Recovery of bioactive phenolic compounds from papaya seeds agroindustrial residue using subcritical water extraction

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ARTICLE INFO

ABSTRACT

The novelty of this study was the use of a subcritical water unit to recover phenolic compounds from papaya seeds, a relevant agroindustrial waste. The effect of temperature conditions on subcritical water extraction (SWE) was compared to Soxhlet extraction (SOX) in terms of total yield, phenolic content (TPC), Maillard reaction products (MRPs), and antioxidant activity, detected by DPPH and β-carotene bleaching methods. TPC and MRPs values were maximized by SWE at 150 °C for 5min, which provided the best antioxidant potential by DPPH method. Liquid chromatography analysis of papaya seed extracts identified 30 phenolic compounds within the samples tested. The main compounds were ferulic acid, mandelic acid, and vanillic acid. It was also found that the subcritical water equipment was successfully applied for the recovery of bioactive compounds from papaya seeds in a simple and effective method.

1. Introduction

Brazil is the second largest world producer of papaya (*Carica papaya* L.), a tropical fruit from *Caricaceae* family, typical from subtropical regions (Dhekney et al., 2016). The fruit weight varies from 200 g to 3000 g, with approximately 15 %–20% of seeds (in wet weight) (Anwar et al., 2018). Since seeds are not consumed, more than 20% of the biomass, including seeds and peel, are discarded (Pathak et al., 2018), generating an enormous amount of wastes and by-products that cause environment organic pollution (Senrayan and Venkatachalam, 2018). Papaya seeds contain around 30% of oil, composed mostly by palmitic, stearic, oleic and linoleic acids, along with tocopherols and carotenoids with nutritional and functional properties (Anwar et al., 2018; Malacrida et al., 2012). This residue has been used as raw material for a various purposes, from biodiesel production and dietary additives (Senrayan and Venkatachalam, 2018) to substrate for yeast (Pathak et al., 2018). Papaya seeds also present in its constitution various phytochemicals such as carpaine, glucotropacolin, benzylisothyocyanate (BITC), caricin (sinigrin) and anzymemyosin (Vij and Prashar, 2015). The natural phytochemicals present in seeds are good source of antioxidant substances, which have been correlated to the plant secondary metabolites such as flavonoids, carotenoids, alkaloids, tannins, tocopherols, phytosterols and phenolic compounds (Alotaibi et al., 2017). Therefore, papaya seeds are a good source of valuable substances that can be used for the production of food additives or supplements (Pathak et al., 2018). Consequently, the development of processes that enable the use of papaya seeds for the production of useful nutraceutical or dietary components and high value food products are potentially prized.

Organic solvent extraction is the commonly used method for extraction of phenolic compounds, however, it requires long extraction time, and consumes high amount of solvent during extraction (Plaza and Turner, 2015). Furthermore, high purity solvents must be applied, which are not often easily available. To overcome this difficulty, innovative extraction methods and/or solvents are becoming especially interesting to promote a more efficient use of energy and resources (Herrero and Ibañez, 2018). Within this framework, water is the "greenest solvent" imaginable: it is readily available at the required purity, it is cheap, it is promptly recycled, non-toxic, non-flammable, and attend ecological requirements. Besides, Subcritical water extraction (SWE) is an attractive process that allow improving the extraction yield and reducing the extraction time using water rather than traditional solvents, extracting important phenolic compounds from diverse

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agricultural and food by-products (Erşan et al., 2018).

SWE uses the liquid solvent (water) under pressure and with a temperature range of 100–374 °C. The increase in temperature, above water boiling point, decreases significantly the subcritical water dielectric constant, which becomes similar to organic solvents, affecting its polarity and dissociation constant (Mazzutti et al., 2017; Plaza and Turner, 2015). These changes in the thermodynamic properties of water enhances the solubility of non-polar substances in water, increasing diffusivity and mass transfer rate due to the reduced viscosity and surface tension of water (Plaza et al., 2010a).

Subcritical water extraction has been increasingly used to extract value-added products (like phenolic compounds) from various plant materials such as pistachio hulls and red ginseng (Erşan et al., 2018; Lee et al., 2018). Bioactive polar compounds such as catechins, phenolic compounds, anthocyanins and flavonoids have been successfully extracted with subcritical water at moderate temperatures (125–175 °C), avoiding plant matter degradation (Plaza et al., 2010a). Nevertheless, to the best of our knowledge, there has been no work carried out to study the effect of SWE process variables to recover phenolic compounds from papaya seeds, a residue that represent near 20% of the fruit. Therefore, the aim of this study was the valorization of papaya seeds by recovering the bioactive phenolic compounds using water as solvent in two techniques, SWE and Soxhlet procedure. The effect of the extraction temperature was investigated on the antioxidant potential of the extracts, by DPPH and β-carotene models, and on Maillard reaction system, associated with the antioxidant compounds formation (Plaza et al., 2010b). The phenolic profile of the extracts was evaluated by liquid chromatographic analysis. Then, the SWE results were compared with traditional extraction methods in terms of product quality, to confirm the process suitability and its value for food process application.

