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High-intensity ultrasound-based process strategies for obtaining edible sunflower (*Helianthus annuus* L.) flour with low-phenolic and high-protein content

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ABSTRACT

The sunflower *Helianthus annuus* L. represents the 4th largest oilseed cultivated area worldwide. Its balanced amino acid content and low content of antinutrient factors give sunflower protein a good nutritional value. However, it is underexploited as a supplement to human nutrition due to the high content of phenolic compounds that reduce the sensory quality of the product. Thus, this study aimed at obtaining a high protein and low phenolic compound sunflower flour for use in the food industry by designing separation processes with high intensity ultrasound technology. First, sunflower meal, a residue of cold-press oil extraction processing, was defatted using supercritical CO_2 technology. Subsequently, sunflower meal was subjected to different conditions for ultrasound-assisted extraction of phenolic compounds. The effects of solvent composition (water: ethanol) and pH (4 to 12) were investigated using different acoustic energies and continuous and pulsed process approaches. The employed process strategies reduced the oil content of sunflower meal by up to 90% and reduced 83% of the phenolic content. Furthermore, the protein content of sunflower flour was increased up to approximately 72% with respect to sunflower meal. The acoustic cavitation-based processes using the optimized solvent composition were efficient in breaking down the cellular structure of the plant matrix and facilitated the separation of proteins and phenolic compounds, while preserving the functional groups of the product. Therefore, a new ingredient with high protein content and potential application for human food was obtained from the residue of sunflower oil processing using green technologies.

1. Introduction

The sunflower *Helianthus annuus* L. is cultivated worldwide due to its short cultivation time and highly adaptability to various climates [1]. Currently, it represents the 4th largest area among oilseeds. In Brazil, there is a great potential for expansion of this crop, driven by the feasible area of sunflower planting, which is performed in succession to soybean harvesting that comprises an area of 33.8 million hectares [2,3].

Sunflower grain has approximately 20% protein, with 11S globulins and 2S albumins being the main components. It has a balanced amino acid profile, low levels of antinutrients, which provides the sunflower protein a high nutritional value and a potential complementary protein source for human nutrition [4,5,6]. The grain also contains around 35–42% oil and is rich in linoleic acid 55–70% [1,7]. The high content of polyphenols (1 to 4%) is outstanding, with a predominance of chlorogenic and caffeic acids. However, a complexion of polyphenols with some amino acids of the protein (lysine and methionine) may occur during processing, resulting in undesirable changes in the color of the bran (greenish). Some authors state that this binding may reduce the digestibility and biological value of the protein, limiting its application in food products for human consumption without a consensus [6,8].

In Brazil, the main sunflower products are the cooking oil and biodiesel. Fig. 1-A shows the process flow diagram for conventional refined sunflower oil processing chain. The extraction process of the lipid

Abbreviations: SM, sunflower meal; DSM, defatted sunflower meal; SF, sunflower flour.

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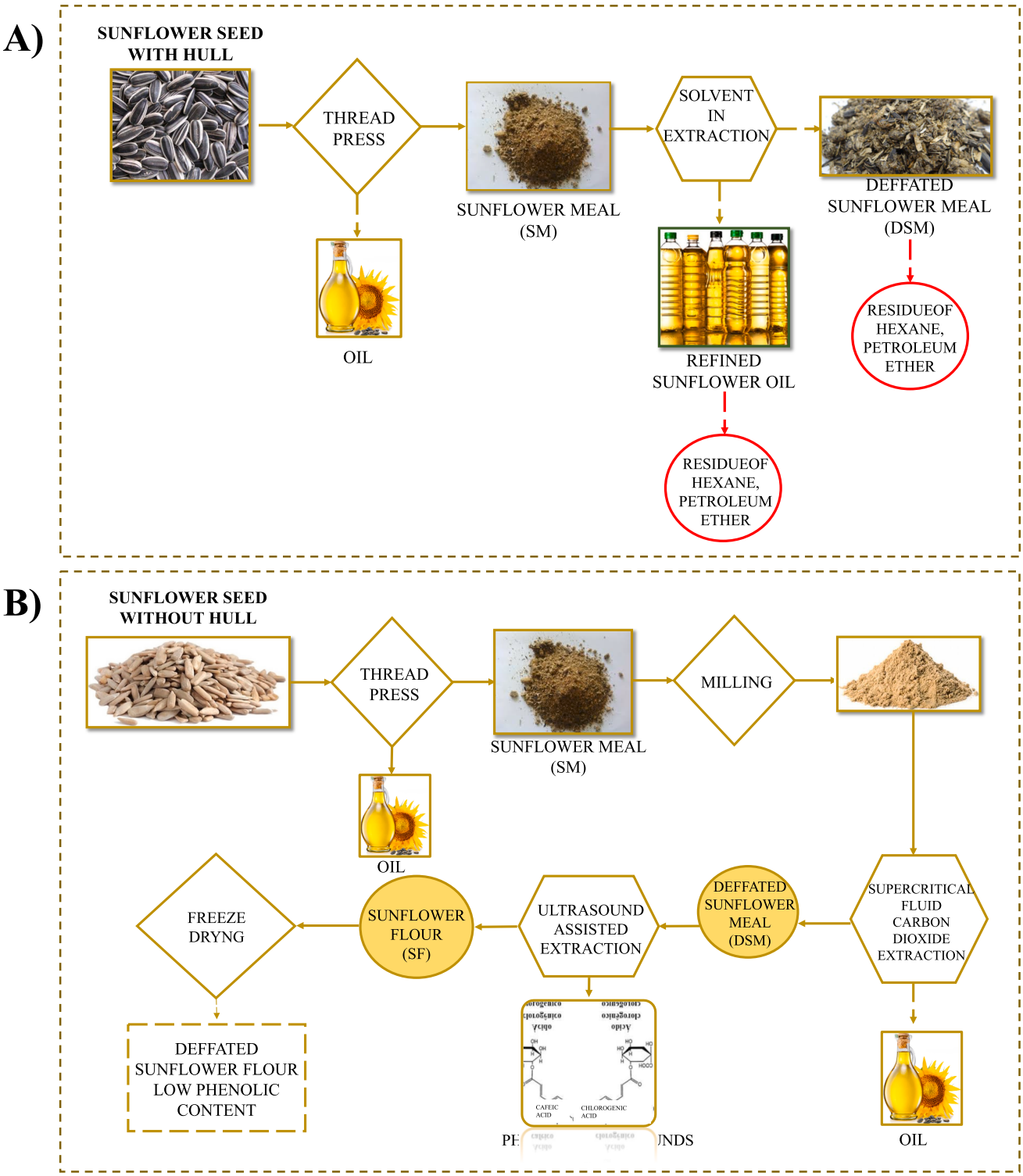

Fig. 1. Synthesis of the sunflower processing chain flow: A) Process flow diagram for conventional refined sunflower oil processing chain; B) Process flow diagram for emerging clean technologies based sunflower oil processing chain.

fraction of the cake produces bran, which is mainly used as animal feed [9]. The commercialization of bran as a protein source is still little explored but with high market potential due to the current demand for new plant-based protein sources. According to the Portuguese vegetarian association, 40% of European consumers have significantly reduced or completely eliminated meat consumption from their diet [10].

