Valorization of sunflower by-product using microwave-assisted extraction to obtain a rich protein flour: Recovery of chlorogenic acid, phenolic content and antioxidant capacity

Grazielle Náthia-Neves*; Esther Alonso

BioEcoUva. Research Institute on Bioeconomy. High Pressure Processes Group, Department of Chemical Engineering and Environmental Technology, University of Valladolid, Valladolid, Spain.

* Corresponding author: grazinathia@yahoo.com.br

Author for correspondence: Grazielle Náthia-Neves (grazinathia@yahoo.com.br)*

High Pressure Processes Group (Department of Chemical Engineering and Environmental Technology).

University of Valladolid, Prado de la Magdalena 5, 47011 Valladolid, Spain

Abstract

The sunflower cake, which is a by-product from sunflower oil refining, is a rich source of chlorogenic acid (CGA), a phenolic compound that must be removed from this by-product before its use for human consumption. This work studied the extraction of CGA from sunflower by-product using microwave-assisted extraction (MAE) and it was divided into two steps. The first step identified the conditions of solvent and solvent-to-feed ratio (S/F) that maximized the CGA extraction. The highest CGA yield was obtained using ethanol 70%, and the S/F did not show statistical significance on the recovery of CGA. In the second stage, ethanol 70% and S/F of 10 were fixed and the effect of power (100, 200 and 300 W) and extraction time (30, 60, 90 and 120 seconds) were evaluated in the extraction of CGA. MAE processes were evaluated in terms of global and CGA extraction yields, total phenolic content (TPC) and antioxidant capacity. The developed process allowed to recover 8.4 ± 0.1 mg CGA / g of raw material in a very short time (30 seconds). ORAC assay revealed that the extracts presented antioxidant capacity. FTIR spectra exhibited no significant differences for all the analyzed samples, and the SEM images showed that the sunflower by-product structure was affected by the irradiation power. Therefore, the MAE process proved to be a fast and efficient method to obtain CGA-rich extracts and a residual solid with a high content of protein (26%) and essential amino acids that allows its usage in products for human nutrition.

Keywords: Emerging technology; by-product valorization; bioactive compounds; polyphenols

1. Introduction

Agricultural production is continuously increasing and as a result, a large amount of wastes/by-products have been generated year by year. One of the most generated industrial waste in the world are oil cakes/meals, which are by-products obtained after the oil extraction from vegetable matrices (Sivaramakrishnan and Gangadharan, 2009). The sunflower is one of the three major predominant oil crops in the world behind soybeans and rapeseed (FAO, 2019). The sunflower oil production has been growing faster than the world population and in this sense, a correct discard of the residue generated during its oil refining is necessary (Carré and Pouzet, 2014). In 2017, 19 million metric tons of sunflower cake were generated (Karefyllakis et al., 2019).

Currently, this by-product is used as feedstuff (ruminants feed) or discarded (Dabbour et al., 2019). Nevertheless, it could be used as feedstock in several industrial processes due to its high nutritional value, high availability, and competitive pricing. For instance, the sunflower by-product is rich in protein (27-63%), ash (6-9.5%), oil (<1.0% to 5.6%), polyphenols (2-5%) among others that make its use interesting for applications in chemical, pharmaceutical and food industries (Wildermuth et al., 2016).

Despite the high protein content, the use of sunflower proteins has been restricted for human consumption due to the presence of relatively high amounts of phenolic compounds. The main polyphenol in sunflower by-product is the chlorogenic acid (CGA). Chemically, phenolic compounds are able to interact with proteins by changing their solubility, digestibility, their shelf life and stability, and their organoleptic properties (green dark color due to oxidation of phenolic compounds) (González-Pérez and Vereijken, 2007; Kroll et al., 2001; Kroll et al., 2000; Sastry and Rao, 1990). Unlike undesirable effects, CGA has biological properties including antioxidant, anti-allergenic, anti-inflammatory, anti-microbial, anti-obesity and anti-thrombotic, and therefore, the CGA extracted from sunflower by-product can be used in the manufacture of pharmaceutical, cosmetic and food ingredients (Daraee et al., 2019; Naveed et al., 2018).

CGA is present in many plant matrices (stem, flowers, seeds, leaves, bark, herbs, fruits, and roots) (Meinhart et al., 2017) and there are many studies in the literature reporting the extraction of this compound using different methods and solvents. For instance, Upadhyay et al. (2012) obtained a maximum CGA yield (8.4 %) from green coffee beans by MAE at 50 °C, 800 W using water for 5 minutes. In another study, 6.14%

of CGA was obtained from flower buds of *Lonicera japonica* Thunb by MAE at 60 °C, 700 W using 50% ethanol for 5 minutes (Zhang et al., 2008).267 µg of CGA / g of potato peels were recovered by ultrasound-assisted extraction (UAE) at 42 kHz using 80% methanol (Kumari et al., 2017). UAE was also used to extract CGA from *Folium eucommiae* herb (0.77 mg/g) using 52% ethanol, at 50 °C and ultrasonic frequency of 5 kHz for 25 minutes (Liu et al., 2010).

The extraction of CGA from sunflower by-product can be performed by different methods. Among existing methodologies to isolate functional ingredients, clean technologies are preferred (Prado et al., 2018) . Microwave-assisted extraction (MAE) is a well-established clean extraction method to obtain bioactive compounds from vegetable matrices (Llompart et al., 2019). During this process, microwave irradiation heats the in-situ water of plant cells increasing the pressure inside them. This increase in pressure promotes the cell wall disruption which allows the release of molecules of interest quickly and efficiently (Vinatoru et al., 2017). The advantages of this method are mainly related to the process intensification in terms of reduction of the extraction time, solvent consumption, energy consumption and the amount of needed raw material (Vinatoru et al., 2017). To the best of our knowledge, there are no previous studies in the literature dedicated to the extraction of CGA from sunflower by-product using MAE. Thus, the purpose of this work was to obtain a CGA-rich extract from sunflower by-product using MAE and to analyze the phenolic content and the antioxidant capacity of the obtained extracts. Moreover, the obtained results are expected to promote the usage of sunflower by-product (high-protein content and low CGA content) in the food industries for human consumption.

2. Material and Methods

2.1. Chemicals

All chemicals and reagents used in this study were of analytical and food grades. For the extractions, absolute ethyl alcohol (99.0% purity) was purchased from a local supplier. The acetonitrile (≥99.0%), formic acid (≥95.0%) and HPLC standard chlorogenic acid (≥99.0%) were provided by Sigma-Aldrich (St. Louis, USA). For assays of total phenolic content Folin-Ciocalteu reagent and sodium carbonate were purchased

from Panreac Química (Barcelona, Spain), and gallic acid was purchased from Sigma-Aldrich (St. Louis, USA). For assaying antioxidant capacity, (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox, 97%), 2,2-Azobis (2-methylpropionamidine dihydrochloride (AAPH) and fluorescein were purchased from Sigma-Aldrich (St. Louis, USA). Sodium phosphate dibasic dihydrate (Sigma-Aldrich; St. Louis, USA) and potassium phosphate monobasic anhydrous (Amresco; Philadelphia, USA) were used for phosphate buffer solution (PBS) preparation in bidistilled water (Milli-Q® Integral).

2.2. Raw material preparation and characterization

The raw material was kindly provided by Sociedad Cooperativa General Agropecuaria (Olmedo, Valladolid - Spain). It is the solid by-product resulting from the sunflower oil extraction process of a mixture of kernel and shells. Only for characterization purposes, the raw material was separated into three fractions by sieving. The main characteristics of each fraction are shown in Table 1. To obtain a higher yield regarding the quantity of residue, the raw material used in this work is formed by the whole sunflower by-product.

The sunflower by-product was milled in a ball mill (PM100, Retsch, Haan, Germany) to reduce particle size and promote the homogenization of the raw material. After milling, the raw material was stored at 4 °C until further chemical analysis and its use in the extractions.

The final particle size of the milled sunflower by-product was determined by light scattering using a Mastersizer 2000 (Malvern Instruments Ltd, Worcestershire, UK.) coupled with a Sirocco dry powder feeder.

Moisture (method n° 931.04), ash (method n° 972.15), lipids (method n° 963.15), and protein (method n° 970.22; using a conversion factor of 6.25) analyses were performed using methodologies recommended by the AOAC (1997). Carbohydrate content was calculated by difference. All analyses were performed in triplicate.

2.3. Extraction

2.3.1. Conventional extraction

The Soxhlet method was selected as the conventional extraction technique for comparison purposes. Five grams of sample was wrapped in filter paper and inserted into the Soxhlet apparatus connected to a solvent flask containing 150 mL of the solvent (water, ethanol, water:ethanol (30:70%w/w) and water:ethanol (50:50%w/w)). After that, the system was heated to boiling. Reflux was continued for 7 h, and then the solvent was evaporated at 105 °C. The assays were performed in triplicate, and the results were expressed as the mean ± standard deviation.

