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Microwave-assisted extraction of bioactive compounds from blueberry (*Vaccinium ashei* Reade) and their antioxidant and antimicrobial capacity

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Abstract

The aim of this study was to evaluate the content of phenolic compounds, flavonoids and total anthocyanins, as well as the antioxidant and antimicrobial activity of blueberry extracts (*Vaccinium ashei* Reade) of the Climax variety. The extracts were obtained by focused microwave extraction at different temperatures (30, 40, 50 and 60°C) and solvent concentrations (60% and 80% hydroethanolic). The antioxidant activity of the extracts was quantified by the DPPH radical sequestration method, inhibition of auto-oxidation of the β-carotene system, ABTS+, FRAP and IC50. The antimicrobial activity was evaluated by the disk diffusion method. The antioxidant activity (FRAP, ABTS+ and DPPH), total phenolics and total anthocyanins showed a positive linear trend in relation to the extraction temperature, with no significant difference between the solvent concentrations (60 and 80%). The IC_{50} and β-carotene showed the greatest inhibition at 60°C at solvent concentrations of 60% and 80%, respectively. The flavonoids showed no significant difference in terms of the extraction temperature or the concentration of solvent that was used. No antimicrobial activity was detected in the extracts in relation to the tested microorganisms. The results showed a positive correlation between the total phenolic content and the determined antioxidant activities. This study confirms the potential of this fruit as a source of phenolic compounds with high antioxidant activity and it also provides a new and efficient technique to extract bioactive compounds using microwaves.

Introduction

Chemical preservatives are commonly added to foods to enhance microbiological food safety. However, consumers are increasingly concerned about the potential harmful effects of chemical preservatives and prefer foods containing no chemical preservatives (Wu *et al*., 2008; Marta *et al.*, 2012). Berries are rich in phenolics and have been reported to protect against cancer and cardiovascular diseases (Almeida *et al.*, 2006; Serafini *et al*., 2009).

Blueberries have been commercially produced for many years in North America, and nowadays blueberries are cultivated worldwide. Many studies reported that blueberries contain health-promoting compounds including phenolic acids and flavonoids, such as chlorogenic acid, ellagic acid, quercetin, and quercetin-3-galactoside (Talcott and Lee, 2002; McDougall *et al.*, 2005; Michelle *et al*., 2011). Natural antioxidants are an alternative to prevent the oxidative deterioration of foods, minimizing the oxidative damage that these compounds cause in humans (Melo and Guerra, 2002).

Several extraction techniques are widely used for the isolation of bioactive substances from natural substances. However, these techniques are generally time-consuming and must be carefully controlled because they are likely to cause degradation or unwanted chemical changes in products (Cravotto *et al*., 2008). Microwave-assisted extraction has been reported to improve the extraction efficiency of trace compounds in foods. It is recognized as a highly efficient technique because it reduces the extraction time and increases the quality of extracts (Cravotto *et al*., 2008).

This study investigated the extraction of bioactive compounds from blueberries using focused microwave with different concentrations of solvent and extraction temperatures to develop the use of blueberries as antioxidants in food.

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Materials and Methods

Raw materials

The blueberry samples (*Vaccinium ashei* Reade) were of the Climax variety and were acquired from the Vale do Dourado Orchard located in the municipality of Erechim (RS), Brazil, where the fruits are grown through an organic production system.

The selected, cleaned and sanitized fruits were dehydrated in an oven with forced air circulation at 50°C for 48 hours. The fruits were then ground in an analytical mill cooled to 4°C (Quimis, Q298A21 model, Brazil) with the aid of an ultrasonic thermostatic bath (Solab, SL-152/10 model) and then stored in amber bottles at -18°C, to obtain the extracts.

Obtaining the extracts – microwave-assisted focused extraction

For the microwave-assisted focused extraction the procedures for the digestion of samples described by Costa *et al.* (2006) were used, with modifications. The extraction time was set at 20 minutes in accordance with Viera (2012). The extractions were performed in a microwave oven with two cavities equipped with glass bottles with a maximum capacity of 180 mL (Star System 2, 800W, CEM, Matthews, N.C., USA).

