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Evaluation of nutritional characteristics and bioactive compounds of soursop-yoghurt and soursop-frozen dessert

Lucila J. Virgen-Ceceña[1] · Luis M. Anaya-Esparza[2] · Ana V. Coria-Téllez[3] · María de Lourdes García-Magaña[1] · Hugo S. García-Galindo[4] · Elhadi Yahia[5] · Efigenia Montalvo-González[1]

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Abstract The nutritional quality, sensory attributes, polyphenols and acetogenins content in yoghurt and frozen dessert formulated with soursop pulp were investigated. The addition of soursop pulp to yoghurt and frozen dessert improved the sensory attributes and the nutritional quality of soursop dairy products resulting in a composition of 0.92 and 2.17% of dietary fiber, 11.25 and 9.84 mg/100 g of vitamin C as well as 243.02 and 490.98 mg/100 g of total polyphenols, respectively. Acetogenins were extracted from both dairy products using maceration, sonication, microwave and Soxhlet. Sonication showed to be faster and safer than the other methods for acetogenins extraction. Higher annonacin (an acetogenin) content was found in yoghurt (38 ng/g) than in frozen dessert (15 ng/g). The quantification of bioactive compounds implied the nutraceutical properties to yoghurt and ice cream when they are added with soursop pulp. The results are useful for the consumers seeking healthier foods.

Keywords Dairy foods · *Annona muricata* · Nutritional quality · Polyphenols · Acetogenins

Introduction

Dairy foods are of great importance world-wide because of their nutritional and organoleptic properties. The consumption of yoghurt is highly recommended by health organizations because of the presence of probiotic bacteria. It is a rich source of bioactive peptides and an excellent source of proteins, minerals and vitamins that positively affect the health of the consumers (Lutchmedial et al.,

✉ Efigenia Montalvo-González
efimontalvo@gmail.com; emontalvo@ittepic.edu.mx

Lucila J. Virgen-Ceceña
lucila_virgen@outlook.com

Luis M. Anaya-Esparza
l_m_ae@hotmail.com

Ana V. Coria-Téllez
anac@colmich.edu.mx

María de Lourdes García-Magaña
mgarciam@ittepic.edu.mx

Hugo S. García-Galindo
hugosgg@gmail.com

Elhadi Yahia
yahia@uaq.mx

[1] Laboratorio Integral de Investigación en Alimentos, Instituto Tecnológico de Tepic, Av Instituto Tecnológico No 2595, Col. Lagos del Country, 63175 Tepic, Nayarit, Mexico

[2] Laboratorio de Microbiología de Alimentos, Universidad de Guadalajara, Centro Universitario de los Altos, Carretera a Yahualica km 7.5, 47630 Tepatitlán de Morelos, Jalisco, Mexico

[3] Laboratorio de Análisis y Diagnóstico del Patrimonio, El Colegio de Michoacán A. C., Cerro de Nahuatzen No. 85, Fracc. Jardines del Cerro Grande, C.P. 59370 La Piedad, Michoacán, Mexico

[4] Unidad de Investigación y Desarrollo de Alimentos, Instituto Tecnológico de Veracruz, Avenue Miguel Angel de Quevedo 2779, 91897 Veracruz, Veracruz, Mexico

[5] Facultad de Ciencias Naturales, Universidad Autonoma de Queretaro, Avenida de las Ciencias S/N, Juriquilla, 76230 Querétaro, Querétaro, Mexico

2004; Nguyen and Hwang, 2016). On the other hand, ice cream or frozen desserts are delicious and nutritious frozen dairy products that provide about 4.9% protein, 13% fat and 20.3% of carbohydrates, and therefore are considered valuable milk products (Bajwa et al., 2003). Although yoghurt and frozen desserts have high nutritional value, they are poor sources of natural antioxidants, dietary fiber and other bioactive compounds (Erkaya et al., 2012). In recent years, consumers have searched for healthier and functional foods with natural antioxidants, dietary fiber, minerals, vitamins and metabolites with healthy biological activity; and therefore, the development of new dairy-product formulations with the addition of ingredients with functional or nutraceutical properties are being explored.

Soursop is considered a healthy fruit because of its bioactive compounds such as polyphenols and acetogenins that offer attractive health benefits such as anti-inflammatory, anti-diabetic, and anti-tumoral activities (Coria-Téllez et al., 2018). Many studies have reported inhibitory effects of acetogenins on several cancer cell lines including prostate, liver, breast, lung, and further (Coria-Téllez et al., 2018).

Some studies have been done where yoghurt and ice cream have been flavored by adding soursop pulp. Kumari et al. (2014) prepared an ice cream added with soursop pulp (40 g/L) aiming to improve the technological properties of the ice cream. They reported that consistency was enhanced by the presence of fruit pulp, but they did not report nutritional quality improvement or bioactive compounds in the ice cream. Lutchmedial et al. (2004) found good acceptance of yoghurt added with 10% and 15% of soursop pulp by panelists, and reported that consumption of this fermented product provided relevant amounts of zinc, phosphorous and calcium, although they did not report the presence of bioactive compounds. On the other hand, the addition of soursop juice enhanced the antioxidant properties of stirred yoghurt (Imanthika-Dias and Niroshan-Jayasooriya, 2017).

Therefore, the objective of this work was to investigate the nutritional quality, content of polyphenols, extraction (different methods), identification and quantification of acetogenins in two dairy foods (soursop yoghurt and frozen dessert) added with soursop pulp.

Materials and methods

Plant material

Fully ripe soursop fruit (*Annona muricata* L.) were harvested from orchards located in the community of "El Tonino" at Compostela, Nayarit, Mexico. The fruit were peeled and seeds were removed. The pulp had a pH of 3.89 ± 0.03, total soluble solids of 15.0 ± 0.01 °Brix and titratable acidity of 0.86 ± 0.07%.