To meet consumer demands for green and sustainable technology, new extraction and purification techniques have been increasingly explored, aiming at using all by-products generated in the food industry. An efficient technique for depleting oil extraction, associated with cold pressing, consists in the use of the supercritical technology. Once standardized for different raw materials, this technique can efficiently use CO_2 as a green solvent, eliminating the risk of contamination with chemical solvents, due to its greater selectivity and higher extraction yield. Supercritical CO_2 has been explored by researchers to obtain

sunflower oil from different varieties around the world [11–15].

Phenolic compounds complex with protein during oil extraction, resulting in an extremely green coloration of the residual bran. As proteins are the most interesting fraction of the residual bran, it is necessary to adopt a procedure for the extraction of phenolic compounds that enables its use [8]. To overcome this limitation, the extraction of phenolics through high-intensity ultrasound-assisted extraction was investigated. The application of ultrasound can intensify the extraction process through the phenomenon of acoustic cavitation, which releases a large amount of energy capable of breaking the cellular matrix. Cavitation produces mechanical effects on the sample, such as cell agitation and cell disruption, resulting in increased mass transfer rate and improved solvent penetration. These conditions favor extraction and enable the use of green solvents, such as water and ethanol, resulting in extract production with competitive yield [16,17].

Considering the low commercialization of sunflower bran for human consumption and the relevance of studying green food manufacturing processes, this study aimed at designing processes based on high-intensity ultrasound technology to obtain sunflower flour (SF) with high content of protein in contrast with a reduced content of phenolic compounds. Partially defatted shelled sunflower meal (SM) (cold extraction) was subjected to a continuous oil extraction process using supercritical CO_2. Then, high-intensity ultrasound was used to remove phenolic compounds, using different process strategies. This treatment enabled the production of a new product, with potential application as a protein ingredient in the development of foods for human consumption. Fig. 1-B presents process flow diagram for emerging clean technologies based sunflower oil processing chain.

2. Material and methods

2.1. Raw material

Commercial sunflower meal (SM) semi defatted by cold oil extraction of previously dehulled sunflower kernels was purchased from Veris Óleos Vegetais Ltda-ME (Veris Brasil), Vinhedo, SP, Brazil.

2.2. Chemical composition characterization

Semi defatted commercial sunflower meal (SM) was crushed in a hammer mill. The sample was stored at −18 °C for until 30 days in sealed plastic protected from light for analyses. For characterization of initial samples and samples obtained after the extraction processes with supercritical CO_2 and high-intensity ultrasound, we analyzed moisture, proteins by Kjeldahl (conversion factor 6.25), lipids and ash [18,19].

2.3. Extraction of residual oil from sunflower meal with supercritical CO_2

Residual oil was extracted from sunflower meal (SM) (*H. annuus* L.) using supercritical CO_2 in a continuous supercritical fluid extraction system (SFE Thar Technologies, model SFE-2×5 LF-2-FMC, Pittsburgh, USA). A total of 3.1 kg of sunflower meal (SM) was defatted in a 5 L capacity extractor at a temperature of 50 °C and pressure of 40 MPa, employing a flow rate of 200 g/min CO_2. The solvent to feed mass ratio (S/F) was 38.4. In this regard, extraction conditions based on high S/F values have their feasibility on larger scale conditioned by processes in which CO_2 is recycled after extraction procedure for consecutive extraction steps.

2.4. High-intensity ultrasound-assisted extraction of phenolic compounds

The ultrasound-assisted extraction was performed with a 13 mm diameter probe with variable nominal input power and constant frequency of 19 kHz (Unique, Disruptor, 500 W, Indaiatuba, Brazil). Calorimetric assays were carried out to determine the acoustic power delivered by each nominal power [20]. For the nominal powers of 200 W, 300 W, and 400 W, acoustic powers of 8.5 ± 0.1 W, 14.5 ± 0.3 W, and 20 ± 1 W were determined, respectively [21].

The extraction experiments were performed with an S/F of 10 and initial temperature of 10 °C. The temperature was maintained with a circulating water bath (10 °C) in a jacketed beaker. The experiments were conducted in 3 steps for process optimization, being the phenolic compound content and the protein content quantified in each step for parameter control.

The effects of solvent composition (water and ethanol) and pH of the medium on ultrasound-assisted extraction on sunflower phenolic compounds and proteins followed a complete factorial design (5 × 5) in duplicate, totaling 50 experiments. The water/ethanol proportions in the extraction solvent were 0 g/100 g, 25 g/100 g, 50 g/100 g, 75 g/100 g, 100 g/100 g. For each solvent composition the pH of the medium was adjusted to 4, 6, 8, 10 or 12. At this stage, the extraction time was set at 10 min and the ultrasound power rating at 300 W.

After the selection of the solvent composition and pH value that had the best efficiency for the extraction of phenolic compounds, we evaluated the impact of the nominal power of ultrasound on the extraction yield. For this, the nominal powers of 200 W, 300 W, and 400 W were compared, while the extraction kinetics were evaluated for a process time of 30 min. The kinetic curve was performed to verify the appropriate time for the extraction of a greater amount of phenolic compounds, minimizing the loss of protein caused by the process.

At last, we evaluated the ultrasound application mode using continuous and pulsed modes. The maximum extraction time was set at 5 min and the ultrasound application time with a maximum of 3 min. The treatments based on the different pulsed application strategies were compared to the 3 min continuous mode because in this process time since it resulted in a higher extraction of phenolic compounds and lower co-extracted protein content.

2.5. Sunflower extracts characterization

2.5.1. Total phenolic content (TPC)

The total phenolic content of the extracts was determined by the Folin-Ciocalteu method using chlorogenic acid as the standard. The sample was diluted to 1:12, using 300 μL of an appropriate dilution of the extracts, 300 μL of Folin-Ciocalteu reagent, and 2400 μL of a 5% (w/v) saturated sodium carbonate solution. The mixture was stirred on a vortex mixer protected from light with a temperature of 21 °C (±2) for 20 min. The absorbance was measured at 760 nm. The results were expressed as mg chlorogenic acid (chlorogenic acid equivalent) per 100 g of sample on a weight basis (mg chlorogenic acid/100 g) [22].

2.5.2. Liquid chromatography triple quadrupole mass spectrometry (LC–MS/MS) polyphenols identification

Samples were analyzed by liquid chromatography with triple quadrupole mass spectrometry (LC-MS/MS), using a column with octadecylsilane stationary phase and the mobile phase was composed of acetonitrile and water (containing 0.1% formic acid). Ionization of the compounds was performed by electrospray in negative mode, ESI(−). Qualitative analysis of the phenolic compounds was performed using multiple reaction monitoring (MRM) through the transitions to the following compounds: Sinaptic acid, Vanillic acid, Cumaric acid, 3,4 Dihydroxybenzoic acid, Quercetin, Gallic acid, Syringic acid, Kampferol, Chlorogenic acid, Caffeic acid, Ferruic acid, Rutin, Cinnamic acid, Apigenin, Gluteonin, Protocatechuic acid, Myricetin.

