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Physicochemical, Functional and Nutritional Properties of Vegetables Proteins and a Novel Mixture of Soybean-Maize as Ingredients for Application in Foods

A dissertation presented by

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Dedication

This research work is dedicated to my brother Jesus Soria Hernández for believing in my dreams, making them his, and supporting me to achieve them. Thank you for your dedication, love, and care of the whole family. I love you

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Physicochemical, Functional and Nutritional Properties of Vegetables Proteins and a Novel Mixture of Soybean-Maize as Ingredients for Application in Foods

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Abstract

Vegetable proteins are an excellent alternative to cope with the high demand for protein worldwide due to their low cost, availability and good nutritional value. In this study, the interactions between the physicochemical parameters and the functional properties of twenty vegetable proteins were determined to facilitate their incorporation into food processes. Likewise, the physicochemical parameters, functional and nutritional properties of isolated and hydrolyzed soybean proteins were compared with isolated and hydrolyzed proteins of a new mixture of soybean-maize to offer the consumer a new protein alternative of high nutritional value and useful functionality. Finally, the most suitable conditions for the hydrolysis of the soybean-maize protein were determined in terms of solubility to modify its functional properties and allow better integration in beverages.

Through this study, it was determined that the pH, electrical conductivity (EC), urease activity (UA) and free alpha-amino nitrogen (FAAN) influenced the functional properties related to the protein-water interactions corresponding to the water solubility index (WSI), nitrogen solubility index (NSI), foaming activity (FA), foam stability (FS), heat coagulation capacity (HCC) and emulsion stability (ES). Likewise, it was observed that the content of soluble solids, which includes reducing sugars (RS), determined the performance of the fat absorption index (FAI), emulsifying activity index (EAI) and foam density (FD) of the twenty vegetable proteins.

On the other hand, the comparison of the physicochemical, functional and nutritional properties of the isolated and hydrolyzed soybean proteins with those of soybean-maize allowed us to determine that the isolate and hydrolysate of the soybean and maize mixture had better functional properties than their analogs of soybeans since they had 10% and 52% more solubility, 47,385.01 (m^2/g) and 12,071.87 (m^2/g) more emulsifying capacity, 4.5% and 4.2% more foam density and 36, 3% and 1.2% more coagulation capacity,

respectively. Besides, the soybean-maize protein mixture had 2.5% and 20.0% more isoleucine and tyrosine, respectively. At the same time, the electrophoretic profile of the protein mixture showed four additional bands to the typical pattern of soybeans with a molecular weight of 56, 55, 52 and 18 kDa, which could correspond to globulins and *β*-zein from maize, respectively.

Regarding the study of enzymatic hydrolysis of soybean-maize protein, it was determined that the most suitable conditions for its hydrolysis were to use neutrase as the catalyst at a concentration of 0.45%, with a hydrolysis time of 30 minutes, at a pH 6.5 and a temperature of 45 °C. Under these hydrolysis conditions, it was determined that the hydrolyzed protein dispersion had free amino nitrogen of 5.03 mg/g, a solubility of 49.99% and a viscosity of 9.6 cP. The hydrolysis process increased the solubility of the soybean-maize protein by 34.33% compared to the unhydrolyzed sample.

Therefore, soybean-maize protein has higher functionality than soybean protein, increasing the possibilities of being used in the food industry and taking advantage of its nutritional value and unit cost advantages.

Keywords: **Vegetable proteins – solubility – coagulation – emulsifying capacity – foaming capacity – hydrolysis.**

Propiedades Fisicoquímicas, Funcionales y Nutricionales de Proteínas Vegetales y de una Nueva Mezcla de Soya-Maíz como Ingredientes para su Aplicación en los Alimentos

por

Cintya Geovanna Soria Hernández

Resumen

Las proteínas vegetales son una excelente alternativa para hacer frente a la elevada demanda de proteína a nivel mundial debido a su bajo costo, disponibilidad y buen valor nutricional. En este estudió se determinaron las interacciones existentes entre los parámetros fisicoquímicos y las propiedades funcionales de veinte proteínas vegetales para facilitar su incorporación en los procesos alimenticios. Asimismo, se compararon los parámetros fisicoquímicos, las propiedades funcionales y nutricionales de proteínas aislada e hidrolizada de soya con proteínas aislada e hidrolizada de una nueva mezcla de soya-maíz, con la finalidad de ofrecer al consumidor una nueva alternativa proteica de alto valor nutricional y buena funcionalidad. Finalmente, se determinaron las condiciones más adecuadas para la hidrólisis de la proteína de soya-maíz en términos de solubilidad para modificar sus propiedades funcionales y permitir una mejor integración en bebidas.

Mediante este estudió se determinó que el pH, conductividad eléctrica (EC), actividad ureásica (UA) y free alpha-amino nitrogen (FAAN) influyeron sobre las propiedades funcionales relacionadas con las interacciones proteína-agua correspondientes a water solubility index (WSI), nitrogen solubility index (NSI), foaming activity (FA), foam stability (FS), heat coagulation capacity (HCC) y emulsion stability (ES). Asimismo, se observó que el contenido de sólidos solubles, que incluye a los azúcares reductores (RS), determinó el rendimiento del fat absorption index (FAI), emulsifying activity index (EAI) y foam density (FD) de las veinte proteínas vegetales.

Por otro lado, la comparación de las propiedades fisicoquímicas, funcionales y nutricionales de las proteínas aislada e hidrolizada de soya con las de soya-maíz, permitió determinar que el aislado e hidrolizado de la mezcla de soja y maíz tuvieron mejores propiedades funcionales que sus análogas de soya, ya que tuvieron 10% y 52% más de solubilidad, 47,385.01 (m^2/g) y 12,071.87 (m^2/g) más de capacidad emulsionante,

4,5% y 4,2% más de densidad de espuma y 36,3% y 1,2% más de capacidad de coagulación, respectivamente. Además, la mezcla de proteínas de soya-maíz tuvo 2.5% y 20.0% más de isoleucina y tirosina, respectivamente. Mientras que el perfil electroforético de la mezcla de proteínas mostró cuatro bandas adicionales al patrón típico de la soya con un peso molecular de 56, 55, 52 y 18 kDa, las cuales podrían corresponder a las globulinas y *β*-zeína del maíz, respectivamente.

En cuanto al estudio de hidrólisis enzimática de la proteína de soya-maíz se determinó que las condiciones más adecuadas para su hidrólisis fueron usar como catalizador a la neutrasa a una concentración de 0.45%, con un tiempo de hidrólisis de 30 minutos, a un pH de 6.5 y una temperatura de 45 °C. Bajo estas condiciones de hidrólisis se determinó que la dispersión de proteína hidrolizada tuvo un amino nitrógeno libre de 5.03 mg/g, una solubilidad de 49.99% y una viscosidad de 9.6 cP. El proceso de hidrólisis incrementó la solubilidad de la proteína de soya-maíz en un 34.33% respecto a la muestra sin hidrolizar. Por lo tanto, la proteína de soya-maíz tiene mayor funcionalidad que la proteína de soya, lo cual incrementa las posibilidades de ser usada en la industria de alimentos y de aprovechar su valor nutrimental y ventajas en costo unitario.

Palabras clave: **Proteínas vegetales – solubilidad – coagulación – capacidad emulsificante – capacidad espumante – hidrólisis.**

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Chapter 1

1. Introduction

Proteins of plant origin vary widely in protein quality determined by the essential amino acid balance and digestibility. However, they represent a low-cost source of energy. Because of this, interest in their isolation and subsequent incorporation as ingredients in food systems has increased. Their physicochemical characteristics and functional properties determine the stability and interaction of proteins with other components during food formulation and processing. Solubility, coagulation, emulsifying, and foaming capacities are some of the functional properties of proteins that impact food processing. These properties are affected by intrinsic factors, such as the structure and molecular size of proteins and extrinsic factors, including extraction method, pH, ionic strength and other food components.

Compared to animal proteins, plant origin has reduced functionalities when used as food ingredients, limiting their industrial application. To improve their characteristics as raw materials, it is necessary to use other treatments such as physical and enzymatic.

Enzymatic hydrolysis is one of the most used methods to improve proteins' solubility; It consists of using a proteolytic enzymes that hydrolyze the polypeptide chains into smaller peptides, which in turn are more soluble than the original molecule. Therefore, the objective of this study was to determine the existing interaction between the physicochemical parameters and the functional properties of twenty vegetable proteins to better understand their use as food ingredients. Likewise, to compare the physicochemical parameters, functional and nutritional properties of isolated and hydrolyzed soybean proteins with isolated and hydrolyzed proteins of a new mixture of soybean-maize to offer the consumer a new protein alternative of good nutritional value and functionality. Finally, to determine the most suitable conditions regarding the type of enzyme, enzyme concentration and hydrolysis time for the soybean-maize protein's

hydrolysis in terms of solubility, to modify its solubility and allow a better incorporation in the development of beverages.

1.1. Justification

Due to population growth, the World Health Organization (WHO) has predicted an increase of protein demand, between 32% and 43% by 2050. Therefore, it is necessary to find new sources of proteins with high nutritional value that allow meeting the demands without compromising the planet such as animal proteins.

Vegetable proteins are potential and viable alternatives to face new challenges in protein deficiency facing the world. However, there is little information on the interaction of the physicochemical parameters and the functional properties of vegetable proteins that allows them to be incorporated more efficiently into food systems.

On the other hand, in the food industry, vegetable proteins use is limited due to their comparatively lower functional characteristics. For this reason, the use of enzymatic treatments to improve functional properties will facilitate their incorporation (in higher percentages) into food and beverage formulations that demand higher solubility.

Therefore, it is necessary to generate basic knowledge of the effects and interactions of vegetable proteins' physical-chemical, functional, and nutritional properties.

1.2. Hypothesis

The detailed study of the influence of the physicochemical parameters on the functional properties of vegetable proteins will allow generating information about existing interactions, to understand their potential use as food ingredients. Likewise, evaluating the functionality and nutritional value of isolated and hydrolyzed soybean-maize proteins will allow determining its convenience of use instead of soybean protein. Finally, soybean-maize protein's hydrolysis process will improve its solubility, facilitating their incorporation into the development of beverages.

1.3. Objectives

1.3.1. General objective

To determine the existing interaction between the physicochemical parameters and the functional properties of twenty vegetable proteins to better understand their potential use as food ingredients. Likewise, to compare the physicochemical parameters, functional and nutritional properties of isolated and hydrolyzed soybean proteins with isolated and hydrolyzed proteins of a new mixture of soybean-maize to offer the consumer a new protein alternative of good nutritional value and functionality. Finally, to determine the most suitable conditions regarding the type of enzyme, enzyme concentration and hydrolysis time for the soybean-maize protein's hydrolysis in terms of solubility, to modify its solubility and allow a better incorporation in the development of beverages.

1.3.2. Specific objectives

- To characterize the physicochemical and functional properties of vegetable and cereal proteins. Furthermore, to explore the correlations between the physicochemical characteristics (pH, electrical conductivity, moisture, protein, reducing sugars, free amino nitrogen and urease activity) and the functional properties (water absorption index, nitrogen solubility index, water solubility index, fat absorption index, emulsifying activity index, emulsion stability, foaming activity, foam stability, foam density and heat coagulation capacity) of proteins, to better understand their potential use as food ingredients.
- To compare the physicochemical (pH, electrical conductivity, moisture, protein, reducing sugars, free amino nitrogen and urease activity), functional (water absorption index, nitrogen solubility index, water solubility index, fat absorption index, emulsifying activity index, emulsion stability, foaming activity, foam stability, foam density and heat coagulation capacity), and nutritional characteristics (amino acid score) of protein isolates and hydrolysates from soybean and soybean-maize mixes, to explore their use as a ingredients for foods.

- To select the most suitable conditions regarding the type of enzyme (neutrase and papain), enzyme concentration (0.064, 0.15, 0.30, 0.45, 0.50 and 1.0%) and reaction time (30, 60 and 90 minutes) to hydrolyze soybean-maize protein and improve protein solubility to facilitate its incorporation in the development of beverages.

1.4. Research Plan

The experimental strategy to develop this research was divided into three stages, presented herein as chapters. It is important to mention that chapters III and IV have already been published in indexed scientific journals, while chapter V is in the editing process to be sent to publication. Therefore, in this document, they will be shown as separate research projects composed of abstract, introduction, materials and methods, results, discussion and conclusions. The summary of each of these stages is briefly described below:

- In Chapter III, statistical correlation of physicochemical parameters (moisture, pH, electrical conductivity, protein content, reducing sugars, free alpha-amino nitrogen and urease activity) of different plant proteins with functional properties (water absorption index, nitrogen solubility index, water solubility index, fat absorption index, emulsifying activity Index, emulsion stability, foaming activity, foam stability, foam density and heat coagulation capacity) was performed.
  For this, the physicochemical parameters and functional properties of a set of 20 vegetable proteins (peas, soybeans and a soybean-maize mixture obtained at the Protein Research and Development Center -CIDPRO-) were characterized. These data were statistically analyzed using one-way analysis of variance, Tukey tests (α=0.05), Pearson's correlations and principal component analyses (PCA). This study intended to find interactions between specific functional properties that would allow to better understand their use as food ingredients.
- Chapter IV compares the physicochemical, functional, and nutritional properties between a commercial soybean protein isolate (native and hydrolyzed) and new

mixtures of soybean proteins with maize germ (-native and hydrolyzed- obtained at the Research Center and Protein Development -CIDPRO-) was made. For which, the physicochemical (moisture, pH, electrical conductivity, protein content, reducing sugars, free alpha-amino nitrogen (FAAN) and urease activity), functional (water absorption index, nitrogen solubility index, water solubility index, fat absorption index, emulsifying activity Index, emulsion stability, foaming activity, foam stability, foam density and heat coagulation capacity) and nutritional characteristics (amino acid composition) of the four proteins were evaluated.

These properties were statistically analyzed using a one-way analysis of variance. 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. This study intended to offer consumers an alternative protein source with good nutritional value and functionality.

- Once the functionality of the soybean-maize protein mixture was evaluated in Chapter IV, Chapter V optimized a hydrolyzed protein mixture in terms of water solubility. This particular functionality is relevant for the incorporation of ingredients into food systems and the production of beverages. The study of the hydrolysis of soybean-maize protein was carried out in two stages: in the first stage they were tested neutrase (45 °C and pH 6.5), papain (70 °C and pH 6.0) and the mixture of these (1:1, 60 °C and pH 6.5) at different concentrations (0.064, 0.50 and 1.0%) and 50 ppm of free cysteine (presence or absence) for 90 minutes.

  In the second stage, the hydrolysis was carried out using only neutrase and varying the concentration (0.064, 0.150, 0.30, 0.45 and 0.50%) and the hydrolysis time (30, 60 and 90 minutes). Likewise, a validation kinetics was performed with 0.45% neutrase at 45 °C, pH 6.5 for 10 hours of hydrolysis with samples every 30 minutes. In each stage the hydrolysis reaction was stopped by adjusting the pH to 5 with 2N hydrochloric acid (HCl). Finally, the samples at a temperature of 25 °C were analyzed for free alpha-amino nitrogen and viscosity.

  To select the most suitable conditions to hydrolyze the soybean-maize protein, a full factorial design was used. The first stage was composed of three factors type

of enzyme (neutrase, papain and the mixture), enzyme concentration (0.064, 0.50 and 1.0%) and the use of cysteine (presence or absence). In the second stage, a full factorial design was used only two factors concentration (0.064, 0.150, 0.30, 0.45 and 0.50%) and reaction time (30, 60 and 90 minutes). In the two stages, the significant differences between means were determined with Tukey's multiple comparison test at a 5% significance level (Minitab 16, USA). The objective of this study was to select the most suitable conditions regarding the type of enzyme, enzyme concentration and reaction time to hydrolyze soybean-maize protein in terms of solubility.

Chapter 2

2. Theoretical Framework

2.1. Vegetable proteins

Legumes and cereals are an excellent source of protein since they contain 18.5 to 50% and 6 to 18% on a dry basis, respectively (Pinciroli *et al*., 2009; De Mesa-Stonestreet *et al*., 2010; Toews y Wang, 2013). According to their solubility, vegetable proteins are classified into albumins: soluble in water, globulins: soluble in saline solutions, glutelins: soluble in alkalis and prolamines: soluble in alcoholic solutions and reducing agents (Cao *et al*., 2009). Vegetable proteins have a low cost, are widely distributed in nature, and are considered good nutritional quality despite being deficient in some amino acids (Lqari, 2002; Rodríguez-Ambriz *et al*., 2005).

Legumes contain proteins deficient in methionine and cysteine, whereas cereals are primarily limited by lysine (Kakade, 1974; De Lumen, 1986). In terms of cereals, this deficiency is aggravated by a second deficiency caused by threonine or tryptophan (Kakade, 1974). Commonly, amino acid deficiencies in plant proteins are resolved by combining legumes with cereals. Nutritionists know this combination as “Complementary effects of proteins”.

A protein is considered nutritionally acceptable when its amino acid profile meets a 2- to 5-year-old child's nutritional needs, such as casein (Wang *et al*., 1999). In this study, a mixture of soybean proteins and maize germ was analyzed. Soybean is the legume with the highest consumption due to its nutritional contribution; its protein has high lysine levels, although it lacks methionine and cysteine (Cao *et al*., 2009). For its part, maize germ is deficient in essential amino acids such as lysine and tryptophan but is very high in sulfur-containing amino acids (Wang *et al.,* 2008; Hasjim *et al.,* 2009). The combination of soybeans with maize germ represents an attractive option to produce nutritionally complete food products.

Recently, vegetable proteins have become the focus of researchers' attention since they represent an alternative to malnutrition problems that afflict a large part of the population (Table 2-1). These problems are mainly due to the low availability of animal origin proteins given their high costs. However, the problem lies in finding alternative protein sources and ensuring that they have functional properties similar to those of animal origin for food application.

Table 2-1. Nutritional composition of some legumes, cereals and other grains.

Vegetable	Moisture (%)	Protein* (%)	Fat* (%)	Ash* (%)	Reference
Castile beans	10.39	27.88	1.27	13.84	Butt and Batool, 2010
Soybean	6.70	50.00	20.00	2.16	Wolf, 1970; Căpriţă *et al*., 2010
Maize	------	12.73	4.61	1.63	Ayala-Rodríguez *et al*., 2009
Peanut	4.62	28.00	55.00	2.50	Mestrallet *et al*., 2008
Canola	7.00	26.00	7.70	3.20	Manamperi *et al*., 2011; Day, 2013
Pea	9.05	22.95	1.41	3.48	Butt and Batool, 2010
Chickpea	------	23.7	0.96	2.72	Kaur *et al*., 2007
Quinoa	------	16.50	6.30	3.80	Vega-Gálvez *et al*., 2010
Lentil	9.39	24.59	0.97	2.88	Hernández-Nava *et al*., 2011
Bean	------	30.57	3.22	3.61	Mortuza *et al*., 2009

* Wet base: wb.

2.2. Functional properties

Functional properties provide information on proteins' physicochemical behavior in food systems (Schmitt *et al.,* 2005; Wu *et al*., 2009). These are affected by factors such as size, shape, chemical composition, amino acid sequence, net charge, hydrophobicity, secondary structure, and molecular rigidity in response to the external environment (pH, temperature, ionic strength, dielectric constant, among others parameters) or the interaction with other food components (Butt and Batool, 2010).

Solubility, coagulation, emulsifying, and foaming capacities are the main functional properties determining vegetable proteins versatility. They can be classified into three groups according to the type of interaction: protein-water interaction (solubility, water absorption, viscosity, and others); protein-protein (coagulation, precipitation, and others), and protein-interface (emulsifying and foaming capacity and others) (Schmitt *et al*., 2005; Castel, 2010).

The functionality of a vegetable protein is limited compared to that of an animal protein (Yin *et al.,* 2011). This is pointed out by Tomotake *et al.* (2002), who compared the functional properties of a buckwheat product, soybean isolate, and casein. These authors observed that casein and soybean isolate presented a similar protein content (85 and 83.3%) and that the solubility of casein was 21.9 and 71.9% higher than soybean and the buckwheat product, respectively. According to Day (2013), the main reason vegetable origin proteins are underutilized is their lack of functionality compared to animal counterparts. Besides, vegetable proteins commonly have a lower nutritional value. In legumes, the high content of polar amino acids with opposite charges in their proteins causes the formation of oligomeric proteins that reduce solubility (Carbonaro *et al*., 1993).

2.2.1. Water solubility

Solubility is the most important functional property of a protein since it influences other characteristics such as coagulation, emulsifying and foaming capacities (Yalçin and Çelik, 2007). Factors such as structure, size, pH, ionic strength, charge, type of solvent, and temperature directly affect proteins' solubility (Day, 2013).

2.2.2. Emulsifying activity

In general, emulsions are thermodynamically unstable systems made up of at least two immiscible liquids. These systems can be kinetically stabilized using emulsifying and stabilizing agents that retard or inhibit destabilization mechanisms (Girón-Calle, 2003). Polysaccharides, surfactants, and proteins are emulsifying agents with a surface activity that forms a protective barrier on the drops preventing aggregation. Polysaccharides are frequently used to delay destabilization and control emulsions' texture (Calero *et al*., 2013).

In proteins, the physicochemical characteristics that determine their emulsifying capacity are hydrophobicity, charge density, pH, ionic strength, structure, size, and conformational freedom (steric hindrance) (Cameron *et al*., 1991). Surface hydrophobicity influences the protein's ability to absorb oil on one side of the water-oil interface, where greater integration leads to greater emulsifying capacity (Matemu *et al*., 2011). In contrast, the surface charge of the protein influences the solubility within the aqueous phase, where high solubility is desired to have higher diffusion rates at the interface.

Once the viscoelastic film is formed, the droplets can assume a negative or positive charge depending on whether the emulsion's pH is above or below the isoelectric point of the protein, respectively (Figure 2-1) (Lad and Murthy, 2012). On the other hand, high electrostatic repulsion between oil droplets tends to lead to greater emulsion stability. At the same time, at low pH close to the isoelectric point, flocculation/aggregation dominates, leading to coalescence and instability (Sikorski, 2002). For its part, steric hindrance physically restricts droplet attachment since depending on the size, structure and

conformational freedom of the protein, the "loops" or "tails" of protein segments can irradiate from the interface generating steric stabilization (Day, 2013).

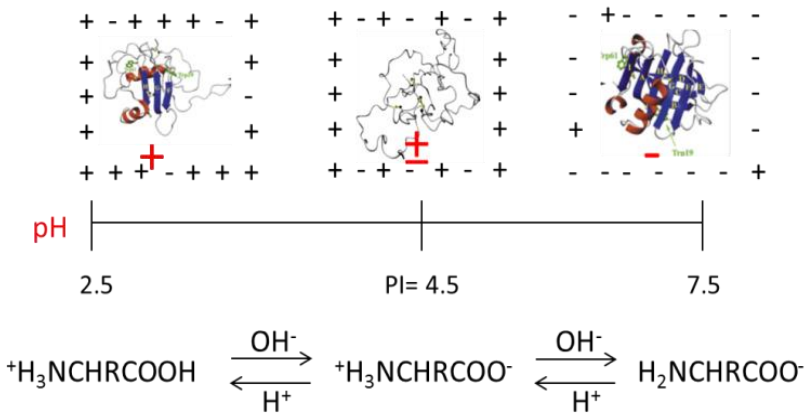

Figure 2-1. Effect of pH on the structure and charge of soy (Sikorski, 2002; Foegeding and Davis, 2011).

2.2.3. Foaming activity

Foams are defined as colloidal systems composed of two dispersed phases, one liquid and the other gas (Abirached *et al.,* 2010). Foam formation is governed by three factors: transport, penetration, and reorganization of the molecules at the air/water interface. These processes depend on the size, surface hydrophobicity and structural flexibility of the surfactants and proteins (Castel, 2010). Proteins are widely used as functional ingredients for the production and stabilization of foams due to the amphiphilic regions that give them an active surface with good steric and electrostatic stability (Rouimi *et al.,* 2005). The ability of proteins to stabilize foams is related to the tendency to be adsorbed on air-water interfaces, with the ability to reduce surface tension and form strong interfacial membranes through protein-protein interactions at air-water interfaces (Day, 2013).

