

Article

Comparison of Physicochemical, Functional and Nutritional Properties between Proteins of Soybean and a Novel Mixture of Soybean-Maize

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Featured Application: The vegetable proteins represent a source of low-cost nutrients, but, unfortunately, not all of them fulfill highly digestible essential amino acids required for proper nutrition. This is why, in this study, a protein extracted from a mixture of soybean meal and maize germ was developed. The combination of soybean proteins with maize germ proteins represents an attractive alternative for elaborating nutritionally complete food products that contribute to the right physical and mental development of consumers.

Abstract: Vegetable proteins are potential low-cost alternatives to solve the protein deficiency of the world population. A protein extracted from a mixture of soybean meal and maize germ was developed to offer more protein alternatives with high nutritional value. In this study, physicochemical, functional, and nutritional characteristics of isolates and hydrolysates of soybean and counterparts extracted from a soybean meal-maize germ were compared. The isolate and hydrolysate of the soybean-maize blend had a protein content of 93.9% and 73.6%, respectively. These protein mixtures contained 10% and 52% more solubility, 303.9%, and 22.7% more emulsifying capacity, 4.5% and 4.2% higher foam density and 36.3% and 1.2% more coagulation capacity compared to the soybean isolate and hydrolysate. Electrophoretic profiles of soybean-maize proteins showed four additional bands to the typical soybean pattern of 56, 55, 52 and 18 kDa, which could correspond to globulins and zeins from maize. The isolate extracted from the mixture of soybean meal and maize is a new alternative to provide the necessary amino acids for proper physical and mental development. Additionally, it has a high potential to be used as an ingredient by the food industry due to its excellent functionality and nutritional value.

Keywords: solubility; emulsifying activity; coagulation capacity; amino acid score

1. Introduction

In some countries such as Mexico, 41.90% and 7.40% of the 130 million people live in poverty and extreme poverty, respectively [1]. These individuals lack the resources to acquire an adequate or permanent supply of foods, and therefore they generally have a low caloric intake and develop nutrient deficiencies, especially in terms of micronutrients and essential amino acids.

Proteins provide the essential amino acids necessary for the construction and maintenance of tissues, organs, muscles, and antibodies, and therefore they are fundamental for the proper physical and mental development of children. Furthermore, the adequate intake of high-quality proteins allows for protection against infectious diseases and is, at the same time, an elementary unit for nutrition in adulthood [2]. Dietary proteins can be obtained from animal or vegetable sources.



Vegetable proteins represent a low-cost energy source, but, unfortunately, not all of them fulfill highly digestible essential amino acids required for proper growth [3]. Their versatility depends on solubility, coagulation, emulsifying and foaming capacities (functional properties that limit their application in the food industry) [4].

The type, size, structure and degree of hydrolysis of the protein fractions significantly influences their functionality [5]. Fractionation studies of soybean proteins show that glycinin (11S) is more soluble than other vegetable proteins. Glycinin provides more emulsifying activity and foam stability than β -conglycinin (7S), while the latter stimulates foaming and gelling activity [6–8].

Legumes and cereals are excellent sources of proteins containing 18.5% to 50.0% and 6.0% to 18.0% of protein (db), respectively [9]. Toews and Wang characterized the physicochemical and functional properties of proteins from peas, lentils, navy beans and chickpeas, and determined that they have the potential to be applied in food due to their excellent functionality [9]. Legume proteins are deficient in methionine, cysteine, and tryptophan, contrary to the cereals where lysine and threonine are usually the limiting amino acids [10]. Commonly, vegetable proteins' amino acid deficiencies are improved through the combination or blending of legumes with cereals. Suri et al. [11] optimized the nutrient content and protein quality of maize, sorghum, and millet combined with cowpeas, peanuts, or soybeans to supplement the diet in Ghana. Whereas Chiweshe et al. [12] mixed millet, rapoko and sorghum with soybeans and groundnuts to develop a high protein-energy cereal blend for the vulnerable population in Zimbabwe.

Soybean is the legume with the highest protein content, with around 50%, while maize germ contains 18.4% [13]. Maize is a staple in the American continent and the most produced cereal worldwide. The combination of soybean with maize proteins represents an attractive alternative for elaborating nutritionally complete food products.

This study's objective was to compare the physicochemical, functional, and nutritional characteristics of protein isolates and hydrolysates from soybean and soybean-maize mixes, exploring their use as a source of potential ingredients for the food industry.

