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Use of jabuticaba (*Plinia cauliflora*) skin in the processing of ice creams

Uso de piel de jabuticaba (*Plinia cauliflora*) en el procesamiento de helados

Bruna Böger[1], Andréa Leal B[2], Luciano Lucchetta[2], Ellen Porto P[2].

1. Departamento de Ciência e Tecnologia de Alimentos, Universidade Estadual de Londrina, Londrina, Paraná.
2. Departamento Acadêmico de Engenharia de Alimentos, Universidade Tecnológica Federal do Paraná, Francisco Beltrão, Paraná.

Corresponding autor: Ellen Porto Pinto.
Departamento de Ciência e Tecnologia de Alimentos,
Universidade Tecnológica Federal do Paraná
Campus Francisco Beltrão.
Linha Santa Bárbara s/n, CEP: Francisco Beltrão, Paraná, Brasil.
Telephone: + 55 4635202641
E-mail: ellenporto@utfpr.edu.br

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ABSTRACT

The aim of this work was to process jabuticaba skin aqueous extract, varying the crushing and sieving time and to develop ice cream with different concentrations of jabuticaba skin extract, evaluate its physicochemical, microbiological and bioactive composition. Different extractive processes of jabuticaba skin were tested. Extract A was crushed for 25 seconds and sieved, extract B was crushed for 25 seconds without sieving, extract C was crushed for 45 seconds and sieved and extract D was crushed during 45 seconds not sifted. From the extract that presented the highest indices of bioactive compounds, formulations of ice cream with concentrations of 5, 10 and 15% were elaborated. Extract B showed the highest content of phenolic compounds (201.81 mg gallic acid. 100 g^{-1} skins), anthocyanins (60.32 mg cyanidin-3 glycoside.100 g^{-1} peels) and significant antioxidant activity (5047.72 g skins. g^{-1} DPPH) and was chosen to be added in the ice cream. The evaluated ice creams met the microbiological standards established by the Brazilian legislation. The use of progressive concentrations of jabuticaba skin extract in the elaboration of ice cream increased the rates of phenolic compounds and antioxidant capacity. The values found were significant and generated an alternative use for jabuticaba skin, which is normally discarded.

Keywords: Antioxidant activity; Ice cream; Phenolic compounds; Plinia cauliflora.

RESUMEN

El objetivo de este trabajo fue elaborar extractos acuosos de piel de jabuticaba, variando el tiempo de trituración y cribado. Además de desarrollar helados con diferentes concentraciones de extracto de piel de jabuticaba, evaluando su composición físico-química, microbiológica y de compuestos bioactivos. Se analizaron diferentes procesos de elaboración de extractos de cáscara de jabuticaba, siendo denominados extracto A - triturado durante 25 segundos y tamizado, extracto B - triturado durante 25 segundos no tamizado, extracto C - triturado durante 45 segundos y tamizado y extracto D - triturado durante 45 segundos sin cribado. A partir del extracto que presentó los índices más elevados de compuestos bioactivos, se elaboraron formulaciones de helado con concentraciones de 5, 10 y 15%. El extracto B fue el que presentó mayor contenido de compuestos fenólicos (201,81 mg ácido gálico. 100 g^{-1} piel), antocianinas (60,32 mg cyanidina-3 glycosideo.100 g^{-1} piels) y significativa actividad antioxidante (5047,72 g piels. g^{-1}DPPH) siendo el elegido para ser añadido en el helado. Los helados evaluados se encuentran dentro de los patrones microbiológicos estabelecidos por la legislación brasileña. La utilización de concentraciones progresivas de extracto de piel de jabuticaba en la elaboración de helado incrementó los índices de compuestos fenólicos y de capacidad antioxidante. Los valores encontrados son significativos y genera una alternativa en el aprovechamiento de la piel de jabuticaba, normalmente descartada.

Palabras clave: Actividad antioxidante; Compuestos fenólicos; Helados; Plinia cauliflora.

