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Total Phenolics and Total Anthocyanins Found in Grape from Benitaka Cultivar (*Vitis vinifera* L.)

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Abstract: The consumption of fruits and fruit derivates has increased significantly in the last years mainly because of the benefits to health, caused by the protective role of functional compounds when included to human daily diet. Due to the several beneficial effects of the functional compound activity to health as antioxidant, anti-inflammatory and anti-carcinogenic and because of the influence in the sensorial quality of many products, the knowledge on the amount of such compounds and their behavior during the storage period is relevant. In this investigation the total phenolics and total anthocyanins were determined in grape juice from Benitaka cultivar (*Vitis vinifera* L.), during a storage period of 210 days. The statistical analysis of total phenolic parameters and total anthocyanins during the storage period was significant ($p \leq 0.05$). The total phenolics varied from 0.72-1.12 g L^{-1} tannin acid, whereas there was a decrease of total anthocyanins from 3.56-1.43 mg/100 mL grape juice, during the storage period. Several factors contributed for obtaining the contents of phenolic compounds in the present study and the most significant were the cultivar and the methods used for processing the juice. It is relevant to emphasize that, during the storage period; the juices investigated presented contents of significant functional compounds.

Key words: Phenolics, anthocyanins, stability, Benitaka cultivar, *Vitis vinifera*, regression analysis

INTRODUCTION

Phenolic compounds are responsible for the antioxidant activity of several vegetables, thus are potential inhibitors of carcinogens, acting in different stages of the pathological process of initiation of tumors and may be relevant in the prevention of cardiovascular disorders (Blum, 1996), besides bringing sensorial quality to fruits. However, the sensorial quality may be affected during the technological process used in the production of juices and other fruit derivates (Vendramini and Trugo, 2004).

Among fruits and vegetables, grapes are seen as having the largest source of phenolic compounds. Such content may vary according to the species, variety, ripeness stage, climatic conditions and cultivar. In order to process juice, grapes are submitted to several treatments and it is known that the extraction method, the squeezing process, the thermal and enzymatic treatments, among others, influence the quality and the amount of phenolic compounds in the final product (Frankel and Meyer, 1998).

The great diversity of cultivars result in grapes with different characteristics both, in flavor and coloration and that is associated with the content and the profile of polyphones. Knowing the contents of those compounds in grapes is relevant, once they are the raw material for the production of wine and juice and as already mentioned, that influences the quality of processed products (Abe *et al*., 2007).

The most important phenolics that are present in grape are the flavonoids, the estilbens, the phenolic acids and the large variety of tannins (Francis, 2000). Anthocyanins belong to the flavonoid family and are glycosides of anthocyanidin, polyhydroxy and polymethoxy, which derive from 2-phenylbenzopirilium or cation flavilium. They are widely spread in the nature, being responsible for most of the blue, violet, rose, red and purple colors of many fruits, juices, flowers and leaves. They are unstable pigments that may be degraded during juice processing and juice storage (Markakis, 1982). Romero-Pérez *et al*. (1999) reported, that the consumption of grapes and mainly of grape juice has been presenting advantages because of their

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antioxidant compounds. Several studies have already evaluated and reported the positive effect of wine in the human organism. Regarding the juice, the absence of alcohol allows its free consumption by most of the people, including children and bearers of diseases such as hepatitis.

Benitaka variety, originated of the somatic mutation occurred in Italian grape variety was discovered in a farm in the municipal district of Florai, located on the North of Parana State. That variety is known due to the intense development of the dark rosy coloration when still not ripen, in any season of the year. The grape bunches are large, with an average weight of approximately, 400 g and the berries are also big (8-12 g). The pulp is firm, having a neutral flavor and presenting good conservation after being harvested. Such characteristics are responsible for the prominent place Benitaka occupies, once that color grape is the one that has been arousing large interest of local grape producers in the last years (Souza-Leao, 2000).

The present study had as objective to determine the content of phenolics and total anthocyanins found in grape juice processed with grapes belonging to Benitaka cultivar, to evaluate its stability during a 210 days storage period and to present the physicochemical characteristics of the grape.

MATERIALS AND METHODS

To carry out the investigation, grape juice was processed with fresh, ripe grapes belonging to Benitaka cultivar and free from pathogen action, which presented 15°Brix on average and 39.7 total relationship °Brix/acidity.

The juice was processed by using the steam extraction method, with the aid of a steam juicer. In the course of the process, the juice was pasteurized at a temperature of 100°C, for 15 min. Following, it was cooled and poured into glass bottles and then maintained at room temperature during the whole period of investigation. The juice was processed at the Food Biochemistry Laboratory of the State University of Maringa-PR and afterwards, it was sent to the Laboratory of Fruits and Vegetables of the Federal University of Ceara to be analyzed.