Preparation of soursop yoghurt and frozen dessert

Yoghurt was prepared according to the procedure of Cinbas and Yazici (2008) with some modifications. Fresh milk was pasteurized at 85 °C for 30 min. The pasteurized milk was inoculated with 2.5% (w/v) of dried yoghurt culture (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*; Chr. Hansen de Mexico SA de CV, Mexico City, Mexico). The inoculated milk was incubated at 42 °C until the pH decreased to 4.5. Based on total mass, 15% of soursop pulp (the pulp was chopped with a blender for 60 s, heat treated at 85 °C for 15 min) and 5% sucrose (heated at 75 °C for 30 min) before adding to the yoghurt. The product was kept at 4 °C until its analysis. For frozen dessert preparation, all the ingredients were weighed: soursop pulp (50% w/w), water (15% v/w), sucrose (15% w/w), dried milk (15% w/w), buttermilk (2% w/w), egg white (3% w/w) and carboxymethyl cellulose (1.0 w/w) as stabilizer. The ingredients were mixed with a blender (International LI-3, Veca International S.A. de C.V., Mexico City). Then the mixture was heated at 60 °C for 5 min in water bath, and stirred manually until a uniform preparation resulted. Then, soursop pulp (50% w/w) was added and mixed, the uniform mixture was pasteurized (70 °C for 30 min), and the product was cooled to room temperature. The mixture was then shaken in an electric mixer (KitchenAid Professional 600, Whirlpool Corporation, Benton Harbor, Michigan, USA) for 4 h, at − 20 °C. The dairy product was stored at − 20 °C for 24 h and then it was freeze-dried (Bajwa et al., 2003).

Physicochemical parameters were measured in fresh and frozen dessert; meanwhile nutritional quality and bioactive compounds were evaluated in the freeze-dried (Labconco Freeze Dryer 77522020, Labconco Corporation, Kansas City, MO) yoghurt and the frozen dessert. The freeze-dried samples were stored at − 20 °C until analysed.

Physicochemical parameters

Titratable acidity (TA, method 942.15), pH (method 981.12) and total soluble solids (TSS, method 932.12) were determined according to the AOAC Official Methods (AOAC, 2005). Color (L, C, Hue values) was measured using a Minolta Colorimeter (Konica Minolta CR-400, Konica Minolta Inc., Osaka, Japan).

Nutritional composition

Protein (method 978.04), ash (method 940.26), fat (method 950.54) and moisture (method 934.06) contents were

determined according to the AOAC Official Methods (AOAC, 2005). Soluble dietary fiber (SDF), insoluble dietary fiber (IDF), and total dietary fiber (TDF), were analyzed using the AOAC (AOAC, 2005) enzymatic–gravimetric method (method 991.42) modified by Mañas and Saura-Calixto (1993). Total soluble carbohydrates were measured by the phenol–sulfuric method (Dubois et al., 1956). All data were expressed as grams per 100 g of fresh weight (g/100 g FW).

Ascorbic acid (AA) content

The content of AA was determined according to the method of Suntornsuk et al. (2002). The samples (10 g) were homogenized with 25 mL of sulfuric acid (1.04 mol/L), 25 mL of distilled water and 3 mL of starch solution (50 g/L) as an indicator. The mixture was titrated with potassium iodide-diiode solution (0.12 mol/L and 0.02 mol/L, respectively) and the results were expressed in milligram per gram (mg/100 g FW).

Soluble (SP) and hydrolysable polyphenols (HP) and antioxidant activity (AOX)

An organic-aqueous extraction was performed on freeze-dried samples (0.5 g) mixed with methanol–acidified water (20 mL) solution (0.8% of HCl 72.8 g/L), methanol/water (100 mL) solution (50:50 v/v) and acetone–water (20 mL) solution (80:20 v/v) (Pérez-Jiménez et al., 2008). The quantification of SP was based on the Montreau (1972) method using the Folin–Ciocalteu's reagent at 750 nm in a multi-mode microplate reader (Synergy HT microplate reader, Biotek Instruments Inc., Winooski, Vermont, USA). HP were determined by hydrolysis with methanol/H_2SO_4, 90:10 (v/v) at 85 °C for 20 h, on the residues obtained in the organic-aqueous extraction (Hartzfeld et al., 2002). Samples were centrifuged (15 min, 25 °C, 3000 g) and HP were determined in the supernatants by the Folin–Ciocalteu's reagent as described above. The results were expressed as milligrams of gallic acid equivalent per 100 g of fresh weight (mg/100 g FW), utilizing a calibration curve of gallic acid as standard.

The AOX were determined in the organic-aqueous extracts. Three methods were implemented: ABTS (2,2-azinobis-3-ethylbenzothiazoline-6-sulfonic acid) assay (Re et al., 1999), DPPH (2,2-diphenyl-1-picrylhydrazyl) assay (Prior et al., 2005) and the ferric-reducing antioxidant power (FRAP) following the method of Benzie and Strain (1996). All AOX analyses were carried out in a multi-detection microplate reader. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was used as standard and the results were expressed as Trolox equivalent millimoles per gram (mmol TE/g FW).

Sensory evaluation

Sensory attributes were evaluated by a panel of 70 non-trained judges. Acceptance tests (taste, color, aroma and consistency) were scored based on a structured hedonic scale (Pedrero and Pangborn, 1989). The hedonic scale was structured with three points; 0 = dislike, 5 = neither like nor dislike, and 10 = like. The same procedure was applied for each product.

Extraction and qualitative identification of acetogenins (ACGs) from soursop yoghurt and frozen dessert

ACGs crude extraction was performed with four extraction methods (maceration, soxhlet, sonication and microwave extractions), using 30 g of sample with 100 mL of chloroform according to León-Fernández et al. (2017). After the extraction by maceration the samples were stored at room temperature during 5 days. The extraction was performed using a Soxhlet equipment (Novatech VH-6, Diseño de Laboratorios Equipamiento de laboratorio QUIMILAB, Guadalajara, Mexico). The extraction started at the boiling temperature (80 °C) of chloroform for 8 h. Sonication extraction was carried out at 25 °C. The samples were treated with 3 cycles (1 cycle for 1 h) at a constant frequency (42 kHz) on an ultrasonic equipment (Cole-Parmer 8891, Cole-Parmer Instrument Company, Vernon Hills, Illinois, USA). For the microwave extraction, samples were placed in the chamber of a microwave oven (Samsung MW830WA, Samsung Electronics Co. LTD., Selangor, Malaysia) operating at 600 W. The extraction was made with irradiation cycles of 10 s for 1 min. All extractions were performed in triplicate and extracts were filtered and concentrated in a rotatory evaporator (Yamato RE300, Yamato Corporation Dataweigh Division, Santa Clara, California, USA) at 30–50 °C and 90 rpm. Each extract was subjected to a thin layer chromatography on 5 × 5 cm silica plates (Merck Millipore Thin Layer chromatography HX312859, Millipore Corporation, Darmstadt, Germany) to determine the presence of acetogenins with Kedde reagent (Champy et al., 2005). Annonacin standard (Biobhiopha-BBP02455, BioBioPha Corporation, Ltd., Yunnan, China) was used as a positive control.

