



Quantification of the major bioactive phytochemicals in the gabirola (*Campomanesia xanthocarpa* Berg) juice

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ABSTRACT. The juice was evaluated regarding yield, turbidity, physicochemical characteristics and acceptability. In order to check the acceptability, three samples were prepared by adding sucrose until reaching 12, 14 and 16°Brix, respectively, once this is the characteristic range of commercial samples for ready-made juices. The use of enzymes increased the juice yield, which showed a good retention of bioactive compounds. The sample with the highest overall acceptance was that of 14°Brix with an overall average of eight, corresponding to the expression 'I liked it very much', suggesting a high market potential.

Keywords: gabirola, juice, phytochemicals, bioactivity.

Quantificação dos principais fitoquímicos bioativos no suco de gabirola (*Campomanesia xanthocarpa* Berg)

RESUMO. O suco de gabirola foi avaliado em relação à turbidez, ao rendimento, às características físico-químicas e à aceitabilidade. A fim de verificar a aceitabilidade do suco, três amostras foram preparadas por adição de sacarose até atingirem 12, 14 e 16°Brix, respectivamente, porque estas são as características de amostras de sucos comerciais disponíveis no mercado. O uso de enzimas elevou o rendimento de suco, que mostrou boa retenção de compostos bioativos. A amostra com a maior aceitação global foi a de 14°Brix, com média global de oito anos, o que corresponde à expressão 'Eu gostei muito', sugerindo elevado potencial de mercado.

Palavras-chave: gabirola, suco, fitoquímicos, bioatividade.

Introduction

Native tropical fruit is becoming customers' preference, mainly with respect to sensory characteristics such as exotic flavor and aroma. The gabirola is a native fruit of the Myrtaceae family, with high acceptability due to its sensory features. Native fruit has received much attention recently, owing their technological potential, and also because they can be used to diversify the fruit production of a certain region. Although gabirola presents a high potential to be used in food industry, data on its cultivation, production and usability features are scarce. Gabirola trees can be naturally found in Southern Brazil, Argentina, Paraguay and Uruguay (BIAVATTI et al., 2004).

The knowledge of physical-chemical and technological features of gabirola represents a remarkable contribution by stimulating the economy of some regions, improving the familiar agro-industry and providing the market with new

aromas and flavors. The use of gabirola pulp in substitution to the whole fruit is promising because it can be used during off season periods for manufacturing of juices, marmalades, ice creams, baby foods etc. The valuation of native fruit contributes to the rational use of this natural resource, which is still underused and most often lost in the field even before harvesting.

Currently several scientific papers have shown the relationship between diet and health (CRUZ et al., 2007). A new tendency in the consumer market increases the demand for foods with a high nutritional value. This increasing interest in healthy products is one of the factors that have contributed for expanding the consumption of fruit-based juices and beverages in general (BELSCAK et al., 2009).

Therefore, the purpose of the present study was to extract and characterize the juice of the gabirola fruit, aiming at introducing an alternative for the technological use of this raw material and adding value to the fruit. The main objectives were: to

check the possibility of extracting the juice through enzymatic treatment and to check its acceptance by means of a sensory analysis, as well as to characterize the juice in terms of physicochemical and nutritional standards. Thereby attempting to encourage sustainable development combined with utilization and enhancement of regional indigenous fruit, a raw material but also provide an alternative source of income for rural producers, these features are less appreciated, and we believe that the use of these native fruit may open space for farmers in agribusiness due to its economic and social potential.

Material and methods

Plant material

Ripe fruit of gabirola (*Campomanesia xanthocarpa* Berg), Family Myrtaceae, were harvested from native plants in the district of Itaiacoca, near the city of Ponta Grossa (Paraná State).

