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Pressurized liquid extraction applied for the recovery of phenolic compounds from beetroot waste

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ABSTRACT

Beetroot (*Beta vulgaris* L.) residues, leaves and stems, normally disposed as animal feed or compost, were investigated for the recovery of valuable compounds by pressurized liquid extraction (PLE) at temperature of 40 °C and pressures of 7.5, 10 and 12.5 MPa, and flow rate of 3 mL min^{-1}. This abundant residue, combined with the lack of related studies of beetroot waste by applying the PLE methods to obtain bioactive extracts is the main novelty of this work. Then, the objective was to explore the PLE process to enhance the value of the residues, evaluating process yield, total phenolic content (TPC) and antioxidant activity (DPPH, ABTS and FRAP methods) of the recovered extracts. Chemical composition was analyzed using LC-ESI-MS/MS and GC-MS analysis. Antioxidant potential results suggest the recovered extracts are comparable with synthetic antioxidant (BHT). Ferulic acid, vitexin and sinapaldehyde, were the most abundant phenolic compounds obtained from the extracts. These results indicate that PLE technique can be a good alternative for extracting phenolic compounds of beetroot resides with high biological potential that could be used in formulations for nutraceuticals, functional food or pharmaceutical industry.

1. Introduction

The beetroot (*Beta vulgaris L.)* is one of the most produced vegetables in Brazil, with 177.154 tons according to Brazilian Census of Agricultural, cultivated primarily for its roots, which have high nutritional value (IBGE, 2006; Lasta et al., 2019). The beetroot leaves and stems are commonly underutilized, at vegetable distribution centers or industrial units, and destined to animal feed, organic fertilizer or discarded as waste, despite their inherent value (Biondo et al., 2014). Then, this agro-industrial residue could be used as source of valuable components, and its recovery aids value to the processing chain, improving the management of an agro-waste (Narnoliya et al., 2017). Normally, beetroot aerial parts, which consist of leaves and stems, have a high content in iron, sodium, potassium, vitamin A, and Complex B, at levels significantly higher than those of roots (Tivelli et al., 2011). These waste contain fatty acids, especially polyunsaturated fatty acids, flavonoids, phytochemicals, carotenoids, triterpene saponins, betalains, betacyanins, betaxanthins, minerals and antioxidant compounds such as phenolic acids (Biondo et al., 2014; Chhikara et al., 2019; Koubaier et al., 2014). Some reports from literature have described that leaves and stems contain phytochemicals which are virus-inducible type 1 ribosome-inactivating proteins, possess diuretic, purgative, and anti-inflammatory activity and are useful in alleviating paralysis, spleen, and liver diseases (Iglesias et al., 2015; Sulakhiya et al., 2016). Therefore, more studies of these aerial parts may show valuable responses regarding the recovery of bioactive compounds with potential use as food additives and/or nutraceuticals.

The bioactivity of extracts recovered from natural products is dependent on its composition and consequently on the extraction process, solvent type and raw material characteristics. Solvents and extraction methods must be carefully selected to maximize yield and selectivity (Azmir et al., 2013). Traditional extraction methods, normally applied to recover bioactive extracts, present drawbacks such as high temperature, energy input and process time, combined with low selectivity, and also the probable use of large amounts of toxic solvents (Mazzutti et al., 2018; Mendiola et al., 2007). In this way, new methods have emerged, such as the pressurized liquid extraction (PLE), which is based on the use of solvents at high pressure and temperature, below the

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critical point to maintain the solvent in its liquid state, with objective to promote the extraction of compounds from solid or semisolid matrices in short time and with small amount of solvents (Viganó and Martinez, 2015). The small amount of solvents associated with a fast processes confer to PLE the recognition of a "green method" (Mendiola et al., 2007). The use of PLE using water:ethanol mixtures as solvent offers the potential to minimize or eliminate the use of toxic solvents and improve sample throughput via reduced extraction time (Armenta et al., 2017). Hence, PLE is an alternative to conventional methods, such as Soxhlet and maceration, since it provides good yields and high extract quality. Besides the recognized importance of *Beta vulgaris* L. waste, there is a lack of studies related PLE for the recovery of bioactive phenolic compounds from beetroot leaves and stems. Therefore, the present work has the objective to enhance the value of *Beta vulgaris* L. residues through the use of PLE at different conditions to obtain extracts with biological activities. The results obtained were evaluated in terms of process yield and product quality, represented by the antioxidant potential of the extracts. The liquid chromatographic analysis was also performed in order to evaluated the chemical profile of beetroot extracts.

2. Material and methods

2.1. Raw material

The beetroot residues, composed essentially by leaves and stems and collected in November and December 2015, were provided by "*Programa dos Trabalhadores Rurais Sem Terra*" (Chapecó, Santa Catarina, Brazil) with specimen deposited at Viveiro Florestal Universitário from UNOCHAPECO (Santa Catarina, Brazil). Leaves and stems were dried in a forced air circulation oven (DL-SE, DeLeo) at 45 °C for 10 h for the leaves and 24 h for the stems, reaching moisture and volatiles content of 8.48 g/100 g and 6.62 g/100 g, respectively, evaluated according to Lasta et al. (2019). Briefly, they were separated, water sanitized, wiped with paper towel and dried in a forced air circulation oven (DL-SE, DeLeo, Porto Alegre/RS, Brazil) up to a moisture content of 8.48 g 100 g^{-1} and 6.62 g 100 g^{-1} for the leaves and the stems, respectively, evaluated by 012 IV method (IAL - Adolfo Lutz Institute, 2008). Then, samples were ground in knife mill (DeLeo, Porto Alegre, RS, Brazil), with particle diameter of 0.258 mm and 0.267 mm, for leaves and stem, respectively, and packed and stored at −18 °C until use.

2.2. Extraction methods

2.2.1. Pressurized liquid extraction (PLE)

A self-assembled PLE apparatus, configured according to information previously presented by Mazzutti et al. (2018), was used for the extracts recovery. A fixed bed of particles was formed with 5 g of samples (beetroot leaves or stems) inside the extraction vessel (138.2 mL, internal diameter of 20 mm and a height of 440 mm). A kinetics evaluation of an overall extraction curve (OEC) from beetroot leaves was performed, at condition of 10 MPa, 40 °C and 3 mL min^{-1} (ethanol 99.8%; NEON, São Paulo, Brazil), in order to establish the extraction time.

Based on the research group expertise (Andrade et al., 2017; Mazzutti et al., 2018), the experiments were divided in three categories: (i) PLE for leaves with ethanol at 7.5, 10 and 12.5 MPa; (ii) PLE for stems with ethanol at 7.5 and 10 MPa; (iii) PLE for leaves at 10 MPa and with ethanol: distillated water mixtures of 90:10 and of 70:30 (v/v). PLE assays were conducted at 40 °C and constant flow rate of 3 mL min^{-1} by means of an HPLC pump. The extract samples, collected in glass flasks, were stored under refrigeration and absence of light for further solvent removal by rotary evaporator (Fisatom, model 801, São Paulo, Brazil). Then, stored at -18 °C in domestic freezer (Freezer 250, Brastemp, São Paulo, Brazil). All extractions were done at least in duplicate and the results expressed as mean ± standard deviation.