Separation of acetogenins by column chromatography

The ACGs crude extract from both dairy foods (1 kg) were obtained using the best extraction method. The extracts were filtered and supernatants were evaporated to dryness. The dried extracts (∼ 6 g) were placed on top of an open

glass column (6.4 × 57.0 cm) that was loaded with 80 g of SiO_2, 60 mesh, elution was made using $CHCl_3/CH_3OH$ (chloroform/methanol), starting with 100% chloroform to 100% methanol, to produce 36 fractions of 200 mL for each product. Two microliters of each fraction were used for the thin layer chromatography (TLC) analysis as described above.

Identification of annonacin using direct analysis in real time-mass spectrometry (DART-MS)

In order to confirm the presence of annonacin from the ACG positive fractions of soursop-yoghurt and soursop-frozen dessert, a chemical characterization profile was obtained using a DART-MS (Direct analysis in real time mass spectrometry) spectrometer (JEOL-AccuTOF JMS-T100LC, JEOL USA Inc., Peabody, Massachusetts, USA) having a DART ion source. The DART ion source was operated with helium gas flowing at approximately 4.0 L/min. The gas heater was set at 400 °C. Exact mass calibration was accomplished by including a mass spectrum of PEG 600 solution. The PEG solution, the annonacin standard and the ACG positive fractions were positioned in the gap between the DART source and the mass spectrometer using a closed end of a borosilicate glass melting point capillary tube until a signal was achieved in total-ion chromatogram (TIC). Each sample was introduced to the DART ion source three times. Using the Mass Centre Main software (version 1.3 m; JEOL USA Inc., Peabody, Massachusetts, USA), the elemental composition could be determined on selected peaks.

Quantification of annonacin by HPLC

Annonacin was quantified in the ACGs positive fractions of both dairy foods by HPLC. Each positive fraction was re-suspended in 1 mL of HPLC-grade acetonitrile and micro filtered (0.45 µm). Then, 10 µL were injected to the HPLC (Agilent Technologies 1260 infinity, Waldbronn, Germany) connected to a PDA detector (Agilent Technologies 1260 DAD, Waldbronn, Germany), fitted with a Waters Spherisorb ODS-2 C18 column (4.6 × 250 mm, 5 µm) and separation was made using an isocratic mobile phase of acetonitrile:water (85:15 v/v) at 0.2 mL/min; Annonacin was detected at 220 nm. The quantification was made using a standard curve of annonacin and the results are presented in nanograms per 100 g of dried weight (ng/100 g DW).

Statistical analysis

All values were obtained from three independent experiments and each sample was performed in triplicate (n = 9). Results were expressed as mean ± standard deviation (SD). A one-way analysis of variance (ANOVA) test was employed to analyze the data, and differences among means were compared by the Tukey´s test with a level of significance of $p < 0.05$, using the statistical software STATISTICA (v. 10 Statsoft®, Tulsa, OK).

Results and discussion

Physicochemical parameters

Data for total soluble solids (TSS), Titratable acidity (TA), pH and color (L, C, *h*) of soursop yoghurt and frozen dessert are given in Table 1. Soursop yoghurt and frozen dessert contained a TSS of 14.5 and 22.7 °Bx, respectively. These values are similar to or smaller than the data previously reported in yoghurt (18 °Bx) and frozen dessert (40 °Bx) with added soursop pulp (15% w/w and 10% w/w, respectively) (Imanthika-Dias and Niroshan-Jayasooriya, 2017). It has been reported that yogurt and ice cream without fruit had 14.2 °Bx and 29.31 °Bx, respectively (Erkaya et al., 2012; Imanthika-Dias and Niroshan-Jayasooriya, 2017). The TSS difference is primarily attributable to the different formulations (Sigdel et al., 2018).

TA and pH are commonly used as quality indicators in dairy products, in particular in yoghurt. According to official regulations (CODEX, 2011) yoghurt without fruit pulp should contain 0.50% of lactic acid and a pH < 4.5. The TA and pH values are significantly different ($p < 0.05$) for soursop yoghurt and frozen dessert. Soursop yoghurt and frozen dessert exhibited values of TA of 0.70% and 0.47% of lactic acid, and a pH of 4.08 and 4.99, respectively. Similar trends were previously reported in yoghurt and frozen dessert added with soursop pulp (Imanthika-Dias and Niroshan-Jayasooriya, 2017; Kumari et al., 2014; Lutchmedial et al., 2004). The addition of soursop pulp influenced the pH and TA of both dairy products, but its behavior is dependent on the amount of soursop pulp added (Erkaya et al., 2012). Sigdel et al. (2018) reported an increase in TA and a decrease in pH in yoghurt added with mulberry, and indicated that changes in these parameters were mainly due to the acidity and low pH values of fruits used. Furthermore, the changes in TA and pH in soursop yoghurt are likely attributable to the fermentation process (Lutchmedial et al., 2004).

Color serves as a quality indicator, especially for marketing purposes. The color value of Luminosity of 113, Chrome of 6.16, *h* of 54.43 measured in soursop yoghurt are similar to those reported for yoghurt without fruit pulp (Nguyen and Hwang, 2016). On the other hand, soursop frozen dessert exhibited an off-white, tan color (Luminosity = 106.86, Chrome = 7.65, *h* = 52.84) attributable to

Table 1 Physicochemical parameters, nutritional composition and antioxidant capacity and of yoghurt and frozen dessert added with soursop pulp

Parameter	Yoghurt	Frozen dessert
Total soluble solids (°Bx)	14.50 ± 0.60	22.70 ± 0.70
Titulable acidity (% lactic acid)	0.70 ± 0.01	0.47 ± 0.01
pH value	4.08 ± 0.05	4.99 ± 0.06
Luminosity	54.43 ± 0.78	52.84 ± 1.22
Chroma	6.16 ± 0.77	7.65 ± 0.51
Hue (*h*)	113.03 ± 3.29	106.86 ± 1.94
Moisture (g/100 g FW)	83.69 ± 0.02	74.07 ± 0.06
Protein (g/100 g FW)	3.14 ± 0.03	3.03 ± 0.04
Fat (g/100 g FW)	5.15 ± 0.24	8.42 ± 0.69
Ash (g/100 g FW)	0.61 ± 0.05	0.54 ± 0.04
Soluble carbohydrates (g/100 g FW)	7.73 ± 0.24	14.22 ± 0.45
Soluble dietary fiber (g/100 g FW)	0.53 ± 0.02	1.03 ± 0.07
Insoluble dietary fiber (g/100 g FW)	0.39 ± 0.11	1.14 ± 0.05
Total dietary fiber (g/100 g FW)	0.92 ± 0.06	2.17 ± 0.06
Calories (Kcal/100 g)	90	145
Ascorbic acid (mg/100 g FW)	11.25 ± 2.44	9.84 ± 2.44
Total soluble phenols (mg/100 g FW)	169.38 ± 6.78	406.37 ± 1.36
Hydrolysable polyphenols (mg/100 g FW)	73.64 ± 3.17	84.61 ± 3.29
Total polyphenols (mg/100 g FW)	243.02 ± 4.24	490.98 ± 2.32
Antioxidant capacity (mmol TE/g FW)		
FRAP	3.01 ± 0.07	6.60 ± 0.41
DPPH	0.15 ± 0.09	6.18 ± 0.17
ABTS	9.91 ± 0.42	15.39 ± 0.11