2.3.2. Microwave extraction (MAE)

MAE of sunflower by-product was performed in a commercial monomode oven working at a frequency of 2.45 GHz, CEM Discover One microwave (CEM Corporation, Matthews, USA).

The MAE process was organized in two steps. In the first step, power (200 W) and time (40 seconds) were fixed and the effect of solvent (water, ethanol, water:ethanol (30:70%w/w) and water:ethanol (50:50%w/w)) and the solvent-to-feed mass ratio (S/F; 5, 10 and 15) were evaluated in the recovery of CGA. In the second step, the effect of power (100, 200 and 300 W) and extraction time (30, 60, 90 and 120 seconds) were evaluated using the selected solvent and S/F ratio obtained in the first set of experiments. All experiments were performed in duplicate. Experimental procedure was the same for both set of experiments: For each experimental point, flasks were filled with the appropriate S/F up to 40 mL and, after being homogenized for 10 minutes (stirring at ambient temperature), the flasks were subsequently placed in the microwave and irradiated under the dynamic mode. Temperature was continuously monitored with an optic fiber thermometer (FoTEMP 4, OPTPcon GmbH). After irradiation, the heated solution was immediately cooled down in an ice bath and the suspensions were centrifuged (100000 rpm for 10 minutes) to separate the residual biomass, which was washed with distilled water and dried at 105 °C until constant weight to determine the global extraction yield. The global extraction yield was calculated as the ratio of the total solid mass extracted to the mass of the raw material feeded to the system on a dry basis. Finally, the liquid extract was filtered through a 0.22-μm cellulose acetate and stored at 4 °C for further analysis.

Finally, in order to maximize the extraction of CGA, the solid material resulting from the MAE process (200 W and 30 seconds) was subjected to further extraction using water:ethanol (30:70%w/w) at 38 °C under the agitation of 200 rpm for 1 hour.

2.4. CGA quantification by high performance liquid chromatography (HPLC)

The extracts were analyzed using the HPLC-PDA (Waters, Alliance E2695, Milford, USA) system, consisting of a separation module (2695) with an integrated column heater, autosampler and photodiode array (PDA) detector. The analysis was performed according to the method described by (Meinhart et al., 2019). Separation of the CGA was carried out on a C18 column (Cortecs T3, 2.7µm, 4.6x100 mm, Waters) using a mobile phase of acetonitrile (A) and water acidified with 0.1% formic acid, (B). Elution was conducted using a linear gradient system starting with 10% A, followed by 40% A at 6 min and 100% A at 6.1 min, which was maintained at 100% until 7.5 min for column cleaning. The column was reconditioned with 10% A for the next injection, from 7.6 min to 16 min. The temperature, flow rate and injection volume were 30 °C, 1.2 mL / min and 30 µL respectively. The identification of the CGA was performed by the comparison with the standard through retention time, at 325 nm. The quantification was performed using an external calibration curve of the analytical CGA standard. The calibration curve of the CGA (R^2 = 0.9999) was obtained in the range of 0.34-100 µg / mL.

2.5. Total phenolic content (TPC) and Antioxidant capacity

TPC was determined using the Folin-Ciocalteau method as described by (Singleton et al., 1999) with some modifications. Each extract was diluted in ethanol 70%. Triplicates of the sample (40 µL), water (3mL) and Folin–Ciocalteu (200 µL) reagent were mixed and incubated at room temperature for 3 min. Afterwards, 600 µL of 20% sodium carbonate were added and the mixture was kept in a water bath at 40 °C for 30 minutes. Then, absorbance was recorded at 765 nm with a Shimadzu UV-2550 spectrophotometer (Shimadzu Co, Kyoto, Japan). The gallic acid standard (53 to 852 µg· mL^{-1}) was used, and the results were expressed in terms of mg gallic acid equivalent (GAE) per g of extract or per g of raw material.

The antioxidant capacity was measured by ORAC (oxygen radical absorbance capacity) method by diluting the extracts and Trolox (12.5 - 200 μmol·L^{-1}) in a 10 mM potassium phosphate buffer at pH 7.4. Pure potassium 10 mM phosphate buffer was used as blank. Triplicates of the diluted sample, standard or blank (25 μL), followed by 150 μL fluorescein working solution were inserted into a black well microplate. Afterwards, the microplate was incubated at 37 °C for 30 min, and subsequently, 25 μL of AAPH solution were added to each well. The fluorescence decrease (excitation at 485 nm; emission at 520 nm) was measured for 130 min at 37 °C in the microplate reader. The results were expressed in μmol of Trolox equivalent (TE) per g of extract or per g of raw material.

2.6. Amino acid quantification by HPLC

The amino acids were quantified both in the raw material and in the residual solid after MAE process. For this purpose, 30 mg of solid were added to 30 mL aqueous solution containing HCl 0.1N and phenol 2% in a 50-mL microwave tube, which was irradiated at 600 W for 10 min. The acidic medium was removed using N_2 and the solid phase was dissolved with HCl 0.1N. The solution was filtered through a microfilter (0.22 μm) into a glass vial (2mL) and analyzed in HPLC (Agilent Technologies 1200 serie, Germany). Chromatographic separations were conducted using Zorbax Eclipse AAA C18 column (4.6 mm × 150 mm, i.d., 3.5 μm) at 40 °C. The flow rate was 2 mL / min. The mobile phase consisted of buffer $NaH_2PO_4.H_2O$ (pH = 7.8) (A) and acetonitrile:methanol:water (45:45:10) (B) with the following gradient elution: 0-18 min, 57% B; 18-26 min, 100% B. The injection volume was 0.3 μL. The quantification was performed using an external calibration curve of the analytical amino acid standards. Amino acid profile was expressed as mg of amino acid per g of raw material.

2.7. Scanning electron microscopy (SEM)

SEM was used to analyze the microstructure of the sunflower by-product surfaces before and after the microwave irradiation at 200 W and 30 seconds. SEM analysis was performed on a FlexSEM1000 (Hitachi, USA) microscope. Samples were prepared by mounting small pieces of film onto a stub using conductive carbon tape. Before the SEM analysis, the samples were coated with a nanometer-thick gold film using an SCD 004 (Balzers, Liechtenstein) sputter coater to improve their conductivity (40 seconds and 30

mA). SEM analysis was performed under vacuum, using a 10 kV acceleration voltage, 100 Pa and magnification of 500×.

2.8. FTIR spectroscopy

The FTIR spectra were recorded on a Fourier Transform InfraRed instrument (Platinum-ATR, Bruker Alpha, Massachusetts, USA) equipped with the software of OPUS Optik GmBH. For all samples, the resolution was 4 cm^{-1}, and 70 scans were acquired and then averaged over the 400-4000 cm^{-1} spectral range.

2.9. Statistical

The analysis of variance (ANOVA) of the results was evaluated using the Minitab 16® software (Minitab Inc., State College, PA, USA) with a 95% confidence level (p-value ≤ 0.05). The significant differences at level of 5% ($p \leq 0.05$) were analyzed through the Tukey's test. In the first step, a randomized full factorial design (4 x 3) was used to evaluate the effect of solvent (water, ethanol, water:ethanol (30:70%w/w) and water:ethanol (50:50%w/w)) and S/F (5, 10 and 15) on global extraction yield and CGA recovery. In the second step, a randomized full factorial design (4 x 3) was used to evaluate the effect of time (30, 60, 90 and 120 seconds) and power (100, 200 and 300 W) on global yield and CGA recovery.

3. Results and Discussion

3.1. Characterization of the raw material

The raw material characterization is shown in Table 2. It can be observed that the moisture, ash, lipid, protein and carbohydrate content are in agreement with those found in literature, which range from 7% to 16.4% for moisture, from 6% to 9.5% for ash, from <1% to 11.6% for lipids, from 22.3% to 63% for protein and from 57.6% to 65.4% for carbohydrates (Carrão-Panizzi and Mandarino, 1994; Pereira et al., 2011; Salgado et al., 2012; Tavernari et al., 2008; Wildermuth et al., 2016). Minor differences in chemical composition between this work and those found in the literature can be explained by differences in climate and soil conditions, variety, agricultural practices, post-harvest

management and genetic characteristics of the analyzed samples. In addition, it is worth mentioning that some sunflower oil processing industries use only kernel and others use the kernel + shells. The raw material used in the present study consisted of the whole sunflower seeds (kernel + shells).