2.2.4. Coagulation capacity

The coagulation capacity determines the potential of proteins to form unordered aggregates produced by denaturation in which aggregation reactions or protein-protein interactions predominate concerning protein-solvent interactions (Tang *et al.,* 2006). Protein aggregation is determined by temperature, polarity, ionic strength, and pH. The protein structure and size also play an important role in the stability, firmness, and elasticity of the aggregates (Day, 2013).

2.3. Modification of vegetable proteins by hydrolysis

The incorporation of plant proteins into food systems is determined by chemical composition, protein content, and functional properties (Lqari, 2002). The low functionality of plant proteins has motivated researchers to develop methods such as hydrolysis to improve their functional properties and food applications.

2.3.1. Limited hydrolysis

Enzymatic hydrolysis is an effective way to improve various functional properties and increase the application of proteins. Proteolysis increases protein-water interactions decreases molecular weight, simplifies the secondary structure, increases the number of ionizable groups, and exposes hydrophobic groups, changing the protein's physicochemical interactions with the medium (Tavano, 2013).

The solubility, emulsifying, and foaming capacity of proteins can be improved with limited hydrolysis. Limited hydrolysis is the proteolysis of the amino acid chain of a native protein by a proteolytic enzyme that modifies its structure under moderate conditions of concentration, time, pH, ionic strength and temperature (Adler-Nissen, 1976). However, excessive hydrolysis often causes loss of some functionalities yielding bitter-tasting peptides that negatively affect sensory properties (Sun *et al*., 2011).

The major applications of protein hydrolysates are for nutritional supplements, functional ingredients, flavor enhancers, coffee whiteners, confectionery products, and fortification

of soft drinks and juices. Besides, they are used in the cosmetic and medical areas (Bandyopadhyay and Ghosh, 2002). Mune *et al*., (2011) hydrolyzed Castile bean protein isolates with pepsin at 25 °C. The degree of hydrolysis was 20.04, 27.08, and 32.56% after 100, 200 and 300 minutes of incubation, respectively. The hydrolysis rate decreased late in the reaction, which is explained by the decrease in the specific peptide chains available for the specific enzymatic activity and/or competition between the native protein and the constantly formed peptides. On the other hand, the highest solubility at pH 7 was 75.24%; this increase could be attributed to the smaller molecular size and greater exposure of charges and polar groups to the polar environment.

The type of enzyme used for protein hydrolysis determines the peptides' functionality due to their cleavage sites (Feng and Xiong, 2003). Papain and neutrase are the most widely used cysteine proteases to produce protein hydrolysates, which is why they are commonly studied (Benítez *et al*., 2008). Hou and Zhao (2011) evaluated the effect of hydrolysis of isolated soybean protein with neutrase on solubility, gelling capacity, and fat absorption. The results indicated that the hydrolysate's solubility increased 84.78%, and the fat absorption decreased 23% compared to the native protein. Regarding the gelling capacity, the hardness of the gels prepared with soy hydrolysate was higher compared to those prepared with native soybean.

Neutrase and papain are the proteases commonly used in the food industry for the protein hydrolysis. Therefore, in the next section we will discuss these enzymes.

2.3.2. Catalytic mechanism of metalloproteases

Metalloproteinases are enzymes that have metal in the active site involved in the enzymatic mechanism, most having zinc (Zn), but some cobalt (Co). Its activity starts with an activated water molecule by an active site histidine attacks the carbonyl of the scissile amide bond (Figure 2-2). The carbonyl of the amide coordinates to the zinc to stabilize the oxyanion. The tetrahedral oxyanion then initiates elimination of the amine of the amide,

which then is protonated by the histidine. The histidine protonation and deprotonation are aided by a neighboring acid (Benítez *et al*., 2008).

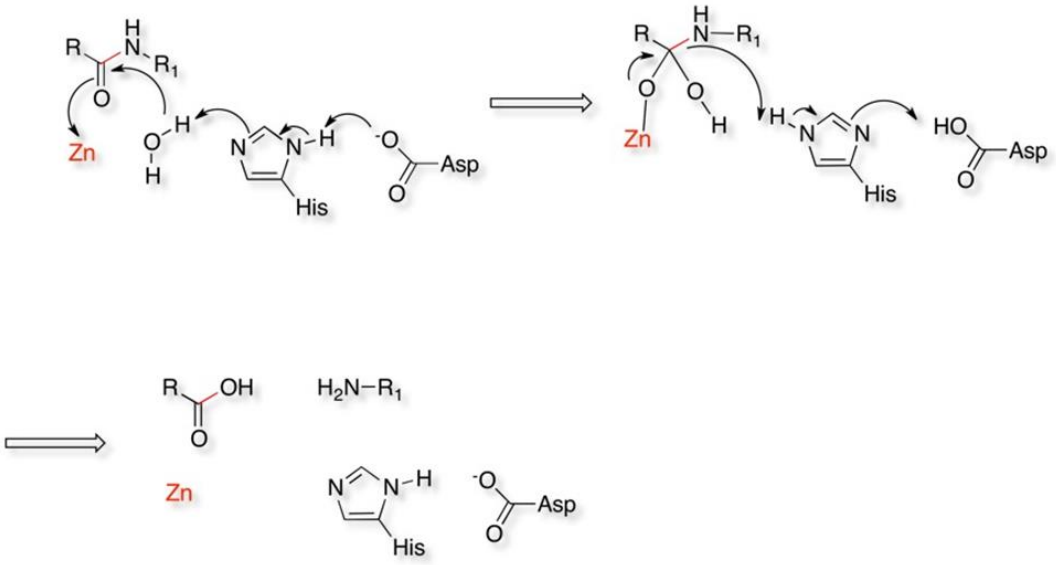

Figure 2-2. Catalytic mechanism of metalloproteases (Benítez *et al*., 2008).

2.3.3. Catalytic mechanism of cysteine proteases

The mechanism of cysteine proteases begins with the activation of a thiol group in the active site of the enzyme, then this thiol acts on the carbonyl group of the peptide (Figure 2-3). Subsequently, the peptide fragment with the amine group is released and the other peptide fragment with the carboxylic terminus is linked via a thioester bond with the sulfur of the enzyme. Finally, the thioester bond is hydrolyzed to generate the peptide fragment with the terminal carboxylic acid and and release the enzyme (Verma *et al.,* 2018).

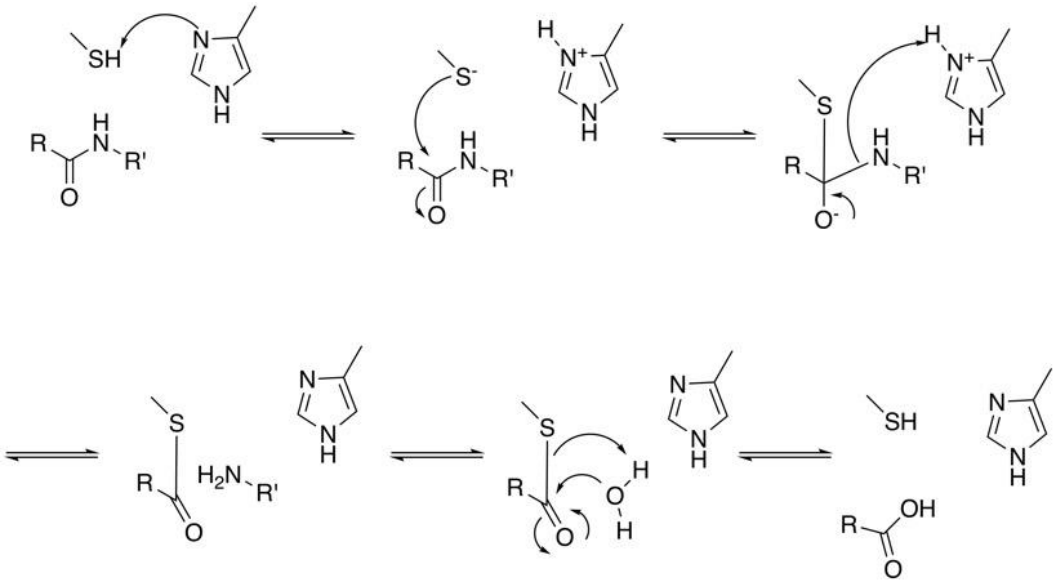

Figure 2-3. Catalytic mechanism of cysteine proteases (Verma *et al.,* 2018).

2.3.4. Neutrase

Neutrase (EC.3.4.24) is a bacterial endoprotease produced by fermentation of a selected strain of *Bacillus subtilis* commercialized by Novozymes (Ortega *et al*., 2009). The enzyme's optimal conditions are at pH 6-9 and temperature between 30 to 65 °C. Neutrase is specific for leucine and phenylalanine and usually generates peptides smaller than 10 kDa (Ou *et al.,* 2010).

2.3.5. Papain

Papain (EC 3.4.22.2) is part of the cysteine proteases and is found in the papaya fruit (*Carica papaya*). Its maximum activity occurs at a temperature of 70 ° C and a pH of 6 to 8, it is specific for arginine, lysine and phenylalanine, and it generates peptides around 10 kDa (LaLonde *et al*., 1998; Benítez *et al.*, 2008).

2.3.6. Free cysteine

Most of the thiols (SH) and disulfides (DS) in cells are found as the amino acid cysteine and its disulfide, cystine (Figure 2-4). The thiol group of cysteine is one of the most reactive functional groups found in proteins. In the food industry, free cysteine is commonly added as a donor of thiol groups to promote the thiol-disulfide exchange reaction and modify the protein structure and functionality. The reaction is initiated by a thiolate nucleophilic attack on an existing disulfide bond, leading to oxidation of the nucleophilic thiol and reducing the leaving group sulfur (Hansen and Winther, 2009).

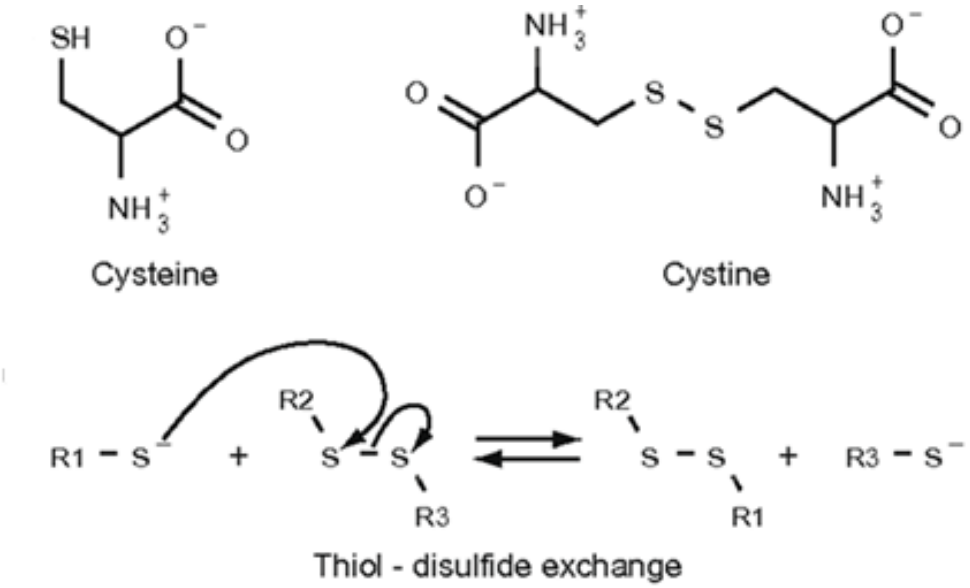

Figure 2-4. Mechanism of thiol-disulfide exchange (Hansen and Winther, 2009).

In this theoretical framework, it was necessary to address the issues of limited hydrolysis and enzymes most used in the food industry, since in this research we worked with a mixture of soybean proteins with maize germ. The solubility of this native protein mixture was relatively less than that of a hydrolyzed soybean protein. Therefore, it was necessary to find the best conditions regarding the type of enzyme, concentration and reaction time to hydrolyze the soybean-maize protein mixture and improve its solubility.

It is important to mention that in this research explored for the first time the use of enzyme mixtures and the use of a thiol donor agent for the hydrolysis of plant proteins. At the moment there is no report on the use of mixtures of proteolytic enzymes or cysteine in the hydrolysis of vegetable proteins. Therefore, the information generated here opens a possibility for the study of enzyme mixtures and facilitating agents for the hydrolysis of vegetable proteins.

2.4. Projection of protein demand

2.4.1. Protein demand by 2050

The proteins are the most relevant of the three fundamental macronutrients because they significantly differ in the nine essential amino acids and digestibility rate. According to the worldwide protein consumption reported by FAO (2001), these are generally obtained from cereals. The United Nations (UN) has projected a world population growth of almost 50%, from 2000 to 2050, from 7 to 9.5 billion, so animal protein demand will face a substantial increase. This demand is determined by the economic and social difference that establishes a marked nutritional inequality. On the one hand, the most vulnerable population demands high energy value proteins and low cost to cope with the high rate of malnutrition. In contrast, another sector of the population demands a greater quantity of proteins due to the change in healthier habits due to chronic diseases such as diabetes, obesity, cholesterolemia and hypertension. Therefore, protein availability becomes complex because there are two extremes of malnutrition and obesity in the same social environment, each with its specific problems and needs.

In some countries such as Mexico, 41.9, and 7.40% of the 130 million people live in poverty and extreme poverty, respectively. These individuals lack the resources to acquire an adequate or permanent supply of food. Therefore, they generally have a low caloric intake and develop nutrient deficiencies, especially micronutrients and essential amino acids. On the other hand, in 2018, it was decreed that the country is experiencing an epidemiological emergency due to an increase to 75.2% of the prevalence of

overweight and obesity. More worrisome, 39.1% and 36.1% of Mexicans over 20 years of age are overweight and obese, respectively. Potentially, 60% of these individuals will need to adopt new dietary patterns to improve their health. Their diets should have more protein and dietary fiber and lower amounts of simple sugars and fats (lower energy density)

The projected demand for animal protein is of particular interest, as it is expected to double by 2050. This higher demand entails food security problems and sustainability since the production of animal proteins yields more greenhouse gases, which harm the environment and are closely associated with climate change.

Therefore, it is necessary to find new sources of proteins that satisfy the nutritional and energy needs of the population, whose production is greener to reduce the impact on the environment. Legumes and cereals are a potential protein alternative, containing around 18 to 50% (Table 2-1). However, these proteins present some nutritional deficiencies according to their nature. Legume proteins are deficient in methionine, cysteine, and tryptophan, whereas proteins of cereals in lysine and threonine. Commonly, the amino acid deficiencies of vegetable proteins are improved by mixing legumes with cereals.

It is important to mention that vegetable proteins have good nutritional value. However, their functionality is reduced compared to animal origin counterparts, limiting their application in food systems. Therefore, it is important to study and generate information on their solubility index and foaming, emulsifying and gelling capacities to propose novel strategies to modify and incorporate them more efficiently into food systems.

Therefore, vegetable proteins are potential alternatives to solve protein security and sustainability problems that humanity will face derived from the high demand for this important macronutrient. The development of new proteins with high nutritional value, useful functionality, and affordable cost should be promoted to satisfy the specific nutritional needs of both sectors facing malnutrition and obesity.

2.4.2. Impact of the COVID-19 pandemic on protein demand

The projections made by the World Health Organization regarding the demand for protein for 2050 did not contemplate the recent impact of the COVID pandemic that has affected the economy of practically all countries. This new reality accelerated the need to produce low-cost and enhanced proteins with a high nutritional value, preventing viral disease by strengthening the immune system.

The World Bank has predicted that the pandemic's impact will push from 88 to 115 million people into extreme poverty, bringing the total amount to between 703 and 729 million globally. These people will demand protein sources of high nutritional value and low cost, given their purchasing power.

The pandemic has affected trade and international markets. For instance, the company Ingredion recently adsorbed 100% of the Canadian company Verdient Foods, which specialized in producing vegetable proteins. This merger announced an investment of 250 million dollars to increase the production of vegetable proteins.

Therefore, the projection made by Tecnologico de Monterrey to anticipate the problem of protein deficiency in 2013 and create the Center for Research and Development of Proteins (CIDPRO) is more accurate than ever. The COVID-19 pandemic added more challenges to the existing ones in terms of protein deficiency, so the research efforts made in the CIDPRO will allow providing alternatives of protein sources of plant origin to the most vulnerable population.

On the other hand, studies have begun to assess the importance of protein consumption to strengthen the human immune system and prevent the SARS-CoV-2 infection. Muscogiuri *et al*. (2020) recommend consuming protein and selected micronutrients during the quarantine to protect the population against COVID-19. Similarly, Naja and Hamadeh (2020) recently concluded that nutritional deficiencies of energy, proteins, and specific micronutrients are associated with a depressed immune function and, therefore, to a higher susceptibility to infections. Therefore, these new challenges to meet the demand for vegetable proteins open opportunities to generate novel proteins with high biological value and low cost to meet the nutritional needs of vulnerable groups.

Chapter 3

3. Physicochemical and Functional Properties of Vegetable and Cereal Proteins as Potential Sources of Novel Food Ingredients

This chapter has been published as:

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Abstract:

Proteins from vegetable and cereal sources are an excellent alternative to substitute animal-based counterparts because of their reduced cost, abundant supply, and good nutritional value. This investigation aimed to characterize the physicochemical and functional properties of vegetable and cereal proteins and explore their possible correlations, to better understand their potential use as food ingredients. Twenty protein sources were studied: five soybean flour samples, one pea flour, and fourteen newly developed blends of soybean and maize germ (five concentrates and nine hydrolysates). The physicochemical characterization included pH (5.63 to 7.57), electrical conductivity (1.32 to 4.32 mS/cm), protein content (20.78 to 94.24 % on dry mass basis), free amino nitrogen (0.54 to 2.87 mg/g) and urease activity (0.08 to 2.20). The functional properties showed interesting differences among proteins: water absorption index ranged from 0.41 to 18.52, the highest being of soy and maize concentrates. Nitrogen and water solubility ranged from 10.14 to 74.89 % and from 20.42 to 95.65 %, respectively. Fat absorption and emulsification activity indices ranged from 2.59 to 4.72 and from 3936.6 to 52 399.2 m^2/g, respectively, the highest being of pea flour. Foam activity (66.7 to 475.0 %) of the soy and maize hydrolysates was the best. Correlation analyses showed that hydrolysis affected solubility-related parameters, whereas fat-associated indices were inversely correlated with water-linked parameters. Foam properties were better in proteins treated with low heat, which also had high urease activity. Physicochemical and functional

characterization of the soy and maize protein concentrates and hydrolysates allowed the identification of differences regarding other vegetable and cereal protein sources such as pea or soybean.

Keywords: vegetable proteins, cereal proteins, functional properties, physicochemical parameters, soy and maize concentrates, and pea flour.

3.1. Introduction

There is a rising interest in protein isolation for their subsequent use as a food ingredient. Sixty percent of Americans consider protein content in food or beverages when making a buying decision (IFICF, 2013). Of the three macronutrients (carbohydrates, proteins, and fats), proteins are the most appealing for consumers concerned about their health. Nearly half of adults perceive proteins as ingredients that increase energy levels, support overall good health, and improve muscle tone. These macronutrients are also considered necessary in diets aimed to complete a weight management program. Despite the awareness of protein importance in a balanced diet, nearly 25% of adults believe that they cannot consume as many proteins as they would like because of the cost (Cheatham, 2014). The protein industry is segmented into animal (gelatin, egg white, casein, and whey) or vegetable, of which soybean is the only source of worldwide relevance. The former has the advantage of being of high nutritional quality, but with a higher cost than the vegetable counterparts, and frequently the supply is irregular and unreliable. The latter is cheaper, abundant and with an excellent nutritional value, mainly when combined, therefore making them a good option as food ingredients. Vegetable proteins, as food ingredients, should perform specific functions within formulations to provide or enhance texture, gelling, emulsifying or foaming characteristics, among others. The best way to test the role of high-protein ingredients in food is in a practical scenario. Unfortunately, this is not always possible, and therefore laboratory procedures for protein characterization are of utmost importance (Kinsella and Melachouris, 1976). Functional tests are required to evaluate and predict how proteins may behave in specific systems,

offering a pre-evaluation of the best application (Moure *et al*., 2010). Thus, the physicochemical and functional characterization should be clear before using proteins as food ingredients (Tomotake *et al*., 2002).

There is currently much information regarding the functional properties of proteins, starting with the overwhelming general data about soybean and oilseed-derived materials (Moure *et al*., 2010; Arrese *et al*., 1991; Kinsella, 1979). Several authors have compared the physicochemical and functional properties of buckwheat protein, soybean protein isolate and casein (Tomotake *et al.,* 2002; Arrese *et al*., 1991; Luo *et al*., 2014). The functional characteristics of pseudocereals as quinoa and amaranth have also been reported in the literature (Tomotake *et al.,* 2002; Shevkani *et al*., 2014; Abugoch *et al.,* 2008). Regarding cereals and other oilseeds, some authors have made comparisons among the protein functional properties of rice cultivars, peanut flour and peanut protein concentrate for their potential use in the food industry (Pinciroli *et al*., 2009; Yu *et al*., 2007). Other high-protein crops, such as pulses, have been explored and characterized: Marama bean (Maruatona *et al*., 2010), cowpea (Khalid *et al*., 2012), pea, lentil, navy bean and chickpea (Toews and Wang, 2013). Despite the high quantity of information about specific crops and high protein materials, specific and novel proteins' characterization is required to determine their physicochemical and functional characteristics. These data are valuable for developing future protein sources through innovation and research, especially new, less expensive materials capable of giving a well-balanced food in terms of health and sensorial characteristics. This work aimed to characterize the physicochemical and functional properties of vegetable and cereal proteins. Furthermore, to explore the correlations between the physicochemical characteristics and the functional properties of proteins, to better understand their potential use as food ingredients.

3.2. Materials and Methods

3.2.1. Materials

The analyzed samples were identified as: pea flour (TECSA, Monterrey, Mexico), soybean flour national (SBFN; Food Proteins Corporation, Mexico City, Mexico), soybean flour 120 (SBF120; Productos Industriales Gaf, Mexico City, Mexico), soybean flour 200/20 (SBF200/20; Food Proteins Corporation), soybean flour Nutrisoy (SBFNutri; ADM, Chicago, IL, USA), soybean flour Ragasa (SBFRagasa; Ragasa, Monterrey, Mexico), concentrates of soybean and maize germ (01 to 05) and hydrolysates of soybean and maize germ (01 to 09). The number at the end of each code represents the sequence in which each protein was produced. All used materials were defatted. The mixtures of soybean and maize proteins were obtained using a standard procedure of alkali extraction followed by acid precipitation (Riaz, 2006). Briefly, the pH of a finely ground mixture of defatted soybean flour and defatted maize germ (proportion 5:1 using 10 parts of water) was adjusted to pH=10 with 50% NaOH. Contents were mixed for 30 min at 50 °C before the separation of bagasse using an industrial centrifuge (Model SA14, GEA Westfalia, Oelde, Germany) operated at 15 L/min and 5500×g. The supernatant was then collected, and the pH was adjusted to 4.5 with 3 M HCl. The curd was separated using the centrifuge operated at the previously described conditions. The resulting product was washed with an equal volume of water, separated by centrifugation, and then the pH was adjusted to 7.0 (with 50% NaOH). The resulting material was dried using an industrial spray dryer designed by Nutrigrains (Monterrey, Mexico) with air inlet and outlet temperatures of 195 and 80 °C, respectively, and an atomization pressure of 1726 N/cm^2. For hydrolyzed proteins, enzymatic hydrolysis was performed before spray drying (Neutrase®, 0.25% of total solids in the curd, 30 min at 40 °C). The spray-dried samples were stored at room temperature in a dry and ventilated place.

3.2.2. Determination of physicochemical parameters

For all samples, moisture (AOAC method 934.06; AOAC, 1996), crude protein (AOAC Method 984.13-1994; AOAC, 1996), reducing sugars (RS; Miller, 1959) and free alpha-amino nitrogen (FAAN; AOAC method 945.30-1945; AOAC, 1945) contents were calculated as well as pH and electrical conductivity (EC; potentiometer model 250, Hanna Instruments, Padova, Italy).