Values are the mean of triplicate determinations from three different experiments (n = 9) ± standard deviation

FW = fresh weight, TE = Trolox equivalent, Luminosity = whiteness or brightness, Chroma = dark/bright ratio, Hue angle (*h*) = location of color, FRAP = Ferric-reducing antioxidant power assay, DPPH = 2,2-diphenyl-1-picrylhydrazyl assay, ABTS = 2,2-azinobis-3-ethylbenzothiazoline-6-sulfonic acid assay

the presence of carotenes from egg yolk employed in its formulation (Barakat and Hassan, 2017). In general, color of yoghurt and frozen dessert was not influenced ($p > 0.05$) by the presence of soursop pulp.

Nutritional composition

The nutritional composition of yoghurt and frozen dessert is showed in Table 1. CODEX (2011) describe the values for moisture (83.69%), protein (3.28%), fat (5.34%), ash (0.64%) and soluble carbohydrates (7.73%) of yogurt without fruit. The soursop yoghurt of this experiment, had similar values; therefore, soursop pulp addition did not change the nutritional composition and it complied with the official regulations for fermented dairy products. Similar results were reported previously by Lutchmedial et al. (2004) in stirred soursop (15 w/w) yoghurt (protein = 4.62%, Ash = 1.01%, total carbohydrates = 8.82%). Also, Imanthika-Dias and Niroshan-Jayasooriya (2017) reported in stirred yoghurt added with 15% (w/w) of soursop nectar, 6.80% of protein, 4.27% of fat, 6.14% of available carbohydrates, 81.87% of moisture and 0.92% of ash content, which provided 91 kcal/100 g. Additionally, in soursop yoghurt evaluated in this work, a total dietary fiber content of 0.92% was measured, of which 0.53% was soluble dietary fiber and 0.39% was insoluble dietary fiber; these components have not been previously determined in soursop yoghurt.

In the case of soursop frozen dessert, the elevated fat (8.42%) and carbohydrates (14.22%) are attributable to fat in the powdered milk and sugar incorporated during its preparation; the soursop pulp addition might influence these parameters, although it has been reported that ice cream without fruit can have more or less fat and carbohydrates depending of their formulation (Erkaya et al., 2012). The total dietary fiber in soursop frozen dessert was 2.14% (soluble dietary fiber of 1.03% and insoluble dietary fiber of 1.15%). It must be noted that soursop frozen dessert had the highest dietary fiber content caused by added fruit pulp. This behavior may be also attributable to the content of pulp added for each dairy product (Agu and Okolie, 2017; Erkaya et al., 2012).

Dietary fiber has nutritional importance because it may affect the rheological properties of the gastrointestinal content, increasing the viscosity of the medium that triggers satiety signals and slows down the gastric emptying. Moreover, soluble dietary fiber may serve as a substrate for the fermentative microbiota producing short-chain fatty acids and it also stimulates the colonic blood flow and electrolyte uptake (Moyano et al., 2016). Thus, the presence of dietary fiber in the prepared dairy foods enhanced their nutritional value (Erkaya et al., 2012). On the other hand, the presence of dietary fiber in yoghurt and frozen desserts aids to improve the stability of these emulsions (Rodríguez et al., 2006). The calculated caloric values were 90 and 145 kcal/100 g for yoghurt and frozen dessert, respectively.

Ascorbic acid content

Ascorbic acid (AA) is the most important water-soluble vitamin, usually present and highly bioavailable in fruits. Table 1 shows the ascorbic acid content of soursop yoghurt (11.25 mg AA/100 g) and frozen dessert (9.84 mg AA/100 g). Bajwa et al. (2003) and Erkaya et al. (2012) reported that the fruit addition can incorporate ascorbic acid in frozen dessert formulations influenced the content of AA. Also, the AA values from soursop yoghurt and frozen dessert were similar to other soursop-based products such as soursop juice (11 mg/100 mL) (Dias et al., 2015). According to the Institute of Medicine, Food and Nutrition Board (IMFNB, 2001), the dietary reference intake (DRI) for vitamin C is 90 mg/day for males and 75 mg/day for females. Therefore, a daily consumption of 100 g/day of soursop yoghurt could ensure an intake of 12.5% and 15% of the DRI of vitamin C for adult males and females, respectively; meanwhile a daily consumption of 100 g/day of soursop-frozen dessert could provide an intake of 10% and 13%, respectively.

Soluble (SP) and hydrolysable (HP) polyphenols content and antioxidant activity (AOX)

Soursop yoghurt contained 169.38 mg/100 g of SP and 73.64 mg/100 g of HP; while soursop frozen dessert had 406.37 mg/100 g of SP and 84.61 mg/100 g of HP. The addition of soursop pulp to yoghurt and frozen dessert is an effective way to improve their functional characteristics, in particular by the presence of phytochemicals such as soluble and hydrolysable polyphenols that provide excellent AOX properties (Agu and Okolie, 2017; Nguyen and Hwang, 2016). Cinnamic acid derivatives and *p*-coumaric acid are the principal phenols that have been reported for soursop pulp (Jiménez et al., 2014).

The AOX of soursop yoghurt was 3.01, 0.15 and 9.91 mmol TE/g by the FRAP, DPPH, and ABTS methods, respectively. However, in soursop frozen dessert it was 6.60, 6.18 and 15.37 mmol TE/g. It has been demonstrated that antioxidants may act via multiple mechanisms of radical-scavenging activity such as metal scavengers, electron transfer or by donating hydrogen ions (Suntornsuk et al., 2002). On the other hand, differences in the antioxidant capacity between assays could be credited to the different concentrations of polyphenols and peptides in the products (Loizzo et al., 2012).

