Article

Ultrasound-Assisted Extraction of *Artocarpus heterophyllus* L. Leaf Protein Concentrate: Solubility, Foaming, Emulsifying, and Antioxidant Properties of Protein Hydrolysates

Julián Vera-Salgado [ID], Carolina Calderón-Chiu, Montserrat Calderón-Santoyo, Julio César Barros-Castillo [ID], Ulises Miguel López-García and Juan Arturo Ragazzo-Sánchez *

Laboratorio Integral de Investigación en Alimentos, Tecnológico Nacional de México/Instituto Tecnológico de Tepic, Av. Tecnológico #2595, Col. Lagos del Country, Tepic 63175, Nayarit, Mexico
* Correspondence: jragazzo@ittepic.edu.mx

Abstract: The impact of ultrasound-assisted extraction (UAE) was evaluated on the functionality of jackfruit leaf protein hydrolysates. Leaf protein concentrate was obtained by ultrasound (LPCU) and conventional extractions by maceration (LPCM). LPCM and LPCU were hydrolyzed with pancreatin (180 min), and hydrolysates by maceration (HM) and ultrasound (HU) were obtained. The composition of amino acids, techno-functional (solubility, foaming, and emulsifying properties), and antioxidant properties of the hydrolysates were evaluated. A higher amount of essential amino acids was found in HU, while HM showed a higher content of hydrophobic amino acids. LPCs exhibited low solubility (0.97–2.89%). However, HM (67.8 ± 0.98) and HU (77.39 ± 0.43) reached maximum solubility at pH 6.0. The foaming and emulsifying properties of the hydrolysates were improved when LPC was obtained by UAE. The IC_{50} of LPCs could not be quantified. However, HU (0.29 ± 0.01 mg/mL) showed lower IC_{50} than HM (0.32 ± 0.01 mg/mL). The results reflect that the extraction method had a significant ($p < 0.05$) effect on the functionality of protein hydrolysates. The UAE is a suitable method for enhancing of quality, techno-functionality, and antioxidant properties of LPC.

Keywords: ultrasound-assisted extraction; enzymatic hydrolysis; protein hydrolysates; antioxidant capacity; techno-functional properties

1. Introduction

Proteins consist of vital amino acids for human beings due to their nutritional value. In addition, they are widely used in the food industry because of their excellent techno-functional properties [1]. Currently, the main sources for obtaining proteins are of animal origin. Moreover, the production processes, as well as the waste generated, have a high environmental impact [2]. Contrarily, the obtention of proteins from vegetal sources represents a lower environmental impact [3].

The supply and consumption of plant-based proteins and dietary transition to plant-based protein consumption patterns are framed among the top global food trends [4]. Additionally, in recent years, efforts have been made to valorize the waste generated by the agroindustry to produce many value-added products [5]. Therefore, becoming the agricultural sector the leading supplier of raw materials for different processes is essential. The circular economy emerges as an agricultural alternative to counteract the production of agro-industrial waste. Thus, by-products gain added value to be used in further processes. Additionally, agro-waste is the biological and techno-functional compounds in agro-waste that could produce suitable profits [6]. Alike, proteins have been used to obtain protein hydrolysates with enzymes to improve the properties of native proteins [7]. Hydrolysates have been associated with multiple benefits to human health, such as antihypertensives [8] and antioxidants [9]. The functionality of hydrolysates is related to their amino acid

composition with diverse applications in the cosmetic and food industry [10]. Although the circular economy has been developed in many fields, new technologies are required to maintain the balance between economic, industrial development, and ecosystem protection with effective resource use [5].

The primary agricultural waste sources are associated with fruit production and crop residues, such as jackfruit. Under conventional harvesting conditions of this crop, it is estimated that leaves produced by tree pruning are around 10,378 tons/ha per year [11]. Recently, phenolic compounds in jackfruit leaves were reported, and extracts showed suitable antioxidant and antimicrobial activity [12]. In another study, the techno-functional properties of protein hydrolysates from jackfruit leaves obtained by high hydrostatic pressures were evaluated. The results evidenced the suitability of peptides for their application as carrier material. As well, the peptides showed suitable foaming and emulsifying properties and high solubility [11]. The quality of proteins and peptides is associated with their physicochemical (color, texture, solubility) and techno-functional (foaming, emulsifying, clarifying, thickening) properties [13]. These features define the possible areas of application, such as the cosmetic, pharmaceutical, and food industries.

Proteins are generally obtained by conventional methods such as maceration. However, this methodology has several disadvantages since it is time-consuming, requires high amounts of solvent, and has high energy consumption [14]. To maximize the protein isolation from plant matrixes, an efficient diffusion of the extraction solvent is crucial to break intramolecular bonds and weaken cellular structures. In addition, alternative extraction methodologies in agreement with principles of green chemistry have been implemented, such as microwave, supercritical fluid, and ultrasound-assisted extractions. Recently, the ultrasound-assisted process was reported as an effective treatment to increase the yield, enzymatic efficiency, amino acid composition, and bio-functionality of hydrolysates [15,16].

The use of ultrasound-assisted extraction (UAE) to obtain proteins is aimed at overcoming the issues of maceration, reducing extraction time, energy costs, and solvent amounts. Moreover, it yields a more homogeneous mixture, higher energy transfer rate, reduced temperature gradients, selective extraction, reduced equipment size, and greater process control [17]. Protein extraction yields more significantly than 30% have been reported from plant sources, including pumpkin seeds [18], bitter melon seeds [19], and soybeans [20]. Until now, reports do not exist about the use of UAE to obtain jackfruit leaves protein and their relationship with the techno-functionality properties of hydrolysates. The research aimed to extract jackfruit leaf concentrate protein from UAE and evaluate the techno-functional and antioxidant properties of hydrolysates.

2. Materials and Methods

2.1. Vegetal Material

Jackfruit leaves were handpicked after trees pruning in the "Tierras Grandes" orchard in Zacualpan, Compostela, Nayarit, Mexico, in May 2022. Then, they were transferred to polyethylene bags and transported to the laboratory. After washing, the leaves were dehydrated in a convective drying oven (Novatech, HS60-AID, Guadalajara, Mexico) for 24 h at 60 °C. Subsequently, they were ground using a high-speed blender (NutriBullet® SERIE 900, Los Angeles, CA, USA) and sieved (#100 mesh, 150 μm diameter). The flour was vacuum-packed and stored at room temperature for subsequent experiments [11].

