

Article

Sequential Processing Using Supercritical Carbon Dioxide and High-Intensity Ultrasound in Sunflower Protein Flour Production: Nutritional Value, Microstructure, and Technological Functionality

Mariana Pacífico dos Santos Friolli ¹, Eric Keven Silva ^{1,*} , Janaíne Chaves ², Marcos Fellipe da Silva ¹, Rosana Goldbeck ¹, Fabiana Andrea Barrera Galland ²  and Maria Teresa Bertoldo Pacheco ² 

¹ School of Food Engineering, University of Campinas (UNICAMP), Campinas 13083-862, Brazil

² Institute of Food Technology (ITAL), Campinas 13070-178, Brazil

* Correspondence: ekeven@unicamp.br or engerickeven@gmail.com

Abstract: Sunflowers are among the world's most widely cultivated oilseeds with an interesting nutritional composition. A biomass composed mainly of carbohydrates, fibers, and proteins is generated from sunflower oil production. In this context, the objective of this study was to investigate the application of emerging technologies to sunflower biomass to obtain an edible protein-rich flour with the potential to be exploited in the food industry. The effects of the optimized conditions for the sequential processing of sunflower meal using supercritical carbon dioxide (SCO₂) and high-intensity ultrasound (HIUS) were investigated. The protein structure was preserved even after the application of HIUS to the flour as verified through differential scanning calorimetry (DSC) and the electrophoresis curves. The fact that the HIUS treatment did not modify the protein structure demonstrates that this emerging technology could be incorporated into the processing chain of this new food ingredient (sunflower flour) without promoting damage to the nutritional value of the product regarding its protein content. At a pH of 7.0, the flour showed only 30% solubility, and HIUS application improved both the formation and the stability of the emulsion when compared to the other samples. The preliminary evaluation of cell viability (caco2 cells) showed its protective potential against reactive oxygen species. Therefore, the flour resulting from the green processes presented the potential to be employed as an ingredient in the food industry, presenting a technological and nutritional potential when considering its chemical composition. In addition to the novel edible flour, the phenolic compounds obtained a present potential as a functional ingredient to be incorporated into foods.

Keywords: sunflower seed; innovative technologies; plant protein; plant-based ingredient; food technology



Citation: dos Santos Friolli, M.P.; Silva, E.K.; Chaves, J.; da Silva, M.F.; Goldbeck, R.; Galland, F.A.B.; Pacheco, M.T.B. Sequential Processing Using Supercritical Carbon Dioxide and High-Intensity Ultrasound in Sunflower Protein Flour Production: Nutritional Value, Microstructure, and Technological Functionality. *Processes* **2023**, *11*, 2407. <https://doi.org/10.3390/pr11082407>

Academic Editors: Néstor Gutiérrez-Méndez, Efrén Delgado, Javier Mateo and Samuel Perez-Vega

Received: 18 July 2023

Revised: 4 August 2023

Accepted: 4 August 2023

Published: 10 August 2023



Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Sunflowers currently compose the fourth largest oilseed planting area in the world. Sunflower grain is composed of about 20% protein, including all the essential amino acids, and 40% lipids, including 31% polyunsaturated fatty acids (55–70% linoleic acid). It has a high content of vitamin E, the B-complex (folate and niacin), and minerals that make up its micronutrients. Additionally, this cultivar presents a high content of phenolic compounds, around 1–4%, mostly chlorogenic and caffeic acids. Sunflower seeds are mainly directed towards obtaining vegetable oil for cooking and biodiesel, also being used in the pharmaceutical and cosmetic industries as well as for animal feed. A large volume of residue is coproduced during the exploitation of refined vegetable oil, composed mostly of shells, carbohydrate fractions, proteins, and plant fibers [1–3].

Due to the change in eating habits over the last few years, there has been a higher demand for plant protein sources to cope with the reduction in animal protein consumption. The plant-based culture is growing in the market, leading to an increasing exploration of foods from pulses (peas, beans, and chickpeas, among others), nuts (both seeds and nuts), nonconventional food plants (NCFPs), and mushrooms as a protein source alternative [4–8]. Sunflowers also present a great potential to be used as an alternative protein source and ingredient in the food industry due to a protein content with a balance of amino acids, a low allergenic potential, and the absence of antinutritional factors [3].

Another advantage of incorporating sunflower protein into the human diet is the use of a byproduct of oil consumption that would normally go to animal consumption. However, after the oil extraction step, the sunflower meal presents a high content of phenolic compounds, which has been associated with undesirable sensory characteristics. In this regard, the investigation of new extraction processes for these compounds could enhance the full use of this food matrix. In addition, within the circular economy, food should be used in its entirety to minimize nutritional losses and to preserve the content and quality of nutrients. This approach leads to improvements in extraction techniques, also resulting in a lower environmental impact during the extraction and purification processes [9,10].

The advantages of applying innovative extraction techniques include a reduction in the number of steps, a reduction in the extraction time, the production of extracts that are free of toxic solvents, and the preservation of heat-sensitive food substrates. In addition, it also results in the selectivity of extracts and the use of nutrients and functional compounds in parts that are difficult to access, such as the cell matrix [9,10].

Innovative technologies can potentially innovate the processing chain of several food matrices, such as the sunflower's, by promoting the full use of the grain in addition to the widely explored vegetable oil. Green technologies are associated with both high-quality products and with a sustainable production that does not result in unwanted residues or environmental waste. Sustainability in the food chain is growing with the increase in the new generation of consumers who seek sustainable products with a lower environmental impact [11]. Thus, innovative techniques such as supercritical carbon dioxide (SCO₂) and high-intensity ultrasound (HIUS) were applied to reduce the lipid content and to extract phenolic compounds from sunflower meal, respectively. SCO₂ technology is a promising extraction technique used to obtain lipids, oils, essential oils, and lipophilic bioactive compounds from food matrices [12]. However, few studies have investigated the impacts of high-pressure CO₂ on biomasses after SCO₂ extraction processing [13]. Likewise, HIUS has attracted an interest for a new food process design due to its action mechanism mainly being applied to the extraction processes of phenolic compounds. In this regard, we evaluated how the sequential processing of sunflower meal using SCO₂ and HIUS, investigated by Friolli et al. [14], could affect the quality and technological attributes of sunflower protein flour.

Therefore, this study explored the application of SCO₂ and HIUS in processing and raising the protein content of sunflower meal. According to Friolli et al. [14], SCO₂ was used to reduce the lipid content, and an HIUS-process-based strategy was investigated to reduce the phenolic content, enhancing the sensory attributes of the novel food ingredient. In this context, the aim of this study was to understand the impact of these innovative technologies in obtaining sunflower flour with a high protein content and a reduced phenolic content from a nutritional and technological point of view.

2. Materials and Methods

2.1. Raw Material

The sunflower meal (SM) used resulted from the cold oil extraction process of previously dehulled sunflower seeds from the company Veris Óleos Vegetais Ltd.a-ME (Veris Brasil/Vinhedo, SP, Brazil). Initially, the SM was crushed in a hammer mill, and then granulometry was performed with a vibrating sieve (Octagon 200, Endecotts Ltd., London,

UK). Particle size analysis allowed the identification of sample composition, which was mostly composed of 0.710 mm (73%) and 0.297 mm (23%) particles. After analysis, the sample was stored at $-18\text{ }^{\circ}\text{C}$ in sealed plastic that was protected from light.

2.2. Sunflower Meal Degreasing Followed by Phenolic Content Extraction

As described by Friolli et al. [14], the residual oil was extracted from SM using supercritical CO_2 in a supercritical fluid extraction system (SFE Thar Technologies, model SFE-2 \times 5LF-2-FMC, Pittsburgh, PA, USA) at $50\text{ }^{\circ}\text{C}$ and 40 MPa with a flow rate of 200 g/min CO_2 . A solvent to feed mass ratio of 38.4 was applied to extract the lipid content from 3.1 kg of SM. The extraction experiments were carried out in triplicate ($n = 3$). A total of 90% of the oil content was recovered in this step, resulting in defatted sunflower meal (DSM). Subsequently, the extraction of phenolic compounds was performed with a 13 mm diameter probe at 300 W and constant frequency of 19 kHz (Unique, Disruptor, 500 W, Indaiatuba, Brazil). A mixture of water and ethanol (1:1, v/v) was used as solvent. The extraction experiments were carried out in quintuplicate ($n = 5$). Approximately 83% of phenolic compounds was recovered, resulting in sunflower flour (SF). The samples were stored at $-18\text{ }^{\circ}\text{C}$ until chemical, structural, thermal, technological, and cell viability analyses were performed.

2.3. Chemical Composition

The chemical composition of the samples was determined by analyzing moisture, proteins (conversion factor of 6.25), lipids, ash, total dietary fiber, and total phenolic content [15,16]. All analyses were performed in triplicate.

2.4. Total Amino Acid Profile

The amino acid profile of the samples was determined after acid hydrolysis (6 M HCl) of proteins (110 $^{\circ}\text{C}/22\text{ h}$), with precolumn derivatization, separation, and identification on reverse-phase high-performance liquid chromatograph (RP-HPLC) with ultraviolet (UV) detector at 254 nm (Shimadzu Corporation, Tokyo, Japan) that was equipped with a Luna/Phenomenex C18 column (250 mm \times 4.6 mm \times 5 μm ; Phenomenex Inc., Torrance, CA, USA). Identification and quantification were performed using a mix of external standards (Pierce/PN 20088), with α -aminobutyric acid (AAAB) being used as an internal standard (Aldrich, Milwaukee, WI, USA) as described by Hagen et al. [17] and White et al. [18]. The analysis was performed in triplicate.

