The Journal of Supercritical Fluids 186 (2022) 105602

Contents lists available at ScienceDirect

The Journal of Supercritical Fluids

journal homepage: www.elsevier.com/locate/supflu

# Green-based processes applied for valorization of peanut by-product: *In vitro* evaluation of antioxidant and enzymatic inhibition capacities

Guilherme Dallarmi Sorita [a], Anielle de Oliveira [b], Thaysa F.M. Moreira [b], Fernanda V. Leimann [b,c], Sandra R.S. Ferreira [a,*,1]

[a] Chemical and Food Engineering Department, Federal University of Santa Catarina, EQA/UFSC, C.P. 476, CEP 88040-900 Florianópolis, SC, Brazil
[b] Post-Graduation Program of Food Technology (PPGTA), Federal University of Technology – Paraná – UTFPR, Campus Campo Mourão, via Rosalina Maria dos Santos, 1233, Caixa Postal: 271, Campo Mourão, CEP 87301-899 PR, Brazil
[c] Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus Santa Apolónia, 5300-253 Bragança, Portugal

## HIGHLIGHTS

- Green processes applied to recover different fractions from peanut by-product.
- High-yield values were achieved.
- SFE recovered the oily fraction rich in unsaturated fatty acids.
- PLE and SWE samples presented strong antioxidant and enzyme inhibition activities.
- Extracts with potential for application in food or pharmaceutical industries.

## GRAPHICAL ABSTRACT


## ARTICLE INFO

*Keywords:*
*Arachis hypogea* L.
Peanut skin
Green extractions
Bioactive components
Alpha-amylase

## ABSTRACT

Peanut (*Arachis hypogea* L.) industrial processing generates underused by-products, which are mostly discharged. In this work, extracts from peanut by-product were obtained by Supercritical Fluid Extraction (SFE), Pressurized Liquid Extraction (PLE) and Subcritical Water Extraction (SWE), and compared to Soxhlet (SOX). Yield values varied from 12.94% to 37.65% for SOX-water and SWE, respectively. The quality of the extracts was evaluated by total phenolic content (TPC), antioxidant and enzymatic inhibition potentials, and fatty acids profile. Best TPC performance and high antioxidant capacity were obtained from samples using ethanol/water by PLE and Soxhlet. High inhibition of human salivary α-amylase was achieved by PLE and Soxhlet samples recovered with ethanol/water mixture. The inhibition of porcine pancreatic α-amylase was higher from samples by SFE and Soxhlet with ethanol. High-pressure methods are appealing alternatives for the recovery of bioactive extracts from peanut by-products, with functional properties and potential inhibitor of digestive enzymes.

## 1. Introduction

Peanuts (*Arachis hypogaea* L.) are important and highly appreciated oilseeds, with increasing worldwide production associated to its appreciable nutrient-rich content and health benefits [1]. Its industrial processing generates underused by-products, such as peanut skin (accompanied by peanut fragments, flour or small pieces) [2] that is predominately used as animal feed or is unproperly discharged. Nevertheless, this by-product is an important source of valuable compounds, such as protein, sugars, fibers and several phytochemical

* Corresponding author.
[1] Sandra Regina Salvador Ferreira

https://doi.org/10.1016/j.supflu.2022.105602

Available online 19 April 2022

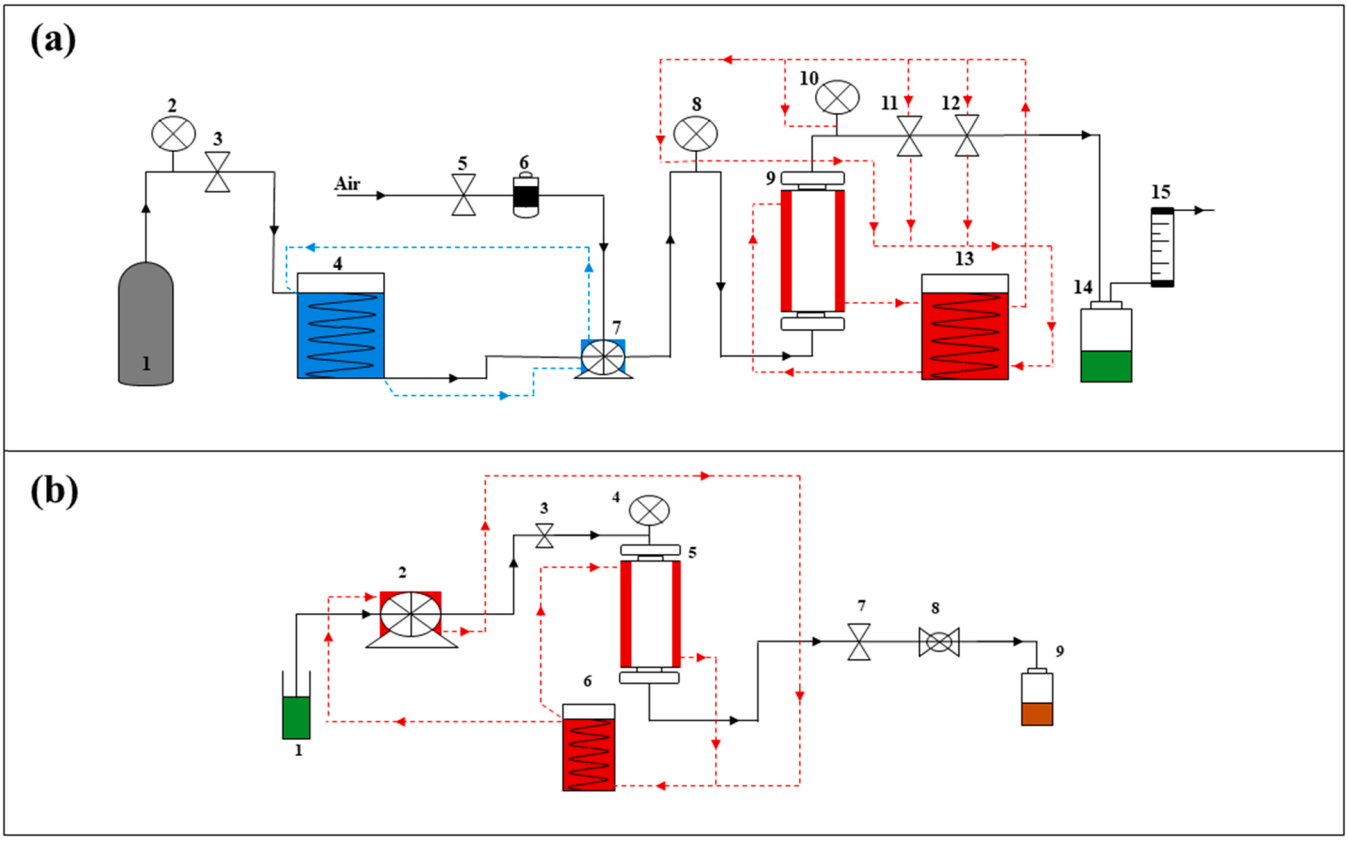


**Fig. 1.** Schematic design of experimental units: (a) SFE apparatus - 1: CO2 cylinder; 2, 8 and 10: Manometers; 3: Block needle valve; 4: Cooling bath; 5: Compressed air valve; 6: Compressed air filter; 7: CO2 pump; 9: Jacketed extraction vessel; 11 and 12: Regulator needle valves; 13: Heating bath; 14: Extract collection flask; 15: Rotameter and $CO_2$ exit; (b) PLE and SWE apparatus - 1: Solvent recipient; 2: HPLC pump; 3: Block needle valve; 4: Manometer; 5: Jacketed extraction vessel; 6: Heating beath; 7 and 8: Regulator needle valves; 9: Extract collection flask.

compounds, such as fatty acids and polyphenols.

Recent investigations demonstrate that peanut skins are rich in phenolic compounds, including phenolic acids, flavonoids, stilbenes and various procyanidins oligomers and proanthocyanidin [3], with potential antioxidant and enzyme inhibitory activities [2]. Oleic acid and linoleic acid (omega 9 and omega 6 fatty acids, respectively) can also be found in peanut by-products, with important applications (especially omega 6), including the prevention of health problems such as heart diseases, hypertension and others [4,5].

Green extraction techniques, such SFE, PLE and SWE have emerged over the last years and decades as viable alternatives for the extraction of different classes of natural compounds. In general, these techniques require less time, energy and solvent, and therefore, are consistent with sustainable development strategies. Moreover, the use of green solvents allows the production of "clean" label compounds which are recognized as safe and are preferred by consumers [6].

SFE presents the advantage of simultaneous combination of gas and liquid properties of the supercritical solvent, with high density and solubilization power for different solutes (liquid-like characteristic) and high diffusivity and low viscosity (gas-like characteristic), facilitating the mass transfer attributes. Carbon dioxide is the most commonly used supercritical solvent due to its moderate critic properties, environmental-friendly characteristics (non-toxic, non-flammable, inert), and low cost, among others [7,8].

The PLE method uses pressurized solvents, in its liquid state, which reduces the solvent's surface tension and viscosity, facilitating the capacity to penetrate in the solid matrix and reducing extraction time and solvent consumption, compared to methods conducted at ambient temperature and atmospheric pressure, such as Soxhlet [8].

The SWE is performed at high pressures and temperatures from 100° to 374°C (below the water critical temperature) and is widely considered a green and environmental-friendly extraction method for bioactive compounds, since it avoids organic solvents. Besides, SWE provides high extraction yields in short processing time, because the high temperatures used decrease significantly the subcritical water dielectric constant [9,10].

In this work, conventional (SOX) and green extraction techniques (SFE, PLE, SWE) were successfully applied to recover different fractions from peanut by-product (peanut skin and seed fragments). The processes were evaluated by yield values and the extracts analyzed in terms of total phenolic content (TPC), antioxidant potential by different methods, and enzyme inhibition ability against salivary and pancreatic α-amylases. The relation between the antioxidant potential with TPC and the fatty acids content from the nonpolar extracts were also observed. Considering the lack of studied using high-pressure methods to recover bioactive compounds from peanut skin, performed only by SFE [11] and PLE [12], and no information regarding the enzyme inhibition potential from peanut by-product, there is an evident novelty in this work concerning the properties of this underestimated material.