2.3. Global yield (X_0)

The global extraction yield (X_0) was calculated as percentage (%) of the mass extract ($m_{extract}$) relative to the total mass of the raw material, in a wet basis (m_{RM}) that was used to perform the extraction, according Eq. (1):

$$X_0(\%) = \frac{m_{Extract}}{m_{RM}} * 100 \quad (1)$$

2.4. In vitro determination

2.4.1. Total phenolic content

The total phenolic content (TPC) of the extracts from beetroot leaves and stem was determined according to Folin-Ciocalteu method (Koşar et al., 2005), with modifications. Briefly, the reaction mixture was composed of 0.1 mL of extracts solutions (concentration of 1667 mg L^{-1}), 7.9 mL of distilled water, 0.5 mL of Folin-Ciocalteu reagent and 1.5 mL of 20% sodium carbonate, in the test tubes. Samples were agitated and allowed to stand 2 h and the absorbance measured at 765 nm in a spectrophotometer (Femto, 800XI, Brazil). TPC was calculated using a standard compound. The assays were performed in triplicates and the average results were expressed as mg of Gallic acid equivalent (GAE) per g of the extract (mg GAE g^{-1}). The TPC values from the extracts were compared to BHT (butylated hydroxytoluene) performance determined by Cruz et al. (2017).

2.4.2. Free radical scavenging potential by DPPH assay

The free radical scavenging of the extracts from beetroot leaves and stems were evaluated using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method, as described by Mensor et al. (2001). Briefly, each extract was mixed with a 0.3 mM DPPH ethanol solution, to give final concentrations from 0 to 500 μg mL^{-1} for leaves and from 0 to 700 μg mL^{-1} for the stem. After 30 min at room temperature (25 °C), the absorbance values were measured at 517 nm and converted into percentage of antioxidant activity (AA%). This activity was also presented as the effective concentration at 50% (EC_{50}), i.e., the concentration of the solution required to give a 50% decrease in the absorbance of the test solution compared to a blank solution, and expressed in μg of extract mL^{-1} DPPH. The EC_{50} values were calculated from the linear regression of the AA% curves obtained for all extract concentrations. The AA% and EC_{50} for all extracts were obtained considering the average of triplicate assays. Also, the antioxidant capacity of the extracts was expressed as antiradical power (ARP), the inverse of EC_{50}, which is used to define antioxidant action of an antioxidant and it is a reciprocal of EC_{50}.

2.4.3. ABTS radical scavenging assay

This ABTS assay was carried out based on the procedure described by Re et al. (1999) with modifications. The synthetic vitamin E, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) (Sigma-Aldrich Co, USA) was used as antioxidant standard. The ABTS [2, 2-azino-bis-(3-ethylbenzotiazoline-6-sulfonic acid)] was dissolved in water to a concentration of 7.0 mMol and submitted to reaction with 140 mMol potassium persulfate to form the radical ABTS•+, that is reduced in the presence of an antioxidant compound hydrogen donor. The absorbance was measured at 734 nm 6 min after the initial mixing of samples and standard with the ABTS solution. Results were expressed as Trolox equivalent antioxidant potential (TEAC) (nM of a Trolox solution, which the AA is equivalent to the activity of 1.0 mgmL^{-1} of the sample solution). In order to find TEAC values, a separate concentration response curve for standard Trolox solutions was prepared. Results are present by average ± standard deviation of triplicate assays.

2.4.4. Ferric reducing antioxidant power (FRAP)

For the determination of the reducing ability of extracts a protocol based on the ferric reducing/antioxidant power (FRAP) assay was

developed, based on the procedure modified by Arnous et al., (2002) with adjustments. The reaction mixture was composed of 0.2 mL of extracts solutions (concentration of 0.002 mg L^{-1}) and 0.2 mL Ferric Chloride anhydrous (3 mM in 5 mM citric acid). For the blanks, 0.2 mL of ethyl alcohol P.A. was added instead of extracts solutions. The tubes with test solutions were manually shaken and placed in a water bath at 37 °C during 30 min and performed in triplicate.

Thereafter, 3.6 mL of TPTZ (2,4,6-tripyridyl-s-triazine, Sigma Aldrich) was added to the test tubes, vortexed and placed in an ice bath for 10 min and the absorbances were read in a spectrophotometer at 620 nm. In order to find the extracts reducing power (P_R) values, a standard curve of Trolox solutions was prepared (0–500 $\mu mol_{Trolox} \cdot g_{extract}$). Results were presented by average ± standard deviation of triplicate assays.

2.5. Chemical profile of extracts by gas chromatography–mass spectrometry

The identification and relative quantification of the volatile compounds present in the extracts were achieved by gas chromatography coupled to mass spectrometry analysis (GC–MS) according to Aguiar et al. (2014). Static headspace analysis was performed using a PAL Syr HS 2.5 mL for combi-PAL. For headspace sampling by syringe, autosampling system was used PAL COMBI-xt sample injector. The analyses were performed in a gas chromatograph coupled with a mass detector (GC 80 789A, MS 5975 MSD) and HP-5 mS 19091S-433 fused-silica capillary column (30 m × 0.250 mm) coated with 5% phenylpolymethylsiloxane (0.25 μm phase thickness) capillary column (Agilent Technologies). The temperature program was started at 35 °C for 2 min, rising to 80 °C at 2 °C.min^{-1}, to 150 °C at 4 °C.min^{-1}, to 230 °C at 8 °C.7 min^{-1}, then 230 °C (2 min). The injector temperature was 250 °C; the carrier gas was helium (1 mL min^{-1}); the injection mode was splitless; the sample volume injected was 1000 μL; the interface temperature 240 °C; and the acquisition mass range was 35–550 mz^{-1}. The identification of compounds extracted from the samples was performed by comparison with a library of spectral data (NIST Willey 2.0 and 7.0).

2.6. Identification and quantification of phenolic compounds by LC-ESI-MS/MS

The extract samples from beetroot leaves and stems, recovered according to procedure described in section 2.3 (by PLE method), were evaluated by chromatographic analysis in order to compared the phenolic profile with that from other extraction methods. The comparative extraction methods were the ones presented by Lasta et al. (2019), i.e., beetroot leaves and stems were submitted do Soxhlet (SOX) and Ultrasound-assisted extraction (UAE) with ethanol and ethanol/water as solvent, and Supercritical Fluid Extraction (SFE) with CO_2/ethanol 10% as solvent.

2.6.1. Sample preparation

The preparation of samples followed the protocol suggested by Schulz et al. (2015), with modifications. Briefly, defatted beetroot leaves and stems extracts were subjected to acid hydrolysis with 5 mL methanol and 5 mL of hydrochloric acid in an oven at 85 °C for 30 min. Then, the pH was adjusted to 2.0 using a 6 mol L^{-1}sodium hydroxide solution. Next, the acidified samples were partitioned with 10 mL of diethyl ether using a centrifuge at 4000 rpm for 10 min. This process was repeated two more times for each sample. The supernatants were combined in a bottom-round flask and the solvent was removed in a rotary evaporator at 40 °C until dryness. Then, the dried sample was resuspended in 1 mL of chromatographic grade methanol and diluted 10 times with methanol:water (30:70 v/v) for the injection in the LC-ESI-MS/MS system.

2.6.2. LC-ESI-MS/MS analysis

The identification and quantification of phenolic compounds were performed by high-performance liquid chromatography (LC) system (1200 Series, Agilent Technologies, Waldbronn-BW, Germany), following the methodology described bySchulz et al. (2015). A Synergi column (4.0 μm, 2.0 × 150 mm d.i.; Phenomenex, Torrance-CA, USA) was used for liquid chromatographic separation, under gradient elution condition. Mobile phases were composed of methanol:water (95.5% v/v – A), and aqueous solution of formic acid (0.1%, v/v - B). The separation was carried out at 30 °C using segmented elution gradient as follows: 0–5 min, 10% A; 5–7 min, 90% A; 7–10 min, 90% A; 10–17 min, 10% A. Between the analyses, the column was conditioned for 5 min with the proportion of the initial mobile phase of the separation. The running flow rate was 250 μL min^{-1}. Sample sizes of 10 μLwere injected. The LC system was coupled to a mass spectrometry system composed by a hybrid triple quadrupole/linear ion trap mass spectrometer (Q Trap 3200 Applied Biosystems/MDS Sciex, Concord-ON, Canada). The mass spectrometer was operated in negative electrospray ionization mode (TurboIonSpray Applied Biosystems/MDS Sciex, Concord-ON, Canada). The MS/MS parameters were: capillary needle maintained at −4500 V; curtain gas at 10 psi; the temperature at 400 °C; gas 1 and gas 2 at 45 psi; and CAD gas, medium. The software Analyst version 1.6.2 was used for the LC–ESI-MS/MS system control and data analysis. Parameters of mass spectrometer for each phenolic compound according to Schulz et al. (2015).