INTRODUCTION

The agro-industry has developed products aimed at obtaining functional foods, which, in addition to meeting normal nutritional needs, have a "protective" effect for health, delaying the onset of diseases[1,2,3]. This functionality comes from a series of compounds that can perform these functions. Such compounds are widely found in plants and are a very diverse group of phytochemicals. In plants, phenolics are essential for plant growth and reproduction, besides acting as anti-pathogenic agents and contributing to the coloration mechanism[4]. Fruit and vegetables are the primary sources of phenolic compounds, which are found in higher quantities in pulp compared to juice[5].

Jabuticaba is a tropical fruit of high nutritional value. It has a higher content of carbohydrates, fiber, vitamins, and minerals compared to other fruits[6,7], and also has a significant content of phenolic compounds, such as anthocyanins and flavonols[13]. Studies indicate that whole jabuticaba has antioxidant activity and anthocyanins in significant amounts[6,8,9,10]. The jabuticaba tree is a native Brazilian fruit tree of the genus Myrtaceae; its fruit is a globose berry, and has an almost black peel, with white, bittersweet pulp[10]. Although it is a popular fruit, the jabuticaba, does not have a very high commercial value because it is perishable and loses moisture and deteriorates quickly.

Most of these phenolic compounds are found in its skin, which is usually wasted[10]. Lima et al.[11] observed a higher content of polyphenols in the fruit peel, being almost 25 times higher than in the pulp. Therefore, it is necessary to seek alternatives for the use of jabuticaba skin by incorporating it into food products[12,15] to use its antioxidant properties[14].

Jabuticaba skin can be subjected to different processes to be used in the processing of food products. Studies have been performed with the use of flour made of jabuticaba skin to make cookies and cereal bars[13,14]. Some authors also report the direct use of its pulp and skin to produce liquor and jellies[16,18]. Other researchers obtained the aqueous extract of its fruit and or skin and used it to produce fermented milk and fruit drinks[19].

Ice cream is one of the products that can be made of jabuticaba skin. It is considered a complete food and has high nutritional value, as it provides proteins, carbohydrates, lipids, vitamins, calcium, phosphorus and other minerals. It is a very popular dessert in Brazil, appealing to all ages and palates, among different social classes. It is considered a good source of energy; thus, for these reasons, it is an appropriate food for incorporating functional ingredients. Therefore, the development of ice cream from jabuticaba skin would be an alternative to add value to the fruit, also making possible its consumption during the inter-harvest period.

This study aimed to elaborate extracts of jabuticaba skin with different formulations of ice cream, evaluating their physical-chemical, microbiological and bioactive composition.

Material and Methods

Jabuticaba (*Plinia cauliflora* (Vell.) Berg, Sabará genotype) skin from the native fruit were obtained in the Federal Technological University of Paraná, Campus Dois Vizinhos, Paraná State, Brazil.

The aqueous extracts of jabuticaba skin were obtained at the concentration of 42% of peel (600 g of jabuticaba and 840 mL of distilled water). The peels were heated at 100°C for 5 minutes, ground in a blender (Philips Blender, Walita Problend 800W, 5 speeds, Philips of Brazil) at speed 1 for 25 and 45 seconds. Two of these extracts were subjected to sieving. Four different extraction methods were tested: Extract A was ground for 25 seconds and sieved, Extract B was ground for 25 seconds and not sieved, Extract C was ground for 45 seconds and sieved, and Extract D was ground for 45 seconds and not sieved. All extracts were thermally processed at 80 °C for 5 minutes and then frozen at -18 °C in sterile plastic bottles. Thawing was done immediately before the beginning of the experiments, at room temperature.