2. Material and methods

2.1. Raw material

Papaya seeds were supplied by *Comercial Kazmierski* (Jaraguá do Sul, SC – Brazil). The raw material received at Laboratory of Thermodynamics and Supercritical Technology from Federal University of Santa Catarina (LATESC/UFSC) was handled according to Hall et al. (2018). The samples, with moisture content of $16.9 \pm 0.1\%$, were particulate and stored until use in polyethylene bags at -18 °C (in a domestic freezer).

2.2. Subcritical water extraction apparatus

A self-assembled SWE apparatus was used for the recovery of extracts from papaya seeds. The customized apparatus was built to perform subcritical water extractions based on Plaza and Turner (2015). The apparatus contains an extraction vessel of 90 mL (internal diameter of 25 mm and a height of 180 mm) made of AISI 316 stainless steel. The vessel was heated using an electrical heating jacket system (a 200 W resistance) and insulated with ceramic fiber. A pre-heater system (a 200 W resistance), with stainless steel coil, brings the water up to the operating temperature before entering the extraction vessel. The temperature of the extraction vessel and the pre-heater were controlled by an automatic temperature control system (model N1030, Novus, Porto Alegre, Brazil). A type-K thermocouple (model TM 201/15104-K, Salvi Casagrande, São Paulo, Brazil) allow the temperature monitoring inside the extraction vessel, by a temperature indicator (model N1500, Novus, Porto Alegre, Brazil). The extraction pressure was monitored by analogical manometers (model 23012, Salvi Casagrande, São Paulo, Brazil). The sonicated water was pumped by an HPLC pump (Waters, model 515, USA) into the extraction cell, where the pressure was controlled by a needle valve (model 20-11LF4, NFA, HIP, USA). The system includes a nitrogen gas (White Martins, Brazil) circuit, with

pressure regulator (model FRN-740, Famabras, Itaquaquecetuba, Brazil), a needle valve (model 15-11AF2, HIP, USA) to purge there maining solvent after the extraction, and a cooling fan (model ASA–12038DV-HB, ASAFAN, Cotia, Brazil), to reduce the water temperature in the vessel.

A schematic diagram of the SWE customized apparatus is illustrated in Fig. 1, containing the basic constituents of a supplying pump, a pre-heating and a temperature controlled system, an extraction vessel, a pressure controlled valve and a collector flask. This equipment was self-assembled with an initial cost of US\$ 7,500, way below a SWE commercial unit. Although this custom-made unit does not contain a variety of process controllers, the equipment is simple, flexible and provide reproducible results. Therefore, the main characteristics of this unit is the low cost associated with an easy operation.

2.2.1. SWE procedure

The SWE was conducted in a continuous flow mode using papaya seeds as raw material. Concisely, each experiment consists of loading 5.0 g of grounded papaya seeds, mixed with 64 g of glass spheres, to form a fixed bed of particle inside the stainless steel extraction vessel. The solvent, sonicated distilled water, was pumped by the HPLC pump directly into the extraction cell. The extraction time was defined by kinetic study, performed to obtain the overall extraction curve (OEC), where extract samples were collected at pre-established time intervals in a process conducted at 10 MPa, 110 °C and solvent flow rate of $4 \, mL \, min^{-1}$. Based on OEC, the SWE time was fixed at 5 min, according to results presented in section 3.1. Following the kinetics study, the effect of extraction temperature was evaluated by the yield assays, performed in triplicate at temperatures of 70, 90, 110, 130 and 150 °C, and at fixed water flow rate at $4 \, mL \, min^{-1}$ and pressure of 10 MPa (Mazzutti et al., 2017). The recovered extracts were collected in glass flasks and rapidly cooled by the cooling fan, and stored under refrigeration and absence of light for further solvent removal by freeze-dryer for 24 h (Liotop, model LD101, São Paulo, Brazil).

2.3. Conventional extraction

A conventional low-pressure extraction was carried out using Soxhlet technique (SOX) with water as the solvent, performed by 920.39C method of AOAC (AOAC, 2005), at least in duplicate. The procedure consisted of 150 mL of solvent recycling over 5 g of dried raw material,
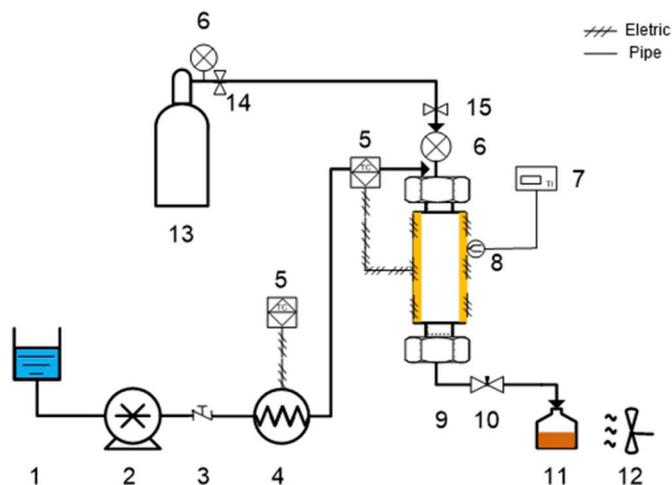

Fig. 1. Schematic design of the customized SWE unit: 1) Water reservoir; 2) Solvent pump; 3) Check Valve; 4) Heat exchanger; 5) Automatic temperature controller; 6) Manometer; 7) Temperature indicator; 8) Thermocouple; Regulator needle valve; 9) Extraction vessel with electrical heating jacket; 10) Regulator needle valve; 11) Glass flask; 12) Cooler; 13) N$_2$ cylinder; 14) Regulator valve; 15) Block valve.

in a Soxhlet apparatus for 6 h at the solvent boiling temperature. Then, the obtained extracts were filtered and the solvent was removed at 40 °C by rotary vacuum evaporator. Extracts were stored in amber flasks at -18 °C prior to analysis.