2. Materials and Methods

2.1. Materials

Analyzed samples were identified as soybean isolate (SI), soybean-maize isolate (SMI), soybean hydrolysate (SH) and soybean-maize hydrolysate (SMH). SI and SH were commercial samples, whereas the proteins developed in this work were SMI and SMH. The commercial soybean isolate (Magic®) was obtained from DVA Mexicana S.A. of C.V. (Naucalpan, Mexico), whereas the soybean hydrolysate (SOYMAX WS[®]) was obtained from Interalimen S.A de C.V. (Mexico, D.F.). The experimental mixtures of soybean-maize proteins were extracted using a standard alkali extraction procedure followed by acid precipitation [14]. Briefly, the pH of a finely ground 30 kg mix of defatted soybean flour: maize germ (in a ratio 5:1 within ten parts of water) was adjusted to pH 10 with sodium hydroxide (NaOH) 50% w/w. Contents were mixed for 30 min at 50 °C before bagasse separation using an industrial centrifuge (Westfalia SA14) operated at 15 L/min and 5,500 g. The supernatant was then collected, and the pH adjusted to 4.5 with 3 N hydrochloric acid (HCl). The coagulated protein or curd was again separated using the Westfalia Centrifuge SA 14 operated at the previously described conditions. The resulting curd was washed with an equal volume of water and separated with centrifuge and pH adjusted to pH 7.0 (NaOH 50% w/w). The obtained material was dried using an industrial spray dryer designed in-house (195 and 80 °C for inlet and outlet air, respectively, with an aspersion pressure of $176 \text{ kg}_{f}/\text{cm}^{2}$). For hydrolyzed proteins, before spray drying, enzymatic hydrolysis was performed using Neutrase[®] (0.011% g per g protein) for 30 min at 40 °C (Figure 1). The samples were stored at room temperature in plastic bags and paper sacks as primary and secondary packaging materials.



Figure 1. Process diagram to analyze the isolates and hydrolysates of soybean and soybean-maize.

2.2. Determination of Physicochemical Parameters

For all samples, moisture, crude protein, reducing sugars (RS), and free alpha-amino nitrogen (FAN) were assessed [15,16]. Electrical conductivity (EC) and pH of the samples were measured using a potentiometer (Hanna-250, Padova, Italy).

2.3. Functional Properties

The water absorption (WAI) and water solubility (WS) indexes were determined on 1 g of sample in 15 mL of distilled water according to procedures by Cheftel et al. [17]. Nitrogen solubility index (NSI) was assayed using 0.5 g of sample dispersed in 50 mL of 0.1M sodium chloride (pH 7.0). Nitrogen was determined with the micro-Kjeldahl method in total and soluble fractions [15]. The fat absorption index (FAI) was performed based on Ahn et al. [18]. The turbidimetric method was used for emulsifying activity index (EAI) in all samples [19]. Emulsion stability (ES) was calculated according to Haque and Kito [20]. Regarding functional properties related to protein–air interaction, foaming properties were evaluated: foaming activity (FA), foam stability (FS), and foam density (FD) over 3% (w/w) protein dispersions in water [20]. Urease activity (UA) was also determined using the American Oil Chemists Society (AOCS) method and heat coagulation capacity (HCC) with the technique proposed by Regenstein and Regenstein (Figure 1) [21,22].

2.4. Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

SDS-PAGE of soybean and soybean-maize proteins was performed on a 5% stacking gel and 10% separating gel with a discontinuous buffer system, according to the method described by Laemmli [23]. Briefly, 2% protein dispersions were dissolved in loading buffer for SDS-PAGE in 1:1 ratio. Electrophoresis was run at 70 V in stacking gel and at 90 V in separating gel until the tracking dye reached the bottom of the gel. Molecular weight standards of 15 to 250 KDa were run with the samples (Bio-Rad Laboratories, Hercules, CA, USA). The gel was stained in 0.25% Coomassie brilliant blue R-250 and destained in a solution containing 10% acetic acid and 45% ethanol. The gels were scanned on an Image Scanner III (GE Healthcare, Amersham, UK). The banding patterns were analyzed by comparing with a reference using the software TotalLab TL120 (version v2006f; Nonlinear Dynamics Ltd., Newcastle upon Tyne, UK).

2.5. Amino Acid Composition

The total amino acid composition was analyzed in isolates of soybean-maize and soybean proteins using the Association of Official Analytical Collaboration (AOAC) method [15].

2.6. Statistical Analysis

Data by triplicate were statistically evaluated using a one-way analysis of variance (Minitab 16, State College, PA, USA). The significant differences between means were determined with Tukey's multiple comparison test at a 5% significance level. The non-parametric Kruskal–Wallis test was used to compare the results among isolates and hydrolysates of soybean and soybean-maize at a 5% significance level (SPSS version 17.0, Inc., Chicago, IL, USA).

