

## Ultrasound-Assisted Extraction and Biological Activities of Extracts of *Brassica oleracea* var. *capitata*

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Received: October 4, 2013

Accepted: January 26, 2015

### Summary

In this work, the antioxidant and antimicrobial activities of *Brassica oleracea* var. *capitata* extracts obtained through ultrasound-assisted extraction are evaluated. The extracts obtained using the best extraction conditions were subjected to different hydrolysis conditions before their use in the biological tests. The crude and hydrolysed extracts were characterized using gas chromatography coupled with a mass detector. The use of ultrasound at 30 °C with 60 % (by volume) solvent enabled obtaining a richer extract. All extracts had antioxidant activities against DPPH (13.0–80.0 %), superoxide (35.2–63.2 %) and peroxy (89.3–99.5 %) radicals, but the use of hydrolysed extracts considerably improved the antioxidant activities. Antimicrobial activities only of the hydrolysed extracts of *Brassica oleracea* var. *capitata* were detected. It was confirmed that antioxidant activity of vegetable extracts can be considerably increased when hydrolysis is applied as a pretreatment to their extraction.

*Key words:* *Brassica oleracea* var. *capitata*, ultrasound-assisted extraction, hydrolysis of extracts, biological activity

### Introduction

*Brassica* sp. is a vegetable belonging to the family Brassicaceae, which is one of the most important families cultivated worldwide. Some species of this family are of pharmacological interest. Several studies have revealed that they exhibit anti-inflammatory, antimycotic, photoprotective, antihyperglycemic, anticarcinogenic and antioxidant activities (1–3). Among the various *Brassica* species, *Brassica oleracea* L. var. *capitata* (cabbage) has been widely studied because of its biological activities (4).

Several authors have reported antioxidant activities of extracts of *Brassica* against different radicals. Among the methods for testing the ability of some compounds to act as free radical scavengers is the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. This procedure has been widely used to evaluate the antiradical activity of several plants, including extracts of *Brassica* sp., because it is considered to be an easy, precise and reproductive test for evaluating the antioxidant activity (5–7). The antioxidant potential of *Brassica* extracts against superoxide radicals (O<sub>2</sub><sup>•-</sup>), which can cause indirect damage because they lead to the for-

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mation of hydroperoxides through the autoxidation of unsaturated fatty acids, was also investigated (5,8,9). However, the use of *Brassica* extracts against the peroxy radical ( $\text{ROO}^\cdot$ ), which is an important intermediary in lipid peroxidation, has not been considered much. Specifically, Zhou and Yu (10) evaluated the scavenging activities of vegetables, including broccoli and kale, against DPPH,  $\text{ROO}^\cdot$  and  $\text{O}_2^\cdot$ , whereas Ou *et al.* (11) determined the antioxidant activity of white cabbage and broccoli extracts against peroxy radicals.

Extracts of *Brassica* are also used as antimicrobial agents. The antibacterial activity of ethanol, methanol and acetone extracts obtained from *Brassica oleracea* was investigated against *Salmonella* Abony, *Pseudomonas aeruginosa*, *Listeria monocytogenes* and *Enterococcus faecalis* (12). Blazevic *et al.* (13) tested the antibacterial activity of aqueous extracts of *Aurinia sinuata* (L.) Griseb. (a genus of flowering plant from *Brassicaceae* family) against *Staphylococcus aureus*, *Bacillus cereus*, *Clostridium perfringens*, *Enterococcus faecalis*, *Micrococcus luteus*, *Aeromonas hydrophila*, *Chryseobacterium indologenes*, *Enterobacter sakazakii*, *Enterobacter cloacae*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Pseudomonas luteola* and *Vibrio vulnificus*.

However, the biological activity of extracts obtained from plant species is related to the extraction method because each method affects the selectivity of the compounds. In this way, novel extraction techniques, such as the ultrasound-assisted method, have been used to obtain plant extracts. The use of ultrasound can increase cell wall destruction, cause leakage of cellular material, enhance the penetration of solvent into plant cells, facilitate hydration and swelling, and improve mass transfer. These phenomena can increase the extraction of antioxidants while significantly reducing the extraction time, thus improving overall efficiency. Ultrasound-assisted extraction (UAE) is versatile and can be performed on small and large scale (14–16). Li *et al.* (17) evaluated ultrasound-assisted extraction of carotenoids, Pingret *et al.* (18) investigated ultrasound-assisted water extraction of polyphenols from apple pomace, while Achat *et al.* (19) evaluated the enrichment of olive oil with oleuropein by ultrasound-assisted maceration.