2.5.3. Chlorogenic acid quantification by UHPLC

Phenolic compounds were analyzed using a UPLC-PDA system (Waters, Acquity H-Class, Liford, Ma, USA) consisting of a quaternary solvent manager, an automatic sample manager, a column manager and PDA detector. An injection volume of 1 μL was used in kinetx 2.6 a C18 (100 mm × 4.6 mm) column at a temperature of 47 °C. As mobile phase, we used the following: (A) water 0.1% acetic acid, (B) acetonitrile 0.1%

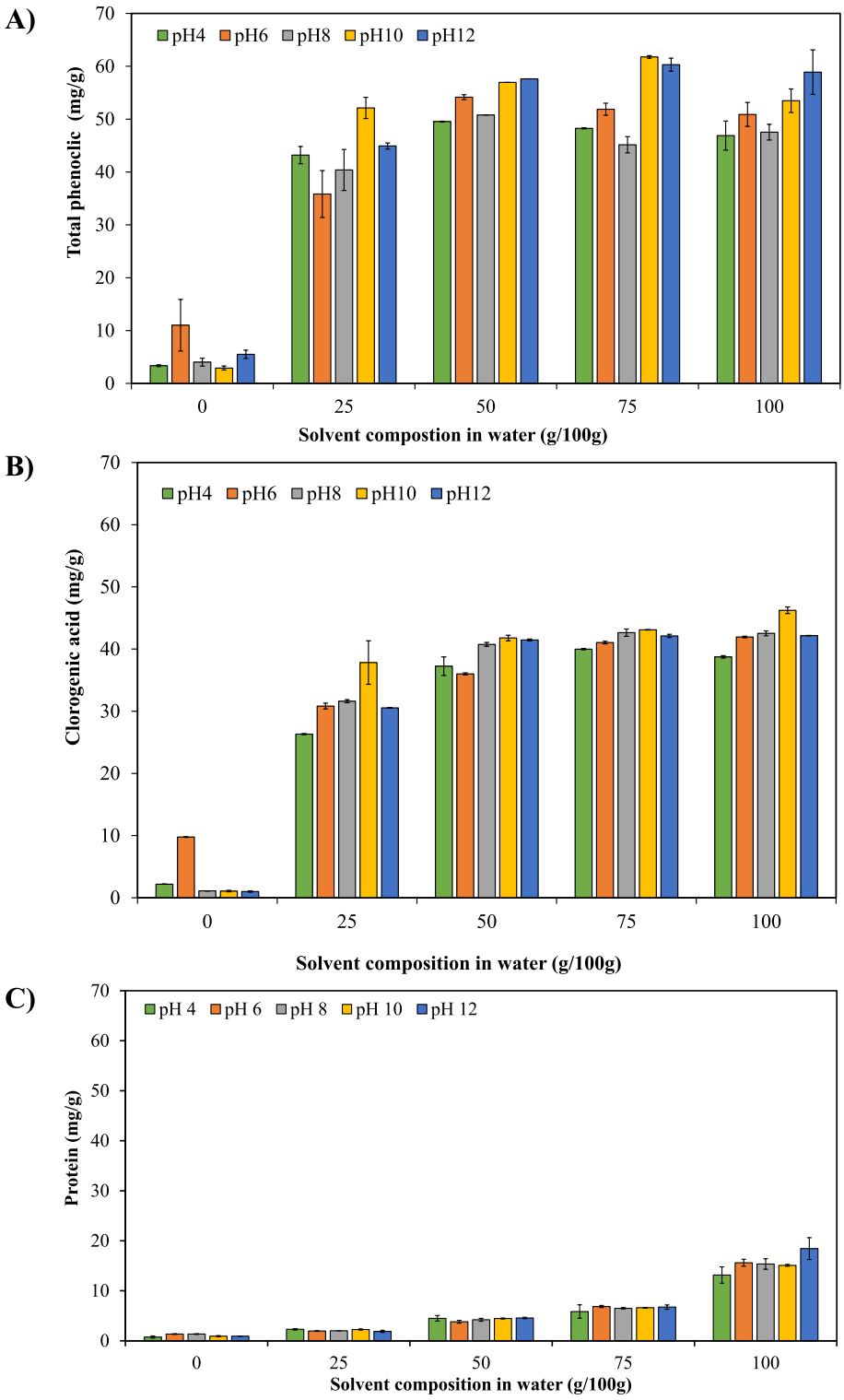

Fig. 2. Phenolics and proteins extracted as a function of pH and solvent composition.

acetic acid at flow rate of 0.5 mL/min. For quantification, a standard curve of chlorogenic acid (Sigma-Aldrich Co., CAS 327-97-9, St Louis, MO, USA) was made at concentrations from 4 mg/mL to 1.29 mg/mL.

2.6. Characterization of sunflower biomass after processing

2.6.1. Chemical stability by Fourier transform infrared (FTIR)

The impact of green processes on the chemical stability of matrix compounds was evaluated by means of Fourier transform infrared (FTIR) spectrum. We used the following equipment: Fourier transform infrared spectrophotometer, model IRPrestige-21, manufacturer Shimadzu (Kyoto, Japan). The acquisition software was IRSolution, version 1.60, with methodology (transmission): KBr pastillation. An approximate ratio of 1:100 (sample: KBr) was used, using 2.0 mg of sample: 200 mg spectroscopic grade KBr. To obtain the pellet, a mixture was dispersed and transferred to a 13 mm diameter stainless steel mold,