Powdered ingredients were weighed and mixed (powdered milk, sugar, and neutral alloy) to prepare the ice cream. The milk cream was added to pasteurized milk, together with the jabuticaba skin extract which presented the highest content of bioactive compounds (Table 1). The ingredients were homogenized in a liquefier (Philips Blender, Walita Problend 800W, 5 speeds, Philips of Brazil) for 5 minutes. The mixture was pasteurized in a water bath at 75 °C for 20 minutes. After, the mixture was matured for 12 hours at 7 °C. Emulsifier was added to the mixture, and it was homogenized again in a blender for 5 minutes. The mixture was placed in a vertical ice-cream maker (Fort Frio V.EXP-5, automatic, 0.84 Kwh, 15L/h) to beat and partially freeze the mixture, allowing the incorporation of air (overrun). The ice cream was frozen in a freezer for 24 hours at -18 °C[20]. Four formulations of ice cream were prepared: standard ice cream (without jabuticaba skin extract), ice cream with 5% jabuticaba skin extract, ice cream with 10% jabuticaba skin extract and ice cream with 15% jabuticaba skin extract (Table 1).

The analyses of pH, titratable total acidity, and total soluble solids were performed in the aqueous extracts of jabuticaba skin, according to A.O.A.C.[21] The color was analyzed using a colorimeter (Minolta CR-300) with diffuse illumination, where the samples were arranged in Petri dishes with 5 cm in diameter and 2 cm in height. We also measured the color angle, using the equation: ºHue = Tan-1 (b*/a)[21].

The ice cream was subjected to the analyses of titratable total acidity and fat by the Rose-Gottlieb method, according to A.O.A.C.[21]. Total and thermotolerant coliforms, *Staphylococcus aureus* analyses and microorganism with growing 0 a 7 ºC (Psychrotrophic) counts were also performed, according to ICMSF[22].

Hydroalcoholic extracts of ice cream were prepared, according to the method described by Vedana[23]. The aqueous extracts of jabuticaba skin and hydroalcoholic extracts of ice

cream were evaluated as the total phenolic compounds and total anthocyanins contents. The phenolic compounds were determined according to the method described by Singleton and Rossi[24]. The results were expressed as milligrams of gallic acid *100 g^{-1} of jabuticaba skin. Total anthocyanins were quantified by the differential pH method described by Lee et al.[25]. The value of total anthocyanins was expressed as mg equivalent to cyanidin-3-glycoside in 100 g^{-1} of jabuticaba skin.

The antioxidant activity of the extracts was determined by the DPPH method (2,2-diphenyl-1-picrylhydrazyl) following the method described by Brand-Williams et al.[26] and modified by Rufino et al.[27]. The result was expressed as EC50 (g peel of jabuticaba. g^{-1} DPPH). Also, the free radical sequestering activity was determined from a standard curve of Trolox-DPPH. The result was expressed as antioxidant activity equivalent relative to Trolox (TEAC) in μM.g^{-1} of jabuticaba skin.

We conducted analysis of variance and Tukey tests at 5% of significance.

RESULTS

The pH of the jabuticaba skin extracts ranged from 6.10 to 6.12, and the acidity content ranged from 1.68 to 1.87 citric acid.100 g^{-1} (Table 2); however, there was no statistically significant difference ($p< 0.05$). Acidity is intimately related to the taste and quality of the extracts, since it interferes with the taste and general characteristics of the extracts that will be used in the elaboration of ice cream. These two parameters maintained characteristics of the milk and, consequently, of the ice cream, demonstrating stability and coherence with the type of product desired.

Regarding the total soluble solids (7.30 to 7.75 ºBrix), there was no statistical difference ($p< 0.05$) between the extracts of jabuticaba skin evaluated here (Table 2). These values were expected since we only used the skin, which contains less soluble solids than the pulp. Previous studies have described higher contents (28.5 °Brix); however, the extracts used in these studies were elaborated from whole fruit[13,16].

Table 1. Formulations of ice cream added with different concentrations of jabuticaba skin extract.

Ingredients (g)	Ice cream formulations (g)			
	Control	5% Extract	10% Extract	15% Extract
Pasteurized and homogenized bovine milk	65.18	53.20	41.23	29.26
Extract of jabuticaba	00.00	11.98	23.95	35.92
Powdered bovine milk	13.29	13.29	13.29	13.29
Sugar	13.29	13.29	13.29	13.29
Cream (42% fat)	6.64	6.64	6 64	6.64
Neutral Binder*	0.80	0.80	0 80	0.80
Emulsifier (Emustab®)**	0.80	0.80	0.80	0.80

*Composition: sugar, guar gum, carboxymethylcellulose, tara gum.
**Composition: water, emulsifiers: monoglycerides of distilled fatty acids, fatty acid salt, sorbitan monostearate and polyoxyethylene sorbitan monostearate.

