

Antioxidant evaluation of food grade extracts obtained from soursop (*Annona muricata*) fruit

Evaluación antioxidante de extractos grado alimenticio obtenidos a partir del fruto de guanábana (*Annona muricata*)

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Abstract

The soursop fruit (*Annona muricata*) is recognized as a source of carbohydrates, vitamins and minerals, although the presence of bioactive compounds such as acetogenins, alkaloids and phenolic compounds has also been reported, with recognized anticancer, antimicrobial and antioxidant activity. However, in the production chain of this fruit, one of the problems is the short post-harvest life of 4-9 days. For this reason, an alternative for the use of its bioactive compounds could be to obtain and use soursop extracts to improve the quality of food products. Based on the above, in the present experiment, the changes in firmness, color and weight of soursop fruits in their ripening stages were determined, as well as the amount of phenolic compounds and antioxidant activity by DPPH and ABTS methods of two different tissues (peel and pulp) at two different stages of maturity (physiological and consumption) using two types of solvents (70% food grade ethanol and water). The results showed that the extract obtained with a 70% ethanol solution from the peel of fruits at the consumption maturity stage presented significantly higher amounts of phenolic compounds, as well as the highest antioxidant activity. The results support that the peel of the fruit, which is preferably consumed or industrialized fresh, may represent a sustainable alternative for the utilization of the bioactive compounds of soursop as a preservative in food products.

Resumen

El fruto de guanábana (*Annona muricata*) es reconocido como fuente de carbohidratos, vitaminas y minerales, aunque también se ha reportado la presencia de compuestos bioactivos como acetogeninas, alcaloides y compuestos fenólicos, a estos se les reconoce actividad anticancerígena, antimicrobiana y antioxidante. No obstante, en la cadena productiva de este fruto, uno de los problemas es la corta vida postcosecha, siendo de 4-9 días. Por esa razón, una alternativa para el aprovechamiento de sus compuestos bioactivos, podría ser la obtención y utilización de extractos de guanábana para mejorar la calidad de los productos alimenticios. Con base en lo anterior, en el presente experimento se determinaron los cambios de firmeza, color y peso que presentan los frutos de guanábana en sus etapas de maduración, también se evaluó la cantidad de compuestos fenólicos y la actividad antioxidante por los métodos de DPPH y ABTS de dos diferentes tejidos (cáscara y pulpa) en dos diferentes etapas de madurez (fisiológica y de consumo) utilizando dos tipos de disolventes (etanol grado alimenticio al 70% y agua). Los resultados mostraron que el extracto que se obtuvo mediante una solución con etanol al 70%, a partir de la cáscara de frutos en etapa de madurez de consumo presentó significativamente la mayor cantidad de compuestos fenólicos, así como la mayor actividad antioxidante. Los resultados sustentan que la cáscara del fruto, el cual se consume o industrializa preferentemente en fresco, puede representar una alternativa sustentable para el aprovechamiento de los compuestos bioactivos de la guanábana como conservador de productos alimenticios.

Soursop, Antioxidant activity, Food Grade

Guanábana, Actividad antioxidante, Grado alimenticio

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Introduction

Soursop fruit, nutritionally, is recognised as a source of carbohydrates, vitamins and minerals, although the presence of bioactive compounds such as acetogenins, alkaloids and phenolic compounds has also been reported, which are recognised as having anticancer, antimicrobial and antioxidant activity (Jiménez et al., 2014., p.44.). Regarding the production of this fruit, the largest production worldwide is attributed to Mexico and, at the national level, Nayarit ranks first in soursop production (SIAP-SAGARPA, 2019). However, in the production and distribution chain of this fruit, one of the problems is its short post-harvest life, which averages 4-9 days at a temperature of 15-20 °C (Berumen-Varela et al., 2019., p.271.).

On the other hand, it is estimated that annually one third of the food produced in the world is wasted, this waste is mainly attributed to factors such as environmental conditions, microbial spoilage, mechanical damage during transport and oxidative processes. In order to reduce the percentage of global food waste, each of the above-mentioned factors must be overcome. In terms of oxidative spoilage of food; oxidative stability has been improved through the strategy of using a wide variety of antioxidants of both synthetic and natural origin (Rangaraj et al., 2021., p.1). However, in recent years, the demand for natural antioxidants, mainly of plant origin, has grown in recent years due to increasing concern among consumers about the presence of toxicological effects of synthetic antioxidants (Falowo et al., 2014., p.177).

In response to the demands of finding natural resources with antioxidant activity and based on the agricultural production of the state of Nayarit, soursop is one of the fruits that could be used as a source of phytochemical compounds that improve the oxidative stability of foods. Therefore, it is necessary to explore different extraction methods and perform antioxidant evaluations on extracts obtained from soursop fruit.

