fukaya-shi, Japan).

Determination of total solids-non-fat

The total solids-non-fat (SNF) of soursop drinking yoghurt was determined according to the following formula by Matela *et al*. (2019).

Solids-non-fat % = Total solids % - Fat content %

The fat content of the drinking yoghurt samples was determined using Rose Gottlieb method as described in AOAC (2012).

The fat content was measured using the phase separation technique.

Briefly, a 10.0 g sample was extracted by a mixture of solvents (10 mL of 95% ethanol, 25 mL of diethyl ether, 25 mL of petroleum ether, and 0.88 mL of ammonia solution). The fat was extracted into the organic phase and solvent was removed under vacuum (200 mbar) at 40 °C using a rotor evaporator (Heidolph, Germany).

Titratable acidity

The titratable acidity of drinking yoghurt samples were measured according to the method described by Olugbuyiro (2011), and expressed as percentage acidity.

A 15 mL sample of the drinking yoghurt was titrated with 0.1 M sodium hydroxide using phenolphthalein as the indicator.

$$\text{Titratable acidity\%} = \frac{\text{NaOH volume} \times \text{M} \times 90 \times 100}{\text{Volume of sample} \times 1000}$$

Where, M = Molar concentration of NaOH

pH of drinking yoghurt

The pH was measured on the same day of preparing the products according to AOAC, 2012, at room temperature (27 °C) using a digital pH meter (Ikmag rec-g, USA).

Syneresis of drinking yoghurt

The syneresis of drinking yoghurt was determined according to the method described by Vahedi *et al*. (2008). The following equation was used to determine the syneresis.

A 10 g sample of drinking yoghurt sample was placed on a filter paper resting on the top of a Buchner funnel. After 10 min of drainage under vacuum, the quantity of remaining yoghurt was weighted and syneresis was expressed as percentage of free whey.

$$\text{Free whey (g/100g)} = \frac{(\text{Weight of initial sample} - \text{Weight of sample after filtration}) \times 100}{\text{Weight of initial sample}}$$

Proximate composition analysis of vacuum-dried soursop powder and soursop drinking yoghurt

Proximate composition of vacuum-dried soursop powder and soursop drinking yoghurt were determined according to the methods described in AOAC (2012) and SLS 824 (2018) respectively.

In brief, the moisture content of both vacuum-dried soursop powder and soursop drinking yoghurt was determined by oven drying at 105 °C for 5 h, applying a gravimetric principle, using a heating oven (Memmert, Schwabach, Germany). The fat content of the vacuum-dried soursop sample was determined by extraction at 150 °C for 2.5 h using petroleum ether (40–60 °C), using a Soxhlet extractor (C. Gerhardt GmbH & Co. KG, Schwabach, Germany) while the fat content of the soursop drinking yoghurt was determined as described in a previous section. The crude fibre content of the vacuum-dried soursop powder and soursop drinking yoghurt were determined using an acid/alkali digestion method, with a Fibretec hot extractor (Fibretec^{TM}M6 1020, Apeldoorn, Netherlands). The ash contents of vacuum-dried soursop powder and soursop drinking yoghurt were determined by a dry ashing method using a furnace (Lenton, Wales, England). The crude protein content of vacuum-dried soursop powder and soursop drinking yoghurt were determined by wet digestion followed by steam distillation using a Kjeldahl apparatus (VELP Scientifica DK 20 and 139, Usmate, Italy). The percentage of carbohydrate (CHO) of the two products was determined using a mathematical equation as described by Matela *et al*. (2019).

$$\text{CHO \%} = 100 - (\text{ash} + \text{crude protein} + \text{crude fat} + \text{crude fibre} + \text{moisture})\ \%$$

Total energy values of vacuum-dried soursop powder and soursop drinking yoghurt were determined by the Atwater method as described by Matela *et al*. (2019). The energy was measured in kilocalories/100 g.

$$\text{Energy value} = (\%\ CP \times 4) + (\%\ CFT \times 9) + (\%\ CHO \times 4)$$

where,% CP = percentage crude protein,% CFT = percentage crude fat,% CHO = percentage carbohydrate

Determination of antioxidant potential

Sample preparation for the determination of antioxidant properties

Determination of antioxidant properties in fresh soursop fruit, vacuum-dried soursop powder and soursop drinking yoghurt was carried out according to the method as described by Agu and Okolie (2017).

A well-mixed fresh edible portion of soursop fruit (25.0 g) and vacuum-dried soursop powder (5.0 g) were separately shaken overnight using an orbital shaker (Ratek-ms 120, India) at 110 rpm with 4 times the sample weight of 80% ethanol. The ethanol was evaporated off in a rotary evaporator (Heidolph, Germany) at a temperature of 45 °C at 110 rpm. Ethanol free extracts were freeze-dried overnight at 20 °C. Freeze-dried extracts were collected into the Eppendoff tubes and refrigerated (4 °C) till samples were analysed for antioxidant properties.