Table 2. Physicochemical composition and bioactive compounds of the different jabuticaba skin extracts.

Parameters	Jabuticaba skin extracts*			
	Extract A	Extract B	Extract C	Extract D
pH	6.10±0.02a	6.12±0.02a	6.10±0.01a	6.10±0.02a
Titratable total acidity (g Citric acid.100g^{-1})	1.70±0.1a	1.68±0.1a	1.87±0.1a	1.83±0.1a
Total soluble solids (ºBrix)	7.75±0.5a	7.58±0.4a	7.30±0.3a	7.59±0.4a
Color (ºHue)	0.90±0.6b	3.47±0.7a	0.17±0.05b	3.85±0.3a
L	18.29±0.3a	17.71±0.3a	18.24±0.04a	18.42±0.1a
a	27.07±0.5a	22.61±0.6c	25.32±0.1b	22.96±0.2c
b	0.53±0.3a	-1.36±0.2b	0.09±0.02a	-1.49±0.1b
Anthocyanins (mg cyanidin-3 glycoside.100g^{-1} skin)	33.6±2.7b	60.3±15.4a	27.0±2.6b	40.7±2.2ab
Phenolics compounds (mg gallic acid.100g^{-1} skin)	121.3±0.6c	201.8±0.8a	102.4±1.7d	178.3±0.5b
EC50 (g skin .g^{-1} DPPH)	4717±132c	5047±106ab	5234 ±79a	4955± 30bc
TEAC (μM Trolox. g^{-1} skin)	10.16±0.6a	9.23±0.4ab	8.29±0.3b	10.19±0.1a

* Means followed by the same small letter in the same line do not statistically differ at 5% probability by Tukey test

Extracts B and D had a higher hue than the other extracts, which represented a more reddish color. Extract A showed a higher intensity of red coloration (chromaticity coordinate "a") followed by extract C. In relation to the "b" coordinate, extracts A and C did not differ from each other, presenting positive values and having a greater intensity of yellow coloration, while the extracts B and D presented negative values, indicating a greater intensity of blue coloration (Table 2).

The results of phenolic compounds and antioxidant activity of jabuticaba skin extracts was presented in Table 2.

In the ice cream with added jabuticaba skin extract, it was observed that, with the increase in the percentage of the extracts, there was a decrease in pH and an increase in acidity, probably due to the organic acids present in the extracts of jabuticaba skin. The content of total soluble solids also increased, since, in addition to the extracts, 13.29% of sugar was added (Table 3).

Regarding the fat content, the ice cream with the highest fat content was the standard (Table 3). In the formulations with jabuticaba skin extract; there was a decrease in fat content, probably due to the dilution of this component with the addition of the extracts in the other formulations. It is important to emphasize that all the formulations of ice creams were within the standard required by the Brazilian legislation: 2.5% of milk fat[28].

The ice cream with a 10 and 15% addition of jabuticaba skin extract presented higher ºHue. The control ice cream presented the highest value of "L", indicating greatest luminosity, which was expected since it did not have the addition of the extract of jabuticaba skin. There was a decrease in luminosity according to the greater addition of extract to the ice cream. The ice cream with 15% of jabuticaba skin extract showed the highest intensity of red coloration (chromaticity coordinate "a") (Table 3).

The results of phenolic compounds and antioxidant activity of ice creams was presented in Table 3.

The elaborated ice creams are within the standards required by the Brazilian legislation, regarding the values of *Staphylococcus aureus* and coliforms at 45 °C, which should be $5x10^2$ CFU.g^{-1} and 5x10 MLN.g^{-1}, respectively (Table 4).