2.5. Carbohydrate Identification and Quantification

The samples were dried at $65\text{ }^{\circ}\text{C}$ for 24 h to a maximum moisture content of 10%. They were characterized with respect to the contents of their main structural carbohydrates, glucans, and xylans using the method proposed by the National Renewable Energy Laboratory (NREL) [15] with modifications. Approximately 0.3 g of samples was subjected to hydrolysis with 72% concentrated sulfuric acid (w/w) for 1 h followed by dilution with distilled water to a final concentration of 4% (w/v). The suspension was autoclaved at $121\text{ }^{\circ}\text{C}$ for 1 h, was cooled and filtered through a syringe filter with a porosity of 0.22 μm , and was stored in vials for determination of the concentrations of cellobiose, glucose, xylose, arabinose, stachyose, and raffinose through HPLC. All carbohydrate standards were obtained from Sigma-Aldrich[®], Milwaukee, WI, USA. For the runs, an Accela chromatograph (Thermo Scientific[®], Waltham, MA, USA) was used that was equipped with an HPX-87 H separation column (Bio-Rad[®], Hercules, CA, USA), and they were maintained at $45\text{ }^{\circ}\text{C}$ and flow rate of 0.6 mL/min and used a sulfuric acid solution with a pH of 2.6 as the phase. After obtaining the compounds, the glucan and xylan contents were estimated according to Equations (1) and (2). The analysis was performed in triplicate.

$$\text{Glucan [\%]} = (((0.90 \times [\text{glucose}] + (0.95 \times [\text{cellobiose}] + (3.53 \times [\text{formic acid}]))) / 0.3) \times 0.087 \quad (1)$$

$$\text{Xylan [\%]} = (((0.88 \times [\text{xylose}] + (0.88 \times [\text{arabinose}] + (0.72 \times [\text{acetic acid}]))/0.3) \times 0.087 \quad (2)$$

2.6. Structure Characterization and Thermal Analysis

2.6.1. Particle Size Distribution

The analysis was performed in a laser diffraction particle size analyzer (Mastersizer 3000 from Malvern Instruments, model: MAZ3000, Worcestershire, UK) with Hydro EV accessory. Sample preparation: dispersion medium was distilled water (IR: 1.333), sample refractive index was 1.5295, sample absorption index was 0.1, and stirring/pumping was at 1750 rpm. Time of 4 s was used to obtain readings and background. The mean diameter was determined based on the average diameter of a sphere of the same volume, the Brouckere diameter ($D_{4,3}$), according to Equation (3). The analysis was performed in triplicate.

$$D_{4,3} = \frac{\sum n_i d_i^4}{\sum n_i d_i^3} \quad (3)$$

where d_i is the average particle diameter and where n_i is the number of particles.

2.6.2. Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis

The soluble protein composition of the samples was detected through sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE). SF samples were diluted to 2 mg/mL homogenate in a sample buffer (0.0625 M tris–HCl, pH of 6.8, 2% (*w/v*) SDS, 5% (*w/v*) b-mercaptoethanol, 10% (*v/v*) glycerol, and 0.002% (*w/v*) bromophenol blue) and then were boiled and centrifuged at $10,000 \times g$ for 5 min. Protein content was measured through the Lowry method, modified by Peterson [19], using bovine serum albumin as standard. Equal amounts (15 μg) of total protein were subjected to 12% (*w/v*) SDS–polyacrylamide gel electrophoresis. The gels were run constantly at 180 V for 1.5 h and were stained with 0.006% (*w/v*) Coomassie blue R and 6% (*v/v*) acetic acid. Decolorizing was carried out with water. Molecular weights of polypeptides were estimated using low molecular weight standards (Sigma–Aldrich[®]) with molecular weight of 6500–66,000 Da.

2.6.3. Differential Scanning Calorimetry (DSC)

A small portion of the sample (~8 mg) was collected with the aid of a metal microspatula and was weighed into an Al crucible (40 μL) using a microanalytical balance. Pressing was performed with the aid of an appropriate piston, and then the crucible was sealed with a lid and taken to the equipment for analysis. Material was then heated from 50 to 130 $^{\circ}\text{C}$ at a rate of 10 $^{\circ}\text{C}/\text{min}$ under an inert atmosphere of N_2 at a flow rate of 50 mL/min in a crucible with a lid without a hole.

2.7. Technological Functionality

2.7.1. Solubility

Sample duplicates (0.5 g) were dispersed in 25 mL of distilled water. The solution was adjusted in a pH range of 4 to 7 using 0.5 mol/L NaOH or 0.5 mol/L HCl [20]. After stirring with a magnetic bar for 1 h at room temperature (22 ± 2 $^{\circ}\text{C}$), samples were centrifuged at 8000 rpm (Type GLC-1, Ivan Sorvall Inc., Norwalk, CT, USA) for 20 min at 24 $^{\circ}\text{C}$ and were filtered (Whatman n^o1) for determination of proteins in the supernatant via the Kjeldahl method. The analysis was performed in duplicate. The solubility profile was obtained by plotting average of protein solubility (%) as a function of pH, where

Solubility (%) = Amount of proteins in the supernatant / Amount of proteins in the sample.

2.7.2. Emulsion Formation and Emulsion Stability

The emulsifying activity (EA) and emulsion stability (ES) of the sunflower samples were determined in duplicate following the methods of Dabbour et al. [21] and Mekala

et al. [22] with modifications. A 2% SF solution (5 mL) in the pH range of 4–7 was thoroughly mixed with 3 mL of sunflower oil in IKA T25 digital Ultra-turrax[®] equipment (IKA Werke, Staufen, Germany) at speed of 15,000 rpm for 5 min. The kinetic stability of the emulsions was analyzed in Turbiscan equipment (LAB[®] Expert—Formulation, Toulouse, France) through the measurement of the backscattering (BS) profile in duplicate at times of 0, 0.25, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, and 6.0 h. The analysis was performed in duplicate. The emulsions were also characterized according to their droplet size distribution, which was performed in a laser diffraction particle size analyzer (Mastersizer 3000 from Malvern Instruments, model: MAZ3000) with Hydro EV accessory (Worcestershire, UK). The size of the oil droplets was expressed as the volume–surface mean diameter ($D_{3,2}$) according to Equation (4). The analysis was performed in triplicate.

$$D_{3,2} = \frac{\sum n_i d_i^3}{\sum n_i d_i^2} \quad (4)$$

where d_i is the average droplet diameter and where n_i is the number of droplets.

2.7.3. Light Microscopy

After manual homogenization of the sample, an in natura portion was placed on the slide using a disposable Pasteur pipette and was covered with a coverslip. A Leica[®] Optical Microscope (DMLM, Cambridge, UK) was used for observation, and images were obtained in different fields of the slides of the in natura sample at magnifications of 50 and 100× in transmitted light mode.

2.8. Cell Viability Evaluation

2.8.1. Viability of Caco-2 Cells Subjected to Oxidative Stress

The human intestinal cell line Caco-2 (American Type Culture Collection, Rockville, MD, USA) was used for in vitro cytotoxicity studies. Briefly, cells were kept at 37 °C in Modified Eagle Medium (MEM) containing 10% (*v/v*) fetal bovine serum, 1% nonessential amino acids, 1 mM L-glutamine, 10,000 U/mL of penicillin, and 10,000 µg/mL of streptomycin in 5% CO₂. Cells were grown in a 25 cm bottle for 6 to 7 days with medium replaced every 2 to 3 days. Oxidative stress was induced in confluent cell cultures through the addition of 1 mM H₂O₂. Caco-2 monolayers were formed from 2 × 10⁴ cells/well, which were grown in 98-well culture plates and were treated after confluence. For treatment, cells were preincubated with two concentrations of SM or SF (0.25 and 0.5 mg/mL), and, after 1 h, cells were stimulated with 1 mM H₂O₂ for another 6 h as a stress agent. The analysis was performed in duplicate.

2.8.2. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium Bromide (MTT) Reduction Assay

After treatment, cells were incubated with 50 µg/mL of MTT for 30 min at 37 °C. Then, the medium was removed, and the MTT crystals were dissolved in dimethylsulfoxide (DMSO). Absorbance values were measured at 560 and 650 nm. MTT reduction was calculated as (absorbance at 560 nm) – (absorbance at 650 nm) and was expressed as a percentage of the baseline [23]. The analysis was performed in duplicate.

2.9. Statistical Analysis

The results were expressed through their mean values and standard deviation. The electrophoresis, light microscopy, differential scanning calorimetry, and backscattering profile results were analyzed descriptively.

3. Results and Discussion

3.1. Chemical Composition

The change in the composition of the samples as a function of processing can be seen in Figure 1 as reported by Friolli et al. [14]. Initially, the SM was obtained from its partially defatted form through mechanical pressing (cold extraction) from shelled grains. The initial

protein content agrees with that found by Salgado et al. [24] in the sunflower cake that resulted from oil extraction.

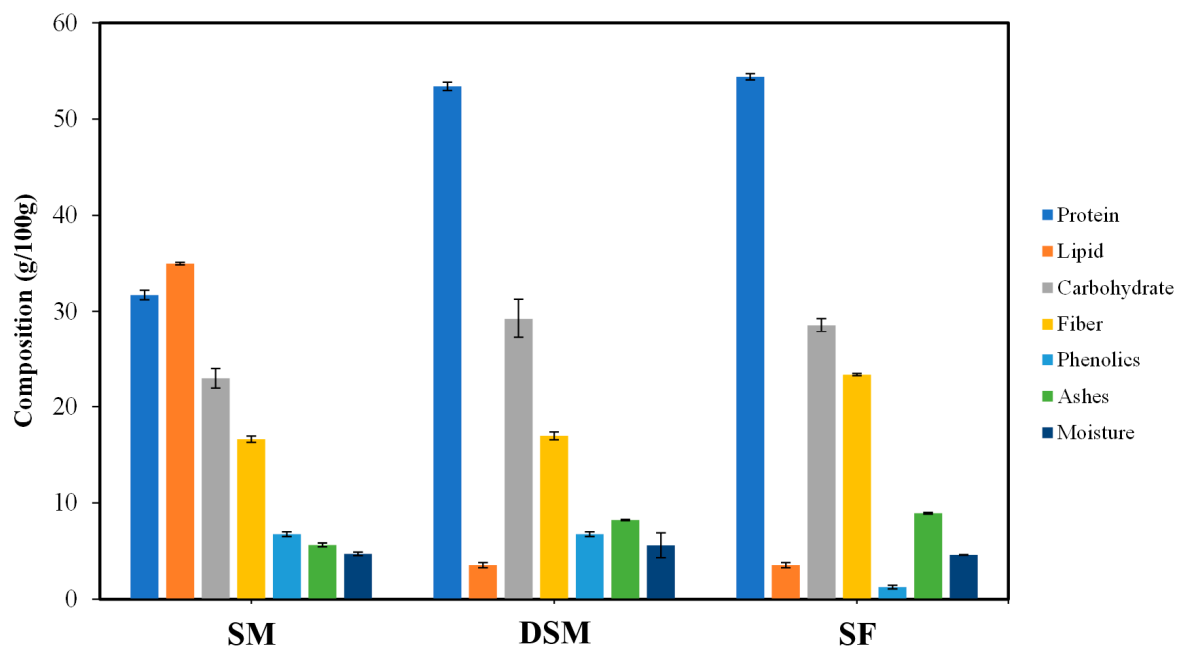


Figure 1. Chemical composition of sunflower meal (SM), defatted sunflower meal (DSM), and sunflower flour (SF) (n = 3).