The milled samples were initially weighed and transferred to the glass jars. The solvent 1:10 (w/v) was added and subjected to heating by microwave radiation for 20 minutes. Eight treatments were performed in three replications, varying the solvent concentration (60% and 80% hydroethanolic) and extraction temperature (30ºC, 40ºC, 50ºC and 60ºC).

After the extraction, the extracts were centrifuged at 3000 rpm for 20 minutes and filtered. The supernatants were then placed in amber vials and stored in a freezer (-18°C) until analysis. At the end of each extraction the apparatus was decontaminated with 10 mL of PA alcohol for 10 minutes.

Determination of total phenolic content (TPC)

The total phenolic content in the extracts was determined by the Folin–Ciocalteu method Roesler (2007). Briefly, 200 µL of extract was mixed with 1000 µL of 1:10 diluted Folin–Ciocalteau reagent. The solutions were mixed thoroughly and incubated at room temperature (27ºC) for 5 min. After incubation, 800 µL of 7.5% sodium carbonate (Na2CO3) solution was added and again incubated in a water bath at 50°C for 5 min. The absorbance of the reaction mixtures was measured at 765 nm using a UV– Vis spectrophotometer (Biospectro, model: SP - 220). The absorbance of the extract was compared with a gallic acid standard curve for estimating the concentration of TPC in the sample. The TPC was expressed as mg of gallic acid equivalents (GAE) per gram of powder on a dry weight (DW) basis.

Determination of flavonoid content

The total flavonoid content was determined using the colorimetric assay developed by Zhishen et al. (1999). A known volume (0.5 mL) of the extract was added to a test tube and at zero time, 150 µL of 5% NaNO2 was added. After 5 min, 150 µL of 10% AlCl3 was added and after 6min, 1 mL of 1 M NaOH was added, followed by the addition of 1.2 mL of distilled water. Absorbance at 510 nm was used for UV/Vis spectra recording, using an spectrophotometer (Biospectro, model: SP - 220). The absorbance of the extract was compared with a quercetin standard curve for estimating the concentration of flavonoid content in the sample. The flavonoid content was expressed as mg of quercetin equivalents (QE) per gram of powder on a dry weight (DW) basis.

Determination of total anthocyanin content

The anthocyanin content was determined by the pH-differential method (Giusti and Wrolstad, 2001). Each extract (0.5 mL) was separately diluted with 2.5 mL of 0.025M potassium chloride buffer, pH 1.0 and 0.4M sodium acetate buffer, pH 4.5. The diluted solutions were then left at room temperature for 15 min and the absorbance of each dilution was read at 520 and 700 nm against a blank cell filled with distilled water. The anthocyanin content was calculated using the following equation:

$$\text{Anthocyaninscontent}_{(mg/100gofdrymatter)} = A \times MW \times DF \div (\varepsilon \times W)$$

Where: A = absorbance $(A_{520nm} - A_{700nm})_{pH1.0}$ - $(A_{520nm} - A_{700nm})$pH4.5, MW= molecular weight of cyanidin-3-glucoside ($C_{15}H_{11}O_6$, 449.2), DF = dilution factor, ε =molar absorptivity (26900), and W= sample weight (g).

In vitro *antioxidant assays*

DPPH assay

The radical scavenging activity of the extracts in relation to the DPPH radical was measured using the method of Brand-Williams *et al.* (1995), modified as follows: an aliquot (0.5 mL) of methanolic solution containing different concentrations was added to 2.5 mL of methanol DPPH solution (0.1 mM). The mixture was shaken gently and left to stand at room temperature in the dark for 30 min. Thereafter, the

absorbance was read at 515 nm. The scavenging activity was measured as the decrease in absorbance of the samples in comparison withthe DPPH standard solution. The results were expressed as radical scavenging activity percentage (%) of the DPPH radical according to the following formula:

$$\%DPHH_{radicalscavenging}=[(A_0-A_s)\div A_0]\times 100$$

Where A_0 is the absorbance of the control and A_s is the absorbance of the sample. The effective concentration had 50% radical inhibition activity (IC_{50}), expressed as mg extract/ mL, which was determined from the graph of the free radical scavenging activity (%) against the extract concentration.