Freshly prepared soursop drinking yoghurt (1 mL) was diluted with 9 mL of distilled water to prepare 1/10 dilution of the initial drinking yoghurt. The diluted yoghurt samples were directly used for the determination of antioxidant properties.

Total polyphenolic content

Total polyphenolic contents (TPC) of sample extracts were determined using the Folin-Ciocalteu reagent as described by Singleton *et al.* (1999).

In brief, each extract was diluted in distilled water

(2 mg/mL), and 20 µL of diluted sample, 110 µL of 10 times diluted Folin-Ciocalteu reagent and 70 µL of 10% sodium carbonate (Na_2CO_3) solution were mixed in a well of a 96-well micro plate. After incubating for 30 min at 25 ± 2°C, the absorbance was measured at 765 nm using a 96-well micro plate reader (SpectraMax Plus 384 Molecular Devices, USA) using gallic acid as the standard. The gallic acid standard solution (1 mg/mL) was prepared by dissolving 1 mg of gallic acid in 1000 µL of distilled water and the standard series was prepared using half dilution.

TPC was expressed as mg gallic acid equivalents (GAE) / g of the sample on a dry weight basis.

Total flavonoid content

Total flavonoid content (TFC) of sample extracts were determined using the methods described by Pourmorad (2005).

In brief, each extract was diluted in methanol (2 mg/mL), and 100 µL of diluted sample and 100 µL of 2% aluminium chloride were added into a well of a 96-well micro plate. After incubating for 10 min at 25 ± 2°C, the absorbance was measured at 415 nm using quercetin as the standard using micro plate reader (SpectraMax Plus 384 Molecular Devices, USA). The standard stock solution (1 mg/mL) was prepared by dissolving 1 mg of quercetin in 1000 µL of methanol and the standard series was prepared by half dilution.

The results were expressed as mg quercetin equivalents (QE)/g of the sample on a dry weight basis.

Ferric reducing antioxidant power assay

Ferric reducing antioxidant power (FRAP) of sample extracts was performed according to the method described by Benzie and Szeto (1999).

In brief, 150 µL of FRAP reagent [a mixture containing 300 mM of acetate buffer at pH 3.6, 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) in 40 mM HCl solution, and 20 mM $FeCl_3.6H_2O$ in a ratio of 10:1:1 followed by incubation at 37°C for 10 min], 30 µL acetate buffer and 10 µL diluted sample (2 mg/mL) were transferred to a micro well. After incubating at 25 ± 2°C for 10 min, the absorbance was measured at 600 nm via 96-well micro plate reader (SpectraMax Plus 384 Molecular Devices, USA). The standard curve was plotted using half dilution series of the standard Trolox stock solution (1 mg/mL) which was prepared dissolving 1 mg of Trolox in 1000 µL of Phosphate Buffer Saline (PBS).

The results were expressed as mg of Trolox equivalents (TE) /g of sample (dry weight basis).

DPPH radical scavenging activity

The DPPH radical scavenging activity of samples was performed according to the method described by Blois (1958).

In brief, 125 µL of DPPH radical (20 mg/100 mL) and 50 µL (2 mg/mL) of diluted sample were mixed in a well and incubated at 25 ± 2°C for 10 min. Absorbance was measured at 517 nm (SpectraMax Plus 384 Molecular Devices, USA). The stock standard solution was prepared by dissolving 1 mg of Trolox in 1000 µL of PBS and standard series was prepared by half dilution.

Results were expressed as mg Trolox equivalent (TE)/g of the sample on dry weight basis.

ABTS radical scavenging activity

The ABTS radical scavenging activity of samples was determined according to the method described by Re *et al*. (1999).

40 µL of seven times diluted ABTS stock solution (10 mg of ABTS in 2.5 mL of 2.5 mM potassium persulphate solution incubating at 37° C for 16 h in the dark), 110 µL phosphate buffer and 50 µL (2 mg/mL) of sample were incubated at 25 ± 2°C for 10 min. The absorbance was recorded at 734 nm using 96-well micro plate reader (SpectraMax Plus384 Molecular Devices, USA). The standard stock solution (1 mg/mL) was prepared by dissolving 1 mg of Trolox in 1000 µL of PBS and then the standard series was prepared by half dilution.

Results were expressed as mg Trolox equivalent (TE)/g of the sample on a dry weight basis.

Statistical analysis

Completely Randomized Design (CRD) was used to analyse the parametric data of drinking yoghurt samples. Data was analysed using Analysis of Variance (ANOVA) in the Statistical Software Analysis (SAS) programme at 95% confidence interval and mean separation was conducted by the Least Significant Difference (LSD) method.

The data obtained for the two treatments of the most preferable fruit content and acceptability of sugar vs. artificial sweetener were statistically analysed by the Friedman method using MINITAB software at 95% confidence interval.