3.2. Conventional extraction

The results obtained by the Soxhlet method using different solvents are shown in Table 3. The global yield ranged from 4.1 to 9.5%. The best solvent to extract CGA was ethanol 70% that extracted 136 ± 2 mg CGA/g of extract. The yield of CGA ranged from 3.2 to 15 mg of CGA /g of raw material, corroborating those results reported in the literature which describes that the amount of CGA in defatted flour sunflower is in the range of 0.5 to 3.6% on a dry mass basis (Wildermuth et al., 2016).

3.3. Effect of the solvent and S/F on the global extraction yield and CGA extraction

Figure 1 shows the global extraction yield obtained by the MAE process. The highest values of global extraction yield were obtained using water (9.9 - 12%) and the mixtures water-ethanol (8.3 - 9.5%) in comparison with pure ethanol (1.9 - 2.6%). S/F did not affect the global extraction yield. The highest yield was obtained using water as a solvent, which may be explained by the high content of hydrophilic compounds such as carbohydrates (66.6%) that are easily extracted with this solvent.

Table 4 shows the results of CGA content in the extracts, indicating the CGA purity and the CGA extraction yield expressed as mg CGA per g of extract and as mg CGA per g of raw material, respectively. The solvents were chosen due to the environmentally friendly effect and non-toxicity for food applications. The extraction of polyphenolic compounds can be affected by the polarity of the solvent (Ye et al., 2015). Polar solvents such as methanol, acetone, ethanol and water are able to efficiently extract this compound class. For instance, (Mokrani and Madani, 2016) studied the extraction of phenolic compounds from peach using different solvents and they observed that the use of 60% acetone improved the recovery of these compounds; 80% methanol showed to be the best solvent to extract phenolic compounds from *Amomum chinense* C. leafs (Butsat

and Siriamornpun, 2016); and the aqueous ethanol (50:50%) was the most effective solvent to extract phenolic compounds from Artichoke waste (Zuorro et al., 2014). From this, it can be inferred that the choice of the solvent depends on the raw material and the target phenolic compound to be extracted. Here, mixtures of water and ethanol favored the extraction of CGA in comparison with the pure solvents, Figure 2. The yield of CGA increased by 90% as the ethanol concentration changed from 100 to 70%. The best solvent to extract CGA was ethanol 70% that extracted 118 ± 3 mg CGA/g of extract or 9.7 mg CGA/g of raw material, Figure 2. Several studies report that mixtures of organic solvents and water are the most promising solvents for an efficient CGA extraction (González-Pérez et al., 2002; Scharlack et al., 2017). Considering the Soxhlet as the most efficient method to recover CGA, the process using MAE with ethanol 70% allowed to recover from 60 to 69% of CGA from sunflower by-product, Table 4. CGA was quantified in extracts obtained from sunflower seed cake using ultrasound-assisted extraction and different ethanol concentrations (0–85%) and a yield ranged from 609 to 1635 mg CGA/100 g of raw material was reported (Zardo et al., 2019). Daraee et al. (2019) obtained a maximum recovery of CGA of 52.08% from sunflower seed kernels using ethanol as co-solvent in the supercritical CO_2 extraction in comparison to Soxhlet method. Weisz et al. (2009) investigated the conventional extraction of CGA using aqueous methanol (60%, v/v) of the shells and of the kernels from sunflower (*Helianthus annuus* L.) and found 59.1 mg/100 g and 3050.5 mg/100 g, respectively. Based on the results of CGA extraction, that was the main goal, aqueous ethanol 30:70% w/w an S/F of 10 were selected for the next steps.

3.4. Effect of the power and time on the global yield and CGA extraction

Power and time are parameters that influence the extraction efficiency using MAE, both in terms of extraction yield and energy consumption. In this study, three values of microwave power (200, 300 and 400 W) and four microwave extraction times (30, 60, 90 and 120 seconds) were evaluated using ethanol 70% and S/F of 10. Statistical analysis (ANOVA, $\alpha = 0.05$) showed that global extraction yield was only significantly affected by power (p-value = 0.008) and that the interaction between power and time significantly affected the CGA extraction (p-value = 0.02). As shown in Figure 3a, the global extraction yield increased with the increase in microwave power from 100 W to

200 W. This phenomenon can be attributed to the fact that higher microwave powers are able to break the cell walls of the sunflower by-product releasing the compounds inside the matrix to the extraction solvent. However, increasing the microwave power to higher levels means increasing the temperature inside the microwave (Alara et al., 2019). In that sense, when the power rises to 300W during 120s of extraction, the temperature has risen to 105 °C, triggering a global yield decrease. The decline of extraction yield with the increase in power and temperature was previously reported (Alara et al., 2019). From this, it may be concluded that the increase in power has a positive effect on the global extraction yield until a certain extent (in this case 200 W) and beyond this point, the increase in the power promotes a negative effect on this response due to the temperature increase. Similar effect of power was observed to the extraction of CGA, Fig 3b. The increase in power from 100 to 200 W promotes an increase in the recovery of CGA, further increase to 300 W has a negative effect on the CGA extraction. The sunflower cell walls tend to absorb microwave energy causing an increase in internal superheating, inducing the cell disruption that facilitates leaching out of CGA molecules from the sunflower by-product. Thus, extending the irradiation power and time levels may lead to thermal degradation of the CGA recovered (Alara et al., 2018). As shown in figure 3b, the increase in the extraction time (30 - 120 seconds) promoted an increase in the temperature from 32 to 69 °C for 100 W, from 38 to 93 °C for 200 W and from 45 to 105 °C for 300 W. This negative effect of the temperature on the CGA recovery was confirmed by the negative Pearson's correlation (r = - 0.8). For every power, CGA extraction yield diminishes with time, being 30s the optimum time for all the tested powers. Considering that 30 seconds is a short time to extract bioactive compounds from a by-product plant matrix, the MAE process (with 200 W) proposed in the present study was efficient to recover CGA (8.4 ± 0.1 mg CGA / g of raw material). Similar effect of the power on the CGA extraction from *Flos Lonicerae* was reported by Yao et al. (2018). In that study, the increase at the power from 100 to 200 W increased the yield of CGA from 26 to 32 mg/g while the increase at the power from 200 to 300 W decreased the yield of CGA from 32 to 26 mg/g. The extraction of CGA from tobacco residues using MAE was studied by Li et al. (2010). These authors observed a positive effect of the increased power from 200 to 400 W in the CGA extraction yield while no significant change was observed when power was increased from 400 to 700 W. The increased in the extraction time also had a negative effect on the recovery of phenolic compounds from tobacco residues (Li et al., 2010), *Phaleria macrocarpa* (Alara et al., 2019), *Myrtus*

communis L. leaves (Dahmoune et al., 2015), citrus mandarin peels (Hayat et al., 2009), and from *Pistacia lentiscus* leaves (Dahmoune et al., 2014). In order to extract the amount of CGA present in the remaining sunflower by-product from the MAE process, the residue obtained at 200 W and 30 seconds was extracted using a stirred flask at 120 rpm for 1 hour using 70% ethanol at 38 °C. The results showed that the combination of the MAE-Shaker processes allowed to recover a total of 9.97 ± 0.12 mg CGA / g of sunflower by-product (which corresponds to 71% of the value obtained by Soxhlet method). By adding this additional stirring time, the recovery of CGA has increased from 8.4 to 9.97 mg CGA / g of sunflower by-product. However, from the authors' point of view, the incorporation of this second step in the extraction is not economically justified at an industrial scale, or at least further economical evaluation must be performed to consider this extraction unit after MAE process.

3.5. TPC and antioxidant capacity by ORAC

The main goal of this work was the recovery of CGA from the sunflower by-product. However, this raw material is also a source of phenolic compounds that can be used as natural antioxidants. Because of that, this section is dedicated to analyzing the TPC and the antioxidant capacity of the obtained extracts.

No significant differences were observed for TPC and antioxidant capacity values in the evaluated extraction conditions, Table 5, probably due to the narrow variation of global extraction yield (8.15 – 8.55%). The TPC values were around 1300 mg GAE / 100 g of raw material on a dry basis that agrees with values in the literature. This value was lower than those reported for the extracts obtained from dehulled sunflower kernels (2938.8 mg / 100 g to 4175.9 mg / 100 g) and higher than those obtained from dehulled sunflower shells (40.8 mg/100 g to 86.0 mg/100 g) (Weisz et al., 2009), since in the present work the mixture kernels+shells is used as raw material. Ye et al. (2015) studied the extraction of TPC in sunflower florets and found 2309 mg GAE / 100 g using ultrasonic cleaner and 90% (v/v) ethanol as extracting solvent. TPC found by Pająk et al. (2014) in sunflower seeds by shaking with methanol was around 400 mg GAE / 100 g.