Therefore, the objective of this study is to evaluate the antioxidant and antimicrobial activities of *Brassica oleracea* var. *capitata* (white cabbage) extracts obtained using ultrasound-assisted extraction. First, the extraction conditions were optimized, and then the extracts were submitted to different hydrolysis conditions before their use in the biological evaluations. The crude and hydrolysed extracts were characterized using gas chromatography coupled with mass spectrometry (GC-MS).

## Materials and Methods

### Chemicals

Sodium hydroxide, 4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid (HEPES), 2,2'-azobis(2-aminodipropyl) hydrochloride (ABAP), 2',7'-dichlorofluorescein (DCFH<sub>2</sub>-DA), xanthine oxidase (XOD) 25 UN, hypoxanthine (HPX), nitrotriazolium blue chloride (NBT) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) were obtained from

Sigma-Aldrich, St. Louis, MO, USA. Dimethyl sulphoxide (DMSO), potassium chloride and magnesium chloride were obtained from Isofar® (Rio de Janeiro, Brazil). Sodium carbonate was obtained from Merck (Darmstadt, Germany). Ethylenediaminetetraacetic acid (EDTA) was obtained from Nuclear® (Diadema, Brazil). Ultrapure water was obtained from a Milli-Q system UV Synergy® (Millipore SA, Molsheim, France). The solvents methanol, acetonitrile and anhydrous ethanol, all of HPLC grade, were obtained from the Tedia Company (Fairfield, OH, USA). The microorganisms used in the tests (*Staphylococcus aureus* ATCC 29213 and *Escherichia coli* ATCC 14948) were obtained from the Laboratory of Immunobiology and Molecular Glycobiology, Federal University of Viçosa, Viçosa, Brazil.

### Samples

*Brassica oleracea* var. *capitata* (white cabbage) was bought in a local supermarket in Santa Maria, Rio Grande do Sul, Brazil, in January 2011. The sample was dried in an oven with air circulation at 60 °C for 72 h, according to Tanongkankit *et al.* (20). Next, the material was ground and stored at room temperature under a nitrogen atmosphere prior to extraction.

### Ultrasound-assisted extraction

Experiments were performed in a reactor with a thermostatic water bath (temperature accuracy of  $\pm 1.0$  °C). The experimental setup consisted of an ultrasonic bath (model USC 1800A, Unique Inc., Indaiatuba, SP, Brazil) equipped with a transducer with longitudinal vibrations. The ultrasonic unit had an operating frequency of 40 kHz and a maximum ultrasound power output of 132 W. The ultrasonic transducer (surface area of 282.2 cm<sup>2</sup>) was fitted at the bottom of the bath horizontally along the length of the bath, corresponding to an ultrasonic intensity of 0.46 W/cm<sup>2</sup>.

For the optimization of the extraction conditions, a central composite rotational design (CCRD) with two independent variables was constructed to evaluate the influence of ethanol fraction and temperature on the extraction (21). Table 1 presents the investigated range for each independent variable. The extractions were performed in the ultrasonic bath for 120 min using an Erlenmeyer flask containing 2 g of dry material and 10 mL of an aqueous solution of ethanol. The same experiment was performed in duplicate in the presence and absence of ultrasound irradiation for comparison. In this paper, the evaluated response was the sum of the area of all compounds identified by HPLC. For comparison, an extraction was performed by substituting ethanol with methanol under the optimized experimental conditions because there is a difference in the polarity of these two solvents.

