

Bioactive compounds and antioxidant activity of blueberry toppings with honey

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Abstract

Blueberry (*Vaccinium* spp.) and honey contain several health-enhancing bioactive compounds. In this study we focus on the main phenolic compounds found in blueberries toppings. Three formulations of blueberry toppings were tested to which honey was added to replace sugar, partially or totally. The pH, soluble solids, total acidity, total sugar, color, phenolic compounds, anthocyanins, flavonoids and antioxidant activity of blueberry toppings were analyzed. Losses of 62.34% in phenolic compounds, 95.66% in anthocyanins, 83.38% in flavonoids and 40% in antioxidant activity occurred when compared to contents in fresh blueberry topping. Toppings with honey showed the best results in the phenolic compounds and flavonoids retention and in reduction of the antioxidant activity. An exception was the anthocyanin content of the formulation with honey which showed the lowest loss rate. Pearson's correlation between anthocyanins and flavonoids content was not reported even though high positive co-relationships occurred between the other variables.

Keywords

Anthocyanins

Flavonoids

Honey

Processing

Phenolic compounds

Toppings

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Introduction

Intake of fruits and vegetables has been extensively enhanced for the best diet (Naczki and Shahidi, 2006; Haraszyn and Oledzi, 2014). It is a well-known fact that food affects health by the prevention of diseases and in their treatment (Terry, Terry and Wolk, 2001; Spagnuolo *et al.*, 2012; Tavares *et al.*, 2012). Some time ago such influence was merely attributed to such nutrients as vitamins, fibers and minerals. However, several studies have shown the influence that secondary metabolism-produced compounds have on the human body (Haraszyn and Oledzi, 2014). Since these compounds are actually produced by plants mainly for the protection against external agents, they normally concentrate on the external rind. Although they do not merely contribute towards sensory characteristics (Alasalvar *et al.*, 2001), they are not considered nutrients (Terry, Terry and Wolk, 2001).

Blueberries make up a group of fruits that should be highlighted with regard to quantity and bio-availability of their phenolic compounds. In fact, the blueberry is a highly relevant fruit (Li *et al.*, 2013; Haraszyn and Oledzi, 2014). The blueberry is native to North America and Europe (Reque *et al.*, 2014). The fruit with its blue-purple color and sweet-acid taste is consumed worldwide (Leiva-Valenzuela *et al.*, 2013). The fruit contains high rates of phenolic

compounds, especially phenolic acids, flavonols, anthocyanins and proanthocyanidins (Naczki and Shahidi, 2006).

Several studies relate the intake of the fruit with the prevention of chronic and degenerative diseases, such as diabetes, hypertension, dyslipidemia, obesity, cancer, neurodegenerative diseases and osteoporosis. The therapeutic properties are associated with anti-proliferative, heart-protecting, antioxidant, anti-inflammatory and antiangiogenesis activities (Tiwari and Cummins, 2013; Mohamed, 2014). Still recently, evidence of its activities in delaying cerebral aging has been registered (Yang *et al.*, 2014; Mohamed, 2014).

However, the blueberry is perishable due to the high moisture content, showing only a brief harvest period (Stojanovic and Silva, 2007; Reque *et al.*, 2014). Therefore, blue blueberries in natura have been submitted to several preservation methods to extend their durability. Feasible and low cost alternatives for a visual attractive product consist in the use of the fruit in the preparation of sweets, jellies, juices and also by the addition to desserts, cereals, bread products and in toppings (Stojanovic and Silva, 2007; Yang *et al.*, 2014).

Toppings may have either the entire fruit or standard pieces of the fruit which are immersed in a viscous liquid phase. The fruit should maintain its sensory characteristics without homogenization or

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transference of color, aroma and taste to the food consumed. The formulation is composed basically of water, sugar, thickener and acidulant (Rodrigues *et al.*, 2010).