2.4. Extraction yield (X_0)

Extraction yield (X_0) was calculated as a percentage of the mass of extract ($m_{Extract}$) relative to total mass of raw material, in wet basis (m_{RM}), according to Equation (1):

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

2.5. Total phenolic content (TPC)

Total phenols content (TPC) of papaya seed extracts was determined by Folin–Ciocalteu method (Koşar et al., 2005), with modifications. Shortly, 10 μL of extract water solution (10 mgmL^{-1}) and 600 μL water were mixed to 50 μL undiluted Folin–Ciocalteu reagent (Sigma-Aldrich, USA). After 1min, 150 μL of Na$_2$CO$_3$20% (*w/v*) was added and the volume completed to 1 mL with water. The samples were incubated for 2 h at 25 °C in darkness. Spectrophotometer absorbance was evaluated at 760 nm, with calibration by a standard curve of Gallic acid solutions. The results were expressed in mg of Gallic acid (GAE) per g of extract, from triplicate measurements.

2.6. DPPH free radical scavenging

The free radical scavenging of the papaya seeds extracts was evaluated by 1,1-diphenyl-2-picrylhydrazil (DPPH) method (Mensor et al., 2001). Briefly, different extract concentrations were tested (five concentrations for each extract) by mixing DPPH solution (975 μL) and 25 μL extract solution. The absorbance was observed at 517 nm after 30min in darkness and at room temperature. The results, mean values of triplicates, were expressed as EC$_{50}$ (test concentration required to reduce 50% in absorbance compared to a blank solution), in μg mL^{-L}.

2.7. β-Carotene bleaching assay

The β-carotene/linoleic acid system followed Matthäus (2002) with modifications. Briefly, 40 μL of linoleic acid and 1 mL of β-carotene solution (1 mgmL^{-1} in chloroform) was mixed with 400 mg Tween 20. Chloroform was removed at 40 °C by vacuum rotary evaporator. Then, the mixture was immediately diluted to 100 mL with aerated distilled water and vigorously shaken for 3 min, establishing an emulsion, and adding 50 μL of extract (5 mg mL^{-1}). A control sample, replacing the extract with water, was prepared. Absorbance at 470 nm was immediately measured. After 120 min at 50 °C the absorbance was once more evaluated. The antioxidant activity of papaya seed extracts was calculated by Equation (2).

$$\text{Antioxidant activity } (\%) = \left[1 - \frac{Abs_{sam0} - Abs_{sam120}}{Abs_{con0} - Abs_{con120}}\right] * 100 \tag{2}$$

where Abs_{sam0} and Abs_{sam120} are the corrected absorbance values for test extract measured at zero time and after incubation, respectively; while Abs_{con0} and Abs_{con120} were corrected absorbance values for the control at zero time and after incubation, respectively.

2.8. Maillard reaction products (MRPs)

The products from the Maillard reaction, correspondent to the browning extent, was evaluated by Samaras et al. (2005) method, with modifications. Papaya seed extracts, obtained by SWE at different temperatures and by SOX, were diluted with water/dimethyl sulfoxide mixture (70:30). The 420 nm absorbance was measured using a 10 mm light path in a cuvette, as an indication of the degree of Maillard reaction. The analysis was performed in duplicate and the results expressed as absorbance ± standard deviation.

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

2.9.1. Sample preparation

The preparation of samples followed the protocol suggested by Schulz et al. (2015), with modifications. Briefly, defatted papaya seed 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.

2.9.2. 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 by Schulz 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 μL were 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).

2.10. Statistical analysis

A one-way analysis of variance (ANOVA) was conducted by Software Statistica (Statsoft Inc., USA) for extraction yield, TPC, antioxidant activity, Maillard reaction product, and phenolics profile. Pearson's test provided the correlation between TPC, antioxidant activity and Maillard reaction, while the Tukey test detected significant differences ($p < 0.05$).

