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Extraction of phenolic compounds and evaluation of the antioxidant and antimicrobial capacity of red onion skin (*Allium cepa* L.)

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Abstract

This study evaluated the content of phenolic compounds, total flavonoids and anthocyanins, as well as the antioxidant and antimicrobial activity, of red onion skin extract (*Allium cepa* L.) that was obtained by conventional extraction at different times (30, 60, 120 and 240 minutes) and using different concentrations of ethanol (20, 40, 60 and 80%). The antioxidant activity of the extracts was evaluated using the following methods: DPPH radical sequestration, the inhibition of the auto-oxidation of the β-carotene system, FRAP and IC_{50}. The antimicrobial activity was evaluated by the disk diffusion method. The best results for phenolics, flavonoids and anthocyanins were found using extraction with 80% solvent at 165, 60 and 120 minutes respectively. In relation to the antioxidant activity using the FRAP, DPPH and IC_{50} methods, the best values were found using 80% solvent at 120, 30 and 30 minutes of extraction respectively. The best value for antioxidant activity determined by the β-carotene method was with 59.32% solvent at 240 minutes of extraction. No antimicrobial activity of the extracts in relation to the tested microorganisms was detected. The results of this study showed that the concentration of 80% ethanol favored the extraction of phenolic compounds, flavonoids and total anthocyanins, as well as producing the highest antioxidant activities found by the different methods. The best extracts in terms of antioxidant activity by the DPPH and IC_{50} methods were obtained with the lowest extraction time of 30 minutes, in contrast to the extraction time of 240 minutes, which produced the highest antioxidant activity by the β-carotene methods.

Introduction

Increasing interest in replacing synthetic food antioxidants by natural food antioxidants has intensified the demand for plant materials for the identification of new antioxidants. Moure *et al.* (2001) studied the extraction of antioxidant compounds from industrial waste (peanut shell, tomato skin, lemon skin, grape seed, etc) and identified important antioxidants. In this context, waste from onions has been shown to be a source of antioxidants and compounds that work to prevent the blackening caused by enzymatic reactions (Roldan *et al.*, 2008).

The onion (*Allium cepa* L.) is a versatile vegetable from the Allium family that is appreciated throughout the world, not only for its taste but also as a significant source of many beneficial compounds. Studies have revealed the presence of flavonoids in various different varieties of onions, along with other bioactive compounds (Lachman *et al.*, 2003), almost all of which are located primarily in the skin. Wiczkowski *et al.* (2008) found a higher concentration of quercetin in onion skin than in the fleshy part of the onion. Nuutila *et al*. (2002) showed that onion skin extracts also have high antioxidant capacity. Study by Cao *et al.* (2013) the mixture of onion extract, garlic and ginger was effective against lipid oxidation of pork meat.

There are several methods for the extraction of antioxidants from plants. One of the most traditional methods (agitation, maceration, etc.) uses organic solvents (such as water, ethanol, ether and methanol) to improve the efficiency, the quality of the extract, the extraction time and the consumption of solvent.

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This study aimed to optimize the extraction of antioxidant compounds from red onion skin using the above-mentioned conventional methods with different concentrations of solvent and times.

Materials and Methods

Raw materials

The red onions were acquired in a commercial establishment located in the city of Santa Maria, Rio Grande do Sul, Brazil. The onions were selected for the absence of defects, pests and diseases. The surfaces were washed with mild detergent to remove dirt and they were sanitized with 200 mg L^{-1} of sodium hypochlorite for 20 min. They were then manually peeled with stainless steel knives. The skins were placed on trays and placed in a forced-air circulation oven (Marconi, MA-035/100, Piracicaba, Brazil) at 50°C for 24 hours. The skins were then crushed in a refrigerated analytical mill at 4°C (Quimis model Q298A21, Brazil) with the aid of an ultra-thermostatic water bath (Solab, model SL-152/10) and stored in amber bottles at a temperature of - 18°C until the extracts were obtained.