Honey is a product mainly composed of water and natural sugar, with several bioactive compounds, such as phenolic compounds (Silva *et al.*, 2013; Escriche *et al.*, 2014). Further, honey sugars are mainly fructose and glucose at a ratio of 50:50, with an advantage for intake by diabetic people, since fructose has a much lower glycemic index than glucose. Since honey is also highly antioxidant, it may be compared to the antioxidant activity found in many fruits and vegetables (Gheldof, Engeseth, 2002). To obtain healthier topping with improved sensory properties, the work aimed to replace sugar by honey, and evaluate its physico chemical and phytochemical composition and the antioxidant activity.

Materials and Methods

Chemicals and reagents

The 2,2-diphenyl-1-picrylhydrazyl (DPPH), gallic acid (Fluka, $\geq 98.0\%$) and quercetin (Sigma, $\geq 98.0\%$) were used to analyze the antioxidant capacity, total phenolic compounds and total flavonoids respectively. The xanthan gum (Sigma Aldrich) was used as the thickening agent and citric acid (Synth) as the acidulating agent. All the other reagents were of the highest analytic purity for spectrophotometry degree.

Samples

Blueberries were obtained from a farm in the city of Morro Redondo (state of Rio Grande do Sul, Brazil) and frozen until the analyzes. Fruits were selected according to their maturation degree and integrity stage and after were washed with a solution of sodium hypochloride at 150 ppm. Honey was obtained from wild botanical origins of Pedro Osório, RS, Brazil. The honey samples were processed in October 2013 and stored in a dry and dark place at a room temperature

Processing of blueberry toppings

Toppings were prepared following an adapted formulation of Rodrigues, Rodrigues and Vendruscolo (2010). Three formulations of blueberry toppings were prepared (Table 1): one containing only sugar (T.S.); other containing sugar and honey (1:1) (T.SH); and another containing only honey (T.H).

A solution of sugar and / or honey, water and xanthan was initially prepared with 50% of soluble

Table 1. Formulations of blueberries toppings

Ingredients	TS	TSH	TH
Sugar	150g	75g	-
Honey	-	75g	150g
Water	150mL	150mL	150mL
Xanthan*	0.5%	0.5%	0.5%
Blueberry	150g	150g	150g
Citric acid**	0.08%	0.08%	0.08%

TS (Topping with sugar), TSH (Topping with sugar and honey), TH (Topping with honey). Reducing sugars (% in glucose). Total sugars (% in sucrose). *Calculated by the product's final weight. **based on final product (m/m).

solid. After blueberry and citric acid were added at known concentrations (Table 1), heated (180°C) and homogenized. The product is packed in glass bottles, followed by heat treatment at 100°C for 15 min. The blueberry toppings were stored for 48 hours to occur the product stabilization.

Physico chemical determinations

The pH was determined by potentiometry at room temperature (AOAC, 2002). Soluble solids were determined by a refractometer (Analytikjena), at 20°C, expressing the results in °Brix (AOAC, 2002). Total acidity was determined by the titration of the sample with sodium hydroxide 0.1N, using a digital potentiometer up to pH 8.1. The results were expressed in percentage (AOAC, 2002).

The color was measured by the colorimeter Minolta (Data Processor DP-301 for chroma meter CR-300 series – Japan), with light source D 65 and 8 mm aperture at standard C.I.E. $L^* a^* b^*$. Colorimeter was calibrated by a standard white plate, according to manufacturer's instructions. Rates a^* and b^* were employed to calculate angle Hue ($^{\circ}h = \arctg(b/a)$) and chrome ($((a^2+b^2)^{1/2})$). Samples were placed in 5 cm diameter and 2 cm height petri dishes and readings were done in quadruplicate.

Total sugars were determined by the method described by DuBois *et al.* (1956). The color intensity formed after sugar degradation by strong acid and/or high temperatures was measured in a spectrophotometer at 415 nm. Reducing sugars were determined following methodology described by Miller *et al.* (1959), by reading the samples at 540 nm. The reddish solution formed was due to the monoamine produced by the oxidation of the aldehyde group of reducing sugars and the simultaneous reduction of 3,5-dinitrosalicylic acid (DNS) in acid 3-amino, 5-nitrosalicylic acid. The both results were expressed in mg of glucose.100g⁻¹ of the blueberry topping.