3. Results

3.1. Physicochemical Characterization

The proximate compositions of extracted proteins are shown in Table 1. The pH for the samples was between 6.83 and 7.66. The pH affects the intermolecular forces and hydrodynamics of proteins and is commonly used to modify their structural conformation and functionality. Another parameter related to the charge of the protein is the electrical conductivity (EC). This characteristic is inherent in each protein, as it will depend on the nature and amount of charged species present. The SMI and SMH proteins had higher EC than the soybean isolate and hydrolysate, likely due to the electric charges that provide germ maize proteins.

	Vegetable Proteins				
Parameter	Soybean Isolate (SI)	Soybean-Maize Isolate (SMI)	Soybean Hydrolysate (SH)	Soybean-Maize Hydrolysate (SMH)	
pН	7.66 ± 0.00^{a}	6.90 ± 0.01 ^c	6.83 ± 0.01 ^d	6.95 ± 0.01 ^b	
EC (mS/cm)	2.48 ± 0.00 ^c	3.41 ± 0.09^{a}	2.24 ± 0.05 ^d	3.11 ± 0.05 ^b	
Moisture (%)	9.25 ± 0.03^{a}	8.87 ± 0.12 ^a	9.30 ± 0.34 ^a	4.91 ± 0.17 ^b	
P (% db)	91.82 ± 3.36 ^a	93.91 ± 0.11 ^a	78.38 ± 1.99 ^b	73.67 ± 1.43 ^b	
Fat (% db)	0.09 ± 0.00 ^b	0.34 ± 0.01 ^{ab}	0.51 ± 0.05 ab	0.79 ± 0.26^{a}	
Ash (% db)	4.68 ± 0.03 ^c	5.23 ± 0.03^{a}	4.16 ± 0.02 ^d	5.05 ± 0.03 ^b	
RS (mg/g)	95.19 ± 0.68 ^a	10.33 ± 0.28 ^d	18.30 ± 0.62 ^c	20.51 ± 0.71 ^b	
FAN (mg/g)	0.855 ± 0.04 ^c	0.935 ± 0.02 ^b	0.715 ± 0.03 ^d	2.131 ± 0.06 ^a	
UA	0.04 ± 0.01 ^b	2.32 ± 0.18 ^a	0.07 ± 0.01 $^{\rm b}$	0.10 ± 0.01 ^b	

Table 1. Physical and chemical characterization of isolates and hydrolysates of soybean-maize and soybean proteins ¹.

¹ Means are the average of at least three replicas \pm standard deviation. Means with different letter(s) (a, b, c, d) within rows are statistically different (p < 0.05). EC: electrical conductivity; P: protein; RS: reducing sugars; FAN: free alpha-amino nitrogen; UA: urease activity.

The isolated soybean and soybean-maize protein content was about 93%, while the hydrolysates were about 76%. This is consistent with data reported by Jambrak et al. [24] soybean isolates and concentrates.

The fat content for all samples was around 0.55%, except for the commercial soybean isolate, which only contained 0.09%. Ash content of samples from soybean- maize was slightly higher than that of soybean due to the maize germ's mineral content that averages 10.5% [6].

Reducing sugars of isolates of soybean-maize and soybean proteins was 10.33 and 95.19 mg/g, respectively, while the hydrolysates of the two samples contained around 19.4 mg/g. Free alpha-amino nitrogen (FAN) of all samples was around 0.835 mg/g, except for the soybean-maize hydrolysate, which had a FAN of 2.13 mg/g.

Heat treatment on proteins destroys anti-nutritional components such as amylase and trypsin inhibitors in legumes, thus improving the bioavailability of nutrients and the rate of protein digestibility.

The urease activity (UA) allows the determination of if the heat treatment used to obtain legume-based protein flours was adequate. The SI and SMI had a UA of 0.04 and 2.32, respectively. As for hydrolysates, the UA was around 0.085. The physicochemical characteristics of a vegetable protein are essential because they influence their functionality and, thus, performance in food systems.

3.2. Functional Analysis of Vegetable Proteins

Table 2 shows the functional characterization of isolates and hydrolysates of soybean proteins and the soybean-maize mixture. Solubility is the property of significant importance as it influences other functional parameters measured by the gravimetric method (water solubility: WS) and spectrophotometric or Kjeldahl methods (nitrogen solubility index: NSI).