2.7. Statistical analysis

Global yield, TPC, antioxidant potential and phenolics profile results were statistically evaluated by a one-way analysis of variance (ANOVA) using the Software Statistica for Windows 7.0 (Statsoft Inc., USA). Pearson's test provided the correlation between TPC and antioxidant activity, whereas the significant differences ($p < 0.05$) were analyzed by Tukey test.

3. Results and discussion

3.1. Global extraction yield (X_0)

The global yield (X_0) results, for extracts from *Beta vulgaris* L. leaves and stems obtained by PLE performed at different conditions, are presented in Table 1. The extraction time was set at 90 min, based on kinetics curve, as defined at section 2.2.1.

The X_0 values for the leaves show the highest yields of 36.03 ± 0.04 and 23.24 ± 0.4 obtained for mixture ethanol:water 70/30 and 90/10, respectively. The highest yield values found for solvent mixtures, compared to pure ethanol as solvent, can be explained by the increase in solvent polarity with adding water to solvent mixture, increasing the solubility of different classes of components (carbohydrates, polyunsaturated fatty acids and other substances) from the raw material (Biondo et al., 2014; Mukhopadhyay and Panja, 2008), and decreasing selectivity. For beetroot leaves, the pressure increase from 7.5 to 10 MPa rise significantly ($p < 0.05$) the extraction yield using ethanol as solvent.

Table 1
Global yield (X0) of Beta vulgaris L. leaves and stem extracts obtained by PLE technique.

Extraction procedure	Pressure (MPa)	Solvent	Leaves	Stems
			X_0 (%, w/w)	
PLE	7.5	EtOH	$7.0^{d} \pm 0.3$	$9.7^{a} \pm 1.5$
40 °C	10	EtOH	$12.3^{c} \pm 0.9$	$9.9^{a} \pm 0.5$
		EtOH + H_2O (90/10)	$23.2^{b} \pm 0.4$	nd
		EtOH + H_2O (70/30)	$36.0^{a} \pm 0.04$	nd
	12.5	EtOH	$13.3^{c} \pm 0.2$	nd

Different superscript letters mean groups statistically different ($p < 0.05$) in each column for each raw material. nd: not determined.

The pressure increase facilitates the extractions of components trapped in the matrix pores, forcing the solvent to penetrate in the solid pores to solubilize the analytes (Richter et al., 1996). However, the pressure increase from 10 to 12.5 MPa showed no significant effect on extraction yield ($p > 0.05$).

Comparing Table 1 data with the previous study by Lasta et al. (2019), the X_0 values for beetroot leaves obtained by PLE with pure ethanol at different pressures were higher than ultrasound assisted extraction (UAE) (up to 3.3 times higher for PLE at 12.5 MPa), although lower than SOX extraction (with X_0 value of 21%).

The effect of water content on PLE solvent mixture was observed at 10 MPa, with an yield increase of 1.9 times from pure ethanol to 90/10 mixture and of 2.9 times using 70/30 ethanol:water mixture. These results may be related to the polarity increase of the solvent mixture with water, enhancing the interaction with the target compounds, which rises the component's solubility and consequently the extraction yield. Similar results for beetroot residues were obtained by Lasta et al. (2019) for UAE and SOX method with ETOH:H_2O mixture. These results are also in agreement with literature data for extraction process with ETOH:H_2O mixtures for different plant materials, which the increase in water concentration in solvent mixture increased the extraction efficiency compared with pure solvents (Machado et al., 2015; Pereira et al., 2016).

On the other hand, the X_0 values for the stems show no significant difference with pressure effect in PLE with ethanol (at 7.5 and 10 MPa). Comparing these data with the yield results by Lasta et al. (2019), the X_0 for PLE method was approximately 2 times higher than UAE values ($X_0 = 4.7\%$) and slightly superior than SOX results ($X_0 = 9.0\%$) for pure ethanol. Then, in general, the yield results of PLE provided good results, which enables this method as a good option to replace processes such as SOX and UAE, for the extraction from beetroot residues.

3.2. Total phenolic content (TPC) and antioxidant activity

Table 2 provides the results of total phenolic content (TPC) and antioxidant activity for all the extracts obtained from this study, and compared the values with obtained by the standard synthetic antioxidant BHT. The antioxidant potential was evaluated by three scavenging methods of DPPH, ABTS and FRAP.

The TPC results from samples obtained by PLE from beetroot leaves varied from 7 ± 1 to 252 ± 2 mg_{GAE} $g^{-1}_{extract}$. Among the different pressures and solvents (Table 2), the PLE with ethanol at 10 MPa showed the best performance (252 ± 2 mg_{GAE} $g^{-1}_{extract}$). The ethanol is more selective than mixtures of ethanol and water, leading to a higher concentration of phenols of moderate polarity in the extracts (de Aguiar et al., 2019). This is in agreement with Lasta et al. (2019) which observed the same behavior for sugar beet leaves and stems in TPC values by Soxhlet, ultrasound and maceration extractions. Moreover, Pearson correlation between TPC and X_0 showed a good correlation (-0.86) for beetroot leaves obtained by PLE. These results are consistent with previous study of Lasta et al. (2019) that reported a good correlation between TPC and X_0 for high-pressure extraction performed by Supercritical Fluid Extraction with CO_2 for beetroot leaves. Otherwise, for beetroot stems, the TPC results for samples obtained by PLE with ethanol show no significant difference with pressure and were much lower (up to 16 ± 1 mg_{GAE} $g^{-1}_{extract}$) than obtained by leaves, although consistent with values reported by Koubaier et al. (2014) for acetonitrile-soluble fraction extract of Tunisian red beet stems (*Beta vulgaris L. conditiva*) (10.4 mg_{GAE} $g^{-1}_{extract}$).

The DPPH, ABTS and FRAP assays were conducted to clarify different aspects of the antioxidant capacity from extracts from beetroot residues. The PLE at 7.5 and 10 MPa with pure ethanol provided good DPPH values (lower EC_{50}), close to the synthetic antioxidant BHT (Table 2) found by Cruz et al. (2017). According to Reynertson et al. (2005), EC_{50} values lower than 50 μg mL^{-1} are considered very active antioxidants. Similar to TPC behavior, the DPPH analyses show that the use of

Table 2
Total phenolic content (TPC) and antioxidant activities (AA) determined by DPPH, ABTS and FRAP methods, for *Beta vulgaris* L. leaves and stems the extracts.