## 2. Materials and methods

### 2.1. Materials

The by-product from the processing of Peanut (*Arachis hypogaea* L.), runner variety, composed essentially by skins, with small seed fragments, was kindly donated by Many Industrial and Commerce of Sweets Ltda. (Itajaí, SC – Brazil), in March 2019. Hexane (Neon, Brazil), ethanol (Dinâmica, Brazil), deionized water and $CO_2$ (White Martins, Brazil) were used as solvents. TPC analysis used gallic acid P.A. (Sigma Aldrich,

USA), sodium carbonate (Dinâmica, Brazil) and Folin-Ciocalteu reagent (Sigma-Aldrich, USA). Trolox (Sigma Aldrich, USA) provided the standard curve for antioxidant activity assays, combined with the radicals DPPH (2,2 -diphenyl-1-picrylhydrazil) and ABTS (2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) (Sigma Aldrich, USA), the $K_2SO_4$ solution (Dinâmica, Brazil), anhydrous sodium acetate (Êxodo, Brazil), cloridric acid P.A. (Synth, Brazil), ferric chloride (Synth, Brazil), TPTZ (2,4,6-Tris(2-pyridyl)-s-triazine) (Sigma Aldrich, USA). Also, β-carotene (Sigma Aldrich, USA), linoleic acid (Sigma Aldrich, USA), chloroform P. A (Synth, Brazil) and tween 20 (Sigma Aldrich, USA) were used for the antioxidant bleaching method. Human salivary enzymes (Type IXA, 87.5 units $mg^{-1}$ of solid, Sigma-Aldrich) and porcine pancreatic (Type VI-B, 10 units $mg^{-1}$ of solid, Sigma-Aldrich), potato starch (Sigma-Aldrich), sodium hydroxide (Isofar), sodium tartrate (P.A., Alphatec), dinitrosalicylic acid (P.A., Inlab), calcium chloride (P.A., Prochemicals) and sodium phosphate (P.A., Vetec), phosphate buffer (40 mM, Vetec), NaCl (13.4 mM, Proquímicos) and 3,5-dinitrosalicylic acid (DNS, Inlab) were used to evaluate α-amylases inhibition. Finally, for fatty acid identification were used KOH (Sigma Aldrich, USA), methanol P.A. (Dinâmica, Brazil) and $H_2SO_4$ (Sigma Aldrich, USA).

### 2.2. Sample preparation

The peanut by-product, with moisture content of 7.99 ± 0.07%, determined according to AOAC [13], and mean particle diameter of 0.39 ± 0.11 mm, evaluated by sieving [14], were conditioned in polyethylene bags and storage until use at − 18 °C in domestic freezer.

### 2.3. Extraction methods

The extraction methods conducted were: (1) SOX with different solvents; (2) SFE with $CO_2$ as solvent and varying the process temperature; (3) PLE using different solvents; and (4) SWE. The extraction methods and the experimental conditions are described below.

#### 2.3.1. Soxhlet extraction (SOX)

The Soxhlet extraction was performed according to [13]. Briefly, 150 mL of solvent recirculated over 5 g of raw material in a Soxhlet apparatus for 6 h at the solvent boiling temperature. The extraction was performed with the solvents separately: hexane (SOX1), ethanol (SOX2), ethanol/water (70:30) (SOX3), and water (SOX4). The residual hexane and ethanol solvents on the extracts were removed by a rotary evaporator (Fisatom, model 801, Sao Paulo, Brazil); the residual ethanol/-water mixture was rotary evaporated followed by lyophilization (L101, Liotop, São Carlos, Brazil), while the residual water was removed by lyophilization. The recovered extracts were conditioned in amber flasks and stored in absence of light at − 18 °C until analysis. Extraction yield ($X_0$) values were determined by the ratio between the mass of recovered extract and mass of raw material. The results are expressed by mean value ± standard deviation from duplicate experiments.

#### 2.3.2. Supercritical fluid extraction (SFE)

SFE assays were conducted in a customized unit described by Michielin et al. [15], Fig. 1(a), which contains a $CO_2$ cylinder, a thermostatic bath (Microquímica, MQBTZ99–20, SC, Brazil), a $CO_2$ pump (Maximator M0111, Nordhausen, Germany), connected to a compressed air driven, for $CO_2$ pressurization and pressure control, and a stainless-steel jacketed extraction vessel (138.2 mL). Regulator needle valves (HIP, model 10–11 NAF-REG, EUA) control the flow rate and $CO_2$ expansion, measured by a rotameter (Swagelok, model VAF-G4–1933–1–0, USA).

The SFE parameters were defined taking into account the work by Putra et al. [16] for the recovery of the non-polar fraction (fatty acids) from peanut by-product. Then, the SFE assays were performed with $CO_2$ at constant pressure of 20 MPa and solvent flow rate of 1 kg $h^{-1}$, with temperatures of 40 and 50 °C, namely SFE1 and SFE2, respectively. According to Teixeira et al. [17] these conditions were adequate for fatty acids recovery.

The extraction yield ($X_0$) was calculated based on the initial mass of peanut by-product. The process time for the SFE was defined through the kinetic overall extraction curve (OEC), performed according to Section 2.3.4. Briefly, approximately 15 g of peanut by-product were placed inside a stainless-steel extraction vessel, Fig. 1(a) – 9, to form the fixed bed of particles, with the empty space filled with cotton and glass beads, followed by the control of the process variables (temperature, pressure and solvent flow rate). Then, the extract samples were collected in amber glass flasks, Fig. 1(a) – 14, weighed and stored in a domestic freezer at − 18 °C for further analyses. All SFE experiments were performed in duplicate.

#### 2.3.3. Pressurized liquid extraction (PLE) and Subcritical water extractions (SWE)

PLE and SWE assays were conducted in a customized unit, Fig. 1(b), as described by Mazzutti et al. [8]. The unit is mainly constituted of a solvent reservoir, a solvent pump (Waters, 515 HPLC, Milford, MA, USA), micrometric valves (HIP, 10–11 NFA, Erie, PA, USA), and a jacket stainless steel extraction cell (30.7 mL) coupled to a thermostatic bath (Microquímica, MQBMP-01, SC, Brazil). An analogic manometer (Wika, Klingenberg am Main, BY, Germany) and a back pressure regulator (Swagelok, KPB series, Solon, OH, USA) complete the unit.

The PLE assays were conducted at 10 MPa and 80 °C with two solvents, ethanol, and ethanol/distilled water mixture of 70:30 (v/v), namely PLE1 and PLE2, respectively. When water was used as solvent, the assay was called SWE, and conducted at 10 MPa and 160 °C. PLE and SWE procedures were performed with solvent flow of 3 mL $min^{-1}$, with the aid of an HPLC pump feed directly into the extraction cell packed with the peanut by-product. The process parameters were selected considering the literature about peanut skin PLE processing [12] and also the objective to recover polar fractions from the raw material, compared to the lipid fraction recovered by SFE. The process time for PLE and SWE assays were defined by the overall extraction curves from the kinetic assays, performed according to Section 2.3.4. Briefly, the procedure consisted of forming a fixed bed of particles was formed with 5 g of peanut by-product inside a stainless-steel vessel and was completed with approximately 30 mL of glass beads, followed by the control of the process variables (temperature, pressure and solvent flow rate) [8].

The extract samples from PLE1 and PLE2 were collected in amber flasks and submitted to solvent evaporation by a rotary evaporator (Fisatom, model 801, São Paulo). The extract samples PLE2 (after ethanol removal) and SWE were submitted to lyophilization (L101, Liotop, São Carlos, Brasil) for water removal. The extracts were stored at − 18 °C in a domestic freezer and absence of light until analysis. All extractions were done in duplicate and the yield results were expressed as mean value ± standard deviation.

#### 2.3.4. Kinetic studies

Kinetic studies were conducted, as described by Oliveira et al. [18] to determine the process time for the yield assays of the procedures SFE, PLE and SWE (Sections 2.3.2 and 2.3.3). The mass transfer mechanisms were evaluated from the overall extraction curve (OEC), as described by Sovová [19] and by Ferreira et al. [20], consisting of three periods: constant extraction rate (CER), falling extraction rate (FER) and diffusion-controlled (DC). The OECs, for SFE, PLE and SWE were obtained from the extract samples collected, in amber flasks, at pre-stablished time intervals, and weighted in analytical balance (Ohaus, Model AS200S, NJ, USA). The SFE kinetics assay was performed at 20 MPa, 50 °C and 1 kg $h^{-1}$, with samples collected at predefined time intervals (up to 6 h). PLE kinetics assay was performed with ethanol at 10 MPa, 80 °C and 3 mL $min^{-1}$ (up to 61 min), while SWE was conducted with subcritical water at 10 MPa, 160 °C and 3 mL $min^{-1}$ (until 96 min), with samples recovered at predefined time

intervals. The kinetics for each procedure were conducted to reach the maximum extraction recovery, i.e., until reaching practically null extraction rate at one interval. Therefore, the extraction time (near null extraction rate) defines the end of the high-pressure processes (SFE, PLE or SWE), conducted in duplicate according to Section 3.1, to provide the yield values (Section 2.4). The solvent from PLE samples were removed in a rotary evaporator (Fisatom, model 801, São Paulo). SWE recovered extracts were frozen and then lyophilized (L101, Liotop, São Carlos, Brasil) for water removal.

After kinetics assays, a Piecewise model was fit to the experimental data of the OECs (for SFE, PLE and SWE) using Origin Pro 2015 software (OriginLab, Northampton, MA, USA), by adjusting three straight lines corresponding to the different extraction periods (CER, FER and DC).

The first line (Eq. 1) represents CER, for $t < t_{CER}$.

$$y = a_1 + k_1 t \quad (1)$$

The second line (Eq. 2) represents FER for $t_{CER} \leq t \leq t_{FER}$.

$$y = a_1 + k_1 t_{CER} + k_2 (t - t_{FER}) \quad (2)$$

The DC period, for $t > t_{FER}$, is described by Eq. 3.

$$y = a_1 + k_1 t_{CER} + k_2 (t_{FER} - t_{CER}) + k_3 (t - t_{FER}) \quad (3)$$

where: y: mass accumulated up to the end of each period (g); t: extraction time (min); $a_1$: linear coefficient of the CER period (g); $k_1$, $k_2$ and $k_3$: gradients that represents the extraction rates of CER, FER and DC, respectively (g $min^{-1}$); $t_{CER}$: time of CER period (min); $t_{FER}$: period between CER and FER (min).