RESULTS AND DISCUSSION

Determination of edible portion of soursop fruit

Soursop fruit is comprised of the thorny pericarp, seeds, central pith, and the soursop pulp. Out of those parts, the fruit pulp is considered to be the edible portion, and hence the other parts of the fruit have to be removed. It was reported that soursop seeds contain annonaceous acetogenins, which cause neurotoxic effects on humans (Badrie & Schauss, 2010). Determination of recovery of edible portion is important in new product development activity in order to determine the production cost and wastage. The physical composition of soursop fruit in percentages were presented in Table 1.

Table 1: Composition of soursop fruit

Parts of the fruit	Composition (%)
Edible portion	71.5 ± 3.60
Peel	17.2 ± 1.21
Seeds	8.3 ± 0.8
Central pith	3.0 ± 0.6

Values are presented as mean ± standard deviations of three replicate measurements

According to the Table 1, the edible portion of soursop fruit was in accordance with the data reported in the previous study by Badrie and Schauss (2010), where the edible portion of an average soursop fruit is 67.5%, the amount of peel is 20%, and seeds and the central pith comprise 8.55% and 4% of the fruit, respectively.

Determination of vacuum-dried recovery percentage

The percentage recovery after vacuum drying will be an important factor in determining the feasibility of utilizing soursop powder in food industry.

According to the present study, the recovery percentage of soursop was 16.36 ± 0.83%. In the process of vacuum drying, a certain quantity of the fruit was wasted due to the sticking of the soursop pulp onto the trays and when converting soursop into powder due to machinery losses.

Sensory evaluation of drinking yoghurts

The ideal fruit content of drinking yoghurts was evaluated with levels of soursop fruit content of 15% w/v, 20% w/v, and 25% w/v, using sensory attributes of odour, appearance, texture, taste, aftertaste, and overall acceptability, according to the nine point hedonic scale. Figure 1 shows the results obtained from the sensory evaluation with reference to varying fruit content. There was a significant difference ($p < 0.05$) in each sensory attribute; odour, appearance, texture, taste, aftertaste and overall acceptability.

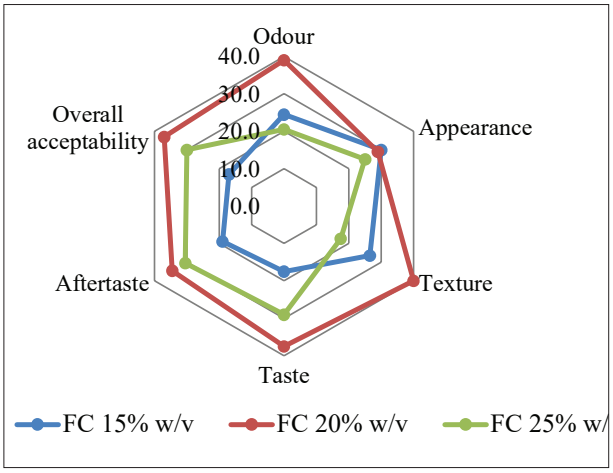

Figure 1: Sensory evaluation of soursop incorporated drinking yoghurts

The 20% w/v soursop incorporated drinking yoghurt showed the highest rank for odour, taste, texture, aftertaste, and overall acceptability while 15% w/v soursop incorporated drinking yoghurt showed the highest rank for appearance. The lowest acceptability regarding the odour, appearance and texture were recorded from 25% w/v soursop incorporated drinking yoghurt, due to the higher content of soursop pulp. It was seen that colour and thickness of the drinking yoghurt increased with increasing incorporation of soursop pulp. Based on the results, the 20% w/v soursop incorporated drinking yoghurt showed the highest overall acceptability in terms of all the attributes, and it was selected as the most preferable fruit content for soursop drinking yoghurt.

The second sensory evaluation trail for the preference of sugar *vs* sucralose was conducted based on the yoghurt with the best level of soursop incorporation (20% w/v). Sucrose added drinking yoghurt was carried out replacing the content of sugar by adding sucralose, 200 mg/kg, according to the Food (Sweeteners) Regulations published by Ministry of Health and Indigenous Medicine (2015). Soursop incorporated drinking yoghurt (20% w/v) with added sugar, soursop incorporated drinking yoghurt (20% w/v) with added sucralose, and drinking yoghurt without incorporation of soursop (0% w/v) with added sugar (control) were evaluated by means of sensory attributes of odour, appearance, texture, taste, aftertaste, and overall acceptability using the nine point hedonic scale. Figure 2 shows the results obtained from the sensory evaluation. There was a significant difference ($p < 0.05$) in all sensory attributes of odour, appearance, texture, taste, aftertaste, and overall acceptability.