Extraction method	Pressure (MPa)	Solvent	Leaves					Stems				
			TPC (mg_{GAE} g^{-1})[1]	DPPH EC_{50} (μG mL^{-1})	ARP^2 (10^{-2})	ABTS TEAC (μmol_{Trolox} g^{-1})[3]	FRAP P_R (μmol_{Trolox} g^{-1})[4]	TPC (mg_{GAE} g^{-1})[1]	DPPH EC_{50} (μG mL^{-1})	ARP^2 (10^{-2})	ABTS TEAC (μmol_{Trolox} g^{-1})[3]	FRAP P_R (μmol_{Trolox} g^{-1})[4]
PLE 40 °C	7.5	EtOH	$196^b \pm 3$	$58^d \pm 6$	1.72	$397^b \pm 6$	$271.5^a \pm 0.4$	$14^a \pm 2$	$394^a \pm 23$	0.25	$15.7^a \pm 1.2$	$18^a \pm 0.5$
	10	EtOH	$252^a \pm 2$	$65^d \pm 2$	1.54	$400^b \pm 14$	$275^a \pm 3$	$16^a \pm 1$	$515^b \pm 89$	0.19	$49.7^b \pm 0.2$	$18^a \pm 0.1$
		EtOH + H_2O (90/10)	$19^c \pm 2$	$470^a \pm 19$	0.21	$252^c \pm 1$	$18^c \pm 2$	nd	nd		nd	nd
		EtOH + H_2O (70/30)	$7^d \pm 1$	$304^b \pm 19$	0.32	$103^d \pm 1$	$10^c \pm 1$	nd	nd		nd	nd
	12.5	EtOH	$159^e \pm 7$	$120^c \pm 3$	0.83	$823^a \pm 48$	$101^b \pm 8$	nd	nd		nd	nd
Standard BHT[5]			$266.4 \pm 0.4^{(5)}$	$67 \pm 0.3^{(5)}$	1.49	$391.9 \pm 0.6^{(6)}$	$215 \pm 2^{(6)}$	$266.4 \pm 0.4^{(5)}$	$67 \pm 0.3^{(5)}$	1.49	$391.9 \pm 0.6^{(6)}$	$215 \pm 2^{(6)}$

Different superscript letters mean groups statistically different ($p < 0.05$) in each column for each raw material. (1) mg_{GAE} $g^{-1}_{extract}$; (2) antiradical power (ARP) inverse of EC_{50}; (3) μmol_{Trolox} $g^{-1}_{extract}$; (4) μmol_{Trolox} $g^{-1}_{extract}$; (5) Reported by (Cruz et al., 2017); (6) Reported by (Lasta et al., 2019); nd: not determined. TPC: Total phenolic content. EC_{50}: Effective concentration at 50% AA referent to DPPH method. TEAC: Trolox-equivalent antioxidant activity. P_R: Reducing power.

ethanol:water mixture in PLE processes for beetroot leaves did not improve the extract antioxidant potential. This could be associated to the presence of water-soluble compounds in the matrix, reducing the selectivity towards antioxidant compounds as provided by pure ethanol. The result of Antiradical Power (ARP) from Table 2, evidently, shown highest values by PLE with ethanol at 7.5 and at 10 MPa in comparison with other results for the beetroot leaves.

The ABTS results for PLE samples lay between 103 and 823 $\mu mol_{Trolox}\ g^{-1}_{extract}$ for beetroot leaves and from 15.7 to 49.7 $\mu mol_{Trolox}\ g^{-1}_{extract}$ for the stems. The highest ABTS value was obtained for PLE with ethanol at the higher pressure, i.e., at 12.5 MPa for leaves (823 $\mu mol_{Trolox}\ g^{-1}_{extract}$) and at 10 MPa for stems (49.7 $\mu mol_{Trolox}\ g^{-1}_{extract}$), probably due of a higher disruption of cellular structures, enhancing the recovery of antioxidant compounds. Additionally, the ABTS result for the leaves, by PLE with ethanol at 12.5 MPa, was higher than the values obtained by Lasta et al. (2019) for the same raw material using SOX and UAE methods ($586 \pm 82\ \mu mol_{Trolox}\ g^{-1}_{extract}$ for SOX with ethanol and $384 \pm \mu mol_{Trolox}\ g^{-1}_{extract}$ for UAE with ethanol), and also higher than the synthetic antioxidant (BHT) (Table 2). These data coincide with those obtained by other researchers that indicated better antioxidant activity of PLE samples compared to traditional methods (Viganó and Martinez, 2015; Wianowska and Gil, 2019). This suggests that the compounds responsible for the antioxidant activity, detected by ABTS analysis, are soluble in ethanol (Andrade et al., 2017). Though the ABTS and DPPH react by similar mechanisms, the reaction rates are different, which affect the antioxidant results, and therefore, it is necessary to combine more than one method for the antioxidant analysis (Rudke et al., 2019).

The antioxidant activity was also measured by FRAP method, which evaluates the antioxidant's ability to reduce ferric iron (Fe^{3+}) (Ozgen et al., 2006). The FRAP values for the samples from beetroot leaves obtained by PLE ranged from 10 to 275 $\mu mol_{Trolox}\ g^{-1}_{extract}$. The PLE results with ethanol at 10 and 7.5 MPa were higher compared to the BHT performance (Table 2) and to the values reported by Lasta et al. (2019) for SOX and UAE, i.e., ranging between 140 and 182 $\mu mol_{Trolox}\ g^{-1}_{extract}$. This behavior was similar to reported by TPC, DPPH and ABTS analyzes, which indicated ethanol as the best solvent for the recovery of antioxidant compounds from beetroot leaves (Lasta et al., 2019).

For extracts from beetroot stems, no significant difference ($p > 0.05$) was detected for FRAP values for PLE at 10 and at 7.5 MPa. These results for beetroot stems (Table 2) were similar to values reported by Lasta et al. (2019) for samples recovered by SOX with ethanol:water mixture (17 $\mu mol_{Trolox}\ g^{-1}_{extract}$). The difference in antioxidant activities for extracts from leaves and stems, obtained by PLE, may be related to the differences in plant matrices. The results from the present study strengthen the literature data in order to define beetroot residues (leaves and stems) as a good source of natural antioxidants (leaves extracts having better antioxidant activity than stems) (Chhikara et al., 2019; Koubaier et al., 2014). These wastes, besides considered as source of minerals and vitamins, may also be an alternative to provide natural antioxidant for further use in foods, food supplements or cosmetics.

The relative importance between phenolic compounds and antioxidant activity was evaluated using Pearson analysis to elucidate the understanding to the biological activity of the extracts. For the leaves extracts obtained by PLE, the results revealed a positive correlation between TPC and FRAP (0.71), suggesting that the antiradical action of these extracts may be associated with the TPC. These results are consistent with Paciulli et al. (2016) that reported a high correlation between FRAP and TPC results from beetroot slices using raw and high-hydrostatic pressure treated samples. Additionally, a low correlation coefficient was found between TPC and DPPH (-0.25) and between TPC and ABTS (-0.19). These low correlation values may be attributed to the complexity of the extracts (from beetroot residues), which present different groups of components that are responsible for the antioxidant activity, besides the antioxidant compounds with capacity for scavenging the DPPH and the ABTS radicals. Examples of these substances from beetroot residues are sugars, carotenoids, ascorbic acids or amine groups. Also, the higher correlation between TPC and FRAP, may suggest that this antioxidant method better elucidates the understanding to the biological activity of the extracts from beetroot residue.

3.3. Chemical profile

3.3.1. Gas chromatography

The gas chromatography analysis was performed for extracts from beetroot leaves obtained by PLE with ethanol at 10 MPa in order to evaluate the profile of the volatile fraction. This extract was selected because it provided the higher TPC result (Table 2). The chromatographic result identified the presence of 2-Ethyl-1-Hexanol (peak relative area of 2.54%), 2,2-dimethoxy-1,2-diphenyl-ethanone (peak relative area of 0.10%) and the 1,1,3-Trimethoxypropane (peak relative area of 0.55%).