3.2.3. Functional properties

The water absorption (WAI) and water solubility (WSI) indices were determined using 1g of sample placed in 15 mL of distilled water, according to Cheftel *et al.* (1989). The nitrogen solubility index (NSI) was assayed using 0.5 g of sample dispersed in 50 mL of 0.1 M sodium chloride (pH=7.0) (Cheftel *et al.,* 1989). Nitrogen was determined with the micro-Kjeldahl method in total and soluble fractions (AOAC Official Method 984.13-1994; AOAC, 1996). Fat absorption index (FAI) was determined based on a previously reported method (Ahn *et al*., 2005). The turbidimetric procedure (Pearce and Kinsella, 1978) was used for determining emulsifying activity index (EAI) in all samples, whereas emulsion stability (ES) was calculated according to Haque and Kito (Haque and Kito, 1983). Regarding functional properties related to protein and air interaction, foaming characteristics were evaluated: foaming activity (FA), foam stability (FS) and foam density (FD) in 3% (by mass) protein dispersions in water (Haque and Kito, 1983). Urease activity (UA) was determined as a change in pH according to AOCS Method Ba 9-58 (AOCS, 1987) and heat coagulation capacity (HCC) with the technique proposed by Regenstein and Regenstein (1984).

3.2.4. Statistical analysis

All determinations were performed in triplicate, and data were analyzed with ANOVA (Minitab Statistical Software v. 16, Minitab Inc., State College, PA, USA). Mean values

were compared with Tukey's test (α=0.05). Pearson's correlations, linear regression, and principal component analysis (PCA) were determined using the same statistical software.

3.3. Results and Discussion

3.3.1. Physicochemical characterization of vegetable and cereal proteins

Tables 3-2 and 3-3 shows the physicochemical properties of the array of analyzed vegetable and cereal proteins. The pH, an important parameter associated with protein solubility, ranged from 6.42 to 7.57, except for the soybean and maize concentrates 01 and 02 with values of 5.63 and 5.89, respectively. At lower or higher values than the isoelectric point, the electrostatic repulsion increases and consequently, the solubility of proteins improves. This parameter is also related to the material's water absorption capacity: ionized amino acid groups bind more water than non-ionized ones. Lowering the pH below 4 changes the carboxyl groups into non-ionized forms, thus reducing the water-binding properties of the protein.

The electrical conductivity (EC) of all samples was between 4.32 and 1.32 mS/cm (soybean and maize hydrolysates 02 and 04). This parameter is a measure of a material's ability to conduct electrical current and is affected by the content of protein, fat, and minerals, among others. Foods with electrolytes such as salts, acids, certain gums, and thickeners contain charged groups that have a notable effect on the EC. Protein charge and amino acid composition also affect this property. Foods such as apples, strawberries and potatoes have EC of 0.7, 1.9 and 0.4 mS/ cm at 25 °C, respectively (Smith, 2011).

The EC of soybean and maize concentrates ranged from 2.47 to 3.64 mS/cm, comparable to the results reported by Jambrak *et al*. (2009) of 3.28 mS/cm of a soybean protein concentrate. The soybean and maize protein hydrolysates 02 and 04 showed the lowest and the highest conductivity of 1.32 and 4.32 mS/cm. Therefore, neither the protein content nor the higher degree of hydrolysis influenced the high EC of the protein, which depended on the charge density of the protein and configuration acquired after a particular process (thermal or enzymatic hydrolysis). EC is important for developing foods or beverages because it influences solubility, emulsifying and foaming activities, and

consequently on the interaction with other ingredients and the protein stability in each food system. EC is also important because it determines the heating rate and effectiveness of novel food processes, such as ohmic heating and pulsed electric-based operations (dehydration, extraction, pasteurization and others) (Rastogi, 2010).

Tables 3-2 and 3-3 shows that the protein content and hydrolysis degree significantly affect the EC ($p<0.05$). The pea flour and soybean and maize protein hydrolysate 04 contained the lowest and the highest protein fractions of 20.78 and 94.24% (on dry mass basis). The concentrates and some soybean and maize hydrolysates contained approx. 70% protein, similar to soybean concentrates available on the market. The free alpha-amino nitrogen (FAAN) content determines free amino acids or small peptides. Therefore, the degree of protein hydrolysis, solubility, and water absorption capacity, ranged between 0.54 and 2.87 mg/g. As expected, the hydrolyzed proteins (treated with protease) had a higher FAAN content (>2.0 mg/g), except for soybean and maize hydrolysate 03, which contained 1.52 mg/g, similar to SBFRagasa and soybean and maize protein concentrate 03. FAAN values between 12 and 27 % in soybean pods, 5 and 12% in spinach and 34 and 56% in potato tubers have been reported (Eppendorfer and Bille, 1996). The percentage of FAAN in total nitrogen ranged between 0.6 and 2.3 % (Tables 3-2 and 3-3), and these values were below those reported by Eppendorfer and Bille (1996) in vegetable protein products. Besides the availability of proteins and amino acids, the differences could be associated with the method used for FAAN determination.

The reducing sugar (RS) assay is highly relevant because the amounts of sugars relate to the stability or retention of protein functionality during storage (Kinsella and Melachouris, 1976). Foods can deteriorate during storage due to both enzymatic and Maillard-type reactions of primary amino groups with RS (Friedman, 1996). Determination of RS by the dinitrosalicylic acid method is a useful index for the characterization of high-protein materials (Tables 3-2 and 3-3). Pea flour had the highest RS of 136.65 mg/g, followed by SBFRagasa with 85.09 mg/g. The lowest RS content was measured in SBF120, SBF200/20 and soybean and maize hydrolysate 02 with only 5 mg of glucose reducing equivalents per gram.

The urease activity (UA) indicates the intensity of heat treatment during the processing of protein meals. A value of 0.3 or less suggests that the protein source retains slight urease

activity but has received sufficient heat treatment for the inactivation of the soybean trypsin inhibitors (Kunitz and Bowman-Birk). A product with a pH increase of 0.02 or less during the urease activity test (AOCS, 1987) was overheated, yielding a material with diminished functional properties. All UA results shown in Tables 3-2 and 3-3 were between 0.08 and 2.20 (soybean and maize protein concentrate 01 and SBFRagasa, respectively), indicating that these protein sources received high and low thermal treatments and thus contained low and high residual enzymatic activity, respectively. In the specific case of pea flour, its UA was similar to that of SBF120, SBF200/20 and soybean and maize protein concentrate 04. Values are also similar to the ones reported by Valencia *et al.* (2008), who compared UA activity of pea protein vs. soybean protein concentrates.

Table 3-2. Physical and chemical characterization of vegetable and cereal proteins (part a).

Sample	pH	EC/(mS/cm)	Moisture/%	Protein/% (db)	RS/(mg/g)	FAAN/(mg/g)	UA
pea flour (PF)	(6.42±0.12)^{h}	(1.35±0.01)^{kl}	(10.54±0.46)^{ab}	(20.78±0.35)^{j}	(136.65±2.11)^{a}	(0.720±0.04)^{gh}	(0.17±0.03)^{kl}
soybean flour national (SBFN)	(6.63±0.01)^{fg}	(2.59±0.01)^{ef}	(10.73±0.02)^{a}	(50.74±2.54)^{h}	(6.07±0.39)^{kl}	(0.545±0.02)^{h}	(0.13±0.01)^{lm}
soybean flour 120 (SBF120)	(6.63±0.00)^{fg}	(2.64±0.04)^{e}	(7.94±0.12)^{d}	(49.61±1.98)^{hi}	(5.26±0.35)^{l}	(0.594±0.01)^{h}	(0.15±0.01)^{kl}
soybean flour 200/20 (SBF200/20)	(6.74±0.01)^{ef}	(2.79±0.03)^{d}	(4.96±0.33)^{f}	(54.20±0.15)^{h}	(5.32±0.30)^{kl}	(0.597±0.02)^{h}	(0.21±0.01)^{k}
soybean flour nutrisoy (SBFNutri)	(6.60±0.01)^{g}	(2.90±0.05)^{d}	(3.97±0.07)^{hi}	(49.63±0.59)^{hi}	(6.19±0.40)^{kl}	(0.569±0.01)^{h}	(0.44±0.01)^{i}
soybean flour ragasa (SBFRagasa)	(6.79±0.02)^{e}	(2.26±0.03)^{g}	(3.58±0.07)^{i}	(45.38±0.12)^{i}	(85.09±0.68)^{b}	(1.404±0.03)^{f}	(2.20±0.03)^{a}
soybean and maize concentrate-01	(5.63±0.01)^{j}	(2.82±0.01)^{d}	(10.09±0.31)^{b}	(69.93±1.73)^{de}	(24.83±0.14)^{d}	(0.653±0.04)^{gh}	(0.08±0.01)^{m}
soybean and maize concentrate -02	(5.89±0.01)^{i}	(2.47±0.05)^{f}	(6.08±0.37)^{e}	(68.38±1.26)^{def}	(35.68±0.70)^{c}	(0.903±0.02)^{g}	(2.07±0.02)^{b}
soybean and maize concentrate -03	(7.54±0.01)^{a}	(3.64±0.02)^{c}	(5.71±0.10)^{e}	(62.62±0.44)^{g}	(19.46±0.43)^{gh}	(1.577±0.06)^{f}	(0.27±0.01)^{j}
soybean and maize concentrate -04	(7.57±0.04)^{a}	(3.54±0.06)^{c}	(4.98±0.04)^{f}	(71.69±1.11)^{d}	(21.07±0.49)^{efg}	(2.114±0.12)^{de}	(0.14±0.02)^{l}

Mean values are the average of at least three replicates ± standard deviation. Mean values with a different letter(s) in superscript within columns are statistically different ($p<0.05$). EC=Electrical Conductivity; RS=Reducing Sugars; FAAN=Free Alpha Amino Nitrogen; UA=Urease Activity; Dry basis= db.

Table 3-3. Physical and chemical characterization of vegetable and cereal proteins (part b).

Sample	pH	EC/(mS/cm)	Moisture/%	Protein/% (db)	RS/(mg/g)	FAAN/(mg/g)	UA
soybean and maize concentrate-05	(6.57±0.06)^{g}	(3.62±0.12)^{c}	(4.11±0.03)^{hi}	(67.13±0.24)^{defg}	(7.26±0.17)^{k}	(1.876±0.04)^{e}	(0.40±0.01)^{i}
soybean and maize hydrolysate-01	(6.60±0.00)^{g}	(4.00±0.06)^{b}	(5.04±0.02)^{f}	(68.38±1.41)^{def}	(5.99±0.07)^{kl}	(2.248±0.07)^{de}	(0.94±0.02)^{g}
soybean and maize hydrolysate-02	(6.43±0.06)^{h}	(4.32±0.04)^{a}	(4.07±0.19)^{hi}	(65.29±1.67)^{efg}	(5.01±0.08)^{l}	(2.678±0.07)^{ab}	(1.14±0.02)^{f}
soybean and maize hydrolysate-03	(7.14±0.01)^{b}	(4.14±0.02)^{b}	(2.18±0.14)^{j}	(64.69±0.10)^{fg}	(16.17±0.79)^{i}	(1.525±0.02)^{f}	(0.31±0.01)^{j}
soybean and maize hydrolysate-04	(6.57±0.01)^{g}	(1.32±0.06)^{l}	(8.31±0.06)^{d}	(94.24±1.61)^{a}	(11.52±0.01)^{j}	(2.793±0.08)^{a}	(1.93±0.02)^{c}
soybean and maize hydrolysate-05	(6.76±0.01)^{e}	(1.47±0.05)^{jk}	(4.70±0.12)^{fg}	(77.37±0.81)^{c}	(11.97±0.25)^{j}	(2.872±0.01)^{a}	(2.10±0.02)^{b}
soybean and maize hydrolysate-06	(7.08±0.01)^{bc}	(1.57±0.02)^{ij}	(4.36±0.07)^{gh}	(78.44±3.76)^{c}	(21.97±0.27)^{ef}	(2.391±0.06)^{cd}	(1.85±0.01)^{d}
soybean and maize hydrolysate-07	(7.05±0.01)^{bcd}	(1.76±0.04)^{h}	(3.79±0.18)^{hi}	(76.53±2.41)^{c}	(18.66±0.49)^{h}	(2.361±0.10)^{bc}	(1.95±0.01)^{c}
soybean and maize hydrolysate-08	(6.94±0.07)^{d}	(1.52±0.03)^{ij}	(7.93±0.07)^{d}	(91.16±0.39)^{ab}	(20.37±0.76)^{fgh}	(2.611±0.08)^{ab}	(1.69±0.01)^{e}
soybean and maize hydrolysate-09	(6.98±0.06)^{cd}	(1.64±0.03)^{hi}	(9.03±0.07)^{c}	(87.96±1.03)^{b}	(22.42±0.20)^{e}	(2.523±0.17)^{bc}	(0.81±0.04)^{h}

Mean values are the average of at least three replicates±standard deviation. Mean values with a different letter(s) in superscript within columns are statistically different ($p<0.05$). EC=Electrical Conductivity; RS=Reducing Sugars; FAAN=Free Alpha Amino Nitrogen; UA=Urease Activity; Dry basis= db.

3.3.2. Functional analysis of vegetable and cereal proteins

Functional characterization of the array of analyzed proteins is summarized in Tables 3-4 and 3-5. The water absorption index (WAI) is one of the most important parameters to consider for product development, particularly for dairy products and foods exposed to thermal treatments such as baking and thermoplastic extrusion (Wolf, 1970). WAI is defined as the water absorbed per gram of tested material, and it is regularly used as synonymous with water-holding, water binding or water retention capacity (Barbut, 1996). WAI values were between 0.41 and 18.52 (Tables 3-4 and 3-5). The protein concentrates exhibited higher WAI values compared to the other vegetable or cereal protein sources (average of 8), followed by the soybean and pea flour (4.31 to 5.38 and 4.97, respectively) and the hydrolysates (around 1.0). Despite the direct relationship between water holding capacity and protein concentration (Kinsella, 1979), the higher protein concentration of hydrolysates did not improve the WAI compared with the other samples. This is influenced by protein structure and composition. According to Barbut (1996), water can be divided into two general types according to its relationship with the protein molecule: absorbed and retained. The first is the water bound to the protein molecule and therefore no longer available for its use as a solvent, whereas the second is trapped within the protein matrix. The first kind depends mainly on the amino acids and pH of the system, and the second is more dependent on the same relationship among protein molecules. Because of the type of water absorption procedure used herein, the second type of water is the one that varied the most among samples (Tables 3-4 and 3-5), which is mainly due to protein structure organization.

On the other hand, the smaller protein molecules that form hydrolysates reduce the interaction among molecules, yielding structures that do not hold water. Nitrogen solubility index (NSI) is another parameter related to the hygroscopic properties of proteins: it is a measurement of the protein dispersibility in a NaCl solution. The NSI values of all the analyzed proteins were between 10.14 (of the SBFNutri) and 74.89 (soybean and maize protein hydrolysate 05). These values were similar to the amounts reported for commercial high-protein soybean products, ranging from 10 to 90% (Wolf, 1970). NSI is generally related to the extent of heating or protein denaturation and is also important

because it affects the solubility of proteins at different ionic strengths. It offers a more realistic approach to the performance of the protein in foods (since these are complex ionic systems). As expected, the hydrolysates showed the highest NSI because these proteins were hydrolyzed with protease beforehand, which, according to Kinsella and Melachouris (1976), markedly improves nitrogen solubility.

The water solubility index (WSI) of the proteins is the most important functional property because it affects other functional characteristics such as EAI, FA and HCC. WSI depends on the protein's ability to interact with water. The WSI of soybean flour and soybean and maize concentrates was around 35%, while hydrolysates (02 and 09) ranged from 32.71 to 95.65%. Arrese *et al.* (1991) reported the WSI values of soybean proteins of 36.3 to 83.6%. According to Tomotake *et al.* (2002), WSI of a buckwheat isolate at a similar pH value as used in our study was around 50%, followed by the soybean protein isolate, with values below 20% and that of peanut flour of 30% (2007). Therefore, WSI values obtained in some analyzed samples (Tables 3-4 and 3-5) are higher than those reported for similar products.

The fat absorption index (FAI) is the ability of the vegetable and cereal proteins to bind fat by capillary attraction physically. This is a parameter of paramount importance in food development because fats act as flavor retainer and increase the mouthfeel of the foods. The FAI of pea, soybean flour and soybean and maize concentrates and hydrolysates ranged from 2.59 to 4.72. Meng and Ma (2002) reported a value of FAI of a commercial soybean protein of 1.52. FAI variation may be due to the different surface hydrophobicities of vegetable proteins because the absorption of fat has been attributed to physical entrapment within the protein and non-covalent bonds such as hydrophobic, electrostatic and hydrogen bonding, the forces involved in lipid-protein interactions (Kaushal *et al*., 2012). Another property related to hydrophobicity is the emulsifying activity index (EAI), *i.e.,* the ability of a protein to form and stabilize the emulsion by creating electrostatic repulsion on the oil droplet surface. The emulsion stability index (ESI) reflects the ability of the proteins to form and maintain a stable emulsion over a period by preventing flocculation and coalescence of the oil globules (Shevkani *et al*., 2014). The soybean and maize protein hydrolysate 05 had the lowest EAI of 3936.62 m^2/g, while the pea flour had the highest, 52 399 m^2/g. These results may be due to the high content of nonprotein

solids in pea flour, favorable for emulsion. The good performance of pea protein as an egg replacer in mayonnaise-like products has received comprehensive coverage in the media (Cheatham, 2014). In general, the differences in protein emulsifying activity may be related to their solubility and conformational stability. This property is widely utilized in totally or partially emulsified foods, such as mayonnaise, cream, sauces, desserts, comminuted meat products and some beverages.

Moreover, the emulsions of a pea, soybean flour and soybean and maize concentrate and hydrolysates were all stable for 24 to 48 h, *i.e.*, they all have a similar capacity to stabilize an emulsion. The high stability of the emulsions of vegetable and cereal proteins is due to their conformation. They are globular protein structures that reduce surface tension and form more rigid interfacial films.

Similar to emulsion characteristics, another related property with two-phase interaction is foaming capacity or activity (FA). Foam can be defined as a two-phase system where a continuous liquid layer separates air cells, and foam stability (FS) is the capacity of a protein to reduce the surface tension forming strong interfacial membranes via protein-protein interactions at the air-water interface. FA values of pea flour and soybean and maize hydrolysate 04 were 75 and 475%, respectively (Tables 3-4 and 3-5). The high content of nonprotein solids of the pea flour may have increased the surface tension of the dispersion, significantly reducing the FA. The FS of pea flour was one of the lowest, with a zero value, and the highest of soybean and maize concentrate 02 (93.20%). The foam density (FD) was similar in all samples. Protein foams can provide unique textures (as in meringue and nougat) associated with many foods such as angel and pound cakes, ice cream and confectionery products.

Heat coagulation capacity (HCC) was also determined, and results are shown in Tables 3-4 and 3-5. Results ranged from 66.35 to 95.50% for pea flour and soybean and maize concentrate 05, respectively. Coagulation is the capacity of the protein to form a clot or a semisolid mass after an initial denaturation (driven by different factors, such as heat). It involves the rupture of hydrogen bonds within peptide chains, and when an advanced state is reached, denaturation becomes irreversible. According to Kinsella (Kinsella, 1979), in soybean proteins, initial heating above 60 °C is necessary to induce dissociation of quaternary globulins. This thermal treatment causes the unfolding of polypeptides of

the protein subunits with an increase in viscosity. Upon cooling, the unfolded polypeptides reassociate via hydrophobic associations, hydrogen bonding, ionic interactions and possibly some disulfide linkages, forming a gel. HCC is thus an essential property in food applications such as processed meat, sausages, and cheese.

Table 3-4. Functional properties of vegetable and cereal proteins (part a).

Sample	WAI	NSI/%	WSI/%	FAI	EAI/(m^2/g)
pea flour (PF)	(4.97±0.04)^{cd}	(18.13±0.71)^{g}	(20.42±0.87)^{i}	(4.72±0.15)^{a}	(52399±2306)^{a}
soybean flour national (SBFN)	(5.38±0.18)^{c}	(14.84±0.80)^{gh}	(23.22±1.42)^{i}	(3.16±0.11)^{cdef}	(8713±146)^{ghij}
soybean flour 120 (SBF120)	(4.92±0.26)^{cde}	(13.42±0.54)^{hi}	(29.05±0.10)^{gh}	(3.01±0.02)^{efgh}	(16747±822)^{e}
soybean flour 200/20 (SBF200/20)	(4.49±0.15)^{def}	(14.07±0.61)^{hi}	(25.07±0.29)^{hi}	(2.89±0.06)^{fghi}	(25157±2084)^{cd}
soybean flour nutrisoy (SBFNutri)	(4.45±0.13)^{ef}	(10.14±0.76)^{i}	(25.47±1.68)^{hi}	(2.77±0.09)^{ghi}	(51300±3325)^{a}
soybean flour ragasa (SBFRagasa)	(4.31±0.10)^{f}	(13.66±0.74)^{hi}	(47.00±1.79)^{d}	(3.10±0.03)^{cdef}	(29293±1575)^{b}
soybean and maize concentrate 01	(18.52±0.52)^{a}	(10.70±0.58)^{i}	(39.19±5.41)^{e}	(2.74±0.03)^{hi}	(10687±680)^{fgh}
soybean and maize concentrate -02	(3.60±0.02)^{g}	(15.38±0.57)^{gh}	(30.00±0.59)^{gh}	(3.07±0.07)^{defg}	(8381±262)^{hij}
soybean and maize concentrate -03	(8.35±0.16)^{b}	(33.84±0.55)^{f}	(33.69±1.37)^{fg}	(3.33±0.11)^{bcde}	(22234±1506)^{d}
soybean and maize concentrate -04	(8.04±0.25)^{b}	(27.61±0.96)^{f}	(37.88±0.66)^{ef}	(2.59±0.10)^{i}	(17491±1188)^{e}
soybean and maize concentrate -05	(8.34±0.08)^{b}	(36.06±1.46)^{e}	(37.82±0.21)^{ef}	(2.76±0.09)^{ghi}	(10147±123)^{fgh}
soybean and maize hydrolysate01	(3.74±0.11)^{g}	(33.09±2.12)^{e}	(39.22±2.13)^{e}	(3.35±0.13)^{bdc}	(12414±145)^{fg}
soybean and maize hydrolysate02	(3.59±0.06)^{g}	(33.65±2.54)^{e}	(32.71±2.32)^{fg}	(2.88±0.09)^{fghi}	(27282±1822)^{bc}
soybean and maize hydrolysate03	(4.82±0.04)^{de}	(34.80±1.14)^{e}	(37.58±1.04)^{ef}	(3.40±0.07)^{bc}	(14055±999)^{ef}
soybean and maize hydrolysate04	(1.05±0.04)^{i}	(40.45±1.55)^{d}	(84.80±1.45)^{bc}	(3.02±0.06)^{efgh}	(5426±435)^{jk}
soybean and maize hydrolysate05	(1.83±0.02)^{h}	(74.89±0.90)^{a}	(81.39±0.89)^{c}	(3.31±0.07)^{bcde}	(3936±75)^{k}
soybean and maize hydrolysate06	(1.13±0.08)^{i}	(45.37±1.68)^{c}	(88.84±1.04)^{b}	(3.20±0.21)^{bcdef}	(8880±466)^{ghij}
soybean and maize hydrolysate07	(1.03±0.06)^{j}	(48.27±2.69)^{c}	(88.63±1.37)^{b}	(3.05±0.00)^{defgh}	(9495±98)^{ghi}
soybean and maize hydrolysate08	(1.12±0.01)^{i}	(56.55±0.82)^{b}	(87.26±0.32)^{b}	(3.51±0.20)^{b}	(605313)^{ijk}
soybean and maize hydrolysate09	(0.41±0.02)^{j}	(55.20±0.53)^{b}	(95.65±0.36)^{a}	(2.89±0.09)^{fghi}	(9346±292)^{ghij}

Mean values are the average of at least three replicates ± standard deviation. Mean values with a different letter(s) in superscript within columns are statistically different ($p<0.05$). WAI= water absorption index; NSI=nitrogen solubility index; WSI= water solubility index; FAI=fat absorption index; EAI=emulsifying activity Index.