Obtaining the extracts – conventional extraction

For the conventional extraction, three grams of sample powder were placed in a 250 mL glass beaker, and 60 mL of cereal alcohol 1:20 w/v (20%, 40%, 60% and 80%) were added. In the extraction by agitation (conventional method) the beaker with the mixture was kept under constant stirring (80 rpm) using a mechanical shaker (Marconi, MA-0.39, Piracicaba, Brazil) for times (30, 60, 120 and 240 min) and temperatures 25+2ºC that were controlled with the aid of ultra-thermostatic water bath (Solab, model SL-152/10). After extraction, the solution was centrifuged at 3000 rpm for 15 min, filtered through qualitative filter paper (No. 1) and then the volume was adjusted to 100 mL. The extracts were stored at -12°C until further analysis.

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 (Na_2CO_3) 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 measured 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% $NaNO_2$ was added. After 5 min, 150 µL of 10% $AlCl_3$ was added and after 6min, 1 mL of 1 M NaOH was added, followed by the addition of 1.2 mL of distilled water. The sample absorbance was read at 510 nm using a UV/Vis spectrophotometer (Biospectro, model: SP - 220). The absorbance of the extract was compared with a quercetin standard curve using concentrations of 0 to 200 mg.L^{-1} 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:

$$Anthocyanins\ content_{(mg/100g\ of\ dry\ matter)} = A \times MW \times DF \div (\varepsilon \times W$$

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 (0.3; 0.6; 1.25; 2.5; 5.0; 10; 15; 20; 25; 30; 35 and 40 mg/mL) 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 with the DPPH standard solution. The results were expressed as radical scavenging activity percentage (%) of the DPPH radical according to the following formula:

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

The TEAC compound (Trolox equivalent antioxidant capacity) (range of 0 to 100 µM) was used for the calibration standard curve. The results were expressed in µmol of trolox equivalents per gram of powder on a dry weight (DW) basis (µmol TE/g^{-1})

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, 10 mM TPTZ and 20 mM 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 TEAC compound (Trolox equivalent antioxidant capacity) (range of 0 to 25 µM) was used for the calibration standard curve. The results were expressed in µmol of trolox equivalents per gram of powder on a 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, 0.01g 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:

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

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 *antimocrobial activity*

The red onion skin extracts 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 enteritidis* clinical 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* sp clinical isolate, 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 µL of 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 µg for gram-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 (Khattree and Naik, 2000).

Results and Discussion

Total phenolic compounds

Methanol, ethanol, acetone, ethyl acetate, and combinations of these, have been frequently used to extract polyphenols using different proportions of water resulting in different conditions of interactions with the matrices (Dai and Mumper, 2010). In this study, grain alcohol (ethanol) was used as solvent because of its extraction power and safety in terms of human consumption, since it is used in the food industry. The extraction temperature was set at 25°C bearing in mind that high temperatures increase the chance of the oxidation of phenolic compounds.

Table 1 shows the average values for the content of phenolic compounds, flavonoids and anthocyanins for the red onion skin extracts obtained by the conventional method under varying conditions of solvent concentration and extraction times. There was an interaction ($P < 0.05$) between the solvent and extraction time for total phenolics. The total phenolic content increased ($P < 0.05$) as the concentration of the solvent was increased for the times of 60, 120 and 240 minutes respectively, as can be seen in the following linear equations; ($r^2=0.89$) $\hat{y}_{T60}=-117.13+8.64S$ ($r^2=0.95$); $\hat{y}_{T120}=5+8.5S$ $\hat{y}_{T240}=-120+9.33S$ ($r^2=0.80$). However, with a 30 minute extraction time, the values for total phenolics were invariant as a function of the solvent concentration.