2.2. Chemical Substances

Pancreatin (EC 232-468-9), amino acids standard (AAS-18), L-norleucine (Nor, ≥98%), *N-tert*-butyldimethylsilyl-*N*-methyltrifluoroacetamide (MTBSTFA, >97%), acetonitrile (ACN), potassium persulfate, 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS+), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), Bradford reagent, and sodium dodecyl sulfate (SDS) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Analytical-grade chemicals such as sodium hydroxide (NaOH) and hydrochloric acid (HCl) were provided from Thermo Fisher Scientific Inc. (Waltham, MA, USA).

2.3. Preparation of Flour for Extraction Procedures

A depigmentation procedure was developed prior to extraction. Briefly, the flour was mixed with acetone in the liquid-to-solid ratio of 1:10 (*w/v*), then the sample was stirred on a magnetic plate for 24 h. Subsequently, the sediment was separated by decantation, and the depigmented flour was dried at room temperature until dry. Samples of leaf flour (30 g) were placed in a 1 L beaker containing 563 mL of distilled water and 188 mL of a solution (0.2 M NaOH), then the mixture was stirred for 10 min on a magnetic stirring plate. The same procedure was conducted for the following extraction approaches.

2.3.1. Maceration Extraction (M)

The homogenized solution was transferred to conical centrifuge tubes (50 mL) and centrifuged at 1500× *g* for 20 min at 10 °C (HERMLE, Z 326K, Waseerburg, Germany). The supernatant was collected and adjusted to pH 4.0 with 1 N HCl until the isoelectric precipitation. The samples were left to stand for 2 h and then centrifuged at 1500× *g* for 20 min at 10 °C [11]. Finally, the precipitate obtained by maceration (LPCM) was collected and used for further analysis.

2.3.2. Ultrasound-Assisted Extraction (UAE)

The homogenized solution was subjected to UAE using an ultrasonic bath (Digital Ultrasonic Cleaner, CD-4820, Guangdong, CHN) at 42 kHz for 20 min. The mixture was centrifuged at 1500× *g* for 20 min at 10 °C (HERMLE, Z 326K, Waseerburg, Germany). The supernatant was recovered, pH was adjusted, proteins were precipitated, and recovered by centrifugation in the same way mentioned above. The pellet of LPC obtained by UAE (LPCU) was used for comparison with the LPCM.

2.4. LPC Hydrolysis

LPCM and LPCU were hydrolyzed with pancreatin enzyme for 180 min [21]. A solution of LPC (1%, *w/v*) was prepared with distilled water and incubated at 37 °C, 115 rpm in a shaking water bath (Shaking Hot Tubs 290200, Boekel Scientific, Feasterville-Trevose, PA, USA). The solution was adjusted to pH 7.0 (1 N NaOH), and the enzyme was added in an enzyme-substrate ratio of 1:100 (*w/w*). The pH was maintained by adding NaOH if necessary. The hydrolysis process was stopped by enzyme inactivation; thus, the mixture was heated at 95 °C for 15 min. The pH 7.0 was adjusted, and the solution was centrifuged at 10,000× *g* for 15 min at 10 °C (HERMLE, Z 326K, Waseerburg, Germany). The supernatant was filtered (0.45 μm) and evaporated in a convective oven at 50 °C until the required concentration for further analysis. The hydrolysates by maceration (HM) and UAE (HU) were obtained. The yield was calculated (Equation (1)).

$$\text{Yield}(\%) = \frac{m_h}{m_{LPC}} \cdot 100 \tag{1}$$

where:

HM: mass of hydrolysate, g;
LPCM: mass of LPC, g.

2.5. Analysis of Amino Acids by Gas-Chromatography Mass-Spectrometry (GC-MS)

The amino acid determination was carried out following the protocol proposed by Brion-Espinoza et al. [7]. Samples of flour, LPC, and hydrolysates were subjected to acid hydrolysis with 6 M HCl for 24 h at 110 °C. Then, they were derivatized with MTBSTFA (*N-tert*-Butyldimethylsilyl-*N*-methyltrifluoroacetamide), a reactant for GC derivatization. Briefly, 100 μL of hydrolysate and 10 μL of L-norleucine (internal standard, 0.2 mg/mL in HCL 0.1 M) were evaporated under nitrogen gas to dryness. The resulting precipitate was dissolved in 200 μL of acetonitrile and 200 μL of MTBSTFA. This solution was incubated at 100 °C for 2.5 h in a glycerol bath. The derivatization reaction was performed in a 2 mL PTFE-lined screw-capped vial. For the L-amino acids standards mixture, the same

procedure was followed. The GC-MS was performed using a GC 7890A coupled to MS 240 Ion Trap (Agilent Technologies; Palo Alto, CA, USA). A capillary column Agilent J&W VF-5ms (30 m × 0.25 mm, i.d., 0.25 μm film thickness) was used for the separation. The carrier gas was helium (99.99%) at a flow rate of 2 mL/min. The oven temperature program was set at 150 °C for 2 min, increased at 3 °C/min to 280 °C. A total of 2 μL were injected with autosampler in split mode (20:1) in the GC injector port at 260 °C. MS parameters were as follows: energy of ionization (70 eV), full scan mode (35–650 m/z), ion trap (150 °C), manifold (80 °C), and transfer line (130 °C). Linear retention indexes were calculated using a mixture of straight-chain alkanes (C_7–C_{30}), injected under the same analysis conditions. The amino acid profile was reported as g of amino acid/100 g of sample.

2.6. Techno-Functional Properties

2.6.1. Solubility

The solubility was carried out following the protocol used by Calderón-Chiu et al. [11]. Samples of LPCs and hydrolysates (10 mg) were placed inside a 3 mL conical tube, and 1 mL of distilled water was added. Then, tubes were vortexed for 30 s to dissolve the sample. Each sample was adjusted at pHs 2.0, 4.0, 6.0, 8.0, and 10.0 with 1 N HCl or NaOH. The solutions were vortexed for 30 min and centrifuged (7500× g, 15 min) (Hettich MIKRO 220R, Tuttlingen, Germany). The protein content of the supernatant recovered was determined by the Bradford method [22]. After the solubilization of the sample in 1 mL of 0.5 N NaOH solution, the total protein content was determined, and solubility (%) was calculated (Equation (2)).

$$\text{Solubility (\%)} = \frac{P_{Snat}}{P_{Total}} \cdot 100 \tag{2}$$

where:

P_{Snat}: protein content in the supernatant, g;
P_{Total}: total protein content in the sample, g.