The study in relation to the grape juice stability began soon after receiving the product and the analysis were accomplished in intervals of 30 days. Several analysis were carried out: the pH was evaluated through Hanna Instrument pH meter, HI 9321 model, gauged after each use with buffer solutions at 4.0 and 7.0 pH, according to the method described by AOAC (1995); the soluble solids were determined by refractometry making use of N^{-1} Atago refractometer, with scale varying from 0-32° Brix, compensating the reading at 20°C; titrated acidity was determined through titration with a solution of NaOH 100 mM and the results were expressed in percentage of tartaric acid; the color was determined by making use of the methodology described by Rangana (1997); total anthocyanins were determined by Francis (1982) methodology in which a sample of 1 mL was homogenized with a solution of HCl (1.5 M) and with ethanol 85% for proceeding the extraction. After a night rest, under cooling system and total absence of light, the extracts were filtered and the reading, through spectrophotometer (λ = 535 nm) was carried out; total phenolics were determined in agreement with the methodology described by Reicher *et al.* (1981), through spectrophotometry (λ = 760 nm), by using Folin-Denis' reagent, having the tannin acid as pattern.

To carry out the statistical analysis, three bottles were collected (three lots) and each replication was accomplished in duplicate. The results of the analysis, obtained during the 210 days of juice storage, were appraised by using the statistical program of SAS Institute (2006) by using regression analysis. Trend lines were made having as base the values estimated for each model.

RESULTS AND DISCUSSION

Table 1 presents the mean values obtained in analysis such as -pH analysis, soluble solids, total acidity and color during the 210 days storage period.

During the total period studied, aspects such as the soluble solid parameters, the total acidity and the color have not presented significant difference ($p>0.05$). The juice analyzed showed, during the period studied, a mean value of 10.6° Brix, 0.66% of tartaric acid and 0.140 for color. According to Chitarra and Chitarra (2005), the composition of the titrated acidity in grape is on average, 0.5% of the tartaric acid in fresh fruit. The statistical analysis on values obtained for pH was significant in the course of the storage period ($p\leq 0.05$), showing a maximum value of 3.65, in a period of 120 days and a minimum value of 3.19, in a period of 180 days.

The statistical analysis for the total phenolic parameters and total anthocyanins was significant ($p\leq 0.05$) with the time of storage (Table 2), however, it was not possible to adjust the equation for total phenolics; consequently, the distribution of the averages was accomplished.

Contents showed, a variation from 0.72-1.12 g L^{-1} grape juice tannin acid (Fig. 1). It was observed an increment in the content of total phenols up to 120 days of storage and that was the period that presented higher content 1.33 L^{-1} grape juice tannin acid. In that period

Table 1: Mean of pH, Soluble Solids (SS), Titrated Acidity (TA) and color, obtained from the grape juice, in the course of the 210 days storage period

Time (days)	pH	SS (°Brix)	Total acidity (Tartaric acid %)	Color (420 nm)
60	3.41±0.09	10.53±1.13	0.67±0.04	0.134±0.07
90	3.39±0.10	10.69±1.03	0.64±0.06	0.146±0.08
120	3.65±0.07	11.10±0.62	0.66±0.04	0.165±0.05
150	3.39±0.07	10.75±1.75	0.68±0.04	0.129±0.04
180	3.19±0.02	10.33±0.52	0.67±0.04	0.149±0.03
210	3.38±0.02	10.20±0.86	0.65±0.07	0.117±0.03

Mean values obtained with the three replications ±SD. That has differed significantly during the storage period ($p \leq 0.05$)

Table 2: Result of regression analysis for the total phenolic parameters and total anthocyanins of grape juice

		Quadrado médio	
Source of variation	df	Total phenolics	Total anthocyanins
Storage period (time)	5	3035.6133	4.04430
Lineal model	1	3612.6934*	16.2801*
LOF	4	2891.3433*	0.9854^{ns}
Quadratic model	2	3742.7439*	-
LOF	3	2564.1929*	-

Significant at the level of 5% probability ($p \leq 0.05$) ^{ns}: Non-significant at 5% level of probability. df: Degree of freedom