Table 2. Physico chemical parameters of fresh blueberry and blueberry topping

Samples	pH	TA (%)	Reducing sugars	Total sugars
Blueberry	3.34 C	0.13 A	2.13 d	11.32 c
T S	3.71 A	0.04 B	2.74 c	36.1 b
T SH	3.5 B	0.05 B	5.36 b	46.06 ab
T H	3.47 B	0.06 B	7.03 a	56.07 a

TA (titratable acidity), SS (soluble solids), TS (Topping with sugar), TSH (Topping with sugar and honey), TH (Topping with honey). Reducing sugars (% glucose). Total sugars (% sucrose). Means with different letters in the same column are statistically significant ($p \leq 0.05$) by Tukey's test.

Phenolic compounds content

The total phenolic content was measured according to the method of Swain and Hillis (1959). Two g of sample was diluted with methanol (15 mL) and of homogenized. The phenol extract was centrifuged for 30 minutes at 7000 rpm and the supernatant was collected. For the quantification step, 250 μ L of phenol extract was removed and mixed with 4 mL of ultrapure water.

Then 250 μ L of this phenol extract was mixed with 250 μ L of 0.25 N Folin-Ciocalteu reagent and homogenized for 3 minutes. After it was added 0.5 mL of 1N of sodium carbonate (Na_2CO_3). After incubation in the dark at room temperature for 2 h, the absorbance was measured at 725 nm in a spectrophotometer (Jenway 6705 UV / Vis). Calibration curve was defined by known concentrations of gallic acid, between 0.00 and 0.20 $\text{mg}\cdot\text{mL}^{-1}$ ($R^2=0,9947$) and the results were expressed in milligrams of gallic acid equivalents ($\text{mg GAE}\cdot 100\text{g}^{-1}$ of sample dry base).

Anthocyanin Content

Total anthocyanin content was determined according to Lee and Francis (1972) with few modifications. One g of sample was added to 25 mL of HCl ethanolic solution (pH 1.0) and allowed to stand for 1 h, stirring every 5 minutes. After, the anthocyanin extract was filtrated and transferred to a 50 mL volumetric flask that was filled with HCl ethanolic solution (pH 1.0). The measurement was done using a spectrophotometer (Jenway 6705 UV/ Vis.) at 520 nm. The anthocyanin measurement was based on the molar extinction coefficient of the cyanidin-3-glucoside (Eq. 1) and the anthocyanins concentration was based on the Beer's Law, expressing the results in milligrams equivalent to cyanidin-3-glucoside ($\text{mg EC}\cdot 100\text{g}^{-1}$ d.b.).

Table 3. Coloring of fresh blueberry and blueberry toppings

Samples	L*	a*	b*	Hue Angle	Chroma
Blueberry	21.75 A	13.59 a	-2.10 b	351.19 a	13.75 b
T S	21.58 A	15.34 c	-2.65 b	349.59 ab	15.61 a
T SH	22.52 A	15.73 c	-1.78 a	353.17 a	15.86 a
T H	19.46 B	12.06 b	-4.14 c	341.05 b	12.75 b

TA (titratable acidity), SS (soluble solids), TS (Topping with sugar), TSH (Topping with sugar and honey), TH (Topping with honey). Means with different letters in the same column are statistically significant ($p \leq 0.05$) by Tukey's test.

$$A = \epsilon \times C \times l \quad (1)$$

where: A= absorbance; ϵ = molar absorption coefficient; C= concentration mol/L; l = path length in cm.

Flavonoid content

Total flavonoid content was assessed by the spectrophotometric method, following the methodology described by Arvouret-Grand *et al.* (1994). Sample solution was prepared by mixing 2 g of sample with 25 mL of methanol 50% and filtered through quantitative filter.

Briefly, 5 mL of aluminum chloride solution (AlCl_3) at 2% with methanol were mixed with the same volume of sample solution (250 μ L sample solution + 4750 μ L of methanol 50%). The absorbance was measured at 415 nm after ten minutes of incubation at room temperature. Standard curve was defined by known concentrations of quercetin, between 0 and 40 $\text{mg}\cdot\text{L}^{-1}$ (R^2 0.9989) and expressed in milligrams of quercetin equivalents ($\text{mg QE}\cdot 100\text{g}^{-1}$ d.b.)