### Hydrolysis of the crude extracts

The hydrolysis of the crude extracts (methanolic and ethanolic) obtained using the best experimental conditions of the previous step was performed following the method proposed by Robbins *et al.* (22) with some modifications. Acid hydrolysis consisted of treating 2 mL of the crude extract with 1 mL of HCl (2 M) and then heating the

Table 1. Experimental conditions used to compare HPLC peak areas after 120 min in the presence and absence of ultrasound

Run	Temperature	$\varphi$ (ethanol) %	Peak area mAU	Peak area <sub>US</sub>	Peak area increase
	°C			mAU	%
1	36	32	107.75	177.81	39.4
2	64	32	105.90	198.99	48.8
3	36	88	73.81	174.51	57.7
4	64	88	67.57	158.17	57.3
5	30	60	237.90	494.25	51.9
6	70	60	186.87	231.89	19.4
7	50	20	74.62	118.87	37.2
8	50	100	35.44	46.71	24.1
9	50	60	126.86	167.33	24.2
10	50	60	142.43	184.39	22.8
11	50	60	137.44	164.59	16.5

$$\text{Peak area increase} = 100 - \left( \frac{\text{Peak area}}{\text{Peak area}_{\text{US}}} \right) \cdot 100$$

US=ultrasound, AU=arbitrary units

mixture to 95 °C for 1.5 h. Alkaline hydrolysis consisted of treating 5 mL of the extract with 5 mL of an alkaline solution (2 M NaOH, 10 mM EDTA and 1 % ascorbic acid). The reaction mixture was maintained at 30 °C under stirring for 30 min. Next, the pH was adjusted to 3.0 with 8 M HCl solution, and extraction with ethyl acetate (two 5-mL portions) using sonication for 20 min was performed. Organic solvent was removed using a rotary evaporator. The solid residue was resuspended in 2 mL of methanol and filtered (polyvinylidene fluoride, particle size 0.22 µm). Prior to testing for antioxidant and antibacterial activities, these extracts were neutralized.

### Chromatographic analysis

In the extraction step, high-performance liquid chromatography (HPLC) was employed to separate the compounds according to the method proposed by Leoni *et al.* (23) with some modifications. For this purpose, a Dionex HPLC (model P680, UV-VIS detector UVD-170 Dionex GmbH, Germering, Germany) equipped with a column ODS-18 (250 mm×5 µm, Thermo Scientific, Waltham, MA, USA) using a CH<sub>3</sub>CN/H<sub>2</sub>O 15:85 mixture as the eluent was used. A flow rate of 1.0 mL/min, wavelengths of 240 and 254 nm and a total run time of 15 min were employed.

The identification of compounds present in the crude and hydrolysed extracts was performed only in the optimized run of the experimental design (methanolic and ethanolic extracts) using gas chromatography coupled with mass spectrometry detector (GC-MS). The extracts were analyzed with a gas chromatograph (HP 6890, Agilent, Ramsey, MN, USA) interfaced with a mass selective detector (HP 5973, Agilent) with an automatic injection system (HP 6890, Agilent), using a capillary column HP-5ms (30 m×0.32 mm×0.25 µm). Helium was the carrier gas with a flow rate of 2 mL/min at a pressure of 0.35 bar and electronic impact mode of 70 eV. Samples of 1 µL were injected at 250 °C interface temperature with the fol-

lowing column temperature gradient programming: 70 °C for 1 min, then 12 °C/min up to 280 °C.

### Antioxidant activities of extracts

#### Radical DPPH scavenging activity

The analytical method by Zhao *et al.* (24), with modifications, used to measure the radical scavenging activity of crude and hydrolysed extracts of *Brassica oleracea* var. *capitata* against DPPH radical was based on the addition of 1500 µL of extract to 1480 µL of DPPH solution plus 20 µL of hydroethanolic solution. A blank assay was performed using 1500 µL of hydroethanolic solution instead of the extract. The resulting solution was left to rest for 30 min. Then, the absorbance of samples was determined at 522 nm using UV-VIS 8453 Hewlett-Packard spectrophotometer (Agilent Technologies, Santa Clara, CA, USA). The antiradical activity against DPPH (AA<sub>DPPH</sub>) was calculated according to the following equation:

$$\text{AA}_{\text{DPPH}} = \left( \frac{A_{\text{DPPH}} - (A - A_{\text{B}})}{A_{\text{DPPH}}} \right) \cdot 100 \quad /1/$$

where  $A_{\text{DPPH}}$ ,  $A$  and  $A_{\text{B}}$  are the absorbance of the DPPH solution, sample and blank, respectively.