Regarding the microorganism with growing 0 a 7 ºC (Psychrotrophic) analysis, only the ice cream with 5% jabuticaba skin extract added presented $2.5x10^3$ CFU.g^{-1}, the other ones had <10 CFU.g^{-1} (Table 4). In Brazil, there is no legislation that defines the maximum limit of psychotropic microorganisms in food, but a low count of psychotropic microorganisms is of fundamental importance for food quality, since the metabolic activity of these microorganisms' results in biochemical changes in the constituents of milk, limiting the shelf life of the products[29].

Table 3. Physicochemical composition of bioactive compounds and antioxidant activity of different concentrations of jabuticaba skin extract added to ice cream.

Parameters	Ice creams*			
	Control	5% Extract	10% Extract	15% Extract
pH	6.8±0.02a	6.55±0.01b	5.97±0.02c	5.65±0.02d
Total soluble solids (ºBrix)	24.7±0.1c	25.6±0.0b	26.5±0.0a	26.5±0.0a
Acidity (%)	0.29±0.00ab	0.21±0.02b	0.29±0.03ab	0.30±0.02a
Fats (%)	13.9±0.25a	12.7±0.11b	12.2±0.07bc	11.7±0.01c
Color (ºHue)	26.0±5.9c	65.3±4.4b	77.5±2.2a	77.4±1.8a
L	81.60±0.7a	68.26±0.3b	53.70±0.6c	45.27±0.5d
a	0.39±0.2d	5.40±0.01c	9.94±0.1b	11.99±0.2a
b	4.10±0.7c	9.33±0.2a	9.70±0.1a	7.29±0.09b
Total anthocyanin (mg cyanidina-3-glycosideo.100g^{-1} skin)	-0.97±0.04d	2.77±0.02c	6.69±0.80b	10.75±1.20a
Total Phenolic Compounds (mg gallic acid.100g^{-1} skin)	103.5±4.2b	111.7±5.3b	170.8±10.1a	188.2±7.3a
EC50 (g skins. g^{-1} DPPH)	104118±236a	36986±243b	11830±139b	7573±125b
TEAC (µM Trolox.g^{-1} skin)	1.49±0.02d	3.1±0.25c	6.67±0.53b	10.51±0.39a

* Means followed by the same small letter in the same line do not statistically differ at 5% probability by Tukey test

Table 4. Microbiological evaluation of ice cream.

Parameters	Ice cream		
	5 % Extract	10 % Extract	15 % Extract
Staphylococcus aureus (CFU.g^{-1})*	1.2 x 10^4	< 100	< 100
Psychrotrophic (CFU.g^{-1})	2.5 x 10^3	< 10	< 10
Total Coliforms a 35 ºC (MPN.g^{-1})**	14	9.2	< 3
Total Coliforms a 45 ºC (MPN.g^{-1})	<3	<3	< 3

*CFU.g-1: Colony Forming Units. **MPN.g^{-1}: Most Probable Number.

DISCUSSION

Jabuticaba skin aqueous extracts B and D presented a value of ºHue higher than the other extracts, which represents a more reddish color. This fact is also due to the higher indices of phenolic compounds retained since no sieving was carried out. Anthocyanin levels were also higher in extracts B and D. The absence of sieving provides a retention of a more significant amount of fibrous material (data not shown) frequently bonded to the matrix of phenolic compounds, which, in contact with the water in the extract, allows the extraction process to continue increasing over time. Consequently, the content of phenolic compounds will be higher in comparison to the extracts with a lower content of fibrous material. Although it has not been analyzed, the non-sieved extract presented a higher fibrous content (soluble and insoluble polysaccharides). Phenolic compounds may be associated with the fibers through covalent bonds with compounds in the cell wall of fruit peels, cellulose, lignin, pectin, and proteins. The soluble fraction of fibers (pectins, beta-glucans, gums, and mucilages) has a high capacity of water retention and gel formation 24 and thus it can retain other soluble compounds such as anthocyanins. In this study, a similar behavior was observed with a higher retention of total phenolic compounds, among them, anthocyanins.