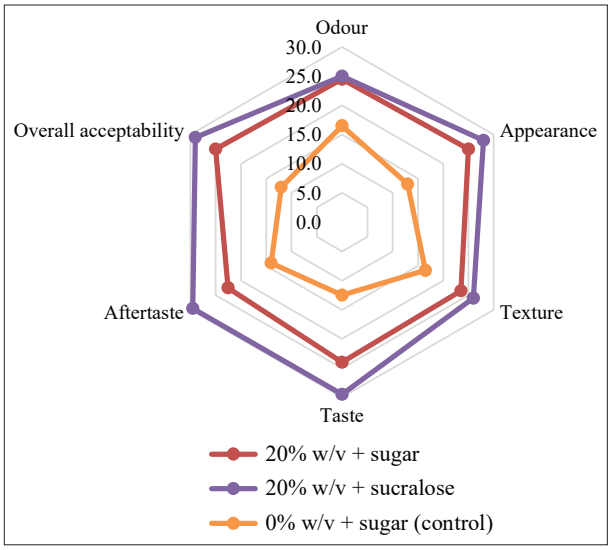

Figure 2: Sensory evaluation of the sugar added, sucralose added 20% Soursop incorporated drinking yoghurts and control drinking yoghurt

The 20% w/v soursop incorporated drinking yoghurt with added sucralose showed the highest ranks for odour, appearance, taste, texture, aftertaste and overall acceptability while control (0% w/v soursop) drinking yoghurt with added sugar showed the lowest ranks for all the attributes. Since soursop fruit is rich in polyphenols, it undergoes enzymatic browning easily and exhibits a pleasant cream colour with milk while the control drinking yoghurt prepared without adding soursop was pure white. In considering the taste, sucralose added soursop drinking yoghurt achieved a higher rank than sugar added drinking yoghurts without bitter or objectionable aftertaste. Since sucralose has an excellent, intense taste profile the sweetness of sucralose inside the mouth was retained longer than the sweetness of sugar.

Even though 20% w/v soursop drinking yoghurt with added sucralose was selected as the most preferable drinking yoghurt, there are some limitations regarding sucralose which cause difficulty in commercializing. The higher price of sucralose and unknown health issues which may occur when overdosing are the main problems in commercializing sucralose added products. Therefore, sugar added 20% w/v soursop drinking yoghurt was selected as the best drinking yoghurt for further studies of functional and physico-chemical analysis, and it was compared with normal drinking yoghurt as a control.

Determination of physico-chemical properties of vacuum-dried soursop powder and drinking yoghurts

Proximate composition of vacuum-dried soursop powder

Proximate composition of moisture, crude fat, crude fibre, ash, crude protein and carbohydrates were determined and results are presented in Table 2.

Table 2: Proximate composition of vacuum-dried soursop powder, soursop incorporated drinking yoghurt and drinking yoghurt

Parameter	Soursop powder	Soursop drinking yoghurt	Control drinking yoghurt
Moisture (%)	4.04 ± 0.05	83.67 ± 0.31^{b}	84.67 ± 0.35^{a}
Fat (%)	1.86 ± 0.04	2.50 ± 0.03	3.43 ± 0.03^{a}
Crude fibre (%)	4.23 ± 0.03	0.14 ± 0.02^{a}	0.07 ± 0.01^{b}
Ash (%)	3.97 ± 0.04	1.04 ± 0.06^{a}	0.79 ± 0.02^{b}
Protein (%)	8.71 ± 0.04	2.96 ± 0.05^{b}	3.24 ± 0.03^{a}
Carbohydrates (%)	77.21 ± 0.06	9.70 ± 0.18^{a}	7.82 ± 0.23^{b}
Energy (kcal/100g)	360.42 ± 0.19	73.10 ± 1.07^{a}	75.26 ± 1.32^{a}

Values are represented in means ± standard deviations of three replicate measurements. Significant different was observed only between the soursop drinking yoghurt and control drinking yoghurt.
Means with the same letters in the same row were not significantly different ($p < 0.05$).

According to the moisture content found in vacuum-dried soursop powder (4.04%), it had less moisture than the freeze-dried soursop powder (8.68 ± 0.07%), as described by Ceballos *et al.* (2012). Similar results were obtained, 4.33% and 4.02%, for the crude fibre and ash content, respectively, of the oven-dried soursop powder according to the study conducted by Badrie and Schauss (2010). In the same study by Badrie and Schauss (2010), the carbohydrate value of oven-dried soursop powder was found to be 84.82% and that value was comparatively higher than the carbohydrate content of vacuum-dried powder (77.21%).

A higher protein value could be observed in vacuum-dried soursop powder as shown in Table 2 when compared to the protein value in oven-dried soursop powder (7.34 ± 0.07%), developed by Badrie and Schauss (2010). The higher content of protein in vacuum-dried soursop powder may due to drying at a lower temperature (60 °C) (Ngamwonglumlert & Devahastin, 2018). It was reported that the protein content of processed products derived from fresh fruits and vegetables is adversely affected by the temperature (Zhang *et al*., 2016).