Studying the influence of the concentration of ethanol (40-60%) in the extraction of phenolic compounds from different parts of onions, Kiassos *et al.* (2009) also reported that increasing the ethanol concentration resulted in a better extraction of these compounds, being 60% more efficient. The solubility of phenolic compounds is influenced by the polarity of the solvents that are used, by their degree of polymerization, and by their interaction with other constituents of the plant (Lyanna-Pathirana *et al.*, 2005; Su *et al.*, 2007). Considering that there are polyphenols with different polarities in plants, the use of a solvent that is highly polar or apolar is not recommended in order to enable an efficient extraction of these constituents. Thus, the results of this study corroborate the above statement, given that the highest values for total phenolic content were obtained using the concentration of solvent with an intermediate polarity (80%). However, according to Chirinos *et al*. (2007) low extraction of phenolic compounds in concentrations of 20 to 40% solvent may be because the solvent has a higher amount of water, and a water is responsible for extracting greater amounts of impurities (organic acids, carbohydrates, polysaccharides) which may interfere with the determination of total phenolic compounds

As regards the type of raw material used in the present study (red onion skin), the soluble phenolic compounds are generally located in compartments within the cell vacuoles (in free form or conjugated) and the insoluble phenolic compounds are generally linked to cell wall structures, requiring a longer time for extraction. This was demonstrated in this study: a quadratic behavior with 80% solvent in function of time $\hat{y}_{S80}=184.01+6.94T-0.021T^2$ ($r^2=0.70$) with a maximum point at 165.24 minutes. Therefore, the extraction of total phenolic compounds was optimized with 80% solvent extraction and 165 minutes extraction time, and was estimated to be 757.38 mg GAE/g of red onion skin. Likewise, Tan *et al.* (2013) found quadratic behavior for the extraction of total polyphenols from the stems of henna plants *(Lawsonia inermis)* in function of the extraction time, with an increase from 30 to 270 minutes followed by a decline until 360 minutes.

Total flavonoid content

There was an interaction ($P < 0.05$) between the concentration of the solvent and the extraction times for the total flavonoid values. The extraction of total flavonoids increased as the concentration of solvent was increased for the extraction times. The following linear equations: $\hat{y}_{T30}=20.39+0.18S$ ($r^2=0.62$); $\hat{y}_{T60}=17.15+0.29S$ ($r^2=0.76$) $\hat{y}_{T120}=19.09+0.21S$($r^2=0.96$); $\hat{y}_{T240}=17.40+0.25S$ ($r^2=0.99$) show that the greatest slope was obtained after 60 minutes of extraction, indicating a higher level of extraction. However, with 80% solvent there was no change in the total flavonoid content as a function of extraction time. Thus, the extraction of flavonoids was optimized at 40.35 mg QE/g of dry onion skin using 80% solvent

Table 1. Total phenolic, flavonoids and anthocyanins content of dry onion skin

Solvent, %	Time, min 30	60	120	240	Mean or Equation	P-value S	T	S×T
	Total phenolics, mg GAE/g dry onion skin					0.001	0.078	0.0003
20	311.0	113.4^{B}	156.4^{B}	138.2^{B}	179.8		Tendency	
40	367.3	188.4^{B}	338.0^{AB}	91.9^{B}	246.4		L	Q
60	404.2	309.0^{B}	585.1^{A}	549.2^{A}	461.8	S	0.001	0.7378
80	300.1^{b}	649.5^{Aa}	640.8^{Aa}	608.4^{Aab}	549.7	T	0.635	0.0602
Mean or Equation	345.6	315.1	430.1	346.9				
	Total flavonoids, mg QE/g dry onion skin					0.001	0.064	0.0048
20	22.7^{C}	22.6^{B}	22.6^{B}	22.8^{C}	22.7		Tendency	
40	26.7^{BC}	26.9^{B}	29.0^{AB}	27.3^{BC}	27.2		L	Q
60	36.2^{Aab}	40.9^{Aa}	33.3^{Ab}	32.2^{ABb}	35.6	S	0.001	0.0021
80	31.4^{AB}	37.4^{A}	35.2^{A}	38.1^{A}	35.5	T	0.828	0.5488
Mean or Equation	29.3	31.9	29.8	30.1				
	Total anthocyanins, mg cyanidin 3-O-glucoside /100g dry onion skin					0.001	0.001	0.0001
20	73.5^{B}	66.5^{C}	55.5^{C}	73.8^{B}	67.3		Tendency	
40	104.3^{AB}	127.7^{BC}	125.9^{BC}	117.0^{AB}	118.7		L	Q
60	227.7^{Ab}	470.2^{Aa}	217.7^{ABb}	217.0^{Ab}	283.2	S	0.0001	0.0010
80	221.1^{A}	202.1^{B}	309.1^{A}	233.4^{A}	241.4	T	0.233	0.1161
Mean or Equation	156.6	216.6	177.0	160.3				