Antioxidant activity

The DPPH radical scavenging activity was determined by the method described by Simirgiotis *et al.* (2013), with few modifications. The appropriate aliquots of solutions prepared with 500 μ L of sample (0.001 and 0.02 $\text{g}\cdot\text{mL}^{-1}$ methanol) and 2 mL of DPPH solution (0.1 mM) were transferred to a flask, homogenized and allowed to stand for 30 minutes. The absorbance was determined at 517 nm in a spectrophotometer. The DPPH scanning effect was calculated as the percentage of DPPH discoloration by using the following equation 2:

$$AA\% = 100 - \frac{(\text{Abs Sample} - \text{Abs Control}) \times 100}{\text{Abs Control}} \quad (2)$$

The results were expressed as percentage of inhibition of 50%, calculated from a calibration curve for each sample, with the percentage of antioxidant activity versus sample extract concentration. The linear regression equation was applied to calculate the concentration capable to inhibit 50% (IC₅₀) of the free radical DPPH.

Statistical analysis

All assays were performed in triplicate and the results underwent analysis of variance. Tukey's test at 5% significance level was employed for comparing means, following procedures of Statistical Analyses System (SAS). Pearson's co-relations were used to determine the association between content of phenolic compounds, anthocyanins, flavonoids and antioxidant activity.

Results and Discussion

Physico chemical determinations

The use of toppings in a great variety of products is highly attractive. Toppings are used as eye-catchers for decoration and taste. A recent study provided the implications that toppings have in the selection of products by consumers; the authors described that there was an increase in perception by consumers when toppings were added to food. In fact, the products' calorie rates are underestimated when they are embellished by fruit toppings, such as berries (Jiang and Lei, 2014).

The physico chemical parameters are important tools for the selection of the process's best conditions. The pH, acidity and soluble solid contents did not change in the different topping formulations and showed the average contents of 3.56, 0.05 and 38.95 respectively (Table 2). The highest variation occurred between the fresh fruit and the processed product. The blueberry was characterized by pH 3.34, acidity 0.13% and soluble solids of 12°Brix. Contents of soluble solids in fresh fruit was equivalent to ones registered by Leiva-Velenzuela *et al.* (2013) who reported contents varying between 6.0 and 18.5 °Brix for commercial highbush blueberry.

Beaudry (1992) suggested that blueberries used for consumption or for the preparation of products should contain at least 10% of soluble solids, between 0.3 and 13% of titratable acidity and pH between 2.25 and 4.25. Therefore, the blueberry cv. Bluegem used in the topping was within these standards. Phenol-sulfuric spectrophotometric tests were employed to determine in a quick, simple and reproductive method the total sugar content in blueberry toppings. Blueberry provided 11.32% of total sugars. The

amount of soluble solids in honey may be reproduced in the total sugar content since the honey composition in solids is made up of carbohydrates.

Honey has a maximum of 10% sucrose and 21% of moisture in its composition. The other percentages comprise glucose, fructose and traces of other compounds (BRASIL, 2000). As expected, toppings with honey showed high rates of reducing sugars. Color is a physical peculiarity which is immediately perceived by the consumer and is highly useful for quality classification in several foods (Boussaid *et al.*, 2014). Table 3 shows the color parameters of the three formulations of the blueberry topping and the fresh blueberry. The colorimeter employed is based on measurements of luminosity index scale (L^*) which vary between 0 (black) and 100 (white), red ($+a^*$) or green ($-a^*$) degree and yellow ($+b^*$) or blue ($-b^*$) degree.

Maturation and maturing phases of blueberry occur according to physiological changes such as changes in color, tissue softening and changes in phytochemical contents. There is an increase in anthocyanin content during the maturing phase, whereas the contents of flavonoids and hydroxycinnamic acids decrease. Consequently, the blueberry turns blue during maturation (Reque *et al.*, 2014).