	Vegetable Proteins				
Parameter	Soybean Isolate (SI)	Soybean-Maize Isolate (SMI)	Soybean Hydrolysate (SH)	Soybean-Maize Hydrolysate (SMH)	
WAI	7.97 ± 0.16 ^a	5.56 ± 0.18 ^b	8.52 ± 0.39^{a}	2.17 ± 0.07 ^c	
NSI (%)	13.82 ± 1.11 ^c	38.71 ± 1.90 ^b	15.07 ± 0.89 ^c	56.05 ± 0.89 ^a	
WS (%)	33.11 ± 1.13 ^c	43.05 ± 1.44 ^b	15.95 ± 0.32 ^d	67.31 ± 2.54 ^a	
FAI	2.66 ± 0.05^{a}	2.78 ± 0.09^{a}	2.86 ± 0.09 ^a	2.78 ± 0.07 ^a	
EAI (m^2/g)	15,587.50 ± 617.70 ^c	62,972.51 ± 3408.02 ^a	53,025.17 ± 3421.29 ^b	65,097.04 ± 3535.64 ^a	
ES 24 h (%)	43.39 ± 0.92 ^c	73.65 ± 1.88 ^b	78.45 ± 2.69 ^a	73.78 ± 0.77 ^b	
ES 48 h (%)	76.39 ± 4.31 ^a	77.14 ± 2.47 ^a	80.80 ± 0.00 ^a	73.33 ± 0.00 ^a	
FA (%)	442.00 ± 15.57 ^{ab}	334.78 ± 0.00 ^c	528.17 ± 65.54 ^a	388.33 ± 2.89 ^{bc}	
FS (%)	90.17 ± 2.42 ^a	83.40 ± 0.35 ^b	79.41 ± 0.00 ^c	85.47 ± 0.31 ^b	
FD (%)	18.46 ± 0.54 ^b	23.00 ± 0.00 ^a	15.80 ± 1.71 ^c	20.00 ± 0.00 ^b	
HCC (%)	40.96 ± 0.25 ^c	77.31 ± 0.58 ^b	81.81 ± 2.59^{a}	83.06 ± 0.79^{a}	

Table 2. Functional properties of isolates and hydrolysates of soybean-maize and soybean proteins ¹.

¹ Means are the average of at least three replicas \pm standard deviation. Means with different letter(s) (a, b, c, d) within rows are statistically different (p < 0.05). WAI: water absorption index; NSI: nitrogen solubility index; WS: water solubility; FAI: fat absorption index; EAI: emulsifying activity index; ES: emulsion stability; FA: foaming activity; FS: foam stability; FD: foam density; HCC: heat coagulation capacity.

The WS determines the amount of protein and non-protein solids soluble in water and is rarely used for functionality-specific studies but is widely used in the food industry for its accessibility. SMI was 10% more hydrosoluble than the commercial soybean counterpart. Interestingly, the SMH was 52% more soluble than soybean because it had a higher degree of hydrolysis or FAN value. The SH has less FAN than the isolate, despite being a hydrolyzed sample, resulting in a protein with lower solubility.

Another property-related to solubility is the nitrogen solubility index (NSI). This parameter determines the percentage of total nitrogen dispersible in a 0.1 M sodium chloride (NaCl) solution corresponding to globulins and small peptides. The solubility of SMI and SMH proteins was 25% and 41% higher than soybean, respectively (Table 2). However, the soybean isolate and hydrolysate solubility did not show significant differences (p > 0.05).

The water absorption index (WAI) is the amount of water absorbed per gram of sample. The structural configuration and environmental factors determine if the interaction of the protein with the water is retention by entrapment or absorption, respectively. As analysis conditions were standard for all samples, the WAI depends on the conformation of each protein. The soybean isolate and hydrolysate did not present significant differences in the WAI (p > 0.05). The soybean-maize samples had significantly lower (p < 0.05) water absorption indexes than the soybean counterparts.

The functional properties associated with the hydrophobicity of the protein are the fat absorption index (FAI), emulsifying activity index (EAI), and emulsion stability (ES). The FAI of a protein depends on intrinsic factors such as amino acid composition, protein conformation, and polarity or hydrophobicity of the surface. The FAI can be achieved by physical entrapment of the oil with the protein by non-covalent interactions (hydrophobic, electrostatic, hydrogen bonding). The isolate

and hydrolysate of soybean and soybean-maize proteins had the same FAI (p > 0.05); therefore, fat absorption was independent of protein content and degree of hydrolysis.

EAI refers to the ability of a protein to establish interactions at the interface water-protein-oil, while the ES determines the resistance of the protein to maintain the emulsion for a specific time [16]. Table 2 shows that SMI and SMH proteins had the highest emulsifying activity and were not significantly different (p > 0.05).

Concerning the ES, the SH was more stable at 24 h of storage followed by the soybean-maize hydrolysate, soybean-maize isolate, and finally, soybean isolates with 43.4% stability. However, at 48 h of storage, all soybean and soybean-maize samples showed a slight stability increase, and statistical analysis indicated no significant differences among proteins (p > 0.05).

The foaming activity (FA) of a protein, related to the EAI, is mainly due to the air–protein–water interactions. FA is related to the ability of a protein to form a two-phase system where the air molecules are separated by a continuous layer of liquid [2]. The FA of both the soybean isolate and hydrolysate were higher than the soybean-maize. Authors have related FA to solubility and the protein's ability to unfold and refold around the air-water interface [25].