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: time; L: linear; Q: quadratic; p-value: probabilistic value; CV (%): coefficient of variation; SEM: standard error of the mean
CV (phenolics) = 58.8; CV (flavonoids) = 21.0; CV (anthocyanins) = 63.3
SEM (phenolics) = 30.5; SEM (flavonoids) = 0.9; SEM (anthocyanins) = 16.2

and 60 minutes extraction time. When Chew *et al.* (2011) evaluated the extraction of total flavonoids in Centella asiatica with 20 to 100% ethanol they found the best values for total flavonoids using 60% ethanol.

In general, longer extraction times facilitate the extraction of bioactive compounds. However, the extraction time with 60% solvent negatively influenced the levels of total flavonoids: $\hat{y}_{S60}=39.03-0.03T$ ($r^2=0.51$). The slope indicated that an increase ın extraction time led to a reduction in the extraction of total flavonoids. This reduction may have occurred due to the exposure of bioactive compounds to oxidative degradation. However, no change in total flavonoid content as a function of extraction time was observed in the other solvent concentrations. Chew *et al.* (2011) used 60% ethanol and times of 60 and 120 minutes for the highest values for the extraction of total flavonoids from *Centella asiatica* and there was no difference (P> 0.05) between the times.

Total anthocyanins

Interaction (P<0.05) was observed between the extraction time and concentration of solvent for the values of total anthocyanins. The linear equations $\hat{y}_{T30}=15.07+2.83S$ ($r^2=0.85$); $\hat{y}_{T120}=-36.07+4.26S$ ($r^2=0.99$); and $\hat{y}_{T240}=15.65+2.89S$ ($r^2=0.93$) showed that at times of 30, 120 and 240 minutes there was a greater extraction of total anthocyanins with an increased concentration of solvent. It can be seen that the slope (4.26) indicated the highest level of extraction, which occurred at 120 minutes. The extraction time of 60 minutes showed quadratic behavior, which was described by the equation: $\hat{y}_{T60}=-382.23+24.32S-0.205S^2$ ($r^2=0.58$), with a peak at 59.32% of solvent. On the other hand, the anthocyanins were not influenced by the extraction times with 20, 40 and 80% of solvent. However, with 60% of solvent the extraction was greatest (P <0.05) at 60 minutes, even without adjusting (P>0.05) the regression. Therefore, considering that increases in solvent concentration increased the level of total anthocyanins, the extraction of anthocyanins was