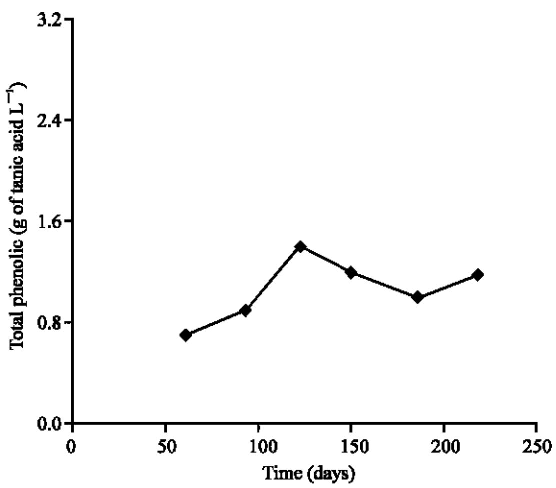

Fig. 1: Mean of total phenol content in grape juice, during a 250 days storage period, at room temperature

of time, the color and the pH have also shown the highest values, thus the behaviors detected may be related to each other. According to Abe *et al.* (2007), more intense the grape coloration, more interesting the functional point of view, once, in his/her study, the grapes with dark coloration showed larger content of phenol compounds and higher antioxidant capacity.

Malacrida and Motta (2006) evaluated the concentration of total phenols in reconstituted and simple grape juices (also named integral juice) of different trademarks available in Belo Horizonte-MG and observed that, in reconstituted grape juices that concentration varied from 0.27-1.32 g L^{-1}, whereas in juices of simple grape there was a variation from 0.60-2.41 g L^{-1}. The researchers above mentioned observed that, the reconstituted grape juice showed inferior mean value if compared to the juice extracted from simple grape. That may have occurred due to the method used for processing the juice. Reconstituted juice was obtained from the dilution of concentrated or dehydrated grape juice till reaching the original concentration of the integral juice (Brazil, 1990), thus the concentration process, that submits the juice to high temperatures, may have caused losses in the compound content during the recovery of aroma and evaporation (Frankel and Meyer, 1998).

The differences in total phenolic mean values are related to the different methods of industrial processing used for producing juice (Sautter *et al.*, 2005). Frankel and Meyer (1998) determined the concentration of total phenolics in commercial grape juices produced from grapes belonging to concord variety and juices made of mixtures with other grape varieties and the averages obtained were 1.79 and 1.47 g L^{-1} gallic acid, respectively. Different samples of grape juice, analyzed by Sautter *et al.* (2005), showed some variations in the averages of total phenolics. The researchers verified the difference of those contents in integral, reprocessed, reconstituted and sweetened juices and also, in the grape nectar produced in several Brazilian states. However, the juices of integral grape showed higher values and varied from 1.6-2.2 g L^{-1} gallic acid. In relation to reprocessed, reconstituted and sweetened juices, there was a variation from 1.5-1.6 and from 0.2-0.9 g L^{-1} gallic acid, respectively. The grape nectar presented a mean value of 1.0 g L^{-1} gallic acid. When, evaluating frozen pulps from different fruits.

Other researchers confirm that the content of phenolic compounds that prevails in products made of grape may depend on relevant factors, the grape variety, the method applied for extracting the compounds and the storage conditions. According to Penna *et al.* (2001), the amount of phenolic compounds vary according to factors such as, climate, soil condition, grape variety, grape ripeness, grape maceration, pH and others. Grapes that are squeezed with husks, peel and seed generate larger amounts of those compounds. In a study accomplished by Baydar *et al.* (2004), on the concentration of total phenolics in grape seeds separately and in pulp together with the grape juice, without seeds, it was verified that the seed showed higher phenolic content (647.92), whereas the husk/juice showed 37.49 mg of gallic acid extract g^{-1}.

Although, vitamin C is considered by some researchers as the largest responsible for the antioxidant activity, the largest responsible for the antioxidant activity in fruits are the phenol compounds. The influence of photochemical compounds in the antioxidant activity, mainly the anthocyanin pigments of wild fruits and frozen fruit pulps. In fruit pulps that hinder the detection of pigments, such as, pineapple, graviola, cupuacu and passion fruit, the antioxidant activity showed smaller values.

Results obtained in the course of the investigation, 0.72-1.33 g L^{-1} tannin acid (Fig. 1), are close to values of total polyphenol found in red wines by several researchers. According to Freitas (2000), red wines produced in the region of Bento Gonçalves-South of Brazil have total polyphenol concentration from 491.4-1722.3 mg L^{-1} for wines of Cabernet cultivar and from 430.3-1992.6 mg L^{-1} for wines of Merlot cultivar; however, for wines of Tannat cultivar the concentration is from 832.9-1932.9 mg L^{-1}. Therefore, juices produced with grapes from Benitaka cultivar have shown to be a good source of polyphenol for consumers that search for a better life quality.

Anthocyanins proved to be very unstable pigments, thus may be degraded through heating during the industrial processing and during the storage period, nevertheless, other factors, such as the pH, exposition to light and to oxygen affect the juice stability. The dark red coloration is a relevant factor in the quality of fruits and fruit derivate. However, the coloration is affected by the total content of anthocyanins and its distribution is affected by other factors: the amount of chloroplasts that store such pigments, the formation of anthocyanin-metal complexes and also by pH (Chitarra and Chitarra, 2005).