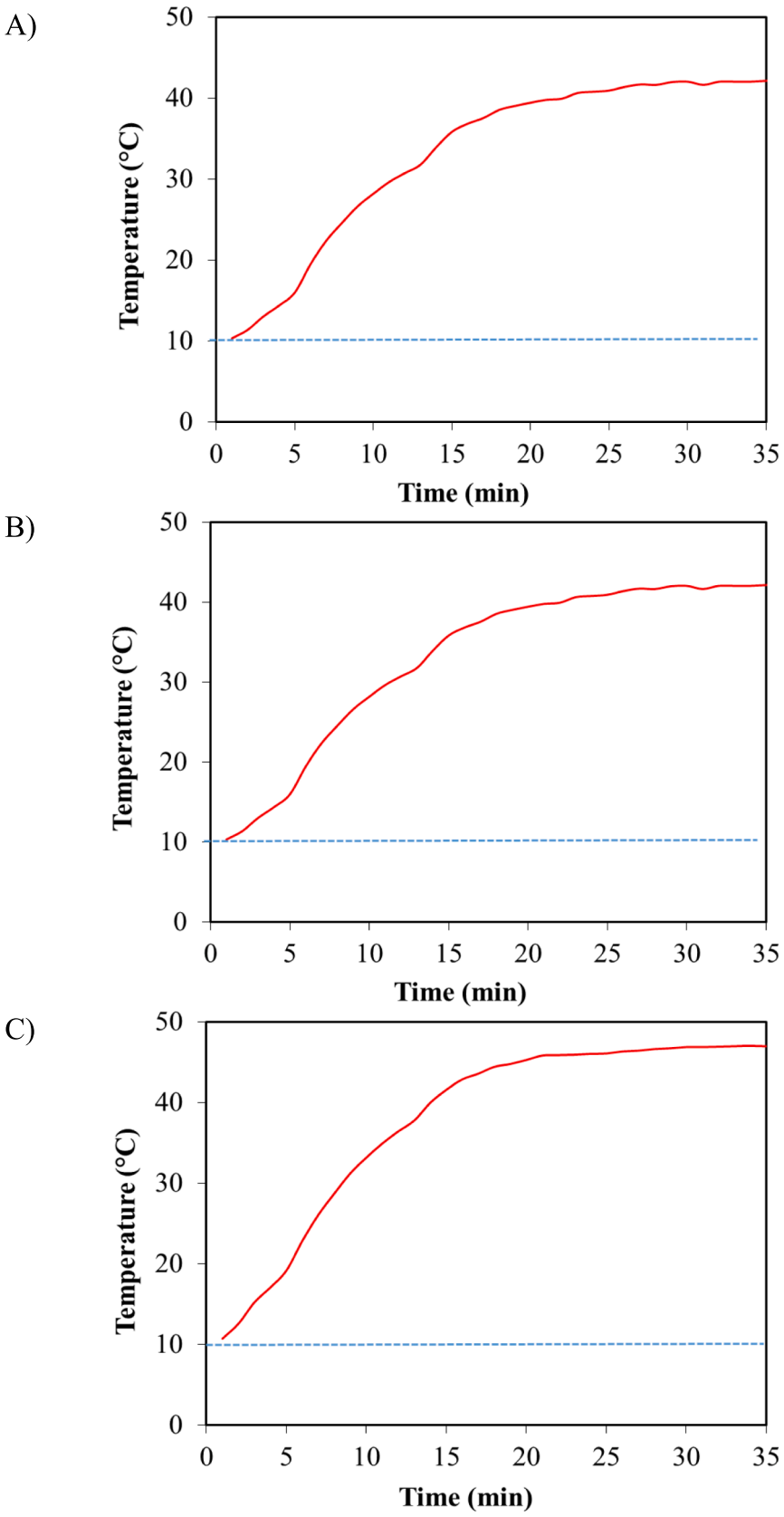

Fig. 3. Influence of ultrasound nominal power on extraction kinetics and process temperature: A) Ultrasound power 200 W; B) Ultrasound power 300 W; C) Ultrasound power 400 W.

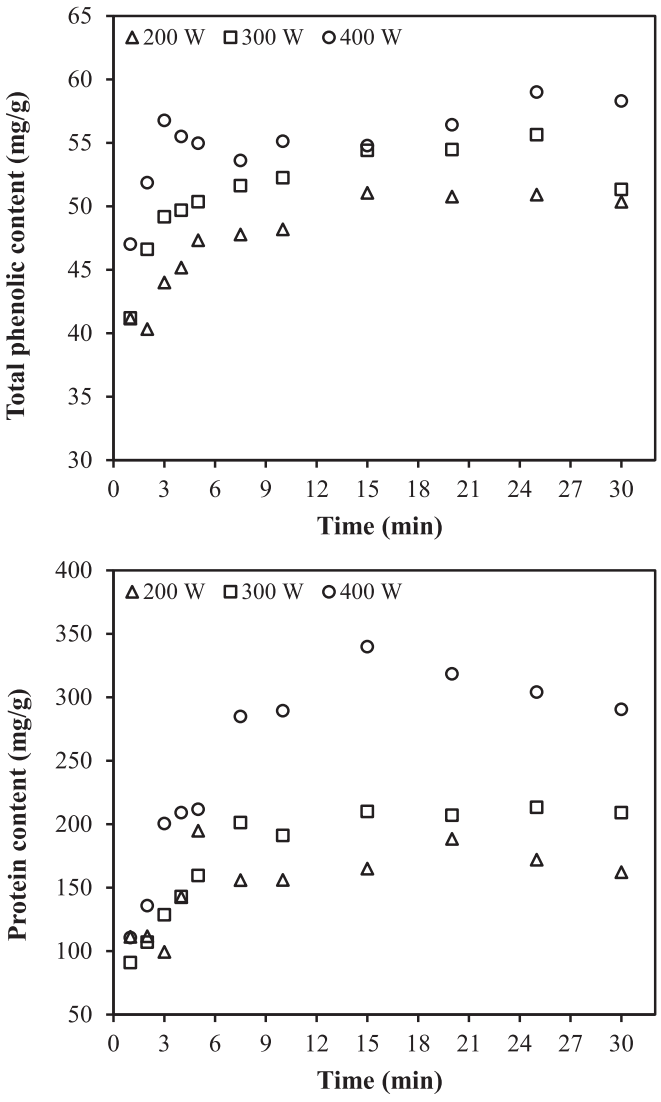

Fig. 4. Extraction kinetics of total phenolic and protein as a function of ultrasound nominal power.

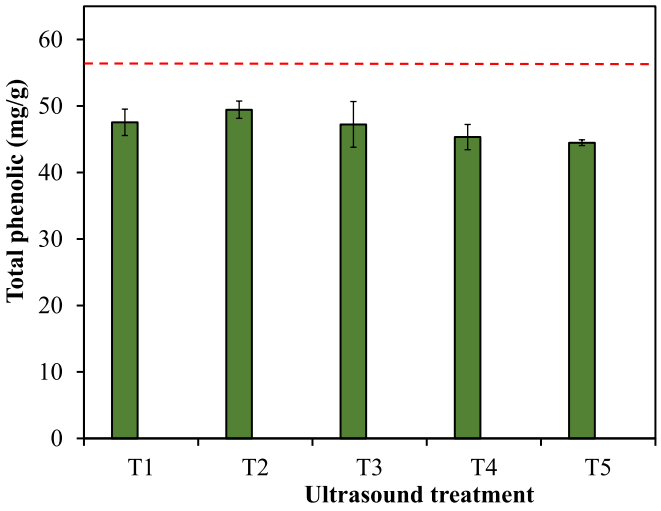

Fig. 5. Amount of total phenolics extracted using pulsed and continuous ultrasound. Dotted line represents the total phenolics extracted in continuous mode.

using a Shimadzu hydraulic press model SSP-10[a] (80 kN force) connected to a vacuum pump for 10 min under vacuum. The spectra were obtained in the range of 400 to 4000 (1/cm), with resolution of 4 (1/cm), 10 scans, Happ-Genzel apodization, using pure KBr pellet as background.

2.6.2. Microstructure by scanning electron microscopy (SEM)

For scanning electron microscopy, we used the Hitach benchtop scanning electron microscope, model TM4000Plus (Tokyo, Japan), with acquisition software TM4000, magnification 10 to ~100,000× and acceleration voltage of 5 kV. BSE (backscattered electron), and eSE (secondary electron) imaging. Maximum sample dimensions were 80 mm (diameter) × 50 mm (thickness).