#### Superoxide anion radical scavenging activity

The antiradical activity of *Brassica oleracea* var. *capitata* extracts, obtained by ultrasound-assisted extraction, against O<sub>2</sub><sup>-</sup> radicals was evaluated by the enzymatic system HPX/XOD (24). For this purpose, 100 µL of EDTA (30 mmol/L), 100 µL of HPX (3 mmol/L) and 200 µL of NBT (1.42 mmol/L) were mixed with 100 µL of extract. After 3 min, 100 µL of enzyme XOD (0.75 U/mL, diluted in phosphate buffer) were added. The final volume of the solution was brought to 3 mL with phosphate buffer (0.05 mol/L, pH=7.4). The blank sample was prepared in the same manner but without the NBT. Additionally, a control test was performed containing all reagents with the solvent employed in the samples and a blank control. After 40 min of reaction, the absorbance of the samples was measured using UV-VIS 8453 Hewlett-Packard spectrophotometer (Agilent Technologies) at 560 nm. The antiradical activity against O<sub>2</sub><sup>-</sup> (AA<sub>O<sub>2</sub><sup>-</sup></sub>) was calculated according to the following equation:

$$\text{AA}_{\text{O}_2^-} = \left( 1 - \frac{(A - A_{\text{B}})}{(A_{\text{C}} - A_{\text{BC}})} \right) \cdot 100 \quad /2/$$

where  $A$  and  $A_{\text{B}}$  are the absorbance of the sample and blank, respectively, and  $A_{\text{C}}$  and  $A_{\text{BC}}$  are the absorbance of the control and blank control, respectively.

#### Peroxy radical anion scavenging activity

The antiradical activity of *Brassica oleracea* var. *capitata* extracts, obtained by ultrasound-assisted extraction, against ROO<sup>-</sup> radicals was evaluated using the fluorimetric method using DCFH2-DA as a substrate (25). A plate containing 96 wells was subdivided into two regions: the first region corresponded to lines A, B, C and D, and the second region corresponded to lines E, F, G and H. In the first three wells of each region 10 µL of solvent were added to the samples. In the remaining wells of the plate, 10 µL of the extract were added. Then, 127.5 µL of buffer were

added to all wells of the plate. Subsequently, 7.5  $\mu\text{L}$  of ultrapure water were added to all wells of region 1, whereas 7.5  $\mu\text{L}$  of ABAP (4 mmol/L) were added to the wells of region 2. Before the analysis, 10  $\mu\text{L}$  of DCFH2-DA (16  $\mu\text{mol/L}$ ) were added. A Vitor 2 fluorimeter (PerkinElmer, Waltham, MA, USA) was programmed to maintain the temperature at 37  $^{\circ}\text{C}$  and to measure the fluorescence at 485 nm (excitation) and 520 nm (emission) in regular time intervals of 5 min for 30 min. The antiradical activity against  $\text{ROO}^{\cdot}$  ( $\text{AA}_{\text{ROO}^{\cdot}}$ ) was calculated after 30 min according to the following equation:

$$\text{AA}_{\text{ROO}^{\cdot}} = \left( 1 - \frac{(F_A - F_{AB})}{(F_S - F_{SB})} \right) \cdot 100 \quad /3/$$

where  $F_A$  and  $F_{AB}$  are the fluorescence of the sample containing ABAP and the fluorescence of the blank without ABAP, respectively, and  $F_S$  and  $F_{SB}$  are the fluorescence of the solvent containing ABAP and the fluorescence of the solvent blank without the ABAP, respectively.