Ferric reducing antioxidant power (FRAP)

The FRAP method was performed according to Benzie and Strain (1996), with modifications proposed by Pulido *et al.* (2000). In this assay, 3.6 mL of FRAP reagent (0.3 M, pH 3.6 acetate buffer, 10mM TPTZ and 20mM ferric chloride) were mixed with 200 µL of extract diluted in distilled water and then incubated for 30 min at 37ºC. The FRAP solution was used as reference reagent and the absorbance was read at 593 nm. The results were expressed in µmol of trolox equivalents per gram of powder on a dry weight (DW) basis (µmol TE/g^{-1}).

$ABTS^+$ method

The ABTS method was performed out according to the methodology described by Re *et al.* (1999), with modifications. The ABTS radical was formed from the reaction of 140 mM potassium persulfate with 7 Mm ABTS stock solution, kept in the dark and at room temperature for 16 h. For the analysis, the ABTS radical was diluted in distilled water until a solution with absorbance of 700 nm ± 0.02 nm at 734 nm was obtained. A 30 µL aliquot of each extract was then homogenized with 3mL of the ABTS radical. The absorbance of the samples was read at 734 nm after 6 min of reaction. The results were expressed in µmol of trolox equivalent per gram of powder on dry weight (DW) basis (µmol TE/g^{-1}).

β-carotene bleaching assay

The assay was performed as described by Cao *et al.* (2009) with some modifications. In brief, 0.01 g of β-carotene dissolved in 10 mL of chloroform was mixed with 40 mg of linoleic acid and 400 mg of Tween 40 in a flask. After removing the chloroform in a rotary vacuum evaporator at 40°C for 10 min, 100 mL of oxygenated distilled water was slowly added to the oily residue with vigorous agitation to form an emulsion. A 4.5 mL aliquot of the emulsion was added to a tube containing 0.5 mL of extract sample solution of different concentrations and the absorbance was measured immediately at 470 nm against a blank consisting of the emulsion without β-carotene. The tubes were placed in a water bath at 50°C and the oxidation of the emulsion was monitored spectrophotometricaly by measuring the absorbance at 470 nm every 15 min until 120 min had passed. The antioxidant activity (AA) of the sample extract was evaluated in terms of the bleaching of β-carotene using the following equation:

$$\text{Inhibition }\%=[(A_t-C_t)\div(C_o-C_t)]\times 100$$

Where A_t and C_t are the absorbance values of the test sample and control, respectively, after a certain time (t) of incubation and C_0 is the absorbance value for the control, measured at the beginning of the experiments.

Determination of in vitro *antimicrobial activity*

The blueberry extracts (*Vaccinium ashei* Reade) were individually tested against *Escherichia coli* ATCC 25922, *Shigella flexneri* ATCC 12022, *Enterobacter aerogenes* ATCC 13048, *Acinetobacter baumannii* ATCC 19606, *Pseudomonas aeruginosa* PA01, *Salmonella cholerestasus* ATCC 10708, *Salmonella enteritidisclinical* isolate, *Listeria monocytogenes* ATCC 7644, *Bacillus cereus* ATCC 9634, *Staphylococcus epidermidis* ATCC 35985, *Enterococcus faecium* ATCC 6569, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Streptococcus spclinical* isolate, *Candidatropicalis* ATCC 66029, *Candida kefyr* ATCC 66028 and *Saccharomyces cerevisiae* (environmental isolate).

For the disk diffusion technique, suspensions of microorganism were prepared in 0.9% NaCl solution and compared to the turbidity of 0.5 on the Mcfarland scale (equivalent to approximately 1.5 x 10^8 colony forming units/mL). Using sterile swabs, the bacterial suspensions were sown on the surface of Petri dishes containing about 15 mL of Mueller-Hinton agar with a thickness of approximately 4 mm.