The effect of the addition of different types of fruit pulp as functional ingredients on the antioxidant properties of yoghurt and frozen dessert has been studied. Imanthika-Dias and Niroshan-Jayasooriya (2017) found that AOX was enhanced in yoghurt when soursop juice was added, and similar data was reported in pumpkin-flavored yoghurt (Barakat and Hassan, 2017). Citta et al. (2017) investigated the oxidative changes during shelf life in lipids, proteins, and antioxidants from yoghurt supplemented with different fruits (berries, pineapple, apricot, strawberries). They reported that the addition of fruits rich in antioxidant compounds provided a protection against lipid peroxidation of yoghurt, thus extending their shelf life in 9 weeks at 4 °C. Additionally, authors reported that measured AOX depends on the added antioxidants contained in fruits plus the bioactive peptides produced during the fermentation process. Therefore, with this work we confirm that the addition of soursop pulp in dairy foods increased their AOX, suggesting that its consumption may promote better health of consumers.

Sensory evaluation

The sensory attributes of products as yoghurt and frozen dessert have been improved by the addition of fruit products (Lutchmedial et al., 2004; Çam et al., 2013). Figure 1 shows the sensory evaluation (aroma, color, taste, texture and overall acceptability) of the soursop yoghurt (Fig. 1A) and frozen dessert (Fig. 1B). The color scores were 8.9 for yoghurt and 9.0 for frozen dessert, which indicates that soursop pulp positively contributed to color acceptability of yoghurt and frozen dessert (Lutchmedial et al., 2004). Aroma and taste were assessed by the judges with scores above 7 for soursop yoghurt and frozen dessert. These scores were in the scale of "I like it". The texture in yoghurt was graded with 6.7; the panelists described that the yoghurt was liquid and not creamy, while soursop frozen dessert was qualified with 9.1, meaning a good acceptability. Addition of soursop pulp into yoghurt and frozen dessert enhanced the stability of these products, apparently caused via the presence of dietary fiber (Rodríguez et al., 2006). In general, both yoghurt and

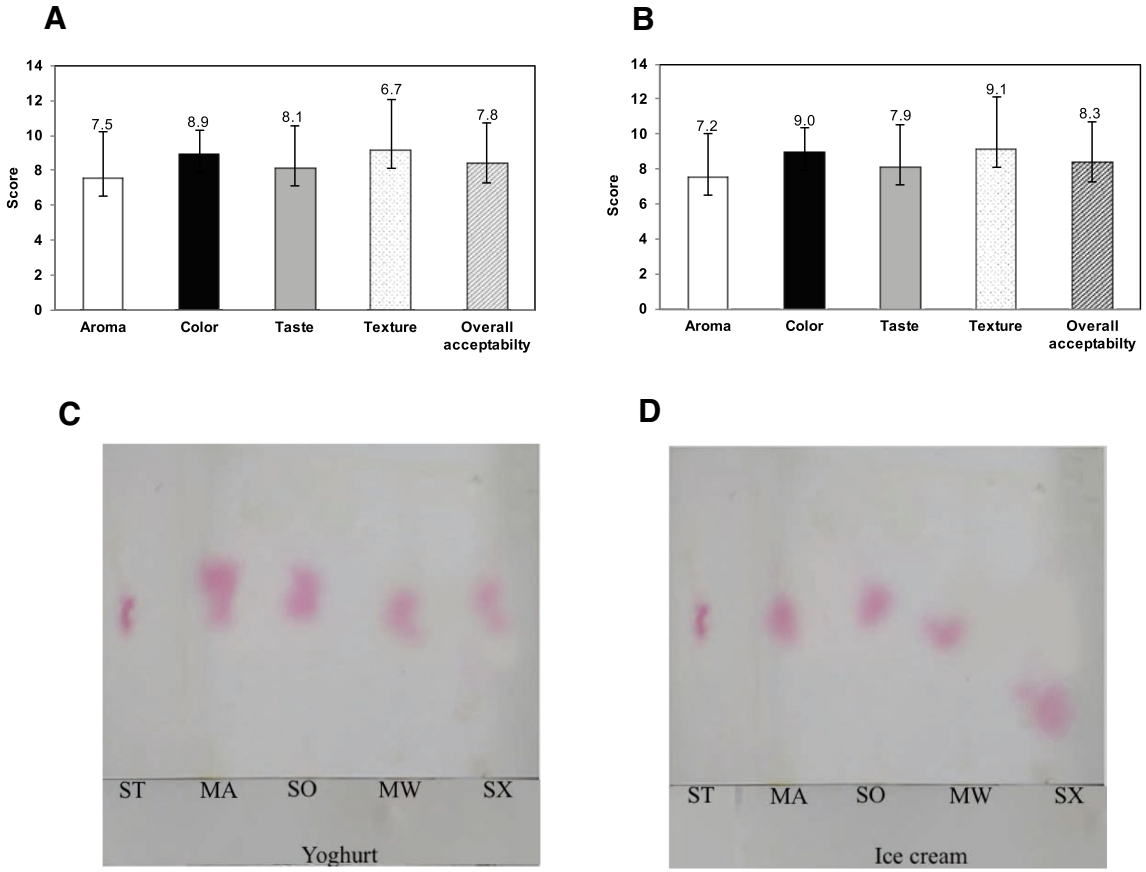

Fig. 1 Sensory evaluation of yoghurt (**A**) and frozen dessert (**B**) with added soursop pulp, and qualitative identification of acetogenins by thin layer chromatography (TLC) obtained from four different extraction methods from yoghurt (**C**) and frozen dessert (D) with added soursop. *ST* Annonacin standard, *MA* Maceration, *SO* sonication, *MW* Microwave, *SX* Soxhlet

frozen dessert added with soursop pulp had good overall acceptability (7.8 and 8.3, respectively), which suggests that the yoghurt and frozen dessert with soursop pulp may have a good opportunity in the market.