Foam stability (FS) refers to the ability of a protein to reduce surface tension and form robust interfacial membranes via air–protein–water interactions [2]. The highest foam stability was observed in the SI > SMH > SMI > SH. Similarly, FA hydrophilic properties of the proteins did not affect the FS, since the proteins of higher solubility (soybean-maize) did not present the highest FS.

Heat coagulation capacity determines the potential of the proteins to form heterogeneous aggregates produced by thermal denaturation, where the protein-protein interactions predominate concerning the protein–solvent interactions [26]. The SMH had the highest coagulating capacity of 83.06% and showed no significant difference with the soybean hydrolysate (p > 0.05). Regarding the isolates, the SMI showed proper coagulation while SI was the lowest with 40%. The low coagulation of SI could be due to the high amount of reducing sugars and non-protein solids that interfered with the protein-protein interactions.

The Kruskal–Wallis test was used to compare the physicochemical characteristics and the functional properties among isolates and hydrolysates of soybean and soybean-maize. This non-parametric test, with a statistical significance of 0.05, determined that the proteins are significantly different (Figure 2).

3.3. Electrophoretic Profile

The isolate and hydrolysate of soybean and soybean-maize proteins were analyzed using SDS-PAGE. As shown in Figure 3, the SI presented the typical pattern of electrophoresis: lipoxygenase was observed at 109.88 kDa and 71.74, 68.80 and 53.33 kDa the α , α' and β units of β -conglycinin, respectively. The acidic subunits (A₃, y A_{1a}, A_{1b}, A₂ y A₄ indicated by the letter A) and basic (B) glycinin at 47.88 and 21.52 kDa appear in the gel, respectively. This pattern is consistent with the reports by Mo et al. [5] on soybean proteins. The SMI presented a profile similar to soybean but exhibited three additional bands to the typical pattern of 56, 55, and 52 kDa that could be proteins provided by the maize germ. The SH protein did not show the typical band related to lipoxygenase, possibly because it was hydrolyzed into lower molecular weight moieties. The SMH showed a hydrolyzed pattern of the subunits of β -conglycinin. Acidic and basic subunits of glycinin were partially hydrolyzed due to a high number of unidentified fragments in the bottom of the gel.

The amino acid profile determines the nutritional quality of a protein. Table 3 shows the amino acid composition of isolates of soybean and soybean-maize proteins and includes the aminogram of casein for comparison since it is considered the standard reference protein. Lysine content of soybean and soybean-maize proteins provided 112% and 104% of the requirement for two to five-year-old infants, respectively [27]. This content is comparatively slightly lower compared to casein.



Figure 2. Comparison of ranges among isolates and hydrolysates of soybean and soybean-maize by the Kruskal–Wallis test (p < 0.05).



Figure 3. Electrophoretic profile of isolates and hydrolysates of soybean-maize and soybean proteins (SDS-PAGE). Reference (REF); soybean isolate (SI); soybean-maize isolate (SMI); soybean hydrolysate (SH); soybean-maize hydrolysate (SMH). A typical electrophoretic pattern for soybean protein: L: lipoxygenase; α , α' and β subunits of β -conglycinin; **A**₃: acidic glycinin subunit; **A**: acidic glycinin subunit;

	Proteins			
Essential Amino Acids (%)	Soybean ^a	Soybean-Maize ^b	Casein ^c	
Histidine (His)	136.84	134.74	142.11	
Isoleucine (Ile)	164.29	166.79	175.00	
Leucine (Leu)	119.70	117.73	127.27	
Lysine (Lys)	112.07	103.97	122.41	
Threonine (Thr)	114.71	99.12	108.82	
Valine (Val)	140.00	138.00	171.43	
Tyrosine (Try)	118.18	138.18	127.27	
Sulfur-containing amino acid				
Methionine (Met) + Cysteine (Cys)	104.00	98.40	105.60	
Aromatic amino acid				
Phenylalanine (Phe) + Tyrosine (Tyr)	130.16	140.48	158.73	

Table 3. The amino acid score of soybean, soybean-maize and casein proteins ¹.

¹ The score was calculated according to suggested requirements by Food and Agriculture Organization (FAO)/ World Health Organization (WHO) (2–5-year-old). ^a Day, 2013; ^b data determined in the study; ^c Standard Tables of Amino Acid Composition of Food in Japan [28].