Sterilized filter paper disks, six mm in diameter, containing 10 µLof distinct compounds were placed on the surface of the plates in contact with the inoculum of the microorganism. The plates were incubated at 37°C for 24 hours (for the bacteria) and for 48 hours at 25°C (for the fungi) in an oven. After the incubation time, the formation of halos was analyzed. The tests were performed in triplicate and

the results were expressed in mm as the arithmetic average of the diameter of the inhibition halos that were formed around the disks. The following were used as inhibition controls: ketoconazole 50 µg (Biorad) for fungi; polymyxin 300 units (Biorad) for gram- negatives; and vancomycin 30 µgforgram-positives.

Statistical analysis

The results were submitted to analysis of variance (ANOVA) and their means were set by the method of ordinary least squares and compared by Tukey's test at a significance level of 5% ($p < 0.05$). Regardless of the significance of the interactions, the trends were tested via contrast from the coefficients for the interpolation of the orthogonal polynomials. Additionally, descriptive statistical analysis and Pearson's simple linear correlation were performed between the variables that were studied.The statistical analyses were performed using the SAS® - Statistical Analysis System,version 9.0 (SAS Institute Inc., Cary, NC, USA) at a 5% significance level.

Results and Discussion

Total Phenolic Compounds, Total Flavonoid Content and Total anthocyanins

Table 1 shows the average values for the content of total phenolics, flavonoids and anthocyanins for the blueberry extracts obtained by the microwave method using different concentrations of solvent and extraction temperatures. The values for total phenolics increased ($P < 0.05$) with increases in the extraction temperature for both solvent concentrations, demonstrating a linear tendency i.e. an increase in the extraction temperature resulted in an increase in the total phenolic content.

Similar behavior was observed by Wu *et al.* (2012), who extracted phenolic compounds from potatoes using focused microwave at temperatures of 50, 60, 70 and 80°C and found a marked increase in the content of total phenolics with increasing temperature. Regarding the solvent, there was no significant difference between the concentrations of solvent that were used. However, Wu *et al*. (2012) found that a concentration of 60% ethanol was more efficient than a concentration of 80%, suggesting that phenolic compounds are more soluble in concentrations of 60%.

Table 1 shows that the extraction of total flavonoids was not affected ($P > 0.05$) by the temperature and concentration of solvent that was used. In contrast, Spagolla *et al.* (2009) found that extractions using 40% and 60% ethanol were more efficient than 80% when extracting total flavonoids from dried blueberry of the Rabbiteye variety *(Vaccinium ashei)*.

Table 1. Total phenolic, flavonoids and anthocyanins content of blueberry extract.

Solvent %	Temperature, °C 30	40	50	60	Mean	P-value S	T	S×T
	Total phenolics, mg GAE/g of dried fruit					0.1751	<0.0001	0.2571
60	60.36	75.18	75.02	97.08	76.91		Tendency	
80	51.15	81.42	88.18	113.38	83.53		L	Q
Mean	55.75^{c}	78.3^{b}	81.60^{b}	105.23^{a}		T	<0.0001	0.9087
	Total flavonoids, mg QE/g of dried fruit					0.2744	0.1389	0.6696
60	3.31	3.91	4.37	7.33	4.73		Tendency	
80	2.74	3.84	3.96	4.56	3.77		L	Q
Mean	3.03	3.87	4.17	5.94		T	0.0291	0.5905
	Total anthocyanins, mg cyanidin 3-O-glucoside /100g of dried fruit					0.4917	0.0075	0.6651
60	13.70	20.00	18.29	21.20	18.30		Tendency	
80	14.23	17.40	19.00	19.35	17.49		L	Q
Mean	13.96^{b}	18.70^{a}	18.65^{a}	20.27^{a}		T	0.002	0.1915

Means with the same small letter in the row do not differ ($P>0.05$) by Tukey test.
Means with the same capital letter in the column do not differ ($P>0.05$) by Tukey test.
GAE = Gallic acid equivalent; QE: Quercetin equivalent; S: solvent; T: temperature; L: linear; Q: quadratic; p-value: probabilistic value; CV (%): coefficient of variation; SEM: standard error of the mean.
CV (phenolics) = 26.4; SEM (phenolics) = 4.3; CV (flavonoids) = 50.8; SEM (flavonoids) = 0.4; CV (anthocyanins) = 19.3; SEM (anthocyanins) = 0.7.