2.6.2. Foaming Properties

The foaming capacity (FC) and foaming stability (FS) of LPCs, and the HM and HU hydrolysates were determined according to the methodology used by Calderón-Chiu et al. [11]. A protein solution at 4.6 mg/mL in distilled water was prepared for each test. Then, an aliquot of 6 mL was placed in a 15 mL conical tube. The sample was homogenized using Ultra-Turrax (IKA T10, Staufen, Germany) at 16,000 rpm for 2 min to incorporate air bubbles, and the test was developed at room temperature. The total solution was immediately transferred to a 15 mL glass graduated cylinder; after 30 s, the total volume was recorded, and FC (%) was calculated (Equation (3)).

$$\text{FC(\%)} = \frac{A_0 - B}{B} \cdot 100 \tag{3}$$

where:

A_0: volume after homogenization, mL;
B: volume before homogenization, mL.

For the FS (%) determination, the same homogenized sample was used, and after 10 min allowed to stand, the volume was recorded and calculated (Equation (4)).

$$\text{FS (\%)} = \frac{At - B}{B} \cdot 100 \tag{4}$$

where:

At: volume after rest, mL;
B: volume before homogenization, mL.

2.6.3. Emulsifying Properties

The turbidimetric method was used for the emulsifying properties. Briefly, in a 15 mL test tube, 2 mL of olive oil were mixed with 6 mL of solution 4.6 mg/mL of LPC or hydrolysates in distilled water. Initially, the samples were homogenized using Ultra-Turrax (IKA T10, Staufen, Germany) at 10,000 rpm for 1 min. Then, aliquots of 50 μL were taken from the bottom of the tube at 0 and 10 min and diluted 100 times separately in 5 mL of 0.1% SDS solution. The sample was stirred for 10 s on a magnetic stirring plate. A spectrophotometer (Cary 50 Bio UV–Visible, Varian, Mulgrave, Australia) was used to measure the absorbance (500 nm) at the time (t) of 0 min (A_0) and 10 min (A_{10}) after emulsion formation. The emulsifying activity index (EAI) and emulsion stability index (ESI) were calculated (Equation (5)) and (Equation (6)) respectively [23].

$$\text{EAI} = \frac{2 \cdot 2.303 \cdot DF \cdot A_0}{c \cdot f \cdot 10,000} \tag{5}$$

$$\text{ESI} = \frac{A_0}{A_0 - A_{10}} \cdot t \tag{6}$$

where:

DF: dilution factor, 100;
c: mass of the sample, g;
f: mass fraction of olive oil in the emulsion, 0.25.

2.7. Antioxidant Properties

To evaluate the radical-scavenging activity (RSA), an $ABTS^+$ stock solution at a concentration of 7 mM $ABTS^+$ in 2.45 mM potassium persulfate was prepared and maintained in total darkness at 25 °C for 15 h. A dilution with distilled water of an aliquot of the stock solution was adjusted to an absorbance of 0.70 ± 0.02 at 734 nm. Then, 50 μL of LPC or hydrolysate solutions (0.3–1 mg/mL) were taken and mixed with 950 μL of $ABTS^+$ radical, shaking vigorously for 10 s. The absorbance was recorded at 734 nm on a spectrophotometer (Cary 50 Bio UV–Visible, Varian, Mulgrave, Australia) after 7 min. $ABTS^+$ RSA was calculated with Equation (7).

$$\text{ABTS}^+\text{RSA (\%)} \frac{Acontrol - Asample}{Acontrol} \times 100 \tag{7}$$

where:

$A_{control}$: absorbance of the $ABTS^+$ solution;
A_{Sample}: absorbance of the reaction ($ABTS^+$ with sample).

Subsequently, curves representing radical-scavenging activity (RSA, %) *y*-axis versus sample concentration (mg/mL) *x*-axis were plotted for each sample. The corresponding point at 50% of antioxidant activity with the x-intercept was defined as the IC_{50} value. A linear regression equation of curves was used for this purpose [11].

2.8. Statistical Analysis

Data obtained in triplicate were analyzed with a one-way analysis of variance (ANOVA), followed by the post hoc Tukey test for the mean comparison ($p < 0.05$) with the STATISTICA software (version 12.0, StatSoft, Inc., 2011, Caty, CN, USA).

3. Results and Discussion

3.1. Yield and Amino Acid Composition

Table 1 showed no significant difference ($p > 0.05$) in the yield between LPCM and LPCU. However, LPCU showed a slightly higher yield than LPCM, which can be attributed to ultrasound. The UAE facilitates the disruption and degradation of the leaf. Then, the extraction solvent penetrates the internal structure of vegetal material, enhancing the mass transfer and increasing yield [24]. These results differ from those reported by Moreno-

Nájera et al. [25], who reported a yield of 9.74% of jackfruit leaf protein extracted by ultrasound. The differences are attributed to the extraction solvent since these authors used a 1 M NaCl solution for the extraction.

Table 1. Amino acid composition and yield of leaf protein concentrates obtained by maceration and ultrasound and their hydrolysates.