A brilliant red-purple coloring in the fruit and in the toppings is desirable in products with blueberry (Mohideen *et al.*, 2014). A dark red color is also related with anthocyanins content. It is indicated by high rates of a^* , as observed in the formulations with sugar (TS). The more negative rates of b^* indicate bluish tones, which was observed in the formulation with honey (TH). On the other hand, a decrease of L^* rates is also associated with reaction to darkening, as in hydroxymethylfurfural (Tiwari *et al.*, 2009; Aguiló-Aguayo *et al.*, 2009), similar to formulation with honey (TH).

The color attribute or hue angle describes the visual sensation of color perceived by the human eye. The lowest color angle rate represents a more bluish tone, whereas higher rates, with a maximum of 360, tend to be reddish (Yemmireddy *et al.*, 2013). Topping with sugar and honey increased the hue angle at a higher rate than that of the fresh blueberry. According to Rein and Heinonen (2004), the above may be attributed to the degradation of phenolic compounds during the process. Toppings with sugars (TS and TSH) had higher chromaticity rates which indicated color saturation. Consequently, higher rates suggest greater intensity coloration.

Chromaticity rates reported in current analysis were higher than those reported by Yemmireddy *et*

Table 4. Contents of bioactive compounds and antioxidant activity in fresh blueberry and blueberry toppings

Samples	Phenolic compounds (mg GAE.100g ⁻¹)	Flavonoids (mg QE.100g ⁻¹)	Anthocyanins (mg cyanidin 3-glucoside.100g ⁻¹)	Antioxidant activity IC50 (µg.g ⁻¹)
Blueberry	1529.13a	264.05a	370.32a	2.98c
T S	621.55b	47.86b	19.44b	16.41a
T SH	469.88c	33.80c	14.21c	14.75a
T H	635.97b	49.97b	14.55c	12.33b

TS : topping with sugar; TSH: topping with sugar and honey; TH: topping with honey. Means with different letters in the same column are statistically significant ($p \leq 0.05$) by Tukey's test. GAE: Gallic acid equivalents. QE: Quercetin equivalents. IC₅₀: Concentration capable of inhibiting 50% of free radicals DPPH

al. (2013) in dehydrated blueberry, 4.10; and they were lower than those found in blueberry extracts plus gum, registered by Jiménez-Aguilar *et al.* (2011), 41.89. The above demonstrates that even with significant differences between the attributes, they do not de-characterize the coloration of the fresh blueberry.

Bioactive compounds

Significant differences between the topping with sugar and honey (T.SH) and topping with sugar (T.S.) and topping with honey (T.H.) occurred in total contents of phenolic compounds (Table 4). Toppings with honey (T.H.) and sugar (TS) showed the highest concentration of phenolic compounds and significantly differed from topping with sugar and honey (T.SH), with a rate of phenolic compounds of 469.88 mg gallic acid.100g⁻¹.

According to earlier work by our research group, the honey used in the preparation of toppings was previously analyzed for total content of phenolic compounds and antioxidant capacity by DDPH method, when was observed 66.98 mg GAE.100g⁻¹ honey and antioxidant capacity of 11.88 mg QE.100g⁻¹ of honey. From the total content of phenolic compounds in blueberry (1529.13 mg GAE.100g⁻¹) 24.20% were anthocyanin-derived compounds, whereas 17.2% were other classes of flavonoids.

Average percentages, 37.66%, 4.34% and 16.62%, respectively for phenolic compounds, anthocyanins and flavonoids in the toppings were observed when compared to contents of phenolic compounds in the blueberry fruit. It should be pointed out that different ingredients and water were added to the toppings which were also thermally treated. However, although there were great amounts of anthocyanins in the blueberry, the phenolic compounds had the lowest quantity in the toppings and thus the highest losses. It is a well-known fact

that anthocyanins are highly unstable bioactive compounds on thermal treatment, and they comprise the majority of the natural pigments. Typically, these compounds are most stable under acidic conditions, but they may degrade by any mechanism leading to the formation of dark and / or insoluble compounds (Min-Sheng and Silva, 2006). On the other hand, total content of phenolic compounds showed the lowest loss perhaps due to the fact that blueberries have not only flavonoids but also tannins and phenolic acids.