#### *Antibacterial activities (S. aureus and E. coli) of extracts*

The antimicrobial activity of crude and hydrolysed extracts of *Brassica oleracea* var. *capitata* was determined using the antibiogram with solid-phase (disc-diffusion) methodology. The extracts were resuspended in the extraction solvent (methanol or ethanol) at a concentration of 100 mg/mL. *Staphylococcus aureus* ATCC 29213 and *Escherichia coli* ATCC 14948 were inoculated on Müller-Hinton agar and kept in an oven maintained at  $(36 \pm 2)^{\circ}\text{C}$  for 24 h. As a positive control for *S. aureus* and *E. coli*, erythromycin (10  $\mu\text{g}/\mu\text{L}$ ) and ciprofloxacin (0.1  $\mu\text{g}/\mu\text{L}$ ), respectively, were applied in the same plate. The antimicrobial activity of the solvents (negative control) was also determined. The degree of sensitivity or resistance of a microorganism was determined by measuring the size of the antimicrobial effect zones (size of halo formed). Samples were examined in duplicate against each bacterium.

#### *Statistical analysis*

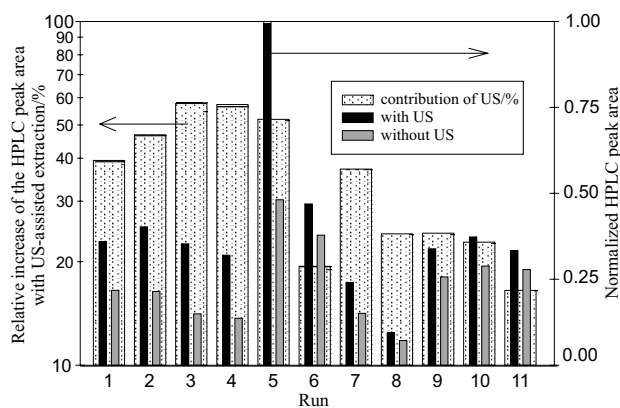
The statistical analysis was performed using ANOVA coupled with the Tukey's test at a 95 % confidence level using STATISTICA<sup>®</sup> v. 8.0. (StatSoft Ltd, Tulsa, OK, USA).

## Results and Discussion

#### *Ultrasound-assisted extractions*

The process variables studied in this work were temperature in the range of 30–70  $^{\circ}\text{C}$  and ethanol volume fraction in the range of 20–100 %. All experiments were performed in the presence and absence of ultrasound irradiation. Table 1 presents the results obtained with the CCRD concerning the total HPLC area of all compounds separated in the sample, which ranged from 35.44 to 237.90 mAU and from 46.71 to 494.25 mAU in the absence and presence of ultrasound, respectively. Considering the mean value obtained in the 11th run, significant differences ( $p < 0.05$ ) were detected between the amounts obtained in the absence and presence of ultrasound because the mean values were 137.44 and 164.59 units, respectively.

Fig. 1 presents the extraction profiles in the presence and absence of ultrasound expressed as normalized HPLC peak area. The right y-axis shows the normalized HPLC peak area for both experimental sets, black bars show ultrasound extraction and grey without ultrasound, whereas the left y-axis indicates the contribution or increase (white dotted column) in percentage when ultrasound was applied. In all runs, a positive effect of ultrasound in comparison with conventional extraction (maceration) is clearly observed, and an average HPLC peak area increase of  $(36.1 \pm 15.5)\%$  was observed.



**Fig. 1.** Comparison between the presence and absence of ultrasound (US) in *Brassica* extraction after 120 min (right Y-axis) and the contribution/increase of the US (left Y-axis)

Note that the ultrasound effect can be associated with an increase in the mass transfer process during the reaction through the formation of cavitation bubbles, which provide an important benefit of opening up the surface of solid substrates. Consequently, at lower temperatures, the energy provided by the ultrasound is responsible for the improvement in the extraction rates, but at higher temperatures thermal energy is sufficient and the ultrasound exhibits a positive effect on the extraction runs.

Based on the results presented in Table 1, the experimental ultrasound conditions for the extraction of bioactive compounds of *Brassica oleracea* var. *capitata* were verified in run 5 (30  $^{\circ}\text{C}$  and 60 %, by volume, of ethanol) in the presence of the ultrasound. Considering this result, an additional extraction was performed in which ethanol was substituted with methanol, and the obtained peak area of the extracted sample was 330.5 mAU. In the following steps, only the crude and hydrolysed extracts obtained using methanol or ethanol were considered.