### 2.4. 2.4Extraction yield ($X_0$)

The extraction yield ($X_0$) was calculated as percentage (%) of mass of extract ($m_{Extract}$) relative to total mass of raw material ($m_{RM}$), according to Eq. (4):

$$X_0 \ (\%) = \left(\frac{m_{Extract}}{m_{RM}}\right) * 100 \quad (4)$$

### 2.5. 2.5Total phenolic content (TPC)

The total phenolic content (TPC) of peanut by-products extracts was performed by Folin-Ciocalteu method described by Singleton and Rossi [21]. Firstly, the extracts were diluted in the solvents used for extraction. The SFE samples, recovered using $CO_2$ as solvent, were solubilized in hexane (also non-polar), with concentrations ranging from 0.02 to 0.04 mg $mL^{-1}$. Then, the reaction mixture was formed mixing 10 μL of extract solution, 600 μL of distilled water and 50 μL Folin-Ciocalteu reagent. After 1 min, 150 μL of aqueous $Na_2CO_3$ 20% (w/v) were added and the volume completed up to 1 mL (90 μL) with distilled water. The solutions were then stirred and stored in the dark for 2 h at ambient temperature. After that, the absorbance was read at 760 nm (TECAN, M2000 model). Gallic acid solution at different concentrations (from 0.03 to 2 mg. $mL^{-1}$) was used as a standard curve. The results were expressed as mg of Gallic acid equivalent (GAE) per g of extract ± standard deviation from triplicate measurements.

### 2.6. Antioxidant potential

#### 2.6.1. DPPH radical scavenging assay

The peanut by-product extracts were evaluated by the 2,2 -diphenyl-1-picrylhydrazil (DPPH) assay, according to Brand-Williams et al. [22]. Briefly, the samples were diluted in their respective solvents, although the SFE samples, recovered using $CO_2$, were solubilized in hexane (concentrations from 0.001 to 0.020 mg $mL^{-1}$). Then, 50 μL of each extract solution was mixed with 250 μL of a 125 μmol $L^{-1}$ DPPH ethanolic solution (in triplicate). After 30 min of reaction at ambient temperature in the absence of light, absorbance was read at 517 nm (TECAN, M2000 model). Trolox solution at different concentrations was used as standard curve. The results were expressed in μmol TE $g^{-1}$ extract ± standard deviation.

#### 2.6.2. ABTS method

The peanut by-products extracts was also evaluated by the ABTS radical method, conducted according to Re et al. [23]. $ABTS^+$ 2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt radical cation was produced by reacting 7 mM ABTS solution and 2.45 mM potassium persulfate solution in the dark at room temperature for 16 h. Then, $ABTS^+$ solution was diluted in distillated water until an absorbance of 0.7 (± 0.05) at 734 nm. Finally, 20 μL of peanut by-product extracts (previously diluted) were added in 280 μL of $ABTS^+$ solution (in triplicate) and incubated in the dark for 30 min for absorbance measurement at 734 nm. Trolox was used as a standard reference and the values were calculated from the standard curve. Results were expressed as by μmol TE $g^{-1}$ extract ± standard deviation from triplicate measurements.

#### 2.6.3. FRAP method

The ferric reducing antioxidant power (FRAP) of peanut by-products extracts was according to Benzie and Strain [24]. Briefly, triplicates of 10 μL of solubilized extracts were added to 290 μL of FRAP reagent (0.3 M, pH 3.6 acetate buffer, 10 mM TPTZ and 20 mM ferric chloride). This solution was kept in absence of light at ambient temperature for 30 min, followed by absorbance measured at 593 nm. The blank was composed by 10 μL of each solvent and 290 μL of FRAP solution. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic) was used as reference and the values were calculated from the standard curve. Results were expressed as mg of Trolox equivalent (TE) $g^{-1}$ of extract ± standard deviation from triplicate measurements.

#### 2.6.4. β-carotene/linoleic acid bleaching method

The antioxidant potential of peanut by-product extracts was also evaluated by β-carotene linoleic acid bleaching method, according to Matthäus [25]. Firstly, linoleic acid (44 μL), Tween 20 (400 mg) and β-carotene (3.4 mg) were mixed with 5 mL of chloroform. Chloroform was removed in a rotary vacuum evaporator at 50 °C (Fisatom, model 801, São Paulo) and 100 mL of water were added. A blank emulsion was prepared without β-carotene. The assay was performed adding one milliliter of β-carotene-linoleic acid emulsion and 40 μL of extract solution previously prepared (16.67 mg $mL^{-1}$ prepared with the same solvent used for extraction) in triplicate. For each sample, a blank was prepared, adding 40 μL of extracts solutions and 1 mL of blank emulsion (without β-carotene). Also, a control sample was prepared adding 40 μL of each solvent and 1 mL of β-carotene emulsion. The flasks were incubated in a water bath at 50 °C for 2 h and the absorbance was monitored (at time 0 and 2 h) at 470 nm. The antioxidant activity was calculated according to Eq. (5), and the results was expressed as percentage of β-carotene bleaching inhibition (AA) ± standard deviation from triplicate measurements.

$$\%AA = 1 - \left[\left(\frac{Abs_{t=2} - Abs_{t=0}}{Abs_{control\ t=2} - Abs_{control\ t=0}}\right) * 100\right] \quad (5)$$

Where: %AA : β-carotene bleaching inhibition; $Abs_{t=2}$: sample absorbance at 2 h of reaction, and $Abs_{t=0}$: sample absorbance at time 0; $Abs_{control\ t=2}$: control absorbance at 2 h of reaction and $Abs_{control\ t=0}$: control absorbance at time 0.

### 2.7. Inhibition of human salivary and porcine pancreatic α-amylases by peanut by-products extracts

Inhibition of salivary and pancreatic α-amylases was evaluated by $IC_{50}$ (extract concentration required to reduce 50% inhibition of enzyme

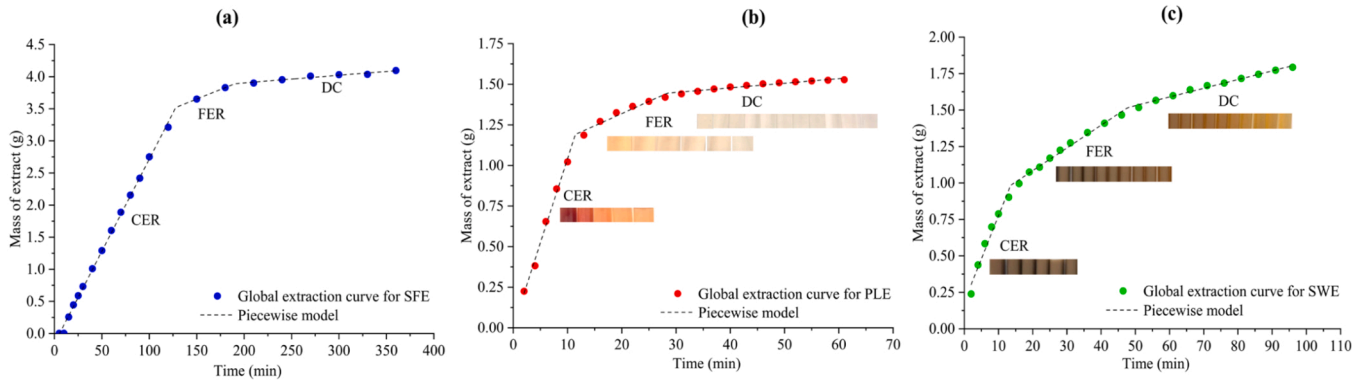


**Fig. 2.** Kinetic assays from peanut by-product by (a) SFE at 20 MPa, 40 °C and 1 kg of $CO_2$ $h^{-1}$; (b) PLE at 10 MPa, 80 °C and 1 mL of ethanol $min^{-1}$; (c) SWE at 10 MPa, 160 °C and 1 mL of water $min^{-1}$ with color palette of the recovered extracts. Kinetic assays from peanut by-product by (a) SFE at 20 MPa, 40 °C and 1 kg of $CO_2$ $h^{-1}$; (b) PLE at 10 MPa, 80 °C and 1 mL of ethanol $min^{-1}$; (c) SWE at 10 MPa, 160 °C and 1 mL of water $min^{-1}$ with color palette of the recovered extracts.

activity) and performed according to Silva et al. [26] with modifications. Porcine pancreatic α-amylase and human salivary α-amylase were solubilized in phosphate buffer and NaCl, pH 6.9. Potato starch was used as substrate (1% in water). Peanut by-products extracts were evaluated as inhibitors at final concentrations of 2000, 800 and 80 μg $mL^{-1}$ (solubilized in ethanol, with exception of SWE1 extract, solubilized in water).

The reaction was conducted in a thermostatic bath (37 °C) for 15 min, starting with the addition of the enzyme (74 U $mL^{-1}$). The produced reducing sugars from starch hydrolysis were assayed by the 3,5-dinitrosalicylic acid (DNS) method at wavelength 540 nm (model USB-650-UV–VIS Red Tide, Ocean Optics, USA) according to Miller [27]. The $IC_{50}$ concentrations were obtained by numerical interpolation of the curves for % inhibition versus concentration of the extracts (2000 – 80 μg $mL^{-1}$), using the Stineman equation [28] by MicroMath Scientific Software (Salt Lake City, UT, USA).

### 2.8. Identification of fatty acids by gas chromatography with flame ionization detector

Fatty acids profile from the nonpolar fraction recovered from peanut by-product by Soxhlet (SOX1) and by SFE (SFE1 and SFE2), were identified by gas chromatography with flame ionization detector, following procedure by Shin et al. [29].