2.7. Statistical analysis

Minitab 18® software was used to verify the effects of solvent composition (water/ethanol) and pH (4, 6, 8, 10, and 12) on the recovery of TPC, chlorogenic acid and protein content according to the full factorial design (5 × 5). Analysis of variance was also carried out using Minitab 18® software to evaluate the impact of different modes of ultrasound application on phenolic extraction. Differences between mean values were compared by Tukey's test of means at a significance level of 5% (p-value < 0.05). The results of the FTIR and SEM were analyzed descriptively.

3. Results and discussion

3.1. Identification and quantification of phenolic compounds

The phenolic compounds chlorogenic acid, caffeic acid, coumaric acid and vanillic acid were identified in extracts obtained by high-intensity ultrasound-assisted extraction processes from SM. Our results agree with those reported in the literature for SM [23]. We found that 66.2% of the phenolic acids present in the extracts are chlorogenic acid, being confirmed as the major phenolic compound of SM [24,25,22].

3.2. Solvent composition and pH influence on the recovery of phenolic compounds

Fig. 2 presents the effects of solvent composition and pH on the extraction of TPC (Fig. 2-A), chlorogenic acid (Fig. 2-B), and sunflower protein co-extraction (Fig. 2-C). The predominant phenolic compound, chlorogenic acid, was quantified as a reference of the resulting behavior of the variables (solvent composition and pH) in relation to the extraction efficiency for obtaining a protein-rich SF with reduced phenolic compound content. This extraction process needs to be designed to favor the extraction of phenolic compounds and minimize the extraction of sunflower protein so that it can be consumed as an ingredient in food formulations.

The increase in the proportion of water within the composition of the solvent used for extraction interfered in the content of phenolic compounds and protein extracted (Fig. 2). In the treatment where only ethanol was used as solvent, it was possible to minimize the losses of protein during the phenolic compound extraction process. In the latter treatment, the amount of extracted phenolic compounds was very small ($p < 0.001$), about 0.54 g/100 g of sample. The low solubility of polyphenols in absolute organic solvents may be due to the strengthening of hydrogen bonds between polyphenols and proteins, with reduced solvent polarity, reflecting on the extraction yield [26,27]. Meanwhile, the addition of water favored an increased ionization of polyphenols with the weakening of hydrogen bonds, an important factor for polyphenol extraction [28,29]. Adding water to the solvent even at the lowest percentage (25 g/100 g water) increased the extraction yield by up to 808% when compared to extraction with ethanol alone (Fig. 2) ($p < 0.001$). Increasing the amount of water in the solvent composition had a

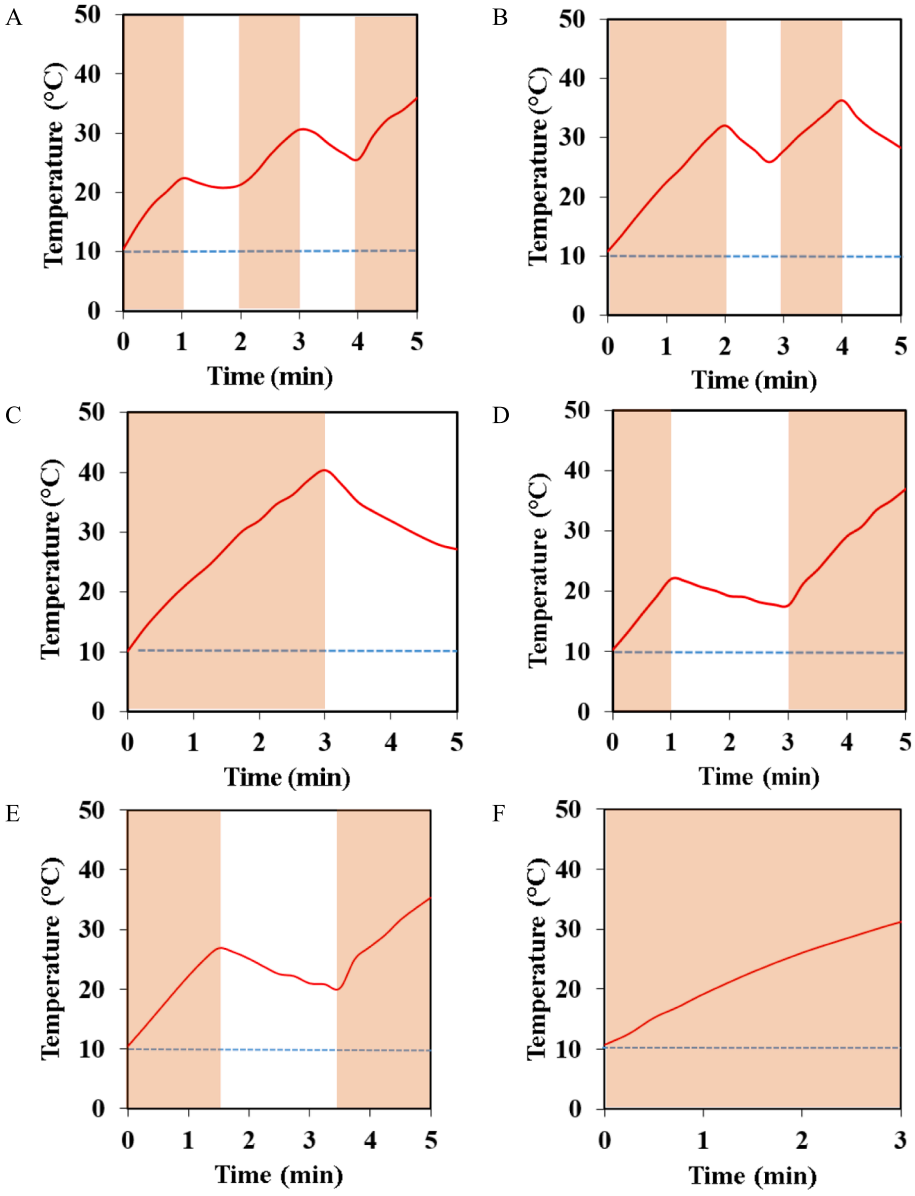

Fig. 6. Influence of pulsed × continuous mode of ultrasound on extraction temperature: A) T1; B) T2; C) T3; D) T4; E) T5; F) Continuous process.

direct impact on the amount of phenolic compounds extracted, reaching a yield of 82% when using 50 g/100 g of water ($p < 0.001$). The removal of chlorogenic acid using this solvent composition was 41.78 mg/g, significantly different from other solvent combinations ($p < 0.001$). When using only water as solvent and pH 12, about 58.29 mg of total phenolic compounds (chlorogenic acid equivalents) were removed ($p < 0.005$). The results found in this work are in line with the results reported by different studies reported in the literature [30,22,25].

Regarding the amount of protein co-extracted with the phenolic compounds, the percentage of water in the solvent composition induced a direct and gradual increase in protein extraction with a variation from 0.11 g/100 g to 1.60 g/100 g ($p < 0.001$). The use of pure water or increased temperature may enhance the extraction of chlorogenic acid. However, employing these extraction conditions will result in considerable protein losses, which are not desirable for obtaining a flour used as a food ingredient [31,32].