This phenomenon is due to the rate of the Maillard reaction at temperatures at or below 60 °C becoming slower, so that amino acid nitrogens are hardly consumed in the Maillard reaction. With increasing temperature (above 60 °C), the rate of the Maillard reaction in food materials is accelerated, leading to a faster consumption of amino acid nitrogen; thereby the protein value of food becomes low. According to a study conducted by Wijewardana *et al*. (2016), vacuum drying was recommended as the most effective drying method to protect the chemical characteristics of fruit powders, compared to the other drying methods tested, such as sun drying, solar drying, freeze-drying, and oven drying.

Proximate composition of drinking yoghurts

Moisture, crude fat, crude fibre, ash, crude protein, and carbohydrates of both soursop drinking yoghurt and control drinking yoghurt are presented in Table 2.

As shown in Table 2, the moisture content of soursop drinking yoghurt was significantly lower ($p < 0.05$) than control drinking yoghurt. However, the soursop drinking yoghurt sample indicated a similar value for moisture content (83.69 ± 0.02) as reported by Virgen-Ceceña *et al*. (2019). The reason for the lower moisture content of soursop drinking yoghurt, compared with control drinking yoghurt, was the significant increase ($p < 0.05$) in total solids of the soursop drinking yoghurt relative to control drinking yoghurt. Incorporation of fruit pulps, fruit pieces or fruit syrups into regular yoghurts resulted in higher total solid contents by lowering the moisture content of the product (Sengupta *et al*., 2014).

The fat content of soursop drinking yoghurt was significantly lower ($p < 0.05$) than that of control drinking yoghurt while both drinking yoghurt samples were in accordance with Sri Lanka Standards (SLS 824, 2018). The fat content of soursop set yoghurt developed by Virgen-Ceceña *et al*. (2019) is comparatively higher (5.15 ± 0.24) than the fat content of soursop drinking yoghurt as shown in Table 2 . Those changes may be due to the initial fat content of the cow's milk and the soursop fruits used (Jafarpour *et al*., 2017).

The crude fibre content of the soursop drinking yoghurt according to Table 2 was significantly ($p < 0.05$) higher than control drinking yoghurt due to the incorporation of fibre rich soursop fruit pulp. A higher crude fibre content (3.13 ± 0.15) was observed in a coconut enriched drinking yoghurt prepared by Sengupta *et al.* (2014), compared to the soursop drinking yoghurt developed in this study.

According to Table 2, the soursop drinking yoghurt exhibited a significantly higher ($p < 0.05$) ash content than control drinking yoghurt due to the incorporation of mineral rich soursop fruit pulp. The total ash content of soursop incorporated set yoghurt developed by Sengupta *et al*. (2014) was comparatively lower (0.61 ± 0.05) than the value of soursop drinking yoghurt in the present study.

The protein content of the soursop drinking yoghurt exhibited a significantly lower value ($p < 0.05$) than the control drinking yoghurt due to the addition of gelatin into the control yoghurt as a stabilizer. However, the stabilizing effect in soursop drinking yoghurt is fulfilled by the pectin, which is naturally present in the soursop fruit (Wijesinghe *et al*., 2018).

Energy value of stirred yoghurts developed by Dias and Jayasooriya (2017) using soursop juice and soursop nectar were higher than the value exhibited in soursop drinking yoghurt as shown in Table 2. The reason may due to the changes in carbohydrates, protein, and fat content in the final product (Sengupta *et al*., 2014).

Evaluation of physico-chemical properties of drinking yoghurts

Titratable acidity, pH, total soluble solids (TSS), milk solids non-fat (MSNF) and syneresis of both (20% w/v) soursop drinking yoghurt and control drinking yoghurt were determined once the products were prepared.

Titratable acidity, pH, total soluble solids, syneresis, and milk solids non-fat of selected soursop drinking yoghurt were 0.85%, 4.5, 16.3 °Brix, 29.3%, and 13.8% respectively. According to the Sri Lanka standards, the minimum amount of MSNF required is 2.2% (w/w) and MSNF values obtained for both soursop drinking yoghurt and control drinking yoghurt satisfied the SLS requirement. The MSNF contents of soursop incorporated set yoghurt developed by Virgen-Ceceña *et al*. (2019) was comparatively lower (10.8%) than the value exhibited in the soursop drinking yoghurt developed in the present study. It may be due to the changes of the quantities of soursop pulp incorporated into the yoghurt.