4. Discussion

The physicochemical and functional properties of vegetable proteins determine their application in the food industry. The pH of the samples was between 6.83 and 7.66. Lawal [29] mentions that the relationship between pH and solubility of a protein depends on the prevailing charge on the amino acids at various pH values and that the balance of charges (+,-) reduces electrostatic repulsion and thus the solubility. On the other hand, Mohamed et al. [30] reported that the hydrophobicity of the surface of a protein is a good indicator of the foaming and emulsifying capacity.

Electrical conductivity, also related to protein charge, was higher in SMI and SMH proteins than their soy analogs. Arzeni et al. [31] reported a conductivity of 2.70 mS/cm for commercial SI (500E), consistent with the results obtained herein related to SI and SH of 2.48 and 2.24 mS/cm, respectively. In the food industry, the EC of a protein is crucial because it determines its stability in different food systems, such as beverages [32]. Additionally, EC in emerging processes such as pulsed electric fields affects the performance of the protein during treatments.

The soybean and soybean-maize samples had similar protein contents: 93% and 76% for isolates and hydrolysates, respectively. However, their protein composition was different because the maize germ contained 18.4% protein, containing 30% albumin, 30% globulins, 25% glutelins, and 5% zein (db), respectively [6]. On the other hand, soybean contains approximately 50% protein, with 90% as globulins [13].

The fat content for all samples was around 0.55% except for the commercial soybean isolate, which only contained 0.09%. This result is in agreement with fat values reported by Wolf, [33], for soybean flour (0.55%), concentrate (0.30%), and isolate (<0.01%).

Ash content of samples from soybean- maize was slightly higher than that of soybean likely due to the mineral content of the maize germ that averages 10.5% [6]. However, the results correspond to previous results reported by Toews and Wang [9] for soybean protein concentrates (4.6%) and other legumes such as pea (5.7%), lentil (5.4%), chickpea (3.8%) and white beans (5.7%).

Reducing sugars of isolates of soybean-maize and soybean proteins was 10.33 and 95.19 mg/g, respectively, while the hydrolysates of the two samples contained around 19.4 mg/g. Sugar presence may influence protein stability, lysine availability, and color of foods after applying thermal treatments due to Maillard reactions [34]. Additionally, it has been reported that sugars may negatively influence some functional properties, such as water absorption [25].

Free alpha-amino nitrogen (FAN) of all samples was around 0.835 mg/g, except for the soybean-maize hydrolysate, which had a FAN of 2.13 mg/g. This parameter allows the determination of the hydrolysis degree of a protein by the content of free ε -amino groups and is closely related to

peptides' size or molecular weights. FAN directly influences the water solubility (WS) of a protein and, thus, the functional properties in general.

Regarding the UA, the ideal for a protein flour is 0.05 to 0.2. An activity higher than 0.2 indicates a poor heat treatment, and lower than 0.05 indicates an over-processing and possible damage to the protein quality [35]. The SI and SMI had a UA of 0.04 and 2.32, respectively, indicating that the SI was over-processed, while the heat treatment applied to SMI was insufficient to inactivate urease. As for hydrolysates, the UA was around 0.085; therefore, the processing was adequate. Vasconcelos et al. [36] reported for soybean proteins a UA of 0.109, and 0.252 for a commercial and a Brazilian variety named Bays.

The functional properties provide information on the physicochemical behavior of the protein in a food system. The SMI was 10% more hydrosoluble than the commercial soybean counterpart. Interestingly, the SMH was 52% more soluble than soybean because it had a higher degree of hydrolysis or FAN value. The SH has less FAN than the isolate, despite being a hydrolyzed sample, resulting in a protein with lower solubility. Another factor that could have influenced the observed higher solubility of soybean-maize proteins is protein composition as they contain higher amounts of albumins from the germ of maize. The protein fractionation studies show that legumes with higher albumin content have better solubility than counterparts with a high concentration in globulins [25].

The NSI of SMI and SMH proteins was 25% and 41% higher than soybean, respectively. The NSI determined for the analyzed proteins coincides with that reported for vegetable proteins. Wolf [33] reported that the NSI values of soybean flours concentrate and isolates ranged between 10% and 90%. Paredes-López et al. [37] reported an NSI of 21.2% for soybean isolate. NSI values of 23.1%, 46.3%, and 50.3%, respectively, were reported for other legumes such as peas, chickpeas, and lentils [38–40]. The higher observed solubility of the soybean-maize proteins can be employed to develop beverages, sauces, dairy analogs, and products in general because it will facilitate its incorporation and homogeneous distribution in food formulations.