Assays

Fruit went through cleaning processes so as to select them according to color consistency, size and absence of injuries. Samples were sanitized using a solution of 500 ppm sodium hypochlorite for 15 minutes, followed by successive washings with potable water. Fruit were then crushed and homogenized in a food processor (WALITA MASTER, RI3414, SER0326) thus obtaining the pulp used in the juice extraction. Juice was obtained through enzymatic extraction using pectinolytic enzymes Pectinex Ultra SP-L KRN05508 donated by the company Novozymes. Preliminary tests were conducted prior to juice extraction in order to establish the best extraction conditions, according to the instructions given by the enzyme supplier. Juice extraction using a 2² factor planning with a center point to fix the incubation temperature (50°C) and having as variables enzyme concentration (0.05%, 0.1, and 0.15%) and time (60, 90, and 120 minutes). After extraction, samples were filtered through a 150 µm Brasholanda synthetic filter obtaining then the depectinized juice, which was bottled in transparent glass vials, hermetically closed and pasteurized at 90°C for 5 minutes followed by a cooling process. Juice was evaluated regarding yield, turbidity, physicochemical characteristics and acceptance. The yield was calculated by the percentage ratio of pulp weight per juice weight obtained after filtration. The pH, content of soluble solids, and total acidity were determined according to AOAC (1995).

The gabirola juice was stored for 90 days and monitored monthly for concentrations of ascorbic

acid, total carotenoid, total phenolic and total flavonoid contents.

The turbidity was measured using the method described by Ranganna (1977) and modified by Qin et al. (2005). Total sugars and reducing sugars were measured by spectrophotometric analysis, phenol-sulphuric and DNS methods (3,5-dinitrosalicylic acid), at 490 and 540 nm, respectively (DUBOIS et al., 1956; MILLER, 1959). Vitamin C content was determined by the method proposed by Nejati-Yazdinejad (2007). Total phenolics in the samples were measured by spectrophotometry in the visible region using the Folin Ciocalteu method (SINGLETON; ROSSI, 1965). For the quantification of phenolic compounds, an aliquot of 100 µL of this solution was placed in 5 mL flasks. 1 mL of distilled water was added to the solution and, later, 0.2 mL of the Folin-Ciocalteu's reagent was added. Finally, 0.6 mL of a 20% solution of Na₂CO₃ was added and the volume was completed with ethanol 80% (v v⁻¹). After 60 min., the absorbance was measured at 760 nm. Total phenols content was determined by interpolation of the sample absorbance against a gallic acid standard curve (50 to 500 µg mL⁻¹) and expressed as mg of gallic acid per mL of juice. The calibration curve of gallic acid was expressed by the equation $y = 0.0025x + 0.0802$ with a correlation coefficient of $R^2 = 0.9968$. All analyses were carried out in triplicate.

For the extraction and quantification of carotenoids, it was used the methodology described by Ferreira and Rodriguez-Amaya (2008). Carotenoids content was determined by interpolation of the sample absorbance against a beta-carotene standard curve (Sigma). The equation used for the calculation was $y = 0.2807x + 0.006$ with $R^2 = 0.9954$.

The sensory analysis of the gabirola juice was carried out by affective test using the 9-point structured hedonic scale ranging from 'I disliked it very much' (1) to 'I liked it very much' (9). 52 volunteer members of the university community of both sexes and of all ages participated in the test. In order to check the juice acceptability, three samples were prepared by adding saccharose until reaching 12, 14 and 16°Brix using the content of soluble solids found in commercial samples of ready-made juices available in local markets as a parameter. Averages obtained in the hedonic scale were subjected to an analysis of variance (ANOVA) and Tukey test ($p = 0.05$ of significance).

The project was approved by the Research Ethics Committee of the Health Sciences Sector at Federal University of Paraná and it is duly registered at SISNEP – National System of Ethics in Research # CAAE-0014.0.091.000-10.

Results and discussion

Yields obtained in the preliminary tests on the gabioba juice extraction ranged from 58.56 to 79.95%. The most influencing factor on juice yield was the extraction time. The enzymatic treatment has improved the yield by approximately 43% and enhanced the extraction of phenolic compounds, which resulted in a final product with remarkable sensory aspects, preserving great part of the nutritional characteristics of the fruit. Averages and standard deviations of physicochemical properties for the enzyme-extracted juice and the control sample without added enzymes are shown in Table 1. The enzymatic extraction preserved 86% of flavonoids, about 80% of carotenoids, 95% of total phenolic compounds and 90% of the vitamin C content compared with the content of those substances in the fruit.

Table 1. Physicochemical composition of the enzyme-treated gabioba juice and control samples without enzymatic treatment.