Phenolic acids rarely occur in a free form and they are usually associated with other types of compounds. When they appear in a simple form, it is due to some process such as microbiological contamination or technological transformation (Cotê *et al.*, 2010). The above may partially explain the low loss of phenolic compounds in the toppings.

However, the low loss of phenolic compounds may also be associated to another factor. According to Giovanelli *et al.* (2013), the method used in the determination of total contents of phenolic compounds is also reactive for partially degraded phenolic compounds and in products of Maillard's reaction. In fact, they may be present (according to the color test) and, consequently, the contents of the phenolic compounds may have been super-estimated.

On an average, topping formulations showed approximately 20% of flavonoid contents in the fruit. Topping with honey (T.H.) showed the highest flavonoid content (49.97 mgQE.100g⁻¹ d.b.) in the three topping formulations, which may be due to the sum of the flavonoid contents in honey and blueberry. In fact, honey has a considerable amount of flavonoids (Pyrzyska and Biesaga, 2009). Flavonoids and other phenolic compounds have been used as markers to identify and authenticate the origin of honey flowering. It is a well-known fact that honey qualities may vary according to flowerings, nectar harvesting and environmental factors, even though they are not

Table 5. Pearson's correlation coefficients between total phenolic compounds, antioxidant activity, total anthocyanins and total flavonoids

	TFC	AA	TA	TF
Total phenolic compounds		-0.95625	0.94964	0.99529
		**	**	**
Antioxidant activity			-0.90322	-0.96604
			**	**
Total anthocyanins				0.94431
Total flavonoids				

Pearson's correlations between total phenolic compounds (TFC), antioxidant activity (AA), total anthocyanins (TA) and total flavonoids (TF).

a95% confidence interval

* significant at $p \leq 0.05$

** significant at $p \leq 0.001$

sufficient to change the quantity of flavonoids and phenolic compounds in the type of honey (Escriche *et al.*, 2014). In their study with long-term usage of honey, sucrose and sweetener and their effects on rats' memory and anxiety, the authors reported that symptoms of anxiety were lower in subjects treated with honey. The rats also had a better spatial memory throughout the 12 months of diet. The above result is related to the fact that honey is a source of phytochemical compounds with antioxidant activity in the organism and to a low glycemic index than that of sucrose (Chepulis *et al.*, 2009).

There was also observed a significant difference in the rate of total anthocyanins between the formulations of toppings. The topping with sugar (T.S.) differed significantly from the others, with high anthocyanin content. Fruits that contains anthocyanins and which are used in food processing showed that the compounds when submitted to temperatures above 25°C (room temperate) undergo high degradation and even severe degradation when pH is increased (above 7) (Castañeda-Ovando *et al.*, 2009).

High temperatures during processing (100°C) may have caused a significant loss of the compounds since anthocyanins are sensitive to light, high temperatures and oxygen (Reque *et al.*, 2014). In fruits with high anthocyanin contents, such as the blueberry, the tannins are bonded to anthocyanins and may form co-polymers (Shahidi and Naczka, 2004). This may be the reason for the reduction of these compounds in the analyzed toppings, since the tannin analysis was not performed in this study.

According to Stojanovic and Silva (2007), citric acid is an acidulant that acts in synergy with phenolic compounds and also as an antioxidant. Results showed that the use of xanthan gum associated with citric acid made possible the development of blueberry topping

with a high retention of anthocyanins.

Antioxidant capacity

The sequester method of the radical DPPH, given in IC_{50} , refers to the concentration of the extract which is capable of inhibiting 50% of the free radicals. Therefore, the higher the antioxidant activity of the fruit, the less is the IC_{50} value. Results showed that the fruit had the lowest IC_{50} (2.98 $\mu\text{g}\cdot\text{mL}^{-1}$ d.b.) and, consequently, the highest antioxidant capacity of the blueberry. Data from literature also showed the same (Vrhovsek *et al.*, 2012; Reque *et al.*, 2014).