#### *Gas chromatography-mass spectrometry analysis of Brassica oleracea var. capitata extracts*

The crude and hydrolysed extracts of *Brassica oleracea* var. *capitata* obtained by ultrasound-assisted extraction (at 30  $^{\circ}\text{C}$  and 60 %, by volume, of ethanol or methanol) were analyzed with a gas chromatograph interfaced with a mass selective detector. The composition of the extracts obtained in this work was expressed as the percentage of the normalized peak areas, which are presented in Table 2. It was possible to identify 10 compounds, including derivatives of fatty acids, sulphur compounds and glyco-

Table 2. Chemical profile of crude and hydrolysed extracts of *Brassica oleracea* var. *capitata* obtained by ultrasound-assisted extraction

Solvent	Extract	<i>t</i> (retention)/min; Normalized peak area/%									
		pentanoic acid	2-furan-carbox-aldehyde	L-glucose	phenol	ethyl $\alpha$ -D-glucopyranoside	desulpho-sinigrin	$\beta$ -D-glucopyranose	D-mannose	hexa-decanoic acid	octa-decanoic acid
Ethanol	crude	–	–	7.78; 49.00	10.32; 9.43	–	11.77; 3.00	12.24; 1.05	12.41; 2.00	–	19.84; 11.64
	acid hydrolysis	5.15; 5.75	7.16; 61.10	–	–	11.37; 8.52	–	–	–	–	–
	alkaline hydrolysis	–	–	11.82; 13.00	–	–	10; 18.95	–	–	14.45; 3.35	–
Methanol	crude	–	–	7.47; 0.50	–	–	–	–	12.5; 0.75	–	–
	acid hydrolysis	–	7.16; 35.10	–	–	10.9; 41.50	–	11.2; 2.50	–	–	–
	alkaline hydrolysis	–	–	–	–	–	–	–	–	14.45; 4.77	–

sides. The crude ethanol was the sample with the highest number of identified compounds. Desulphosinigrin and octadecanoic acid are among the compounds of interest.

Although flavonoids and glucosinolates, which are usually found in *Brassica* sp., were not determined in the extracts, a significant amount of glycosides, which are associated with flavonoids and phenolic acids, were found. The presence of these glycosides can indicate the presence of flavonoids in the sample. The hydrolysis of the extracts led to differences in the chemical profiles of the samples. For example, in both acid-hydrolysed extracts, the presence of 2-furancarboxaldehyde, which is a degradation product formed during the acid hydrolysis of glucose, was detected. The degradation of compounds when ultrasound is employed was well reported in the studies of Pingret *et al.* (26,27), who stated that acoustic cavitation might be responsible for initiating the formation of degradation products, which can trigger radical chain reactions and provoke substantial quality impairments in those products. The use of ultrasound can intensify the hydrolysis of flavonoids, leading to the identification of glycosides in the extract.

In addition, in these same samples, the presence of ethyl  $\alpha$ -D-glucopyranoside, which is a compound with a positive effect on the prevention of skin barrier disruption (28), was detected. In the crude and alkaline hydrolysis of ethanolic extracts, the presence of desulphosinigrin, a glucosinolate, was observed. Considering the content of fatty acids present in the extracts, hexadecanoic acid was the compound with the highest fraction, 3.35 and 4.77 % in runs 5 and 6, respectively. Fatty acids showed antioxidant (29) and antimicrobial activities (30), which were also evaluated in this study.

#### *Antioxidant activity of ultrasound extracts of Brassica oleracea* var. *capitata*

Table 3 presents the results concerning the antioxidant activities of the crude and hydrolysed extracts of *Brassica oleracea* var. *capitata* obtained using ultrasound-assisted extraction (at 30 °C and 60 %, by volume, of eth-

Table 3. Antioxidant activities of crude and hydrolysed extracts of *Brassica oleracea* var. *capitata* obtained by ultrasound-assisted extraction