Evaluated parameters	Control sample*	Enzyme-treated juice
pH	3.76 ± 0.04 ^a	3.72 ± 0.02 ^a
Titrate acidity (mg 100 mL ⁻¹ Citric acid)	1.39 ± 0.12 ^a	1.23 ± 0.05 ^b
Soluble solids (g%)	13.77 ± 0.65 ^a	11.97 ± 0.18 ^b
Total sugars (g%)	7.93 ± 0.90 ^a	7.84 ± 0.08 ^a
Reducing sugars (g%)	6.73 ± 0.12	6.59 ± 0.25
Glucose (g%)	2.45 ± 0.12 ^a	2.40 ± 0.12 ^a
Fructose (g%)	4.28 ± 0.41 ^a	4.20 ± 0.23 ^a
Vitamin C (mg 100 mL ⁻¹ AA)	237.20 ± 5.04 ^b	285.50 ± 1.04 ^a
Total phenolic compounds (mg 100 mL ⁻¹ EGA)	111.97 ± 4.87 ^b	125.08 ± 2.46 ^a
Total carotenoids (μg g ⁻¹ Beta-carotene)	195.65 ± 5.16 ^b	233.18 ± 1.23 ^a

Note: EGA = Gallic Acid; EQ = Quercetin, AA = Ascorbic Acid. Averages ± standard deviation. *Without enzymatic treatment. Values in the same column followed by different letters are significantly (p < 0.05) different.

By evaluating the effect of enzyme application on the gabioba juice, it is observed a decrease in pH and total sugar when compared with the fresh pulp. The decrease of pH and the increase of titratable acidity in samples with enzyme can be justified because, according to Sarioglu et al. (2001), the enzymatic treatment increases the galacturonic acid content of the juice. During the process, protopectin

and pectin are degraded into galacturonic acid, increasing thus the content of uronic acid and methanol as well as the total acidity of the product.

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The content of vitamin C observed for the gabioba (*Campomanesia xanthocarpa* Berg) juice was higher than found by Rufino et al. (2010) for Bacuri (*Platonia insignis*) 2.4 ± 0.3, Carnaúba (*Copernicia prunifera*) 78.1 ± 2.6, Cajá (*Spondias mombin*) 26.5 ± 0.5, Jabuticaba (*Myrciaria cauliflora*) 238 ± 2.2 and Jambolão (*Syzygium cumini*) 112 ± 5.8, exotic tropical fruit.

Variations verified in the content of phytochemical compounds during the storage time (90 days) for the juice extracted with 0.15% enzyme and incubated at 50°C for 120 minutes are listed in Table 2.

During storage time, the total loss of vitamin C for the enzyme-extracted gabioba juice was 16.11%, lower than reported by Yamashita et al. (2003), which mentioned a reduction of 32% vitamin C for the acerola juice after four months of storage time. Comparing carotenoids content obtained at zero and 90th day, a decrease of about 18.41% in the carotenoid content was observed. The highest instability of total carotenoids in samples can be associated to the continued light exposure due to the nature of the package – a transparent glass vial.

The total retention of the content of phenolic compounds and flavonoids in the juice during the storage time was 78.38 and 91.78%, respectively. Flavonoids are effective antioxidants owing their properties of scavenging free radicals and chelating metal ions (LUXIMON-RAMMA et al., 2003). Phenolic compounds usually have high redox potentials, which allow them to act as reducing agents with a metal-chelating potential (RICE-EVANS; MILLER, 1996). A high content of phenolic compounds results in a high total antioxidant activity (SUN; HO, 2005). Glucose procyanidins and quercetins are known to be the best antioxidants (SUN; HO, 2005).

Table 2. Average content of ascorbic acid, total carotenoids, total phenolics, and total flavonoids in the gabioba juice.