An Agilent 7890 A GC System gas chromatographic system with flame ionization detection (Agilent 5975 C inert MSD with Triple Axis Detector) equipped with an Agilent 19091S-433HP-5MS (30 m x 250 μm x 0.25 μm) column was utilized for fatty acids identification. Initially, the column was heated at 130 °C and maintained for 5 min, with a heating ramp of 4 °C $min^{-1}$ up to 240 °C, keeping it constant for 15 min. Helium was used as carried gas at 2.7 mL $min^{-1}$. Fatty acids were identified by comparing the peak retention times observed in the samples with standards (NIST 11 - *National Institute of Standards and Technology,* USA). Fatty acids were quantified (comparative quantification) using the peak area normalization method, and the results were expressed as mass percentage.

### 2.9. Statistical analysis

The results were expressed as the averaged followed by standard deviation. One-way analysis of variance (ANOVA) was conducted to determine significant differences between treatments ($p < 0.05$). In addition, Pearson's correlation test was used to evaluate the relation between TPC and antioxidant activity. All statistical analyses were performed by Software Statistica (Statsoft Inc., USA).

**Table 1**
Parameters of Piecewise model by fitting SFE (20 MPa, 50 °C and 1 kg $h^{-1}$), PLE (10 MPa, 80 °C and 3 mL $min^{-1}$) and SWE (10 MPa, 160 °C and 3 mL $min^{-1}$) curves of peanut skin extracts.

| Kinetic parameters | SFE | PLE | SWE |
|---|---|---|---|
| $a_1$ (g) | -1.556 | 0.008 | 0.185 |
| $k_1$ (g $min^{-1}$) | 0.029 | 0.103 | 0.059 |
| $t_{CER}$ (min) | 128 | 11 | 13 |
| $k_2$ (g $min^{-1}$) | 0.006 | 0.015 | 0.016 |
| $t_{FER}$ (min) | 190 | 28 | 48 |
| $k_3$ (g $min^{-1}$) | 0.001 | 0.003 | 0.060 |
| $R^2$ | 0.999 | 0.998 | 0.996 |

Where: $a_1$ (in grams) is the linear coefficient of the CER period; $k_1$, $k_2$ and $k_3$ (in g min-1) are the gradients representing the extraction rates of CER, FER and DC, respectively; $t_{CER}$ (minutes) represents time of CER period; $t_{FER}$ (minutes) represents period between CER and FER.

## 3. Results and discussion

### 3.1. Kinetic assays

The OECs from the kinetics of the high-pressure methods are presented at Fig. 2 providing the mass of extract versus extraction time, for: (a) SFE, (b) PLE, and (c) SWE. The color bars that accompanied the OECs represent the color of the recovered extracts for each time interval for PLE and SWE. Also, Table 1 presents the kinetics parameters obtained from experimental data and by Eqs. (1), (2) and (3), accompanied by the fitting $R^2$ values.

From the OECs it was possible to observe the three extraction periods, CER, FER and DC. The CER period was detected up to 128 min for SFE, up to 11 min for PLE (samples with intense red color), and up to 13 min for SWE (samples with intense brown color), as provided at Fig. 2(a), (b) and (c), respectively. In sequence, FER period is between 128 and 191 min, 11–28 min and 13–48 min, for SFE, PLE and SWE, respectively. Finally, Diffusion controlled (DC) step takes place above 191 min, 28 min and 48 min for SFE, PLE and SWE, respectively. The CER period represents the extraction of the easily accessible solute that covers the particle surface due to cell walls rupture during griding, and the convection is the mass transfer mechanism dominant. Then, the FER period, is characterized by convection and diffusion mechanisms due to partial solute exhaustion from particles surface. The last DC period has diffusion-controlled mechanism, where the extraction is close to the maximum content of extractable solute, with the solute exhaustion from particle surface [19,20]. Then, based on the kinetics assays, the total extraction time, defined for each process, was selected as the second point of the DC period since it reaches near null extraction rate, and

**Table 2**
Process conditions, extraction yield (%), total phenolic content and antioxidant activity of peanut by-product extracts obtained by different extraction techniques (Soxhlet – SOX; Supercritical fluid extraction – SFE; Pressurized liquid extraction - PLE and Subcritical water extraction – SWE) evaluated by DPPH, ABTS, FRAP and β-carotene/linoleic acid bleaching methods.

| Extract | Extraction conditions | [5,6]Yield (%) | [5,6]TPC (mg GAE $g^{-1}$) | [5,6]DPPH (μmol TE $g^{-1}$) | [5,6]ABTS (μmol TE $g^{-1}$) | [5,6] FRAP (μmol TE $g^{-1}$) | [5,6] AA (%) |
|---|---|---|---|---|---|---|---|
| SOX1 | [1]Hex | $37.65^{a} \pm 0.04$ | $1.09^{f} \pm 0.000$ | $2.32^{g} \pm 0.17$ | $3.77^{g} \pm 0.16$ | $4.44^{e} \pm 0.05$ | $25.18^{f} \pm 5.72$ |
| SOX2 | [2]EtOH | $35.30^{a} \pm 0.38$ | $58.80^{d} \pm 0.54$ | $152.17^{c} \pm 0.93$ | $58.08^{e} \pm 1.15$ | $108.24^{c} \pm 4.11$ | $59.20^{b,c} \pm 2.35$ |
| SOX3 | [2]EtOH/[3]$H_2O$ (70:30 v/v) | $19.62^{c,d} \pm 0.50$ | $77.17^{c} \pm 0.67$ | $280.70^{b} \pm 0.83$ | $695.62^{a} \pm 19.67$ | $97.36^{c} \pm 0.92$ | $39.95^{e} \pm 0.99$ |
| SOX4 | [3]$H_2O$ | $12.94^{d} \pm 1.19$ | $86.00^{b} \pm 2.05$ | $133.45^{e} \pm 0.67$ | $286.43^{d} \pm 2.66$ | $300.05^{a} \pm 9.64$ | $66.98^{a,b} \pm 0.00$ |
| SFE1 | [4]$CO_2$. 20 MPa. 40 °C | $26.11^{b,c} \pm 0.74$ | $1.61^{f} \pm 0.10$ | $1.95^{g} \pm 0.11$ | $1.76^{g} \pm 0.16$ | $1.39^{e} \pm 0.17$ | $15.75^{g} \pm 1.02$ |
| SFE2 | [4]$CO_2$. 20 MPa. 50 °C | $20.94^{c,d} \pm 1.30$ | $1.94^{f} \pm 0.03$ | $2.84^{g} \pm 0.35$ | $2.09^{g} \pm 0.03$ | $1.10^{e} \pm 0.03$ | $20.26^{f,g} \pm 0.86$ |
| PLE1 | [2]EtOH. 10 MPa. 80 °C | $29.75^{a,b} \pm 4.82$ | $24.61^{e} \pm 0.04$ | $23.19^{f} \pm 0.75$ | $35.73^{f} \pm 0.82$ | $44.17^{d} \pm 2.65$ | $44.49^{d,e} \pm 1.02$ |
| PLE2 | [2]EtOH:[3]$H_2O$ (70:30 v/v). 10 MPa. 80 °C | $16.97^{d} \pm 0.34$ | $109.52^{a} \pm 2.37$ | $319.12^{a} \pm 4.62$ | $542.43^{b} \pm 9.09$ | $185.16^{b} \pm 2.58$ | $66.71^{a,b} \pm 1.52$ |
| SWE | [3]$H_2O$. 10 MPa. 160 °C | $37.63^{a} \pm 3.18$ | $84.47^{b} \pm 2.83$ | $138.32^{d} \pm 0.87$ | $360.17^{c} \pm 7.13$ | $305.53^{a} \pm 14.05$ | $52.50^{c,d} \pm 3.25$ |
| BHT | – | – | – | – | – | – | $76.03^{a} \pm 0.77$ |

[1]Hexane; [2]Ethanol; [3]Water; 4Carbon Dioxide; 5Mean±standard variation; 6Different superscript letters mean groups statistically different ($p < 0.05$) in each column by Tukey's test.

because CER and FER periods can recover from 70% to 90% of the total extractable solute [30]. Therefore, the extraction times were defined as 270 min, 40 min and 80 min, for SFE, PLE and SWE, respectively. These total extraction times represent more than 95% of the total amount of each extract recovered by the kinetics assays (Fig. 2), confirming the near null extraction rate.

### 3.2. Extraction yield ($X_0$) for conventional and green extractions

The values of extraction yield ($X_0$) obtained by SOX, SFE, PLE and SWE applied for peanut by-product are shown at Table 2. The maximum yield values were obtained by SOX1, SOX2 and SWE (37.65%, 35.30% and 37.63%, respectively), with no significant difference. The behavior, provided by these solvents with different polarities, 0.0 (hexane), 5.2 (ethanol) and 9 (water) [31], probably indicate the large diversity of compounds from the raw material, with high content of fatty acids (lipidic fraction), soluble in non-polar solvents; but also rich in more polar compounds (such as procyanidins, catechin and epicatechin, contributing to extraction yield [4]), soluble in solvents as ethanol and water (from intermediate to high polarity values).

Comparing SOX1 with SFE (yield up to 26.11%), both using non-polar solvents, the highest yield from Soxhlet is due to the use of higher temperature (the hexane boiling temperature, 68.65 °C [32]), the solvent recycling and solvent/solute interactions, which enhances the solute solubilization. Also, the surface tension and viscosity of the solvents increase with temperature, facilitating the solute solubilization within the solid material, increasing yield [33]. Although, Soxhlet extraction presents drawbacks compared to SFE, for example, high temperatures can cause thermal decomposition of thermolabile compounds, and the solvent used (hexane) must be separated from solute, after extraction, contrasting with SFE which provides a solvent-free extract.

Related to the use of ethanol as solvent by Soxhlet and by PLE, no significant differences in yield were detected between with SOX2 (35.30%) and PLE1 (29.75%). The results agree with obtained by Putra et al. [16] which reached $X_0$ of 36.28% for Soxhlet with ethanol recovered from peanut skin.

The use of the solvent mixture ethanol/water (70:30 v/v) also provided yield values without significant difference between Soxhlet and PLE methods (SOX3 of 19.62% and PLE2 of 16.98%). Although the yield values are statistically equal, it is noted that PLE was performed in only 40 min, while SOX was conducted for 6 h; and PLE used 120 mL of solvent while SOX used 150 mL. Lower extraction time can be associated with low energy consumption, decreasing the process costs.