Table 3: Physico-chemical properties of drinking yoghurts

Parameter	Soursop drinking yoghurt	Control drinking yoghurt
Total soluble solids (TSS) (°Bx)	16.33 ± 0.31^{a}	15.33 ± 0.35^{b}
Milk solids non-fat (MSNF)	13.83 ± 0.29^{a}	11.90 ± 0.36^{b}
Titratable acidity (%)	0.85 ± 0.01^{a}	0.8 ± 0.02^{b}
pH	4.45 ± 0.01^{b}	4.52 ± 0.01^{a}
Syneresis (%)	29.33 ± 1.17^{b}	33.5 ± 1.04^{a}

Values represented are means ± standard deviations of three replicate measurements. Means with the same letters in the same row are not significantly different ($p < 0.05$)

The yoghurt starter culture (*Lactobacillus bulgaricus* and *Streptococcus thermophiles*) ferments milk sugar (lactose) to produce lactic acid which is essential for retaining the texture, taste, and shelf life of yoghurts in acidic environments to inhibit pathogen growth. Higher lactic acid content makes the yoghurts sour in taste and less palatable (Chandan & Kilara, 2013). Therefore, it is essential to maintain an optimum lactic acid content when developing a drinking yoghurt. The lactic acid content can be determined by measuring titratable acidity of drinking yoghurts as shown in Table 3. The titratable acidity of both soursop drinking yoghurt and control drinking yoghurt are in accordance with the Sri Lanka standards where the range is defined as 0.8–1.25% (SLS 824, 1989). The titratable acidity of soursop drinking yoghurt was significantly higher ($p < 0.05$) than control drinking yoghurt (Table 3). The titratable acidity of soursop incorporated set yoghurt (0.70 ± 0.01) developed by Virgen-Ceceña *et al*. (2019) and that of soursop incorporated stirred yoghurt (0.68 ± 0.01) developed by Dias and Jayasooriya (2017) are less in value than that obtained for the soursop drinking yoghurt developed in the present study. This may due to the acidity of the fruit, changes in the chemical composition of cow milk, and the fermentation conditions practised in developing the yoghurts.

The pH of the soursop incorporated drinking yoghurt exhibited a significantly lower value ($p < 0.05$) than control drinking yoghurt due to the inherent acidity of the soursop pulp. Espinosa *et al*. (2013) reported that just 3–4 days after harvesting of ripe soursop, malic acid increased by sevenfold, ascorbic acid increased by eleven fold and citric acid increased by three fold. Erkaya *et al.* (2012) reported that the amount of soursop pulp added and the fermentation process influenced both the pH and titratable acidity of dairy products. According to SLS 824 (2018), drinking yoghurts should have a pH of 4.5, and both soursop incorporated yoghurt and control drinking yoghurt developed in this study complied with this standard.

Total soluble solids (TSS) of soursop drinking yoghurt and control drinking yoghurt exhibited a significant difference ($p < 0.05$). Brix value (20.85 ± 0.25) of soursop nectar incorporated stirred yoghurt developed by Dias and Jayasooriya (2017) was higher than the Brix value of soursop drinking yoghurt in the present study due to the fruit content and changes in chemical composition in milk (Calvacanti *et al*., 2006).

Syneresis is the process of collecting whey on the surface of yoghurt and it is considered as a textural defect in yoghurt. As shown in Table 3, the soursop incorporated drinking yoghurt exhibited a significantly lower ($p < 0.05$) syneresis than the control drinking yoghurt. According to Vahedi *et al*. (2008), the reduction of syneresis in fruit incorporated yoghurts is due to absorption of unbound water molecules by fruit pulp. Further, the degree of homogenization, milk composition, type of culture, acidity resulting from the growth of bacterial cultures, and heat treatment of milk are also responsible for the occurrence of syneresis in yoghurt (Aswal *et al*., 2012).

Determination of functional properties of fresh soursop fruit, vacuum-dried powder and drinking yoghurts

Determination of total polyphenolic content (TPC), total flavonoid content (TFC) and ferric reducing antioxidant power (FRAP) in soursop fruit and the products

TPC, TFC, and FRAP of fresh soursop fruit, vacuum-dried powder and the drinking yoghurts were determined and results are presented in both dry weight and fresh weight basis in Table 4.

Total polyphenolic content (TPC)

In the present study, comparisons were made only between fresh soursop fruit and the drinking yoghurts. According to Table 4 there was a significant difference ($p < 0.05$) in the TPC between the fresh fruit and the drinking yoghurts. The phenolic content of soursop drinking yoghurt had a significantly ($p < 0.05$) higher value than that of fresh soursop fruit and control. It was seen that when incorporating the fresh soursop fruit (11.91 ± 1.80 mg GAE/g on dry weight basis) in developing the soursop drinking yoghurt, the TPC had increased to 14.13 ± 0.64 mg GAE/g. This increment may be due to the breakdown of soursop cell walls and subsequent activities of enzymes that lead to the liberation of bound polyphenolic compounds during fermentation (Adebo & Medina-Meza, 2020).