The component 2-Ethyl-1-Hexanol is also called as isooctanol, an important substance used as a fragrance ingredient (McGinty et al., 2010), while 2,2-dimethoxy-1,2-diphenyl-ethanone (Benzil dimethyl ketal) is a highly efficient photoinitiator used for the photo polymerization of unsaturated prepolymers, e. g., using in UV curing technology (Prospector, 2019). Comparing with data from Lasta et al. (2019) for extracts from beetroot leaves obtained by SOX-EtOH, UAE-ethanol and SFE - 7% ethanol:water, it is observed that the results from the present work (by PLE) showed similar behavior to obtained by the polar extracts by SOX and UAE (all using ethanol as solvent). These results show low relation between volatile fractions and polar compounds, which suggest that the GC-MS analysis was not able to identify the components from the beetroot leaves extracts that contribute to its antioxidant activity. Therefore, the extract samples were submitted to liquid chromatographic analysis, as presented in the following section. The samples from beetroot leaves and stems analyzed by liquid chromatography (LC) were: PLE performed at 10 MPa with ethanol (best overall antioxidant performance), which were compared to the extracts from UAE (with ethanol:water), SOX (with ethanol:water) and SFE (with CO_2 and 10% ethanol:water) recovered by Lasta et al. (2019).

3.3.2. Identification and quantification of phenolic compounds by LC-ESI-MS/MS

Tables 3 and 4 shows the phenolic profile of extracts from beetroot leaves and stems, respectively. The results from leaves extracts, from Table 3, evidenced the presence of 33 phenolic compounds, mainly phenolic acids, within the 46 standard phenolics tested in the analysis, performed according to section 2.6. The PLE sample was the richest one, with 30 identified compounds, followed by UAE sample with 27, SFE sample with 10 and finally SOX sample with 7 phenolics compounds. On the other hand, the UAE sample provided higher amount of quantified phenolics (3.2958 mg $100\ g^{-1}$) compared to samples from other extraction methods. The main phenolic compound quantified in the samples, in relation to the total amount of compounds identified and quantified, was the Ferulic acid **(8)**, with 42.9% from UAE sample, 50.4% at PLE sample and 49.9% at SFE sample. The compounds Vitexin **(38)**, Isoquercetin **(28)**, Quercetin **(35)** and Sinapaldehyde **(42)** are also among the major compounds detected.

The results from stems extracts (Table 4) show that 24 phenolic compounds were detected, within the 46 standard phenolics tested in the analysis. PLE sample was the richest one, with 22 noticed compounds and with the higher amount of phenolics detected (0.6128 mg $100\ g^{-1}$), compared to samples from other extraction methods. The major compounds recovered from stems extracts were Resveratrol **(39)**, with 57.6% of total phenolic content from SFE sample, Ferulic acid **(8)** with 22.9% of total phenolic content from SFE sample, and Isoquercetin **(28)** with 37.8% of total phenolic content from UAE sample. The SOX method provided lower amount of phenolics identified by LC-ESI-MS/MS analysis for leaves and stems (0.1283 and 0,1332 mg. g^{-1}, respectively), probably due to the high temperatures used in the SOX, compared to other procedures. SFE also provided lower amount of phenolic

Table 3
Phenolic compounds (mg g^{-1} of extract) extracted of beetroot leaves obtained by different techniques.

	Phenolic Compounds	PLE 10 MPa EtOH (mg g^{-1})	UAE EtOH + H2O (mg g^{-1})	SOX EtOH + H2O (mg g^{-1})	SFE 25 MPa/40 °C 10% EtOH + H2O (mg g^{-1})
	Phenolic acids				
1	3.4 dihydroxybenzoic acid	0.0184 ± 0.0052^{a}	0.0170 ± 0.0010^{a}	nd	nd
2	4- aminobenzoic acid	<LOQ	nd	nd	nd
3	4-hydroxymethylbenzoic acid*	0.0079 ± 0.0012^{a}	0.0080 ± 0.0002^{a}	0.0147 ± 0.0004^{b}	0.0069 ± 0.0001^{a}
4	Caffeic acid	0.0600 ± 0.0101^{a}	0.0516 ± 0.0042^{a}	nd	nd
5	Cinnamic acid	nd	nd	nd	nd
6	Chlorogenic acid	0.0047 ± 0.0006^{a}	0.0046 ± 0.0004^{a}	0.0083 ± 0.0002^{b}	0.0043 ± 0^{a}
7	Ellagic acid	0.0430 ± 0.0084	<LOQ	<LOQ	nd
8	Ferulic acid	1.2781 ± 0.0916^{a}	1.4141 ± 0.1368^{a}	nd	0.0682 ± 0.0043^{b}
9	Gallic acid	<LOQ	0.0539 ± 0.0024	nd	nd
10	Mandelic acid	nd	nd	nd	nd
11	Methoxyphenylacetic acid*	nd	0.0359 ± 0.0586	nd	nd
12	p-Anisicacid	nd	nd	nd	nd
13	p-Coumaric acid	nd	nd	nd	nd
14	Rosmarinic acid	0.0035 ± 0.0053^{a}	0.0011 ± 0.0019^{a}	nd	nd
15	Salicylic acid	0.0466 ± 0.0023^{a}	0.0761 ± 0.0090^{b}	nd	nd
16	Sinapic acid	0.0663 ± 0.0044^{a}	0.0874 ± 0.0133^{a}	nd	nd
17	Syringic acid	0.0471 ± 0.0158^{a}	0.0442 ± 0.0026^{a}	nd	nd
18	Vanillic acid	0.0552 ± 0.0038^{a}	0.0792 ± 0.0088^{b}	0.0333 ± 0.0032^{a}	0.0032 ± 0.0044^{c}
	Flavonoids				
19	Apigenin	nd	nd	nd	nd
20	Aromadendrin	nd	nd	nd	nd
21	Catechin	nd	nd	nd	nd
22	Chrysin	nd	nd	nd	nd
23	Epicatechin	<LOQ	nd	nd	nd
24	Eriodictyol*	0.0001 ± 0.0001	<LOQ	nd	nd
25	Fustin	nd	nd	nd	nd
26	Galangin*	0.0017 ± 0.0033	<LOQ	nd	nd
27	Hispidulin*	0.0105 ± 0.0044	nd	nd	nd
28	Isoquercetin*	nd	0.3125 ± 0.0844	nd	nd
29	Isorientin*	nd	0.0095 ± 0.0014	nd	nd
30	Kaempferol	<LOQ	nd	nd	nd
31	Myricetin	0.0085 ± 0.0043^{a}	0.0073 ± 0.0021^{a}	0.0108 ± 0.0007^{a}	0.0054 ± 0.0001^{a}
32	Naringenin*	0.0006 ± 0.0006^{a}	0.0008 ± 0.0001^{a}	nd	nd
33	Naringin	nd	nd	nd	nd
34	Pinocembrin	nd	nd	nd	nd
35	Quercetin	0.0168 ± 0.0120^{a}	0.1793 ± 0.0146^{b}	<LOQ	<LOQ
36	Rutin	<LOQ	<LOQ	nd	nd
37	Taxifolin*	0.0046 ± 0.0002	nd	nd	nd
38	Vitexin	0.6557 ± 0.0024^{a}	0.6626 ± 0.0168^{a}	nd	0.0056 ± 0.0024^{b}
	Stilbene				
39	Resveratrol	0.0318 ± 0^{a}	0.0317 ± 0^{b}	0.0612 ± 0^{c}	0.0320 ± 0^{d}
	Phenolic Aldehydes				
40	Coniferaldehyde*	0.0009 ± 0.0001^{a}	0.0018 ± 0.0014^{a}	nd	nd
41	Sinapaldehyde*	0.1661 ± 0.0007^{a}	0.2094 ± 0.0312^{a}	nd	0.0076 ± 0.0009^{b}
42	Syringaldehyde	nd	nd	nd	nd
43	Vanillin	0.0083 ± 0.0017^{a}	0.0072 ± 0.0022^{a}	nd	0.0033 ± 0.0001^{a}
	Coumarin				
44	Scopoletin	nd	nd	nd	nd
45	Umbelliferone	0.0024 ± 0.0002^{a}	0.0006 ± 0.0018^{a}	nd	nd
	Phenolic Diterpene				
46	Carnosol*	<LOQ	nd	nd	nd
Total Phenolic Content (mgg^{-1})		**2.5317**	**3.2958**	**0.1283**	**0.1365**

<LOQ – not quantifiable. Results followed by the same letter in the row do not differ significantly (*t*-test, $p < .05$). nd: detected; * Reported for the first time.

identification, which is most probably associated to the lower solvent polarity, compared to solvents from other procedures.