The antioxidant capacity of the obtained extracts from sunflower by-product was assessed using ORAC, Table 5. ORAC consists to be the most effective method to

evaluate the antioxidant capacity in biological samples by changing the fluorescent molecule when it suffers oxidative damage caused by a source of peroxyl radicals (Kumar, 2015; Zulueta et al., 2009). The antioxidant capacity by ORAC ranged from 180 to 266 μmol TE/g raw material. Antioxidant capacity by ORAC of 454 μM TE/ g of sunflower florets was reported by Ye et al. (2015) using 50% ethanol as solvent. These differences observed in TPC and antioxidant capacity can be explained by differences in analytical methodologies, in the extraction methods and in the sample material and origin. The antioxidant capacity in extracts from sunflower by-product assessed by ORAC can be compared with extracts obtained from *Abelmoschus moschatus* seeds (213 ± 4 μmol TE/g), *Juniperus communis* fruit (183 ± 18 μmol TE/g) and *Ceratonia silique* pod (225 ± 11 μmol TE/g) (Dudonne et al., 2009).

3.6. Protein content and amino acid profile in the sunflower by-product before and after the MAE process

The content of proteins in the raw material was 27.1 ± 0.5% while the solid residue derived from the microwave process presented 26 ± 1% of proteins. This result indicates that by removing the CGA, the main compound that hinders the use of sunflower protein in human applications, the generated by-product still exhibits a high content of protein. Table 6 shows the amino acid profile of the sunflower by-product before and after the MAE process. It can be observed that the treatment with microwave irradiation does not change the amino acid profile since no significant differences in the profiles were observed, that is, in both samples, the same amino acids were found. However, the employed treatment increased the content of all amino acids present in sunflower by-product (third column), probably due to their concentration in the solid associated to the selective extraction of other compounds, such as CGA. For instance, an increase over 66% was observed for proline, an increase over 41% was observed for aspartate and an increase over 25% was observed for threonine, valine, isoleucine and lysine. This same amino acid profile was reported in the literature for the sunflower by-product (Akande, 2011; Azam et al., 2019; Dadalt et al., 2016). Glutamate was the major amino acid found in the samples followed by arginine and glycine. Rosa et al. (2009) also identified glutamate as the major amino acid present in defatted sunflower meal (7.72 g / 100g of dehulled defatted sunflower meal) followed by aspartate (3.28 g / 100g of dehulled defatted sunflower meal) and arginine (3.00 g / 100g of dehulled defatted sunflower

meal). These differences among the amino acid profiles found in this study and the profiles reported in the literature may be due to variations in the raw materials and differences in the analytical methods. It is worth highlighting the presence of essential amino acids in the sunflower by-product as isoleucine, leucine, lysine, methionine, phenylalanine, threonine, histidine and valine. These amino acids cannot be synthesized by animals and humans i.e., they are essential in the human diet as playing important roles in the maintenance of health (Mitsuhashi, 2014). Therefore, this residue generated by the MAE process could be applied to products intended for human consumption as protein supplements, emulsifiers and carriers of bioactive compounds (microencapsulation matrix for antioxidants).

3.7. FTIR

FTIR spectrometry is a physico-chemical technique that has been extensively used as a tool to provide qualitative information about functional groups (Davis and Mauer, 2010). The FTIR spectra of the extract obtained by MAE, and those of the sunflower by-product before and after the MAE process are shown in Figure 4. The same peak shape was detected in all samples; however, the absorbance (intensity) was different. This shows that no remarkable changes in the chemical structure of the raw material, such as new bonds or degradation along the MAE processing occurred. The sunflower by-product and its extract have a complex chemical composition, and therefore the determination of their band correspondences can be a hard task. The peak identified at ∼3300 cm^{-1} shows the stretching vibration of OH group or OH wagging of phenol functional group (Alara et al., 2018). The bands in the region of 2986-2906 cm^{-1} are due to CH stretching of CH_3 and CH_2. The identified peak at 1517 cm^{-1} shows the presence of CH_2, CH_3, flavonoids, and aromatic rings. Likewise, the peak at ∼1660 cm^{-1} is attributed to the vibrational frequency of carbonyl group of amides present in the protein portion and to the presence of aromatic ring deformations and CC groups, which could be associated with the presence of polyphenols and flavonoids (Alara et al., 2018; Rai et al., 2016). The peak at ∼1040 cm^{-1} revealed the presence of stretching vibrations of CO group which suggest the presence of ethers, carboxylic, esters, and alcoholic compounds (Afolabi et al., 2018).

3.8. SEM

Figure 5 shows SEM images of sunflower by-product: (a), (b), and (c) prior to MAE process; and after MAE process at 200 W and 30 seconds (d), (e) and (f). Despite the short process time, the sample submitted to the MAE process presented morphological differences compared to the untreated raw material. As shown in Figure 5 (a) and (d) no difference could be noted in the scale bar of 1mm, however comparing the Figure 6 (b) and (e) (scale bar of 200 μm) and Figure 5 (c) and (f) (scale bar of 20 μm) it could be observed that the cell walls of treated sample were ruptured. This rupture resulted in the effective and easy extraction of CGA from sunflower by-products and it can be associated with the effect of the rapid heating and the internal pressure increase caused by the microwave power.

4. Conclusions

The sunflower by-product is a source of CGA and protein that its main destination till now is for animal feeding, because its use for human consumption is restricted by the CGA content.

In the present study, MAE process was investigated in the extraction of CGA from sunflower by-product. The results showed that CGA could be successfully extracted from sunflower by-product using MAE technology in a much faster process (30 seconds) compared with Soxhlet extraction method (7 hours). This efficiency exhibited by MAE process is related to the cell rupture of solid matrix by microwave irradiation as it is evidenced by SEM images. The experimental results also indicated the high content of phenolic compounds and the high antioxidant capacity of the obtained extracts, confirming that the sunflower extract could be used as a natural antioxidant in several products (cosmetics, food and pharmaceuticals).

The MAE process did not change the protein content of the sunflower by-product, therefore these results support the utility of MAE technology to obtain two high value-added products, a rich CGA extract (8.4 ± 0.1 mg CGA / g of raw material) with antioxidant capacity (222 ± 5 μmol TE/g of raw material) and a flour rich in protein (26%) and essential amino acids (as isoleucine, leucine, lysine, methionine, phenylalanine,

threonine, histidine and valine) that promotes the use of this by-product for human nutrition.

Acknowledgements

This work was supported by the project VA277P18 financed by Junta Castilla y León and FEDER.

References

Afolabi, H.K., Mudalip, S.K.A., Alara, O.R., 2018. Microwave-assisted extraction and characterization of fatty acid from eel fish (Monopterus albus). Beni-Suef University Journal of Basic and Applied Sciences 7, 465-470.

Akande, K., 2011. Proximate and Amino acid Analyses of Full-fat Sunflower (Helianthus annuus L.) Seed Meal.

Alara, O.R., Abdurahman, N.H., Ukaegbu, C.I., Azhari, N.H., 2018. Vernonia cinerea leaves as the source of phenolic compounds, antioxidants, and anti-diabetic activity using microwave-assisted extraction technique. Industrial Crops and Products 122, 533-544.

Alara, O.R., Mudalip, S.K.A., Abdurahman, N.H., Mahmoud, M.S., Obanijesu, E.O.-O., 2019. Data on parametric influence of microwave-assisted extraction on the recovery yield, total phenolic content and antioxidant activity of Phaleria macrocarpa fruit peel extract. Chemical Data Collections 24, 100277.

AOAC, 1997. Official methods of analysis of the Association of Official Analytical Chemistry, 16th ed, AOAC International, Gaithersburg, USA (1997).

Azam, F., Qaisrani, S.N., Khalique, A., Bibi, F., Akram, C.A., Naveed, S., Pasha, T.N., 2019. Exploring nutritive profile, metabolizable energy, protein, and digestible amino acids contents of indigenous protein sources of different locations for male broilers. Poultry Science 98, 4664-4672.

Butsat, S., Siriamornpun, S., 2016. Effect of solvent types and extraction times on phenolic and flavonoid contents and antioxidant activity in leaf extracts of Amomum chinense C. International Food Research Journal 23, 180.

Carrão-Panizzi, M., Mandarino, J., 1994. Girassol: derivados protéicos. Embrapa Soja-Documentos (INFOTECA-E).

Carré, P., Pouzet, A., 2014. Rapeseed market, worldwide and in Europe. OCL 21, D102.

Dabbour, M., He, R., Mintah, B., Xiang, J., Ma, H., 2019. Changes in functionalities, conformational characteristics and antioxidative capacities of sunflower protein by controlled enzymolysis and ultrasonication action. Ultrasonics sonochemistry 58, 104625.

Dadalt, J.C., E. Velayudhan, D., Neto, M.A.T., Slominski, B.A., Nyachoti, C.M., 2016. Ileal amino acid digestibility in high protein sunflower meal and pea protein isolate fed to growing pigs with or without multi-carbohydrase supplementation. Animal Feed Science and Technology 221, 62-69.