3. Results and discussion

3.1. Kinetics assay

A kinetic study was conducted to define the time for the SWE. Fig. 2 shows the overall extraction curve (OEC) representing the accumulated mass with the extraction time, for an extraction performed at 110 °C, 10 MPa and flow rate of 4 mL min^{-1}. The condition of 10 MPa was kept constant, based on Mazzutti et al. (2017), since for SWE, the pressure has lower influence compared to temperature on solvent characteristics, and
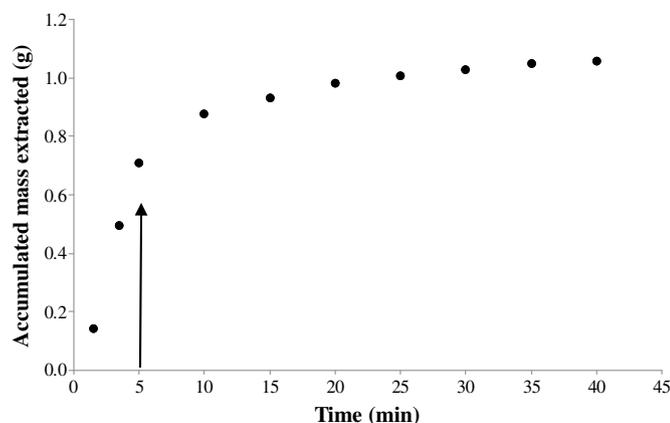
Fig. 2. Kinetics assays of papaya seeds at 10 MPa, 110 °C and 4 mL min^{-1}.

consequently, on process selectivity and efficiency. This behavior is explained because water is incompressible at temperatures below 300 °C, without significant pressure effect on the liquid physical properties (Gilbert-López et al., 2017; Plaza and Turner, 2015).

As can be observed from the OEC (Fig. 2), different mechanisms control the mass transfer. The extraction starts with convection as the dominant mechanism, with a constant extraction rate due to solute available on particle's surface, which is easily recovered by the solvent (fast desorption). With solute depletion from the particle's surface, compounds from inside the particles start to be extracted, and the diffusion mechanism takes place (Sovová, 1994). Considering the kinetics behavior, the extraction time for the group of yield assays was set at 5 min, representing the first linear part of the OEC, controlled by the convection mechanism (Ferreira and Meireles, 2002) due to the recovery of the easily accessible extract (on particles surface). This fast process, besides extracting around 70% of the solute (Brunner, 1994), also enables a process cost reduction, as an analogy from supercritical fluid extraction with CO_2. In addition, the constant extraction rate period enables the estimation of solubility of papaya seed extract in subcritical water, representing the slope of the linear line adjusted to experimental data which was defined as 0.013 $g_{extract}$ g_{water}^{-1}, performed according to Kitzberger et al. (2009). Therefore, SWE yield assays were conducted for 5 min of a dynamic process. After that, the flow rate was interrupted and the system drained, enabling the recovery of the extract inside the vessel and within the particles.

3.2. Extraction yield and TPC results

The yield results (X_0, w.b.) for the SWE performed at 10 MPa, different temperatures (70, 90, 110, 130, 150 °C) and extraction time of 5 min are presented in Table 1, together with the TPC values obtained by the different extracts. The results from SWE are also compared with Soxhlet (SOX) performance. Comparing Table 1 result at 110 °C (18.5%) with the OEC yield at 5 min (14%), we detect that the higher yield assay value is due to the drainage of the solute inside the vessel, after the 5 min

Table 1
Extraction yield and total phenol content (TPC) of papaya seed extracts obtained by different extraction techniques (Subcritical Water Extraction – SWE, Soxhlet – SOX). Different superscript letters mean groups statistically different ($p < 0.05$) in each column.

Extraction method		Extraction yield (%)	TPC (mg GAE g^{-1})
SWE	70 °C	14. 7 ± 0.6[a]	34.7 ± 0.4[a]
	90 °C	15.7 ± 0.4[a]	38.4 ± 2.0[a]
	110 °C	18.5 ± 0.4[b]	47.7 ± 1.0[b]
	130 °C	22.5 ± 0.4[c]	61.5 ± 4.0[c]
	150 °C	26.3 ± 0.6[d]	91.6 ± 3.0[d]
SOX		15.03 ± 0.01[a]	34.0 ± 1.0[a]

dynamic extraction.

From Table 1, SWE yield increases with temperature, reaching a maximum X_0 of 26.3 ± 0.6%, at 150 °C. On the other hand, SOX provided extraction yield of 15.03 ± 0.01%, a performance statistically similar ($p > 0.05$) to SWE at 70 and 90 °C. Therefore, in terms of yield, SWE at 70 and at 90 °Care analogous to SOX, although more efficient because SWE was performed in 5 min while SOX method took 6 h. Senrayan and Venkatachalam (2018) studied the solvent-assisted extraction of oil from papaya seeds using n-hexane. The results show the yield varying from 4.84 to 20.56%. Hall et al. (2018) verified yield up to 21.6% using supercritical $CO_2$for the extraction from papaya seeds.

As a polar solvent at room temperature, water solubilizes mostly polar compounds. In subcritical conditions, higher temperatures cause the drop of water polarity, increasing its ability to solvate compounds of medium or low polarity. The water dielectric constant decreases with enhancing temperature and, at 250 °C, it is close to methanol and ethanol values at 25 °C. At high temperatures, the diffusion rate and desorption kinetics also increase. Insoluble components, normally consisted of complex polymeric structures (proteins, polysaccharides etc.), can also be hydrolyzed during SWE, producing water-soluble products (amino acids, sugars), which increase the overall extraction yield (Ibañez et al., 2003). Therefore, these results show the ability of water at different temperatures toward the extraction of different compounds.