Values found at the beginning of this study showed that there was a content decrease, varying from 3.56 mg/100 mL to an average of 1.43 mg/100 mL grape juice, measured at the end of the storage period (Fig. 2).

At the end of the study, a reduction of 59.83% in the content of anthocyanins, in relation to the initial time of storage, was verified. The grape juice studied has undergone a thermal treatment (100°C, 15 min^{-1}) and it was conditioned in glass bottles, what might have contributed to a larger degradation of pigment. Another factor that might have favored the high loss of anthocyanins in the samples would be the pH that varied from 3.19-3.65 during the period studied.

However, Freitas *et al.* (2006), when studying the stability of acerola juice, obtained by juice Hot Fill Process and stored in glass bottles for 350 days, have not observed anthocyanin losses at the end of the storage period. On the other hand, a significant loss of that content was observed already in the aseptic process, what, according to the researchers, might have been caused by a regeneration of juice enzymes.

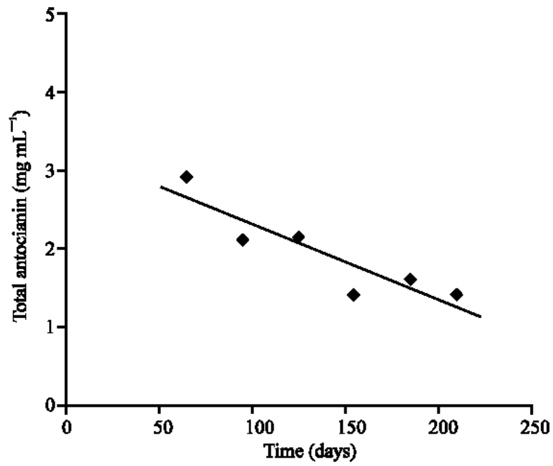

Fig. 2: Average of total anthocyanins in grape juice during the 210 days storage period, at room temperature (Y = 3.25567-0.00956x, R^2 = 0.90178)

Hofsommer (1995) investigated alterations in the anthocyanic constitution of 60 samples of red grape juice during the processing stages. Most of losses were verified during the pasteurization process carried out at 90°C, for 10 min. The decrease in the amount of anthocyanins was close to 50%. Both, the time and the storage temperature lead to changes in the amount of anthocyanins in juice. Larger losses were verified in larger storage time. The decrease in anthocyanin content during the storage period is due to the formation of polymeric pigments, which are less sensitive to pH changes and more resistant to discoloration by Sulfur dioxide (SO_2).

Anthocyanins are promptly destroyed by heating, during the processing and storage of food in general. Many studies showed logarithmic relationship between destruction of anthocyanins and arithmetic increase of temperature. Processes using lower period of time in high temperatures have been recommended for a better retention of pigments. Regarding red fruit juices, anthocyanin losses were insignificant for thermal treatments inferior to 12 min at 100°C (Markakis, 1982).

Malacrida and Motta (2006) evaluated the content of monomer anthocyanins in reconstituted and simple grape juices of different trademarks and obtained a mean content of 17.31 mg L^{-1} for reconstituted grape juice and 28.70 mg L^{-1} for simple juice. Provenzi *et al.* (2006) and Falcao *et al.* (2004) when analyzing the concentration of total anthocyanins in Cabernet Sauvignon grape raw extract obtained a concentration of anthocyanins of 95 and of 237 mg/100 g of grape peel.

Bordô and Jacquez varieties are the main grape varieties, rich in anthocyanic pigments. Such varieties are the most frequently used in the production of juices in Brazil, followed by Concord and Isabel varieties (Rizzon *et al.*, 1998). The contents found in grape juice from Benitaka cultivar are equivalent to the contents of anthocyanins found by several other researchers.

Kirca *et al.* (2006) investigated the stability of carrot anthocyanins added to juices (apple, orange, grape, grapefruit, tangerine and lemon) and to nectars (apricot, peach and pineapple), during heating at 70-90°C and in storage system at 4-37°C. Results showed the great effect of the storage temperature in the anthocyanin stability in all of juices and nectars investigated, thus showing that the faster degradation occurred during storage at 37°C. However, anthocyanins showed smaller stability during heating and storage in orange juice. Regarding heating, anthocyanins found in apple and grape juices and in the apricot and peach nectars, showed larger stability at 70 and 80°C.

CONCLUSION

Several factors contributed for obtaining the contents of phenolic compounds in the present study and the most significant were the cultivar and the methods used for processing the juice. It is relevant to emphasize that, during the storage period, the juices investigated presented contents of significant functional compounds.

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