The sunflower flour (SF) sample obtained after the extraction of the oil and phenolic compounds presented approximately 55% proteins, representing a 71.6% increase from the initial protein value. A similar result was found by Alexandrino et al. [25], who obtained 61% proteins using Soxhlet extraction as the lipid extraction method and using n-hexane as the solvent. The phenolic compounds were reduced by approximately 82% (Figure 2).

3.1.1. Amino Acid Profile

Table 1 shows the order of the major amino acids (AAs) identified in the samples, such as glutamic acid, aspartic acid, arginine, and leucine. Acid hydrolysis possibly converted asparagine and glutamine to aspartic and glutamic acids [26].

The AA composition shows that the applied treatments, despite having a concentrated protein content, did not change the AA composition of the proteins from the samples. The content of hydrophobic, hydrophilic, and neutral AAs was not different between treatments.

On average, neutral AAs represented 18.8% of the total AAs, hydrophobic AAs represented 32.8%, and hydrophilic AAs represented 48.4%. The content of hydrophilic AAs has the potential to improve the technological quality of the flour [27], especially regarding the interfacial properties in protein functionality.

The essential AAs in the sunflower cake, such as sulfur (cysteine and methionine), branched AAs (leucine, valine, and isoleucine), tryptophan, alanine, and phenylalanine, represented 297 mg/g–29.7% on average of the total AA profile [26]. By evaluating the amino acid score in Table 2, it is possible to confirm lysine as the limiting amino acid, with an inadequate concentration of approximately 13.8% to 11.1% of the recommended amount, in the SM and SF samples, respectively. An increase in the lysine content of about 2.7% was observed from SM to SF, which is similar to the results of Alexandrino et al. [24]. All the other essential AAs reached the score recommended by FAO/ONU, RDC n° 54 [28].

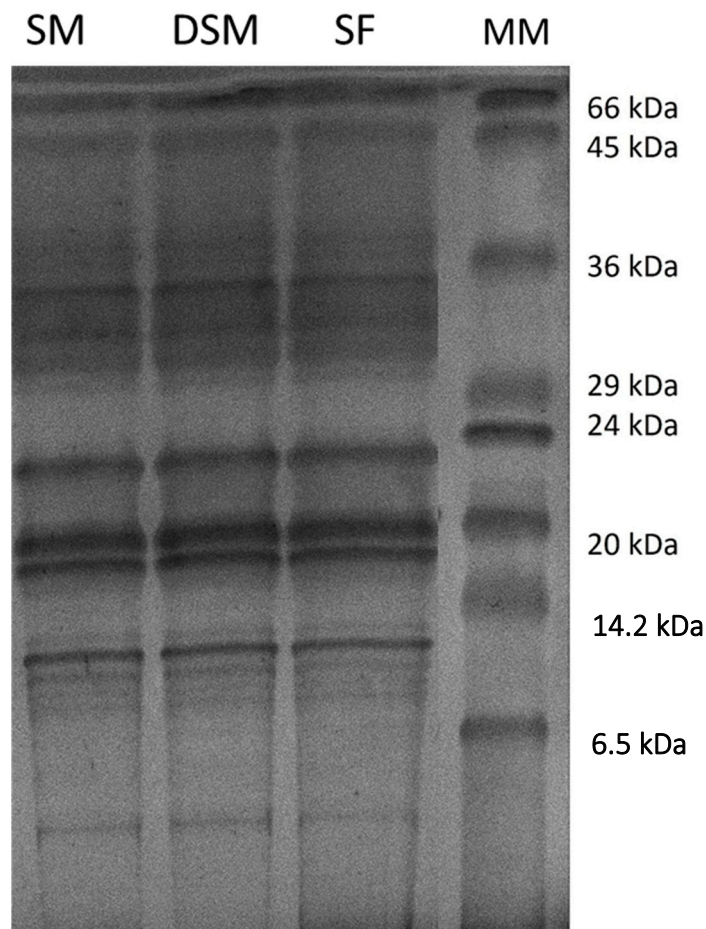


Figure 2. Electrophoresis of sunflower meal (SM), defatted sunflower meal (DSM), and sunflower flour (SF). MM—molecular weight marker.

Table 1. Amino acid composition of sunflower meal (SM), defatted sunflower meal (DSM), and sunflower flour (SF) (n = 3).

Amino Acids (mg/g of Protein)	SM	DSM	SF
Aspartic acid	106.7 ± 0.1	102.8 ± 0.1	97.6 ± 0.2
Glutamic acid	222.3 ± 0.2	222.2 ± 0.1	215.5 ± 0.5
Serine	46.2 ± 0.2	47.2 ± 0.0	48.4 ± 0.1
Glycine	57.9 ± 0.1	65.0 ± 0.2	63.9 ± 0.2
Histidine	27.1 ± 0.0	28.8 ± 0.0	30.9 ± 0.1
Arginine	90.8 ± 0.2	99.6 ± 0.1	92.2 ± 0.2
Threonine	35.9 ± 0.1	39.3 ± 0.0	42.8 ± 0.1
Alanine	44.5 ± 0.1	46.9 ± 0.0	44.8 ± 0.1
Proline	38.3 ± 0.0	47.6 ± 0.0	47.8 ± 0.2
Tyrosine	28.0 ± 0.0	28.1 ± 0.0	29.8 ± 0.1
Valine	58.2 ± 0.1	55.5 ± 0.1	52.0 ± 0.1
Methionine	22.2 ± 0.1	21.8 ± 0.1	23.1 ± 0.1
Cystine	15.9 ± 0.0	21.0 ± 0.0	11.9 ± 0.0
Isoleucine	45.6 ± 0.1	42.5 ± 0.1	42.4 ± 0.3

Table 1. *Cont.*

Amino Acids (mg/g of Protein)	SM	DSM	SF
Leucine	64.2 ± 0.2	66.9 ± 0.0	66.9 ± 0.3
Phenylalanine	47.4 ± 0.1	47.4 ± 0.1	49.8 ± 0.3
Lysine	38.8 ± 0.1	36.4 ± 0.2	40.0 ± 0.2
Hydrophobic	327.0	328.6	326.8
Hydrophilic	485.7	489.8	476.2
Neuter	183.9	181.7	196.8

Mean values of replicate amino acids and standard deviation; hydrophobic = Ala, Val, Met, Phe, Leu, Ile, Pro, and Trp; hydrophilic = Arg, Asp, His, Lys, and Glu; neuter = Ser, Gly, Tyr, and Cys.

Table 2. Amino acid score of sunflower meal (SM), defatted sunflower meal (DSM), and sunflower flour (SF) according to FAO/RDC n° 54 [28].

Total Amino Acids	FAO/RDC 54, 2007	mg/g of Protein		
		SM	DSM	SF
Histidine	15	27.1	28.8	30.9
Isoleucine	30	45.6	42.5	42.4
Leucine	59	64.2	66.9	66.9
Lysine	45	38.8	36.4	40.0
Methionine + Cystine	22	38.1	42.8	35.0
Cystine + Tyrosine	43.9	49.1	65.91	41.7
Threonine	23	35.9	39.3	42.8
Tryptophan	6	10.1	10.3	11.1
Valine	39	58.2	55.5	52.0

3.1.2. Carbohydrate Composition Profile

The samples presented two types of polysaccharides, which constituted reserve and structural material. Among the reserve polysaccharides were galactomannans, xyloglucans, glucans, and mannans. The xyloglucans presented D-glucose units in their cellulose chain, while the galactomannans, obtained mainly from the endosperm, presented α -D-mannose units [29–31].

The SF showed a higher content of identified carbohydrates. Xylan (from the xylose content), a cell wall polysaccharide, was mainly present in the SF sample (Table 3). According to the literature, SFs have a higher content of di- and oligosaccharides, while a reduced portion (0.6%) is represented by monosaccharides [32]. The glucan that was present in the grains was a polysaccharide with an interesting health potential due to its action in reducing cholesterol and blood glucose [3].

Table 3. Characterization of carbohydrates obtained from acid hydrolysis of sunflower meal (SM) and sunflower flour (SF) (n = 3).

Carbohydrate	SM	SF
Glucans:	3.66 ± 0.02	5.19 ± 0.01
Glucose (%)	1.89 ± 0.01	1.86 ± 0.01
Cellobiose (%)	1.77 ± 0.01	3.33 ± 0.00
Xylan (%)	6.9 ± 0.03	9.76 ± 0.05
Stachyose (%)	1.63 ± 0.02	1.86 ± 0.02

In a study by Bishop [33], the hydrolyzed polysaccharides from sunflower seeds consisted of about 23.5% D-glucose (xyloglucans) and 16.2% D-galactose (galactomannans),

with traces of arabinose and raffinose below the detection limit of the method, as in the present work. Meanwhile, Sosulki et al. [32] were able to identify 2.5% raffinose in defatted sunflower flour.