Storage time (days)	Vitamin C (mg 100 mL ⁻¹ AA)	Beta-carotene (μg mL ⁻¹)	Flavonoids (mg 100 mL ⁻¹ EQ)	Phenolic Compounds (mg 100 mL ⁻¹ EGA)
zero	289.56 ± 1.32 ^a	233.18 ± 3.02 ^a	58.94 ± 0.77 ^a	125.08 ± 2.46 ^a
30	273.21 ± 3.02 ^b	223.14 ± 4.52 ^b	56.40 ± 0.98 ^a	119.69 ± 1.82 ^a
60	251.12 ± 3.02 ^c	203.47 ± 2.32 ^c	55.43 ± 1.01 ^a	109.14 ± 0.95 ^b
90	242.89 ± 3.02 ^d	190.24 ± 4.28 ^d	54.09 ± 0.82 ^a	98.05 ± 1.01 ^c

Note: Juice extracted using 0.15% of enzyme and 120 min. of incubation. EGA = Gallic Acid; EQ = Quercetin, AA = Ascorbic Acid. Averages ± standard deviation. Values in the same column followed by different letters are significantly (p < 0.05) different.

Hertog et al. (1993) determined the quercetin content in some fruit and vegetables and found contents ranging from 32 to 45 mg kg⁻¹ for peas, 30 mg kg⁻¹ for broccoli, 14 mg kg⁻¹ for cabbage and 8mg kg⁻¹ for tomato. Among fruits studied, average quercetin content was 15 mg kg⁻¹, with the highest contents found in apples with values ranging from 21 to 72 mg kg⁻¹. In beverages, such as beer, coffee, instant chocolate mix and white wine, the content was approximately 1 mg L⁻¹. In turn, in the red wine the content ranged from 4 to 16 mg L⁻¹, 7 mg L⁻¹ for lemon juice and 3 mg L⁻¹ for tomato juice. Black tea was the beverage with the highest quercetin content, around 10-25 mg L⁻¹. Gabiroba juice showed a content of 54.08 mg 100 mL⁻¹ after 90 storage days.

Sensory characteristics of a food product play an important role in the overall quality and the acceptance of the product in the market depends on these characteristics (COSTA et al., 2001). Results of the sensory evaluation for the gabiroba juice formulas are illustrated in Figure 1. The gabiroba juice had a good acceptance, since the highest frequency of responses from the raters was between 7 and 8, corresponding to 'I fairly liked it' and 'I liked it very much'.

The Figure 1 shows the profile of the acceptance test, in which the selection has been influenced by the content of soluble solids included in the sample.

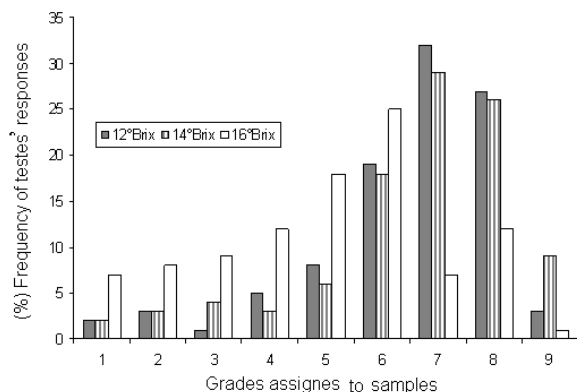


Figure 1. Histogram of results of the gabiroba juice sensory analysis with respect to the frequency of hedonic values.

Evaluating the results of the acceptability test and intention of purchase, no significant statistical difference ($p \leq 0.05$) was observed among the three gabiroba juice formulas. The sample with 14°Brix had the highest overall acceptance, once 83% have evaluated this sample between 'I fairly liked it' and 'I liked it very much'. The sample with the lowest acceptance was that with 16° Brix, presenting 45% acceptability.

As a response to the question about the intention of purchasing the new product, 5% of the raters said they would not buy the product, 10% said they would probably buy the product and 85% said they would buy the product.

Conclusion

The use of enzymes with the purpose of increasing juice extraction was effective. The enzyme-extracted juice showed a good retention of bioactive compounds. The juice also presented a good sensory acceptance.

Physicochemical and nutritional characteristics and the considerable yield of the gabiroba (*Campomanesia xanthocarpa* Berg) juice are indicators of the industrial potential of this fruit. The processing of regional native fruit like gabiroba to obtain byproducts may be a new commercial alternative, besides contributing to the use of this natural resource, which is still underused, and can collaborate for the development of new production standards, which are primarily grounded on extractive alternatives, seeking to combine the preservation of the environment with obtaining new sources of supplementary income for farmers.

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