Qualitative identification of acetogenins

The presence or absence of acetogenins by TLC obtained by maceration, sonication, microwave and soxhlet extraction from soursop yoghurt and frozen dessert is shown in Fig 1C, D, respectively. The retention factor (Rf) values of acetogenins for standard and crude extracts of soursop yoghurt and frozen dessert are shown in Table 2. The standard used was annonacin, which is the major ACG found in soursop pulp, that exhibited a Rf of 0.36 (Champy et al., 2005). Significant differences ($p < 0.05$) were observed among extraction treatments where maceration, sonication and microwave were the most effective methods to extract ACGs (Rf = 0.36–0.38) compared to that carried out by Soxhlet (Rf = 0.13–0.18) for both soursop yoghurt and frozen dessert. Similar trends were reported by León-Fernández et al. (2017) when comparable extraction procedures for extracting acetogenins from soursop pulp (Rf = 0.36) were evaluated. However, because of intrinsic characteristics of each extraction method as long extraction

Table 2 Retention factor (Rf) values of acetogenins from crude extract of yoghurt and frozen dessert added with soursop pulp obtained by maceration, sonication, microwave and soxhlet

Product	Extraction method	Rf
Annonacin standard	–	0.36 ± 0.01^{a}
Yoghurt	Maceration	0.38 ± 0.01^{a}
	Sonication	0.38 ± 0.02^{a}
	Microwave	0.36 ± 0.01^{a}
	Soxhlet	0.18 ± 0.02^{b}
Frozen dessert	Maceration	0.36 ± 0.01^{a}
	Sonication	0.35 ± 0.01^{a}
	Microwave	0.36 ± 0.01^{a}
	Soxhlet	0.13 ± 0.02^{b}

Values are the mean of triplicate determinations from three different experiments (n = 9) ± standard deviation

Means in a column with different letters are significantly different ($p < 0.05$)

Table 3 Annonacin content from fractions obtained during the separation and purification the acetogenins from the crude extract of yoghurt and frozen dessert added with soursop pulp

No. fraction	Yoghurt	Frozen dessert
F7	4.49 ± 1.02	ND
F8	3.02 ± 0.98	ND
F9	2.99 ± 0.13	ND
F11	10.78 ± 1.73	0.58 ± 0.02
F12	7.98 ± 0.72	0.37 ± 0.01
F13	1.48 ± 0.14	14.45 ± 0.24
F14	2.64 ± 0.01	ND
F15	ND	0.16 ± 0.01
F17	2.40 ± 0.56	0.30 ± 0.01
F18	2.49 ± 029	ND
Total concentration (ng/g DW)	38.30	15.86
Yoghurt portion (250 g FW)	1.62 ng per portion	–
Frozen dessert portion (30 g FW)	–	0.12 ng per portion

Values are the mean of triplicate determinations from three different experiments (n = 9) ± standard deviation

ND not detected, *DW* dry weight, *FW* fresh weight

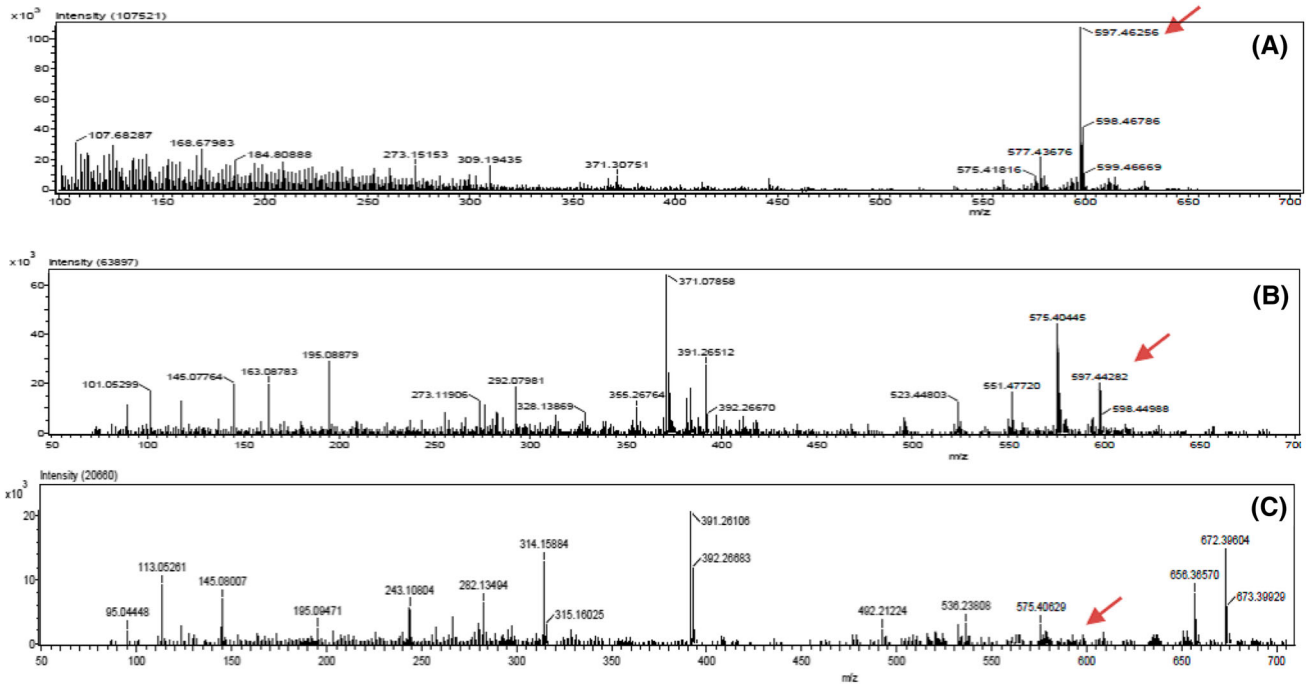

Fig. 2 Identification of annonacin by direct introduction of samples in direct analysis in real time mass spectrometry (DART-MS) (**A** standard), from yoghurt (**B**) and frozen dessert (**C**) with soursop pulp added

times for maceration or the use of solvents for microwave extraction, we decided to use sonication, since it is regarded as safe, rapid, and a low cost method for ACGs extraction, including separation and quantification of annonacin in soursop yoghurt and frozen dessert.

ACGs qualitative identification (Rf = 33–36) from soursop yoghurt was obtained in fractions 7–18, while from soursop frozen dessert the fractions with ACGs were 11–18, without significant differences ($p > 0.05$) between both dairy products (Table 3). Differences in obtained ACG-positive fractions may be attributable to the complexity of frozen dessert matrix (Melot et al., 2009).