3.2. Structural Characterization

3.2.1. Particle Size Distribution

Table 4 presents the results of the particle size distribution of the flour samples. In all the treated samples, there was a significant reduction in particle size compared to the original untreated SM. The particle size of the DSM sample was reduced, and the reduction was even more evident in the SM subjected to the SF treatment for the extraction of residual oil and phenolic compounds (Table 4).

Table 4. Particle size distribution of sunflower meal (SM), defatted sunflower meal (DSM), and sunflower flour (SF) (n = 3).

	$D_{4,3}$ (μm)	d_{10} (μm)	d_{50} (μm)	d_{90} (μm)
SM	764	34.4	551	1860
DSM	260	12.1	174	655
SF	231	10.3	159	571

The treatment with SCO_2 promoted the removal of about 90% of the oil present in the sample, which possibly caused the reduction in particle size. Therefore, the conditions under which the sample was submitted during oil extraction did not cause major conformational changes, but the change in granule composition evidently contributed to the reduction in particle size.

Although the HIUS treatment aimed at extracting phenolic compounds that were present in small proportions when compared to the oil content, it also resulted in a greater reduction in particle size. The HIUS probably exerted great shear activity that was capable of breaking the plant matrix and reducing the volume of the granule due to the presence of numerous cavities [34,35].

Our results agree with those published by Zhang et al. [36], who observed a particle size reduction in the samples subjected to the sonication of peanut proteins, with a progressive size reduction with increasing HIUS exposure time. However, Malik, Sharma, and Saini [37] found that SF exposed to HIUS for a prolonged time of 30 min resulted in an increased particle size, possibly due to aggregate formation.

Regardless, the reduction in particle size influences the technological properties of SF. The increase in the surface area of the flour particles causes a greater interaction between the plant matrix and the medium, interfering with the formation properties of colloidal systems. Thus, these changes may promote conditions that enable the interaction between flour and a complex food system solubilized in a solvent, which confers its potential application as an ingredient in the food industry [38].

3.2.2. Electrophoresis

We did not observe differences in the protein profile of the three fractions (Figure 2), indicating that the process of SCO_2 and HIUS did not alter the molecular weight of the protein units present in the SF.

Malik et al. [37] observed a variation in the electrophoretic profile of sunflower protein isolates in samples that were ultrasonicated. The authors associate the change in protein structure with the effect of sonication. However, Sullivan et al. [39], Xiong et al. [40], and Sullivan et al. [41] reported that HIUS application did not induce an alteration in the molecular weight of wheat protein isolates, soybean, ovalbumin, animal proteins, or plant proteins (pea, soybean, and rice) under the conditions of an intensity of 20 kHz and a time of 2–40 min. These studies differ in terms of the complexity of the sample composition submitted to the HIUS system. For instance, the study of Malik et al. [37]

used isolated proteins, while our sample was more complex, containing carbohydrates, fibers, and phenolics, among other elements, in addition to proteins. The complexity of the structure probably exerted a different exposure to ultrasonication, protecting and giving a greater stability to the protein structure during the ultrasonic process.

Electrophoresis with the denaturing agent SDS and heat promoted the separation of the intramolecular bonds of the oligomers, undoing the quaternary structure and separating the proteins into units. The highest-molecular-weight protein observed was 66 kDa, and the lowest was 6.5 kDa (Figure 2), which is somewhat different from the values reported by Rodríguez et al. [42], who found sunflower proteins with bands between 11.5 and 94.5 kDa. Regarding the sunflower proteins' composition, globulins constituted the majority of the proteins, ranging from 40 to 90%, while about 10 to 30% consisted of albumins and glutelins, the prolamins. Two major classes of globulin proteins were present: globulin 11 S (heliantin) and albumins, including albumin 2 S and 7 S fractions [43].

Heliantin (11 S globulin) is a globular oligomeric protein with a molecular weight of 300–350 kDa [44] with respect to the undenatured protein. The 2 S albumin fraction, on the other hand, has molecular weights ranging from 10 to 18 kDa and can even be found in the range of 11.5 to 20.1 kDa [43]. It has been shown that the formation of high-molecular-weight aggregates (15–18 S) of heliantin is favored at high protein concentrations, becoming the largest fraction at moderate alkaline pH values (pH of 8.5–9), probably due to electrostatic interactions [45].

3.2.3. Differential Scanning Calorimetry

Through the DSC analysis, it was possible to observe the thermal behavior of the flour samples (Figure 3). With the application of heat to different samples, the transition temperature (T_d) was observed, represented by a variation in the curve of all the samples at 100.7 °C for the SM, 100.9 °C for the DSM, and 100.2 °C for the SF.

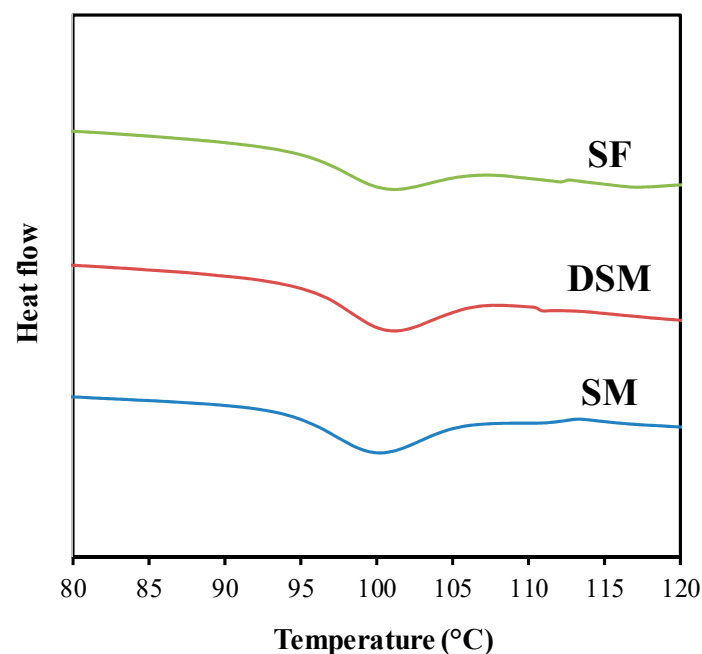


Figure 3. Differential scanning calorimetry (DSC) of sunflower meal (SM), defatted sunflower meal (DSM), and sunflower flour (SF).

In the range of 110 °C to 115 °C, a small thermal event could be observed. With a change in the energy movement from DSF to SF, we speculate that the chemical composition and the applied HIUS treatment possibly influenced the thermal behavior of the SF at higher temperatures. The 2 S fraction of the sunflower protein corresponding to albumin presented a denaturation temperature that was higher than 100 °C, reaching up to 118 °C [42]. As the

molecular weights of the 2 S fraction range from 10 to 18 kDa, the well-marked band near 20 kDa in our electrophoresis indicates an important concentration of the 2 S fraction.

In studies performed with isolated proteins, such as those of Malik et al. [37], there is a change in enthalpy values, which is attributed to possible molecular changes in the protein structure after ultrasonication application. As a result of ultrasonication, proteins are probably more prone to heat treatment, as pointed out by the decrease in the denaturation enthalpy. Alexandrino et al. [25] were able to observe values ranging from 108.7 °C to 113.0 °C.

When considering that denaturation precedes the protein gelation process, some changes in the protein structure and the exposure of the functional groups are correlated with the transition temperature, which is studied in DSC. Salgado et al. [24] found that the denaturation temperature of their sunflower cake was close to 100 °C. The authors proposed that the globulin 11 S at a neutral to an alkaline pH shows its Td at 100 °C, with the occurrence of an increase in beta sheets, which is indicative of the denaturation and aggregation of the protein since high protein concentrations favor protein gelation [24,38,46].

The functional property of protein gelling is of great interest in the plant-derived market, as there is a growing interest in replacing animal protein with plant-derived sources, with a potential application in the production of second-generation meat substitutes. For this application, the fat content should be reduced, and the sensory characteristics should be similar to those of animal meat to reaching not only vegetarians but also flexitarians [46].

Through the behavior of the Td, it is possible to deduce the occurrence of the gelation phenomenon of sunflower proteins since proteins are the major component in the flour. This behavior could potentially be applied in the food industry, probably as a fat mimicking ingredient used in sausage foods to confer sensory quality to the product. The use of plant proteins as an ingredient considers, in addition to the nutritional quality, the functional technological performance, such as the gelling property, in the constitution of products with a health promotion appeal [38,46,47].

3.3. Functionality

3.3.1. Solubility

Figure 4 shows the evident effect of pH on the solubility of all the samples as well as the variation in solubility among the samples. As expected, an increase in pH improved the percentage of soluble proteins in all the samples, especially at a pH of seven, reaching a solubility of around 30% in the DSM and SF samples. Rodrigues et al. [42], Sripad and Narasinga Rao [48], and Bau et al. [49] found a solubility of 30%, 50%, and 55%, respectively, at a pH of eight.

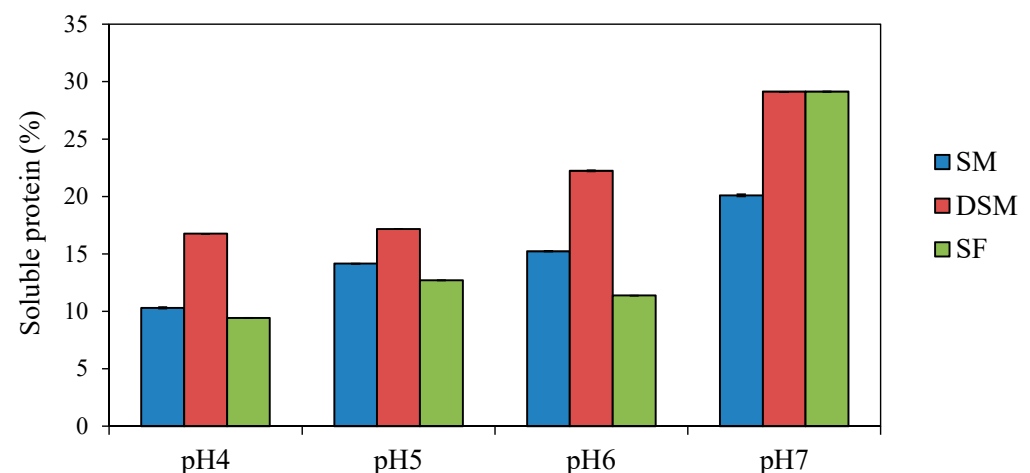


Figure 4. Solubility of sunflower meal (SM), defatted sunflower meal (DSM), and sunflower flour (SF) as a function of pH (n = 2).