The impact of solvent on protein functionality is an important response to consider due to the potential damages that different solvents can promote on matrix structure. The use of absolute ethanol can make the matrix dense and compact and probably may difficult the extraction of polyphenols [31]. The choice of extraction solvent should be made based on the polarity and chemical composition of the compound to be extracted. However, the solvent selection should also consider its surface tension and viscosity. These physical properties affect the acoustic cavitation phenomenon and may compromise the performance of high-intensity ultrasound as an enhancing tool for the extraction of phenolic compounds from sunflower matrix [33]. The use of solvents such as water and ethanol in the ultrasound-assisted extraction involves the increase of mass transfer coefficients, contributing to the reduction of extraction time with the application of ultrasound, minimizing eventual losses and degradation of target compounds.

The pH had a less pronounced effect on the extraction of phenolic compounds and SM protein compared to extracting solvent composition (Fig. 2). The pH 8 resulted in the lowest content of phenolics extracted ($p < 0.001$). The highest amount was extracted at pHs 10 and 12, which were not significantly different from each other. The amount of protein co-extracted with the phenolic compounds was influenced by the increase in pH. There was no difference between the effects of pHs 6, 8, and 10 (p-value < 0.944) on protein co-extraction, but an increase of 24.75% was observed at pH 12.

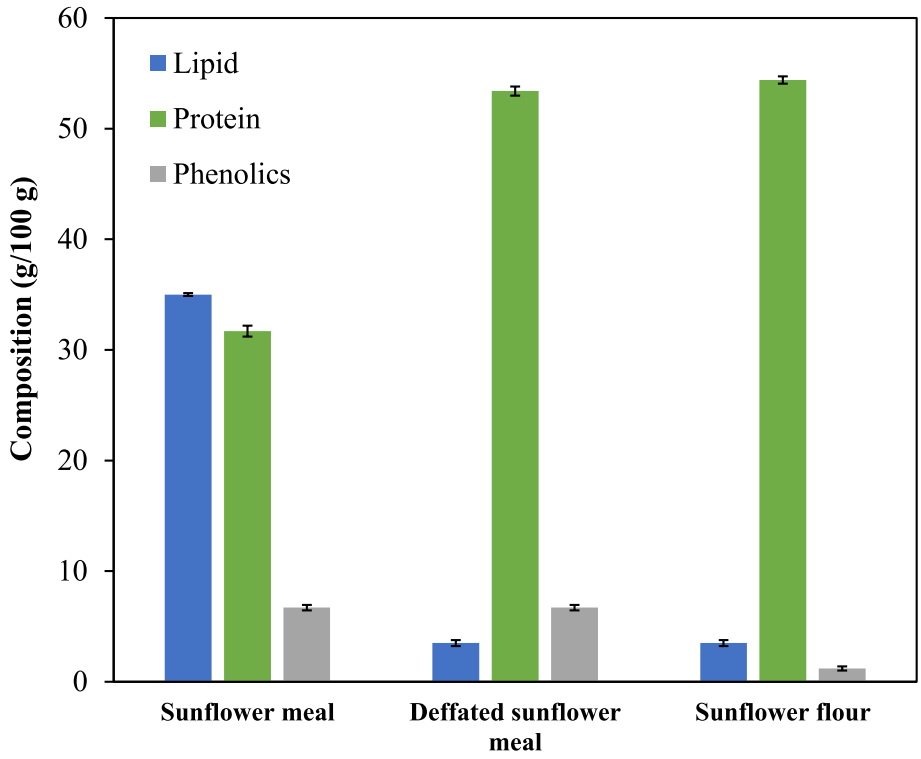

Fig. 7. Lipid, protein, and phenolic content of sunflower samples.

Higher pH values and neutral pH were better for chlorogenic acid extraction when compared to acidic pH [27]. More acidic pH solvents resulted in lower extraction of chlorogenic acid, probably due to greater interaction between the phenolic compounds and the low solubility protein, as they are close to the isoelectric point [31]. Similar behavior can be seen in protein co-extraction. In the pH range 4 to 6, there was no loss of protein during the ultrasonic extraction process due to the low solubility of the protein. The globulin fraction of sunflower protein presents the lowest solubility in the aforementioned range [31].

At the isoelectric point, the protein–protein interaction increases and the electrostatic forces are weakened with little interaction with the solvent (water). With pH other than the protein's PI, there is interaction between the protein and the water molecules, making the protein soluble in the medium. Phenolics can interact with proteins through hydrogen bonds, ionic and hydrophobic interactions, or are oxidized and bind to proteins at extreme alkaline pH [32].

Modulation of the pH of the solvent used in the extraction of phenolic compounds is also a variable that can be employed to minimize protein loss during extraction with high-intensity ultrasound. The use of solutions with acidified water contributes to the reduction in protein losses. Thus, the increased solubility at alkaline pH consequently leads to greater protein loss [32].

3.3. Influence of ultrasound power on the extraction kinetics of phenolic compounds

The initial extraction temperature was standardized as 10 °C. There was a gradual and proportional increase in system temperature with increasing ultrasound power. Acoustic cavitation promotes temperature increase through the conversion of acoustic energy into thermal and mechanical energy due to the hatching of cavitation microbubbles [34]. Maximum registered temperature for each nominal power applied was around 36 °C at 200 W, up to 42 °C at 300 W, and up to 47 °C at 400 W. Fig. 3 shows the thermal history of the ultrasound-assisted extraction process.

The extraction rate of phenolic compounds reached a plateau with increasing extraction time, resulting in increased sample core temperature. The exacerbated increase in sonication temperature can lead to a reduction in the extraction yield of bioactive compounds as they degrade by oxidation mechanisms induced by thermal, mechanical and chemical stresses that occur during acoustic cavitation [35,36].

The phenolic compounds and sunflower protein extraction yields were directly affected by increasing temperature over processing time (Fig. 4). Kinetic curves and analytical results indicated an equilibrium between phenolic compounds extraction yields and co-extracted protein yields at the 3 min time. When comparing the nominal powers of ultrasound during extraction, the yield at 200 W was 74.1% of phenolic compounds over 30 min and 64.8% at 3 min. For 300 W nominal power, the yield was 72.4% in 3 min and 75.5% in 30 min, with important yield losses along the phenolic compound extraction kinetic curve. At 400 W power, phenolic extraction yield increased to 83.6% at 3 min time and was also accompanied by fluctuations along the kinetic curve (Fig. 4). According to the literature, increasing ultrasound power favors chlorogenic acid extraction [37,38]. However, we observed that prolonged extraction time (30 min) reduces phenolic compound extraction yield, probably due to chlorogenic acid degradation induced by oxidative mechanisms associated with ultrasound action [35,39,40,37].

Protein content co-extracted with the phenolics was more impacted by varying ultrasound power and extraction time. Increasing ultrasound power induced an increase of 29.4% at 300 W and 55.9% at 400 W. Extracted protein amounts also fluctuated more along the kinetic curve. It is worth noting that the increase in temperature raises the possibility of protein denaturation, possibly leading to exposure of hydrophilic groups, therefore reducing the interaction of the protein with water [32].