As verified above, the extraction yield increases with temperature. Nevertheless, at very high temperatures, target compounds are degraded or lost (Mazzutti et al., 2017). Also, because yield results are quantitative, and not qualitative, further analyses are necessary for the characterization of papaya seed extracts. Therefore, it is very important to optimize the extraction temperature in SWE, considering yield performance of target components. Phenolic compounds are a well-known class of antioxidants due to the hydroxyl groups in their structure, which stabilize the intermediate radicals by donating an electron and hydrogen atom (Cheynier, 2012). The TPC values from papaya seed extracts (Table 1) shown, at first glance, a positive influence of SWE temperature. Increasing temperature from 70 °C to 150 °C the TPC enhanced 2.6 times (from 34.7 ± 0.4 to 91.6 ± 3.0 mg GAE g^{-1}). Comparing SWE with SOX we observe that, resembling yield results, the traditional method (SOX) provided TPC value similar to SWE only at lower temperatures (70 °C and 90 °C). Also, TPC from SWE at 150 °C was 2.7 times higher than that obtained by SOX (34.0 ± 1.0), showing the good performance of this alternative method. Hall et al. (2018)using CO_2 supercritical for papaya seeds extraction verified TPC values up to 11 mg GAE g^{-1}.

3.3. Antioxidant activity

In order to evaluate the functional properties of the papaya seed extracts, the antioxidant activity was assessed using two commonly employed assays, namely DPPH and β-carotene bleaching methods, with results presented in Table 2. Also, the antioxidant activities of the

Table 2
Antioxidant activity of papaya seed extracts obtained by different extraction techniques (Subcritical Water Extraction – SWE, Soxhlet – SOX) evaluated by DPPH and β-Carotene bleaching methods. Different superscript letters mean groups statistically different ($p < 0.05$) in each column for each raw material.

Extraction method and temperature		DPPH EC$_{50}$ (µg mL^{-1})	β-Carotene bleaching Antioxidant activity (%)
SWE	70 °C	4.1 ± 0.1[a]	92 ± 3[a,b]
	90 °C	3.4 ± 0.4[c]	96 ± 3[a,b]
	110 °C	2.9 ± 0.4[d]	98 ± 2[a]
	130 °C	2.0 ± 0.3[e]	98 ± 2[a]
	150 °C	1.67 ± 0.01[f]	87 ± 4[b]
SOX		3.74 ± 0.01[b]	95 ± 4[a,b]
BHT		0.305	100

papaya seeds extracts were compared with those from butylated hydroxytoluene (BHT), a synthetic antioxidant standard. Considering that there are several oxidative mechanisms involved in most systems (Granato et al., 2018), it is necessary to combine different procedures, such as DPPH and β-carotene bleaching methods, to represent the antioxidant potential from a sample (or extract).

The results from Table 2 suggest that antioxidant activity by DDPH increased with extraction temperature. These results may be related to the water diffusivity increase, which improves the mass transfer and the compounds recovery (higher extraction yields) as showed in Table 1. Several SWE applications have also shown an antioxidant capacity enhancement with increasing extraction temperature probably due to formation of new antioxidants from caramelization or Maillard reactions, occurred at SWE conditions (Plaza et al., 2010a, 2010b). This behavior of an increase in the DPPH performance with the extraction temperature was reported by Gilbert-López et al. (2017); Plaza et al. (2010b), 2010a.

From Table 2, the extract obtained at 150 °C resulted in higher antioxidant performance by DPPH essay (EC$_{50}$ of 1.67 ± 0.01 μg mL^{-1}), although the samples that presented higher antioxidant potential by β-Carotene bleaching assay were obtained with SWE temperatures up to 130 °C ($98 \pm 2\%$). One possible explanation of these difference is due to multiple reactions and mechanisms for the determinations of antioxidant activity, according to the samples particular composition (Craft et al., 2012). A single method does not reflect accurately all the antioxidant activity from a complex system and, in this context, DPPH and β-carotene radicals can be an appropriate for practical/analytical evaluation of the radical scavenging potential due to experimental feasibility and low cost associated to these essays (Craft et al., 2012; Matthäus, 2002; Mensor et al., 2001). The β-Carotene bleaching results show no significant variation with SWE temperature (Table 2), with values ranging from 87 ± 4 to $98 \pm 2\%$. However, these values were close to verified by the synthetic antioxidant BHT (100%). Also, no significant difference ($p > 0.05$) was detected for β-carotene bleaching method between SOX sample and SWE at 70 or at 90 °C. Therefore, SWE can be used as an alternative to SOX with water, providing similar extracts in a much shorter period of time (5 min).

3.4. Maillard reaction products (MRPs)

Maillard reactions are common during food processing and storage. They consist of a natural condensation of carbonyl group from sugars, aldehydes or ketones with the amine group of amino acids, peptides, proteins or nitrogenous substance and resulting invarious compounds called Maillard reaction products (MRPs) (Al-Baarri et al., 2018). These reaction products enhance the antioxidant activities of various foods and food products (Plaza et al., 2010b).