Solubility is influenced by the process used in the extraction of protein and phenolic compounds [45]. The HIUS treatment promotes a reduction in the particle size of the molecules, possibly leading to a greater interaction between the compounds present in the cell matrix and the solution, with an increased solubility of ultrasonicated samples. The HIUS treatment can enhance the solubility of plant proteins through a process known as ultrasonic-assisted extraction or sonoextraction. When plant materials containing proteins are subjected to HIUS, the mechanical energy generated by the sound waves creates cavitation bubbles in the liquid medium. Cavitation refers to the formation, growth, and implosion of tiny bubbles in the liquid caused by the rapid pressure changes induced by ultrasound. During the implosion of these bubbles, localized high pressures and temperatures are reached, leading to the disruption of cell walls and the release of intracellular components, including proteins [9,11,14]. However, it is necessary to consider the pH of the solution in addition to the sonication time, a variable that may cause the denaturation of proteins and the formation of protein aggregates that reduce solubility [42,50].

We demonstrated a reduction in particle size in the samples that underwent SCO₂ and HIUS, which reached a higher solubility at a pH of seven when compared to the SM. However, at pH < 7, the ultrasonicated sample showed a lower solubility. One hypothesis is that there was a change in the isoelectric point (IP) of the SF protein with a variation around a pH of six, justifying the low solubility at this pH that was not observed for the SM or the DSM. The reduction in the hydrophobic lipid fraction can probably also be used to explain the better performance of the DSM flour at all the pH values. Another variable to be considered is the particle size. It is possible that the SF flour was much more exposed to pH variations in the solution medium. The larger surface promoted greater contact between the cell matrix, its compounds, and the solution, so the pH of the solution to which the SF was subjected had a greater influence when compared to the SM and DSM (a flour with a larger structure size).

3.3.2. Emulsion Characterization Backscattering Profile

The backscattering (BS) profile obtained from the turbidimetric measurements was performed to evaluate the kinetic stability of the emulsions and to explore the emulsifying potential of the protein-rich flour. The technique allowed for the detection of the destabilization phenomenon of the emulsions before any visual observation. The measurement of the BS profiles was obtained from the fresh emulsions at the times 0.25, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, and 6.0 h (Figure 5). In Figure 5, it is possible to see that there was emulsion formation in the wavelength range of the tube subjected to the equipment, with a difference in emulsion formation observed according to the flour sample and the applied pH.

The emulsion obtained for the SF sample at a pH of seven showed less phase separation according to infrared light backscattering. In general, it was observed that the BS profile values decreased at the bottom and increased at the top of the measuring tube. This result indicates that the application of HIUS to the flour sample improved both the formation and stability of the emulsion when compared to the other samples. Increasing the pH (pH of seven) improved the kinetic stability of the emulsions of all the samples.

The emulsions presented two phases, sediment formation at the bottom of the tube and the aqueous phase. However, there was no such formation in the SF sample at a pH of seven, which is considered the most suitable for technological applications that rely on emulsion formation.

By correlating the pH applied in the formation of the emulsion with solubility, it is possible to infer that a pH of seven presented the highest percentage of solubility (30%). At a pH of seven, sunflower proteins were soluble in the medium and interacted at the water–oil interface, justifying the positive result in all the samples. However, the SF presented the smallest particle size in the particle size distribution analysis, corresponding to the largest contact surface and thus favoring the solute–solvent interaction [43,45].

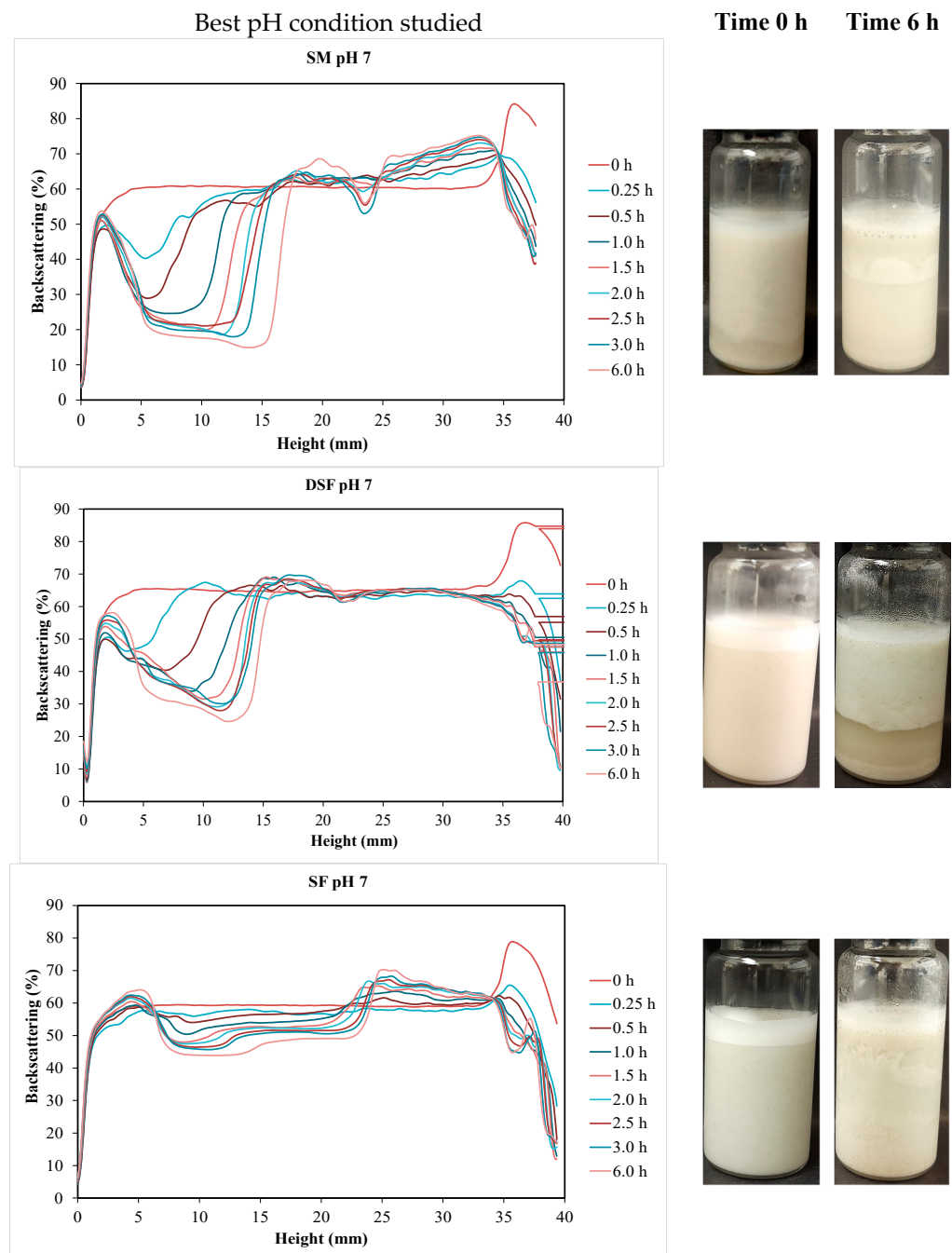


Figure 5. Backscattering profile of the emulsions produced with sunflower meal (SM), defatted sunflower meal (DSM), and sunflower flour (SF) at pH of 7 ($n = 2$).

Regarding the emulsifying property, the composition of the flour should be considered since it was rich in proteins as well as other compounds, such as polysaccharides, that may favor the formation of colloidal systems. In addition, phenolics, even at lower concentrations, such as in the SF samples, had the ability to bind to the proteins and compromise its functionality [49].

Regardless of the degree of solubility, even insoluble proteins can contribute to the stability of colloidal systems such as emulsions, with possible actions such as pickering. Moreover, both proteins and other compounds, such as lipids, polysaccharides, and surface-aggregated particles, although they are not integrated or solubilized in these systems, have the function of maintaining the arrangement of the colloidal system. Possibly due to their particle size, other components that do not participate in the formation of the micelle

structure may contribute by preventing the flow of water or even the destabilization of the system, preventing exchanges with the environment [51].

Studying rice flour, Tahmasebi et al. [52] found a higher amount of fiber and starch in its composition. There was a reduction in the moisture content that restricted the water mobility, with an improved stability in the emulsions. The addition of proteins regardless of origin will likely contribute to improved emulsion stability results. Plant proteins can improve the incorporation of fat into an emulsion through the presence of apolar AA chains, which favor the binding of the hydrocarbon side chain of the oil to the flour [52,53].

Therefore, the application of a flour rich in sunflower proteins, based on the conditions studied in the present work, can be explored in its application as an ingredient with the function of favoring the stability of colloidal systems such as emulsions.

Droplet Size Distribution

Table 5 shows the droplet size distribution of the emulsions obtained from the application of mechanical stirring at different pH values (four to seven). The droplet size distribution varied as a function of the flour composition and applied pH, with droplets having larger sizes in the DSM at a pH of seven and a smaller size in the SF at a pH of seven. The results agree with the more homogeneous emulsion profile depicted in Figure 6. When relating the BS values to the droplet size of the emulsions, the BS values were inversely proportional to the droplet size, with larger BS values and smaller values of diameter. The variables involved in the formation of the emulsions were the emulsifying agents, oil percentage, and agitation. Therefore, when you have a smaller particle size range, the closer the droplet size distribution is associated with the uniformity and the formation of a colloidal system [54].