Table 3-5. Functional properties of vegetable and cereal proteins (part b).

Sample	ES_1 /%	ES_2 /%	FA/%	FS/%	FD/%	HCC/%
egg albumin*	33 ± 2	- - - - -	200 ± 10	- - - - -	- - - - -	- - - - -
pea flour (PF)	(72.73±2.26)^{abc}	(73.69±0.31)^{ab}	(75.00±0.00)^{k}	(0.00±0.00)^{g}	(57.14±0.00)^{a}	(66.35±2.08)^{i}
soybean flour national (SBFN)	(73.24±0.15)^{ab}	(73.33±0.00)^{ab}	(108.33±0.00)^{ij}	(50.67±7.51)^{f}	(35.00±0.00)^{b}	(84.05±1.10)^{d}
soybean flour 120 (SBF120)	(71.19±1.61)^{bc}	(71.81±1.32)^{ab}	(275.00±0.00)^{gh}	(0.00±0.00)^{g}	(24.23±0.48)^{e}	(75.89±0.47)^{gh}
soybean flour 200/20 (SBF200/20)	(75.71±0.00)^{a}	(77.62±1.65)^{a}	(261.90±0.00)^{h}	(77.16±0.81)^{cde}	(27.25±0.00)^{d}	(78.72±1.35)^{ef}
soybean flour nutrisoy (SBFNutri)	(73.33±0.00)^{ab}	(73.33±0.00)^{ab}	(288.00±0.00)^{gh}	(0.00±0.00)^{g}	(20.77±0.88)^{fg}	(77.76±0.39)^{fg}
soybean flour ragasa (SBFRagasa)	(69.23±0.00)^{c}	(57.14±0.00)^{d}	(375.00±0.00)^{e}	(89.40±0.00)^{ab}	(20.00±0.00)^{gh}	(95.16±0.29)^{a}
soybean and maize concentrate 01	(73.41±2.00)^{ab}	(76.92±0.00)^{a}	(376.19±0.00)^{de}	(74.05±0.10)^{de}	(20.84±0.00)^{fg}	(84.95±0.20)^{cd}
soybean and maize concentrate -02	(72.60±0.00)^{abc}	(73.33±0.00)^{ab}	(397.58±39.92)^{cde}	(93.20±9.95)^{a}	(19.42±1.10)^{gh}	(93.39±0.41)^{a}
soybean and maize concentrate -03	(74.71±2.8)^{ab}	(63.57±1.01)^{cd}	(84.55±0.00)^{jk}	(0.00±0.00)^{g}	(29.45±0.43)^{c}	(75.55±0.53)^{gh}
soybean and maize concentrate -04	(72.85±1.37)^{abc}	(68.57±8.92)^{bc}	(66.67±0.00)^{k}	(84.72±1.27)^{abc}	(33.33±0.00)^{b}	(73.87±0.31)^{h}
soybean and maize concentrate -05	(73.33±0.00)^{ab}	(73.33±0.00)^{ab}	(113.00±0.00)^{i}	(73.77±1.68)^{de}	(24.20±2.30)^{e}	(95.50±0.22)^{a}
soybean and maize hydrolysate01	(73.17±0.27)^{ab}	(73.65±0.55)^{ab}	(298.61±2.41)^{g}	(79.72±0.43)^{cd}	(25.09±0.15)^{e}	(87.65±0.16)^{b}
soybean and maize hydrolysate02	(73.97±0.36)^{ab}	(75.34±0.00)^{ab}	(328.33±2.89)^{f}	(80.67±0.22)^{bcd}	(22.22±0.00)^{f}	(86.97±0.08)^{bc}
soybean and maize hydrolysate03	(72.84±1.60)^{abc}	(73.33±0.00)^{ab}	(300.00±0.00)^{g}	(68.17±0.29)^{e}	(24.62±0.00)^{e}	(77.62±1.59)^{fg}
soybean and maize hydrolysate04	(74.68±1.80)^{ab}	(78.10±0.82)^{a}	(475.00±0.00)^{a}	(82.78±0.19)^{bcd}	(16.67±0.00)^{i}	(94.11±0.40)^{a}
soybean and maize hydrolysate05	(73.61±0.00)^{ab}	(75.24±0.82)^{ab}	(423.33±2.89)^{bc}	(83.42±3.24)^{bc}	(18.24±0.10)^{hi}	(93.95±0.37)^{a}
soybean and maize hydrolysate06	(73.33±1.65)^{ab}	(73.33±1.65)^{ab}	(375.00±0.00)^{e}	(79.60±1.44)^{cd}	(20.00±0.00)^{gh}	(80.69±0.55)^{e}
soybean and maize hydrolysate07	(75.71±0.00)^{a}	(71.43±0.00)^{ab}	(375.00±0.00)^{e}	(79.00±1.00)^{cd}	(20.00±0.00)^{gh}	(84.00±1.52)^{d}
soybean and maize hydrolysate08	(73.66±0.57)^{ab}	(74.32±0.00)^{ab}	(428.33±2.89)^{b}	(80.73±0.31)^{bcd}	(18.18±0.00)^{hi}	(93.14±0.23)^{a}
soybean and maize hydrolysate09	(73.38±0.82)^{ab}	(74.74±2.86)^{ab}	(403.81±0.00)^{bcd}	(80.67±0.10)^{bcd}	(19.09±0.00)^{gh}	(85.26±0.21)^{bcd}

Mean values are the average of at least three replicates ± standard deviation. Mean values with a different letter(s) in superscript within columns are statistically different ($p<0.05$). $ES_1$and ES_2=emulsion stability at 24 and 48h; FA=foaming activity; FS=foam stability; FD=foam density; HCC=heat coagulation capacity.*(Komakina and Míková, 2005).

3.3.3. Correlation analysis between physicochemical and functional properties of vegetable and cereal proteins.

Results of correlation analyses between functional properties and physicochemical parameters are summarized in Table 3-6. Protein content was positively correlated with the FAAN (R=0.75), NSI (R=0.69), WSI (R=0.78), FA (R=0.59) and FS (R=0.62) because higher protein mass fraction was observed in the hydrolyzed samples (last nine rows in Tables 3-2 to 5). On the other hand, protein mass fraction correlated negatively with RS (R=–0.56), EAI (R= –0.77) and FD (R=–0.72), which means that a higher protein content lowered EAI and FD, even though high-protein materials are suitable emulsifiers (Cabra *et al*., 2008). This result is reinforced by the fact that EAI showed an inverse relationship with WSI. Protein content was also positively correlated with FS and FA, and as expected, inversely with FD.

Regarding water-related properties, WSI correlated positively with protein content (R=0.78), FAAN (R=0.77), UA (R=0.65), NSI (R=0.81) and FA (R=0.65), and negatively with EC (R=–0.62) and EAI (R=–0.56) as previously stated. WSI had a good correlation coefficient with FA, perhaps because of the reduced size of the protein molecules that favored protein-water interaction, increasing the water-air interface. NSI also correlated positively with protein and FAAN content (R=0.69 and 0.87). The positive and highly significant relationships among WSI and NSI with FAAN clearly indicate that the degree of hydrolysis of a protein promotes solubility. Proteolysis enhances protein-water interactions because as the molecular mass decreases, it simplifies the secondary structure, increases the number of ionizable groups and exposes the hydrophobic groups, changing the physicochemical interactions of the protein with the medium (Tavano, 2013). WAI showed an inverse relationship with UA. Since the latter is an indicator of thermal treatment or heating index, it would be expected that the denaturation of high-protein materials affected its water absorption capacity. The other significant group of functional properties is the fat-associated indices (FAI and EAI). EAI was correlated with foam capacity and, as described previously, inversely with protein. The EAI had a positive correlation with FD (R=0.55) and a negative with FS (R=–0.63) and HCC (R=–0.57).

These correlations refer to the balance between hydrophilic and hydrophobic groups exposed on the surface of vegetable and cereal proteins.

On the other hand, FA and FS were positively correlated with UA, with correlation values of R=0.73 and 0.56, respectively. These correlations can be associated with the heating used during the extraction process and not directly with the residual enzyme activity. FA and FS could then be associated with the degree of denaturation of the protein structure. HCC, the only functionality evaluated for protein-protein interaction, showed a good correlation coefficient with UA (R=0.67), which meant that high UA increased HCC values. Therefore, proteins with lower denaturation due to lower exposure to heat treatments were more prone to coagulation.

Table 3-6. Pearson's correlation coefficients between physicochemical parameters and functional properties of vegetable and cereal proteins.

Parameter	pH	EC	Moisture	P	RS	FAAN	UA	WAI	NSI	WSI	FAI	EAI	ES_1	ES_2	FA	FS	FD
pH	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	----
EC	0.075	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	----
Moisture	-0.384	-0.439	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	----
P	0.177	-0.178	-0.093	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	----
RS	-0.132	-0.374	0.265	-0.564	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	----
FAAN	0.391	-0.167	-0.281	0.749	-0.230	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	----
UA	-0.037	-0.499	-0.285	0.466	0.068	0.582	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	----
WAI	-0.352	0.431	0.221	-0.263	0.029	-0.498	-0.596	-----	-----	-----	-----	-----	-----	-----	-----	-----	----
NSI	0.409	-0.330	-0.175	0.694	-0.228	0.871	0.495	-0.517	-----	-----	-----	-----	-----	-----	-----	-----	----
WSI	0.280	-0.620	-0.024	0.777	-0.145	0.773	0.654	-0.529	0.814	-----	-----	-----	-----	-----	-----	-----	----
FAI	0.002	-0.333	0.297	-0.418	0.699	-0.076	0.015	-0.213	0.089	-0.059	-----	-----	-----	-----	-----	-----	----
EAI	-0.045	0.121	-0.045	-0.773	0.527	-0.481	-0.357	0.100	-0.530	-0.558	0.330	-----	-----	-----	-----	-----	----
ES_1	0.117	-0.021	-0.015	0.312	-0.347	0.219	-0.050	-0.083	0.277	0.204	-0.085	-0.154	-----	-----	-----	-----	----
ES_2	-0.399	-0.132	0.287	0.291	-0.339	0.082	-0.120	-0.059	0.176	0.128	-0.016	-0.206	0.403	-----	-----	-----	----
FA	-0.290	-0.429	-0.100	0.590	-0.191	0.417	0.729	-0.392	0.386	0.653	-0.185	-0.404	0.017	0.245	-----	-----	----
FS	-0.082	-0.058	-0.255	0.620	-0.207	0.541	0.559	-0.178	0.384	0.473	-0.361	-0.629	0.059	0.132	0.516	-----	----
FD	-0.061	0.042	0.380	-0.719	0.615	-0.430	-0.565	0.209	-0.359	-0.542	0.641	0.553	-0.049	-0.068	-0.780	-0.517	----
HCC	-0.304	-0.152	-0.139	0.541	-0.258	0.453	0.673	-0.199	0.358	0.420	-0.318	-0.569	-0.066	0.029	0.594	0.671	-0.689

EC=electrical conductivity, P=protein, RS=reducing sugars, FAAN=free alpha amino nitrogen, UA=urease activity, WAI=water absorption index, NSI=nitrogen solubility index, WSI=water solubility index, FAI=fat absorption index, EAI=emulsifying activity index, ES_1 and ES_2=emulsion stability at 24 and 48 h respectively, FA=foaming activity, FS=foam stability, FD=foam density, HCC=heat coagulation capacity.

3.3.4. Principal component analysis

Principal component analysis (PCA) was used to visualize the correlation among the physicochemical and functional properties of the twenty vegetable proteins. A 3D graphic presentation of the first three components (PC1, PC2 and PC3) described 67.3 % of the variance. The PCA showed two correlated clusters, as shown in Figure 3-5. The first group (A) is formed by pH, EC, UA, protein content (P), WSI, NSI, FAAN, FA, FS, HCC and ES after 24 and 48 h, whereas the second group (B) includes FAI, EAI, RS, soluble solids (SS) and FD.

Group A is characterized by the association with the charging properties of the protein (pH, EC, UA and FAAN) that influenced the protein-water interactions. Group B included properties related to solid content. Aluko *et al*. (2001) reported a significant effect of the content of soluble solids on the emulsifying activity of coriander flour and protein concentrate. This is similar to the previously discussed results regarding the high emulsification capacity (52 399 m^2/g) observed in pea flour, mainly due to its high RS mass fraction (136.65 mg/g).

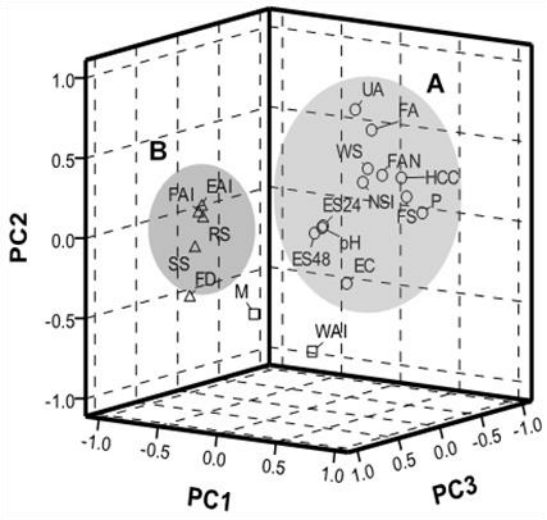

Figure 3-5. Graph of principal components (PC1, PC2 and PC3) in a rotated matrix. Properties associated with solubility (o, A), solid content (∆, B) and intermediate (□). This plot describes 67.3% of the variance.

3.4. Conclusions

This research characterized and compared the chemical and functional properties of some vegetable and cereal proteins, including commercial and new protein concentrates and hydrolysates obtained from a mixture of soybean and maize germ. Correlations were obtained between physicochemical and functional properties to better understand vegetable and cereal proteins as food ingredients. Water-related properties, such as WSI and NSI, in the soybean and maize hydrolysates were higher, making them good options for use as ingredients in beverages. WAI was better in soybean and maize concentrates, indicating their best suitability as extenders for sausages and related products. Fat-related properties (mainly FAI and EAI) were better in the pea flour, making it a good emulsifier option for dressings and other high-fat formulations. On average, FA and FS were better in the soybean and maize hydrolysates, which also had the best air trapping or foaming properties. The degree of protein hydrolysis was positively correlated with solubility-related parameters. Fat-associated characteristics were inversely correlated with water-associated characteristics. Foam and coagulation properties were better in low-heat-treated materials, which had high UA. The PCA of pea flour and soybean and maize concentrates and hydrolysates was linked within two groups, the first mainly associated with foam and coagulation properties and the second related to emulsification characteristics. This research characterized a set of vegetable and cereal proteins from a wide range of samples of raw materials and demonstrated relationships among their physicochemical and functional properties.

Chapter 4

4. Comparison of Physicochemical, Functional and Nutritional Properties Between Proteins of Soybean and a Novel Mixture of Soybean-Maize

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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, 47,385.01 (m^2/g) and 12,071.87 (m^2/g) 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 and amino acid score.

4.1. Introduction

In some countries such as Mexico, 41.90% and 7.40% of the 130 million people live in poverty and extreme poverty, respectively (CONEVAL, 2020). 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 are, at the same time, elementary units for essential nutrition in adulthood (Day, 2013). Dietary proteins can be obtained from animal or vegetable sources. Vegetable proteins represent a source of low-cost energy, but unfortunately, not all of them fulfill the highly digestible essential amino acids required for proper growth (Wu *et al*., 2009). Their versatility depends on solubility, coagulation, emulsifying and foaming capacities (functional properties that limit their application in the food industry) (Schmitt *et al*., 2005).

The type, size, structure, and degree of hydrolysis of the protein fractions significantly influence their functionality (Mo *et al*., 2011). 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 (Shukla and Cheryan, 2001; Khatib *et al*., 2002; Parris *et al*.,2006).

Legumes and cereals are excellent sources of proteins containing 18.5 to 50.0% and 6.0 to 18.0% protein (db), respectively (Toews and Wang, 2013). Toews and Wang characterized the physicochemical and functional properties of proteins from peas, lentils, navy beans and chickpeas, and determined that they can be applied in food due to their good functionality (Toews and Wang, 2013). Legume proteins are deficient in methionine, cysteine, and tryptophan, contrary to the cereals where lysine and threonine are usually the limiting amino acids (De Lumen *et al*., 1986). Commonly the amino acid deficiencies

of vegetable proteins are improved through the combination or blending of legumes with cereals. Suri *et al*., (2014) optimized the nutrient content and protein quality of mixtures of maize, sorghum and millet combined with cowpeas, peanuts or soybeans to supplement the diet in Ghana. In comparison, Chiweshe *et al*., (2014) mixed millet, rapoko and sorghum with soybeans and groundnut to develop a high protein-energy cereal blend for the vulnerable population of Zimbabwe.

Soybean is the legume with the highest protein content, with around 50%, while maize germ contains 18.4% (Kinsella, 1979). The maize is a staple in the American continent and the most produced cereals worldwide. The combination of soybean with maize proteins represents an attractive alternative for elaborating nutritionally complete food products.

The objective of this study was to compare the physicochemical, functional and nutritional characteristics of protein isolates and hydrolysates from soybean and soybean-maize mixes, to explore their use as a ingredients for foods.

4.2. Materials and Methods

4.2.1. 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®) from Interalimen S.A de C.V. (Mexico, D.F.). The experimental mixtures of soybean-maize proteins were extracted using a standard procedure of alkali extraction followed by acid precipitation (Riaz, 2006). Briefly: the pH of a finely ground 30kg mix of defatted soybean flour: maize germ (in a proportion 5:1 within ten parts of water) was adjusted to pH 10 with NaOH 50% w/w. Contents were mixed for 30 minutes at 50 °C before the separation of bagasse using an industrial centrifuge (Westfalia SA14) operated at 15 L/min and 5,500

xg. The supernatant was then collected, and the pH was adjusted to 4.5 with 3 N 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 kg_f/cm^2). Before spray drying, enzymatic hydrolysis was performed for hydrolyzed proteins using Neutrase® (0.011%) for 30 minutes at 40 °C (Figure 4-6). The samples were stored at room temperature in plastic bags and paper sacks as primary and secondary packaging materials, respectively.

4.2.2. Determination of physicochemical parameters

For all samples, moisture, crude protein, reducing sugars (RS), and free alpha-amino nitrogen (FAAN) were assessed (AOAC, 1990; Miller, 1959). The electrical conductivity (EC) and pH of the samples were measured using a potentiometer (Hanna-250, Padova, Italy).

4.2.3. Functional properties

The Water Absorption (WAI) and Water Solubility (WSI) indexes were determined on 1 g of sample in 15 mL of distilled water according to procedures by Cheftel *et al.* (1989). 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 in total and soluble fractions (AOAC, 1990). Fat Absorption Index (FAI) was performed based on Ahn *et al.* (2005). The turbidimetric method was used for Emulsifying Activity Index (EAI) in all samples (Pearce, 1978). Emulsion Stability (ES) was calculated according to Haque and Kito (1983). 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 (Haque and Kito, 1983). Urease Activity (UA) was also determined using the AOCS Official Method and Heat Coagulation

Capacity (HCC) with the technique proposed by Regenstein and Regenstein (AOCS,1983; Regenstein and Regenstein, 1984-).

4.2.4. 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 (1970). Briefly, the dispersions to 2% were dissolved protein loading buffer for SDS-PAGE in the ratio 1:1. 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). 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, UK).

4.2.5. Amino acid composition

The total amino acid composition was analyzed in isolates of soybean-maize and soybean proteins using the AOAC official method 994.12 (AOAC, 2005).

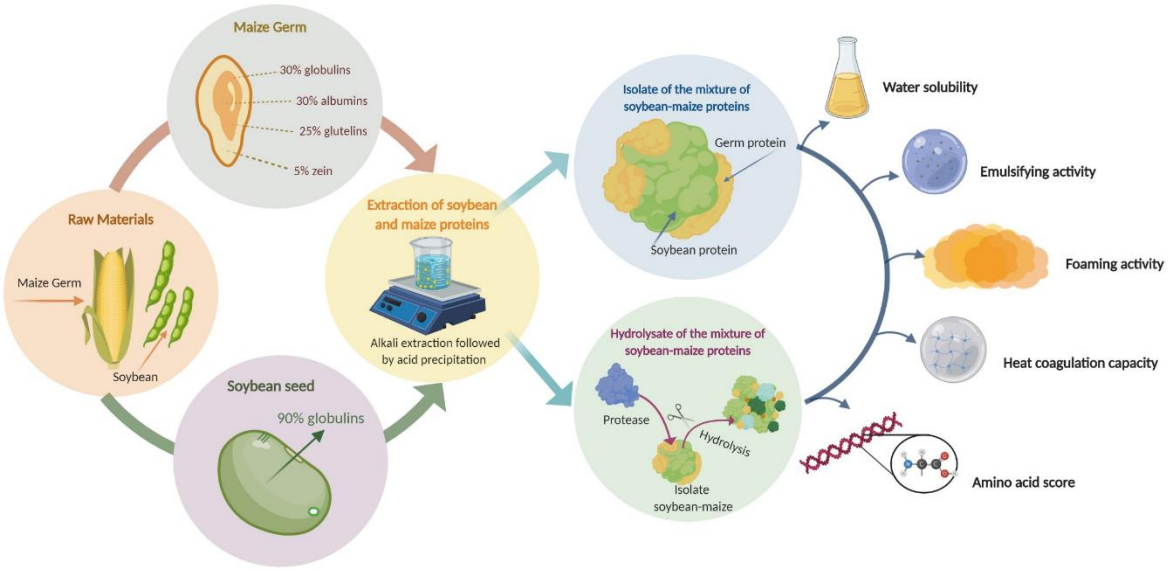

Figure 4-6. Process diagram to analyze the isolates and hydrolysates of soybean and soybean-maize. The soybean-maize protein was hydrolyzed with a neutrase concentration of 0.011% for 30 minutes at 40 ° C.

4.2.6. Statistical analysis

Data by triplicate were statistically evaluated using a one-way analysis of variance (Minitab 16, 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, Illinois).

4.3. Results and discussion

4.3.1. Physicochemical characterization

The proximate compositions of extracted proteins are shown in Table 4-7. pH for the samples was between 6.83 and 7.66. The pH affects the intermolecular forces and hydrodynamics of proteins. Commonly is used to produce proteins with different structural conformations and functionality. Lawal (2004) mentions that the relationship between pH and solubility of a protein depends on the prevailing charge on the amino acids at various pH values, that the balance of charges (+,-) reduces electrostatic repulsion and thus the solubility. On the other hand, Mohamed *et al*., (2005) reported that the hydrophobicity of the surface of a protein is a good indicator of the foaming and emulsifying capacity.

Another parameter related to the charge of the protein is the electrical conductivity (EC). This characteristic is inherent to 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 charges that provide germ maize proteins. Arzeni *et al*., (2012) 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 (Sharma *et al*., 1998). Besides, EC in emerging processes such as pulsed electric fields affects the performance of the protein during treatments.

The isolated soybean and soybean-maize protein content was about 93%, while the hydrolysates about 76%. This is consistent with Jambrak *et al*., (2009) data for soybean isolates and concentrates. The samples of soybean and soybean-maize had similar protein contents; 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 (Shukla and Cheryan, 2001). On the other hand, soybean contains approximately 50% protein, of which 90% are globulins (Kinsella, 1969).

The fat content for all samples was around 0.55% except for the commercial soybean isolate, which only contained 0.09%. This result agrees with fat values reported for soybean flour (0.55%), concentrate (0.30%), and isolate (<0.01%; Wolf, 1970).

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% (Shukla and Cheryan, 2001). However, the results correspond to previous results reported by Toews and Wang (2013) 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 0.010 and 0.095 mg/g, respectively, while the hydrolysates of the two samples contained around 0.020 mg/g. Sugar presence may influence protein stability, lysine availability, and color of foods after applying thermal treatments due to Maillard reactions (Rhee and Rhee, 1981). Also, it has been reported that sugars may negatively influence some functional properties, such as water absorption (Kaur and Singh, 2007).