Amino Acid (mg/100 g Protein)	Leaf Protein Concentrate (LPC)		Hydrolysate (H)		Suggested Intake (%) [1]
	Maceration (M)	Ultrasound (U)	Maceration (M)	Ultrasound (U)	
Yield (%)	6.76 ± 0.60 [a]	7.04 ± 0.29 [a]	38.80 ± 0.92 [b]	41.38 ± 4.14 [b]	NA
Alanine	2.06 ± 0.12 [a]	1.89 ± 0.45 [a]	3.78 ± 0.48 [b]	3.16 ± 0.07 [ab]	NA
Glycine	2.10 ± 0.11 [a]	1.67 ± 0.53 [a]	3.70 ± 0.39 [b]	3.79 ± 0.19 [b]	NA
Valine	2.15 ± 0.14 [a]	2.26 ± 0.46 [a]	4.72 ± 0.0 [b]	3.67 ± 0.02 [c]	3.9
Leucine	2.81 ± 0.10 [a]	2.89 ± 0.61 [a]	6.76 ± 0.23 [c]	4.48 ± 0.04 [b]	5.9
Isoleucine	1.60 ± 0.08 [a]	1.80 ± 0.33 [a]	3.52 ± 0.13 [c]	2.71 ± 0.07 [b]	3.0
Proline	1.54 ± 0.06 [a]	2.23 ± 0.36 [ab]	4.40 ± 0.67 [c]	3.96 ± 0.59 [bc]	NA
Methionine	0.37 ± 0.02 [a]	0.55 ± 0.08 [ab]	0.71 ± 0.06 [b]	0.61 ± 0.08 [ab]	2.2
Serine	1.15 ± 0.03 [a]	1.46 ± 0.28 [ab]	1.54 ± 0.48 [ab]	2.35 ± 0.10 [c]	NA
Threonine	1.12 ± 0.04 [a]	1.44 ± 0.26 [a]	1.88 ± 0.05 [a]	2.16 ± 0.23 [b]	2.3
Phenylalanine	1.56 ± 0.08 [a]	2.01 ± 0.41 [ab]	3.20 ± 0.32 [c]	2.83 ± 0.22 [bc]	3.8
Aspartic acid	2.99 ± 0.08 [a]	3.11 ± 0.84 [a]	3.18 ± 0.58 [a]	5.68 ± 0.40 [b]	NA
Glutamic acid	3.19 ± 0.26 [a]	4.45 ± 1.01 [a]	3.19 ± 0.56 [a]	6.69 ± 0.77 [b]	NA
Lysine	2.02 ± 0.31 [ab]	9.03 ± 0.30 [b]	nd	7.02 ± 1.12 [c]	4.5
HAA	12.09 ± 0.61 [a]	13.63 ± 1.69 [a]	27.10 ± 1.11 [b]	21.42 ± 0.22 [c]	NA
AAA	1.56 ± 0.08 [a]	2.01 ± 0.41 [ab]	3.20 ± 0.32 [c]	2.83 ± 0.22 [bc]	NA
EAA	10.51 ± 0.73 [a]	18.54 ± 1.18 [ab]	18.91 ± 0.96 [ab]	21.32 ± 2.56 [b]	NA
NCAA	6.18 ± 0.34 [a]	7.56 ± 0.86 [a]	6.36 ± 0.14 [a]	12.37 ± 1.17 [b]	NA
TAA	24.65 ± 1.44 [a]	34.78 ± 2.93 [ab]	40.58 ± 2.16 [b]	49.11 ± 2.55 [c]	NA

Hydrophobic amino acids: HAA; aromatic amino acids: AAA; essential amino acids: EAA; negatively charged amino acids: NCAA; total amino acid: TAA; nd: not detected. [a–c] Different small letters in the same row indicate significant differences among the treatment ($p < 0.05$). Suggested profile of EAA requirements for an adult human by FAO/WHO [26] [1].

Likewise, the extraction of the LPC by UAE and NaOH solution could lead to protein structural damage, which produces aggregates that do not solubilize with the extraction solvent. These protein aggregates possibly remain in the centrifugation residue, leading to low yield [27]. Then, the results could suggest that less extraction time is required to improve yield. Therefore, in subsequent studies, it is recommended to evaluate shorter extraction times. On the other hand, the hydrolysates showed a higher yield than the concentrates, which suggests high cleavage of peptide bonds by the enzyme [11]. However, no significant differences ($p > 0.05$) were shown between HM and HU. The above indicates that the extraction method did not influence the yield.

Regarding the amino acid profile (Table 1), although the LPCU presents a higher content of the total amino acid (TAA), essential amino acids (EAA), and hydrophobic amino acids (HAA) than LPCM, no significant differences were observed between both treatments ($p > 0.05$). These concentrates showed high contents of lysine, glutamic acid, aspartic acid, leucine, valine, and proline. Concerning the hydrolysates, HM and HU showed significantly ($p < 0.05$) higher content of TAA, EAA, and HAA than LPCs, which indicates that the release of amino acids during the enzymatic process with pancreatin was successful. HU showed higher content of lysine, glutamic acid, aspartic acid, glycine, serine, and threonine, whereas HM showed high content of leucine, valine, proline, alanine, isoleucine, and phenylalanine. Notwithstanding, HM showed EAA such as valine, leucine, isoleucine, and methionine, which are found in the requirements suggested by FAO/WHO [26]. For its part, HU presented methionine and lysine at levels required too. Lysine is a vital amino acid from a nutritional point of view since its deficiency in children is responsible for retarded growth. Likewise, it is important to note that the content of valine, leucine, isoleucine, and lysine of HU was higher than those reported for *Spirulina platensis* protein hydrolysates obtained by enzymatic hydrolysis with pancreatin [28].

Hence, HU could be used as an alternative source for plant-based foods that are low in lysine [29]. This suggested that UAE changed the molecular structure of LPC. This trend has been reported by Sun et al. [30] for peanut protein isolate extracted by ultrasound. The results showed that the extraction method affected the amino acid profile of the samples. Therefore, this confirms that UAE could maintain the quality of the protein concentrate and hydrolysates. Thus, UAE could be considered an alternative technique to obtain LPC with better quality, which would affect the functionality of the protein hydrolysates.

3.2. Techno-Functional Properties

3.2.1. Solubility

The functional properties of food proteins are fundamental in food processing, which can influence food texture and organoleptic characteristics [31]. In general, the solubility of the samples was dependent on the pH (Table 2). The LPCM and LPCU did not present solubility at acidic pH (2.0–4.0), which is attributed to proximity to the isoelectric point (pI) of the samples [28]. Subsequently, the increase in pH to 6.0–8.0 improved the solubility in both treatments. The LPCU exhibited slightly higher solubility than LPCM; however, this was not significant ($p > 0.05$).

Table 2. Solubility of protein concentrates and hydrolysates at different pH.

pH	Leaf Protein Concentrate		Hydrolysate	
	Maceration	Ultrasound	Maceration	Ultrasound
2	0 ± 0 [a]	0 ± 0 [a]	1.00 ± 0.02 [b,A]	3.83 ± 0.23 [c,B]
4	0 ± 0 [a]	0 ± 0 [a]	60.14 ± 0.10 [d,A]	72.12 ± 3.05 [ab,B]
6	0.97 ± 0.18 [ab,A]	2.59 ± 0.1 [a,A]	67.8 ± 0.98 [a,B]	77.39 ± 0.43 [b,C]
8	2.95 ± 0.18 [b,A]	2.89 ± 0.91 [a,A]	65.27 ± 1.35 [a,B]	66.26 ± 1.70 [a,B]
10	0 ± 0 [a]	0 ± 0 [a]	52.74 ± 0.10 [c,A]	66.16 ± 0.14 [a,B]

[A–C] Different capital letters in the same row indicate significant differences among the treatment ($p < 0.05$). [a–d] Different small letters in the same column indicate significant differences in the concentration of the sample ($p < 0.05$). nd, not detected.