Dahmoune, F., Nayak, B., Moussi, K., Remini, H., Madani, K., 2015. Optimization of microwave-assisted extraction of polyphenols from Myrtus communis L. leaves. Food chemistry 166, 585-595.

Dahmoune, F., Spigno, G., Moussi, K., Remini, H., Cherbal, A., Madani, K., 2014. Pistacia lentiscus leaves as a source of phenolic compounds: Microwave-assisted

extraction optimized and compared with ultrasound-assisted and conventional solvent extraction. Industrial Crops and Products 61, 31-40.

Daraee, A., Ghoreishi, S.M., Hedayati, A., 2019. Supercritical CO2 extraction of chlorogenic acid from sunflower (Helianthus annuus) seed kernels: modeling and optimization by response surface methodology. The Journal of Supercritical Fluids 144, 19-27.

Davis, R., Mauer, L., 2010. Fourier transform infrared (FT-IR) spectroscopy: a rapid tool for detection and analysis of foodborne pathogenic bacteria. Current research, technology and education topics in applied microbiology and microbial biotechnology 2, 1582-1594.

Dudonne, S., Vitrac, X., Coutiere, P., Woillez, M., Merillon, J.-M., 2009. Comparative study of antioxidant properties and total phenolic content of 30 plant extracts of industrial interest using DPPH, ABTS, FRAP, SOD, and ORAC assays. Journal of agricultural and food chemistry 57, 1768-1774.

FAO, 2019. Food Agriculture Organization of the United Nations (2019) Food outlook: biannual report on global food markets. Rome.

González-Pérez, S., Merck, K.B., Vereijken, J.M., van Koningsveld, G.A., Gruppen, H., Voragen, A.G.J., 2002. Isolation and Characterization of Undenatured Chlorogenic Acid Free Sunflower (Helianthus annuus) Proteins. Journal of Agricultural and Food Chemistry 50, 1713-1719.

González-Pérez, S., Vereijken, J.M., 2007. Sunflower proteins: overview of their physicochemical, structural and functional properties. Journal of the science of food and agriculture 87, 2173-2191.

Hayat, K., Hussain, S., Abbas, S., Farooq, U., Ding, B., Xia, S., Jia, C., Zhang, X., Xia, W., 2009. Optimized microwave-assisted extraction of phenolic acids from citrus mandarin peels and evaluation of antioxidant activity in vitro. Separation and Purification Technology 70, 63-70.

Karefyllakis, D., van der Goot, A.J., Nikiforidis, C.V., 2019. Multicomponent emulsifiers from sunflower seeds. Current Opinion in Food Science 29, 35-41.

Kroll, J., Rawel, H.M., Rohn, S., Czajka, D., 2001. Interactions of glycinin with plant phenols–Influence on chemical properties and proteolytic degradation of the proteins. Food/Nahrung 45, 388-389.

Kroll, J., Rawel, H.M., Seidelmann, N., 2000. Physicochemical properties and susceptibility to proteolytic digestion of myoglobin− phenol derivatives. Journal of agricultural and food chemistry 48, 1580-1587.

Kumar, S., 2015. Analytical Techniques for Natural Product Research. CABI.

Kumari, B., Tiwari, B.K., Hossain, M.B., Rai, D.K., Brunton, N.P., 2017. Ultrasound-assisted extraction of polyphenols from potato peels: profiling and kinetic modelling. International Journal of Food Science & Technology 52, 1432-1439.

Li, Z., Huang, D., Tang, Z., Deng, C., Zhang, X., 2010. Fast determination of chlorogenic acid in tobacco residues using microwave-assisted extraction and capillary zone electrophoresis technique. Talanta 82, 1181-1185.

Liu, Q., Yang, X., Zhang, L., Majetich, G., 2010. Optimization of ultrasonic-assisted extraction of chlorogenic acid from Folium eucommiae and evaluation of its antioxidant activity. Journal of Medicinal Plants Research 4, 2503-2511.

Llompart, M., Celeiro, M., Dagnac, T., 2019. Microwave-assisted extraction of pharmaceuticals, personal care products and industrial contaminants in the environment. TrAC Trends in Analytical Chemistry 116, 136-150.

Meinhart, A.D., Damin, F.M., Caldeirão, L., da Silveira, T.F.F., Filho, J.T., Godoy, H.T., 2017. Chlorogenic acid isomer contents in 100 plants commercialized in Brazil. Food Research International 99, 522-530.

Meinhart, A.D., Damin, F.M., Caldeirao, L., de Jesus Filho, M., da Silva, L.C., da Silva Constant, L., Filho, J.T., Wagner, R., Godoy, H.T., 2019. Chlorogenic and caffeic acids in 64 fruits consumed in Brazil. Food chemistry 286, 51-63.

Mitsuhashi, S., 2014. Current topics in the biotechnological production of essential amino acids, functional amino acids, and dipeptides. Current Opinion in Biotechnology 26, 38-44.

Mokrani, A., Madani, K., 2016. Effect of solvent, time and temperature on the extraction of phenolic compounds and antioxidant capacity of peach (Prunus persica L.) fruit. Separation and Purification Technology 162, 68-76.

Naveed, M., Hejazi, V., Abbas, M., Kamboh, A.A., Khan, G.J., Shumzaid, M., Ahmad, F., Babazadeh, D., FangFang, X., Modarresi-Ghazani, F., WenHua, L., XiaoHui, Z., 2018. Chlorogenic acid (CGA): A pharmacological review and call for further research. Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie 97, 67-74.

Pająk, P., Socha, R., Gałkowska, D., Rożnowski, J., Fortuna, T., 2014. Phenolic profile and antioxidant activity in selected seeds and sprouts. Food chemistry 143, 300-306.

Pereira, E.S., Pimentel, P.G., Bomfim, M.A.D., Carneiro, M.S.d.S., Cândido, M.J.D., 2011. Torta de girassol em rações de vacas em lactação: produção microbiana, produção, composição e perfil de ácidos graxos do leite. Acta Scientiarum. Animal Sciences 33, 387-394.

Prado, J.M., Veggi, P.C., Náthia-Neves, G., Meireles, M.A.A., 2018. Extraction Methods for Obtaining Natural Blue Colorants. Current Analytical Chemistry 14, 1-28.

Rai, A., Mohanty, B., Bhargava, R., 2016. Supercritical extraction of sunflower oil: A central composite design for extraction variables. Food chemistry 192, 647-659.

Rosa, P., Antoniassi, R., Freitas, S., Bizzo, H., Zanotto, D., Oliveira, M., Castiglioni, V., 2009. Chemical composition of brazilian sunflower varieties/composición química de las variedades de girasol brasileñas/composition chimique de sortes de tournesol brésiliennes. Helia 32, 145-156.

Salgado, P.R., Drago, S.R., Molina Ortiz, S.E., Petruccelli, S., Andrich, O., González, R.J., Mauri, A.N., 2012. Production and characterization of sunflower (Helianthus annuus L.) protein-enriched products obtained at pilot plant scale. LWT - Food Science and Technology 45, 65-72.

Sastry, M.S., Rao, M.N., 1990. Binding of chlorogenic acid by the isolated polyphenol-free 11 S protein of sunflower (Helianthus annuus) seed. Journal of agricultural and food chemistry 38, 2103-2110.

Scharlack, N.K., Aracava, K.K., Rodrigues, C.E., 2017. Effect of the type and level of hydration of alcoholic solvents on the simultaneous extraction of oil and chlorogenic acids from sunflower seed press cake. Journal of the science of food and agriculture 97, 4612-4620.

Singleton, V.L., Orthofer, R., Lamuela-Raventós, R.M., 1999. [14] Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent, Methods in Enzymology. Academic Press, pp. 152-178.

Sivaramakrishnan, S., Gangadharan, D., 2009. Edible Oil Cakes, in: Singh nee' Nigam, P., Pandey, A. (Eds.), Biotechnology for Agro-Industrial Residues Utilisation: Utilisation of Agro-Residues. Springer Netherlands, Dordrecht, pp. 253-271.

Tavernari, F., Albino, L.F.T., Dutrajunior, W.M., Lelis, G.R., Nery, L.R., Maia, R., 2008. Farelo de girassol: composição e utilização na alimentação de frangos de corte. Revista Eletrônica Nutritime 5, 638-647.

Upadhyay, R., Ramalakshmi, K., Jagan Mohan Rao, L., 2012. Microwave-assisted extraction of chlorogenic acids from green coffee beans. Food chemistry 130, 184-188.

Vinatoru, M., Mason, T.J., Calinescu, I., 2017. Ultrasonically assisted extraction (UAE) and microwave assisted extraction (MAE) of functional compounds from plant materials. TrAC Trends in Analytical Chemistry 97, 159-178.