The MRPs from papaya seed extracts were monitored by spectral analysis (section 2.8) in order to investigate the thermal influence of the extraction processes. The color of the extracts obtained by SWE and by SOX was spectrophotometrically investigated at 420 mn, indicating the Maillard reaction progress, and the results are showed in Fig. 3. Temperature is an important variable of Maillard reaction, which occurs at low (storage) and at high temperatures (processing) (Caballero et al., 2015). As observed from Fig. 3, the MRPs increased with SWE temperature, and the SOX performance was statistically similar to values by SWE at 70 °C and at 90 °C, which were very low compared to SWE at higher temperatures. The extent of the Maillard reaction is affected by the heating intensity. A temperature increase induces the reactivity between the sugar and the amino groups from the raw material, leading to the formation of neoantioxidant compounds (Plaza et al., 2010a, 2010b).

The heating time also affects the Maillard reaction (Caballero et al., 2015). The products from Maillard reaction for samples from SWE obtained at 110, 130 and 150 °C (5 min) were higher ($p < 0.05$) than samples from SOX (6 h). The Maillard reaction obtained during a
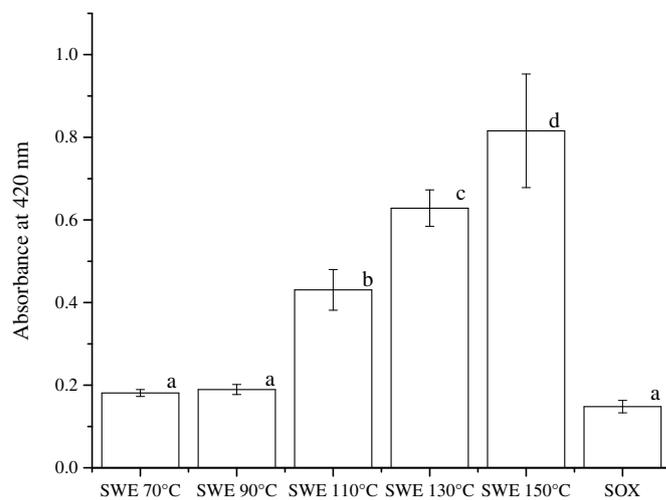

Fig. 3. Effect of the temperature of SWE and convention extraction (SOX) on the Maillard reaction product level.

high-intensity short time heating can be equivalent to the one obtained by a mild treatment for a long time (Caballero et al., 2015). For instance, in the present work, there was no significant difference ($p > 0.05$) between the absorbance values obtained for SOX extract (6 h) and those obtained by SWE at 70 and 90 °C (5 min).

3.5. Correlations between antioxidative properties

The results of TPC, DPPH, β-carotene and MRP were correlated using Pearson analysis to investigate their influence on the antioxidant activity of papaya seed extracts. From that, DPPH and MRPs shown a strong linear correlation with TPC (-0.93 and 0.96, respectively). The results also revealed that DPPH showed a strong linear correlation with Maillard reaction product level (0.97). These data are in agreement with Lee et al. (2018), which found better DPPH values for extracts with a higher phenolic content and higher MRP level for red ginseng extracts provided by SWE. Similarly, Lachos-Perez et al. (2018) described a significant positive correlation between TPC and DPPH activity for defatted orange peel extracts recovered by SWE. Moreover, from the present work, a low correlation coefficient was found between the values of β-Carotene bleaching assay with TPC (-0.56) and with MRP (0.36). These behaviors suggest that the TPC and MRP values are associated to compounds that offer hydrogen donation that causes DPPH radical discoloration, providing better correlation compared to β-carotene bleaching method.

3.6. Chromatographic analysis of papaya seed extracts

Table 3 shows information of concentrations of phenolic compounds extracted from papaya seeds using SWE at 10 MPa in 70, 110 and 150 °C and SOX technique. For SWE, these three temperatures were selected to optimize the chromatographic analyses and in order to evaluate the influence of extreme levels (70 and 150 °C) and an intermediate level (110 °C) of temperature towards the composition of the extracts obtained. Then, among the 46 phenolics tested, a total of 30 were detected throughout SWE at 70, 110 and 150 °C and for SOX method, which are predominantly composed of phenolic acids and flavonoids, although other phenolic classes were also detected. SOX was the richest one, with 25 noticed compounds, followed by SWE 150 °C with 22 and SWE 70 °C and SWE 110 °C with 21 phenolics compounds each sample. The higher number of phenolic compounds obtained by SOX, compared to SWE, may be explained probably due to the acidic hydrolysis of phenolic acids and tannins, which can promote the decomposition of these structures and release phenolic acids, with the long extraction time of the SOX

Table 3
Phenolic compounds (mg g^{-1} of extract) extracted with SWE at different temperatures and SOX.