The total anthocyanin values (Table 1) showed an increase with a linear trend, i.e. increased extraction temperature in the solvent concentrations (60% and 80%) produced a higher extraction of total anthocyanins. Similar results were found by Zheng *et al*. (2013), who used microwave-assisted extraction in blueberries at different temperatures (30, 50, 70, 90 and 110°C) and observed that the total anthocyanin content significantly increased in the range of 30-50ºC. This phenomenon can probably be attributed to the increased diffusion rate of the anthocyanins within the particles and the solubility capacity in the solvent extracted with increasing temperature.

However, in the present study significant difference was not observed between the concentrations of solvent used in the extraction process. In contrast, Zheng *et al.* (2013) used microwave-assisted extraction in blueberry, varying the concentrations of ethanol (20%, 40%, 60%, 80% and 100%), and they found the best extraction condition using 60% ethanol.

Antioxidant activity

Different techniques are used to determine the antioxidant activity in vitro in order to allow the selection of pure substances and matrices with

Table 2. Antioxidant activities of blueberry extract.

Solvent	Temperature, °C				Mean	P-value		
%	30	40	50	60		S	T	S×T
	Ferric reducing activity, µmol TEAC/g of dried fruit					0.5138	<0.0001	0.3563
60	2.73	4.14	5.86	6.84	4.89		Tendency	
80	3.62	3.88	6.29	6.50	5.07		L	Q
Mean	3.17^{c}	4.01^{c}	6.07^{a}	6.67^{a}		T	<0.0001	0.6694
	ABTS$^{+\cdot}$ radical-scavenging activity, µmol TEAC/g of dried fruit					0.8291	0.001	0.9873
60	2.55	3.90	2.32	4.04	3.20		Tendency	
80	2.42	3.81	2.15	4.17	3.13		L	Q
Mean	2.48^{c}	3.85^{a}	2.24^{c}	4.10^{a}		T	0.0351	0.4407
	DPPH$^{\cdot}$ radical-scavenging activity, µmol TEAC/g of dried fruit					0.1224	0.0179	0.864
60	4.91	5.05	5.63	8.46	6.01		Tendency	
80	3.93	4.44	4.98	6.48	4.96		L	Q
Mean	4.42^{c}	4.74^{c}	5.30^{bc}	7.47^{a}		T	0.004	0.1743
	IC_{50}% inhibition of DPPH radical, mg/mL					0.0401	0.0038	0.4454
60	8.17	9.11	7.57	6.63	7.87^{B}		Tendency	
80	10.07	9.27	8.92	7.01	8.82^{A}		L	Q
Mean	9.12^{a}	9.19^{a}	8.24^{ab}	6.82^{c}		T	0.0008	0.0975
	β-Carotene bleaching inhibition activities, %					0.0016	0.0199	0.0473
60	50.02^{B}	62.23	57.94	63.73^{B}	58.48		Tendency	
80	72.44^{A,ab}	61.78^{c}	62.84^{c}	85.28^{A,a}	70.59		L	Q
Mean	61.23	62.00	60.39	74.5		T	0.0167	0.0533

Means with the same small letter in the row do not differ (P>0.05) by Tukey test.
Means with the same capital letter in the column do not differ (P>0.05) by Tukey test.
TEAC = trolox equivalent antioxidant capacity; S: solvent; T: temperature; L: linear; Q: quadratic; p-value: probabilistic value; S: solvent; CV (%): coefficient of variation; SEM: standard error of the mean.
CV (ferric reducing activity) = 32.0; SEM (ferric reducing activity) = 0.3; CV (ABTS+.)= 33.3; SEM (ABTS+.) = 0.2; CV (DPPH.) = 34.5; SEM (DPPH.) = 0.4; CV (IC50) = 17.3; SEM (IC_{50}) = 0.3; CV (β-Carotene) = 18.5; SEM (β-Carotene) = 2.4.

this property. In the present study the antioxidant activities of blueberry extracts were evaluated by the following four methods: FRAP, $ABTS^{+}$, DPPH and β- carotene (Table 2).