On the other hand, the protein hydrolysates showed a significant ($p < 0.05$) increase in solubility from pH 2 (concerning LPCs), reaching the maximum solubility at pH 6.0 for both hydrolysates. The increase in solubility at acid pH could be due to a shift in the isoelectric point (pI) [32]. The shift in the pI after hydrolysis has also been attributed to differences in the types and numbers of charged groups on the proteins after hydrolysis. This trend was reported by Xu et al. [33] in protein hydrolysates of rice glutelin.

The HU showed significantly ($p < 0.05$) higher solubility than HM. This indicates that the ultrasonic cavitation derived from the collapse of gas bubbles, high-intensity shock waves, shear forces, and turbulence caused structural changes and denaturation of the protein substrates, decreasing the particle size of substrates and consequently exposing more enzymatic cleavage sites [34].

Therefore, in enzymatic hydrolysis of LPCU, there were more digested proteins than short-chain peptides. This meant that the molecular weight of polypeptide chains decreased, and the hydrophilic property was enhanced by increasing the number of polar functional groups ($-NH_2^+$ and $-COO^-$). These groups played a key role in developing the overall hydration of proteins [34,35]. The negatively charged amino acids (NCAA), such as glutamic and aspartic acid in HU ($12.37 \pm 1.17\%$), were higher than HM ($6.36 \pm 3.14\%$). These NCAA, in an alkaline environment, provide a strong net negative charge leading to more interaction with an aqueous environment; hence the solubility is increased [36,37]. This trend was similar to that reported by Chen et al. [15], who observed that UAE improved the enzymatic accessibility of soy protein isolate, allowing the protein to be easily hydrolyzed and rendered soluble.

3.2.2. Foaming Properties

The property of proteins to form stable foams is important in producing various foods [38]. The FC and FS of LPCs and hydrolysates were dependent on the extraction method (Table 3). The LPCs did not exhibit desirable foaming properties. This behavior is due to the low solubility that the LPCs presented (Table 2) since the stable foams are formed with soluble proteins, which can interact and form thick viscous films [38]. The aggregation of LPC interfered with interactions between the protein and water, which is needed to form foam [39].

Table 3. Functional properties of leaf protein concentrate obtained by maceration and ultrasound and their hydrolysates.

Functionals Properties	Leaf Protein Concentrate		Hydrolysate	
	Maceration	Ultrasound	Maceration	Ultrasound
Foaming capacity (FC, %)	0 ± 0 [a]	0 ± 0 [a]	15.56 ± 2.55 [b]	35 ± 1.67 [c]
Foaming stability (FS, %)	0 ± 0 [a]	0 ± 0 [a]	0 ± 0 [a]	21.11 ± 4.19 [b]
Emulsifying activity index (EAI, m^2/g)	9.59 ± 1.10 [a]	33.99 ± 0.91 [b]	67.57 ± 1.31 [c]	78.28 ± 0.03 [d]
Emulsion stability index (ESI, min)	32.74 ± 3.7 [a]	46.19 ± 4.81 [b]	200.34 ± 11.44 [c]	480.89 ± 10.77 [d]

[a–d] Different small letters in the same row indicate significative differences ($p < 0.05$) between treatments.

Conversely, the hydrolysates significantly ($p < 0.05$) increased the FC concerning the LPCs. Nevertheless, the FS only improved in the HU. This behavior is because several molecular properties influence the FC and FS. The FC is affected by the adsorption rate, flexibility, and hydrophobicity. In contrast, the FS depends on the rheological properties of films, such as hydration, thickness, protein concentration, and favorable intermolecular interactions [11,40]. As mentioned above, HU showed high content of NCAA, which are responsible for the hydration properties, an essential requirement for foam stability. Hence, these findings indicate that although the extraction method did not improve foaming properties in LPCs, the UAE contributed to the partially unfolded structures of proteins. This behavior improved the enzymatic process, releasing protein hydrolysates with better molecular flexibility and solubility [39], which increased the FC and FS, as reported in previous studies [39,41,42].

3.2.3. Emulsifying Properties

The EAI and ESI were higher ($p < 0.05$) in the LPCU than LPCM. On the other hand, the hydrolysates showed significantly ($p < 0.05$) better emulsifying properties than LPCs (Table 3). HU showed better ($p < 0.05$) EAI and ESI than HM, this indicates that the extraction method influences functionality. This observation is mainly attributed to the cavitation and mechanical effects of UAE on LPC. These mechanisms play an essential role in changing the molecular structure of the substrate, reducing the substrate particle size, and making it more sensitive to enzymolysis [43–45], which allows the release of peptides with different characteristics from those obtained by maceration.

According to Table 1, LPCU hydrolysis allowed the release of protein hydrolysates with lower HAA content and higher NCAA levels than LPCM. This behavior could indicate that the HU presents a better hydrophobic-hydrophilic balance, given that the high content of HAA in HM could limit protein-lipid interactions [45]. Consequently, protein molecules could not be more effectively adsorbed at the interface O/W, decreasing the emulsifying properties [44]. Similar trends were observed by Chen et al. [15] for protein hydrolysates of soy protein.

However, the results of the emulsifying properties of HU are better than those obtained for hydrolysates of jackfruit leaf protein (under the same hydrolysis conditions). However, the LPC was extracted by hydrostatic pressure [11]. These hydrolysates exhibited EAI

and ESI values of ~56.25 m^2/g and ~88.79 min, respectively. Furthermore, it must be emphasized that the concentrations used to evaluate the emulsifying properties of HU were lower than those used by the authors mentioned above. The preceding indicates that the choice of a suitable method for extraction is essential before enzyme hydrolysis. Because the functionality of the protein hydrolysates depends on their structure unfold generated during the UAE.

3.3. Antioxidant Properties

In general, the $ABTS^+$ radical-scavenging activity of LPCs and hydrolysates depends on the extraction method and sample concentration of the sample (Table 4). LPCs showed significantly lower antioxidant capacity than protein hydrolysates. However, HU showed significantly better $ABTS^+$ radical-scavenging activity than HM at 0.5–0.8 mg/mL concentrations, reaching the highest antioxidant capacity (99%) at a concentration of 1 mg/mL for both treatments.

Table 4. $ABTS^+$ radical-scavenging activity (%) at different concentrations and IC_{50} (mg/mL) of leaf protein concentrates obtained by maceration and ultrasound and their hydrolysates.