According to a study conducted by Adefegha *et al*. (2015), the TPC of fresh soursop fruit showed a lower value (4.30 ± 0.16 mg GAE/g) than the value in Table 4. According to Padmini *et al*. (2014), the TPC of fresh fruit pulp of the Sri Lankan *A. muricata* was reported as 0.22 mg/g, which was quite lower than the value obtained for the present study. The reason for those variations may due to the differences in methodologies and geographical locations (Babbar *et al*., 2011). The TPC of soursop incorporated set yoghurt developed by Virgen-Ceceña *et al*. (2019) had a lower value (2.43 ± 0.04 mg GAE/g of fresh weight) than the value obtained in the soursop incorporated drinking yoghurt developed in the present study. It may due to the differences in the incorporated flesh weight of soursop fruit into the drinking yoghurt in both studies.

Table 4: TPC, TFC and FRAP contents of the fresh soursop fruit and the products

Product	TPC (mg GAE/g)		TFC (mg QE/g)		FRAP (mg TE/g)	
	DW basis	FW basis	DW basis	FW basis	DW basis	FW basis
Fresh soursop fruit	11.91 ±1.80^{b}	1.85± 0.22^{b}	0.57 ± 0.12^{b}	0.09 ± 0.01^{b}	0.92 ± 0.03^{b}	0.15 ± 0.02^{ab}
Vacuum dried soursop powder	1.80 ± 0.09^{c}	1.73 ± 0.07^{b}	0.07 ± 0.01^{b}	0.07 ± 0.01^{b}	0.12 ±0.01^{d}	0.12 ±0.01^{b}
Control drinking yoghurt (0% soursop)	13.03 ± 0.88^{ab}	1.93 ± 0.10^{b}	0.66 ± 0.04^{b}	0.10 ± 0.05^{b}	0.51 ±0.02^{c}	0.07 ±0.00^{c}
Soursop drinking yoghurt (20% soursop)	14.13 ± 0.63^{a}	2.49 ± 0.09^{a}	3.39 ± 0.36^{a}	0.59 ± 0.14^{a}	1.32 ± 0.30^{a}	0.16 ± 0.00^{a}

Values are means ± standard deviations of three replicate measurements. Means with the same letters in the same column are not significantly different ($p < 0.05$).
TPC- total polyphenolic content; TFC- total flavonoid content; FRAP- ferric reducing antioxidant power; FW- fresh weight; DW- dry weight

The TPC of fresh fruit was significantly reduced when developing vacuum-dried soursop powder. It was described by Pokorný and Schmidt (2001), that the heat treatment causes the reduction of antioxidant content by as much as 60%.

Total flavonoid content (TFC)

When comparing the TFC of drinking yoghurts, soursop drinking yoghurt gave the highest value ($p < 0.05$), even higher than the TFC value of the fresh soursop fruit.

As described by Kwak *et al*. (2007), the reason for the increase in TFC value is the production of aglycone isoflavone and malonylglycoside isoflavone compounds during fermentation. The TFC value of fresh soursop fruit (0.09 ± 0.01 mg QE/g) is slightly lower than the value reported by Adefegha *et al*. (2015) in the fresh fruit (1.00 ± 0.08 mg QE/g). The TFC value of soursop incorporated set yoghurt (0.32mg QE/g) developed by Virgen-Ceceña *et al*. (2019) was lower than the value obtained in the present study (0.59 ± 0.14 mg QE/g) as shown in Table 4. The reason for such a change in TFC level may the changes in the quantity of soursop fruit content incorporated into the yoghurt and the fermentation conditions (Kwak *et al*., 2007).

Ferric reducing antioxidant power (FRAP)

Comparisons were discussed between fresh soursop fruit and the drinking yoghurts. According to the results obtained, the FRAP of soursop fruit is significantly lower ($p < 0.05$) than the FRAP of soursop drinking yoghurt. This suggests that consumption of soursop drinking yoghurt has more possibility in fighting cancer cells as a potent antioxidant than consuming soursop fruit. Further, the FRAP of fresh soursop fruit is significantly higher than that of the control drinking yoghurt which indicates that consuming soursop fruit is more beneficial than consuming regular drinking yoghurt with respect to ferric reducing antioxidant power. The FRAP of soursop drinking yoghurt is significantly higher than that of the control drinking yoghurt.

The FRAP values obtained in previous research cannot be compared with the present study due to the differences in the units used to express the results. The FRAP of vacuum-dried powder had a low value as presented in Table 4 due to the reduction of antioxidant compounds like polyphenols, tannins, coumarin, flavones, and flavonoids at higher temperatures, as mentioned by Anaya Esparza and Montalvo-González (2020).

Determination of DPPH and ABTS radical scavenging activity

DPPH and ABTS radical scavenging activities of fresh soursop fruit, vacuum-dried powder and the drinking yoghurts were determined and presented in Table 5 in both wet and dry weight basis.