The SWE yield was three times higher than Soxhlet with water (SOX4) and more than two times higher than PLE-EtOH/$H_2O$ (PLE2), indicating the high influence of the water at subcritical condition (SWE conduced at 160 °C), which uses water above its boiling point. Then, the temperature increase provides a significant decrease in subcritical water dielectric constant, increasing the solubility of different non-polar substances in water (phenolic compounds, sugars, proteins, among others) due to increase in solvent diffusivity and viscosity decrease, improving the solid penetration and contributing to the mass transfer [34].

Also, changing the solvent polarity it is possible to obtain different extracts (polar and nonpolar). The higher yields obtained for all techniques evaluated in this work can be linked to the rich nonpolar and polar composition of peanut by-product.

### 3.3. Total phenolic content (TPC)

The total phenolic content (TPC) from the recovered extracts from peanut by-product obtained by various extraction methods are also present in Table 2.

The TPC results ranged from 1.09 to 109.52 mg GAE $g^{-1}$, where SFE1, SFE2 and SOX1 samples provided lowest TPC values. This behavior was expected as these methods recover the oily fraction from the by-product and suggests low solubility of the phenolic compounds (phenolic fraction) on the non-polar solvents such as $CO_2$ (SFE1 and SFE2) and hexane (SOX1). Besides, the temperature increase, from 40 °C (SFE1) to 50 °C (SFE2), had no significant effect ($p < 0.05$) on TPC values from the recovered extracts.

Mainly, phenolic compounds are soluble in more polar solvents, as observed in Table 2, where the highest TPC results were obtained by extracts recovered by PLE2, SOX4 and SWE, with the polar solvents ethanol/water (70:30 v/v) and water. PLE2 provided the highest TPC value (109.52 mg GAE $g^{-1}$), with significant difference to obtained by SOX3, performed with the same solvent mixture (77.17 mg GAE $g^{-1}$), emphasizing the relevance of the high pressures to recover bioactive compounds. The good performance of the ethanol/water mixture as solvent can be explained by the fact that while water enhances the solute desorption from the solid matrix, the ethanol favors the solute solubility, improving the extraction efficiency [35].

Also, high TPC values were observed using water as solvent, by SOX4 and SWE, with statistically equal values ($p < 0.05$). This behavior is probably due to fragmentation or hydrolysis of polymeric compounds from the peanut by-product (e.g., proteins, polysaccharides, or others) producing water-soluble products (such as amino acids and sugars) due to elevated process temperature (SWE) or extraction time (SOX). The hydroxyl groups from these compounds could react with Folin-Ciocalteu reagent (Section 2.5), enhancing the TPC value of these extracts [6,36].

### 3.4. Antioxidant capacity

Due to multiple reactions and mechanism, a single method does not reflect accurately the antioxidant capacity of a complex material. Therefore, four antioxidant assays (DPPH, ABTS, FRAP and β-carotene/

linoleic acid system) were performed to provide the different aspects of antioxidant capacity from the peanut by-product extracts, as presented in Section 2.6. The results from all methods and extracts evaluated are also presented in Table 2.

In general, extracts recovered by ethanol, ethanol/water mixture and water (solvents from medium to high polarity) presented higher antioxidant capacity values compared to samples recovered by $CO_2$ or hexane, within the evaluated methods. The antioxidant performance agrees with the TPC values, suggesting that the antioxidant potential from the peanut by-product is due to the presence of phenolic compounds.

The best DPPH values were provided from the extract recovered by PLE2 (319.12 μmol TE $g^{-1}$), followed by the SOX3 (280.70 μmol TE $g^{-1}$), both recovered by ethanol/water 70:30 (v/v). For the ABTS method, the extracts that provided the best performances were also recovered by ethanol/water, i.e., SOX3 (695.62 μmol TE $g^{-1}$), and PLE2 (542.43 μmol TE $g^{-1}$). Both extracts presented higher values by ABTS assay compared to DPPH assay. Camargo et al. [3] reported lower antioxidant activity by ABTS from peanut skin extracts recovered by acetone/water (70:30 v/v) as solvent at 30 °C, reaching a value of 483.40 μmol TE $g^{-1}$, compared to SOX3 and PLE2 samples with ethanol/water mixture (695.62 and 542.43 μmol TE $g^{-1}$, respectively).

The best FRAP results (Table 2) were provided by the hydrophilic extracts, with values of 300.05 and 305.53 μmol TE $g^{-1}$ for the samples SOX4 and SWE, respectively, with no significant difference ($p < 0.5$). The best results of SWE and SOX4 from FRAP (compared to DPPH and ABTS methods) can be explained by different mechanisms of those methods, while FRAP measure the ability of ability of antioxidants to reduce ferric ($Fe^{3+}$) to ferrous ($Fe^{2+}$), DPPH and ABTS are based on hydrogen atom donation and singlet electron transfer mechanism [37].

The higher temperatures and solvent polarity (water), for SOX4 and SWE, probably contributed to increase the recovery of phenolic compounds and others antioxidant substances from the peanut by-product. Also, high extraction temperatures contributed to the formation of new compounds with antioxidant characteristics (neoantioxidants) through Maillard reaction, caramelization and/or thermooxidantion in SWE and SOX4 extracts [34]. Besides, the brown color of Fig. 1(c) is an indicative of Maillard reactions at SWE sample, with extraction conducted at 160 °C, a high temperature, as recognized to intensify the formation of Maillard reaction products, which brown color intensification depends on the intensity of the thermal treatment, intensified about 125 °C [38]. Probably, the new formed compounds have strong ability to reduce ferric ($Fe^{3+}$) to ferrous ($Fe^{2+}$), justifying the high antioxidant activity detected by FRAP assay for SWE and SOX4 samples, conducted at higher temperatures.

The antioxidant capacity of the extracts from peanut by-product was also evaluated by the β-carotene/linoleic acid bleaching assays, with results compared to the synthetic antioxidant BHT (butylated hydroxytoluene), used as standard. The results varied from 15.75% to 66.98% of antioxidant capacity, comparable with the results provided by the DPPH and ABTS methods. The highest values were provided by the high polar solvents (ethanol, ethanol/water mixture and water), with the best performances detected by SOX4 and PLE2 samples (66.98% and 66.71%, respectively), with no significant difference with BHT performance of 76.03%. Otherwise, the extracts obtained by SFE (SFE1 and SFE2) and by SOX1 presented the lower antioxidant performance, probably due to the non-polar aspect of the solvents ($CO_2$ and hexane).

Therefore, based on TPC and antioxidant performance from peanut by-product, the methods of PLE and SWE are viable alternatives with greener attributes comparable with Soxhlet method for the recovery of valuable compounds from peanut by-product with high antioxidant activity.

### 3.5. Pearson's correlation: TPC and AA

The Pearson coefficient (r) was performed to observe the phenolic compounds influence (by TPC data) on the antioxidant potential (DPPH, ABTS, FRAP and AA), and the results are presented at Table 3. This correlation may contribute to elucidate the bioactivity of the extracts from peanut by-products.

**Table 3**
Pearson correlation coefficients (r) between the total polyphenols (TPC) content and antioxidant activity (DPPH, ABTS, FRAP and β-carotene/linoleic acid bleaching methods) for peanut by-product extracts.

| | TPC | DPPH | ABTS | FRAP | β-carotene/linoleic acid |
|---|---|---|---|---|---|
| TPC | 1.00 | – | – | – | – |
| DPPH | 0.90 | 1.00 | – | – | – |
| ABTS | 0.83 | 0.91 | 1.00 | – | – |
| FRAP | 0.84 | 0.53 | 0.55 | 1.00 | – |
| β-carotene | 0.88 | 0.68 | 0.51 | 0.79 | 1.00 |

All correlations are significant at level of 5% ($p < 0.05$).

**Table 4**
Process conditions and effective concentration required for 50% of enzyme activity inhibition at 50% (IC50, mg $mL^{-1}$) of porcine pancreatic and human salivary α-amylases.

| Extracts | Process conditions | [5,6]Porcine pancreatic α-amylase [7]$IC_{50}$ [mg $mL^{-1}$] | [5,6]Human salivary α-amylase |
|---|---|---|---|
| SOX1 | [1]Hex | > 2 | $1.13^{c,d} \pm 0.03$ |
| SOX2 | [2]EtOH | $1.58^{a} \pm 0.87$ | $1.67^{a,b} \pm 0.34$ |
| SOX3 | [2]EtOH/[3]$H_2O$ (70:30 v/v) | > 2 | $0.74^{d} \pm 0.37$ |
| SOX4 | [3]$H_2O$ | – | – |
| SFE1 | [4]$CO_2$, 20 MPa, 40 °C | $1.65^{a} \pm 0.12$ | > 2 |
| SFE2 | [4]$CO_2$, 20 MPa, 50 °C | > 2 | $1.82^{a} \pm 0.27$ |
| PLE1 | [2]EtOH, 10 MPa, 80 °C | > 2 | $1.30^{b,c} \pm 0.87$ |
| PLE2 | [2]EtOH:[3]$H_2O$ (70:30 v/v), 10 MPa, 80 °C | $1.52^{a} \pm 0.06$ | $0.96^{c,d} \pm 0.89$ |
| SWE1 | [3]$H_2O$, 10 MPa, 160 °C | – | $1.66^{a,b} \pm 0.16$ |

[1]Hexane; [2]Ethanol; [3]Water; 4Carbon Dioxide 5Mean±standard variation; 6Different superscript letters mean groups statistically different ($p < 0.05$) in each column by Tukey's test; 7Effective concentration at 50% (at concentrations ranging from 0.08 to 2 mg mL-1)

Positive correlations were found between TPC and all antioxidant methods evaluated, with values higher than 0.80, indicating strong relation of antioxidant capacity with phenolic compounds. The highest correlation values were between TPC and DPPH, followed by TPC and β-carotene/linoleic acid, with data of 0.90 and 0.88, respectively. Lower result was observed by Nepote et al. [39] evaluating the relation between antioxidant activity by DPPH method and TPC from peanut skin extracts obtained by maceration with ethanol/water, with value of $r = 0.78$. Moderated correlations were also found by Win et al. [40] involving TPC values from peanut skin methanolic extracts. Then, the strong correlation values found in the present work between TPC and antioxidant methods support the role of the phenolic compounds as antioxidants from the peanut by-product.