Table 5. Droplet size distribution of the emulsions produced with sunflower meal (SM), defatted sunflower meal (DSM), and sunflower flour (SF) at pH of 7 and with SF at different pH conditions (n = 3).

Treatment	$D_{3,2}$ (μm)	d_{10} (μm)	d_{50} (μm)	d_{90} (μm)
SM at pH of 7	15 \pm 1	8 \pm 2	35 \pm 2	498 \pm 24
DSM at pH of 7	15 \pm 3	8 \pm 2	44 \pm 6	638 \pm 11
SF at pH of 4	16.9 \pm 0.3	7.5 \pm 0.3	42.5 \pm 0.4	458 \pm 2
SF at pH of 5	24.8 \pm 0.4	13.9 \pm 0.5	62 \pm 1	500 \pm 12
SF at pH of 6	33 \pm 3	18 \pm 2	88 \pm 6	549 \pm 36
SF at pH of 7	13 \pm 1	5.4 \pm 0.6	40 \pm 3	425 \pm 17

3.4. Cell Viability

The SM and SF samples were tested for their ability to protect against an oxidative stimulus with peroxide (H_2O_2) in the Caco-2 cells. This strain is used as a model of colonocytes or enterocytes, which are the first cells to come into contact with food and which play a key role in nutrient absorption and immunity. Figure 7 shows cell viability as a response to cytotoxicity in the Caco-2 cells when pretreated with different concentrations of the flours (the SM and SF) as well as their cell protective action against the oxidative stimulus with peroxide. The cytotoxic stimulus with peroxide reduced cell viability, while the SM sample did not compromise cell viability.

It was interesting to observe that the pretreatment of the cells with the SF sample and the subsequent stimulation with peroxide for 6 h did not protect the cells from the cytotoxic action of peroxide at any of the tested concentrations. However, the pretreatment with the SM sample and subsequent stimulation for an additional 6 h with peroxide protected against cytotoxicity at all the concentrations.

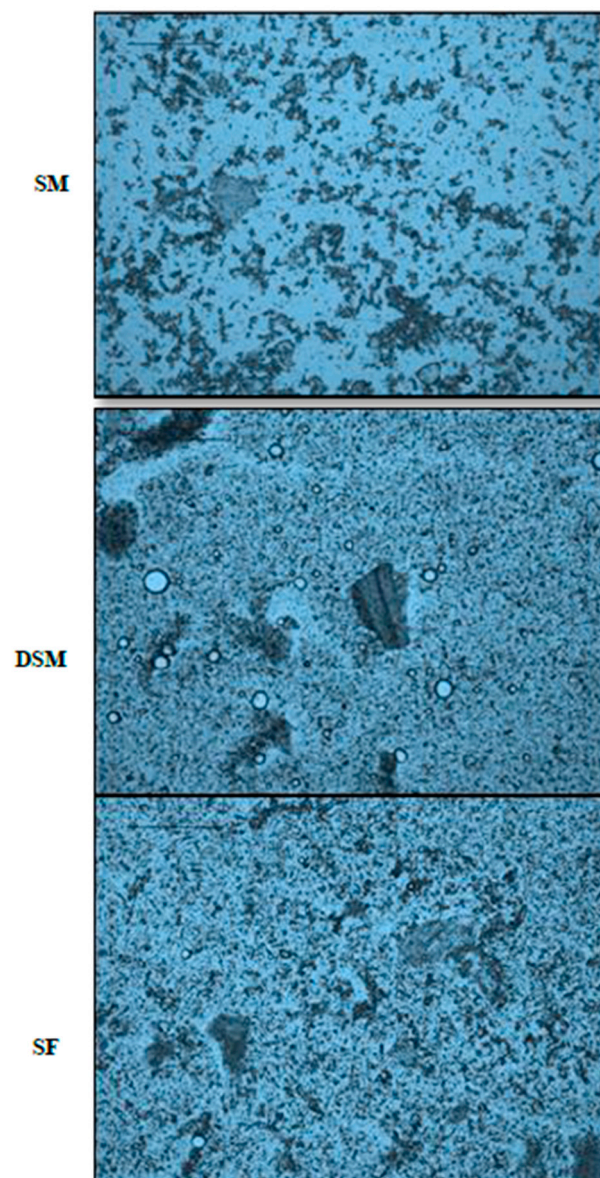


Figure 6. Microstructure of the emulsions produced with sunflower meal (SM), defatted sunflower meal (DSM), and sunflower flour (SF) at pH of 7 (n = 2).

By correlating the composition of the two samples submitted to the cytotoxicity test, it is possible to infer that the protection observed was mainly due to the presence of the phenolic compounds in the SM sample since the phenolic compounds present in the sample, mostly chlorogenic acid and caffeic acid, exert antioxidant properties that act on the control of the nonspecific reactions of several oxidant species that are produced in normal metabolism [55].

The presence of phenolic compounds in the SF can bind to proteins through covalent and noncovalent bonds. The complexation of the phenolics to the sunflower proteins can reduce the quality of the sunflower proteins by inhibiting digestibility, causing undesirable browning and altering the functional properties and behavior of the proteins in various food matrices [55]. Therefore, the extraction of these phenolic compounds allows them to be used in an inactivated or encapsulated form in order to exert the potential benefits already evidenced in the role of cellular protection and antioxidants without prejudice to the plant protein fraction. In the study by Barsby et al. [56], sunflower seeds also proved to be safe, did not compromise cell viability, and still protected the cell exposed to cytotoxicity from oxidative stress.

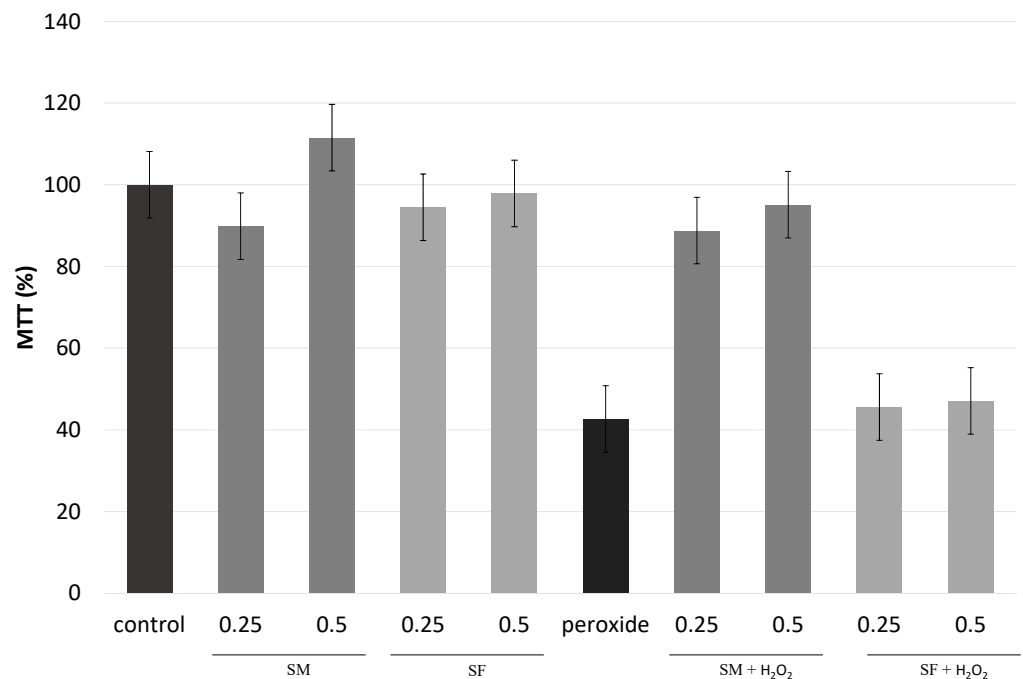


Figure 7. MTT test for sunflower meal (SM) and sunflower flour (SF) (n = 2).

3.5. Impact of Green Technologies on Food Process Chain

The process design of sustainable and innovative technologies, such as SCO₂ and HIUS, with the aim of simplifying and improving the obtainment of high-quality byproducts with commercial value is in line with the principles of biorefinery. In this regard, biorefinery integrates the process of transforming biomass into chemical inputs, materials, food, and energy, and it is used to add value to the production chain while reducing the environmental impact. It aims at improving the use of biomass, minimizing losses, and increasing profits and benefits. The integrated systems of raw material, processes, and inputs are thought up in a sustainable way considering the generation and consumption of products, regional socioeconomic development, and the environmental impacts that permeate production [57].

By combining these two technologies, we have been able to optimize the production of edible sunflower flour from sunflower meal (byproduct), enhancing their nutritional profile and functional properties. These ecofriendly techniques offer potential solutions for valorizing agricultural residues, reducing waste, and promoting sustainable practices in the food industry. The resulting edible sunflower flour can be used as a functional ingredient in various food formulations, adding value to byproducts that would otherwise be discarded and contributing to a more circular and sustainable food system. SCO₂ operates at relatively low temperatures, minimizing the heat-induced degradation of thermally sensitive components and resulting in a higher product quality. On the other hand, HIUS involves the application of intense sound waves to the material, leading to cavitation and the generation of mechanical forces that facilitate various processes, including extraction.

The circular economy is an economic system that favors sustainable development; through the circular flow of resources, it is capable of redesigning the production chain to exhaust the use of matter in all stages of production in order to value natural resources and minimize their use, aiming at a lower environmental impact and better health conditions for humans along with economic growth [58].

Presently, it is of great importance that studies enable and explore food in its integrity, aiming at the production of new products and potential ingredients to supply the market's demand. HIUS is a technology that has demonstrated its potential both in the extraction of nutrients and in its technological application as exploited in the formation of emulsions and other encapsulating systems.