Solvent extraction	Extract	Antioxidant activity/%		
		DPPH	O <sub>2</sub> <sup>•-</sup>	ROO <sup>•</sup>
Ethanol	crude	(13.0±0.4) <sup>ef</sup>	(35.6±4.0) <sup>ef</sup>	(98.6±0.1) <sup>a</sup>
	acid hydrolysis	(74.0±3.7) <sup>abcd</sup>	(45.3±2.9) <sup>d</sup>	(98.5±0.3) <sup>a</sup>
	alkaline hydrolysis	(70.8±2.1) <sup>cd</sup>	(55.1±4.1) <sup>c</sup>	(99.5±0.1) <sup>a</sup>
Methanol	crude	(14.0±0.6) <sup>ef</sup>	(61.1±5.0) <sup>ab</sup>	(97.4±0.2) <sup>a</sup>
	acid hydrolysis	(78.9±3.1) <sup>abc</sup>	(35.2±3.9) <sup>ef</sup>	(97.8±0.2) <sup>a</sup>
	alkaline hydrolysis	(80.0±4.0) <sup>abc</sup>	(63.1±3.2) <sup>ab</sup>	(89.3±2.8) <sup>b</sup>

a,b,c,d,e,f significant differences at 95 % (p<0.05, Tukey's test)

anol or methanol) against DPPH, O<sub>2</sub><sup>•-</sup> and ROO<sup>•</sup> radicals. All samples showed antioxidant activities, which ranged from 13.0 to 80.0, 35.2 to 63.1 and 89.3 to 99.5 % towards DPPH, O<sub>2</sub><sup>•-</sup> and ROO<sup>•</sup> radicals, respectively.

Concerning the antioxidant activity against DPPH radicals, both the methanolic and ethanolic hydrolysed extracts presented higher activities than the crude extracts. These results were statistically evaluated using the Tukey's test (p<0.05), and the hydrolysed extracts of both methanolic and ethanolic fraction did not present significant differences, even though the hydrolysis process was different.

Against the O<sub>2</sub><sup>•-</sup> radical, the crude methanolic extract had a 1.7-fold higher antioxidant activity than the crude ethanolic extract, which was significantly different (p<0.05). This result indicates that the solvent used in the extraction has an influence on the antioxidant activities of the extracts, consistent with the results obtained by Zhao *et al.* (24), who reported that extracts from a barley variety using 80 % methanol possessed the highest O<sub>2</sub><sup>•-</sup> scaveng-

ing activity, followed by water, 80 % acetone and 80 % ethanol extracts. The hydrolysis of ethanolic extracts had higher activity than the crude extract; however, the acid hydrolysis extracts had a lower activity than alkaline hydrolysis extracts, with the results being statistically significant ( $p < 0.05$ ). Considering the methanolic extracts, the acid hydrolysis extract had a lower activity than the crude extract, but the alkaline hydrolysis extract had a similar activity as the crude extract, where no significant difference was detected.

The crude and hydrolysed extracts of *Brassica oleracea* var. *capitata* obtained by ultrasound-assisted extraction showed the highest antioxidant activity against ROO<sup>•</sup> radicals, which ranged from 89.3 to 99.5 %. Only the alkaline hydrolysis of methanolic extract showed a significant difference compared to other extracts. The high inhibitory activity against ROO<sup>•</sup> radicals verified in all the tested extracts can be attributed to the fact that this radical is less reactive than the other extracts that have been studied (31). Another possibility is related to the high fractions of the extracts (20 %).

Vrchovská *et al.* (8) observed the antioxidant activity of aqueous extracts of *Brassica oleracea* var. *costata* against DPPH and O<sub>2</sub><sup>-</sup> radicals. The antioxidant activity of *Brassica oleracea* species (white cabbage and broccoli) obtained using different extraction methods, such as acetone/water 50:50 by volume (11), water extraction in combination with hexane extraction (32) and hexane/dichloromethane (1:1 by volume) in combination with acetone/water/acetic acid (70:29.5:0.5 by volume) (33), also presented activities against ROO<sup>•</sup> radicals. Podsedek (34) reported that the contribution of *Brassica* spp. vegetables to better health may be related to their antioxidant capacity.