Theoretical framework

The soursop tree is an evergreen tree belonging to the Annonaceae family, native to Mexico, Central America, the West Indies and northern South America.

This plant produces oval or cone-shaped fruits with a dark green colour, characterised by a set of thorns with a shell-like appearance all over the skin. These fruits weigh between 0.5 and 4 kg. The pulp represents most of the fruit (approximately 80 %) and is white, juicy and has a sweet and sour taste. The soursop also contains black or brown seeds (127-170), which can measure 1.25 to 2 cm in length. Regarding the composition of the fruit, water (80 %) and carbohydrates (15-17 %) are the most abundant; it is also rich in vitamins C and A, and minerals such as calcium, iron and phosphorus. In addition, the presence of bioactive compounds such as acetogenins, alkaloids and phenolic compounds has been reported, which are recognised to have anticancer, antimicrobial and antioxidant properties (Jiménez et al., 2014, p.45; Leite-Neta et al., 2019, p.72; Berumen-Varela et al., 2019, p.270).

A nivel internacional México es el principal productor de guanábana. A nivel nacional, en el año 2018, el estado de Nayarit fue el principal productor del país, con una producción de 21,860.02 ton; seguido en menor escala por los estados de Colima con 2,933.31 ton, Michoacán con 2,395.80 ton, Guerrero con 1,070.66 ton y Veracruz con 511.06 ton (SIAP-SAGARPA, 2019).

In recent years, the soursop has been the most important member of the anonaceae family, due to the discovery of potential applications of its pulp at nutritional, medicinal and industrial levels. Currently, soursop is consumed as a whole fruit and its pulp is marketed natural or frozen for the preparation of aguas frescas, as well as for the production of ice cream, nectar, jellies, popsicles, liqueurs, jams, jellies, yoghurts and purees (Sanusi and Abu Bakar, 2018, p.393.). However, no references have been documented on the use of soursop extracts as additives in food products.

On the other hand, it is estimated that every year 33 % of the food produced in the world is wasted, this waste is mainly attributed to factors such as environmental conditions, microbial spoilage, mechanical damage during packaging or transport and oxidative processes (Rangaraj et al., 2021., p.1).

With regard to oxidative food spoilage, a clear example can be observed in meat and meat products. The second most relevant cause affecting the quality of meat and meat products are the oxidative processes of both lipids and proteins. The deterioration caused by these oxidative processes is reflected through the following phenomena; development of unpleasant odours and flavours caused by volatile oxidation products, changes in colouring caused by the oxidation of myoglobin, an increase in water loss due to dripping, and a decrease in nutrients due to protein denaturation. There is also a shortening of shelf life and the emergence of oxidation by-products with toxic effects for the consumer, which have been associated with diseases such as arteriosclerosis, neurodegenerative diseases and some types of cancer (Falowo et al., 2014., p.177.; Papuc et al., 2016., p.100.; Pellissery et al., 2019., p.318).

To counteract the oxidative deterioration of food products; the food industry has improved the oxidative stability of foods through the strategy of using a wide variety of antioxidants (Falowo et al., 2014., p.177). In terms of antioxidants, antioxidants are defined as compounds that can neutralise free radicals by accepting or donating electrons to eliminate an overload of oxygen-reactive substances (Bielli et al., 2015., p.212). Antioxidants can be of natural or synthetic origin. Synthetic antioxidants such as hydroxybutylbutylanisole, butylated hydroxytoluene, tertiary butyl hydroquinone and propyl gallate have been widely used in food products. However, in recent years, the demand for natural antioxidants, mainly of plant origin, has grown in recent years due to increasing concern among consumers about the presence of potential toxicological effects of synthetic antioxidants (Falowo et al., 2014., p.178).

The main source of bioactive substances with antioxidant role for humans are plants, therefore, different plant products have been evaluated as natural antioxidants to preserve and improve food quality. These natural antioxidants derived from the plant kingdom are extracted from different agricultural products such as fruits (grapes, pomegranate, date, tangerines, avocado, among others), vegetables (broccoli, potato, pumpkin, curry, among others), herbs and spices (tea, rosemary, oregano, nettle, cinnamon, mint, sage, thyme, ginger, clove, among others) using different methods and solvents (Falowo et al., 2014., p.180).