Table 2. Antioxidant activities of dry onion skin

Solvent, %	Time, min				Mean or Equation	P-value		
	30	60	120	240		S	T	S×T
	Ferric reducing activity (FRAP), µmol TEAC/g dry onion skin					0.0001	0.1480	0.0001
20	18.3^{B}	12.5^{C}	14.4^{B}	21.0^{C}	16.5		Tendency	
40	27.0^{B}	27.3^{CB}	28.1^{B}	24.3^{BC}	26.7		L	Q
60	120.6^{Aab}	152.5^{Aa}	101.5^{Abc}	64.8^{ABc}	109.9	S	0.0001	0.0001
80	54.2^{Bb}	60.5^{Bb}	108.5^{Aa}	97.6^{Aab}	80.2	T	0.2617	0.0580
Mean or Equation	55.0	63.2	63.1	51.9				
	DPPH· radical-scavenging activity, µmol TEAC/g dry onion skin					0.0001	0.3123	0.0663
20	28.8	27.3	22.2	26.4	26.2^{B}		Tendency	
40	33.1	30.2	44.4	50.0	39.4^{B}		L	Q
60	61.4	35.9	83.6	100.1	70.2^{A}	S	0.0001	0.8588
80	97.4	81.3	95.4	50.2	81.1^{A}	T	0.4435	0.6547
Mean or Equation	55.2	43.7	61.4	56.7				
	IC_{50} % inhibition of DPPH radical, mg/mL					0.0001	0.2440	0.0047
20	1.9^{AB}	2.7^{A}	2.4^{A}	2.4^{A}	2.4		Tendency	
40	2.5^{Aa}	1.6^{ABab}	1.3^{ABab}	1.1^{Bb}	1.6		L	Q
60	1.0^{BC}	1.2^{B}	0.6^{B}	0.5^{B}	0.9	S	0.0001	0.0034
80	0.6^{C}	0.7^{B}	0.9^{B}	1.1^{B}	0.8	T	0.0780	0.5318
Mean or Equation	1.5	1.6	1.3	1.3				
	β-Carotene bleaching inhibition activities, % (12.5 mg/mL)					0.0001	0.8288	0.0001
20	49.9^{a}	24.8^{Bab}	25.7^{Bab}	11.1^{Cb}	27.9		Tendency	
40	57.5	60.8^{A}	52.7^{A}	49.6^{B}	55.1		L	Q
60	75.2	67.0^{A}	73.4^{A}	84.2^{A}	74.9	S	0.0001	0.0001
80	55.9^{b}	77.4^{Aab}	74.0^{Aab}	85.7^{Aa}	73.2	T	0.6834	0.4202
Mean or Equation	59.6	57.5	56.4	57.7				

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: time; L: linear; Q: quadratic; p-value: probabilistic value; CV (%): coefficient of variation; SEM: standard error of the mean. CV (FRAP) = 77.5; CV (DPPH.) = 62.2; CV (IC50) = 56.2; CV (β-Carotene) = 39.0 SEM (FRAP) = 6.5; SEM (DPPH.) = 4.9; SEM (IC50) = 0.1; SEM (β-Carotene) = 3.3

optimized at 304.73 mg QE/g of dry onion skin using 80% solvent and 120 minutes extraction time.

The best extraction of anthocyanins, which was caused by the increase in the ethanol concentration, may have been due to the polarity of the anthocyanin molecules, which contain residual groups hydroxyl, methoxyl and that glicosilas that are attached to their aromatic rings, thus making them more soluble in polar solvents such as ethanol. Also, as Dai and Mumper (2010) have indicated, ethanol plays an important role in breaking the hydrogen bonds and hydrophobic bonds that exist between anthocyanin-protein and anthocyanin-cellulose in the water-ethanol system, thereby increasing the power of extraction. Based on the results of the present study, to obtain the highest anthocyanin content a combination of a higher ethanol concentration and a longer extraction time was necessary. This result was probably associated with the use of a low temperature (25°C), which caused a delay in the diffusion of the compounds desired by the extraction liquid. Studies have reported that for the efficient extraction of anthocyanins the process should be conducted at temperatures ranging from 20 to 50°C because temperatures above 70°C have been shown to cause rapid degradation of anthocyanins.

In contrast, Khiari *et al.* (2009) extracted bioactive compounds from brown onion residue and obtained higher anthocyanin results with a longer extraction time (6 hours). Cacace and Mazza (2003) found higher levels of anthocyanins in ethanol concentrations of 39 and 67%. In concentrations between 67-95% there was a reduction in the diffusion coefficient.