Weisz, G.M., Kammerer, D.R., Carle, R., 2009. Identification and quantification of phenolic compounds from sunflower (Helianthus annuus L.) kernels and shells by HPLC-DAD/ESI-MSn. Food chemistry 115, 758-765.

Wildermuth, S.R., Young, E.E., Were, L.M., 2016. Chlorogenic Acid Oxidation and Its Reaction with Sunflower Proteins to Form Green-Colored Complexes. Comprehensive Reviews in Food Science and Food Safety 15, 829-843.

Yao, X.-H., Xu, J.-Y., Hao, J.-Y., Wan, Y., Chen, T., Zhang, D.-Y., Li, L., 2018. Microwave assisted extraction for the determination of chlorogenic acid in Flos Lonicerae by direct analysis in real time mass spectrometry (DART-MS). Journal of Chromatography B 1092, 82-87.

Ye, F., Liang, Q., Li, H., Zhao, G., 2015. Solvent effects on phenolic content, composition, and antioxidant activity of extracts from florets of sunflower (Helianthus annuus L.). Industrial Crops and Products 76, 574-581.

Zardo, I., de Espíndola Sobczyk, A., Marczak, L.D.F., Sarkis, J., 2019. Optimization of Ultrasound Assisted Extraction of Phenolic Compounds from Sunflower Seed Cake Using Response Surface Methodology. Waste and Biomass Valorization 10, 33-44.

Zhang, B., Yang, R., Liu, C.-Z., 2008. Microwave-assisted extraction of chlorogenic acid from flower buds of Lonicera japonica Thunb. Separation and Purification Technology 62, 480-483.

Zulueta, A., Esteve, M.J., Frígola, A., 2009. ORAC and TEAC assays comparison to measure the antioxidant capacity of food products. Food chemistry 114, 310-316.

Zuorro, A., Maffei, G., Lavecchia, R., 2014. Effect of solvent type and extraction conditions on the recovery of phenolic compounds from artichoke waste. Chem. Eng. Trans 39.

Figure captions

Figure 1. Total extraction yield of the extracts obtained from sunflower by-product by MAE using different solvents and S/F. Results expressed on a dry basis.
Different letters indicate significant difference ($p < 0.05$).

Figure 2. Effect of the solvent on the CGA extraction from sunflower by-product by MAE using different solvents. Results expressed on a dry basis.

Figure 3. A) Effect of the power on global yield; B) Effect of the interaction between power and time on the CGA extraction from sunflower by-product by MAE. Results expressed on a dry basis.

Figure 4. FTIR spectrum of sunflower by-product before MAE process (fresh raw material), after MAE process and the extract obtained by MAE at 200W and 30 seconds.

Figure 5. Images obtained by SEM on the surface of sunflower by-product analyzed before (row 1) and after the MAE process at 200 W °C and 30 seconds (row 2) at different magnifications. Scale bar: 1mmm (column a and d); 200 μm (column b and e); 20 μm (column c and f).

Figure 1

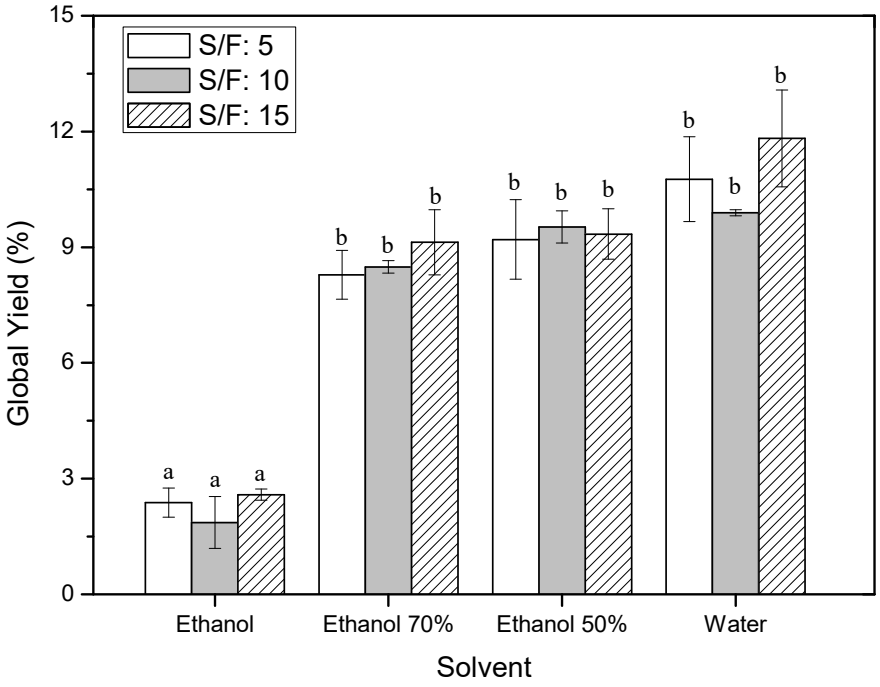

S/F: 5
S/F: 10
S/F: 15
Global Yield (%)
Solvent
Ethanol
Ethanol 70%
Ethanol 50%
Water
0
3
6
9
12
15
a
a
a
b
b
b
b
b
b
b
b
b

Figure 2

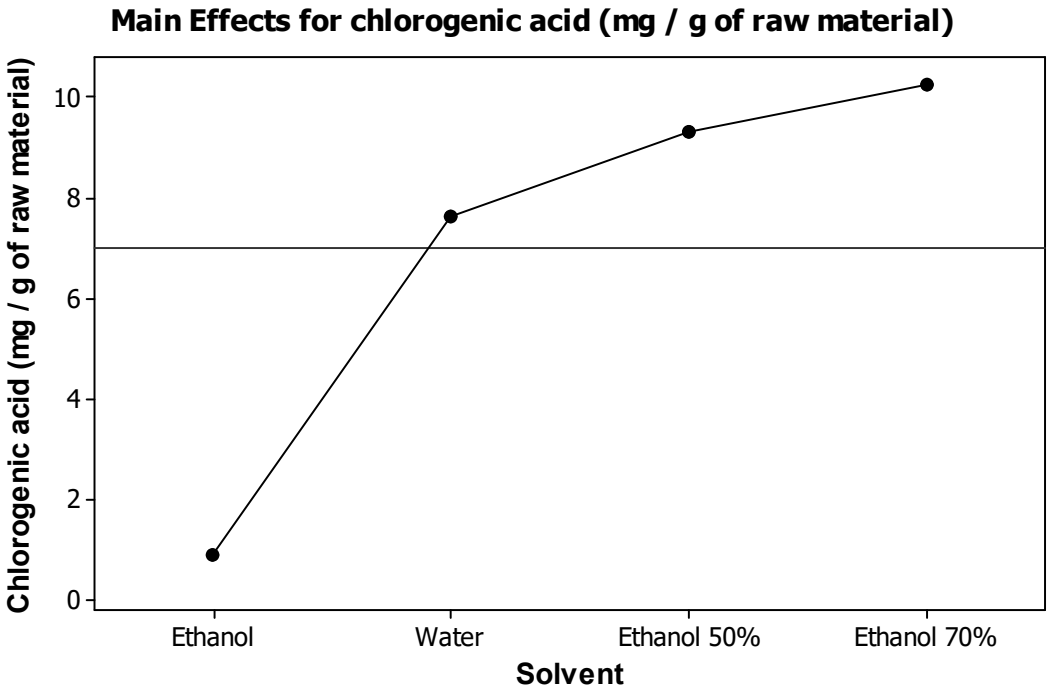

Main Effects for chlorogenic acid (mg / g of raw material)
Chlorogenic acid (mg / g of raw material)
0
2
4
6
8
10
Ethanol
Water
Ethanol 50%
Ethanol 70%
Solvent