Concentration (mg/mL)	Leaf Protein Concentrate		Hydrolysate	
	Maceration	Ultrasound	Maceration	Ultrasound
0.3	11.52 ± 0.59 [a,A]	18.09 ± 2.44 [a,B]	13.88 ± 0.42 [a,AB]	12.21 ± 0.83 [a,A]
0.5	11.97 ± 0.14 [a,B]	23.38 ± 1.06 [a,A]	54.58 ± 1.02 [b,C]	59.41 ± 3.04 [b,C]
0.8	21.25 ± 2.61 [b,B]	30.91 ± 0.17 [b,A]	75.28 ± 3.62 [c,D]	85.59 ± 1.37 [c,C]
1.0	20.37 ± 0.69 [b,B]	38.15 ± 0.39 [c,A]	99.68 ± 0.23 [d,C]	99.63 ± 0.26 [d,C]
IC_{50}	nd	nd	0.32 ± 0.01 [B]	0.29 ± 0.01 [A]

[A–D] Different large letters in the same row indicate significant differences among the treatment ($p < 0.05$).
[a–d] Different small letters in the same column indicate significant differences in the concentration of the sample ($p < 0.05$). IC_{50}, concentration of sample (mg/mL) required to achieve 50% of antioxidant activity.

The antioxidant capacity of ABTS can be reported in IC_{50} values. Low IC_{50} values mean better activity. The LPCs did not reach 50% antioxidant capacity by ABTS; therefore, their IC_{50} could not be quantified. On the contrary, HU showed lower IC_{50} than HM, reflecting that the extraction method had a significant ($p < 0.05$) effect. The UAE breaks Van der Waals forces, hydrogen bonds, and other non-covalent bonds of LPC [46]. The above-mentioned changes the structural configuration of proteins, increasing the accessibility of the proteases, which influences the chain length of peptides and amino acids released in the enzymatic process [47].

The amino acid composition showed that HU had higher contents of the acidic amino acids (glutamic and aspartic) and lysine (Table 1). The acidic amino acids can donate electrons and act as metal chelating agents. Likewise, positively charged amino acids, such as lysine, can bind and neutralize negatively charged free radicals [48], which would explain the high antioxidant properties of HU. The same trend was observed by Fadimu et al. [49] in lupin protein hydrolysates. The results demonstrated the potential of the UAE as a suitable method for enhancing the release of novel bioactive peptides with better antioxidative properties.

4. Conclusions

The UAE of LPC was evaluated on the functionality of protein hydrolysates of jackfruit compared to conventionally extracted LPC. LPCs presented did not show desirable techno-functional and antioxidant properties. Enzymatic hydrolysis with pancreatin improved the techno-functional and antioxidant properties, but it was dependent on the extraction method. The results indicated that UAE of LPC improves the enzymatic hydrolysis process. This was decisive during the hydrolysis since protein hydrolysates were obtained with a different amino acid composition concerning the HM, which evidences changes in the structure of LPC by ultrasound. The above resulted in better solubility, foaming, and emulsifying properties and antioxidant capacity of HU. Ultrasound-assisted extraction

could be an excellent method for obtaining plant proteins, such as leaf protein, since it leads to desirable modifications that improve enzymatic hydrolysis. This has a significant impact on the functionality of the hydrolysates since multiple properties, such as emulsifying, foaming, and antioxidant, are improved. Obtaining these multifunctional ingredients is of great interest in the food industry. However, the influence of concentration, molecular weight, and functional group charges on the functionality of protein hydrolysates will continue to be investigated in future research.

References

1. Tahergorabi, R.; Hosseini, S.V. Proteins, Peptides, and Amino Acids. In *Nutraceutical and Functional Food Components: Effects of Innovative Processing Techniques*; Elsevier Science: Amsterdam, The Netherlands, 2017; ISBN 9780128052570.
2. Deprá, M.C.; Dias, R.R.; Sartori, R.B.; de Menezes, C.R.; Zepka, L.Q.; Jacob-Lopes, E. Nexus on Animal Proteins and the Climate Change: The Plant-Based Proteins Are Part of the Solution? *Food Bioprod. Process.* **2022**, *133*, 119–131. [CrossRef]
3. Detzel, A.; Krüger, M.; Busch, M.; Blanco-Gutiérrez, I.; Varela, C.; Manners, R.; Bez, J.; Zannini, E. Life Cycle Assessment of Animal-Based Foods and Plant-Based Protein-Rich Alternatives: An Environmental Perspective. *J. Sci. Food Agric.* **2021**, *102*, 5098–5110. [CrossRef] [PubMed]
4. Estell, M.; Hughes, J.; Grafenauer, S. Plant Protein and Plant-Based Meat Alternatives: Consumer and Nutrition Professional Attitudes and Perceptions. *Sustainability* **2021**, *13*, 1478. [CrossRef]
5. Yaashikaa, P.R.; Senthil Kumar, P.; Varjani, S. Valorization of Agro-Industrial Wastes for Biorefinery Process and Circular Bioeconomy: A Critical Review. *Bioresour. Technol.* **2022**, *343*, 126126. [CrossRef] [PubMed]
6. Yaashikaa, P.R.; Senthil Kumar, P.; Varjani, S.J.; Saravanan, A. Advances in Production and Application of Biochar from Lignocellulosic Feedstocks for Remediation of Environmental Pollutants. *Bioresour. Technol.* **2019**, *292*, 122030. [CrossRef] [PubMed]
7. Brion-Espinoza, I.A.; Iñiguez-Moreno, M.; Ragazzo-Sánchez, J.A.; Barros-Castillo, J.C.; Calderón-Chiu, C.; Calderón-Santoyo, M. Edible Pectin Film Added with Peptides from Jackfruit Leaves Obtained by High-Hydrostatic Pressure and Pepsin Hydrolysis. *Food Chem. X* **2021**, *12*, 100170. [CrossRef]
8. Fadimu, G.J.; Gill, H.; Farahnaky, A.; Truong, T. Improving the Enzymolysis Efficiency of Lupin Protein by Ultrasound Pretreatment: Effect on Antihypertensive, Antidiabetic and Antioxidant Activities of the Hydrolysates. *Food Chem.* **2022**, *383*, 132457. [CrossRef]
9. Karkouch, I.; Tabbene, O.; Gharbi, D.; Ben Mlouka, M.A.; Elkahoui, S.; Rihouey, C.; Coquet, L.; Cosette, P.; Jouenne, T.; Limam, F. Antioxidant, Antityrosinase and Antibiofilm Activities of Synthesized Peptides Derived from Vicia Faba Protein Hydrolysate: A Powerful Agents in Cosmetic Application. *Ind. Crops Prod.* **2017**, *109*, 310–319. [CrossRef]
10. Halim, N.R.A.; Azlan, A.; Yusof, H.M.; Sarbon, N.M. Antioxidant and Anticancer Activities of Enzymatic Eel (*Monopterus* Sp) Protein Hydrolysate as Influenced by Different Molecular Weight. *Biocatal. Agric. Biotechnol.* **2018**, *16*, 10–16. [CrossRef]
11. Calderón-Chiu, C.; Calderón-Santoyo, M.; Herman-Lara, E.; Ragazzo-Sánchez, J.A. Jackfruit (*Artocarpus heterophyllus* Lam) Leaf as a New Source to Obtain Protein Hydrolysates: Physicochemical Characterization, Techno-Functional Properties and Antioxidant Capacity. *Food Hydrocoll.* **2021**, *112*, 106319. [CrossRef]
12. Vázquez-González, Y.; Ragazzo-Sánchez, J.A.; Calderón-Santoyo, M. Characterization and Antifungal Activity of Jackfruit (*Artocarpus heterophyllus* Lam.) Leaf Extract Obtained Using Conventional and Emerging Technologies. *Food Chem.* **2020**, *330*, 127211. [CrossRef] [PubMed]