Regarding the antioxidant activity of the extracts of jabuticaba skin, there were significant differences in the analyses of DPPH and TEAC. The DPPH analysis expressed as EC50 indicates the amount of sample needed to reduce the initial concentration of DPPH by 50%. Thus, there was an inversely proportional relationship between the DPPH and TEAC analyses, in which the lower the value of EC50, the higher the antioxidant activity because a lower amount of extract was required to react with DPPH. Although there are higher levels of phenolic compounds in extracts B and D, the antioxidant activity potential was not directly correlated. In the same way that we disbelieve that phenolic compounds may be bound to a fibrous matrix, we believe it may also be limiting the reactions of antioxidant potential *in vitro* (DPPH). Phenolic compounds can be bound to fibrous matrices, thus they may be limiting the reactions of antioxidant potential *in vitro* (DPPH).

We considered that the extracts with the most relevant or high indices would be chosen to be used in the formulation of ice cream. Based on the results obtained, extract B was selected to prepare the ice cream, since it had the highest levels of phenolic compounds and significant antioxidant activity.

The formulations of ice cream with concentrations of 10 and 15% jabuticaba skin extract were not significantly different with respect to ºHue. This parameter is essential to demonstrate that, with the addition of extract in the ice creams, there was an increase in the characteristic color of the fruit, which is a positive factor in the visual aspect of the product.

The ice creams formulated with the highest concentration of jabuticaba skin extract presented the highest levels of phenolic compounds and anthocyanins, and consequently, they had the highest antioxidant activity potential.

The formulation of ice cream with no addition of jabuticaba skin extracts also presented phenolic compounds. This fact can be explained by the presence of phenolic compounds in bovine milk, according to data described by Connell and Fox[30], in studies carried out with bovine, goat, and sheep milk. According to these authors, the phenolic compounds thiophenol, phenol, o-cresol, p-cresol, m-cresol, 2-ethylphenol, 3,4-dimethylphenol, 2-isopropylphenol, thymol, and carvacrol are found in bovine milk.

The ice cream with 15% jabuticaba peel extract added presented the highest antioxidant activity equivalent relative to Trolox of 10.51 $\mu M.g^{-1}$ of peel. This fact is related to the higher content of phenolic compounds and anthocyanins found in this ice cream. The antioxidant potential of phenolic compounds from fruits such as jabuticaba[5] provided a significant increase in these indices in the ice cream. The interaction between food ingredients such as ice cream allows the bioavailability and bioactivity (functional compounds) in this food. The main interactions occur through polyphenols with proteins and polysaccharides and may interfere in the absorption, called indirect effects of diet on intestinal physiology. The interaction between milk proteins and polyphenols increases the bioavailability and the antioxidant potential of the plasma[31].

The pasteurization process to which the ice cream was subjected contributed to a decrease in the content of these compounds in comparison to the content observed in aqueous extracts of jabuticaba peel. Anthocyanins are thermolabile, that is, they are rapidly destroyed by heat[31]. Even so, their values have remained significant. Regarding the type of food used to add jabuticaba peel extract, considering that ice cream is preserved at low temperature, these phenolic compounds, often lost by oxidation and temperature oscillations, are preserved[32]. The polyphenols present in the extract of jabuticaba used as an ingredient in the ice cream, provided an increase of phenolic compounds (functional compounds) and other nutritional compounds and the characteristic color of the fruit (Table 3). According to Shahidi and Naczk[4], the use of natural ingredients allows the increase of nutritional, sensorial and visual characteristics inherent in natural foods.

CONCLUSION

It was possible to elaborate aqueous extracts from jabuticaba skin, with the best condition for its production being a process of grinding for 25 seconds without sieving (Extract B). This extract showed the highest levels of phenolic compounds, which theoretically contributed to a higher antioxidant activity. It also showed high values of anthocyanins, essential phenolic compound for the antioxidant potential.

The use of increasing concentrations of jabuticaba skin extract in the ice cream formulation promoted an increase in the phenolic compound indices and, consequently, in

the antioxidant capacity potential.

The values found in the present study are significant and represent an interesting alternative for the use of jabuticaba skin, which is usually wasted in the consumption of this fruit and is completely natural.

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