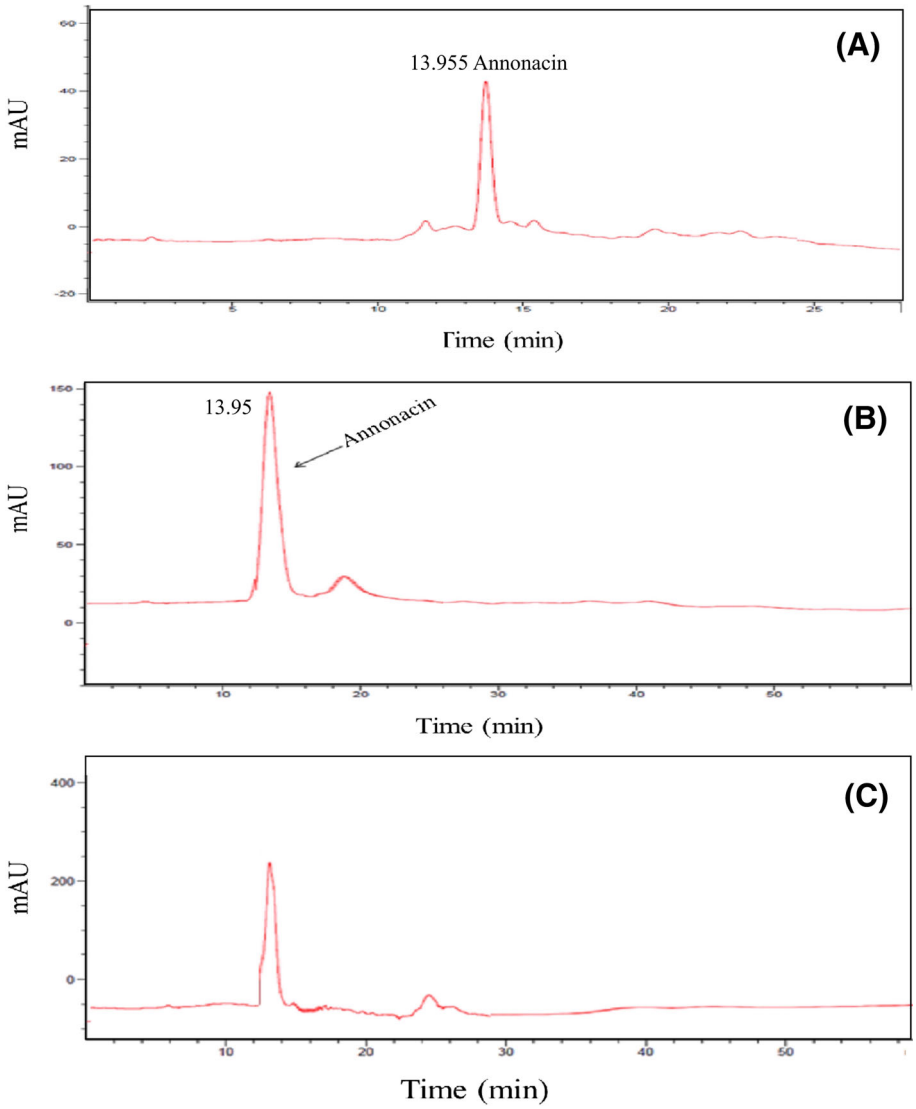

Fig. 3 High pressure liquid chromatography (HPLC) identification of annonacin (**A** standard) from yoghurt (**B**) and frozen dessert (**C**) with soursop pulp added

Identification of annonacin by DART-MS in fractions

Direct introduction of the ACG positive crude extracts of soursop yoghurt and frozen dessert into DART-MS provided the identification of the annonacin by its m/z. Figure 2 shows examples of the DART mass spectra obtained by the direct introduction of the annonacin standard (Fig. 2A), and the crude extracts yoghurt (Fig. 2B) and frozen dessert (Fig. 2C) added with soursop pulp. In the crude extract spectra, it is possible to observe the characteristic signal at a m/z 597 (M + 1) of the annonacin. The annonacin signal is more intense in yoghurt than in frozen dessert, whereas the DART-MS signal was proportional to the compound concentration, and therefore, it is safe to assume that there was more annonacin in yoghurt than in frozen dessert. The same conclusion was found in the quantification of annonacin by HPLC as discussed below.

Quantification of annonacin by HPLC

For annonacin quantification, positive fractions were injected into the HPLC. Figure 3A–C shows examples of the HPLC chromatograms with a single peak at a retention time of 13.95 min, indicating the corresponding annonacin. Annonacin quantification was calculated for each fraction of both products (Table 3). Final annonacin concentration shows that soursop yoghurt contained more annonacin (38 ng/g) than the frozen dessert (15.88 ng/g). The presence (León-Fernández et al. 2017; Melot et al., 2009) and quantification of annonacin in soursop pulp (Champy et al., 2005; Potts et al., 2012) have been previously reported. However, we found no reports of the presence of ACGs in processed foods such as those evaluated in this study.

Quantification of annonacin from soursop yoghurt and frozen dessert is meaningful because it is one of the most important bioactive compounds in soursop that has been reported with outstanding biological properties that improve human health. Annonacin has been reported to have anti-tumor properties in several cancerous cell lines such as prostate, liver, and lung, and anti-tumor activity in murine models (Coria-Téllez et al., 2018).

The addition of soursop pulp did not affect the physicochemical variables and sensory attributes of yoghurt and frozen dessert. Adding soursop pulp to yoghurt and frozen dessert enhanced the technological and health benefits of these products, in particular by the presence of dietary fiber as well as ascorbic acid and polyphenols, with elevated antioxidant activity. Also, we found acetogenins, reported as the main bioactive compound of soursop. Extraction of acetogenins from yoghurt and frozen dessert was achieved by sonication, which is a rapid, safe and a low-cost alternative to obtain acetogenins from soursop-based food products. We have identified and quantified only annonacin but we believe that other acetogenins should be present in soursop-based food products as the literature suggest.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

References

Agu KC, Okolie PN. Proximate composition, phytochemical analysis, and in vitro antioxidant potentials of extracts of *Annona muricate* (Soursop). Food Sci. Nutr. 5: 1029–1036 (2017)

AOAC. Official Methods of Analysis of the Association of Official Analytical Chemists. 15th ed. Washington DC, USA (2005)

Bajwa UA, Huma N, Ehsan B, Jabbar K, Khurram A. Effect of different concentration of strawberry pulp on the properties of ice cream. Int. J. Agric. Biol. 5: 635–637 (2003)

Barakat H, Hassan MFY. Chemical, nutritional, rheological, and organoleptical characterizations of stirred pumpkin-yoghurt. Sci. Res. Publ. 8: 746–759 (2017)

Benzie I, Strain J. The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: the FRAP assay. Anal. Biochem. 239: 70–76 (1996)

Çam M, Erdoğan F, Aslan D, Dinç M. Enrichment of functional properties of ice cream with pomegranate by-products. Food Sci. Nutr. 78:C1543-C1550 (2013)

Champy P, Melot A, Guérineau V, Gleye C, Fall D, Hoglinger G, Hocquemiller R. Quantification of acetogenins in *Annona muricata* linked to atypical parkinsonism in Guadeloupe. Mov. Disorders 20: 1629–1633 (2005)

Cinbas A, Yazici F. Effect of the addition of blueberries on selected physicochemical and sensory properties of yoghurts. Food Technol. Biotechnol. 46: 434–441 (2008)

Citta A, Folda A, Scalcon V, Scutari G, Bindoli A, Bellamio M, Feller E, Rigobello MP. Oxidative changes in lipids, proteins, and antioxidants in yogurt during the shelf life. Food Sci. Nutr. 5: 1079–1087 (2017)

CODEX ALIMENTARIUS-FAO. Milk and Dairy Products (2011). Available from: http://www.fao.org/docrep/015/i2085e/i2085e00.pdf. Accessed May 15, 2018.