Free Alpha Amino Nitrogen (FAAN) of all samples was around 0.835 mg/g, except for the soybean-maize hydrolysate, which had a FAAN of 2.13 mg/g. This parameter allows the determination of the hydrolysis degree of a protein by the content of free *α*-amino groups and is closely related to the size or molecular weights of peptides. FAAN directly influences the water solubility (WS) of a protein and, thus, the functional properties in general.

Heat treatment on proteins destroys anti-nutritional components such as amylase and trypsin inhibitors in legumes, thus improving the bioavailability of nutrients and mainly the rate of protein digestibility. The urease activity (UA) allows determining if the heat treatment used to obtain legume-based protein flours was adequate.

The ideal UA of a protein flour is 0.05 to 0.2 units. An activity above 0.2 indicates a poor heat treatment when the material will be used as food or food ingredient, whereas the activity of less than 0.05 indicates that the samples were over-processed, and possibly the protein quality of the protein is damaged (WHO, 2014). 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, so the processing was adequate. Vasconcelos *et al.* (1997) reported

for soybean proteins, a UA of 0.109 and 0.252 for a commercial and a Brazilian variety named Bays, respectively.

The physicochemical characteristics of a vegetable protein are essential because they influence their functionality and, thus, performance in food systems.

Table 4-7. Physical and chemical characterization of isolates and hydrolysates of soybean-maize and soybean proteins [1].

Parameter	Vegetable proteins			
	Soybean isolate (SI)	Soybean-maize isolate (SMI)	Soybean hydrolysate (SH)	Soybean-maize hydrolysate (SMH)
pH	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)	0.095±0.001^{a}	0.010±0.00^{d}	0.018±0.001^{c}	0.021±0.001^{b}
FAAN (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^{b}	0.10±0.01^{b}

[1]Means are the average of at least three replicas ± standard deviation. Means with different letter(s) within rows are statistically different ($P < 0.05$). EC= Electrical Conductivity; P = Protein; RS = Reducing Sugars; FAAN = Free Alpha Amino Nitrogen; UA = Urease Activity. Dry basis= db.

4.3.2. Functional analysis of vegetable proteins

Table 4-8 shows the functional characterization of isolates and hydrolysates of soybean proteins and the soybean-maize mixture. The functional properties provide information on the physicochemical behavior of the protein in a food system. 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-).

The WS determines the amount of protein and non-protein solids soluble in water 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 FAAN value. The SH has less FAAN 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 a higher albumin content have better solubility than counterparts with a high concentration in globulins (Kaur and Singh, 2007).

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 NaCl solution corresponding to globulins and small peptides. The solubility of SMI and SMH proteins was 25% and 41% higher than soybean, respectively (Table 4-8). However, the soybean isolate and hydrolysate solubility did not show significant differences ($p>0.05$). The NSI determined for the analyzed proteins coincides with that reported for vegetable proteins. Wolf, (1970) reported that the NSI values of soybean flours concentrate and isolates ranged between 10% and 90%. Paredes-López *et al.,* (1991) 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 (Sánchez-Vioque., 1999, Boye et al., 2010; Joshi *et al*., 2011). 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.

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. Because 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. 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*., (1991) values for a soybean isolate (5.7 mL/g protein). For other legumes such as peas, lentils, chickpeas, and Navy beans, WAI values of 3.7, 3.7, 2.9, and 3.8 g H_2O/g protein were reported, respectively (Toews and Wang, 2013). Proteins with low water absorption are ideal for developing products with high lipid interactions, such as dressings.

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. Paredes-López *et al.,* (1991) previously reported an FAI of 1.9 mL/g for a soybean protein isolate, whereas values obtained herein were higher likely due to the nature of the sample. Toews and Wang, (2013) reported for peas, lentils, chickpeas, and Navy beans, FAI 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 yield, texture, mouthfeel, and flavor retention of foods.

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 (Pearce and Kinsella, 1978). Table 4-8 shows that 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 (1989) report, who observed that the high carbohydrate content of maize germ protein favored the EAI. Concerning the ES, the SH was more stable at 24 hours of storage, followed by the soybean-maize hydrolysate, soybean-maize isolate, and finally, soybean isolates with 43.4% stability. However, at 48 hours 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 (Wilde 2000; Ma *et al*., 2011). Given the EAI of soybean-maize proteins, these are interesting alternatives to replace active tension agents used in the food industry for beverages, sauces, dressings, meat analogs, and others.

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 (Day, 2013). The FA of both the soybean isolate and hydrolysate were higher than the soybean-maize. Some authors have related the FA with solubility and the ability to unfold and refold a protein around the air-water interface (Kaur and Singh, 2007). However, the less soluble soybean proteins had higher foaming capacity, possibly due to the structure, flexibility, charge density, and electrostatic repulsions (Kinsella, 1969). Susheelamma and Rao (1974) reported that the foaming activity of Black Gram (*Phaseolus mungo*) proteins depended on the globulin nature.

Foam stability (FS) refers to the ability of a protein to reduce surface tension and form robust interfacial membranes via air-protein-water interactions (Day, 2013). 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. Toews and Wang (2013) observed that like 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, besides

excellent stability, presented higher density than soybean, *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.

Heat Coagulation Capacity (HCC) determines the potential of the proteins to form aggregates unorganized produced by denaturation, where the protein-protein interactions predominate concerning the protein-solvent interactions (Tang *et al.,* 2006). The SMH had the highest coagulating capacity of 83.06% and showed no significant difference with the soybean hydrolysate ($p>0.05$). Factors as temperature, polarity, ionic strength, and pH influence coagulation; however, these parameters remained standard for all samples. Therefore, the coagulation of soybean-maize and soybean proteins depended on the structure, size, and type of interaction (electrostatic and hydrophobic; Ma *et al*., 2011).

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*., (2012), 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 suited to develop analogs of cheeses, yogurts, creams, beverages, dressings, puddings, jellies, jams, and others.

Table 4-8. Functional properties of isolates and hydrolysates of soybean-maize and soybean proteins[1].

Parameter	Vegetable proteins			
	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}
WSI (%)	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)	15587.50±617.70^{c}	62972.51±3408.02^{a}	53025.17±3421.29^{b}	65097.04±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}

[1]Means are the average of at least three replicas ± standard deviation. Means with different letter(s) 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. Albumin Foaming Activity = 200±10% and Albumin Foam Stability= 33±2% (Komakina and Míková, 2005).

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 4-7). However, although they are different, they present slight similarities in the ranges distribution between samples from the same treatment for example: soybean isolates with soybean-maize isolates and soybean hydrolysates with soybean-maize hydrolysates.

Figure 4-7. Comparison of ranges among isolates and hydrolysates of soybean and soybean-maize by the Kruskal–Wallis test ($p < 0.05$).

4.3.3. Electrophoretic profile

The isolate and hydrolysate of soybean and soybean-maize proteins were analyzed by SDS-PAGE. As shown in Figure 4-8, 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 (A_3, y A_{1a}, A_{1b}, A_2 y A_4 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*., (2011) on 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*., (2002) reported that the glycinin fraction had higher solubility than *β*-conglycinin and that soybean proteins provided better foaming stability as determined for this sample.

The SMI presented a profile similar to soybean but exhibited four additional bands to the typical pattern of 56, 55, 52 and 18 kDa that could be proteins provided by the maize germ. According to Parris *et al*., (2006), bands around 50 kDa correspond to maize globulins. Nakai (1980) 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 soybean-maize proteins. This result is consistent with reported by Zayas and Lin (1989), 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*., (2002), 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.

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 intensity of the acid subunits 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. The acidic and basic subunits of glycinin were partially hydrolyzed due to the presence of a higher number of unidentified fragments in the bottom of the gel. This hydrolysate had the highest solubility, and EAI was 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 (Khatib *et al*., 2002).

The profile of a model protein, such as soy protein, provides enough information to estimate its functionality, especially in terms of *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.

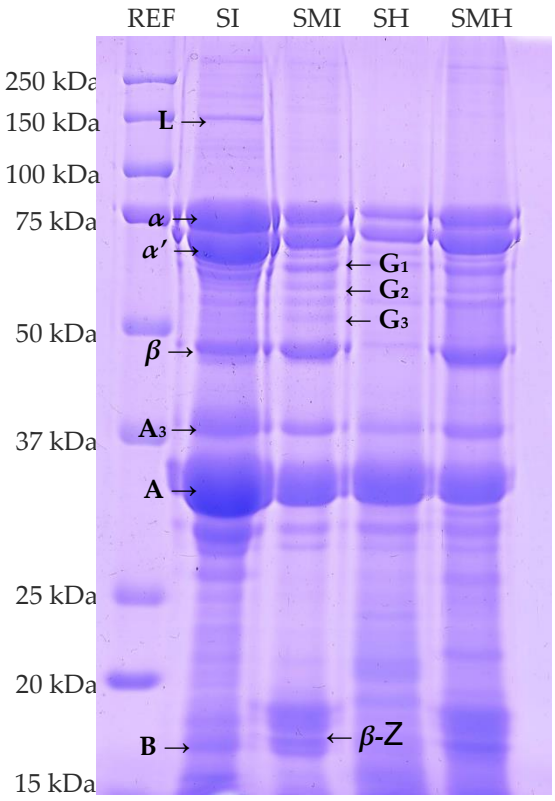

Figure 4-8. Electrophoretic profile of isolates and hydrolysates of Soybean-Maize and Soybean proteins (SDS-PAGE). Reference (REF); Soybean isolate (SI); Soybean-Maize isolate (SMI); Soybean hydrolysate (SH); Soybean-Maize hydrolysate (SMH). Typical electrophoretic pattern for Soybean protein: **L:** lipoxygenase; ***α, α'*** and ***β*** subunits of ***β***-conglycinin; **A_3:** acidic glycinin subunit; **A:** acidic glycinin subunits (A_{1a}, A_{1b}, A_2, A_4); **B:** basic glycinin subunit; **G_{1-3}:** globulins from maize germ; **Z:** *β*-Zein.

4.3.4. Amino acid composition

The amino acid composition determines the quality of proteins, as amino acids are the basic units of proteins. Table 4-9 shows the amino acid composition of soybean and soybean-maize proteins and included for comparison the aminogram of casein since it is considered the standard reference protein. Lysine content of soybean and soybean-maize proteins provided 112% and 104% of the requirement for 2 to 5-year-old infants, respectively. This content is comparatively slightly lower compared to casein. However, the experimental proteins complied with the FAO/WHO/UNU (1985) requirement for 2-5-year-old infants. The content of sulfur amino acids (Met and Cys) of soybean-maize protein is slightly below 100%, 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 a good nutritional quality and represented low-cost alternatives to provide amino acids necessary for adequate physical and mental development (Soria-Hernández *et al*., 2015).

Table 4-9. The amino acid score (AAS, %) of soybean, soybean-maize and casein proteins[1].

Essential amino acids (%)	Proteins Amino acid score (AAS, %)		
	Soybean[a]	Soybean-maize[b]	Casein[c]
His	136.84	134.74	142.11
Ile	164.29	166.79	175.00
Leu	119.70	117.73	127.27
Lys	112.07	103.97	122.41
Thr	114.71	99.12	108.82
Val	140.00	138.00	171.43
Try	118.18	138.18	127.27
Sulfur containing amino acid (Met + Cys)	**104.00**	**98.40**	**105.60**
Aromatic amino acid (Phe + Tyr)	130.16	140.48	158.73

[1]The score was calculated according to suggested requirements by FAO/WHO (2-5-year-old infants). [a] Day, 2013; [b] Data determined in the study; [c] Standard Tables of Amino Acid Composition of Food in Japan (The Resources Council, Science and Technology Agency of Japan, 1986).

4.4. Conclusions

The soybean-maize proteins are potential alternatives for application in food systems and for the development of high-protein food, as they showed better solubility and general functionality compared to the pure soybean counterparts. Besides, the soybean-maize protein mixture had 2.5% and 20.0% more isoleucine and tyrosine, respectively. In solubility, the isolate and the protein hydrolysate of the soybean-maize blend had higher solubility (10% and 52%) compared to the soybean counterparts. Emulsifying activity indexes were also higher in the soybean-maize isolate and hydrolysate (47,385.01 m^2/g and 12,071.87 m^2/g), whereas the emulsion stability at 48 hours 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%), whereas heat coagulation capacity of the soybean-maize isolate and hydrolysate was higher (36.3% and 1.2%) compared to 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 profiles showed four additional bands compared to the typical soybean pattern of 56, 55, 52 and 18 kDa. These corresponded to globulins associated with maize germ, which completed the amino acid score (AAS) of the isolates and hydrolysates, reaching the requirements established by the FAO/WHO/UNU (1985) for 2-5-year-old infants.

The functionality and nutritional value of this protein blend could be adequate for the development of products for children under 5 years of age, as breakfast drinks, for example.

The soybean-maize protein blend has the potential to meet nutritional requirements. However, new blends of cereals and other grains such as soybean and quinoa can be explored to provide better protein options to the consumer.

Chapter 5

5. Selection of Most Appropriate Conditions to Hydrolyze a Novel Mixture of Soybean-Maize Protein in Terms of Solubility

Draft in the editing process to send for publication

Abstract

Enzymatic hydrolysis is an efficient process to improve various functional properties and increase the field of application of plant proteins. In this study, the most suitable conditions were determined regarding the type of enzyme, enzyme concentration and reaction time for the hydrolysis of the soybean-maize protein in terms of solubility. For this, the process was carried out in two stages: in the first dispersions of native soybean-maize protein at 3% were hydrolyzed using neutrase (45 °C and pH 6.5), papain (70 °C and pH 6.0) and the mixture of them (60 °C and pH 6.0) in different concentrations (0.064, 0.5 and 1%) during 90 minutes with cysteine (absence or presence). In the second stage the protein was hydrolyzed with neutrase at different concentrations (0.064, 0.15, 0.30, 0.45 and 0.5%) and hydrolysis times (30, 60 and 90 minutes). This study determined that cysteine had no significant effect on the degree of hydrolysis and that neutrase is the most convenient enzyme to hydrolyze soybean-maize protein in terms of solubility. Using neutrase at a concentration of 0.45% and a reaction time of 30 minutes, the highest solubility of 49.99% was obtained. In these conditions, the protein dispersion had free amino nitrogen of 5.03 mg/g and a viscosity of 9.6 cP. Therefore, the hydrolysis with neutrase increased the solubility of the soybean-maize protein by 34.33% compared to the unhydrolyzed sample, expanding the possibilities of being integrated in beverage development.

Keywords: solubility, hydrolysis, neutrase, papain and soybean-maize.

5.1. Introduction

Plant proteins, particularly those from cereals and legumes, are nutritive and less expensive than animal sources. Its use as ingredients in food products relies on various functional properties (Toews and Wang, 2013). Solubility may be the most relevant property because of its significant influence on other functional characteristics such as hydration and foaming (Yalçin and Çelik, 2007). These features affect the stability and behavior of the food system. Some plant proteins display a limited solubility, making necessary structural modification through enzymatic, physical and/or chemical treatments (Yin *et al*., 2011). Enzymatic hydrolysis is a more suitable treatment than the chemical counterpart because of milder process conditions. Also, enzyme catalysis is more specific and reduces the yield of byproducts. Enzymatic hydrolysis is an effective way to improve various functional properties and increase the application of proteins. Proteolysis increases protein-water interactions decreases molecular weight, simplifies the secondary structure, increases the number of ionizable groups, and exposes hydrophobic groups, which can change the physicochemical interactions of the protein with the medium (Tavano, 2013). This process increases a protein's negative surface charge and increases solubility and improves other functional properties such as foaming, emulsifying and coagulating capacity (Sun *et al*., 2011).

The neutrase and papain are some of the most used proteases in the food industry due to their easy operation, low cost, and specificity. Neutrase is specific for hydrophobic amino acids such as leucine and phenylalanine and is active at 40-50 °C and pH 6-7, while papain shows an affinity for lysine, arginine and phenylalanine, and is active at 70 °C and pH 6 (Benítez *et al*., 2008; Ou *et al.,* 2010). Some research shows the effect of hydrolysis on solubility, and such is the case of Hou and Zhao (2011), who hydrolyzed a soy protein using neutrase and determined that the solubility increased 84.78%. At the same time, Schlegel *et al.* (2019) hydrolyzed lupine protein with alcalase and determined an increase in solubility of 12%.

The peptides obtained by proteolysis have a smaller molecular size and less secondary structure than native protein, and therefore they have enhanced solubility. The

applications of protein hydrolysates are very varied such as nutritional supplements, functional ingredients, flavor enhancers, coffee whiteners, confectionery products and fortification of soft drinks and juices. Besides, they are used in the cosmetic and medical areas (Bandyopadhyay and Ghosh, 2002).

Therefore, the objective of this study was to select the most suitable conditions regarding the type of enzyme (neutrase and papain), enzyme concentration and reaction time to hydrolyze soybean-maize protein in terms of solubility.

5.2. Materials and Methods

5.2.1. Protein extraction and hydrolysis

Analyzed samples were soybean-maize protein hydrolysates. The experimental mixtures of soybean-maize proteins were extracted using a standard procedure of alkali extraction followed by acid precipitation (Riaz, 2006). Briefly: the pH of a finely ground 30 kg mix of defatted soybean flour: maize germ (in a proportion 5:1 within ten parts of water) was adjusted to pH 10 with NaOH 50% w/w. Contents were mixed for 30 minutes at 50 °C before the separation of bagasse using an industrial centrifuge (Westfalia SA14) operated at 15 L/min and 5,500 xg. The supernatant was then collected, and the pH was adjusted to 4.5 with 3 N 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 kg_f/cm^2). For the hydrolysis of soybean-maize protein, enzymatic hydrolysis was performed in two stages: in the first was used neutrase (from *Bacillus subtilis*; Novozymes A/S, Bagsvaerd, Denmark), papain (from *Carica papaya*; Cosmopolitan drugstore, Benito Juárez, Mexico City) and mixture (in a proportion 1:1) at different enzyme concentrations (0.064, 0.50 and 1.0%), and 50 ppm of free cysteine (presence or absence) for a reaction time of 90 minutes. The soybean-maize protein dispersions in water were at 3% (w/v). The hydrolysis process was carried out with

neutrase (45 °C and pH 6.5), papain (70 °C and pH 6.0) and the mixture of these (60 °C and pH 6.5). The hydrolysis was stopped with pH adjustment to 5 with 2 N hydrochloric acid (HCl). Finally, the samples at a temperature of 25 °C were analyzed by free alpha-amino nitrogen and viscosity with methodologies described in the next sections. In the second stage, hydrolysis was carried out using neutrase only and varying the concentration (0.064, 0.150, 0.30, 0.45 and 0.50%) and the hydrolysis time (30, 60 and 90 minutes) to make the analysis more precise. After hydrolysis, the reaction was stopped and analyzed as mentioned above.

Finally, a validation kinetics of the best hydrolysis conditions was carried out. For this validation, 3% soybean-maize protein dispersions were hydrolyzed with 0.45% neutrase at 45 °C, pH 6.5 for 10 hours of hydrolysis with samplings every 30 minutes.

5.2.2. Determination of physicochemical parameters

Moisture (AOAC method 934.06), crude protein (AOAC Method 984.13-1994), reducing sugars (RS; Miller, 1959), and free alpha-amino nitrogen (FAAN; AOAC method 945.30-1945) were measured in soybean-maize protein hydrolysates. The electrical conductivity (EC) and pH of the samples were measured using a potentiometer (Hanna-250, Padova, Italy).

5.2.3. Amino acid composition

The total amino acid composition was analyzed in soybean-maize protein hydrolysates using the AOAC official method 994.12 (AOAC, 2005).

5.2.4. Viscosity

To measure the viscosity of the soybean-maize protein mixture, 20 mL of 3% protein dispersion (w/v) were placed in a 50 mL beaker. Viscosity was measured at 25 °C in a

Brookfield Viscometer RVT using a Helipath stand, RV-4 spindle model, at 100 rpm. Values were expressed as centipoises (cP).

5.2.5. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE of soybean-maize protein hydrolysates was performed on a 5% stacking gel and 10% separating gel with a discontinuous buffer system according to the method described by Laemmli (1970). Briefly, the dispersions to 2% were dissolved protein loading buffer for SDS-PAGE in the ratio 1:1. 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). 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, UK).

5.2.6. Statistical analysis

The hydrolysis process of soybean-maize protein was analyzed using a full factorial design. The first stage was composed of three factors with different levels each: type of enzyme with three levels (neutrase, papain and the mixture), enzyme concentration with three levels (0.064, 0.50 and 1.0%) and the use of cysteine with two levels (presence or absence). In the second stage, a full factorial design was used only two factors concentration with five levels (0.064, 0.150, 0.30, 0.45 and 0.50%) and reaction time with three levels (30, 60 and 90 minutes). In the two stages, the significant differences between means were determined with Tukey's multiple comparison test at a 5% significance level (Minitab 16, USA).

5.3. Results and discussion

5.3.1. Physicochemical characterization of soybean-maize concentrate

The physicochemical characteristics of the soybean-maize protein concentrate used for enzymatic hydrolysis are presented in Table 5-10. The protein and free alpha-amino nitrogen (FAAN) content were 68.38±1.260% (db) and 0.903±0.017 mg/g, respectively. The electrical conductivity of the concentrate was 2.47±0.050 mS/cm, while the concentration of reducing sugars was 0.036±0.001 mg/g. The concentration, type of enzyme, and cysteine use as a facilitating agent of hydrolysis were evaluated to select the ideal conditions to hydrolyze the soybean-maize protein mixture.

Table 5-10. Physicochemical characterization of native soybean-maize protein concentrate[1].

Parameter	Value
Protein (% db)	68.38 ± 1.260
Moisture (%)	6.08 ± 0.370
pH	5.89 ± 0.007
Electrical conductivity (mS/cm)	2.47 ± 0.050
Reducing sugars (mg/g)	0.036 ± 0.001
Free Alpha Amino Nitrogen (mg/g)	0.903 ± 0.017

[1]Means are the average of at least three replicas ± standard deviation. Db: dry basis

5.3.2. Amino acid composition of soybean-maize concentrate

The soybean-maize concentrate has a good amino acid profile since the content of lysine, threonine, leucine and tryptophan was 6.03%, 3.37%, 7.77% and 1.52%, respectively (Table 5-11). Wang *et al*., (1999) reported 7.10%, 3.70%, 8.40% and 1.40% for casein for the same amino acids, and it was observed that the soybean-maize protein had a higher tryptophan content compared to casein. The content of methionine and cysteine was 2.46% for soybean-maize protein, while for casein, it was reported 2.64%; these sulfur amino acids are essential for the proper functioning of the skin, hair, ligaments, and tendons since sulfur is a mineral necessary for the formation of collagen, keratin, mucopolysaccharides, and others. (Wang *et al.,* 1999).

Table 5-11. Amino acid profile of soybean-maize protein*.

Essential amino acids (%)		Non-essential amino acids (%)	
Arginine	7.48	Alanine	3.98
Histidine	2.56	Aspartic acid	11.13
Isoleucine	4.67	Glutamic acid	17.15
Leucine	7.77	Glycine	4.05
Lysine	6.03	Proline	4.91
Methionine	1.29	Serine	3.94
Cystine	1.17	Taurine	0.01
Phenylalanine	5.21	Lanthionine	0.09
Tyrosine	3.64	Hydroxylysine	0.04
Threonine	3.37	Ornithine	0.15
Tryptophan	1.52	- - - - -	- - - - -
Valine	4.83	- - - - -	- - - - -
Total sulfur-	2.46	- - - - -	- - - - -
Total aromatic	8.85	- - - - -	- - - - -
Total	49.54	**Total**	45.45

* All values are expressed as % (g of amino acid per 100 g of protein).

5.3.3. Selection of proteolytic enzyme, concentration range and feasibility of the use of cysteine

The hydrolysis of the soybean-maize protein was carried out with neutrase, papain and a mixture of these at 50% (w/v) in concentrations of 0.00%, 0.064%, 0.50% and 1.0% (w/v) with a hydrolysis time of 90 minutes. To evaluate the degree of hydrolysis of each enzyme, the free alpha-amino nitrogen was measured, which determines the free amino acids or small peptides and, therefore, the degree of hydrolysis, solubility, and water absorption capacity of the proteins. Hydrolysis was carried out in the presence and absence of cysteine (Figure 5-9).