It is worth mention that the **TPC** results for PLE 10 MPa EtOH samples (Leaves = 252 mg_{GAE} g^{-1}; Stems = 515 mg_{GAE} g^{-1}) (Table 2), estimated by Folin-Ciocalteu method, are higher than LC-ESI-MS/MS analysis (Leaves = 2.53 mg g^{-1}; Stems = 0.61 mg g^{-1}) (Tables 3 and 4). These results coincide with those reported by da Silva and Jorge (2017) and Lima et al. (2018) that observed the spectrophotometric analysis detects all the phenolic groups present in the sample, including extractable proteins.

According to the literature, a human diet including beetroot leaves and stems presents health benefits due to physiological action in disease prevention (Chhikara et al., 2019). Koubaier et al. (2014) reported the presence of the following phenolic compounds, gallic, vanillic, ellagic, chlorogenic, ferulic, protocatechuic, caffeic and syringic acids, as well as myricetin, quercetin and rutin (flavonoids), present in beetroot leaves and stems. According to Lorizola et al. (2018), the presence of different phenolic compounds confers the antioxidant properties of beetroots waste. Therefore, the higher number of phenolics detected in the present work, mostly the components in higher amounts like Ferulic acid **(8)**, Vitexin **(38)**, Isoquercetin **(28)** and Quercetin **(35)**, are also associated with prevention of chronic diseases (Inokuchi et al., 2016), chronic inflammation (Lorizola et al., 2018), cardiovascular disorders, diabetes, allergic reactions (Valentová et al., 2014), and can shield the liver from hepatotoxins damage (El-Gengaihi et al., 2016). Moreover, extracts from beetroot leaves and stems from the present work provided considerable amount of Resveratrol **(39)**, previously detected in beetroot leaves by Jacks et al. (2013). Several studies have demonstrated that resveratrol could have beneficial effects on cardiovascular diseases and anti-inflammatory and antioxidant properties (Frombaum et al., 2012).

Table 4
Phenolic compounds (mg g^{-1} of extract) extracted of beetroot stems obtained by different techniques.

	Phenolic Compounds	PLE 10 MPa EtOH (mg g^{-1})	UAE **EtOH + H2O (mg g^{-1})**	SOX **EtOH (mg g^{-1})**	SFE 10% **EtOH (mg g^{-1})**
	Phenolic acids				
1	3.4 dihydroxybenzoic acid	0.0205 ± 0.0091	nd	nd	nd
2	4- aminobenzoic acid	nd	nd	nd	nd
3	4-hydroxymethylbenzoic acid*	0.0166 ± 0.0001[a]	0.0068 ± 0.0001[b]	0.0190 ± 0.0006[a]	0.0681 ± 0.0030[c]
4	Caffeic acid	<LOQ	<LOQ	nd	nd
5	Cinnamic acid	nd	nd	nd	nd
6	Chlorogenic acid	0.0104±0[a]	0.0044 ± 0.0002[b]	0.0118 ± 0.0001[c]	0.0416 ± 0.0002[d]
7	Ellagic acid	<LOQ	0.0318 ± 0.0024	nd	nd
8	Ferulic acid	0.1405 ± 0.0324	nd	nd	nd
9	Gallic acid	<LOQ	0.0038 ± 0.0032	nd	nd
10	Mandelic acid	nd	nd	nd	nd
11	Methoxyphenylacetic acid*	0.0735 ± 0.0141	<LOQ	nd	nd
12	p-Anisic acid	nd	nd	nd	nd
13	p-Coumaric acid	nd	nd	nd	nd
14	Rosmarinic acid	nd	nd	nd	nd
15	Salicylic acid	nd	nd	nd	nd
16	Sinapic acid	0.0221 ± 0.0012	nd	nd	nd
17	Syringic acid	0.0572 ± 0.0114	nd	nd	nd
18	Vanillic acid	0.0772 ± 0.0287[a]	0.0193 ± 0.0011[a]	nd	0.0663 ± 0.0109[a]
	Flavonoids				
19	Apigenin	nd	nd	nd	nd
20	Aromadendrin	nd	nd	nd	nd
21	Catechin	nd	nd	nd	nd
22	Chrysin	nd	nd	nd	nd
23	Epicatechin	<LOQ	nd	nd	nd
24	Eriodictyol*	<LOQ	<LOQ	nd	nd
25	Fustin	nd	nd	nd	nd
26	Galangin	nd	nd	nd	nd
27	Hispidulin*	nd	nd	nd	nd
28	Isoquercetin	nd	0.1169 ± 0.0310	nd	nd
29	Isorientin*	0.0310 ± 0.0040	<LOQ	nd	nd
30	Kaempferol	nd	nd	nd	nd
31	Myricetin	0.0136 ± 0.0007[a]	0.0057 ± 0.0005[b]	0.0147±0[a]	0.0521±0[c]
32	Naringenin	<LOQ	nd	nd	nd
33	Naringin	nd	nd	nd	nd
34	Pinocembrin	nd	nd	nd	nd
35	Quercetin	nd	<LOQ	nd	nd
36	Rutin	<LOQ	nd	nd	nd
37	Taxifolin*	nd	nd	nd	nd
38	Vitexin	0.0473 ± 0.0006[a]	0.0766 ± 0.0059[b]	nd	nd
	Stilbene				
39	Resveratrol	0.0781±0[a]	0.0318±0[b]	0.0877±0[c]	0.3109±0[d]
	Phenolic Aldehydes				
40	Coniferaldehyde*	0.0019±0[a]	0.0008 ± 0.0001[b]	nd	nd
41	Sinapaldehyde	0.0187 ± 0.0055	nd	nd	nd
42	Syringaldehyde	nd	nd	nd	nd
43	Vanillin	0.0042 ± 0.0022[a]	0.0111 ± 0.0035[a]	nd	nd
	Coumarin				
44	Scopoletin	nd	nd	nd	nd
45	Umbelliferone	nd	nd	nd	nd
	Phenolic Diterpene				
46	Carnosol	nd	nd	nd	nd
Total Phenolic Content (mgg^{-1})		**0.6128**	**0.3090**	**0.1332**	**0.5390**

< LOQ – not quantifiable. Results followed by the same letter in the row do not differ significantly (*t*-test, $p < .05$). nd: detected; * Reported for the first time.

Furthermore, as observed in Tables 3 and 4, the chemical profile of the extracts from beetroot leaves and stems resulted in the detection of 19 phenolics compound by LC-ESI-MS/MS analysis, for the first time associated with both these materials. Among these 19 phenolic compounds the main found for leaves was Isoquercetin (**28**) (0.31 mg g^{-1}) by UAE EtOH + H_2O process and for stems was Methoxyphenylacetic acid (**11**) (0.07 mg g^{-1}) by PLE 10 MPa EtOH process. Additionally, the components 3,4 dihydroxybenzoic acid **(1)**, salicylic acid **(15)**, sinapic acid **(16)**, epicatechin **(23)**, vanillin **(43)**, and umbelliferone **(45)** were previously association with beetroot plant, particularly for the pomace, pulp, peel root and seeds (Chhikara et al., 2019).