Coria-Téllez AV, Montalvo-González E, Yahia EM, Obledo-Vázquez EN. *Annona muricata*: A comprehensive review on its traditional medicinal uses, phytochemicals, pharmacological activities, mechanisms of action and toxicity. Arab. J. Chem. 11: 662–691 (2018)

Dias DDRC, Barros ZMP, Carvalho CBO, De Honorato FA, Guerra NB, Azoubel PM. Effect of sonication on soursop juice quality. LWT—Food Sci. Technol. 62: 883–889 (2015)

Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28: 350–356 (1956)

Erkaya T, Dağdemir E, Şengül M. Influence of cape gooseberry (*Physalis peruviana* L.) addition on the chemical and sensory characteristics and mineral concentrations of ice cream. Food Res. Int. 4: 331–335 (2012)

Hartzfeld PW, Forkner R, Hunter MD, Hageman AE. Determination of hydrolyzable tannins (*gallotannins and ellagitannins*) after reaction with potassium iodate. J. Agric. Food Chem. 50: 1785–1790 (2002)

Imanthika-Dias G, Niroshan-Jayasooriya M. Enhancing the physiochemical and antioxidant properties of stirred yoghurt by incorporating soursop (*Annona Muricata*). Int. J. Life Sci. Res. 5: 69–77 (2017)

Institute of Medicine, Food and Nutrition Board (IMFNB). Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc. National Academies Press, Washington, DC (2001)

Jiménez VM, Gruschwitz M, Schweiggert RM, Carle R, Esquivel P. Identification of phenolic compounds in soursop (*Annona muricata*) pulp by high-performance liquid chromatography with diode array and electrospray ionization mass spectrometric detection. Food Res. Int. 65: 42–46 (2014)

Kumari KMGA, Herath HMGP, Rupasinghe RAJNL. Impact of fruits (soursop, papaya, wood apple and bael fruit) with medicinal value on organoleptic, physio-chemical and microbiological properties of ice cream. In abstract: International Symposium on Agriculture and Environment 2014, pp. 215–218, Ruhuna, Sri Lanka. Available from: https://www.cabdirect.org/cabdirect/abstract/20153384238

León-Fernández AE, Obledo-Vázquez EN, Vivar-Vera M, Sáyago-Ayerdi S, Montalvo-González E. Evaluation of emerging methods on the polyphenol content, antioxidant capacity and qualitative presence of acetogenins in soursop pulp (*Annona muricata* L.). Rev. Bras. Frut. 39: 1–8 (2017)

Loizzo MR, Tundis R, Bonesi M, Menichini F, Mastellone V, Avallone L, Menichini F. Radical scavenging, antioxidant and metal chelating activities of *Annona cherimola* Mill. (cherimoya) peel and pulp in relation to their total phenolic and total flavonoid contents. J. Food Compos. Anal. 25: 179–184 (2012)

Lutchmedial A, Ramlal R, Badrie N, Chang-yen I. Nutritional and sensory quality of stirred soursop (*Annona muricata* L.) yoghurt. Int. J. Food Sci. Nutr. 55: 407–414 (2004)

Mañas, E, Saura-Calixto F. Ethanolic precipitation: A source of error in dietary fibre determination. Food Chem. 47: 351–355 (1993)

Melot A, Fall D, Gleye C, Champy P. Apolar annonaceous acetogenins from the fruit pulp of *Annona muricata*. *Molecules* 14: 4387–4395 (2009)

Montreau F. Sur le dosage des composés phénoliques totaux dans les vins par la methode Folin-Ciocalteau. Connaiss Vigne Vin 24: 397–404 (1972)

Moyano G, Sáyago-Ayerdi SG, Largo C, Caz V, Santamaria M, Tabernero M. Potential use of dietary fibre from *Hibiscus sabdariffa* and *Agave tequilana* in obesity management. J. Funct. Foods 21: 1–9 (2016)

Nguyen L, Hwang ES. Quality characteristics and antioxidant activity of yoghurt supplemented with aronia (*Aronia Melanocarpa*) juice. Prev. Nutr. Food Sci. 21: 330–337 (2016)

Pedrero F, Pangborn R. Evaluación sensorial de los alimentos: Métodos analíticos. Editorial Alhambra, DF, México, p. 251 (1989)

Pérez-Jiménez J, Arranz S, Tabernero M, Días-Rubio M, Serrano J, Goñi I, Saura- Calixto F. Updated methodology to determine antioxidants capacity in plant foods, oils and beverages: Extraction, measurement and expression of results. Food Res. Int. 41: 274–285 (2008)

Potts LF, Luzzion FA, Smith SC, Hetman M, Champy P, Litvan I. Annonacin in *Asimina triloba* fruit: Implication for neurotoxicity. *Neurotoxicology* 33: 53–58 (2012)

Prior RL, Wu X, Schaich K. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. J. Agric. Food Chem. 53: 4290–4302 (2005)

Re R, Pellegrini A, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic. Biol. Med. 26: 1231–1237 (1999)

Rodríguez R, Jiménez A, Fernández-Bolaños J, Guillén R, Heredia A. Dietary fibre from vegetable products as source of functional ingredients. Trends Food Sci. Technol. 17: 3–15 (2006)

Sigdel A, Ojha P, Karki TB. Phytochemicals and syneresis of osmodried mulberry incorporated yoghurt. Food Sci. Nutr. 6: 1045–1052 (2018)

Suntornsuk L, Gritsanapun W, Nilkamhank S, Paochom A. Quantitation of vitamin C content in herbal juice using direct titration. J. Pharm. Biomed. Anal. 28: 849–855 (2002)