Table 2 shows that there was no interaction (P> 0.05) between the extraction temperature and the concentration of solvent for the antioxidant activity values measured by the FRAP, $ABTS^{+}$ and DPPH tests. The solvent concentrations of 60 and 80% showed no difference (P> 0.05) in relation to the extraction temperatures. However, the extraction temperature was significant in relation to the values for antioxidant activity, showing a positive linear trend (P <0.05) that resulted in an extract with higher antioxidant activity in line with increases in the extraction temperatures. Corroborating this study, Li *et al.* (2012) extracted antioxidant compounds from tomatoes using microwave and found no interaction (P> 0.05) between the ethanol concentration and temperature for FRAP. An increase in temperature resulted in extracts with higher FRAP values.

Moura (2016) studied the extraction temperatures (30 and 60°C) and the organic solvent (80% ethanol and water) of bioactive compounds present in samples of açaí, blueberry and gojiberry pulp and achieved the best results for antioxidant activity by the DPHH method for blueberry at a temperature of 60°C using 80% ethanol as the extraction solvent.

Several studies have reported that heat improves the efficiency of extracting phenolic compounds and also provides an increase in antioxidant activity (Benmeziane *et al.*, 2013; Vatai *et al.*, 2009). Conducting the extraction process at higher temperatures results in an increase in phenolic solubility, the rate of diffusion, and the extraction rate, due to reduced viscosity and surface tension of the solvent (Ju and Howard., 2003).

Another parameter used to quantify antioxidant properties is the IC_{50} value, which represents the concentration of antioxidants that provide 50% inhibition of the initial amount of free radicals; the lower the IC_{50}, the higher the antioxidant activity. Table 2 shows that there was no interaction (P>0.05) between the extraction temperature and solvent concentration for the IC_{50} values. The IC_{50} values decreased (P <0.05) with increases in the extraction temperature and decreases in the solvent concentration, showing a linear trend and indicating that the extract with the highest inhibitory capacity was found using 60% solvent at 60°C.

The β-carotene/linoleic acid system is a spectrophotometric method based on the oxidation (discoloration) of β-carotene, which is induced by the products of the oxidative degradation of linoleic acid, i.e. the method assesses the ability to inhibit free radicals during the peroxidation of the linoleic acid (Duarte-Almeida *et al*., 2006).

Regarding the β-carotene/linoleic acid system, Table 2 shows that the blueberry extracts underwent an interaction between the extraction temperature and the solvent concentration (P <0.05). The extracts obtained with 80% solvent at temperatures of 30°C and 60°C showed the highest percentage of inhibition; 72.44% and 85.28% respectively.

Pearson correlation coefficient

Table 3 shows the correlations between total antioxidant activity and total phenolic, flavonoid and anthocyanin content. The results in Table 3 show a positive correlation between the total phenol

Table 3. Pearson correlation coefficients.

	TPC	TFC	FRAP	ABTS	IC_{50}	DPPH	BCB	ANT
TPC		0,48*	0.70***	0.47*	-0.61**	0.49*	0.43*	0.64**
TFC			0.42*	0.26ns	-0.42*	0.09ns	0.24ns	0.33ns
FRAP				0.27ns	-0.49*	0.49*	0.35ns	0.57**
ABTS					-0.25ns	0.27ns	0.25ns	0.37ns
IC_{50}						-0.72***	-0.02ns	-0.62**
DPPH							0.11ns	0.61**
BCB								0.18ns
ANT								

nsno significant difference (p>0,05); *significant difference (p<0,05);**significant difference(p<0,01);***significant difference (p<0,001).
TPC: total phenolic content; TFC: total flavonoid content; FRAP: Ferric reducing antioxidant power; ABTS: ABTS assay; IC_{50}: inhibition activity; DPPH: DPPH assay; BCB: β-carotene bleaching assay; ANT: Total anthocyanins.