On the other hand, soybean isolate and hydrolysate did not present significant differences in the WAI (p > 0.05). The soybean-maize samples had significantly lower (p < 0.05) water absorption indexes than the soybean counterparts. This result is attributable to the low availability of polar amino acid residues, the higher degree of hydrolysis, and the size of the peptides that reduced the entrapment of water. In the specific case of soybean-maize hydrolysate, this was more evident. However, the WAI of the soybean-maize proteins is consistent with Paredes-López et al.'s values, [37], for a soybean isolate (5.7 mL/g protein). Other legumes such as peas, lentils, chickpeas, and navy beans, WAI values of 3.7, 3.7, 2.9, and 3.8 g water (H₂O)/g protein were reported, respectively [9]. Proteins with low water absorption are ideal for developing products with high lipid interactions such as dressings.

Regarding the properties associated with hydrophobicity, the isolate and hydrolysate of soybean and soybean-maize proteins had the same FAI (p > 0.05); therefore, fat absorption was independent of protein content and degree of hydrolysis. Paredes-López et al. [37] previously reported an FAI of 1.9 mL/g for a soybean protein isolate, whereas values obtained herein were higher, likely due to the sample's nature. Toews and Wang [9] reported an FAI for peas, lentils, chickpeas, and navy beans of 1.9, 2.1, 2.0, and 1.6 g oil/g protein, respectively. Given the adequate capacity of soybean-maize proteins to trap fat, these could be used as ingredients for vinaigrettes, sauces, sausages, ice creams, and bakery products. Furthermore, these proteins can improve yields, texture, mouthfeel, and flavor retention of foods.

The SMI and SMH proteins had the highest emulsifying activity and were not significantly different (p > 0.05). The SI showed the lowest EAI, possibly due to its lower solubility and higher content of reducing sugars that interfered with establishing interactions at the water–protein–oil interface. This result is consistent with Zayas and Lin [41], who observed that the high carbohydrate content of maize germ protein favored the EAI. About the ES, the SH was more stable at 24 h of storage followed by the soybean-maize hydrolysate, soybean-maize isolate, and finally, soybean isolates with 43.4% of stability. However, at 48 h of storage, all soybean and soybean-maize samples showed a slight

stability increase, and statistical analysis indicated no significant differences among proteins (p > 0.05). According to different authors, it is due to the high hydration and unfolding of globular proteins that improved surface tension by making a more rigid water–protein–oil interface [42,43]. Given the EAI of soybean-maize proteins, these are exciting alternatives to replace active tension agents used in the food industry for beverages, sauces, dressings, meat analogs, and others.

The FA of both the soybean isolate and hydrolysate were higher than the soybean-maize. However, the less soluble soybean proteins had a higher foaming capacity, possibly due to the structure, flexibility, charge density, and electrostatic repulsions [13]. Susheelamma and Rao [44] reported that the foaming activity of black gram (Phaseolus mungo) proteins depended on the globulin nature.

The highest foam stability was observed in the SI > SMH > SMI > SH. Toews and Wang [9] observed that, similar to soybean-maize proteins, the proteins of lentils, peas, and chickpeas with good solubility did not have high foaming activity but showed adequate FS due to the flexibility of proteins and electrostatic repulsions. The foams of soybean-maize proteins, in addition to excellent stability, presented higher density than soybeans, i.e., involving a smaller amount of gas per amount of protein. This characteristic makes foams more compact, homogeneous, and very attractive for bakery products, ice creams, and high-quality confection items.

Regarding coagulation, the SMH had the highest HCC of 83.06% and showed no significant difference with the soybean hydrolysate (p > 0.05). Factors such as temperature, polarity, ionic strength, and pH influence coagulation; however, these parameters remained standard for all samples. Therefore, soybean-maize and soybean proteins' coagulation depended on the structure, size, and type of interaction (electrostatic and hydrophobic) [43]. Regarding the isolates, the SMI showed proper coagulation while SI was the lowest with 40%. The low coagulation of SI could be due to the high amount of reducing sugars and non-protein solids that interfered with protein-protein interactions. This result agrees with findings reported by Kaushal et al., [45], who observed that the protein coagulation capacity of taro, pigeon pea, and rice was reduced by the presence of carbohydrates, fiber, and other solids. The coagulation characteristics that possessed the soybean-maize proteins make them well suited to develop analogs of cheeses, yogurts, creams, beverages, dressings, puddings, jellies, jams, and others.

The Kruskal–Wallis test determined that the isolates and hydrolysates of soybean and soybean-maize have different physicochemical characteristics and functional properties.

Regarding the electrophoretic profile, the SI presented the typical pattern of electrophoresis: lipoxygenase was observed at 109.88 kDa and 71.74, 68.80 and 53.33 kDa the α , α' and β units of β -conglycinin, respectively. The acidic subunits (A₃, y A_{1a}, A_{1b}, A₂ y A₄ indicated by the letter A) and basic (B) glycinin at 47.88 and 21.52 kDa appear in the gel, respectively. This pattern is consistent with the reports by Mo et al. [5] to soybean proteins. This isolate showed the lowest solubility (NSI), and this could also be due to the factors mentioned above and the low amount or partial hydrolysis of glycinin basic (B), as shown in the gel. Khatib et al. [7] reported that the glycinin fraction had higher solubility than β -conglycinin and also that soybean proteins provided better foaming stability as determined for this sample.