### 3.6. Enzyme assays

The $IC_{50}$ (mg $mL^{-1}$) for each extract and α-amylase type are shown at Table 4. Additionally, the samples with the best enzymes inhibition activity (%), caused by the peanut by-product extracts (at concentrations of 0.08, 0.8 and 2 mg $mL^{-1}$), are presented at Fig. 3 for porcine pancreatic α-amylase (a) and for human salivary α-amylase (b).

According to Fig. 3, the extracts samples from peanut by-product presented inhibition activity against porcine pancreatic and human salivary α-amylases. In general, the samples were more effective, at higher concentrations of extracts. For the human salivary enzyme, the polar extracts were more efficient at higher concentration, together with SOX1 sample, reaching more that 80% inhibition for PLE2, SOX3 and SOX1 samples. Otherwise, for porcine pancreatic α-amylase, the extracts

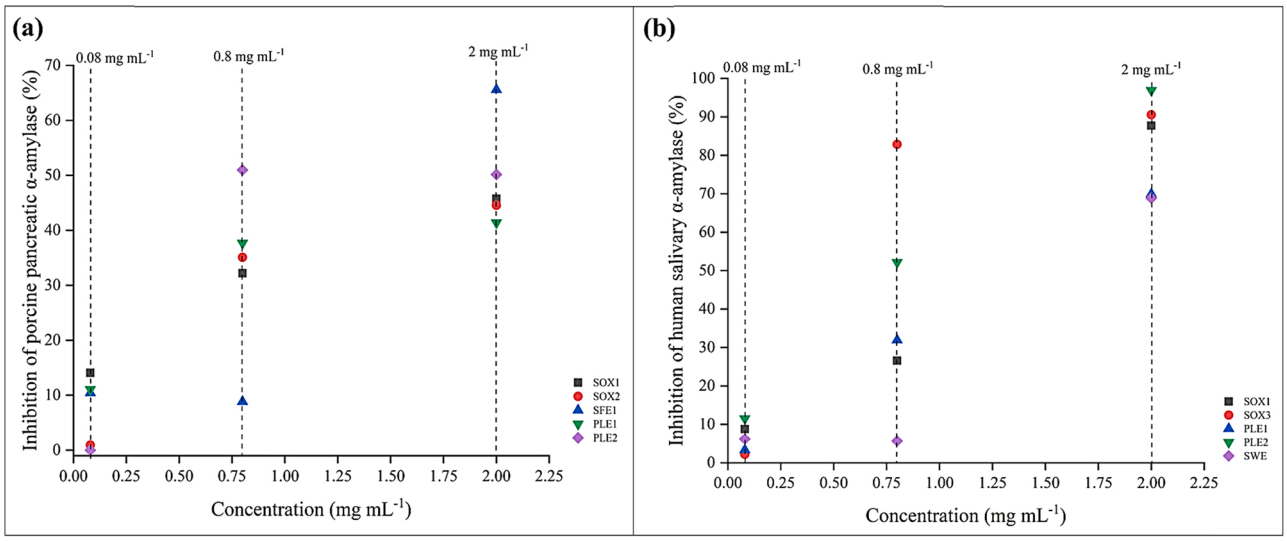


**Fig. 3.** Enzyme inhibition (%) of (a) porcine pancreatic α-amylase and (b) human salivary α-amylase of peanut by-product extracts at concentrations of 0.08, 0.8 and 2 mg $mL^{-1}$.

by non-polar solvents (hexane and $CO_2$) also provided good inhibition, with SFE1 providing the best inhibition performance, near 70% inhibition, followed by PLE2 and SOX1, as very effective extracts at the concentration of 2 mg $mL^{-1}$.

The behavior of the extracts from peanut by-product, obtained by different methods and solvents, to inhibit α-amylases enzymes, is also provided by Table 4 data. The water extracts, obtained by Soxhlet (SOX4) and SWE were no effective to prevent the enzymes activity, except for the SWE inhibition to human salivary α-amylase (1.66 mg $mL^{-1}$). In addition, samples by SOX1, SOX3, SFE2 and PLE1 presented high $IC_{50}$ values, not detectable by the highest α-amylases concentrations tested (up to 2 mg $mL^{-1}$). It is also clear the effect of solvent type and extraction method on $IC_{50}$ values for both enzymes, as observed TPC and antioxidant capacity (Table 2). The best $IC_{50}$ performances (lower values) for porcine pancreatic α-amylase were provided by PLE2 (1.52 mg $mL^{-1}$) and SOX2 (1.58 mg $mL^{-1}$), while for the human salivary α-amylase, the samples with best inhibition performance were SOX3 (0.74 mg $mL^{-1}$) and PLE2 (0.96 mg $mL^{-1}$), all samples with also the best performance in terms of TPC and antioxidant capacity.

The non-polar extracts (SOX1, SFE1 and SFE2) also provided good inhibitory activity of α-amylase enzymes. Literature reports that oleic and linoleic acids, present in peanut oilseeds, mostly recovered by non-polar solvents, may complex with α-amylase and α-glucosidase enzymes, resulting in static quenching, reducing their activity. Although, the action mechanism of the unsaturated fatty acids (namely, oleic and linoleic acids) to inhibit the enzyme activity is still not completely elucidated [41].

Fernandes et al. [42] observed 94.38% inhibition of porcine pancreatic α-amylase using 10 mg $mL^{-1}$ of ethanolic extract from peanut skins, which, according to the authors, is due to the presence of catechin, gallic acid and procyanidin B2 on the ethanolic extract. Otherwise, the ethanolic extracts from the present work (SOX2 and PLE1) provided porcine pancreatic α-amylase inhibition of 44.60% and 41.44%, respectively (Fig. 3), achieved using 2 mg $mL^{-1}$ of extract. Comparing these results, we observe that half inhibition was obtained using 5 times less amount of extract, evidencing the quality of the recovered material from peanut by-product.

Due to the ability to form quinones or lactones, phenolic compounds (such as phenolic acids and flavonoids) can bind covalently to α-amylase, reacting with nucleophilic groups on the enzyme molecule, changing the enzyme activity [43]. Therefore, high inhibitory effect of peanut by-product extracts is due to their polyphenolic composition,

**Table 5**
Lipidic fraction profiles of peanut by-products extracts obtained using SFE and SOX (with hexane).

| Fatty Acid content (%, mass basis) | Experimental assay | | |
|---|---|---|---|
| | SOX1 (hexane) | SFE1 (40 °C, 20 MPa) | SFE2 (50 °C, 20 MPa) |
| Palmitic Acid (C16:0) | 7.60 | 8.67 | 7.65 |
| Linoleic acid (C18:2) | 6.41 | 5.43 | 6.53 |
| Oleic acid (C18:1) | 77.97 | 74.31 | 77.23 |
| Stearic acid (C18:0) | 2.39 | 4.07 | 2.54 |
| Eicosenoic acid (C20:1) | 1.57 | 1.31 | 1.76 |
| Arachidic acid (C20:0) | 0.83 | 0.87 | 1.03 |
| Behenic acid (C22:0) | 1.38 | 2.05 | 2.05 |
| Lignoceric acid (C24:0) | – | 0.85 | 0.70 |
| $\Sigma^1$SFA | 12.20 | 16.52 | 13.97 |
| $\Sigma^2$MUFA | 79.54 | 75.62 | 78.99 |
| $\Sigma^3$PUFA | 6.41 | 5.43 | 6.53 |

[1]SFA: saturated fatty acid; [2]MUFA: monounsaturated fatty acid; [3]PUFA: polyunsaturated fatty acid

rich in proanthocyanidins, catechins and resveratrol, identified in peanut skin extracts [44]. Tsujita et al. [45] detected inhibitory enzyme activity of 32.4 U $mg^{-1}$ (lower than found by PLE2, SOX2 and SFE1 samples, with concentrations of 48.55, 46.83 and 44.79 U $mL^{-1}$, respectively: Table 4) for in vitro assay for porcine pancreatic α-amylase, using peanut skin extracts. The authors attributed this behavior to the presence of proanthocyanidins and catechin on the extracts.

Therefore, considering the results from Table 4, the extracts recovered from peanut by-product that provided high α-amylase inhibition can be considered promising alternatives to synthetic commercial drugs for diabetic control, such as Acarbose, which provides $IC_{50}$ of 0.50 mg $mL^{-1}$ [46], close to that from the peanut extract samples. These results support the quality attributes of the extracts from peanut by-product, also evidencing the good performance of the high-pressure methods, for instance PLE2 sample reached an $IC_{50}$ of 0.96 mg $mL^{-1}$, close to acarbose value.

Besides, good antioxidant amylases inhibition performances from the peanut by-product extracts allows its use as functional ingredient for food industries. Also, since the peanut by-product extracts were efficient to inhibit the carbohydrate hydrolysis by α-amylase, probably due to the presence of procyanidins, anthocyanidins, catechin and others, they can decrease postprandial hyperglycemia (high blood glucose level after a meal) in the digestive system [1].