Antioxidant activity

Due to the fact that reactions involving antioxidant activity are complex, antioxidant activity should not be judged by a single method. For this reason, in the present study the antioxidant activity

of red onion skin extracts were evaluated by different methods, i.e., FRAP, DPPH, IC_{50} and β-carotene (Table 2). There was an interaction (P<0.05) between the extraction time and the solvent concentration for the FRAP values. The times of 30 and 60 minutes showed quadratic behavior: $\hat{y}_{T30}=-89.23+5.70S-0.047S^2$ ($r^2=0.53$) and $\hat{y}_{T60}=-137.76+8.02S-0.066S^2$ ($r^2=0.55$) respectively, with peak concentrations of extraction using 60.6 and 60.7% solvent. For the times of 120 and 240 minutes, as the solvent concentration increased [demonstrated by the linear equations $\hat{y}_{T120}=-25.82+1.78S$ ($r^2=0.89$) and $\hat{y}_{T240}=-15.65+1.35S$ ($r^2=0.92$) respectively there was an increase in the FRAP values.

The solvent concentrations of 20 and 40% showed no difference (P>0.05) for FRAP in terms of the extraction times. However, there was an interaction (P<0.05) between the solvent concentration and extraction time for the solvent concentrations of 60 to 80%, which could be represented by the linear equations $y_{S60}=148.71-0.34T$ ($r^2=0.76$) and $y_{S80}=55.79+0.22T$ ($r^2=0.56$), respectively. Through the coefficient it was noticed that the time had a negative influence on FRAP at a 60% concentration of ethanol. This same behavior was also observed in relation to flavonoids, demonstrating that the latter were responsible for the reducing power of these extracts. Using an 80% concentration of ethanol, an increased extraction time resulted in a higher reducing power of the red onion skin extract. The extract with the highest reducing power (116.58 µmol TEAC/g of red onion skin) was found using the conditions of 80% solvent and 120 minutes for extraction.

According to Jayanthi and Lalitha (2011), compounds with reducing power indicate that they are electron donors and they can reduce the oxidizing form of intermediate processes of lipid peroxidation, so that they can act as primary and secondary antioxidants. For the values of antioxidant activity (DPPH) there was no interaction (P> 0.05) between the extraction time and the concentration of the solvent, which was represented by the following general equation for linear behaviour: $\hat{y}_{DPPH}=5.37+0.98S$ ($r^2=0.96$). The antioxidant activity increased in line with increases in the concentration of solvent, demonstrating behavior with a linear trend. However, there was no difference (P> 0.05) between the concentrations of 60 and 80% solvent. The extraction time did not influence the DPPH values of the extracts. Given the above, the extract with the best antioxidant capacity was that with 80% solvent and 30 minutes extraction time.

In a study by Thoo *et al.* (2013), the factor of time influenced the antioxidant capacity of extracts of *Andrographis paniculata* (a medicinal plant). There was difference (P <0.05) between the times (60, 120, 180, 240 and 300 minutes) and the extract with the best antioxidant capacity was found using an extraction time of 60 minutes; at an extraction time of 120 minutes there was a reduction in antioxidant capacity. This phenomenon has also been confirmed by Liyana-Pathrina and Shahidi (2005), where prolonged extraction caused the decomposition of active compounds in wheat.

There was interaction (P<0.05) between the extraction time and concentration of solvent for the IC_{50} values. The IC_{50} values reduced (P<0.05) with increasing concentration of solvent, as shown in the linear equations $\hat{y}_{T30}=2.87-0.03S$ ($r^2=0.67$); $\hat{y}_{T60}=3.21-0.03S$ ($r^2=0.95$) for the times of 30 and 60 minutes. The times of 120 and 240 minutes showed quadratic behavior $\hat{y}_{T120}=4.38-0.11S+0.0008S^2$ ($r^2=0.99$; $\hat{y}_{T240}=4.82-0.14S+0.001S^2$ $r^2=0.99$), indicating that the minimum points of extraction were found in concentrations of 68.75 and 70% respectively. According to Negri *et al.* (2009), the lower the IC_{50} value the higher the antioxidant power of an extract.

The interaction (P <0.05) between ethanol concentration and extraction time also occurred at concentrations of 40 and 60% ethanol as shown in the linear equations $\hat{y}_{T30}=2.87-0.03S$ ($r^2=0.67$); $\hat{y}_{T60}=3.21-0.03S$ ($r^2=0.95$). The angular coefficients (-0005) and (-0.003) indicated that as the time increased, the IC_{50} values of these concentrations reduced, which was a favorable result considering that the lower the IC_{50} the higher the ability to inhibit the extract. However, the increased time of extraction using 80% ethanol concentration ($\hat{y}_{S80}=0.59+0.002T$ $r^2=0.96$) produced extractswith lower inhibition capacity, necessitating a higher concentration of extract to inhibit 50% of the free radical DPPH.