Figure 3

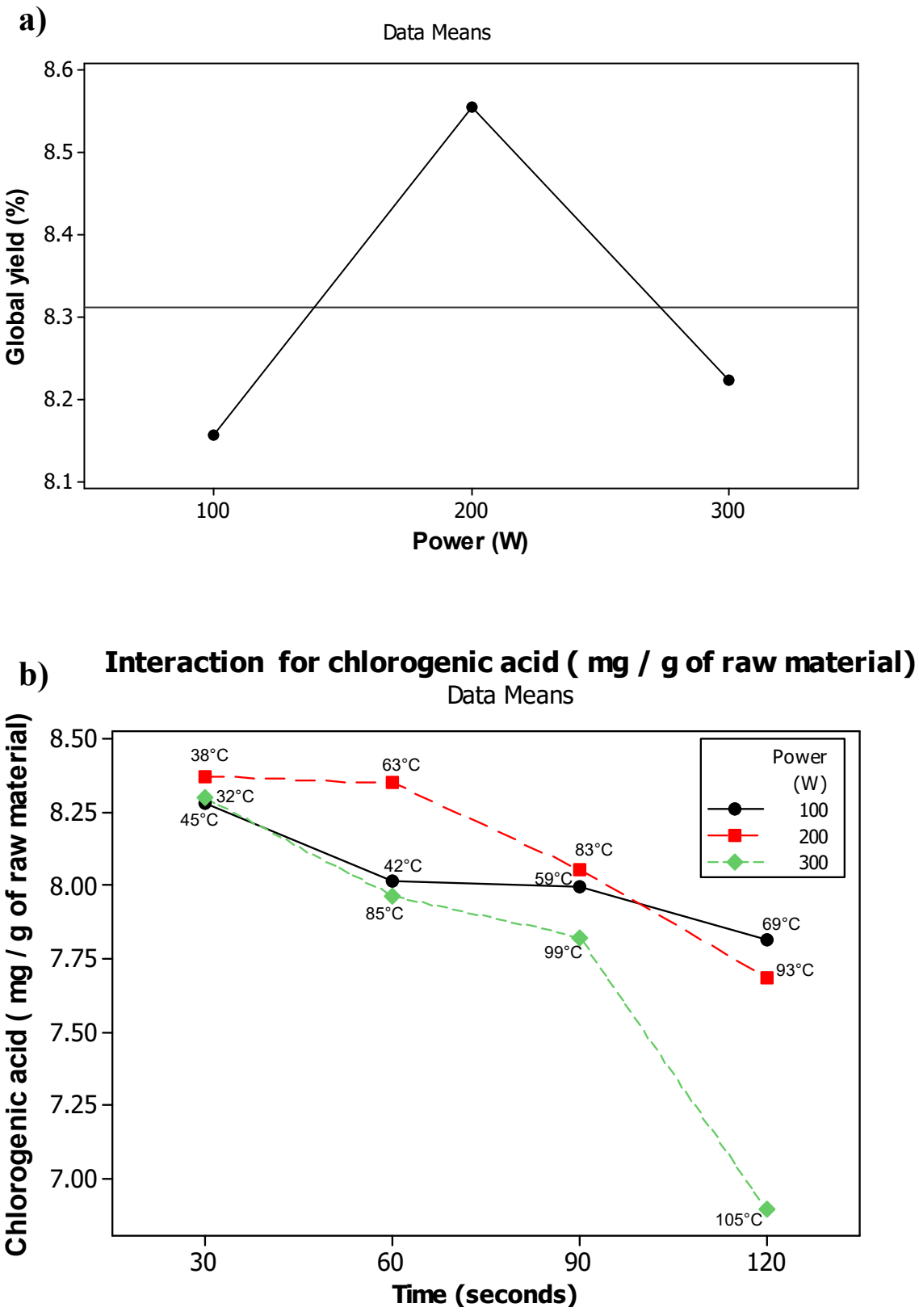

a)
Data Means
Global yield (%)
8.6
8.5
8.4
8.3
8.2
8.1
100
200
300
Power (W)
b)
Interaction for chlorogenic acid (mg / g of raw material)
Data Means
Chlorogenic acid (mg / g of raw material)
8.50
8.25
8.00
7.75
7.50
7.25
7.00
30
60
90
120
Time (seconds)
Power
(W)
100
200
300
38°C
63°C
32°C
45°C
42°C
83°C
59°C
85°C
69°C
99°C
93°C
105°C

Figure 4

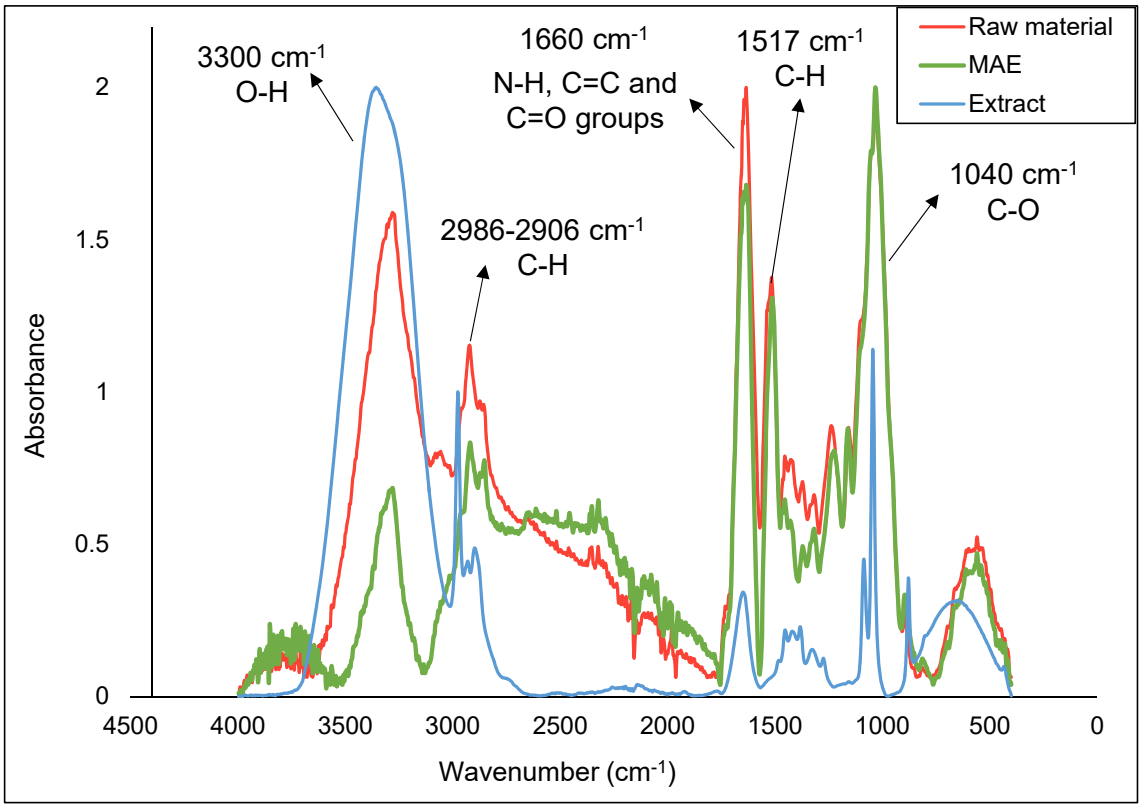
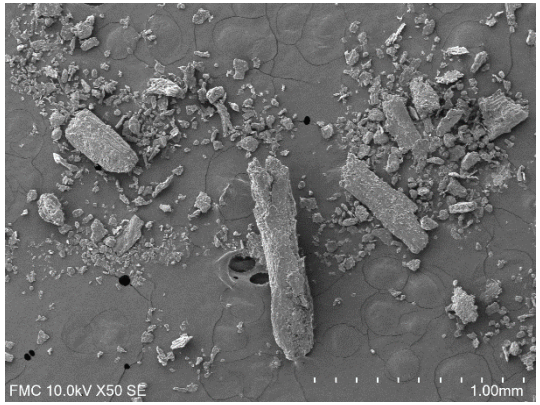
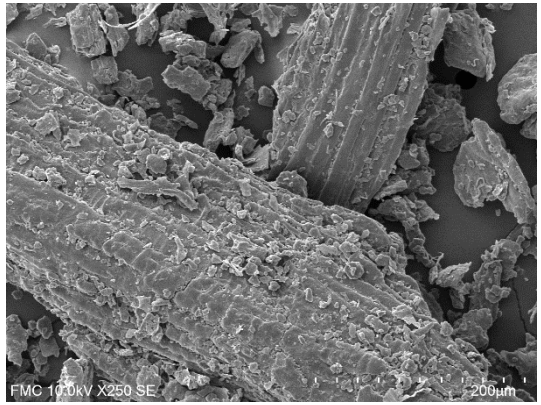

3300 cm-1
O-H
1660 cm-1
N-H, C=C and
C=O groups
1517 cm-1
C-H
1040 cm-1
C-O
2986-2906 cm-1
C-H
Raw material
MAE
Extract
Absorbance
2
1.5
1
0.5
0
4500
4000
3500
3000
2500
2000
1500
1000
500
0
Wavenumber (cm-1)