13. Gigliotti, J.C.; Jaczynski, J.; Tou, J.C. Determination of the Nutritional Value, Protein Quality and Safety of Krill Protein Concentrate Isolated Using an Isoelectric Solubilization/Precipitation Technique. *Food Chem.* **2008**, *111*, 209–214. [CrossRef]

14. Kumar, M.; Tomar, M.; Potkule, J.; Verma, R.; Punia, S.; Mahapatra, A.; Belwal, T.; Dahuja, A.; Joshi, S.; Berwal, M.K.; et al. Advances in the Plant Protein Extraction: Mechanism and Recommendations. *Food Hydrocoll.* **2021**, *115*, 106595. [CrossRef]

15. Chen, L.; Chen, J.; Ren, J.; Zhao, M. Effects of Ultrasound Pretreatment on the Enzymatic Hydrolysis of Soy Protein Isolates and on the Emulsifying Properties of Hydrolysates. *J. Agric. Food Chem.* **2011**, *59*, 2600–2609. [CrossRef]

16. Wu, Q.; Zhang, X.; Jia, J.; Kuang, C.; Yang, H. Effect of Ultrasonic Pretreatment on Whey Protein Hydrolysis by Alcalase: Thermodynamic Parameters, Physicochemical Properties and Bioactivities. *Process Biochem.* **2018**, *67*, 46–54. [CrossRef]

17. 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. Sonochem.* **2017**, *34*, 540–560. [CrossRef]

18. Das, M.; Devi, L.M.; Badwaik, L.S. Ultrasound-Assisted Extraction of Pumpkin Seeds Protein and Its Physicochemical and Functional Characterization. *Appl. Food Res.* **2022**, *2*, 100121. [CrossRef]

19. Naik, M.; Natarajan, V.; Modupalli, N.; Thangaraj, S.; Rawson, A. Pulsed Ultrasound Assisted Extraction of Protein from Defatted Bitter Melon Seeds (*Momardica charantia* L.) Meal: Kinetics and Quality Measurements. *LWT* **2022**, *115*, 112997. [CrossRef]

20. Ding, Y.; Ma, H.; Wang, K.; Azam, S.M.R.; Wang, Y.; Zhou, J.; Qu, W. Ultrasound Frequency Effect on Soybean Protein: Acoustic Field Simulation, Extraction Rate and Structure. *LWT* **2021**, *145*, 111320. [CrossRef]

21. Calderón-chiu, C.; Calderón-santoyo, M.; Damasceno-gomes, S.; Ragazzo-Sánchez, J.A. Use of Jackfruit Leaf (*Artocarpus heterophyllus* L.) Protein Hydrolysates as a Stabilizer of the Nanoemulsions Loaded with Extract-Rich in Pentacyclic Triterpenes Obtained from *Coccoloba uvifera* L. Leaf. *Food Chem. X* **2021**, *12*, 100138. [CrossRef]

22. Bradford, M. A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. *Anal. Biochem.* **1976**, *72*, 248–254. [CrossRef]

23. Pearce, K.N.; Kinsella, J.E. Emulsifying Properties of Proteins: Evaluation of a Turbidimetric Technique. *J. Agric. Food Chem.* **1978**, *26*, 716–723. [CrossRef]

24. Kingwascharapong, P.; Chaijan, M.; Karnjanapratum, S. Ultrasound-Assisted Extraction of Protein from Bombay Locusts and Its Impact on Functional and Antioxidative Properties. *Sci. Rep.* **2021**, *11*, 17320. [CrossRef] [PubMed]

25. Moreno-Nájera, L.C.; Ragazzo-Sánchez, J.A.; Gastón-Peña, C.R.; Calderón-Santoyo, M. Green Technologies for the Extraction of Proteins from Jackfruit Leaves (*Artocarpus heterophyllus* Lam). *Food Sci. Biotechnol.* **2020**, *29*, 1675–1684. [CrossRef] [PubMed]

26. WHO/FAO World Health Organization/Food and Agricultural Organization. *Protein and Amino Acid Requirement in Human Nutrition: Report of a Joint WHO/FAO/UNU Expert Consultation*; World Health Organization: Geneva, Switzerland, 2007.