Free cysteine was proposed as a donor agent for thiol groups to induce the oxidation of the disulfide bonds between the cysteine's anchored in the amino acid chain of the soybean-maize protein through a thiol-disulfide exchange reaction, this to denature and hydrolyze the protein and increase the degree of hydrolysis and therefore the solubility (Nielsen *et al*., 2018).

Figure 5-9 shows the degree of hydrolysis of the soybean-maize concentrate hydrolyzed with neutrase, papain and the 50% mixture. In the hydrolysis with neutrase, the FAAN increased from 0.86 to 5.91 mg/g as the enzyme concentration increased from 0.0% to 1.0%. The samples hydrolyzed with neutrase showed the highest degree of hydrolysis compared to papain and mixed in all concentrations in cysteine presence and absence.

The highest degree of hydrolysis achieved with neutrase was 5.91 mg/g of FAAN in the absence of cysteine; in all other concentrations, there was no significant difference ($p<0.05$). However, at 1% neutrase and in the presence of cysteine, FAAN was slightly reduced, possibly because the higher concentration of enzyme generated peptides that, when reacting with free cysteine, highly reactive, promoted the polymerization of the amino acid chain instead of its hydrolysis. Several authors have already mentioned this type of aggregation; Zhu and Labuza (2010) reported the aggregation of whey proteins by the thiol-disulfide exchange reaction and/or non-covalent interactions at high enzymatic concentrations. This reduction in the degree of hydrolysis could also be due to interactions between the peptides generated. Xu *et al.*, (2011) reported for a casein

hydrolysate that due to the specificity of neutrality, some hydrophobic amino acids such as leucine and phenylalanine in some peptides could be covalently bound to other peptides by transpeptidation or condensation.

According to Zhao and Hou (2009), the higher degree of hydrolysis generated by neutrase could be due to the number of related amino acids that the soybean-maize concentrate has since its cutting site is on hydrophobic amino acids such as leucine and phenylalanine. This protein has 12.98% of these amino acids available for enzyme-protein interaction (Table 5-11).

Regarding the soybean-maize protein concentrates hydrolyzed with papain, the highest degree of hydrolysis was 4.76 mg/g of FAAN at an enzymatic concentration of 0.064% (w/v) in the presence of cysteine. The samples with and without cysteine did not show significant differences ($p<0.05$). At low concentrations, papain had a degree of hydrolysis similar to that of neutrase; this could be because soybean-maize protein contains 18.73% of amino acids derived from *L*-acyl-N such as *L*-arginine, *L*-lysine and *L* -phenylalanine, for which papain is specific (Wan *et al.,* 2013).

The hydrolyzed concentrates treated with 0.5% and 1% (w/v) of papain presented around 50% less FAAN, and there were no significant differences between concentrations with/without cysteine ($p>0.05$). This considerable reduction in FAAN, as the enzyme concentration increased, was possibly due to product inhibition. When the product concentration increases, a more significant proportion of enzyme is immobilized as an enzyme-product complex, decreasing the reaction rate. Several authors have reported this kinetic effect; Humiski and Aluko, (2007) reported that when using a high concentration of papain to hydrolyze pea proteins, the degree of hydrolysis was reduced due to inhibition because of the high peptides concentration.

Regarding the mixture of neutrase and papain in a 1:1 ratio, the highest degree of hydrolysis was 6.25 mg/g of FAAN at a concentration of 1% of a mixture of neutrase-papain in the absence of cysteine. Using cysteine, this same condition showed a 25.88% reduction in the degree of hydrolysis, possibly due to a protein aggregation promoted by cysteine as mentioned previously in the hydrolysis with neutrase.

Of the three enzymatic treatments, the highest degree of hydrolysis obtained was that of the soybean-maize protein concentrate hydrolyzed with the 1% neutrase-papain mixture without cysteine. However, this treatment did not show a significant difference with the degree of hydrolysis obtained only with 1% neutrase without cysteine ($p>0.05$).

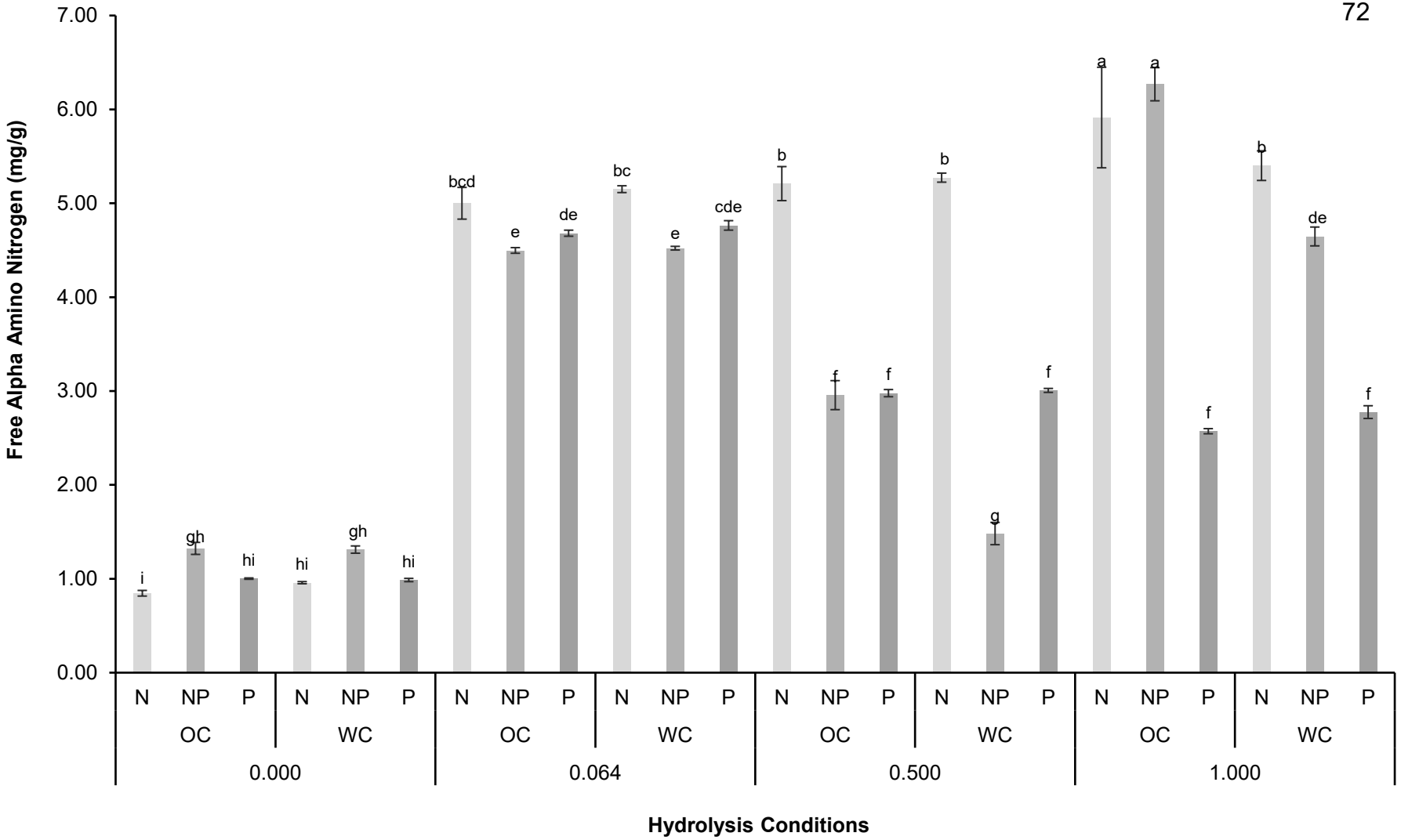

Figure 5-9. Free alpha-amino nitrogen content of soybean-maize protein hydrolyzed with neutrase (N), neutrase-papain mixture (NP) and papain (P) at different enzymatic concentrations (0.0, 0.064, 0.50 and 1%) in the absence and presence of cysteine (without/ with cysteine -OC/WC-) for 90 minutes. Mean values are the average of at least three replicates ± standard deviation. Mean values with a different letter(s) on the bar are statistically different ($p<0.05$).

The solubility is the most important functional property of proteins since it determines the performance of the protein with respect to foaming, emulsifying and coagulating capacity. The proportion and distribution of surface hydrophobic (protein-protein) and hydrophilic (protein-solvent) interactions with water determine the solubility of proteins. The generation of small peptides increases the hydrophilic ratio and, therefore the solubility (Jung *et al*., 2004).

The solubility of the soybean-maize concentrates treated with 0.064%, 0.50%, and 1% of neutrase showed an increase of 44.69%, 163.78% and 142.59%, respectively. This increase in solubility was probably because when the enzyme concentration increased, the degree of hydrolysis increased; the molecular weight of the protein was reduced, the load increased, and the exposure of hydrophilic groups increased, favoring the solubility of the proteins (Ou *et al*., 2010).

The highest solubility of the soybean-maize hydrolysate proteins was 52% and was obtained at a neutrase concentration of 0.50% and in the absence of cysteine (Figure 5-10). In the soybean-maize samples hydrolyzed with neutrase, the use of cysteine did not present a significant difference ($p>0.05$), so its application is not necessary. Likewise, it was observed that the solubility of the 1% neutrase hydrolyzed concentrate was lower despite having a higher degree of hydrolysis; this was probably due to the aggregation of proteins promoted by cysteine.

Regarding the soybean-maize concentrates hydrolyzed with papain, the highest solubility was 52.97% and 51.13%, and they were obtained with 0.50% enzyme in the presence and absence of cysteine, respectively, and there was no significant difference between the samples ($p>0.05$). The samples treated with 0.50% and 1% papain presented higher solubility despite having a lower degree of hydrolysis; this may be due to the size of the peptides generated and the polarity exposed (Noman *et al*., 2017).

The highest solubility obtained from the hydrolyzed soybean-maize concentrates with the neutrase-papain mixture was 49% with 0.50% enzyme concentration and in the absence of cysteine. In the samples hydrolyzed with the mixture, the solubility was reduced in all samples assisted with cysteine with respect to its homology; this probably due to the aggregation of peptides promoted by cysteine (Niesel *et al*., 2018).

The soybean-maize hydrolysate with the highest solubility was obtained with the enzyme neutrase at a concentration of 0.50% in the absence of cysteine; this could be because the specificity of the neutrase generates smaller peptides less than 10 KDa, which increases the interaction with water (Ou *et al*., 2010).

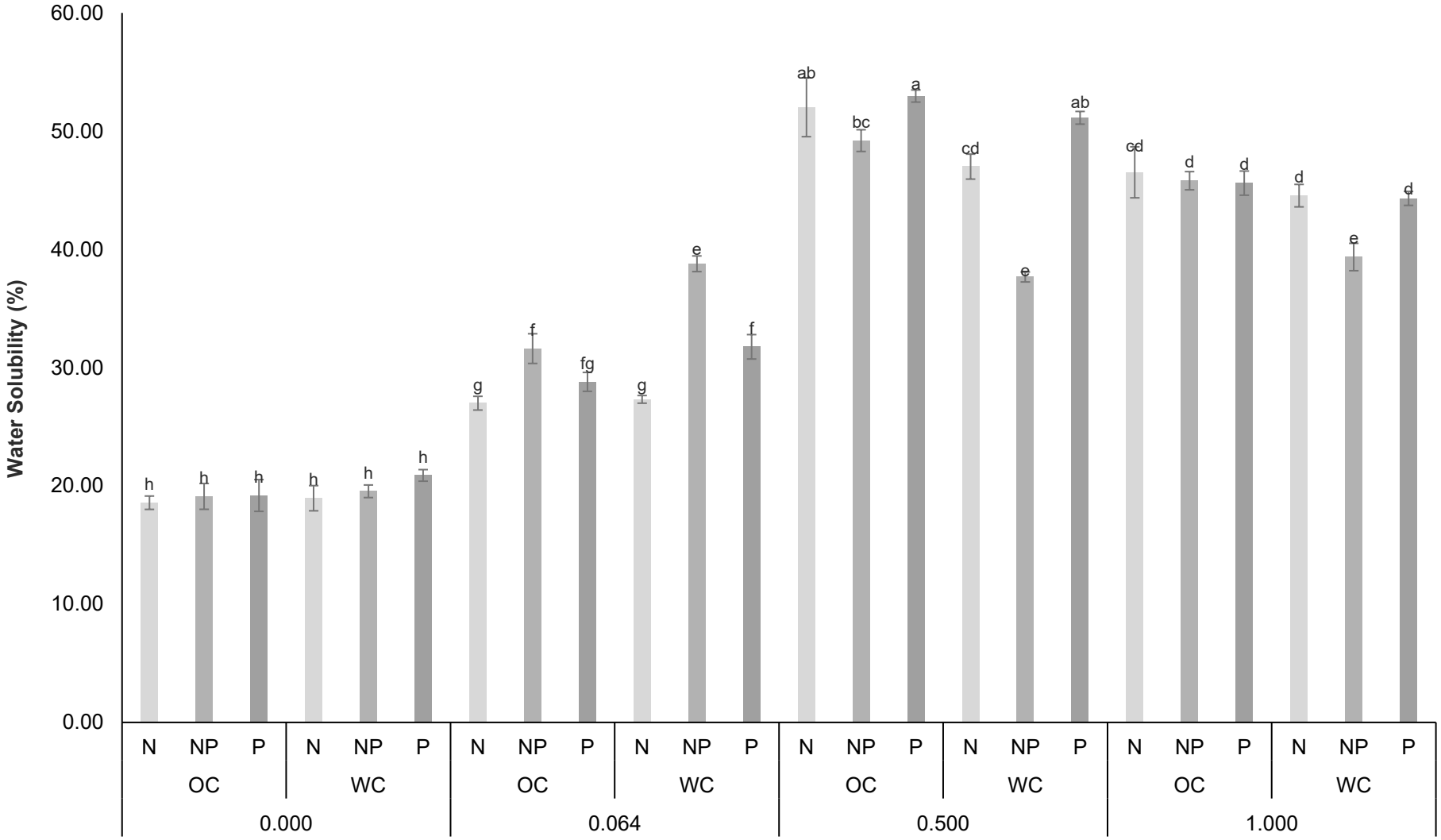

Figure 5-10. Water solubility of soy-maize protein hydrolyzed with neutrase (N), neutrase-papain mixture (NP) and papain (P) at different enzymatic concentrations (0.0, 0.064, 0.50 and 1%) in the absence and presence of cysteine (without/ with cysteine -OC/WC-) for 90 minutes. Mean values are the average of at least three replicates ± standard deviation. Mean values with a different letter(s) on the bar are statistically different ($p<0.05$).

Figure 5-11 shows the viscosity of the soybean-maize concentrates hydrolyzed with neutrase, papain and the mixture of both. The viscosity of the concentrates with neutrase and papain at time zero was around 18.0 cP for all controls with and without cysteine. In the case of concentrates treated with the neutrase-papain mixture, the viscosity at time zero was 16.0 and 15.0 cP with and without cysteine, respectively.

In all the soybean-maize concentrates treated with 0.064%, 0.50% and 1.0% of neutrase and the enzyme mixture, the viscosity decreased as the enzyme concentration increased. This decrease in viscosity was also observed in the concentrates hydrolyzed with papain in the concentrations of 0.064% and 0.50%, but not in the concentration of 1.0% of papain. In the samples treated at 1%, the viscosity was maintained at 81.05% and 77.74% with and without cysteine, with respect to the unhydrolyzed sample; this probably due to an inhibition by product since the viscosity was maintained in both conditions. Statistically, the viscosity of the soybean-maize concentrates hydrolyzed with neutrase, papain, and the enzyme mixture showed significant differences ($p<0.05$), the viscosity of the samples treated with papain being higher (Figure 5-11).

The change in viscosity of soybean-maize hydrolysates can be attributed to the rearrangement of proteins and/or destruction of the original structural configuration caused by enzymes. Puski *et al*., (1975) reported that the decrease in viscosity of a soybean isolate hydrolyzed with pepsin was related to the decrease in the molecular weight of the peptides.

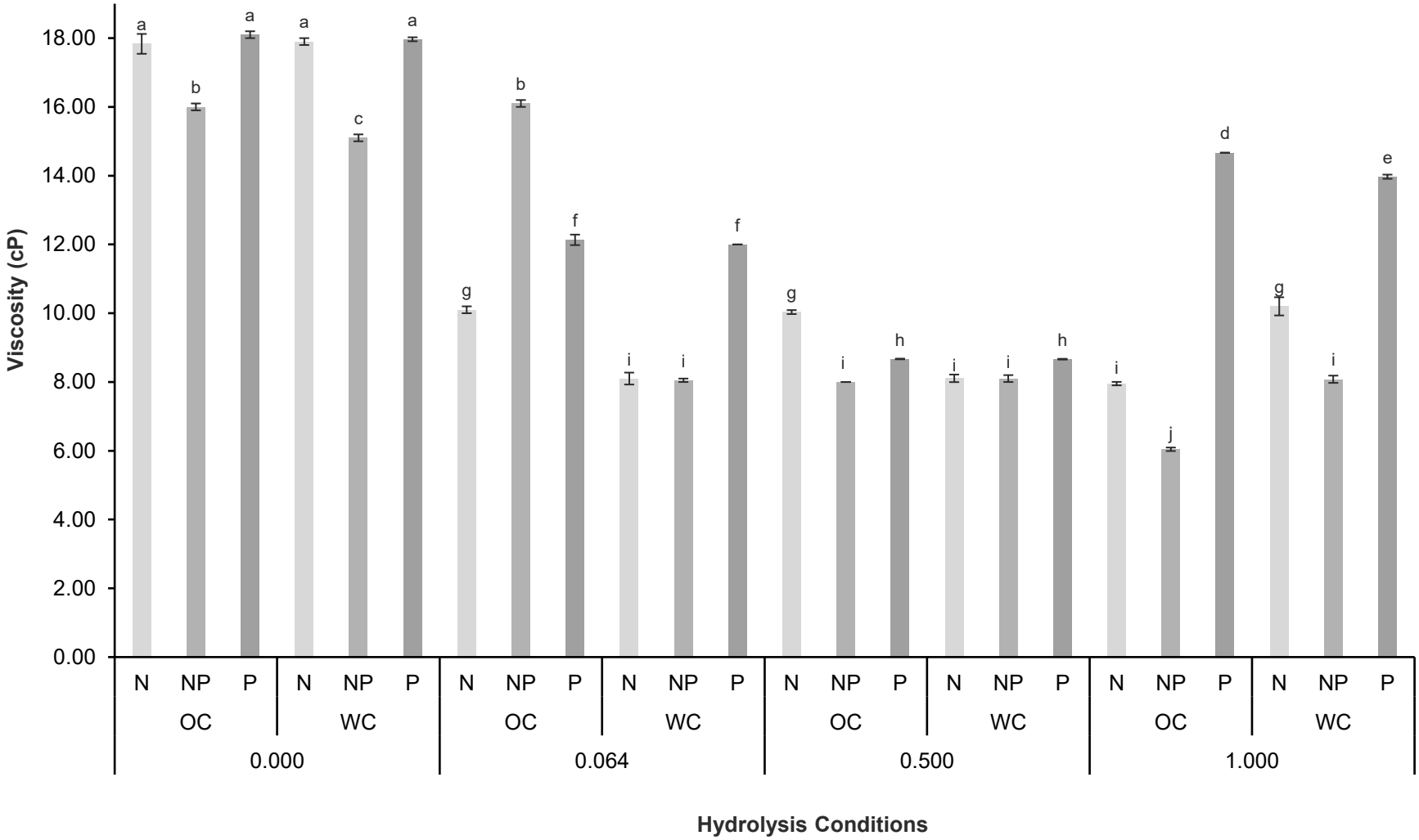

Figure 5-11. Viscosity of soy-maize protein hydrolyzed solutions treated with neutrase(N), neutrase-papain mixture (NP) and papain (P) at three different concentrations: 0.0, 0.064, 0.50 and 1% in the absence and presence of cysteine (without/ with cysteine -OC/WC-) for 90 minutes. Mean values are the average of at least three replicates ± standard deviation. Mean values with a different letter(s) on the bar are statistically different ($p<0.05$).

5.3.4. Selection of adequate enzymatic concentration and reaction time

According to the analysis of the type of enzyme (neutrase, papain or the mixture), the enzyme concentration (0.0%, 0.064%, 0.5% and 1%) and the feasibility of using cysteine to hydrolyze a soybean-maize concentrate, it was determined that the highest solubility was obtained at 0.5% of neutrase and papain in the absence of cysteine and that there was no significant difference between the samples. However, the degree of hydrolysis under these conditions was higher in the samples treated with neutrase. Therefore, neutrase was selected as the working enzyme as it did not present a difference in solubility with papain, it is easier to handle and more economical.

The hydrolysis time was 90 minutes for all tests, so it was decided to evaluate other reaction times, for which hydrolysis was carried out at 0, 30, 60 and 90 minutes. Likewise, it was decided to evaluate more ranges of enzymatic concentration below 0.5% of neutrase (0.0, 0.064%, 0.15%, 0.30%, 0.45% and 0.50%) in order to find the best conditions to hydrolyze the soybean-maize concentrate.

Figure 5-12 shows the degree of hydrolysis of the soybean-maize concentrate measured by free alpha-amino nitrogen. The highest degree of hydrolysis of the soybean-maize concentrate was 5.88 mg/g and was obtained at a neutrase concentration of 0.50% and a hydrolysis time of 30 minutes. The FAAN increased as the concentration of the neutrase increased from 0.064% to 0.50%.

Regarding the hydrolysis time, not always the most extended time generated the highest FAAN, only in the concentration of 0.064% that was 5.03 mg/g was obtained at 90 minutes, and this was similar to that obtained with 0.45% and 30 minutes of hydrolysis. Therefore, at low concentrations of enzymes, the degree of hydrolysis is higher at a longer time; however, at high concentrations of enzymes, it tends to cause inhibition by product, reducing the degree of hydrolysis and solubility ($p<0.05$). Kılıç and Özbek, (2007) determined that the best neutrase concentration to hydrolyze maize gluten was 0.40% for 1% protein for 120 minutes. In comparison, Jung *et al*., (2007) reported a degree of hydrolysis of 2% to 4% for soybean proteins hydrolyzed with 0.30% neutrase for 20 minutes.