Methodology

In a commercial soursop orchard located in the locality of Venustiano Carranza, Mpio. de Tepic, Nayarit (21° 30' N, 104° 54' W, 920 masl), 30 fruits were collected at physiological maturity stage. Subsequently, this raw material was transferred to the laboratory of the Specialised Unit for Food Quality and Natural Products of CENITT-UAN, where the weight, colour and firmness of the fruits were determined. Subsequently, 15 fruits were stored at room temperature until the fruits reached consumption maturity and 15 more fruits were stored at physiological maturity vacuum packed at a temperature of -20°C. For the fruits that reached eating maturity, the weight was determined, the peel was removed by hand and the peel weight was determined. These fruits were stored under vacuum wrapped in aluminium foil at a temperature of -20 °C until further analysis.

The weight was determined using a Torrey LEQ-5 scale. Surface firmness was determined using a SHIMPO digital penetrometer model FGV-50XY, which recorded the force necessary to cause a slight pressure on the surface of the fruit. The colour of the fruit peel was determined by a Minolta R-400 colorimeter. The extracts were obtained using two different solvents (70% food grade ethanol and water) for the peel and pulp of the fruit at two different stages of maturity (physiological maturity and consumption maturity).

To obtain the extracts, 3 g of sample were homogenised in a 50 mL Falcon tube with 15 mL of the solvent according to each treatment. After homogenisation, the tubes were centrifuged at 2500 rpm for three minutes and the liquid fraction was recovered in a ground-glass flask through Whatman filter paper no. 54. The resulting residue was treated again with 15 mL of the same solvent used, applying the same homogenisation and centrifugation conditions. The supernatant obtained was then added to the first one using the same filter paper. Once the extract was completely filtered, it was subjected to rotaevaporation of the solvent at a maximum temperature of 42 °C. The residue obtained was diluted in water and diluted in a solution. The residue obtained was diluted in 25 mL of water and kept refrigerated (4 °C) until further analysis.

From the extracts obtained, aliquots of the extracts were prepared with the following dilutions: 1:4 for pulp extracts and 1:10 for peel extracts in order to perform the following analyses under these conditions. First, the total content of phenolic compounds in the extracts was determined by the Folin-Ciocalteu method following the procedure described by Soong & Barlow (2004); where an aliquot of 200 μL of the previous dilution of the extracts was mixed with 1000 μL of 10 % Folin-Ciocalteu reagent and 800 μL of 7.5 % sodium carbonate. They were left to stand in the dark for 30 minutes until a bluish colour was obtained and then the absorbance was read at 765 nm in a Unico spectrophotometer, mod UV-2150.

The concentration of phenolic compounds in each extract was calculated using a regression equation obtained from a standard curve of gallic acid with a concentration range of 5 to 100 mg gallic acid/L. The total amount of phenolic compounds expressed was calculated using a regression equation. The total amount of phenolic compounds was expressed as milligrams of gallic acid equivalents (GAE) per 100 grams of fresh matter.

Subsequently, each of the extracts was also tested for antioxidant activity equivalent to 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) by 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2-azinobis-3-ethylbenzothiazolin-6-sulphonic acid (ABTS) spectrophotometric methods.

To determine the inhibitory capacity of the extracts by the DPPH technique, a 0.1 mM DPPH solution in methanol was prepared according to the method described by Ganhão et al. (2010). Once the diluted extracts were prepared, 33 μL of each sample was mixed with 2000 μL of the DPPH free radical (initial absorbance $\sim 1,200$), allowed to stand for 6 min in the dark and absorbance was measured at a wavelength of 517 nm. To express the results, a standard curve of Trolox was prepared at different concentrations (0.25, 0.5, 1, 1.5, and 2 mM). The antioxidant capacity was determined in Trolox equivalents and expressed in mM Trolox equivalents per gram of fresh fruit (mM Trolox/g fresh sample).

Finally, the in vitro antioxidant activity of the extracts was determined by testing for inhibition of the chromogenic ABTS+ radical measured by the method described by Re et al. (1999). The ABTS radical was generated by mixing 2,2-azinobis-3-ethylbenzothiazolin-6-sulphonic acid 7 mM with 2.45 mM potassium persulphate, which was allowed to stand for 15 hours at room temperature.

The radical was then diluted in ethanol to an initial absorbance of ~ 0.700 at a wavelength of 734 nm. For the development of the technique, 10 μL of each sample was mixed with 1000 μL of the ABTS+ radical (ABS ~ 0.700) and allowed to stand in the dark for 6 minutes to subsequently measure absorbance at a wavelength of 734 nm. To express the results, a standard curve of Trolox was prepared at different concentrations (0.25, 0.5, 1, 1.5, and 2 mM). The antioxidant capacity was established in Trolox equivalents and expressed in mM Trolox equivalents per gram of fresh fruit (mM Trolox/g fresh sample).