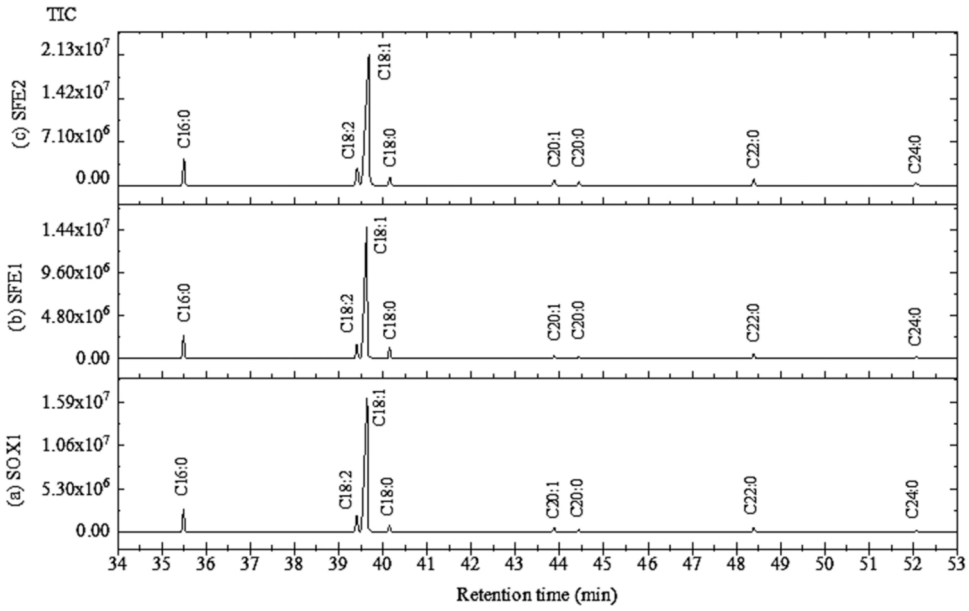


**Fig. 4.** Chromatograms (GC-FID) from peanut by-product samples recovered by: (a) Soxhlet-Hexane (SOX1); (b) SFE at 40 °C and 20 MPa (SFE1); (c) SFE at 50 °C and 20 MPa (SFE2).

### *3.7. Fatty acids chemical profiles*

The fatty acids profile for the oily samples (SOX1, SFE1 and SFE2) was investigated because, although they provided low performance of TPC and antioxidant potential (Table 2), these samples were also promising in terms of enzyme inhibition performance (low $IC_{50}$ values according to Table 4). Therefore, the fatty acids profile obtained by GC-FID for the samples SOX1, SFE1 and SFE2 are compared at Table 5, with the respective chromatograms shown at Fig. 4.

The lipid fraction from the peanut by-product shows similar profile than peanut oil [29] mainly with the fatty acids palmitic, linoleic, oleic and stearic, showing that the studied peanut by-product is a potential source of unsaturated fatty acids. The oleic (C18:1), palmitic (C16:0) and linoleic acid (C18:2) constituted the majority (%, mass basis) of the identified compounds, corresponding to 91.98%, 88.43% and 91.41% of the samples SOX1, SFE1 and SFE2, respectively. A small variation in fatty acids composition was observed among the extracts. Also, the results from Table 5 and Fig. 4 confirm the unsaturated fatty acids (mono and poly) as the main class of lipids from peanut by-products, recovered by SOX1 with 85.95%, by SFE1 with 81.05% and by SFE2 with 85.52%.

Therefore, SFE is a green technique that can be successfully applied for the recovery of unsaturated fatty acids from peanut by-product. The advantages of the SFE, an ambiently-friendly method, shows that greener process can successfully replace conventional extractions methods with similar product quality.

Essential fatty acids, such as omega 6 (linoleic acid, C18:2) and omega 9 (oleic acid, C18:1) have numerous functions in human body and must be included in human diet. Besides, they are associated with several bioactivities, preventing cancer [47], neurodegenerative disorders [48], cardiovascular diseases [49] and others. In addition, oleic acid can also be a precursor of various health-promoting compounds, such as sterol, vitamin E, choline, and others [47,48].

Therefore, peanut by-product can emerge as a promising source of essential fatty acids (as linoleic and oleic acids), that can be further utilized in food industries, enriching food matrices, such as mayonnaise [50], combined with the other valuable fractions, with antioxidant potential and enzyme inhibitors. These different fractions stimulate the promotion of a circular economy approach for peanut processing chain [1].

## 4. Conclusions

In this study, the peanut by-product, composed mainly by skins with small fragments of the oilseed, showed as promising source of high value substances, such as fatty acids and phenolic compounds with strong antioxidant and enzyme inhibition capacities. Greener extraction process (SFE, PLE and SWE) with ecologic solvents were successfully applied to obtain extracts with different characteristics, providing high yield values (up to 26.11% for SFE, to 29.75% for PLE and 37.63% for SWE). The TPC performance of the extract samples was strongly dependent on solvent polarity and extraction method, i.e., the best TPC value was provided by PLE-EtOH/$H_2O$ (PLE2), with 109.52 mg GAE $g^{-1}$. PLE samples provided the best antioxidant and enzyme inhibition activities. The fatty acids profile of the nonpolar samples (SFE and SOX with hexane) evidenced the presence of mainly unsaturated fatty acids. Therefore, the peanut by-product is an interesting raw material suitable as a rich source of valuable compounds capable to increase the aggregated value from peanut processing industry.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgements

The authors wish to thank Brazilian founding agencies for the financial support and fellowship, CNPq (National Council of Technological and Scientific Development, Brazil), Project 404347/2016-9, and CAPES (Coordination for the Improvement of Higher-Level Personnel, Brazil), projects CAPES/PROEX-1624/2018 and CAPES/PRINT88887.310560/2018-00. We are also grateful to LAMEB (Laboratório Multiusuário de Estudos em Biologia), from Biological Sciences Center (CCB) of the Federal University of Santa Catarina (UFSC).

## References

[1] G.D. Sorita, F.V. Leimann, S.R.S. Ferreira, Biorefinery approach: is it an upgrade opportunity for peanut by-products? Trends Food Sci. Technol. 105 (2020) 56–69, https://doi.org/10.1016/j.tifs.2020.08.011.