content and the determined antioxidant activities, with the highest correlation shown by the FRAP test (r = 0.70). This was a moderate positive correlation (0.5 <r <0.8) in accordance with the concepts of the Pearson correlation coefficient. The existence of a positive correlation between the FRAP assay and the levels of phenolics was also verified by Bunea *et al.* (2011), who investigated varieties of cultivated and wild blueberries in Romania. They attributed this correlation to the fact that the tests had the same reaction mechanism.

Likewise, the interactions of total anthocyanins in relation to TPC (r = 0.64), DPPH (r = 0.61) and FRAP (r = 0.57) were moderately positive (0.5 <r <0.8). This can be explained by the fact that anthocyanins are compounds that have antioxidant activity, i.e., the higher the content of these compounds in the extracts, the higher the antioxidant activity. Kuskoski *et al.* (2006) also observed a positive correlation between anthocyanins and antioxidant activity (DPPH) when they quantified various frozen fruit pulps marketed in southern Brazil. These authors also reported a direct correlation between phenolic compounds and antioxidant activity, which was also observed in the present study (Table 3).

Kalt *et al.* (1999) also found a positive correlation between total antioxidant capacity and total anthocyanin content and total phenolic compounds in small fruits such as strawberry, raspberry and blueberry. Regarding IC_{50}, all the parameters correlated inversely and DPPH antioxidant activity (r = -0.72), ANT (anthocyanins) (r = -0.62) and TPC (r = -0.61) presented the highest correlation with the minimum concentration of extract to inhibit 50% of the free radical DPPH, indicating that the higher the content of DPPH, ANT and TPC in the extracts, the lower the IC_{50}, and consequently, the greater the efficiency of capturing free radicals.

Antibacterial and antifungal activity

No activity was found in relation to Gram-positive bacteria, Gram-negative bacteria and fungi in all the blueberry extracts. These results contradicted those of previous studies reported in the literature. Lacombe *et al*. (2012) found that blueberry extract formed inhibition zones in relation to *E. coli, Salmonella* Typhimurium, *L. monocytogenes, Staphylococcus aureus* and *Yersinia enterocolitica.* Silva *et al*. (2013) studied infusion of blueberry fruit *(Vaccinium corymbosum)*, and found antimicrobial activity in relation to *S. aureus, S. entenritidis, E. faecium, L. immocua, B. cereus* and *P. aeruginosa* but no inhibition in relation to *C. albicans* and *E. coli.*

In the present study, despite the fact that the blueberry extracts possessed phenolic compounds, flavonoids and anthocyanins, antimicrobial activity was not confirmed. It is noteworthy that these contradictory results may be linked to the extraction methodology, the origin of the fruits, and the tested microorganisms. Therefore, in this study we observed the absence of antimicrobial and antifungal activity in blueberry extracts extracted by focused microwave.

Conclusion

The results of this study demonstrate that the highest extraction values for phenolic compounds, anthocyanins and antioxidant activity (FRAP, $ABTS^+$ and DPPH) were obtained at a temperature of 60°C and did not differ significantly in relation to the concentrations of solvent. The temperature of 60°C was most efficient for the IC_{50} and β-carotene tests and the latter showed the best values when using solvent concentrations of 60% and 80%, respectively. The total flavonoids did not have significant differences in relation to the temperature and solvent used in the extraction.

Consequently, the temperature of 60°C and 60% solvent concentration were the most efficient in terms of microwave-assisted extraction of bioactive compounds in blueberry, as well as being the most economical. The extracts showed no antifungal or antibacterial activity in relation to the different tested microorganisms. These results show that the proposed microwave-assisted extraction procedure can be considered suitable for obtaining blueberry extracts that are rich in bioactive compounds with high antioxidant activity, and with the possibility of being used as a natural antioxidant.

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