Ultrasound power induces increased shear force, reduced particle size, and increased surface area of exposure of the matrix for the extraction of target compounds [31]. Increased ultrasound power leads to increased process temperature, directly impacting solubility, viscosity, and solvent vapor pressure. These factors combined promote an increase in solvent diffusivity, improving the solubility of target compounds [33]. Harmful effects of cavitation on the plant matrix promote

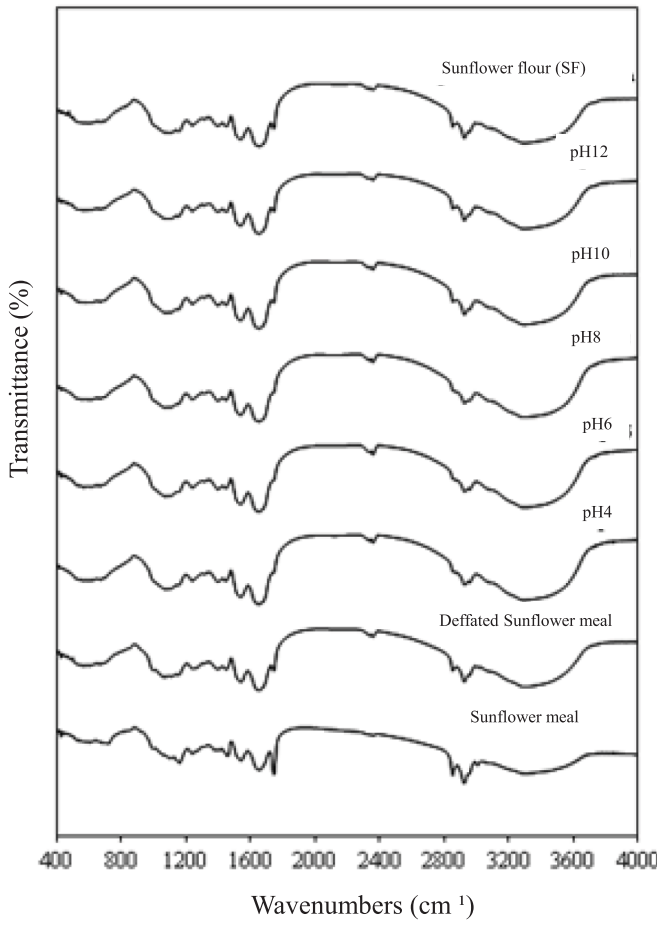

Fig. 8. Fourier-transform infrared spectroscopy (FTIR) of sunflower samples: Sunflower meal (SM); Defatted sunflower meal (DSM); Sunflower flour (SF) in different pH conditions.

heat release at the cell matrix solvent interface and expose cell compounds to possible degradation through both thermal effect and free radical formation. It should be considered that the use of ultrasound impacts extraction at the level of thermal stress (with increased extraction temperature), mechanical stress (shearing the particles), and chemical stress (produced by the formation of free radical during the extraction time). Therefore, temperature control during sonication can minimize the effect of degradation of phenolic compounds and proteins. Thus, ultrasound-assisted extraction processes should be designed individually for different matrices considering processing time and temperature according to the sensitivity of the target compounds [33,32].

3.4. Ultrasound-assisted extraction in pulsed mode

Ultrasound-assisted extraction in pulsed mode is a strategy to deliver acoustic energy discontinuously through an on/off cycle. Some studies suggest that this ultrasound application strategy is better than continuous mode because it contributes to energy savings and preserves temperature sensitive compounds. In theory, it is possible to maintain a high extraction rate even with the equipment off [41].

Ultrasound-assisted extraction processes in pulsed mode were set to a maximum time of 3 min with the equipment on. However, the extraction with pauses resulted in a reduction in the amount of removed phenolic compounds. The extraction in pulsed mode was up to 27.6% lower than in continuous mode. Fig. 5 presents the extraction yield of phenolic compounds using different strategies for pulsed mode extraction. Fig. 6 presents the thermal history of the phenolic compounds extraction processes from defatted sunflower meal (DSM) using ultrasound in pulsed mode. The sharp drop in temperature throughout the extraction process influenced the extracted amount of phenolic compounds. The variation was most evident at T4 and T5, which resulted in the worst extraction yields. Although the different strategies fractionated the optimized extraction time of 3 min, cooling system pauses in the pulsed mode contributed to a lower temperature increase of the extraction medium. As previously discussed, temperature affects the mass transfer mechanisms of sunflower phenolic compounds. Temperature directly impacts the convective mass transfer coefficients. When relating temperature to the extraction potential of a system, it is observed a change in solubility and diffusibility of solvent in contact with the matrix [33]. The temperature reduction in ultrasound-assisted extractions in pulsed mode can interfere with solvent solubility, acoustic cavitation performance, and shear of the plant matrix particles, thus reducing the extraction yield of phenolic compounds [33,42].

According to Kumar, Rao, 2020 [41], when comparing the optimized continuous mode extraction with the pulsed mode, applying the pulsed mode of ultrasound resulted in better yield of compounds extracted from pomegranate. Some authors point out the need to evaluate the combination of pulse duration and pulse interval, however using a short time interval may not be sufficient to complete the mass transfer, resulting in low extraction yield [43]. Therefore, it is necessary to evaluate the continuous extraction duration and cycle duration in pulsed mode extraction it should be noted that in some research occurred in the cycle time of the extraction, not only in the pulse duration [44]. Therefore, the literature differs from the present study due to the use of standardized total cycle time and temperature control with circulating water bath at 10 °C.

3.5. Chemical composition of sunflower biomass

Designing processes that use emerging and clean technologies (Fig. 1-B) is a promising strategy to fully use the by-products from the sunflower processing chain. The use of supercritical CO_2 extraction reduced the oil content of SM by 90%. Therefore, SM had its protein content increased by 58% after the defatting step. Fig. 7 presents the lipid, protein and phenolic contents of sunflower biomass after each process step using green technologies. The extraction process with high-intensity ultrasound reduced the phenolic compound content of SM by up to 82%. The complete centesimal composition of sunflower biomass at each step of the process is presented in Table S1 (supplementary material).

3.6. Process conditions effect on defatted sunflower flour with low phenolic

FTIR spectrometry is a physicochemical technique with wide application to obtain qualitative information about functional groups [45] such as the compounds present in the sample. Fig. 8 presents the FTIR spectra of the ultrasound treated flour at optimized condition, that were modified pH (4–12), SM and DSM using supercritical CO_2. The obtained spectra show the same pattern of peaks in all samples but with different absorbance (intensity). This indicates there were no notable changes in the chemical structure of sunflower meal, such as new bonds or degradation due to the treatment with supercritical CO_2 or high-intensity ultrasound at different pH conditions. Therefore, no variation in band readings was observed, suggesting that the processes did not promote changes in the organic compounds of sunflower flour and possibly no denaturation of sunflower proteins during the emerging treatments evaluated.