[2] J. Peng, Y. Jia, X. Du, Y. Wang, Z. Yang, K. Li, Study of physicochemical stability of anthocyanin extracts from black peanut skin and their digestion enzyme and adipogenesis inhibitory activities, Lwt 107 (2019) 107–116, https://doi.org/10.1016/j.lwt.2019.03.016.
[3] A.C. Camargo, M.A.B. Regitano-d'Arce, C.R. Gallo, F. Shahidi, Gamma-irradiation induced changes in microbiological status, phenolic profile and antioxidant activity of peanut skin, J. Funct. Foods 12 (2015) 129–143, https://doi.org/10.1016/j.jff.2014.10.034.
[4] J.L. Dias, S. Mazzutti, J.A.L. de Souza, S.R.S. Ferreira, L.A.L. Soares, L. Stragevitch, L. Danielski, Extraction of umbu (Spondias tuberosa) seed oil using $CO_2$, ultrasound and conventional methods: evaluations of composition profiles and antioxidant activities, J. Supercrit. Fluids 145 (2019) 10–18, https://doi.org/10.1016/j.supflu.2018.11.011.
[5] R. Muñoz-Arrieta, D. Esquivel-Alvarado, E. Alfaro-Viquez, V. Alvarez-Valverde, C. G. Krueger, J.D. Reed, Nutritional and bioactive composition of Spanish, Valencia, and Virginia type peanut skins, J. Food Compos. Anal. 98 (2021), 103816, https://doi.org/10.1016/j.jfca.2021.103816.
[6] R. Gallego, M. Bueno, M. Herrero, Sub- and supercritical fluid extraction of bioactive compounds from plants, food-by-products, seaweeds and microalgae – an update, TrAC Trends Anal. Chem. 116 (2019) 198–213, https://doi.org/10.1016/j.trac.2019.04.030.
[7] D.M. Ferro, D.A. Mayer, C.M. Oliveira Müller, S.R.S. Ferreira, Scale-up simulation of PLE process applied to recover bio-based materials from Sida rhombifolia leaves, J. Supercrit. Fluids 166 (2020), https://doi.org/10.1016/j.supflu.2020.105033.
[8] S. Mazzutti, L.G.G. Rodrigues, N. Mezzomo, V. Venturi, S.R.S. Ferreira, Integrated green-based processes using supercritical $CO_2$ and pressurized ethanol applied to recover antioxidant compouds from cocoa (Theobroma cacao) bean hulls, J. Supercrit. Fluids 135 (2018) 52–59, https://doi.org/10.1016/j.supflu.2017.12.039.
[9] L.G.G. Rodrigues, S. Mazzutti, I. Siddique, M. da Silva, L. Vitali, S.R.S. Ferreira, Subcritical water extraction and microwave-assisted extraction applied for the recovery of bioactive components from Chaya (Cnidoscolus aconitifolius Mill.), J. Supercrit. Fluids 165 (2020), 104976, https://doi.org/10.1016/j.supflu.2020.104976.
[10] M. Álvarez-Viñas, P. Rodríguez-Seoane, N. Flórez-Fernández, M.D. Torres, B. Díaz-Reinoso, A. Moure, H. Domínguez, Subcritical water for the extraction and hydrolysis of protein and other fractions in biorefineries from agro-food wastes and algae: a review, Food Bioprocess Technol. 14 (2021) 373–387, https://doi.org/10.1007/s11947-020-02536-4.
[11] N.R. Putra, A. Hazim Abdul Aziz, L. Nian Yian, W. Diyana Ramli, M. Azizi Che Yunus, Optimization of supercritical carbon dioxide and co-solvent ethanol extraction of wasted peanut skin using response surface methodology, MATEC Web Conf. 156 (2018), https://doi.org/10.1051/matecconf/201815602005.
[12] R. Bodoira, Y. Rossi, M. Montenegro, D. Maestri, A. Velez, Extraction of antioxidant polyphenolic compounds from peanut skin using water-ethanol at high pressure and temperature conditions, J. Supercrit. Fluids 128 (2017) 57–65, https://doi.org/10.1016/j.supflu.2017.05.011.
[13] AOAC, Official Methods of Analysis of the AOAC International, AOAC. 18 th ed. (2012).
[14] A.S. Pessoa, R. Podestá, J.M. Block, E. Franceschi, C. Dariva, M. Lanza, Extraction of pequi (Caryocar coriaceum) pulp oil using subcritical propane: determination of process yield and fatty acid profile, J. Supercrit. Fluids 101 (2015) 95–103, https://doi.org/10.1016/j.supflu.2015.03.006.
[15] E.M.Z. Michielin, L.P. De Lemos Wiese, E.A. Ferreira, R.C. Pedrosa, S.R.S. Ferreira, Radical-scavenging activity of extracts from Cordia verbenacea DC obtained by different methods, J. Supercrit. Fluids 56 (2011) 89–96, https://doi.org/10.1016/j.supflu.2010.11.006.
[16] N.R. Putra, D. Nur, R. Ahmad, S. Zaini, M. Azizi, C. Yunus, S. Machmudah, M. Syafiq, H. Ruslan, Eff. Part. size yield Extr. Antioxid. Act. Peanut Ski. Using Modif. Supercrit. Carbon Dioxide soxhlet Extr. (2018) 1–9, https://doi.org/10.1111/jfpp.13689.
[17] G.L. Teixeira, L.G. Maciel, S. Mazzutti, R.C.T. Barbi, R.H. Ribani, S.R.S. Ferreira, J. M. Block, Sequential green extractions based on supercritical carbon dioxide and pressurized ethanol for the recovery of lipids and phenolics from Pachira aquatica seeds, J. Clean. Prod. 306 (2021), https://doi.org/10.1016/j.jclepro.2021.127223.
[18] D.A. Oliveira, M. Angonese, C. Gomes, S.R.S. Ferreira, Valorization of passion fruit (Passiflora edulis sp.) by-products: Sustainable recovery and biological activities, J. Supercrit. Fluids 111 (2016) 55–62, https://doi.org/10.1016/j.supflu.2016.01.010.
[19] H. Sovová, Rate of the vegetable oil extraction with supercritical c02–i. modelling of extraction curves, Chem. Eng. Sci. 49 (1994) 409–414.
[20] S.R.S. Ferreira, M.A.A. Meireles, Modeling the supercritical fluid extraction of black pepper (Piper nigrum L.) essential oil, J. Food Eng. 54 (2002) 263–269, https://doi.org/10.1016/S0260-8774(01)00212-6.
[21] V.L. Singleton, J.A. Rossi, Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents, Am. J. Enol. Vitic. 16 (1965) 144–158.
[22] W. Brand-Williams, M.E. Cuvelier, C. Berset, Use of a free radical method to evaluate antioxidant activity, LWT Food Sci. Technol. 28 (1995) 25–30, https://doi.org/10.1016/S0023-6438(95)80008-5.
[23] R. Re, N. Pellegrini, A. Proteggente, A. Pannala, M. Yang, C. Rice-Evans, Antioxidant activity applying an improved ABTS radical cation decolorization assay, Free Radic. Biol. Med. 26 (1999) 1231–1237.
[24] I.F.F. Benzie, J.J. Strain, The ferric reducing ability of plasma (FRAP) as a measure of "'antioxidant power"': the FRAP assay, Anal. Biochem. 239 (1996) 70–76.
[25] B. Matthäus, Antioxidant activity of extracts obtained from residues of different oilseeds, J. Agric. Food Chem. 50 (2002) 3444–3452, https://doi.org/10.1021/jf011440s.
[26] S.M. Silva, E.A. Koehnlein, A. Bracht, R. Castoldi, G.R. de Morais, M.L. Baesso, R. A. Peralta, C.G.M. de Souza, A.B. de Sá-Nakanishi, R.M. Peralta, Inhibition of salivary and pancreatic α-amylases by a pinhão coat (Araucaria angustifolia) extract rich in condensed tannin, Food Res. Int. 56 (2014) 1–8, https://doi.org/10.1016/j.foodres.2013.12.004.
[27] G.L. Miller, Use of dinitrosalicylic acid reagent for determination of reducing sugar, Anal. Chem. 3 (1959) 426–428.
[28] S. Wagon, Mathematica in action & Business Media, Springer Science, 1999.
[29] E.C. Shin, B.D. Craft, R.B. Pegg, R.D. Phillips, R.R. Eitenmiller, Chemometric approach to fatty acid profiles in Runner-type peanut cultivars by principal component analysis (PCA), Food Chem. 119 (2010) 1262–1270, https://doi.org/10.1016/j.foodchem.2009.07.058.
[30] G. Brunner, Gas extraction. An Introduction to Fundamentals of Supercritical Fluids and the Application to Separation Processes, Springer,, New York, NY, 1994.
[31] L.R. Snyder, Classification of the solvent properties of common liquids, J. Chromatogr. A. 92 (1974) 223–230.
[32] G.C. Sekhar, M.J. Lee, Phase equilibria for binary mixtures of hexane with orthoxylene, metaxylene, and nonane with hexane at 101.3 kPa, Results Chem. 1 (2019), 100005, https://doi.org/10.1016/j.rechem.2019.100005.
[33] G. Ferrentino, J. Ndayishimiye, N. Haman, M. Scampicchio, Functional activity of oils from brewer's spent grain extracted by supercritical carbon dioxide, Food Bioprocess Technol. 12 (2019) 789–798, https://doi.org/10.1007/s11947-019-02249-3.
[34] L.G.G. Rodrigues, S. Mazzutti, L. Vitali, G.A. Micke, S.R.S. Ferreira, Recovery of bioactive phenolic compounds from papaya seeds agroindustrial residue using subcritical water extraction, Biocatal. Agric. Biotechnol. 22 (2019), 101367, https://doi.org/10.1016/j.bcab.2019.101367.
[35] A. Mustafa, C. Turner, Pressurized liquid extraction as a green approach in food and herbal plants extraction: a review, Anal. Chim. Acta 703 (2011) 8–18, https://doi.org/10.1016/j.aca.2011.07.018.
[36] E. Karacabey, G. Mazza, L. Bayındırlı, N. Artık, Extraction of bioactive compounds from milled grape canes (vitis vinifera) using a pressurized low-polarity water extractor, Food Bioprocess Technol. 5 (2012) 359–371, https://doi.org/10.1007/s11947-009-0286-8.
[37] H.S. Kareem, A. Ariffin, N. Nordin, T. Heidelberg, A. Abdul-Aziz, K.W. Kong, W. A. Yehye, Correlation of antioxidant activities with theoretical studies for new hydrazone compounds bearing a 3,4,5-trimethoxy benzyl moiety, Eur, J. Med. Chem. 103 (2015) 497–505, https://doi.org/10.1016/j.ejmech.2015.09.016.
[38] M. Plaza, M. Amigo-Benavent, M.D. del Castillo, E. Ibáñez, M. Herrero, Neoformation of antioxidants in glycation model systems treated under subcritical water extraction conditions, Food Res. Int. 43 (2010) 1123–1129, https://doi.org/10.1016/j.foodres.2010.02.005.
[39] V. Nepote, N.R. Grosso, C.A. Guzmán, Optimization of extraction of phenolic antioxidants from peanut skins, J. Sci. Food Agric. 85 (2005) 33–38, https://doi.org/10.1002/jsfa.1933.
[40] M.M. Win, A. Abdul-Hamid, B.S. Baharin, F. Anwar, M.C. Sabu, M.S. Pak-dek, Phenolic compounds and antioxidant activity of peanut's skin, hull, raw kernel and roasted kernel flour, Pak. J. Bot. 43 (2011) 1635–1642.
[41] C.H. Su, C.H. Hsu, L.T. Ng, Inhibitory potential of fatty acids on key enzymes related to type 2 diabetes, BioFactors 39 (2013) 415–421, https://doi.org/10.1002/biof.1082.
[42] A.C.F. Fernandes, I.M. Martins, D.K.T. Moreira, G.A. Macedo, Use of agro-industrial residues as potent antioxidant, antiglycation agents, and α-amylase and pancreatic lipase inhibitory activity, J. Food Process. Preserv. 44 (2020) 1–12, https://doi.org/10.1111/jfpp.14397.
[43] C. Jiang, Y. Chen, X. Ye, L. Wang, J. Shao, H. Jing, C. Jiang, H. Wang, C. Ma, Three flavanols delay starch digestion by inhibiting α-amylase and binding with starch, Int. J. Biol. Macromol. 172 (2021) 503–514, https://doi.org/10.1016/j.ijbiomac.2021.01.070.
[44] P.E.S. Munekata, R.P.P. Fernandes, M.P. Melo, M.A. Trindade, J.M. Lorenzo, Influence of peanut skin extract on shelf-life of sheep patties, Asian Pac, J. Trop. Biomed. 6 (2016) 586–596, https://doi.org/10.1016/j.apjtb.2016.05.002.
[45] T. Tsujita, T. Shintani, H. Sato, Preparation and characterisation of peanut seed skin polyphenols, Food Chem. 151 (2014) 15–20, https://doi.org/10.1016/j.foodchem.2013.11.072.
[46] Y. Xiong, K. Ng, P. Zhang, R.D. Warner, S. Shen, H.Y. Tang, Z. Liang, Z. Fang, In vitro α-glucosidase and α-amylase inhibitory activities of free and bound phenolic extracts from the bran and kernel fractions of five sorghum grain genotypes, Foods 9 (2020), https://doi.org/10.3390/foods9091301.
[47] S.A. Krapf, J. Lund, M. Lundkvist, M.G. Dale, T.A. Nyman, G.H. Thoresen, E. T. Kase, Pancreatic cancer cells show lower oleic acid oxidation and their conditioned medium inhibits oleic acid oxidation in human myotubes, Pancreatology 20 (2020) 676–682, https://doi.org/10.1016/j.pan.2020.04.014.
[48] M.A.M. Kojour, S.E. Barough, H.M. Kouchesfehani, H. Jalali, M.H.K. Ebrahim, Oleic acid promotes the expression of neural markers in differentiated human

endometrial stem cells, J. Chem. Neuroanat. 79 (2017) 51–57, https://doi.org/10.1016/j.jchemneu.2016.11.004.

[49] H. Hioki, T. Miura, Y. Miyashita, S. Ebisawa, H. Motoki, A. Izawa, J. Koyama, U. Ikeda, Circulating eicosapentaenoic acid to oleic acid ratio and risk for cardiovascular events in patients with coronary artery disease: a sub-analysis of the SHINANO registry, IJC Metab. Endocr. 10 (2016) 1–6, https://doi.org/10.1016/j.ijcme.2015.11.001.

[50] V.M. Rojas, L.F. da, C.B. Marconi, A. Guimarães-Inácio, F.V. Leimann, A. Tanamati, Â.M. Gozzo, R.H.B. Fuchs, M.F. Barreiro, L. Barros, I.C.F.R. Ferreira, A.A. C. Tanamati, O.H. Gonçalves, Formulation of mayonnaises containing PUFAs by the addition of microencapsulated chia seeds, pumpkin seeds and baru oils, Food Chem. 274 (2019) 220–227, https://doi.org/10.1016/j.foodchem.2018.09.015.