27. Aguilar-Acosta, L.; Serna-Saldivar, S.; Rodríguez-Rodríguez, J.; Escalante-Aburto, A.; Chuck-Hernández, C. Effect of Ultrasound Application on Protein Yield and Fate of Alkaloids during Lupin Alkaline Extraction Process. *Biomolecules* **2020**, *10*, 292. [CrossRef]

28. Mohammadi, M.; Soltanzadeh, M.; Ebrahimi, A.R.; Hamishehkar, H. Spirulina Platensis Protein Hydrolysates: Techno-Functional, Nutritional and Antioxidant Properties. *Algal Res.* **2022**, *65*, 102739. [CrossRef]

29. Zou, Y.; Li, P.P.; Zhang, K.; Wang, L.; Zhang, M.H.; Sun, Z.L.; Sun, C.; Geng, Z.M.; Xu, W.M.; Wang, D.Y. Effects of Ultrasound-Assisted Alkaline Extraction on the Physiochemical and Functional Characteristics of Chicken Liver Protein Isolate. *Poult. Sci.* **2017**, *96*, 2975–2985. [CrossRef]

30. Sun, X.; Zhang, W.; Zhang, L.; Tian, S.; Chen, F. Effect of Ultrasound-assisted Extraction on the Structure and Emulsifying Properties of Peanut Protein Isolate. *J. Sci. Food Agric.* **2021**, *101*, 1150–1160. [CrossRef]

31. Zhao, Y.; Wen, C.; Feng, Y.; Zhang, J.; He, Y.; Duan, Y.; Zhang, H.; Ma, H. Effects of Ultrasound-Assisted Extraction on the Structural, Functional and Antioxidant Properties of *Dolichos lablab* L. Protein. *Process Biochem.* **2021**, *101*, 274–284. [CrossRef]

32. Jamdar, S.N.; Rajalakshmi, V.; Pednekar, M.D.; Juan, F.; Yardi, V.; Sharma, A. Influence of Degree of Hydrolysis on Functional Properties, Antioxidant Activity and ACE Inhibitory Activity of Peanut Protein Hydrolysate. *Food Chem.* **2010**, *121*, 178–184. [CrossRef]

33. Xu, X.; Liu, W.; Liu, C.; Luo, L.; Chen, J.; Luo, S.; McClements, D.J.; Wu, L. Effect of Limited Enzymatic Hydrolysis on Structure and Emulsifying Properties of Rice Glutelin. *Food Hydrocoll.* **2016**, *61*, 251–260. [CrossRef]

34. O'Sullivan, J.; 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]

35. Hu, Y.; Wu, Z.; Sun, Y.; Cao, J.; He, J.; Dang, Y.; Pan, D.; Zhou, C. Insight into Ultrasound-Assisted Phosphorylation on the Structural and Emulsifying Properties of Goose Liver Protein. *Food Chem.* **2022**, *373*, 131598. [CrossRef] [PubMed]

36. Wu, D.; Tu, M.; Wang, Z.; Wu, C.; Yu, C.; Battino, M.; El-Seedi, H.R.; Du, M. Biological and Conventional Food Processing Modifications on Food Proteins: Structure, Functionality, and Bioactivity. *Biotechnol. Adv.* **2020**, *40*, 107491. [CrossRef] [PubMed]

37. Xu, X.; Qiao, Y.; Shi, B.; Dia, V.P. Alcalase and Bromelain Hydrolysis Affected Physicochemical and Functional Properties and Biological Activities of Legume Proteins. *Food Struct.* **2021**, *27*, 100178. [CrossRef]

38. Zayas, J.F. Foaming Properties of Proteins. In *Functionality of Proteins in Food*; Springer: Berlin/Heidelberg, Germany, 1997; pp. 260–309.

39. Zou, Y.; Wang, L.; Cai, P.; Li, P.; Zhang, M.; Sun, Z.; Sun, C.; Xu, W.; Wang, D. Effect of Ultrasound Assisted Extraction on the Physicochemical and Functional Properties of Collagen from Soft-Shelled Turtle Calipash. *Int. J. Biol. Macromol.* **2017**, *105*, 1602–1610. [CrossRef]

40. Damodaran, S. Amino Acids, Peptides, and Protein. In *Fennema's Food Chemistry*; CRC Press: Boca Raton, FL, USA, 2017; pp. 235–356. ISBN 9781315372914.
41. Dong, Z.Y.; Li, M.Y.; Tian, G.; Zhang, T.H.; Ren, H.; Quek, S.Y. Effects of Ultrasonic Pretreatment on the Structure and Functionality of Chicken Bone Protein Prepared by Enzymatic Method. *Food Chem.* **2019**, *299*, 125103. [CrossRef]
42. Lv, S.; Taha, A.; Hu, H.; Lu, Q.; Pan, S. Effects of Ultrasonic-Assisted Extraction on the Physicochemical Properties of Different Walnut Proteins. *Molecules* **2019**, *24*, 4260. [CrossRef]
43. Li, S.; Yang, X.; Zhang, Y.; Ma, H.; Liang, Q.; Qu, W.; He, R.; Zhou, C.; Mahunu, G.K. Effects of Ultrasound and Ultrasound Assisted Alkaline Pretreatments on the Enzymolysis and Structural Characteristics of Rice Protein. *Ultrason. Sonochem.* **2016**, *31*, 20–28. [CrossRef]
44. Wang, Q.; Wang, Y.; Huang, M.; Hayat, K.; Kurtz, N.C.; Wu, X.; Ahmad, M.; Zheng, F. Ultrasound-Assisted Alkaline Proteinase Extraction Enhances the Yield of Pecan Protein and Modifies Its Functional Properties. *Ultrason. Sonochem.* **2021**, *80*, 105789. [CrossRef] [PubMed]
45. Akbari, N.; Mohammadzadeh Milani, J.; Biparva, P. Functional and Conformational Properties of Proteolytic Enzyme-modified Potato Protein Isolate. *J. Sci. Food Agric.* **2020**, *100*, 1320–1327. [CrossRef] [PubMed]
46. Wen, C.; Zhang, J.; Zhang, H.; Duan, Y.; Ma, H. Effects of Divergent Ultrasound Pretreatment on the Structure of Watermelon Seed Protein and the Antioxidant Activity of Its Hydrolysates. *Food Chem.* **2019**, *299*, 125165. [CrossRef] [PubMed]
47. Kadam, S.U.; Álvarez, C.; Tiwari, B.K.; O'Donnell, C.P. Extraction and Characterization of Protein from Irish Brown Seaweed Ascophyllum Nodosum. *Food Res. Int.* **2017**, *99*, 1021–1027. [CrossRef] [PubMed]
48. Aluko, R.E. *Amino Acids, Peptides, and Proteins as Antioxidants for Food Preservation*; Elsevier Ltd.: Amsterdam, The Netherlands, 2015; ISBN 9781782420972.
49. Fadimu, G.J.; Gill, H.; Farahnaky, A.; Truong, T. Investigating the Impact of Ultrasound Pretreatment on the Physicochemical, Structural, and Antioxidant Properties of Lupin Protein Hydrolysates. *Food Bioprocess Technol.* **2021**, *14*, 2004–2019. [CrossRef]