Although several works have reported the antioxidant activities of extracts from different species of *Brassica* obtained using different extraction methods (5,7), there is no report on common ultrasound method for obtaining hydrolysed extracts. This paper demonstrates that the hydrolysis process can considerably improve the antioxidant activities of the extracts obtained with ultrasound-assisted extraction. For example, the antioxidant activity against DPPH radicals increased from 13 to 74 % after the acid hydrolysis of the extracts. A similar trend was observed against the O<sub>2</sub><sup>-</sup> radical, which increased from 35.6 to 55.1 % after the alkaline hydrolysis of the extracts. In this sense, the main contribution of this work was to demon-

strate that the hydrolysis of extracts from vegetable matrices can considerably enhance their antioxidant activity.

#### *Antimicrobial activity of crude and hydrolysed extracts of Brassica oleracea var. capitata*

The antimicrobial activity of the crude and hydrolysed extracts (methanolic and ethanolic) was determined against Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli*. Table 4 presents the experimentally determined antimicrobial activities of the extracts obtained in this work. Only the hydrolysed extracts showed antimicrobial activity, where the alkaline hydrolysis extracts showed activity against *Escherichia coli*, whereas only the acid-hydrolysed methanolic extract had the activity against *Staphylococcus aureus*. However, the inhibitory halo cannot be considered as a confirmation of antimicrobial activity unless its diameter is  $\geq 8$  mm (35).

The results obtained in this study are in good agreement with the literature; Gram-positive bacteria are more resistant than Gram-negative ones because they have thicker cell walls, ranging from 20–80 nm, whereas Gram-negative bacteria have a maximum wall thickness of 20 nm (36). Other authors have also evaluated the antimicrobial activity of *Brassica oleracea* extracts. Ayaz *et al.* (37) evaluated the antimicrobial activity of methanolic extracts of *Brassica oleracea* L. var. *acephala* using the same antimicrobial procedure and microorganisms as employed in this work, and verified that the extracts had better activity against *S. aureus* than against *E. coli*.

As verified from the antioxidant activities of the extracts, the hydrolysed extracts of *Brassica oleracea* var. *capitata* had higher antimicrobial activities than the crude extracts. This result can be attributed to a difference in the chemical profiles of the samples of the crude and hydrolysed extracts, as shown in Table 2. For example, alkaline hydrolysis enabled the identification of hexadecanoic acid, which shows antimicrobial activity (30), justifying the highest antimicrobial activity of this extract. Another reason for better performance of the hydrolysed extracts is that the extraction procedure used in this work resulted in the extraction of many glycosidic compounds, which have greater activity than the crude extracts after hydrolysis.

Table 4. Antimicrobial activities of crude and hydrolysed extracts of *Brassica oleracea* var. *capitata* obtained by ultrasound-assisted extraction

Solvent extraction	Extract	<i>S. aureus</i> halo/mm				<i>E. coli</i> halo/mm			
		1:1	1:2	1:4	1:8	1:1	1:2	1:4	1:8
Ethanol	crude	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	acid hydrolysis	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	alkaline hydrolysis	0.0	0.0	0.0	0.0	6.3±0.3	0.0	0.0	0.0
Methanol	crude	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	acid hydrolysis	4.5±0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	alkaline hydrolysis	0.0	0.0	0.0	0.0	8.5±1.4	5.3±0.3	0.0	0.0
No solvent	positive control	25.0	nd	nd	nd	18.0	16.0	nd	nd

nd=not determined

## Conclusions

This work presents important contribution to studying antioxidant activity using a combination of different extraction conditions which allow the identification of different compounds from *Brassica oleracea* var. *capitata*. Specifically, the optimization of the ultrasound-assisted extraction of *Brassica oleracea* var. *capitata*, the chemical characterization as well as the determination of antioxidant and antimicrobial activities of crude and hydrolysed extracts were successfully done. All extracts showed antioxidant activity against DPPH, O<sub>2</sub><sup>-</sup> and ROO<sup>·</sup> radicals; however, the hydrolysed extracts had considerably greater antioxidant activity. Antimicrobial activity was only detected in the hydrolysed extracts of *Brassica oleracea* var. *capitata*. Investigation of different ultrasound intensities and the possibility of combining them with other extraction methods is proposed for future investigation.

## Acknowledgements

The authors thank CNPq/Brazil, CAPES and FA- PERGS for financial support and scholarships.

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