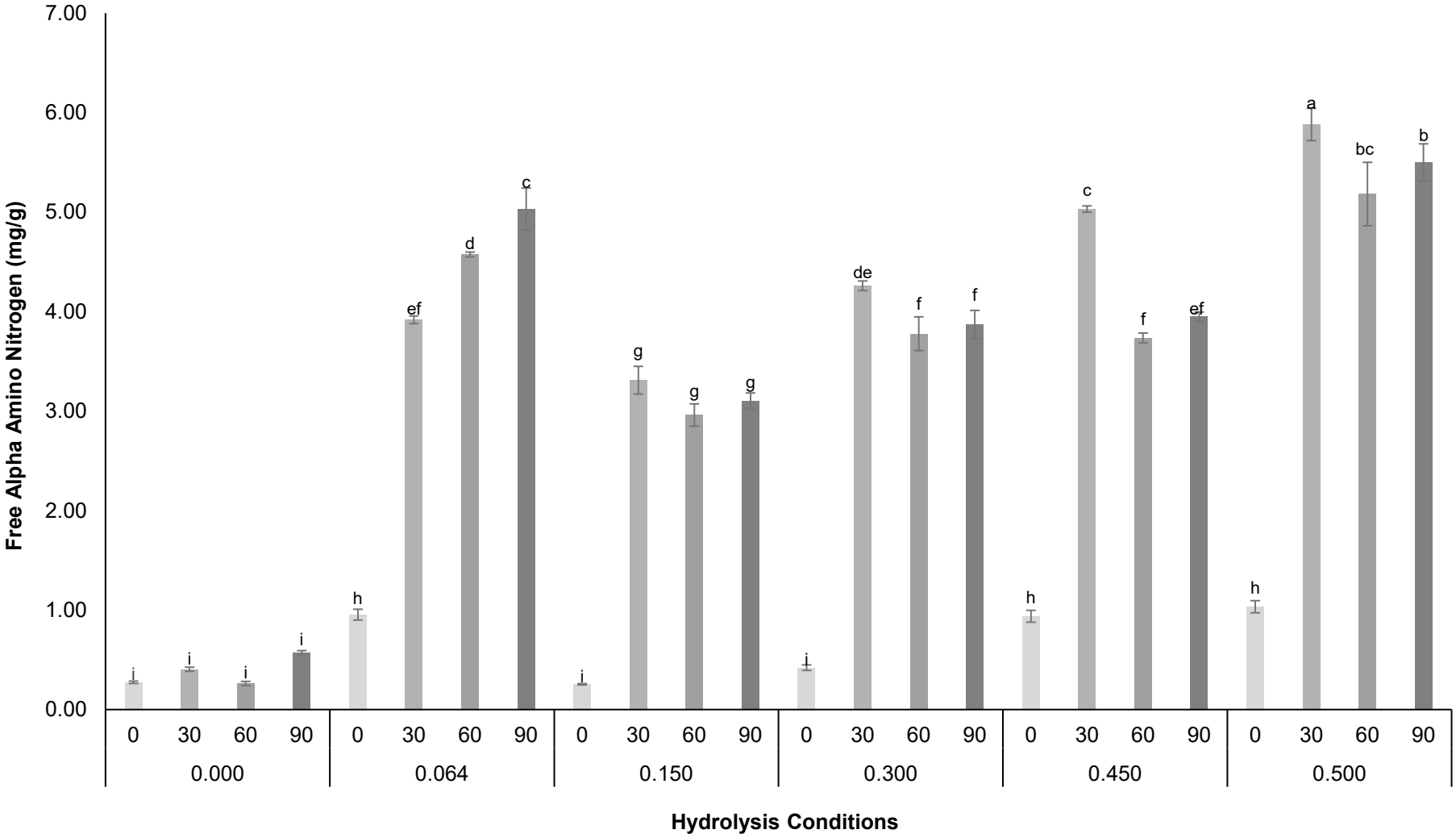

Figure 5-12. Free alpha-amino nitrogen content of soy-maize protein hydrolyzed with different concentrations of neutrase (0.0, 0.064, 0.15, 0.30, 0.45 and 0.50%) and hydrolysis times (0.0, 30, 60 and 90 minutes). Mean values are the average of at least three replicates ± standard deviation. Mean values with a different letter(s) on the bar are statistically different ($p<0.05$).

The highest solubility of the hydrolyzed soybean-maize concentrate was 52.82% and was obtained at an enzymatic concentration of 0.50% and 90 minutes of hydrolysis (Figure 5-13). However, this value did not present a significant difference with the one obtained at 0.45% neutrase and 30 minutes of hydrolysis, which was 49.99% ($p>0.05$). In general, the solubility increased as the enzymatic concentration increased; this can be attributed to increased protein hydrolysis and therefore a greater reduction of the molecular weight of the proteins, the formation of smaller peptides and to the release of carboxylic and amino groups from amino acids, which increase the hydrophilicity of soybean-maize protein (Noman *et al.,* 2017).

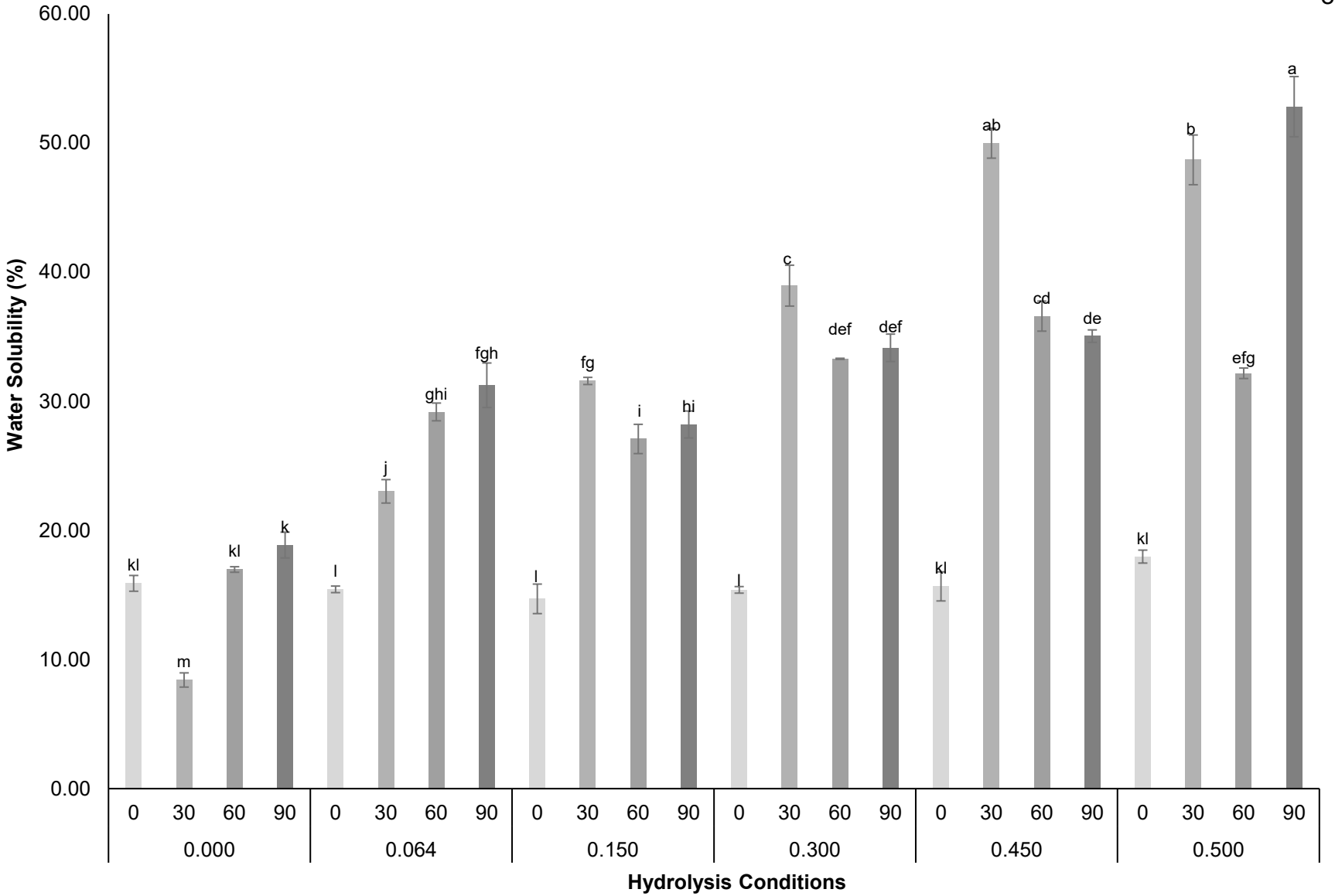

Figure 5-13. Water solubility of soy-maize protein hydrolyzed with different concentrations of neutrase (0.0, 0.064, 0.15, 0.30, 0.45 and 0.50%) and hydrolysis times (0.0, 30, 60 and 90 minutes). Mean values are the average of at least three replicates ± standard deviation. Mean values with a different letter(s) on the bar are statistically different ($p<0.05$).

The viscosity of hydrolyzed soybean-maize protein was generally higher at all hydrolyzed enzyme concentrations at 60 minutes of reaction time. This was because the structural changes in the proteins directly influence the viscosity, such as the case of the cut of the neutrase on the amino acid chain of the soybean-maize protein, which probably caused a reduction in the size of peptides generated and allowed more hydrophilic groups exposed, greater solubility and lower viscosity (Figure 5-14). The samples treated for 30 minutes did not show a significant difference in all enzyme concentrations ($p>0.05$). Proteins hydrolyzed for 90 minutes at all concentrations had higher viscosity than their homologs, possibly due to the inhibition of the enzymatic reaction by product.

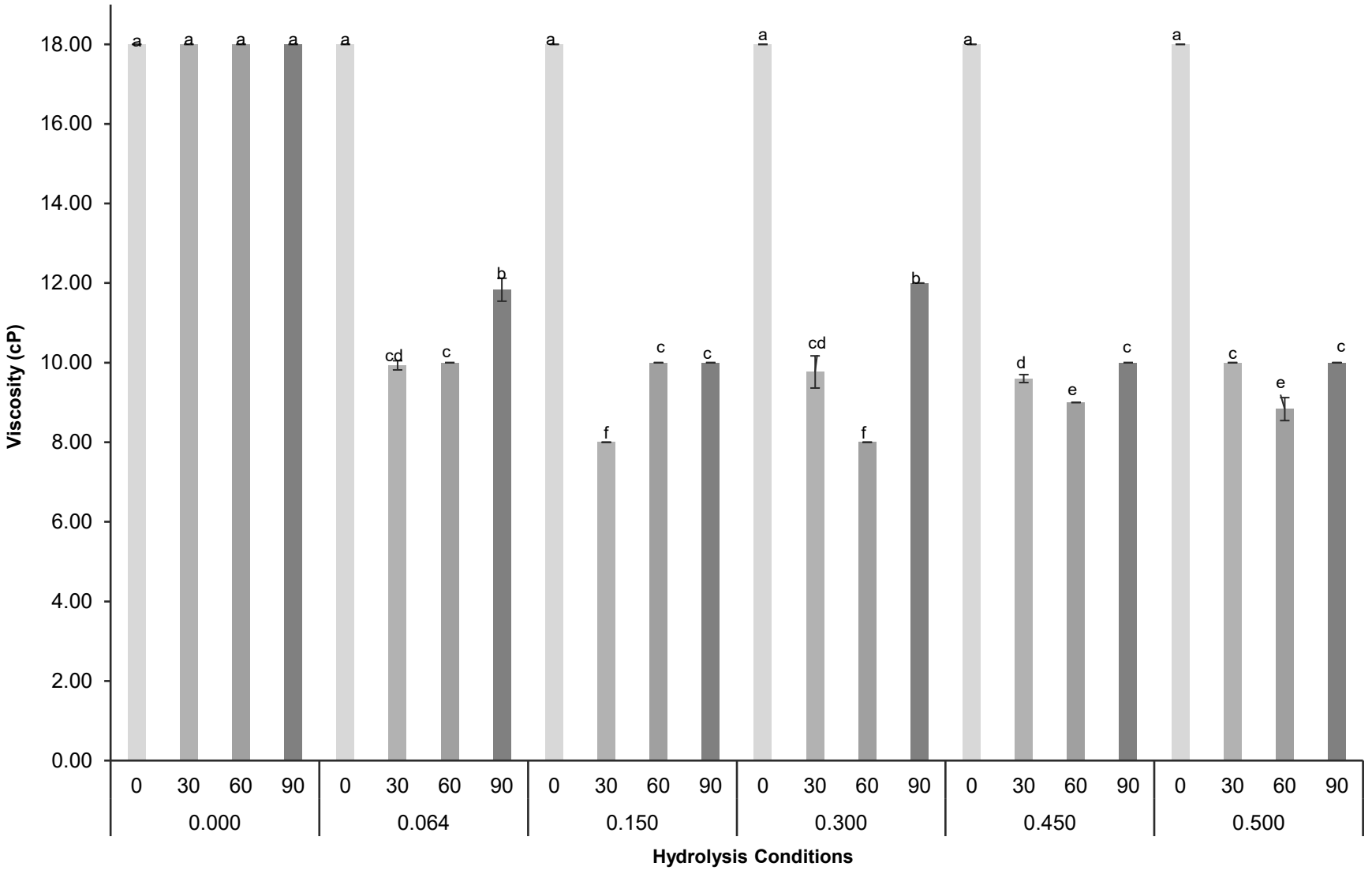

Figure 5-14. Viscosity of soy-maize protein hydrolyzed solutions treated with different concentrations of neutrase (0.0, 0.064, 0.15, 0.30, 0.45 and 0.50%) and hydrolysis times (0.0, 30, 60 and 90 minutes). Mean values are the average of at least three replicates ± standard deviation. Mean values with a different letter(s) on the bar are statistically different ($p<0.05$).

For all the above, the most suitable concentration to obtain a soybean-maize hydrolysate with good solubility was 0.45% neutrase with a hydrolysis time of 30 minutes because there was no significant difference between the solubility obtained in these conditions and with 0.50% at 90 minutes. Therefore, a lower enzyme concentration and a shorter hydrolysis time make it possible to obtain a soybean-maize hydrolysate with adequate solubility.

The determination of these parameters is essential to reduce production costs and make resources more efficient during the production of hydrolyzed proteins in the food industry.

5.3.5. Validation of the adequate conditions to hydrolysis

The most suitable conditions to hydrolyze a soybean-maize protein in terms of solubility were an enzymatic concentration of 0.45% of neutrase, a 30-minutes hydrolysis time, a temperature of 45 °C and a pH of 6.5. However, it was necessary to validate these conditions to confirm these parameters or rectify them.

Hydrolysis kinetics was carried out using a 0.45% neutrase concentration during 10 hours of hydrolysis, with measurements every 30 minutes. In Figure 5-15, it was observed that the FAAN at 30 minutes of hydrolysis was 7.08 mg/g, and the maximum was 10.91 mg/g that occurred at 10 hours of hydrolysis, and if there was a significant difference between the samples ($p<0.05$). In a hydrolysis time range of 3 to 8.5 hours, the FAAN did not show significant differences ($p>0.05$), so it can be seen that the degree of hydrolysis reached the kinetic plateau and remained stable for 5.5 hours.

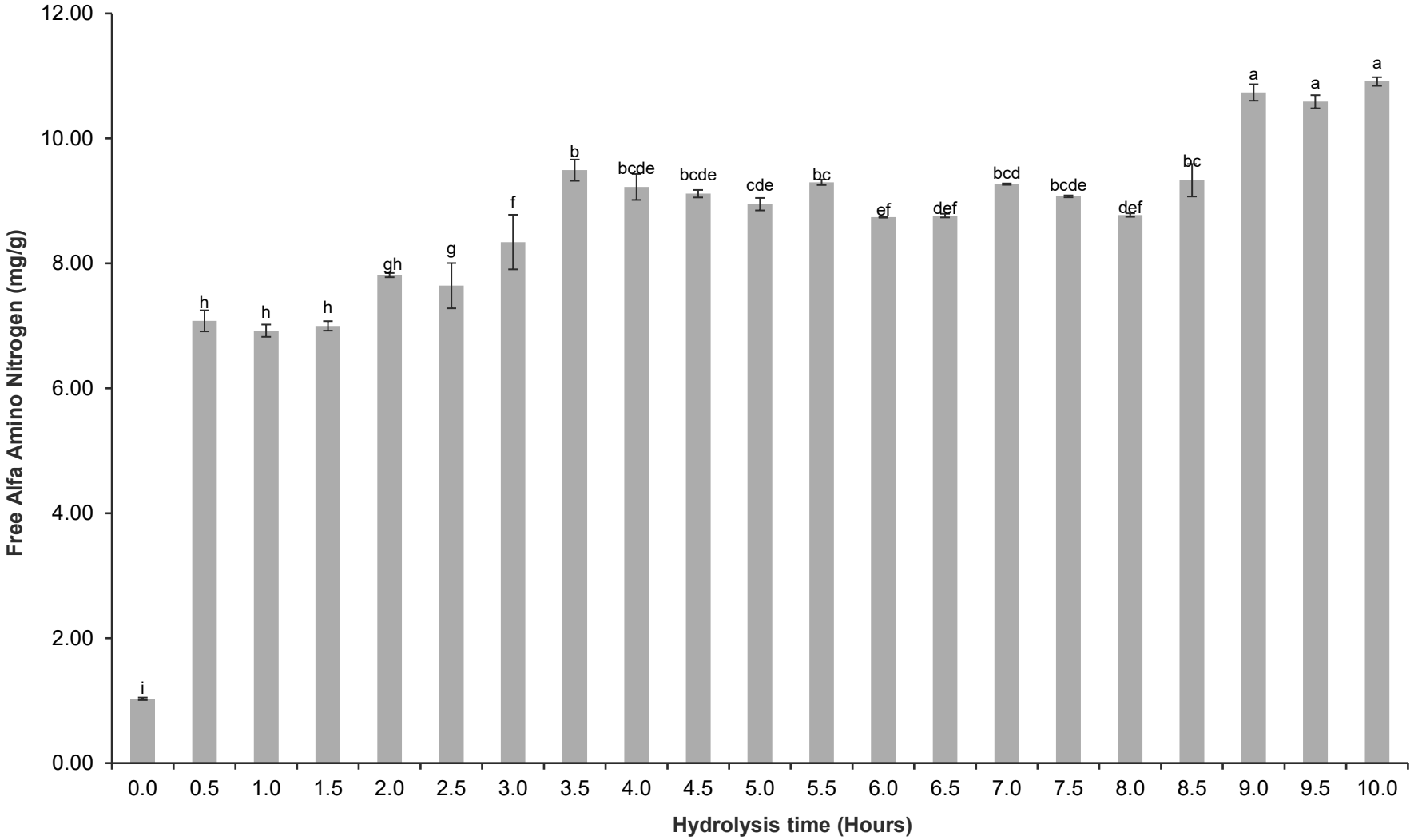

Figure 5-15. Effect of the hydrolysis time over free alpha-amino nitrogen content of soybean-maize protein hydrolyzed with neutrase at 0.45% (w/v) for 10 hours . Mean values are the average of at least three replicates ± standard deviation. Mean values with a different letter(s) on the bar are statistically different ($p<0.05$).

Regarding solubility, it ranged between 45.76% and 51.53% for 30 minutes and 10 hours of hydrolysis, respectively (Figure 5-16). This value agrees with the results observed in selecting the hydrolysis conditions for the soybean-maize concentrate, which was 49.99% water solubility during 30 minutes of hydrolysis (Figure 5-13). The solubility reached the plateau and remained constant during the 10 hours of hydrolysis since it did not show significant differences between the solubility during the 10 hours of treatment ($p>0.05$).

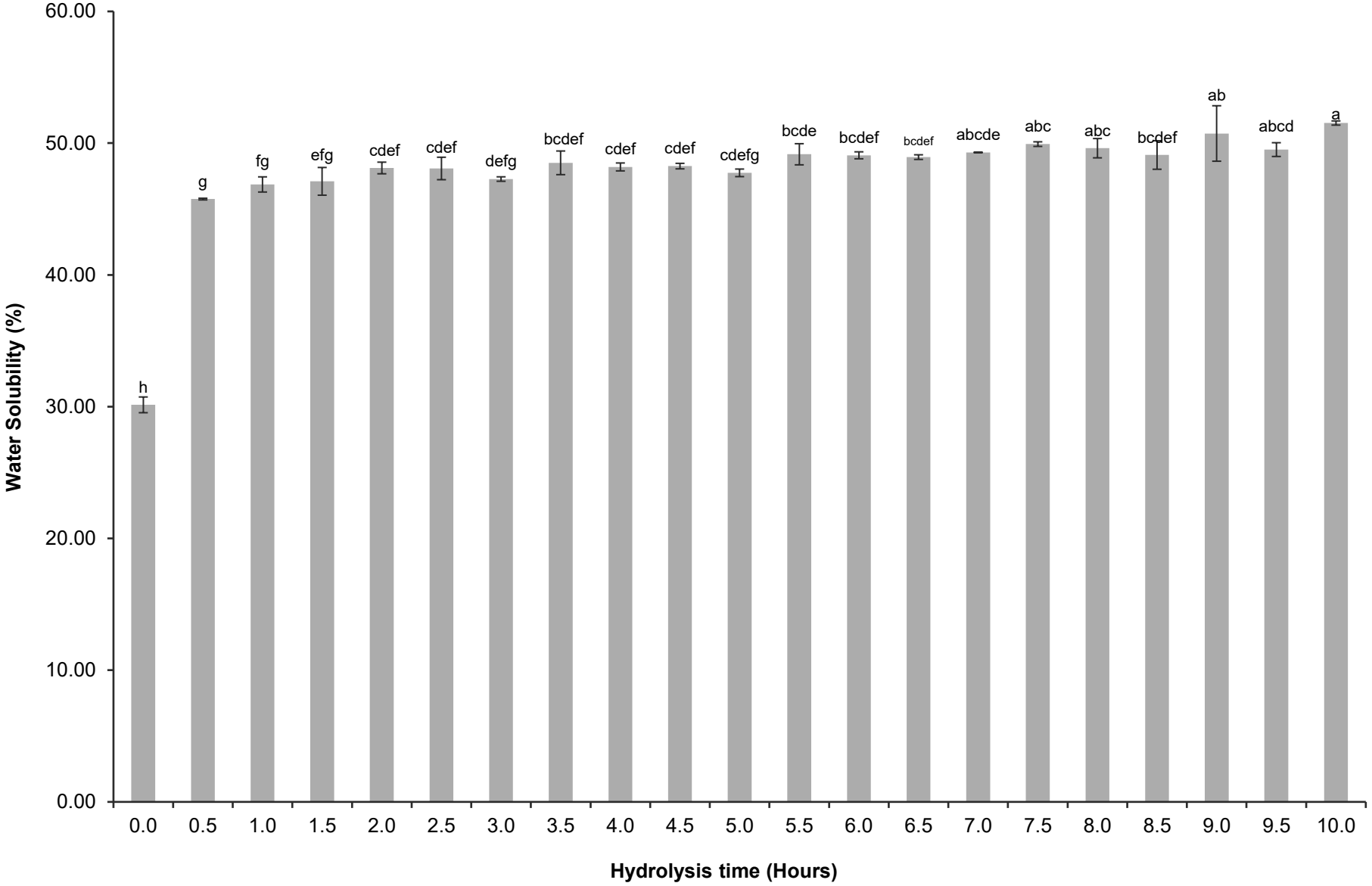

Figure 5-16. Effect of the hydrolysis time over water solubility of soybean-maize protein hydrolyzed with neutrase at 0.45% (w/v) for 10 Mean values are the average of at least three replicates ± standard deviation. Mean values with a different letter(s) on the bar are statistically different ($p<0.05$).

The viscosity of the soybean-maize hydrolysates decreased as the hydrolysis time increased (Figure 5-17). The initial viscosity of the sample was 17.83 cP and the final value after 10 hours of hydrolysis was 4 cP; in this case, the viscosity was reduced by 77.57%. This decrease was gradual, as the neutrase reduced the size of the peptides, and there was no significant difference between the viscosity obtained at 7 and 10 hours ($p>0.05$).