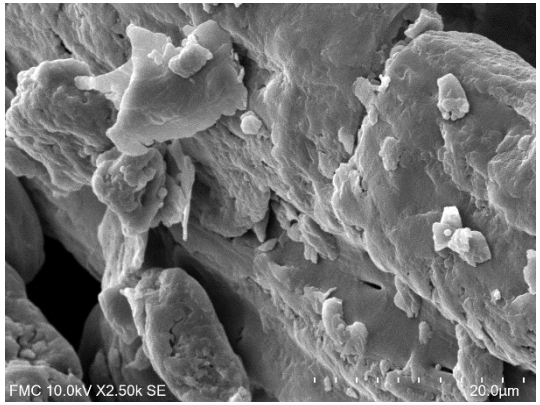
Figure 5

A) **B)** **C)**

1

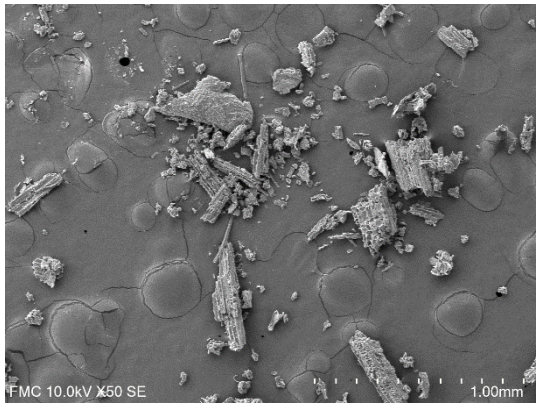


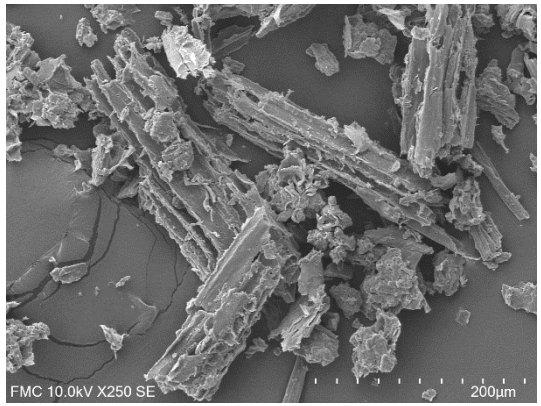




D) **E)** **F)**

2





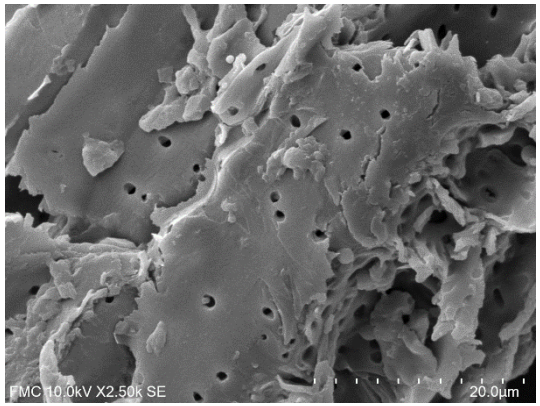


Table 1. The main characteristics of each sunflower by-product fraction.

	Fractions		
	Sieve bottom	0.05 - 0.5 mm	1 - 2 mm
Proportion (%)	0.9	23	76
Extraction yield (%)	7.0 ± 0.1	7.2 ± 0.5	8.2 ± 0.1
Purity (mg of CGA /g of extract)	76 ± 5	70.3 ± 0.4	68 ± 8
Yield (mg of CGA/g of raw material)	5.3 ± 0.4	5.1 ± 0.5	6 ± 1
			

Table 2. Chemical composition (% w/w) of sunflower by-product

Parameter	Results	Units
Mean particle diameter	139 ± 2	μm
Moisture	10.9 ± 0.1	%
Ash	5.2 ± 0.2*	%
Protein	27.1 ± 0.5*	%
Lipids	1.1 ± 0.1*	%
Carbohydrates	66.6 ± 1.1*	%

*Results expressed on a dry basis. Results are mean ± standard deviation of experiments performed in triplicate.

Table 3. Conventional extraction of sunflower by-product using different solvents (results expressed on a dry basis).

Solvent	Global extraction yield (%)	Purity (mg of CGA /g of extract)	Yield (mg of CGA/g of raw material)
Water	8 ± 1	62 ± 6	10 ± 1
Ethanol	4.1 ± 0.1	55 ± 6	3.2 ± 0.6
Ethanol 50%	9.0 ± 0.5	111 ± 2	15 ± 2
Ethanol 70%	9.5 ± 0.1	136 ± 2	14 ± 1

Table 4. Extraction of CGA from sunflower by-product by MAE using different solvents (results expressed on a dry basis).

Solvent	S/F	Temperature (°C)	Purity mg of CGA/g of extract	Yield mg of CGA/g of raw material	CGA Recovery (%)
Water	5	64.2 ± 8	72 ± 5^{a}	7.5 ± 0.2^{cd}	75
	10	65.5 ± 4.9	72 ± 5^{a}	7.1 ± 0.5^{d}	71
	15	72.4 ± 4.1	69 ± 1^{a}	8 ± 1^{bcd}	80
Ethanol	5	78.5 ± 0.7	42 ± 5^{b}	0.99 ± 0.01^{e}	31
	10	76.7 ± 0.3	41 ± 2^{b}	0.87 ± 0.01^{e}	27
	15	77.6 ± 0.6	37 ± 2^{b}	$0.96 \pm 0{,}01^{e}$	30
Ethanol 50%	5	71 ± 2.8	98 ± 3^{c}	8.5 ± 0.4^{abcd}	57
	10	75.6 ± 0.5	97 ± 5^{c}	8.2 ± 0.3^{abcd}	55
	15	76.2 ± 1	97 ± 3^{c}	9 ± 1^{abcd}	60
Ethanol 70%	5	73.5 ± 0.7	118 ± 3^{d}	9.7 ± 1^{a}	69
	10	78.3 ± 0.9	110 ± 2^{cd}	8.4 ± 0.5^{ab}	60
	15	78.3 ± 0.9	107 ± 1^{cd}	9.5 ± 0.5^{ab}	68

CGA Recovery was calculated relative to the Soxhlet method.

Same letters on the same column indicate no significant difference ($p < 0.05$).

Table 5. TPC and antioxidant capacity of sunflower by-product extracts (results expressed on a dry basis).

Power (W)	Time (seconds)	TPC mg GAE of / g of raw material	ORAC μmol TE / g of raw material
100	30	12.9 ± 0.4^{a}	234 ± 32^{ab}
	60	12.5 ± 0.6^{a}	205 ± 18^{ab}
	90	12.5 ± 0.2^{a}	266 ± 1^{a}
	120	12.61 ± 0.04^{a}	216 ± 3^{ab}
200	30	14 ± 1^{a}	222 ± 5^{ab}
	60	13 ± 1^{a}	222 ± 19^{ab}
	90	13 ± 1^{a}	217 ± 28^{ab}
	120	13 ± 1^{a}	227 ± 13^{ab}
300	30	12.3 ± 0.1^{a}	203 ± 21^{ab}
	60	12.7 ± 0.1^{a}	208 ± 29^{ab}
	90	12.00 ± 0.01^{a}	213 ± 31^{ab}
	120	12.1 ± 0.2^{a}	180 ± 20^{b}

Different letters indicate significant difference ($p < 0.05$).

Table 6. Amino acid profile in the sunflower by-product before and after the MAE process.

Amino acids	Sunflower by-product		
	Before MAE process	After MAE process	Increase (%)
	mg of amino acid / g of sample		
Aspartate	8.2 ± 0.1	14 ± 1	41.9 ± 3.7
Glutamate	24.1 ± 0.3	36 ± 3	33.6 ± 0.8
Asparagine	-	-	-
Serine	7.85 ± 0.05	10 ± 1	20.9 ± 2.5
Glutamine	-	-	-
Histidine	5.9 ± 0.1	6.1 ± 0.5	3.7 ± 0.5
Glycine	13.4 ± 0.3	14.4 ± 0.6	6.9 ± 1.7
Threonine	4.7 ± 0.1	6.4 ± 0.7	26.7 ± 2.1
Arginine	14.7 ± 0.1	16.1 ± 0.8	8.4 ± 1.8
Alanine	6.91 ± 0.05	9.2 ± 0.6	25.4 ± 1.2
Tyrosine	2.84 ± 0.01	4.12 ± 0.4	31.1 ± 2.2
Cysteine	1.9 ± 0.1	2.2 ± 0.7	14.4 ± 1.3
Valine	4.7 ± 0.1	6.7 ± 0.5	29.2 ± 0.9
Methionine	3.57 ± 0.01	4.2 ± 0.4	15.1 ± 3.7
Tryptophan	-	-	-
Phenylalanine	6.15 ± 0.1	8.4 ± 0.5	27.3 ± 1.8
Isoleucine	3.97 ± 0.01	6.1 ± 0.5	35.5 ± 1.3
Leucine	9.54 ± 0.1	13.0 ± 0.8	26.7 ± 1.8
Lysine	5.12 ± 0.3	6.87 ± 1	25.5 ± 1.8
Proline	0.9 ± 0.1	2.86 ± 0.6	66.3 ± 2.0
Total	124.63 ± 0.8	167.3 ± 4	25.5 ± 1.2