Given these results, it can be inferred that onion skin can be used as a good alternative as a primary antioxidant to act in the inhibition of oxidation reaction, combining with free radicals or reacting with hydrogen peroxide. The extract with the highest inhibition capacity was found using 80% solvent and 30 minutes of extraction. Table 2 shows that red onion skin extract can inhibit the oxidation of linoleic acid at a concentration of 12.5 mg/mL. There was interaction (P<0.05) between the solvent concentration and extraction time for β-carotene. At times of 60, 120 and 240 minutes the following quadratic equations: $\hat{y}_{T60}=-15.53+2.42S-0.016S^2$ ($r^2=0.96$), $\hat{y}_{T120}=-17.97+2.48S-0.016S^2$ ($r^2=0.99$) and $\hat{y}_{T240}=-53.04+3.60S-0.023S^2$ ($r^2=0.99$) showed that the greatest potential inhibitors were found using respective concentrations of 75.63, 77.50 and 59.32%.

The interaction (P <0.05) between time and

Table 3. Pearson correlation coefficients

	TPC	TFC	FRAP	IC_{50}	DPPH	BCB	ANT
TPC		0.66***	0.51***	-0.53**	0.56***	0.69***	0.48***
TFC			0.82***	-0.68***	0.51***	0.75***	0.85***
FRAP				-0.55***	0.39**	0.65**	0.84***
IC_{50}					-0.76***	-0.72***	-0.56***
DPPH						0.50***	0.39**
BCB							0.57***
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; IC_{50}: inhibition activity; DPPH: DPPH assay; BCB: β-carotene bleaching assay; ANT: Total anthocyanins.

concentration of solvent could also be observed in the solvent concentrations of 20 and 80% through the linear behavior of the following equations: $\hat{y}_{S20}=44.45-0.15T$ ($r^2=0.72$) and $\hat{y}_{S80}=60.95+0.11T$ ($r^2=0.64$). In terms of solvent at a concentration of 20%, the slope (-0.15) indicated that an increase in extraction time had a negative influence, which resulted in a reduction in the inhibiting power of the extracts. In contrast, the extraction performed with 80% solvent revealed that increased extraction time resulted in an extract with a higher inhibition potential.

According to Porter (1993), hydrophilic antioxidants are more effective than hydrophobic antioxidants in oil emulsion because they act at the oil-water interface. In turn, hydrophobic antioxidants inhibit oxidation in the lipid phase of emulsions. In fat emulsions, the phenolics are generally balanced in water, emulsifier (Tween 20), micelles and lipid phase, which increases their hydrophilic property, with a consequent increase in lipid oxidation. It is possible that the phenolic compounds of the extracts with 60 and 80% ethanol and higher extraction times (60, 120 and 240 minutes) achieved this balance, while the other extracts remained primarily in the lipid phase of the system, thus explaining the greater inhibiting power shown by these extracts. Given the studied conditions, the extract that was obtained with 59.32% solvent and an extraction time of 240 minutes showed the highest inhibition percentage (79.53%).

Pearson correlation coefficients

The correlations between total antioxidant activity and phenolic content, total flavonoids and anthocyanins activity can be seen in Table 3. The results showed a positive correlation between the content of total phenols and total flavonoids ($R^2=0.66$). This was expected because flavonoids are a sub-group of polyphenols. However, a positive and strong correlation was found between total flavonoids and anthocyanins ($R^2= 0.85$), indicating that high concentrations of total flavonoids were accompanied by high concentrations of anthocyanins in the red onion skin extracts.