Despite the benefit evidences attributed to beetroot leaves and stems, few studies have investigated the effects of these mostly discharged parts of the vegetable, not only in terms of its inherent nutritional value, but also to reduce food waste (Lorizola et al., 2018). Therefore, to the best of our knowledge, no studies have been found regarding the recovery of phenolic compound from beetroot leaves and stems by means of PLE, an alternative greener technique, compared to traditional methods. In this context, the present study provides valuable information in compounds identification and antioxidant activity in association with beetroot aerial parts, which extracts may be further applied in nutraceuticals, functional foods and herbal formulations.

4. Conclusions

In this work, the bioactive compounds of extracts obtained by PLE at different conditions from beetroot residues was evaluated. In general, PLE was an efficient method in comparison to SOX, UAE and SFE

methods for the recovery of valuable components from beetroot leaves and stem. The PLE provided higher global extraction yield, phenolic content and antioxidant activity, especially for beetroot leaves. It was possible to identified, by liquid chromatography analysis, 33 and 24 phenolic compounds from beetroot leaves and stems, respectively, for the different extracts compared. The most abundant compounds recovered from beetroot residues were ferulic acid, vitexin, and sinapaldehyde, with potential application in nutraceuticals, functional food and herbal formulations. Considering that, the PLE enable the recovery of high amounts of phenolic compounds from beetroot residues, and it is a promising technique for the extraction of valuable substances, enhancing the value of beetroot residues.

5. Practical application

The practical application of the present study is related to the evaluation of valuable components recovered from beetroot waste using pressurized liquid extraction. This technique is a promising extraction method that use a green solvent for the extraction of bioactive compounds from an industrial residue. Beetroot leaves and stems are usually discarded resulting from the indoor markets, vegetable distribution centers and industrial production of beetroot canned, snacks or production of food colorings. Then, this work is a great alternative for adding value to waste, to obtain valuable phenolic components, beginning with the agricultural producer and going through the whole chain productive.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bcab.2019.101353.

References

Aguiar, M.C.S., Silvério, F.O., de Pinho, G.P., Lopes, P.S.N., Fidêncio, P.H., Ventura, S.J., 2014. Volatile compounds from fruits of Butia capitata at different stages of maturity and storage. Food Res. Int. 62, 1095–1099. https://doi.org/10.1016/j.foodres.2014.05.039.

Andrade, K.S., Trivellin, G., Ferreira, S.R.S., 2017. Piperine-rich extracts obtained by high pressure methods. J. Supercrit. Fluids 128, 370–377. https://doi.org/10.1016/j.supflu.2017.05.001.

Armenta, S., Esteve-Turrillas, F.A., Garrigues, S., Guardia, M. de la, 2017. Green Analytical Chemistry: the Role of Green Extraction Techniques, Comprehensive Analytical Chemistry. Elsevier Ltd. https://doi.org/10.1016/bs.coac.2017.01.003.

Arnous, A., Makris, D.P., Kefalas, P., 2002. Correlation of pigment and flavanol content with antioxidant properties in selected aged regional wines from Greece. J. Food Compos. Anal. 655–665.

Azmir, J., Zaidul, I.S.M., Rahman, M.M., Sharif, K.M., Mohamed, A., Sahena, F., Jahurul, M.H.A., Ghafoor, K., Norulaini, N.A.N., Omar, A.K.M., 2013. Techniques for extraction of bioactive compounds from plant materials: a review. J. Food Eng. 117, 426–436. https://doi.org/10.1016/j.jfoodeng.2013.01.014.

Biondo, P.B.F., Boeing, J.S., Barizão, É.O., Souza, N.E. De, Matsushita, M., Oliveira, C.C. De, Boroski, M., Visentainer, J.V., 2014. Evaluation of beetroot (Beta vulgaris L.) leaves during its developmental stages: a chemical composition study. Food Sci. Technol. 34, 94–101. https://doi.org/10.1590/s0101-20612014005000007.

Chhikara, N., Kushwaha, K., Sharma, P., Gat, Y., Panghal, A., 2019. Bioactive compounds of beetroot and utilization in food processing industry: a critical review. Food Chem. https://doi.org/10.1016/j.foodchem.2018.08.022.

Cruz, P.N., Pereira, T.C.S., Guindani, C., Oliveira, D.A., Rossi, M.J., Ferreira, S.R.S., 2017. Antioxidant and antibacterial potential of butia (Butia catarinensis) seed extracts obtained by supercritical fluid extraction. J. Supercrit. Fluids 119, 229–237. https://doi.org/10.1016/j.supflu.2016.09.022.

da Silva, A.C., Jorge, N., 2017. Bioactive compounds of oils extracted from fruits seeds obtained from agroindustrial waste. Eur. J. Lipid Sci. Technol. 119, 1–5. https://doi.org/10.1002/ejlt.201600024.

de Aguiar, A.C., da Fonseca Machado, A.P., Figueiredo Angolini, C.F., de Morais, D.R., Baseggio, A.M., Eberlin, M.N., Maróstica Junior, M.R., Martínez, J., 2019. Sequential high-pressure extraction to obtain capsinoids and phenolic compounds from biquinho pepper (Capsicum chinense). J. Supercrit. Fluids 150, 112–121. https://doi.org/10.1016/j.supflu.2019.04.016.

El-Gengaihi, S.E., Hamed, M.A., Aboubaker, D.H., Mossa, A.T.H., 2016. Flavonoids from sugar beet leaves as hepatoprotective agent. Int. J. Pharm. Pharm. Sci. 8, 281–286.

Frombaum, M., Le Clanche, S., Bonnefont-Rousselot, D., Borderie, D., 2012. Antioxidant effects of resveratrol and other stilbene derivatives on oxidative stress and NO bioavailability: potential benefits to cardiovascular diseases. Biochimie 94, 269–276. https://doi.org/10.1016/j.biochi.2011.11.001.

IAL - Adolfo Lutz Institute, 2008. Physical and Chemical Methods for Food Analysis. São Paulo, Brazil.

IBGE, 2006. Censo agropecuário [WWW document]. Censo agropecuário. https://sidra.ibge.gov.br (accessed 5.9.19).

Iglesias, R., Citores, L., Di Maro, A., Ferreras, J.M., 2015. Biological activities of the antiviral protein BE27 from sugar beet (Beta vulgaris L.). Planta 241, 421–433. https://doi.org/10.1007/s00425-014-2191-2.

Inokuchi, R., Takaichi, H., Kawano, T., 2016. Fluorometric quantification of ferulic acid concentrations based on deconvolution of intrinsic fluorescence spectra. Environ. Control Biol. 54, 57–64. https://doi.org/10.2525/ecb.54.57.

Jacks, A., Toikkanen, S., Pihlajasaari, A., Johansson, T., Hakkinen, M., Hemminki, K., Hokkanen, P., Käpyaho, A., Kärnä, A., Valkola, K., Niskanen, T., Takkinen, J., Kuusi, M., Rimhanen-Finne, R., 2013. Raw grated beetroot linked to several outbreaks of sudden-onset gastrointestinal illness. Finland 2010. Epidemiol. Infect. 141, 1640–1646. https://doi.org/10.1017/S0950268812002233.

Koşar, M., Dorman, H.J.D., Hiltunen, R., 2005. Effect of an acid treatment on the phytochemical and antioxidant characteristics of extracts from selected Lamiaceae species. Food Chem. 91, 525–533. https://doi.org/10.1016/j.foodchem.2004.06.029.