Sunflowers have great potential in the food industry due to their nutritional value beyond the extraction of vegetable oil. Studies have reported the potential for their application in breads [59], cookies [60], gluten-free breads [61], cakes [62], plant milk [63], and sausages [47]. Many studies [47,59–63] have proposed the production of a new ingredient with a good technological quality and acceptance from the sensory point of view for the elaboration of foods with better nutritional quality and a higher content of vitamins and minerals that are gluten-free and rich in phenolics.

The feasibility of the processes used to obtain potential ingredients for the food industry when exploring plant sources, especially those from residues, and using sustainable technologies is necessary to enable broad trade and even to develop high-value products with greater reach/popularity and accessibility to people from different socioeconomic and cultural backgrounds.

4. Conclusions

Our findings have demonstrated the potential of using innovative technologies, such as SCO₂ and HIUS, to obtain new food ingredients from sunflower byproducts. By combining these two technologies, we have successfully described and evaluated the production of edible sunflower flour from sunflower meal (byproduct). These environmentally friendly approaches present promising opportunities for utilizing agricultural residues effectively, reducing waste, and fostering sustainable practices within the food industry, which consequently result in an edible sunflower flour as a valuable functional ingredient for diverse food formulations, add worth to otherwise discarded byproducts, and contribute to the establishment of a more circular and sustainable food ecosystem. The analysis of the composition showed that the product has an interesting amino acid composition as well as reduced phenolic and oil contents. The process by which the flour was subjected improved the final solubility and emulsion formation performance. The preliminary evaluation of cell viability revealed no toxicity and a protective potential against reactive oxygen species through the action of the phenolic compounds present in the sunflower seeds. Therefore, this study supports the production of an ingredient with a technological potential for the plant-based food industry based on sustainable green technologies, demonstrating the potential of the full use of sunflowers.

Author Contributions: Conceptualization, M.P.d.S.F., E.K.S. and M.T.B.P.; methodology, M.P.d.S.F., E.K.S., F.A.B.G., M.F.d.S., J.C., R.G. and M.T.B.P.; software, M.P.d.S.F. and E.K.S.; investigation, M.P.d.S.F., E.K.S., M.T.B.P. and F.A.B.G.; resources, M.T.B.P. and E.K.S.; writing—original draft preparation, M.P.d.S.F., M.F.d.S. and J.C.; writing—review and editing, M.P.d.S.F., E.K.S., M.T.B.P. and F.A.B.G.; project administration, M.P.d.S.F. and M.T.B.P.; funding acquisition, M.T.B.P. All authors have read and agreed to the published version of the manuscript.

Funding: This work was funded by the Fundação de Amparo à Pesquisa do Estado de São Paulo (Fapesp), process number 2018/12546-1, and by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), a doctoral scholarship. Eric Keven Silva thanks FAPESP (2023/01876-9) for the Young Investigator Fellowship.

Data Availability Statement: Data not available.

Acknowledgments: We are grateful to the Instituto de Tecnologia de Alimentos (ITAL) for providing facilities for the composition and solubility analyses.

Conflicts of Interest: The authors declare that there are no conflict of interest. Funders had no role in designing the study; in the collection, analysis, or interpretation of the data; in the writing of the manuscript; or in the decision to publish the results.

References

1. Oliveira, M.F.; Vieira, O.V. *Sunflower Oil Extraction Using Mini Press*; Embrapa Soja: Londrina, Brazil, 2004.
2. Li, S.-T.; Duan, Y.; Guo, T.-W.; Zhang, P.-L.; He, P.; Majumdar, K. Sunflower response to potassium fertilization and nutrient requirement estimation. *J. Integr. Agric.* **2018**, *17*, 2802–2812.
3. Adeleke, B.S.; Babalola, O.O. Oilseed crop sunflower (*Helianthus annuus*) as a source of food: Nutritional and health benefits. *Food Sci. Nutr.* **2020**, *8*, 4666–4684. [[CrossRef](#)] [[PubMed](#)]
4. Mengue, P. *14% of Brazilians Declare Themselves Vegetarian, Shows Ibope Survey*; Estadão, Caderno: São Paulo, Brasil, 2018; Available online: <https://www.svb.org.br/vegetarianismo1/mercado-vegetariano> (accessed on 10 March 2019).
5. Turbiani, R. Consumers Change Eating Habits and Heat Up the Plant-Based Market in Brazil. *Época Negócios*, São Paulo. 2020. Available online: <https://epocanegocios.globo.com/Sustentabilidade/noticia/2020/12/consumidor-muda-habitos-alimentares-e-aquece-mercado-plant-based-no-brasil.html> (accessed on 10 March 2021).
6. Seixas, M.; Contini, E. India: Outlook for pulse and grain markets. Brasília, DF: Embrapa. Secretariat of Intelligence and Strategic Relations. Strategic Dialogues-Observatories Series. Technical Note, 5). 2017.
7. Seixas, M.; Contini, E. India: Pulses: Markets and opportunities for Brazil and Embrapa. Brasília, DF: Embrapa (Embrapa. Secretariat of Intelligence and Strategic Relations. Strategic Dialogues-Observatories Series. Technical note, 9). 2018.
8. Ministry of Agriculture, Livestock and Supply. *National Plan for the Development of the Bean and Pulse Chain*; Ministry of Agriculture, Livestock and Supply: Brasília, Brazil, 2018.
9. Chemat, F.; Rombaut, N.; Sicaire, A.G.; Meullemiestre, A.; Fabiano-Tixier, A.S.; Abert-Vian, M. Ultrasound assisted extraction of food and natural products. Mechanisms, techniques, combinations, protocols and applications. A review. *Ultrason. Sonochemistry* **2017**, *34*, 540–560.
10. Linhares, G.; Roias, M.L. Ultrasound-Assisted Extraction of Natural Pigments from Food Processing By-Products: A Review. *Front. Nutr.* **2022**, *9*, 891462.
11. Zobot, G.L.; Viganó, J.; Silva, E.K. Low-Frequency Ultrasound Coupled with High-Pressure Technologies: Impact of Hybridized Techniques on the Recovery of Phytochemical Compounds. *Molecules* **2021**, *26*, 5117. [[CrossRef](#)]
12. Silva, E.K.; Martelli-Tosi, M.; Vardanega, R.; Nogueira, G.C.; Zobot, G.L.; Meireles, M.A.A. Technological characterization of biomass obtained from the turmeric and annatto processing by using green technologies. *J. Clean. Prod.* **2018**, *189*, 231–239. [[CrossRef](#)]
13. Tejedor-Calvo, E.; García-Barreda, S.; Sánchez, S.; Morales, D.; Soler-Rivas, C.; Ruiz-Rodríguez, A.; Sanz, M.A.; Garcia, A.M.; Marco, P. Supercritical CO₂ extraction method of aromatic compounds from truffles. *LWT* **2021**, *150*, 111954. [[CrossRef](#)]
14. Friolli, M.P.S.; Silva, E.K.; Napoli, D.C.S.; Sanches, V.L.; Rostagno, M.A.; Pacheco, M.T.B. High-intensity ultrasound-based process strategies for obtaining edible sunflower (*Helianthus annuus* L.) flour with low-phenolic and high-protein content. *Ultrason. Sonochemistry* **2023**, *97*, 106449. [[CrossRef](#)]
15. Latimer, G.W., Jr. (Ed.) *Official Methods of Analysis of AOAC International*, 19th ed.; AOAC International: Gaithersburg, MD, USA, 2007.
16. Firestone, D. (Ed.) *Official Methods and Recommended Practices of the AOCS*, 6th ed.; AOCS: Urbana, IL, USA, 2013.
17. Hagen, S.R.; Frost, B.; Augustin, J. Precolumn phenylisothiocyanate derivatization and liquid chromatography of aminoacids in food. *J. AOAC* **1989**, *72*, 912–916. [[CrossRef](#)]
18. White, J.A.; Fry, J.C.; Hart, R.J. An evaluation of the Waters Pico-Tag system for the amino-acid analysis of food materials. *J. Autom. Chem.* **1986**, *8*, 170–177.
19. Peterson, G.L. A simplification of the protein assay method of lowry which is more generally applicable. *Anal. Biochem.* **1977**, *83*, 346–356. [[CrossRef](#)]
20. Lawal, O.S.; Adebawale, K.O.; Adebawale, Y.A. Functional properties of native and chemically modified protein concentrates from bambarra groundnut. *Int. Food Res. J.* **2007**, *40*, 1003–1011. [[CrossRef](#)]
21. Dabbour, M.; He, R.; Ma, H.; Musa, A. Optimization of ultrasound assisted extraction of protein from sunflower meal and its physicochemical and functional properties. *J. Food Process. Eng.* **2018**, *41*, e12799. [[CrossRef](#)]
22. Mekala, S.; Silva, E.K.; Saldaña, M.D.A. Mechanism, kinetics, and physicochemical properties of ultrasound-produced emulsions stabilized by lentil protein: A non-dairy alternative in food systems. *Eur. Food Res. Technol.* **2022**, *248*, 185–196.
23. Hansen, M.B.; Nielsen, S.E.; Berg, K. Re-examination and further development of a precise and rapid dye method for measuring cell growth/cell kill. *J. Immunol. Methods* **1989**, *119*, 203–210. [[CrossRef](#)] [[PubMed](#)]
24. Salgado, P. Sunflower Protein Concentrates and Isolates Prepared from Oil Cakes Have High Water Solubility and Antioxidant Capacity. *J. Am. Oil Chem. Soc.* **2011**, *88*, 351–360. [[CrossRef](#)]
25. Alexandrino, T.D.; Ferrari, R.A.; Oliveira, L.M.; Ormenese, R.C.S.C.; Pacheco, M.T.B. Fractioning of the sunflower flour components: Physical, chemical and nutritional evaluation of the fractions. *LWT* **2017**, *84*, 426–432. [[CrossRef](#)]
26. Petraru, A.; Ursachi, F.; Amariei, S. Nutritional Characteristics Assessment of Sunflower Seeds, Oil and Cake. Perspective of Using Sunflower Oilcakes as a Functional Ingredient. *Plants* **2021**, *10*, 2487. [[CrossRef](#)]
27. Oseyko, M.; Romanovska, T.; Shevchyk, V. Justification of the amino acid composition of sunflower proteins for dietary and functional products. *Ukr. Food J.* **2020**, *9*, 394–403. [[CrossRef](#)]
28. WHO. Report of a joint FAO/WHO/UNU expert consultation. In *Protein and Amino Acid Requirements in Human Nutrition*; WHO: Geneva, Switzerland, 2007.