The reducing power of the extract that was provided by the FRAP method had a positive correlation with the total flavonoids ($R^2=0.85$), total anthocyanins ($R^2=0.84$) and total phenolics ($R^2=0.51$). This trend indicates the implication of total flavonoids and the sub-class of anthocyanins in the donation of electrons. Confirming the correlation results of this study demonstrated that the conditions that favored the maximum extraction of anthocyanins were the same as for the extracts with higher reducing capacity (FRAP).

The correlation value indicated that the DPPH antioxidant activity was weakly related to the anthocyanins ($R^2=0.39$) and moderately with the total flavonoids ($R^2=0.51$) and phenolics ($R^2=0.56$). Cheng *et al*. (2013) studied red and yellow onions and found a strong positive correlation between the total flavonoid content ($R^2=0.82$) and antioxidant activity (DPPH). However, the correlation between total phenolics and DPPH ($R^2=0.62$) was similar to that found in this study. In contrast, Nuutila *et al.* (2003) reported a smaller correlation between antioxidant activity and the TPC of different species of garlic. Regarding the IC_{50}, it was observed that that the anthocyanins, flavonoids and phenolics were negatively-correlated, and that the flavonoids (-0.68) were most strongly correlated to the minimum concentration of extract to inhibit 50% of the free radical DPPH.

The self-oxidation of β-carotene/linoleic acid is a test that differs from the other two methods of evaluation of antioxidant activity (DPPH) because its reaction matrix is an emulsion. In this analysis, the

total flavonoids indicated that they were responsible for the inhibition of auto-oxidation of β-carotene, showing a moderate positive correlation (R^2=0.75), which was higher than for the phenolics (R^2=0.69) and anthocyanins (R^2=0.57). According to Lanzotti (2006), flavonoids are known to be good inhibitors of lipid oxidation in onions.

In this study, the weak correlation (R^2=0.39) between the average values of TEAC DPPH and TEAC FRAP showed that the compounds present in the extracts indicated that antioxidants are reducing agents due to their ability to donate a single hydrogen atom or electron for reduction; however, not all reducing agents are antioxidants.

Antibacterial and antifungal activity

No antibacterial (gram-positive and gram-negative bacteria) and antifungal activity was found for all the extracts of red onion skin. Some reported data on antibacterial activity contradicts the results found in the present study (Najjaa *et al*., 2011). The latter authors reported strong antibacterial activity in relation to *E. coli, Pseudomonas aeruginosa, Staphylococcus epidermidis, Micrococcus luteus* and *Staphylococcus aureus* in extract of *Allium roseum.* Dziri *et al.* (2011) confirmed the antimicrobial activity of extract of pink garlic (*Allium roseum* var. *odoratissimum*) in relation to *Enterococcus faecium, S. aureus, Bacillus subtilis* and *Escherichia coli*. Boo *et al.* (2012) investigated different natural pigments and found low antimicrobial activity in onion skin (*Allium cepa* L.) in relation to *Escherichia coli*; most of the other pigments showed high antimicrobial activity against this bacteria.

Despite the fact that red onion skin extracts contain a high content of phenolics, flavonoids and anthocyanins, antimicrobial activity does not seem to be closely related to these pigments. The cause of these contrary results may have been due to the extraction process, the source of the tested plants, and the microorganisms that were tested. However, our findings reported the absence of antimicrobial and antifungal activity in red onion skin extracts.

Conclusion

The results of this study demonstrated that the content of phenolic compounds, total flavonoids and anthocyanins, as well as the antioxidant activities of the extracts, had higher values when subjected to extraction with 80% ethanol. With respect to the extraction time, it was concluded that the shortest extraction time of 30 minutes favored the extraction of compounds with greater antioxidant activity using the DPPH and IC_5 methods. The longest extraction time of 120 and 240 minutes obtained extracts with the highest antioxidant activities using the FRAP and β-carotene respectively methods. The extracts did not show antifungal or antibacterial activity for the different tested microorganisms. Extracts of red onion skin can be a viable technological alternative to be used as a natural antioxidant in the preparation of meat products because they have high levels of antioxidant activity, thereby prolonging the shelf life of such products.

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