DPPH radical scavenging activity

The DPPH free radical method is an antioxidant assay based on electron-transfer activity that produces a violet coloured solution in ethanol. The antioxidant activity was expressed as the trolox equivalent and the sample extracts which showed high inhibitory activities in the screening study were studied for dose response relationship. The results were expressed as IC_{50} (µg/mL), which is the concentration required to cause a 50% inhibition of DPPH activity (Garcia *et al*., 2012).

Significant differences ($p < 0.05$) in DPPH radical scavenging activity could be observed between the fresh soursop fruit and drinking yoghurts. The DPPH radical scavenging activity of fresh soursop fruit has been significantly increased when incorporating into soursop drinking yoghurt. The enhancement of antioxidant activity of fresh produce due to the fermentation process was described by some researchers (Kwak *et al*., 2007). Fermentation leads to release of phenolic acids and improved bio-accessibility and colonic metabolism of phenolic acids in fermentation (Verni *et al*., 2019). According to a study conducted by Xu *et al.* (2020) the thermal drying of fruits and vegetables, including vacuum drying, results in a notable decrease in DPPH values.

Table 5: DPPH and ABTS radical scavenging activities of the fresh soursop fruit and the products

Product	DPPH radical scavenging activity		ABTS radical scavenging activity	
	mg TE/g	IC 50 (µg/mL)	mg TE/g	IC 50 (µg/mL)
Fresh soursop fruit	17.49 ± 1.01^{b}	360 ± 21^{b}	17.23 ± 0.38^{b}	321 ± 7^{b}
Vacuum dried soursop powder	0.919 ± 0.14^{c}	557 ± 17^{a}	2.0 ± 0.09^{d}	176 ± 6^{c}
Control drinking yoghurt (0% soursop)	15.28 ± 2.79^{b}	345 ± 25^{b}	9.50 ± 1.55^{c}	482 ± 24^{a}
Soursop drinking yoghurt (20% soursop)	25.68 ± 3.38^{a}	176 ± 18^{c}	30.15 ± 3.24^{a}	129 ± 11^{c}

Values are presented as mean ± standard deviations of three replicate measurements. Means with the same letters in the same column are not significantly different ($p < 0.05$).

The radical scavenging rate at the concentration of a 50% inhibition (IC_{50}) was used as the indicator to evaluate the DPPH radical scavenging activity of fresh soursop and its products. The smaller the IC_{50} value, the higher the scavenging rate (Su *et al*., 2017). It was seen that the significantly highest ($p < 0.05$) IC_{50} value was obtained in soursop drinking yoghurt (Table 5).

The DPPH radical scavenging activity of fresh soursop fruit (6.45 ± 0.07 mg TE/g) reported by Paz *et al*. (2015) was comparatively lower than the value obtained for the DPPH radical scavenging activity (Table 5). The reasons may be the varietal differences, soil conditions, maturity stage, and changes in the extraction method. The DPPH radical scavenging of soursop set yoghurt developed by Virgen-Ceceña*et al*. (2019) was lower (0.15 ± 0.09 mmol TE/g) than the DPPH radical scavenging activity of soursop drinking yoghurt studied in the present study. This may be due to the difference in quantity of the soursop incorporated. The IC_{50} value of control drinking yoghurt in relation to DPPH radical scavenging activity reported by Su *et al*. (2017) agrees with the IC_{50} value presented in Table 5. However, the DPPH radical scavenging activity assay has some limitations, such as steric hindrance, variability of DPPH radical interactions with other radicals, and the relations between antioxidants and DPPH (Xu *et al*., 2020).

ABTS radical scavenging activity

Significant differences ($p < 0.05$) in ABTS radical scavenging activity were observed between the fresh soursop fruit and the drinking yoghurts. In comparison with the fresh fruit and the control drinking yoghurt, soursop fruit itself exhibited a significantly higher ($p < 0.05$) ABTS radical scavenging activity, due the presence of phyto-chemicals in the fruit. ABTS radical scavenging activity of soursop fruit had increased significantly ($p < 0.05$) when developing the soursop drinking yoghurt (Table 5) due to the fermentation process (Adebo & Medina-Meza, 2020). When comparing the two yoghurts, ABTS radical scavenging activity of soursop drinking yoghurt was significantly higher than the control drinking yoghurt. This is due to the incorporation of antioxidant rich soursop fruit pulp into regular drinking yoghurt followed by a fermentation process (Sah *et al*., 2014).

CONCLUSION

According to the present study, the value added soursop drinking yoghurt could be developed by incorporating fresh soursop fruit pulp into the regular drinking yoghurt. The soursop (20% w/v) incorporated drinking yoghurt with added sugar was chosen as the most feasible product and it showed acceptable physico-chemical and organoleptic properties with the best functional properties.

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Competing interests

The authors have declared that there are no competing interests

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