Koubaier, H.B.H., Snoussi, A., Essaidi, I., Chaabouni, M.M., Thonart, P., Bouzouita, N., 2014. Betalain and phenolic compositions, antioxidant activity of Tunisian red beet (Beta vulgaris L. conditiva) roots and stems extracts. Int. J. Food Prop. 17, 1934–1945. https://doi.org/10.1080/10942912.2013.772196.

Lasta, H.F.B., Lentz, L., Mezzomo, N., Ferreira, S.R.S., 2019. Supercritical CO 2 to recover extracts enriched in antioxidant compounds from beetroot aerial parts. Biocatal. Agric. Biotechnol. 19, 101169. https://doi.org/10.1016/j.bcab.2019.101169.

Lima, R. da S., Ferreira, S.R.S., Vitali, L., Block, J.M., 2018. May the superfruit red guava and its processing waste be a potential ingredient in functional foods? Food Res. Int. 0–1. https://doi.org/10.1016/j.foodres.2018.10.053.

Lorizola, I.M., Furlan, C.P.B., Portovedo, M., Milanski, M., Botelho, P.B., Bezerra, R.M.N., Sumere, B.R., Rostagno, M.A., Capitani, C.D., 2018. Beet stalks and leaves (Beta vulgaris L.) protect against high-fat diet-induced oxidative damage in the liver in mice. Nutrients 10. https://doi.org/10.3390/nu10070872.

Machado, A.P.D.F., Pasquel-Reátegui, J.L., Barbero, G.F., Martínez, J., 2015. Pressurized liquid extraction of bioactive compounds from blackberry (Rubus fruticosus L.) residues: a comparison with conventional methods. Food Res. Int. 77, 675–683. https://doi.org/10.1016/j.foodres.2014.12.042.

Mazzutti, S., Rodrigues, L.G.G., Mezzomo, N., Venturi, V., Ferreira, S.R.S., 2018. Integrated green-based processes using supercritical CO2and pressurized ethanol applied to recover antioxidant compouds from cocoa (Theobroma cacao) bean hulls. J. Supercrit. Fluids 135, 52–59. https://doi.org/10.1016/j.supflu.2017.12.039.

McGinty, D., Scognamiglio, J., Letizia, C.S., Api, A.M., 2010. Fragrance material review on 2-ethyl-1-hexanol. Food Chem. Toxicol. 48, S115–S129. https://doi.org/10.1016/j.fct.2010.05.042.

Mendiola, J.A., Herrero, M., Cifuentes, A., Ibañez, E., 2007. Use of compressed fluids for sample preparation: food applications. J. Chromatogr. A 1152, 234–246. https://doi.org/10.1016/j.chroma.2007.02.046.

Mensor, L.L., Menezes, F.S., Leitão, G.G., Reis, A.S., Santos, T.C. dos, Coube, C.S., 2001. Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. Phyther. Res. 15, 127–130. https://doi.org/10.1002/ptr.687.

Mukhopadhyay, M., Panja, P., 2008. Recovery of phytochemicals from kokum (Garcinia indica choisy) using pressurized hot water. Int. J. Food Eng. 4. https://doi.org/10.2202/1556-3758.1327.

Narnoliya, L.K., Jadaun, J.S., Singh, S.P., 2017. Management of agro-industrial wastes with the aid of synthetic biology, pp. 11–28. https://doi.org/10.1007/978-981-10-7434-9_2.

Ozgen, M., Reese, R.N., Tulio, A.Z., Scheerens, J.C., Miller, A.R., 2006. Modified 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) method to measure antioxidant capacity of selected small fruits and comparison to ferric reducing antioxidant power (FRAP) and 2,2′-diphenyl-1- picrylhydrazyl (DPPH) methods. J. Agric. Food Chem. 54, 1151–1157. https://doi.org/10.1021/jf051960d.

Paciulli, M., Medina-Meza, I.G., Chiavaro, E., Barbosa-Cánovas, G.V., 2016. Impact of thermal and high pressure processing on quality parameters of beetroot (Beta vulgaris L.). LWT - Food Sci. Technol. (Lebensmittel-Wissenschaft -Technol.) 68, 98–104. https://doi.org/10.1016/j.lwt.2015.12.029.

Pereira, R.G., Garcia, V.L., Nova Rodrigues, M.V., Martínez, J., 2016. Extraction of lignans from Phyllanthus amarus Schum. & Thonn using pressurized liquids and low pressure methods. Separ. Purif. Technol. 158, 204–211. https://doi.org/10.1016/j.seppur.2015.12.007.

Prospector, 2019. No Title [WWW Document]. Irgacure® 651. https://www.ulprospector.com/pt/eu/Adhesives/Detail/4958/203146/Irgacure-651 (accessed 5.9.19).

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans, C., 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic. Biol. Med. 26, 1231–1237. https://doi.org/10.1016/S0891-5849(98)00315-3.

Reynertson, K.A., Basile, M.J., Kennelly, E.J., 2005. Antioxidant potential of seven myrtaceous fruits. Ethnobot. Res. Appl. 3, 025. https://doi.org/10.17348/era.3.0.25-36.

Richter, B.E., Jones, B.A., Ezzell, J.L., Porter, N.L., Avdalovic, N., Pohl, C., 1996. Accelerated solvent extraction: a technique for sample preparation. Anal. Chem. 68, 1033–1039. https://doi.org/10.1021/ac9508199.

Rudke, A.R., Mazzutti, S., Andrade, K.S., Vitali, L., Ferreira, S.R.S., 2019. Optimization of green PLE method applied for the recovery of antioxidant compounds from buriti (Mauritia flexuosa L.) shell. Food Chem. 298, 125061. https://doi.org/10.1016/j.foodchem.2019.125061.

Schulz, M., Borges, G. da S.C., Gonzaga, L.V., Seraglio, S.K.T., Olivo, I.S., Azevedo, M.S., Nehring, P., de Gois, J.S., de Almeida, T.S., Vitali, L., Spudeit, D.A., Micke, G.A., Borges, D.L.G., Fett, R., 2015. Chemical composition, bioactive compounds and antioxidant capacity of juçara fruit (Euterpe edulis Martius) during ripening. Food Res. Int. 77, 125–131. https://doi.org/10.1016/j.foodres.2015.08.006.

Sulakhiya, K., Patel, V., Saxena, R., Dashore, J., Srivastava, A., Rathore, M., 2016. Effect of Beta vulgaris Linn. leaves extract on anxiety- and depressive-like behavior and oxidative stress in mice after acute restraint stress. Pharmacogn. Res. 8, 1. https://doi.org/10.4103/0974-8490.171100.

Tivelli, S.W., Factor, T.L., Teramoto, J.R.S., Fabri, E.G., Moraes, A.R.A. de, Trani, P.E., May, A., 2011. Beterraba: do plantio à comercialização. Boletim Técnico IAC, n, p. 210.

Valentová, K., Vrba, J., Bancířová, M., Ulrichová, J., Křen, V., 2014. Isoquercitrin: pharmacology, toxicology, and metabolism. Food Chem. Toxicol. 68, 267–282. https://doi.org/10.1016/j.fct.2014.03.018.

Viganó, J., Martinez, J., 2015. Trends for the application of passion fruit industrial by-products: a review on the chemical composition and extraction techniques of phytochemicals. Food Public Health 5, 164–173. https://doi.org/10.5923/j.fph.20150505.03.

Wianowska, D., Gil, M., 2019. Accepted Manuscript Critical approach to PLE technique application in the analysis of secondary metabolites in plants. Trends Anal. Chem. 114, 314–325. https://doi.org/10.1016/j.trac.2019.03.018.