The SMI presented a profile similar to soybean but exhibited three additional bands to the typical pattern of 56, 55, and 52 kDa, which could be proteins provided by the maize germ. According to Parris et al., [8], bands around 50 kDa correspond to maize globulins. Nakai [46] reported that solubility, surface hydrophobicity, and molecular flexibility influence the emulsifying capacity of globular proteins. Therefore, the maize germ globulins are probably responsible for the high emulsifying capacity and excellent emulsion stability presented by the soy-maize proteins. This result is consistent with reports by Zayas and Lin [41], who determined a high emulsifying capacity and emulsion stability for proteins extracted from maize germ. The band of glycinin was evident, and its presence, according to Khatib et al. [7], justifies the excellent solubility. The same authors reported that glycinin possesses better emulsifying activity (EAI) because it has more exposed hydrophobic residues than β -conglycinin.

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The SH protein did not show the typical band related to lipoxygenase, possibly because it was hydrolyzed into lower molecular weight moieties. Moreover, the β -conglycinin subunits were slightly hydrolyzed because the band intensity of the subfractions α , α' slightly decreased while the β almost disappeared. Regarding glycine, the acid subunits' intensity decreased while the basic subunits were not observed in the SH. This sample also showed low NSI possibly related to the absence of basic glycinin, and in contrast, the isolate showed less FS possibly related to the hydrolysis of the fraction β of β -conglycinin.

The SMH showed a hydrolyzed pattern of the subunits of β -conglycinin. Acidic and basic subunits of glycinin were partially hydrolyzed due to a higher number of unidentified fragments in the gel's bottom. This hydrolysate had the highest solubility, and EAI possibly related to the presence of glycinin and its hydrolyzed products. Interestingly, the SMH was the sample with the highest coagulating ability, possibly due to the breakdown of glycinin and β -conglycinin [7].

A model protein profile, such as soy protein, provides enough information to estimate its functionality, especially in β -conglycinin and glycinin subunits. However, the functionality relies not only on their nature but also on the pH, temperature, ionic strength, and dielectric constant.

Regarding the amino acid content, lysine content of soybean and soybean-maize proteins provided 112% and 104% of the requirement for two to five-year-old infants, respectively. This content is comparatively slightly lower compared to casein. However, the experimental proteins complied with the Food and Agriculture Organization (FAO)/World Health Organization (WHO) [27] requirements for 2–5-year-old infants. Regarding the sulfur-containing amino acid (methionine –Met- and cysteine -Cys-), the soybean-maize protein also met the FAO requirement, whereas the commercial soybean and casein exceeded only 4% and 5.6%, respectively. As a result, it can be said that all amino acids, except for threonine, of soybean-maize protein mixtures comply with FAO requirements.

Therefore, the soybean and soybean-maize proteins had good nutritional quality and represented low-cost alternatives to provide amino acids necessary for good physical and mental development [47].

5. Conclusions

The soybean-maize proteins are potential alternatives for applying food systems and the development of high-protein food, as they showed better solubility and general functionality compared to the pure soybean counterparts. Additionally, they were nutritionally more complete proteins. In solubility, the isolate and the protein hydrolysate of the soybean-maize blend had higher solubility (10% and 52%) than the soybean counterparts. Emulsifying activity indexes were also higher in the soybean-maize isolate and hydrolysate (303.9% and 22.7%), whereas the emulsion stability at 48 h was similar compared to the soybean samples. Foaming properties of the soybean-maize proteins were lower than soybean, but the stability was excellent, and the density was slightly higher (5%). Heat coagulation capacity of the soybean-maize isolate and hydrolysate was higher (36.3% and 1.2%) than the soybean proteins. The electrophoretic profile of soybean and soybean-maize proteins showed typical bands of lipoxygenase, β -conglycinin, and glycinin. In the case of a soybean-maize mixture, the electrophoretic profile showed three additional bands compared to the typical soybean pattern of 56, 55, and 52 kDa, which correspond to the globulins of maize germ. These proteins completed the isolates' amino acid content and hydrolysates, reaching the requirements established by the FAO/WHO [27] for 2–5-year-old infants, yielding a protein with better nutritional quality. This protein blend's functionality and nutritional value could be adequate for the development of products for children under five years of age, as breakfast drinks, for example, opening the potential to meet nutritional requirements. Additionally, new blends of cereals and legumes such as soybeans and quinoa can be explored to provide better protein alternatives for the consumer.

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