29. Stephen, A.M.; Phillips, G.O.; Williams, P.A. (Eds.) *Food Polysaccharides and Their Applications*; CRC Press: Boca Raton, FL, USA, 2006; Chapter 6.
30. Edwards, M.; Bulpin, P.V.; Dea, I.C.M.; Reid, J.S.G. Biosynthesis of legume-seed galactomannans in vitro. *Planta* **1989**, *178*, 41–51. [[CrossRef](#)]
31. Cunha, P.L.; Paula, R.C.; Feitosa, J.P.A. Polysaccharides from Brazilian biodiversity: An opportunity to transform knowledge into economic value. *Química Nova* **2009**, *32*, 649–660. [[CrossRef](#)]
32. Sosulski, F.; Fleming, F. *Chemical, Sunflower Functional, and Nutritional Protein Products Properties*; Department of Crop Science, University of Saskatchewan: Saskatoon, SK, Canada, 1970.
33. Bishop, C.T. Carbohydrates of sunflower heads. *Can. J. Chem.* **1955**, *33*, 1521–1529. [[CrossRef](#)]
34. Rostagno, M.A.; Prado, J.M. Natural product extraction: Principles and applications. *R. Soc. Chem.* **2013**, *21*, 516.
35. Ávila, N.S.; Capote, F.P.; Castro, M.L. Ultrasound-assisted extraction and silylation prior to gas chromatography-mass spectrometry for the characterization of the triterpenic fraction in olive leaves. *J. Chromatogr. A* **2007**, *1165*, 158–165. [[CrossRef](#)]
36. Zhang, Q.T.; Tu, Z.C.; Xiao, H.; Wang, H.; Huang, X.Q.; Liu, G.X.; Liu, C.M.; Yan, S.; Fan, L.L.; Lin, D.R. Influence of ultrasonic treatment on the structure and emulsifying properties of peanut protein isolate. *Food Bioprod. Process.* **2014**, *92*, 30–37. [[CrossRef](#)]
37. Malik, M.A.; Sharma, H.K.; Saini, C.S. High intensity ultrasound treatment of protein isolate extracted from dephenolized sunflower meal: Effect on physicochemical and functional properties. *Ultrason. Sonochem.* **2017**, *39*, 511–519. [[CrossRef](#)]
38. Gonzalez-Pérez, S.; Vereijken, J.M. Review Sunflower proteins: Overview of their physicochemical, structural and functional properties. *J. Sci. Food Agric.* **2007**, *87*, 2173–2191. [[CrossRef](#)]
39. Sullivan, J.O.; Park, M.; Beevers, J. The effect of ultrasound upon the physicochemical and emulsifying properties of wheat and soy protein isolates. *J. Cereal Sci.* **2016**, *69*, 77–84. [[CrossRef](#)]
40. Xiong, W.; Wang, Y.; Zhang, C.; Wan, J.; Shah, B.R.; Pei, Y.; Zhou, B.; Li, J.; Li, B. High intensity ultrasound modified ovalbumin: Structure, interface and gelation properties. *Ultrason. Sonochem.* **2016**, *31*, 302–309. [[CrossRef](#)]
41. Sullivan, J.O.; Murray, B.; Flynn, C.; Norton, I. The effect of ultrasound treatment on the structural, physical and emulsifying properties of animal and vegetable proteins. *Food Hydrocoll.* **2016**, *53*, 141–154. [[CrossRef](#)]
42. Rodríguez, D.J.; Romero, J.; Rodríguez-García, R.; Sánchez, J.L.A. Characterization of Proteins from Sunflower Leaves and Seeds: Relationship of Biomass and Seed Yield. In *Trends in New Crops and New Uses*; ASHS Press: Alexandria, VA, USA, 2002.
43. González-Pérez, S.; Vereijken, J.M.; Merck, K.B.; Koningsveld, G.A.; Gruppen, H.; Voragen, A.G. Conformational states of sunflower (*Helianthus annuus* L.) helianthinin: Effect of heat and pH. *J. Agric. Food Chem.* **2004**, *52*, 6770–6778. [[CrossRef](#)]
44. Ivanova, P.; Chalova, V.; Koleva, L.; Pishtiyanski, I. Amino acid composition and solubility of proteins isolated from sunflower meal produced in Bulgaria. *Int. Food Res. J.* **2013**, *20*, 2995–3000.
45. González-Pérez, S. Physico-Chemical and Functional Properties of Sunflower Proteins. Ph.D. Thesis, Wageningen University, Wageningen, The Netherlands, 2003. Chapters 3–4.
46. Pena Franca, P.A.; Duque-Estrada, P.; Sa, B.F.F.; Goot, A.J.; Pierucci, A.P.T.R. Meat substitutes—Past, present, and future of products available in Brazil: Changes in the nutritional profile. *Future Foods* **2022**, *5*, 100133. [[CrossRef](#)]
47. Grasso, S.; Pintado, T.; Pérez-Jiménez, J.; Ruiz-Capillas, C.; Herrero, A.M. Potential of a sunflower seed byproduct as animal fat replacer in healthier Frankfurters. *Foods* **2020**, *9*, 445. [[CrossRef](#)] [[PubMed](#)]
48. Sripad, G.; Narasinga Rao, M.S. Effect of methods to remove polyphenols from sunflower meal on the physicochemical properties of the proteins. *J. Agric. Food Chem.* **1987**, *35*, 962–967. [[CrossRef](#)]
49. Bau, H.M.; Mohtadi-Nia, D.J.; Mejean, L.; Debry, G. Preparation of colorless sunflower protein products: Effect of processing on physicochemical and nutritional properties. *J. Am. Oil Chem. Soc.* **1983**, *60*, 1141–1148. [[CrossRef](#)]
50. Arzeni, C.; Martínez, K.; Zema, P.; Arias, A.; Pérez, O.; Pilosof, A. Comparative study of high intensity ultrasound effects on food proteins functionality. *J. Food Eng.* **2012**, *108*, 463–472. [[CrossRef](#)]
51. Karefyllakis, D.; Octaviana, H.; van der Goot, A.J.; Nikiforidis, C.V. The emulsifying performance of mildly derived mixtures from sunflower seeds. *Food Hydrocoll.* **2019**, *88*, 75–85. [[CrossRef](#)]
52. Tahmasebi, M.; Labbafi, M.; Emam-Djomeh, Z.; Yarmand, M.S. Manufacturing the novel sausages with reduced amount of meat and fat: The product development, formulation optimization, emulsion stability and textural characterization. *LWT—Food Sci. Technol.* **2016**, *68*, 76–84.
53. Adebowale, K.O.; Lawal, O.S. Comparative study of the functional properties of bambarra groundnut (*Voandzeia subterranean*), jack bean (*Canavalia ensiformis*) and mucuna bean (*Mucuna pruriens*) flours. *Food Res. Int.* **2004**, *37*, 355–365. [[CrossRef](#)]
54. Silva, E.K.; Gomes, M.T.M.S.; Hubinger, M.D.; Cunha, R.L.; Meireles, M.A.A. Ultrasound-assisted formation of annatto seed oil emulsions stabilized by biopolymers. *Food Hydrocoll.* **2015**, *47*, 1–13. [[CrossRef](#)]
55. Guo, S.; Ge, Y.; Jom, K.N. Review of phytochemistry, metabolite changes, and medicinal uses of the common sunflower seed and sprouts (*Helianthus annuus* L.). *Chem. Central J.* **2017**, *11*, 1–10.
56. Barsby, J.P.; Cowley, J.M.; Leemaqz, S.Y.; Grieger, J.A.; McKeating, D.R.; Perkins, A.V.; Bastian, S.E.P.; Burton, R.A.; Bianco-Miotto, T. Nutritional properties of selected superfood extracts and their potential health benefits. *PeerJ* **2021**, *9*, e12525. [[CrossRef](#)] [[PubMed](#)]
57. Alvim, J.C.; Alvim, F.A.L.S.; Sales, V.H.G.; Oliveira, E.M.; Sales, P.V.G.; Costa, A.C.R. Biorefineries: Concepts, classification, raw materials and products. *J. Bioenergy Food Sci.* **2014**, *1*, 61–77.

58. Oliveira, F.R.; França, S.L.B.; Rangel, L.A.D. *Principles of Circular Economy for the Development of Products in Industrial Clusters*; INTERACTIONS: Campo Grande, Brazil, 2019; Volume 20, pp. 1179–1193.
59. Skrbic, B.D.; Filipčev, B. Nutritional and sensory evaluation of wheat breads supplemented with oleic-rich sunflower seed. *Food Chem.* **2008**, *108*, 119–129. [[CrossRef](#)]
60. Puraikalan, Y.; Sabitha, N. Development and objective evaluation of sunflower seed fortified cookies. *Int. J. Curr. Res.* **2015**, *7*, 19927–19930.
61. Zorzi, C.Z.; Garske, R.P.; Flores, S.H.; Thys, R.C.S. Sunflower protein concentrate: A possible and beneficial ingredient for gluten-free bread. *Innov. Food Sci. Emerg. Technol.* **2020**, *66*, 102539.
62. Salem, E.M.; Hamed, N.A.; Awlya, O.F. Implementation of the sunflower seeds in enhancing the nutritional values of cake. *J. Appl. Sci. Res.* **2012**, *8*, 2626–2631.
63. Kuru, C.; Tontul, I. Optimisation of Plant-based Milk Formulation using Hazelnut, Sunflower Seed and Pumpkin Seed by Mixture Design. *Food Sci. Technol.* **2020**, *8*, 2441–2448. [[CrossRef](#)]

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.