In the present project, a 2 x 2 x 2 x 2 factorial design was used for the analysis of the soursop extracts, the sources of variation of the factorial model being: two types of solvents (70 % food grade ethanol and water), two tissues from the soursop fruit (peel and pulp) and two stages of fruit maturity (physiological and consumption). All analytical techniques were performed in triplicate for each sample and each fruit was considered as a replicate.

The statistical analysis was carried out by processing the data of the individual variables, for which an analysis of variance was performed. The data of the quantitative variables were studied by descriptive statistics. In cases where significant differences were found ($p < 0.05$), a comparison of means test was carried out using Tukey's test to contrast differences between treatments. For these analyses, the SAS programme for Windows version 9.0 was used.

Results

Tables 1 and 2 show the quantitative values of colour (in CIE $L^*a^*b^*C^*h^*$ scale), firmness and weight of soursop at physiological maturity and consumption maturity, respectively.

	L	a*	b*	C*	h*	Weight (kg)	Firmness (N)
Average	39.69	-7.01	16.73	18.73	116.92	1.28	31.73
S	4.07	5.25	3.69	4.17	2.58	0.45	8.30
CV (%)	10.25	74.89	22.09	22.29	2.21	35.46	26.17

L*: Luminance; a*: red/green co-ordinates; b*: yellow-blue co-ordinates; C*: Chroma; h*: hue; S: standard deviation; CV: coefficient of variation; S: standard deviation.

Table 1 Colour, weight and firmness values of guanábanas collected at physiological maturity

	L	a*	b*	C*	h*	Weight (kg)	Firmness (N)	TM (d)
Average	36.09	-3.61	13.50	14.10	104.05	1.39	3.68	7.00
S	4.14	2.47	2.89	3.24	8.25	0.23	1.42	2.49
CV (%)	11.48	-68.29	21.40	23.02	7.93	16.71	38.68	35.63

L*: Luminosity; a*: red/green coordinates; b*: yellow-blue coordinates; C*: Chroma; h*: hue; S: standard deviation; CV: coefficient of variation; TM: ripening time. Regarding the composition of soursop fruits at eating maturity, the peel represented on average 13.25 % of the total weight of the fruit (159 ± 34 g).

Table 2 Colour, weight and firmness values of soursops collected at eating maturity

Table 3 shows the amount of total phenolic compounds obtained from the different extracts.

Tissue	Solvent	Maturity	CFT
Pulp	Agua	Physiological	23.71 ^f
Pulp	Ethanol 70 %	Physiological	26.08 ^f
Shell	Agua	Physiological	46.12 ^{d,e}
Peel	Ethanol 70 %	Physiological	90.50 ^b
Pulp	Agua	Consumption	32.97 ^{e,f}
Pulp	Ethanol 70 %	Consumption	52.74 ^d
Peel	Agua	Consumption	72.56 ^c
Peel	Ethanol 70 %	Consumption	195.37 ^a
E.E.			3.67

S.E.: Standard error of the mean; CFT: Total phenolic compounds expressed in mg GAE/100 g fresh matter; Different literals per column denote significant statistical differences ($p < 0.05$).

Table 3 Total phenolic compounds determined by the FOLIN CIOCALTEU method

Table 4 shows the data obtained from the analyses of antioxidant activity obtained by the DPPH and ABTS methods, respectively.

Tissue	solvent	Maturity	DPPH	ABTS
Pulp	Agua	Physiological	0.25 ^e	0.28 ^d
Pulp	Ethanol 70 %	Physiological	0.24 ^e	0.26 ^d
Shell	Agua	Physiological	0.54 ^{c,d}	0.57 ^{c,d}
Peel	Ethanol 70 %	Physiological	1.06 ^b	1.02 ^b
Pulp	Agua	Consumption	0.39 ^{d,e}	0.42 ^{c,d}
Pulp	Ethanol 70 %	Consumption	0.61 ^c	0.73 ^{b,c}
Peel	Agua	Consumption	0.70 ^c	1.02 ^b
Peel	Ethanol 70 %	Consumption	2.26 ^a	2.46 ^a
E.E.			0.05	0.06

S.E.: Mean standard error; DPPH: Antioxidant activity expressed mg Trolox/g sample by DPPH method; ABTS: Antioxidant activity expressed mg Trolox/g sample by ABTS method; Different literals per column denote significant statistical differences ($p < 0.05$).