The toppings with honey (T.H.) showed the highest antioxidant capacity (12.33 $\mu\text{g}\cdot\text{mL}^{-1}$) (Table 3) and differed significantly from the others. There were no significant differences between topping with sugar and honey (T.SH) and topping with sugar (T.S), even though topping with sugar and honey (T.SH) showed a higher rate than topping with sugar (T.S.). The highest activity occurred probably due to the presence of the honey, which one contain several phytochemicals that may act as antioxidant (Weston, 2000).

Phenolic acids and flavonoids are some of the main causes of the qualities attributed to honey (Estevinho *et al.*, 2008). Estevinho *et al.* (2008) employed DPPH and reported EC_{50} of 27.24 $\text{mg}\cdot\text{mL}^{-1}$ for dark color honey and 68.17 $\text{mg}\cdot\text{mL}^{-1}$ for light colored honey, from Portugal. Slight increases in IC_{50} values for toppings occurred, or rather, they had a lower antioxidant capacity, with a mean difference of 40% when compared to the fruit.

Antioxidant activity had the lowest difference with blueberry among the determinations carried out with toppings. It showed the presence of compounds that affected the antioxidant capacity but which were not analyzed in current study. Processing and high temperatures could break the cells of the fruit and

release the juice. The juice, honey, sugar, xanthan, citric acid and water, could be the factors that affected DPPH rates since phytochemicals present were diluted.

In their study on osmo-dehydrated blueberries, Giovanelli, Brambilla and Sinelli (2013) reported 50% losses in antioxidant activity after processing. The loss was higher than that reported in the determination of total anthocyanin and flavonoid contents. Contrastingly, current analysis showed that the greatest losses occurred in the contents of the phytochemicals under analysis (phenolic compounds, flavonoids and anthocyanins). On the other hand, thermal treatment may have increased the antioxidant capacity of honey which would be beneficent to humans by the products generated by Maillard's reaction (Turkmen *et al.*, 2006).

Few studies deal with the effects of thermal treatments on the antioxidant properties of processed blueberry. Up to the present, studies on the thermal treatment with blueberry have been focused on dehydration or on osmotic dehydration coupled to artificial drying processes (Lohachoompol *et al.*, 2004; Macgregor *et al.*, 2005; Giovanelli *et al.*, 2013). As reported in current analysis, there was also greater loss of osmosis-dehydrated blueberry in compounds of anthocyanins origin (43%) than in loss rate (25%) of the phenolic compounds (Giovanelli *et al.*, 2013).

Study on co-relationships

Possible co-relationships between the evaluated parameters were assessed by Pearson's correlation coefficients (Table 5). Among the possible co-relationships among the variables, only the co-relation between the contents of anthocyanins and flavonoids was not reported. High positive co-relationships were registered between the contents of the phenolic compounds and the contents of anthocyanins and flavonoids. Highly negative co-relationships were reported between the antioxidant activity and the contents of anthocyanins, flavonoids and phenolic compounds.

The analysis of antioxidant activity was given in EC_{50} , which means the concentration that inhibits 50% of free radicals. The lower the EC_{50} , the higher will be its capacity to sequester free radicals. Consequently, a strong negative co-relationship occurred. It may be perceived that the high antioxidant capacity of the blueberry is due to the contents of its bioactive compounds.

Many studies report that the phenolic compounds which infer the fruit's antioxidant activity do not have good stability at high temperatures (Hamama and Nawar, 1991). Temperatures above 60°C are

not recommended due to loss in antioxidant activity (Min-Sheng and Silva, 2006). Current analysis showed that, since topping is a product made from the whole fruit, part of these compounds still persists. In fact, the rind of the blueberry has a great amount of phenolic compounds and, consequently, also preserves antioxidant activities.

Conclusions

The topping with the addition of honey was the one that showed the best results concerning the phytochemical content and antioxidant capacity, which could be explained by the presence of the both honey and blueberry in the formulation.

Functional food is highly promising and further research should be undertaken to underscore the development and commercialization of products that improve health, such as blueberry and honey. In fact, they are a source of new discoveries and improvements on the food market. Adaptations in topping processing are also recommended to lessen the loss of the blueberries phytochemicals which are its main beneficent health factors.

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