	Phenolic Compounds	SWE 70 °C (mgg^{-1})	SWE 110 °C (mgg^{-1})	SWE 150 °C (mgg^{-1})	SOX (mgg^{-1})
Phenolic acids					
1	3.4 dihydroxybenzoic acid*	0.0415 ± 0.0053[a]	0.0230 ± 0.0079[a,b]	0.0163 ± 0.0056[b]	0.0371 ± 0.0007[a]
2	4- aminobenzoic acid*	nd	nd	nd	< LOQ
3	4-hydroxymethylbenzoic acid*	0.0075 ± 0.001[a]	0.0073 ± 0.0004[a]	0.0145 ± 0.0016[b]	0.0242 ± 0.0007[c]
4	Caffeic acid	0.0202 ± 0.0079[a,b]	0.0126 ± 0.0042[b]	0.0038 ± 0.0018[b]	0.0365 ± 0.0069[a]
5	Cinnamic acid	nd	nd	nd	nd
6	Chlorogenic acid	0.0159 ± 0.0001[a]	0.0161±0[a]	0.0159 ± 0.0001[a]	0.0158±0[b]
7	Ellagic acid*	< LOQ	< LOQ	< LOQ	< LOQ
8	Ferulic acid	0.1087 ± 0.0485[a]	0.0712 ± 0.0030[a]	0.0227 ± 0.0059[a]	0.3667 ± 0.0422[b]
9	Gallic acid*	0.0079 ± 0.0028[a]	0.0171 ± 0.044[a]	0.0070 ± 0.0073[a]	0.0042 ± 0.0054[b]
10	Mandelic acid*	nd	nd	0.1227 ± 0.0154	nd
11	Methoxyphenylacetic acid*	nd	0.0253 ± 0.0100[a]	nd	0.0634 ± 0.0057
12	p-Anisic acid*	nd	< LOQ	nd	0.0229 ± 0.0045
13	p-Coumaric acid	0.0105 ± 0.0020[a]	0.0018 ± 0.0025[a,b]	nd	0.0102 ± 0.0015[b]
14	Rosmarinic acid	nd	nd	nd	nd
15	Salicylic acid*	0.0620 ± 0.0128[a,b]	0.0343 ± 0.0076[b]	0.0427 ± 0.0091[b]	0.0981 ± 0.0044[a]
16	Sinapic acid*	nd	nd	0.0121 ± 0.0020	0.0048 ± 0.0015
17	Syringic acid*	0.0012 ± 0.0020[a]	0.0012 ± 0.0002[a]	nd	0.0011 ± 0.0002[a]
18	Vanillic acid	0.0413 ± 0.0087[a]	0.0503 ± 0.0122[a]	0.1080 ± 0.0133[b]	0.0176 ± 0.0006[a]
Flavonoids					
19	Apeginin	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	< LOQ
24	Eriodictyol*	< LOQ	< LOQ	< LOQ	< LOQ
25	Fustin	nd	nd	nd	nd
26	Galagngina	nd	nd	nd	nd
27	Hispidulin*	0.0017 ± 0.0002[a]	nd	0.0002 ± 0.0001[b]	nd
28	Isoquercetin	nd	nd	nd	nd
29	Isorientin*	< LOQ	< LOQ	< LOQ	< LOQ
30	Kaempferol	nd	nd	nd	nd
31	Myricetin*	0.0133±0[a]	0.0135±0[a]	0.0135 ± 0.0002[a]	0.0130±0[a]
32	Naringenin	nd	nd	nd	nd
33	Naringin	nd	nd	nd	nd
34	Pinocembrin	nd	nd	nd	nd
35	Quercetin	nd	nd	nd	nd
36	Rutin*	nd	nd	< LOQ	nd
37	Taxifolin*	nd	nd	< LOQ	nd
38	Vitexina*	< LOQ	< LOQ	< LOQ	< LOQ
Stilbene					
39	Resveratrol*	0.0314±0[a]	0.0318±0[b]	0.0315±0[a,b]	0.0305 ± 0.002[c]
Phenolic Aldehydes					
40	Coniferaldehyde*	0.0025±0[a]	0.0009±0[b]	0.0017±0[c]	0.0008±0[d]
41	Sinapaldehyde	nd	nd	nd	nd
42	Syringaldehyde*	nd	nd	nd	0.0107 ± 0.0120
43	Vanillin*	0.0012 ± 0.009[a]	0.0047 ± 0.0003[b]	0.0047 ± 0.0007[b]	0.0138 ± 0.0008[c]
Coumarin					
44	Scopoletin*	nd	nd	< LOQ	nd
45	Umbelliferone*	0.0018 ± 0.0003[a,b]	0.0011 ± 0.0002[b]	nd	0.0057 ± 0.0016[c]
Phenolic Diterpene					
46	Carnosol	nd	nd	nd	nd
	Total Phenolic Content (mg g^{-1})	0.3686	0.3123	0.4173	0.7772

<LOQ – not quantifiable. Results followed by the same letter in the row do not differ significantly (*t*-test, p < 0.05). nd: detected; * Reported for the first time.

technique. On the other hand, this study showed that SWE provided extracts with higher DPPH performance and similar β-carotene behavior, compared to extract obtained by SOX technique (Table 2). As described before, a single method does not reflect accurately all the antioxidant activity from a complex system, but the two assays presented in this study, along with the results of the LC-ESI-MS/MS analysis, demonstrate a notable contribution of the SWE for the recovery of antioxidant components. According to Dias et al. (2019) differences in phenolic profiles (type of phenolics present and their relative proportions), synergism/antagonism among antioxidants, make the antioxidant activity of some extracts not dependent only on phenolic concentration, but also on the structure and interaction between antioxidants. Another key factor for the SWE, compared to SOX method, is the high process time reduction, from 360 min for SOX to 5 min for SWE, with extracts of similar quality behavior. This result reinforces some advantages of the SWE technique such as the use of a faster and greener

method, with higher yields compared to conventional solvent extraction.