Table 4 Antioxidant activity obtained from soursop extracts by DPPH and ABTS methods

Discussion and conclusions

A large variation in colour, firmness and weight variables can be observed in both soursops at eating maturity and those at physiological maturity, such variation among soursop fruits was also previously described by Terán-Erazo et al. (2019), and by Jiménez-Zurita, et al. (2019), who report a variation of more than 42 % for fruit weight, a variation of 28 % in chromaticity or even a variation of more than 71 % in fruit firmness.

On the other hand, the peel weight of the fruit at eating maturity represented 13.25 % of the total fruit weight. This value is up to 6 percentage points lower than that reported by other authors. Regarding the proportion of the peel compared to the whole fruit Terán-Erazo et al. (2019), reported a proportion of pulp, peel and seed weight of 73.2 %, 19.5 % and 7.3 % respectively. Similarly, Solís et al. (2010) evaluated soursop fruits from an area of Actopan, Veracruz, and determined that the proportions of pulp, peel and seed were 70.3 %, 18.8 % and 5.4 %, respectively.

During the consumption maturity process of the fruits, a slight decrease in the green colouring of the surface, as well as in the luminosity, could be observed, dropping from -7.01 to -3.61 for the a* (red/green) coordinates and from 39.69 to 36.09 for the luminosity. The described phenomenon of change in fruit colouring at physiological maturity and eating maturity has been reported previously.

These changes are attributed to the rupture of chloroplasts, which causes the release of enzymes such as polyphenol oxidase and the degradation of chlorophyll during the last stage of ripening (Jiménez-Zurita et al., 2016., p.1160). Regarding the information provided by the different soursop fruit extracts, it is important to highlight that the extract that had a higher concentration of total phenolic compounds and greater antioxidant activity was the one obtained using 70 % ethanol solvent, from the peel of fruits at the consumption maturity stage.

This could be attributed to the softening of the fruit, which is caused by the enzymatic cellular deterioration that the fruit undergoes when they reach the consumption maturity stage, which could facilitate the extraction of secondary metabolites with antioxidant activity. This softening and cell deterioration was reflected in the firmness of the fruits studied, which went from 31.73 N at physiological maturity of the fruit to 3.68 when the fruit reached consumption maturity. It is worth mentioning that the firmness reported for soursop fruit at eating maturity is 8.3 N (Berumen-Varela et al., 2019., p.270).

The softening that occurs in the ripening process of soursop fruit is associated with the presence and action of different enzymes; among the enzymes that are most involved in this process are: Pectinmethyl esterase (PME) and pectolytic enzymes. PME is related to the degradation of pectic substances in the middle lamella of the cell, which is a component of the cell wall that acts as a cementing or binding agent between cells and can also control the movement of soluble materials. Regarding this enzyme; its activity has been quantified, indicating a considerable increase of this enzyme in short periods and presenting activity 23 times higher in the fruit at consumption maturity with respect to the fruit at physiological maturity (Jiménez-Zurita et al., 2016., p.1162.). It should be noted that some authors have suggested that the function of PME is to promote the de-esterification of galacturonans in order to allow the action of pectolytic enzymes (PGs). These PGs have been directly related to the softening of fruits with high ethylene production. PGs are pectolytic enzymes of which endo-PG (EC 3.2.1.15) and exo-PG (EC 3.2.1.67) have been identified; endo-PG catalyses the random hydrolytic cleavage of the α -(1-4) bonds of galacturonans; exo-PG hydrolyses releasing galacturonic acid.

These enzymes have been described more extensively in fruits such as mango and durian. However, in soursop fruit, a sudden increase in the presence of these enzymes has been reported at the stage coinciding with the climacteric phase of the fruit. PGs promote the degradation of the middle lamella of parenchyma cells, resulting in softening of the fruit (Jiménez-Zurita et al., 2016., p.1163).

It is also important to mention that the extract obtained using 70% ethanol from the peel of fruits at the consumption maturity stage had 135.97 mg GAE per 100 g of fresh matter. However, this concentration is not similar to that obtained by Terán-Eraza et al. (2019), who had an average of 64.2 mg GAE g⁻¹ fresh weight. The discrepancy in the concentration of phenolic compounds in soursop fruits is reported by Coria-Telles et al. (2018, p.665.).

In conclusion, it is important to consider obtaining ethanolic extracts of soursop peel at consumption maturity as an alternative to take advantage of the bioactive compounds with antioxidant activity of a fruit that is highly perishable and that is preferably consumed fresh and the peel is discarded. It would also be interesting to use technologies such as ultrasound to compare the quantity of bioactive compounds extracted and their antioxidant activity with the conventional method.

It is also necessary to challenge ethanolic extracts of soursop peel at consumption maturity in food matrices, particularly in meat matrices, and it would be interesting to evaluate their antioxidant effect and their impact on the oxidative stability of meat.

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