SEM results showed the effects of green processes on the microstructure of the sunflower flour. Granule integrity was observed with no breakage or spacing in the particle (Fig. 9-A). However, there was a small spacing in the granule structure where greater exposure of the

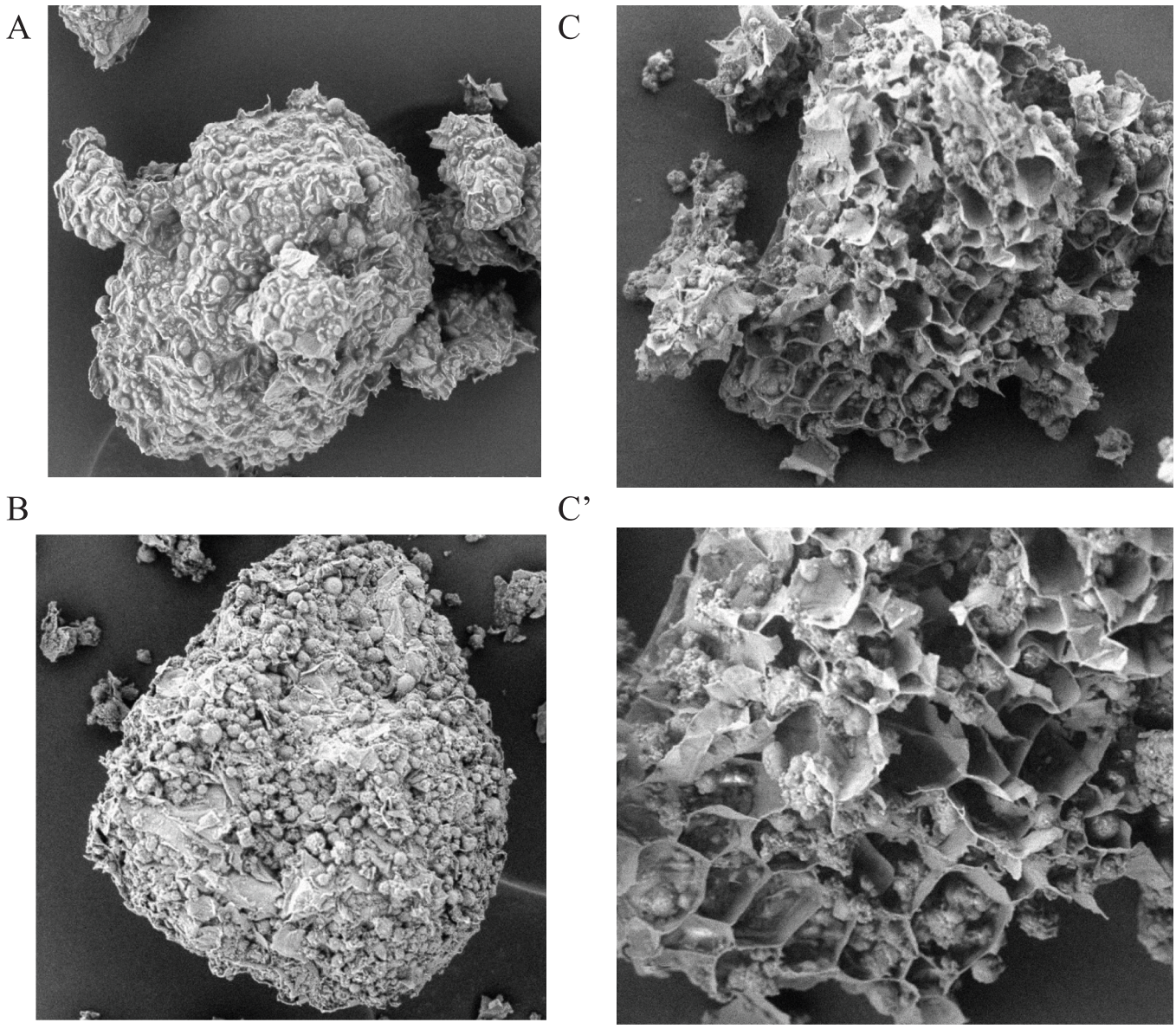

Fig. 9. Scanning electron microscopy (SEM) of sunflower samples: A – Sunflower meal (SM); B – Defatted sunflower meal (DSM); C and C' – Sunflower flour (SF).

particle occurred after removal of the oil with supercritical CO_2 (Fig. 9-B). In the ultrasound treatment, there was both a reduction in the volume of the granule and the presence of disruption of the plant matrix, with the presence of numerous cavities (Fig. 9-C and 9-D). The images clearly illustrate the effect of emerging technologies on SM and SF, corroborating the results obtained in relation to the extraction of phenolic compounds from the plant matrix. The images clearly indicate a good performance of ultrasound in the disruption of cellular structures of the sunflower matrix, which favored the access of the extraction solvent to the interior of the cells promoting the removal of phenolic compounds by diffusion. Moradi, et al, 2017 and Ponmurugan, et al, 2017 [46,47] observed increased pore formation, structural breakdown of the plant tissue, destruction of the cell wall of the sample through SEM. Such modification was not seen in samples that were not treated with ultrasound. Instead, we observed an integrated smooth surface without the presence of pores in these samples.

The fragmentation of plant tissue may occur due to the shear stress caused by the cavitation process in the ultrasound-assisted extraction. The phenomenon of erosion can also be seen during the use of ultrasound, which facilitates the diffusion of the solvent in the sample, acting directly on the mass transfer [33,48].

4. Conclusion

There is a search for new plant protein sources by the world market, as well as for the use of sustainable and environmentally friendly processes in the food industry. The SM was explored through green and sustainable technologies to obtain a protein ingredient with reduced phenolic content, further valuing the production chain from the exploitation of vegetable oil. By using standardized conditions for the employed technologies, the oil extraction yield was optimized in 90%. Likewise, phenolic compounds were recovered up to 83% from the plant matrix. Furthermore, ultrasound promoted the rupture of the cellular structure without degrading the functional groups of the matrix. Our results showed that a promising new food product was obtained to be incorporated as a food ingredient, following a sustainable model of production and plant protein market chain.

CRediT authorship contribution statement

Mariana Pacífico dos Santos Friolli: Investigation, Conceptualization, Methodology, Formal analysis, Writing – original draft. **Eric Keven Silva:** Conceptualization, Visualization, Methodology, Writing – review & editing, Supervision. **Daniele Cristina da Silva Napoli:** Investigation, Methodology. **Vítor Lacerda Sanches:** Methodology. **Maurício Ariel Rostagno:** Methodology. **Maria Teresa Bertoldo Pacheco:** Conceptualization, Methodology, Writing – review & editing, Funding acquisition, Supervision, Project administration.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Maria Teresa Bertoldo Pacheco reports administrative support and equipment, drugs, or supplies were provided by Institute of Food Technology. Maria Teresa Bertoldo Pacheco reports financial support was provided by State of Sao Paulo Research Foundation. Mariana Friolli reports financial support was provided by Coordination of Higher Education Personnel Improvement. Maria Teresa Bertoldo Pacheco reports a relationship with Institute of Food Technology that includes: employment. No has patent No pending to No. There are no additional relationships or activities to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ultsonch.2023.106449.

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