With respect of SWE methods, overall, the result showed no significant difference (p > 0.05) for temperature effect on the 46 phenolic compounds content when the extraction temperature increased from 70 to 150 °C (Table 3). However, the SWE 150 °C resulted in higher amounts of total phenolics (0.4173 mg 100 g^{-1}) compared to SWE 70 °C and SWE 110 °C. Individually, the most abundant compounds found were ferulic acid (47.2% of total phenolic content in SOX), mandelic acid (29.4% of extract sample in SWE 150 °C) and vanillic acid (25.8% of total phenolic compounds detected by SWE 150 °C).

Dias et al. (2015) reported that in the structures of papaya seeds there are phenolic compounds, mainly in the sarcotesta, which is a gelatinous film that protect the seeds and contain a higher concentration of phenotypes in relation to the other structures. Phenolic compounds such as caffeic acid, chlorogenic acid, p-coumaric acid, ferulic acid and

vanillic acid are known to contribute to high antioxidant activity in papaya seeds (Alotaibi et al., 2017), and both were present in papaya seeds extracts obtained in the present study.

In addition, as observed in Table 3, 25 phenolics compound were detected for the first time, in association with papaya seeds, by LC-ESI-MS/MS analysis, among them, 16 compounds were quantified (component numbers **1, 3, 9, 10, 11, 12, 15, 16, 17, 27, 31, 39, 40, 42, 43** and **45**). It should be noted that the compounds 3.4 dihydroxybenzoic acid**(1)**, sinapic acid **(15)**, rutin **(36)**, myricetin **(31),**and umbelliferone **(45)** had been previously detected in the literature in association with papaya extracts, particularly for the pulp, peel and leaves (Vij and Prashar, 2015). The use of phenolic compounds from natural sources for food formulations is an interesting opportunity to value their biological potential to improve the shelf life of perishable food products, without the use of synthetic additives (Martillanes et al., 2017).

The phenolic compounds detected from papaya seed extracts, presented in Table 3, have been used as functional food ingredients due to its ability to prevent oxidation (Rivera-Pastrana et al., 2010), enhance the oxidative stability from vegetable oils and lipid-rich foods (Ribeiro and Jorge, 2017; Pereira et al., 2017), and also retard microbial degradation in fruits and vegetables (Martillanes et al., 2017).

The bioactive phenolic compounds have attracted the attention of the scientific community because of its great antioxidant activities in vitro and *in vivo* and, as previously highlighted.

In this context, the papaya seeds have potential use for food process applications. For instance, Veronezi and Jorge (2018) reported that the high amounts of bioactive compounds from papaya seed extracts increase the nutritional value and the oxidative stability of soybean oil. Therefore, the recovery of bioactive compounds from papaya seeds reduces the environmental impact of this agro-industrial residue and provides a valuable product with potential use also for food production.

It is also worth for notice that, although SWE at temperatures above 110 °C provided values of TPC in vitro higher than SOX (Table 1), the total amounts of the identified phenolics (Table 3) were lower than SOX. This may be caused by hydrolysis processes as a result of the high temperatures applied at SWE, heading to the formation of new components with antioxidant activity and by reactions that occurred with increasing temperature, such as Maillard reaction, as stated previously, which resulting products were not detected by LC-ESI-MS/MS analysis (Gilbert-López et al., 2017). The same tendency was showed by da Silva and Jorge (2017) and by Granato et al. (2018) which reported that the spectrophotometric analysis is a nonselective methodology detecting all the phenolic groups present in the sample, including extractable protein, overestimating the results comparing with LC-ESI-MS/MS data. However, the usefulness of in vitro results, despite their imperfect nature, cannot be ruled out (Granato et al., 2018).

4. Conclusions

In the present work, a customized subcritical water unit allowed the study of temperature influence on extraction from papaya seeds. The SWE unit was successful in recovering extracts with different attractive compositions and represent a fast option for papaya seeds processing. The results reported that global yield, TPC and the antioxidant activity were positively affected by SWE temperature. TPC and MRPs values were maximized at 150 °C for 5 min of SWE, which provided the best antioxidant potential by DPPH method. SWE technique recovered high amounts of phenolic compounds from papaya seeds, faster and with less solvent volume, compared to SOX method. Moreover, liquid chromatography analysis identified 30 phenolic compounds from the papaya seed extracts, within, 25 phenolics were detected for the first time in papaya seeds. In this context, the most abundant compounds were ferulic (SOX), mandelic (SWE 150 °C) and vanillic acids (SWE 150 °C), with potential applicability as nutraceuticals for food and supplement industries. Therefore, this study has shown an important contribution to

the knowledge of this promising extraction technology, particularly for the use of the papaya fruit waste as a valuable source of natural antioxidants.

5. Practical application

The practical application of the present research is related to the evaluation of valuable components recovered from papaya seeds using subcritical water extraction. This technique is a promising extraction method that use a green solvent for the extraction of bioactive compounds from an industrial residue. Papaya seeds resulting from the industrial production of papaya juice, ice cream or jelly are a promising waste, normally underestimated, but very useful as a raw material to obtain valuable phenolic components.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bcab.2019.101367.

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