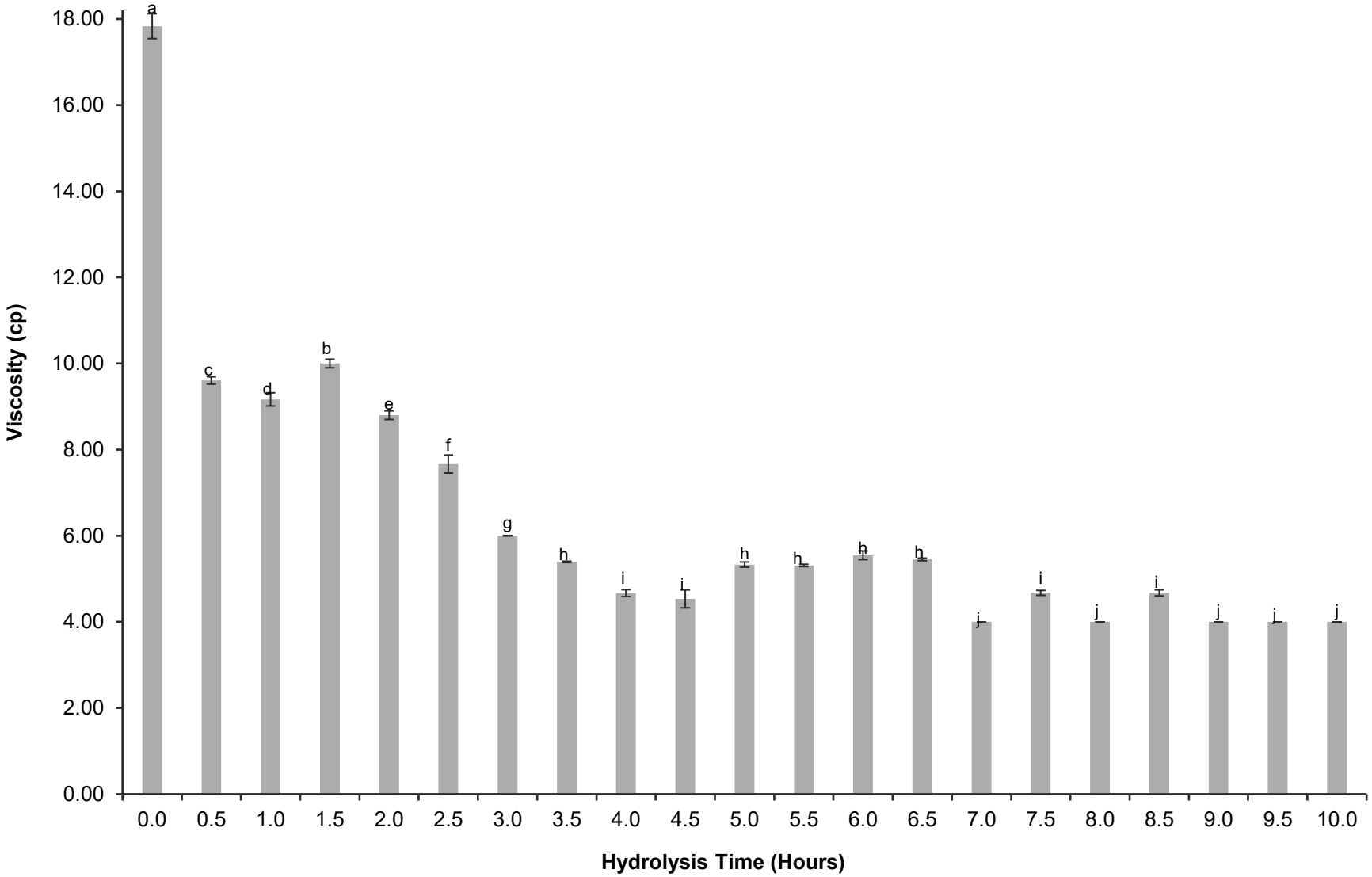

Figure 5-17. Effect of hydrolysis time on the viscosity of soybean-maize protein hydrolyzed solutions treated with neutrase at 0.45% (w/v) for 10 hours. Mean values are the average of at least three replicates ± standard deviation. Mean values with a different letter(s) on the bar are statistically different ($p<0.05$).

The hydrolysis kinetics of the soybean-maize concentrate was followed by SDS-PAGE electrophoresis to evaluate the size of peptides generated during the treatment. In the dispersion in water and at time zero, the typical electrophoresis pattern for a soy protein can be seen (Figure 5-18): 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 (A_3, y A_{1a}, A_{1b}, A_2 y A_4 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*., (2011) on soybean proteins.

In the electrophoresis gel, it was possible to determine how the degree of hydrolysis increased and, therefore, how the size of the peptides was reduced. Lipoxygenase was hydrolyzed at 30 minutes since the corresponding band disappears in the gel at this time. At 30 minutes of hydrolysis, it can be observed that the *α*, *α'* and *β* units of *β*-conglycinin subunits were hydrolyzed as well as the acidic subunits (A_3, and A_{1a}, A_{1b}, A_2 and A_4) and basic of the glycinin.

After 7 hours of hydrolysis, two very marked areas of high saturation were observed, in which it can be observed that the neutrase generated peptides of the soybean-maize protein less than 10 kDa. This coincides with that published by Ou *et al.,* (2010), who reported that the hydrolyzed whey protein with neutrase presented peptides with a molecular weight of less than 10 kDa after 5 hours incubation.

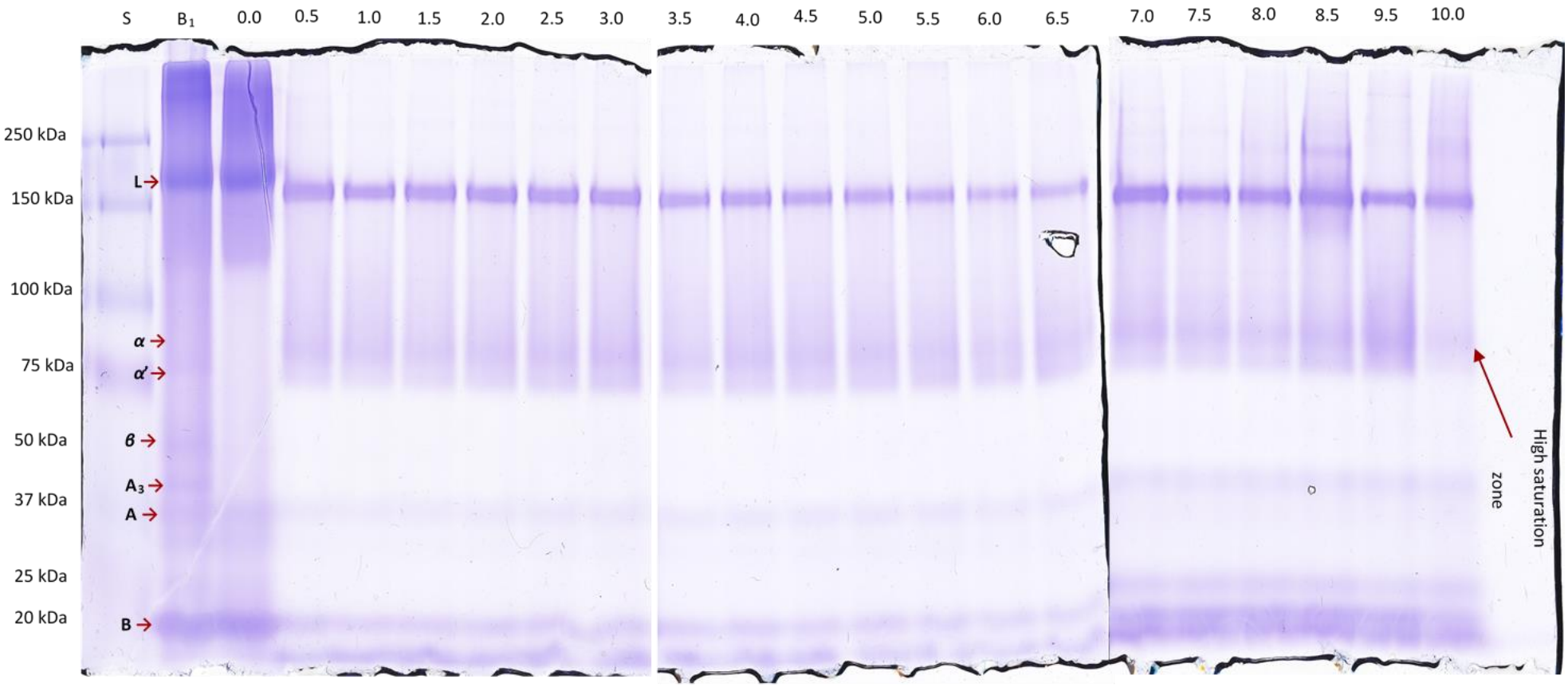

Figure 5-18. Electrophoretic profile of soybean-maize protein hydrolyzed with neutrase at 0.45% (w/v) for 10 hours (NATIVE-PAGE)

S: protein standards; B_1**:** dispersion in water; Hydrolysis time: 0.0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5 and 10.0 hours. Typical electrophoretic pattern for soy protein: **L**: lipoxygenase; ***α, α'*** and ***β*** subunits of ***β***-conglycinin; $\mathbf{A_3}$: acidic glycinin subunit; **A**: acidic glycinin subunits (A_{1a}, A_{1b}, A_2, A_4); **B**: basic glycinin subunit.

5.4. Conclusions

In the first stage of the hydrolysis process of soybean-maize protein, it was determined that the highest solubility was around 52% in the samples treated with neutrase and papain at 5% during 90 minutes and that cysteine had no significant effect on hydrolysis ($p>0.05$). These samples did not present significant differences in terms of solubility ($p>0.05$), for which the neutrase was selected as the working enzyme because it is easier to handle and cheaper. In the second stage of the hydrolysis process, the highest solubility was 52.82% in the sample treated with 0.5% neutrase for 90 minutes. However, this did not present a significant difference with the solubility of 49.99% of the sample treated at 0.45% neutrase for 30 minutes ($p>0.05$). Likewise, it was determined that the degree of hydrolysis is reduced as the enzyme concentration and hydrolysis time increases, and that the viscosity was reduced by 44.8%, 50.0% and 44.4% at 30, 60 and 90 minutes, respectively. So that, it was assumed that there is an inhibition per product that affects the efficiency of the reaction, since, at high enzyme concentrations and long reaction times (0.50% and 1.0% at 90 minutes), the degree of hydrolysis is lower and the viscosity is reduced less. Therefore, the most suitable conditions to hydrolyze the soybean-maize concentrate were to use neutrase at a concentration of 0.45%, during 30 minutes of hydrolysis, at 45 °C and a pH of 6.5 in terms of solubility.

The validation of the adequate conditions for the hydrolysis of soybean-maize protein allowed reaffirming them and making the process robust to be scaled up on a pilot and industrial level.

Determining the most suitable kinetic parameters for the hydrolysis of soybean-maize protein was essential to reduce production costs and make resources more efficient during the production of hydrolyzed proteins.

6. General Conclusions

Physicochemical parameters as pH, electrical conductivity (EC), urease activity (UA) and free alpha-amino nitrogen (FAAN) influenced the functional properties related to protein-water interactions corresponding to water solubility index (WSI), nitrogen solubility index (NSI), foaming activity (FA), foam stability (FS), heat coagulation capacity (HCC) and emulsion stability (ES). Likewise, the soluble solids content, which includes reducing sugars (RS), favors the performance of properties related to protein-interface interactions such as fat absorption index (FAI), emulsifying activity index (EAI) and foam density (FD). Regarding hydrolysis, this affected the parameters related to the solubility of the vegetable proteins, while the indices associated with fat were inversely correlated with the parameters related to water. Foam properties were better in low-temperature treated proteins, which also had high urease activity.

Comparison of the physicochemical, functional and nutritional properties of the native and hydrolyzed soybean proteins with their soybean-maize analogs showed that the soybean-maize protein had better functional properties since they had 10% and 52% more solubility, 47,385.01 m^2/g and 12,071.87 m^2/g more emulsifying capacity, 4.5% and 4.2% more foam density and 36.3% and 1.2% more coagulation capacity, respectively. In addition, the soybean-maize protein blend had 2.5% and 20.0% more isoleucine and tyrosine than soybeans. The electrophoretic profile of the protein mixture showed four additional bands to the typical pattern of soybean with a molecular weight of 56, 55, 52 and 18 kDa, which could correspond to globulins and *β*-zein from maize, respectively. Therefore, soybean-maize protein is suitable for integration into food.

Regarding the study of enzymatic hydrolysis of soybean-maize protein, it was determined that neutrase at 0.45% concentration, 30 minutes' reaction time, a pH 6.5 and a 45 °C temperature were the most suitable conditions to hydrolyze the protein in terms of solubility. Under these process conditions, it was determined that the hydrolyzed protein dispersion had free amino nitrogen of 5.03 mg/g, a solubility of 49.99% and a viscosity of 9.6 cP. The hydrolysis process increased the solubility of the soybean-maize protein by 34.33% compared to the unhydrolyzed sample. Therefore, the hydrolysis process

increases the possibilities of soybean-maize protein to be used as an ingredient in the formulation of beverages.

Determining the most suitable kinetic parameters for the hydrolysis of soybean-maize protein was essential to increase protein solubility, reduce production costs and make resources more efficient during the production of hydrolyzed proteins in the CIDPRO.

Appendix A: Abbreviations

Table A. 1. Abbreviations.

Abbreviation	Description
AOAC	Association of Official Analytical Collaboration
DS	Disulfides
EC	Electrical conductivity
EAI	Emulsifying activity index
ES	Emulsion stability
FAI	Fat absorption index
FD	Foam density
FS	Foam stability
FA	Foaming activity
FAAN	Free alpha-amino nitrogen
HCC	Heat coagulation capacity
pH	Hydrogen potential
NSI	Nitrogen solubility index
PCA	Principal component analysis
P	Protein
RS	Reducing sugars
REF	Reference
SDS-PAGE	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
SH	Thiols
SS	Soluble solids
SBF120	Soybean flour 120
SBF200/20	Soybean flour 200/20
SBFN	Soybean flour national
SBFNutri	Soybean flour Nutrisoy
SBFRagasa	Soybean flour Ragasa
SH	Soybean hydrolysate
SI	Soybean isolate
SMH	Soybean-maize hydrolysate
SMI	Soybean-maize isolate
UA	Urease activity
WAI	Water absorption index
WS	Water solubility
WSI	Water solubility index

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Published papers

The scientific publications presented here correspond to three publications of my thesis work and other research carried out in parallel during my doctoral studies.

- **Soria-Hernández C.G.,** Serna-Saldívar S.O. and Chuck-Hernández C. (2020). Comparison of Physicochemical, Functional and Nutritional Properties between Proteins of Soybean and a Novel Mixture of Soybean-Maize. *Appl. Sci.* 10: 1-14. This article corresponds to chapter 4 of this thesis.
- Gaxiola-Cockburn R., Martínez-Romero O., Elías-Zúñiga A., Olvera-Trejo D., Reséndiz-Hernández J.E. and **Soria-Hernández C.G**. (2020). Investigation of the Mechanical Properties of Parts Fabricated with Ultrasonic Micro Injection Molding Process Using Polypropylene Recycled Material. *Polymers*. 12:1-23.
- **Soria-Hernández C.G,** Palacios-Pineda LM, Elías-Zúñiga A, Perales-Martínez IA, Martínez-Romero O. (2019). Investigation of the Effect of Carbonyl Iron Micro-Particles on the Mechanical and Rheological Properties of Isotropic and Anisotropic MREs: Constitutive Magneto-Mechanical Material Model. *Polymers*.11(10):1705.
- **Soria-Hernández C.**, Serna-Saldívar S. and Chuck-Hernández C. (2015). Physicochemical and functional properties of vegetable and cereal proteins as potential sources of novel food ingredients. *Food Technol. Biotechnol*. 53(3): 269-277. This article corresponds to chapter 3 of this thesis.
- Chuck-Hernández C., Pérez-Carrillo E., **Soria-Hernández C.** and Serna-Saldívar S. (2015). Functionality and organoleptic properties of maize tortillas enriched with five different soybean proteins. *Cereal Chem*. 92(4): 341-349.
- Gutiérrez-Uribe J.A., **Soria-Hernández C**., Rosales-Serna R., García-Lara S., Serna-Saldívar S.R.O. and Reyes-Barraza E. (2015). Enzyme activity in slow darkening pinto common bean cultivars under accelerated aging treatment. *LVIII Annual Report of the Bean Improvement Cooperative*. 58: 7-9.
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original scientific paper

Physicochemical and Functional Properties of Vegetable and Cereal Proteins as Potential Sources of Novel Food Ingredients

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Summary

Proteins from vegetable and cereal sources are an excellent alternative to substitute animal-based counterparts because of their reduced cost, abundant supply and good nutritional value. The objective of this investigation is to study a set of vegetable and cereal proteins in terms of physicochemical and functional properties. Twenty protein sources were studied: five soya bean flour samples, one pea flour and fourteen newly developed blends of soya bean and maize germ (five concentrates and nine hydrolysates). The physicochemical characterization included pH (5.63 to 7.57), electrical conductivity (1.32 to 4.32 mS/cm), protein content (20.78 to 94.24 % on dry mass basis), free amino nitrogen (0.54 to 2.87 mg/g) and urease activity (0.08 to 2.20). The functional properties showed interesting differences among proteins: water absorption index ranged from 0.41 to 18.52, the highest being of soya and maize concentrates. Nitrogen and water solubility ranged from 10.14 to 74.89 % and from 20.42 to 95.65 %, respectively. Fat absorption and emulsification activity indices ranged from 2.59 to 4.72 and from 3936.6 to 52 399.2 m^2/g respectively, the highest being of pea flour. Foam activity (66.7 to 475.0 %) of the soya and maize hydrolysates was the best. Correlation analyses showed that hydrolysis affected solubility-related parameters whereas fat-associated indices were inversely correlated with water-linked parameters. Foam properties were better of proteins treated with low heat, which also had high urease activity. Physicochemical and functional characterization of the soya and maize protein concentrates and hydrolysates allowed the identification of differences regarding other vegetable and cereal protein sources such as pea or soya bean.

Key words: vegetable proteins, cereal proteins, functional properties, physicochemical parameters, soya and maize concentrates, pea flour

Introduction

Currently there is a rising interest in protein isolation for their subsequent use as food ingredient. Sixty percent of Americans take into consideration protein content in food or beverages when making a buying decision (*1*). Of the three macronutrients (carbohydrates, proteins and fats), proteins are the most appealing for consumers concerned about their health. Nearly half of adults perceive proteins as ingredients that increase energy levels, support overall good health and improve muscle tone. These macronutrients are also considered important in diets aimed to complete a weight management program. Despite the awareness of protein importance in a balanced diet, nearly 25 % of adults believe that they cannot consume as much proteins as they would like because of the cost (*2*). The protein industry is segmented into animal (gelatin, egg white, casein and whey) or vegetable, of which soya bean is the only source of worldwide relevance. The former has the advantage of being of high nutritional quality, but with higher cost than the vegetable counterparts and frequently the supply is irregular and unreliable. The latter

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applied sciences

MDPI

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.

Functionality and Organoleptic Properties of Maize Tortillas Enriched with Five Different Soybean Proteins

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Cereal Chem. 92(4):341–349

ABSTRACT

Soybean proteins are ideally suited to enhance the essential amino acid balance of cereal-based foods. The aim of this investigation was to assess the functionality of different soybean proteins in maize tortillas with yield and with sensorial and textural shelf-life characteristics as criteria to select the best supplement. Four different defatted soybean flours (SBF1, SBF2, SBF3, and SBF4) and one soybean protein concentrate (SBC) were added to increase protein content of dry masa flour between 25 and 30%. The evaluated soybean ingredients displayed urease activity of 0.1–2.25, water absorption index of 4.02–8.34, protein dispersibility index of 23–75%, and fat absorption index ranging from 2.5 to 3.1. Moisture, crude protein, crude fat, and rollability were not different among enriched tortillas, but maximum force after five days of storage was higher for SBF1 and lower for SBF 3. The control and SBF1 followed by SBC were the best evaluated overall according to the most relevant parameters for consumers and producers. Correlation analyses displayed a negative association among yield-related parameters and protein dispersibility index, urease activity, and water solubility, opposite to the relationship for texture-related properties. The best soybean proteins to be used in maize tortilla supplementation should have, preferably, reduced water solubility, urease activity, and protein dispersibility index.

Protein malnutrition is still an endemic problem in many developing countries around the globe. It is known to inhibit growth and have a profound and significant effect on physical development, susceptibility to infectious diseases, and brain maturity. Early malnutrition reduces both the brain and cerebellum sizes (Chase et al. 1967; Culley and Lineberger 1968), the number of neurons and synapsis (Warren and Bedi 1984; Stylianopoulos et al. 2002), and the neuron myelization process mainly because of the lower synthesis of proteolipids, cerebrosides, sulfatides, and plasmalogen of the white matter. Functionally speaking, this reduction is relevant because myelinized axons transmit information faster than nonmyelinized fibers (Wiggins 1982). Postmortem inspection of children that were severely affected by marasmus found significantly lower levels of brain cholesterol, phospholipids, RNA, and DNA (Rosso et al. 1970).

Mexico has one of the highest per capita consumptions of maize (*Zea mays* L.), and the main food product from this cereal is the tortilla. The lower the socioeconomic status, the greater the dependence on tortillas. Unfortunately, tortillas are not a perfect food because they lack two essential amino acids, lysine and tryptophan, and adequate levels of iron, zinc, and vitamins A, D, E, and B_{12}. That is the reason why tortillas are ideally suited for nutritional enrichment and protein fortification (Serna-Saldivar et al. 1988; Stylianopoulos et al. 2002; Amaya-Guerra et al. 2004, 2006).

Soybean (*Glycine max*) is the most important legume seed planted worldwide. The estimated world production in 2013 was 276.4 million metric tons. The main country producers are the United States, Brazil, Argentina, and China, with estimated productions of 89.4, 81.7, 49.3, and 12.5 million metric tons. Soybeans are mainly channeled to the oil crushing industries to extract the oil and the defatted soybean meal. The full-fat meal, defatted meal, concentrates, and isolates contain about 42, 49, 65, and 90% protein, respectively. These products are widely applied in the food industry as important ingredients because of their highly nutritious and desirable functional properties. However, in many cases, the application of these types of proteins is limited because of incompatibility between their solubility and other properties (Wang et al. 2008). The enhancement of protein quality and nutritional value of soybean-fortified maize tortillas was first documented with laboratory animals in the 1970s (Bressani et al. 1974, 1979; Del Valle and Perez-Villaseñor 1974). These early investigations clearly indicated that it was feasible to produce fortified tortillas with enhanced protein quality. Amaya-Guerra et al. (2004, 2006) determined the physiological development and brain development of rats fed with soybean-fortified tortilla-based diets for two generations. Growth, reproductive performance, brain development, and short- and long-term memory of rats fed with soybean-fortified tortillas were significantly higher than counterparts fed with regular tortillas. Chavez and de Chavez (2004) conducted a two-year blind study with humans that evaluated the effect of soybean fortification and enrichment with selected micronutrients of dry masa flours in two neighboring rural communities located in Mexico. Infants and children who received the soybean-fortified masa flour grew 49% more than counterparts fed with a regular diet. Clinical tests showed that subjects consuming the fortified and enriched tortillas improved their hair, nail, and skin conditions. Pregnant women who consumed fortified tortillas gave birth to newborns with significantly higher weights, and only 3% of the babies had lower birth weights than 2.5 kg. Interestingly, the infants performed approximately 10 points better in the Bayley Scale of Infant Development test, which assesses motor, language, and cognitive development. Even with the great nutritional benefits of fortified tortillas, the industry has not adopted this technology because the soybean flour affects the organoleptic properties. To our knowledge, there are not investigations aimed at comparing functional properties of soybean proteins in the maize tortilla system. Therefore, the aim of this research was to evaluate the functionality of five different commercial types of soybean proteins in maize tortillas. The yield and physical, chemical, sensorial, and textural shelf life during storage at room temperature were used as the criteria to select the best soybean protein.

MATERIALS AND METHODS

Maize and Soybean Flour Samples. Composite flours were obtained by mixing commercial nixtamalized maize flour (control treatment, Maseca Premium Plus high-yield dry masa flour supplied with added gums and emulsifiers) with about 6% (6 g for each 100 g of nixtamalized maize flour) of four different defatted soybean flours (SBF1 [Industrial de Alimentos], SBF2 [GAF 120], SBF3 [ADM], and SBF4 [Ragasa]) or 4% (4 g for each 100 g of nixtamalized

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Participation in International Congresses

Enzymatic hydrolysis optimization of a modified soy-maize protein in terms of solubility

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Introduction

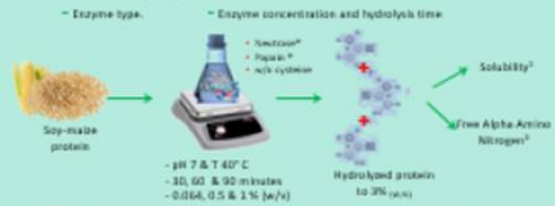
Plant proteins, particularly from cereals and pulses, are nutritious and less expensive than animal. However, its functionality is not as good as animal-based within food products. Enzymatic hydrolysis is the most used treatment to improve the functional properties and increase the field of application of plant proteins[1].
The use of proteins as ingredients in food is based mainly on its solubility, because this characteristic influences other important parameters as hydration and foaming. Altogether affect protein stability and behavior in a food system.

Objective

The objective of this research was to optimize the limited hydrolysis of modified soy-maize protein regarding solid content in the supernatant and Free Alfa Amino Nitrogen (FAN).

Methodology

- **Limited hydrolysis.**



- For hydrolysis optimization factorial designs were used (2^3 x 2^1 & 2^2). Statistical analysis was performed using Design Expert 7.1.5.

Figure 1. Methodology for hydrolysis optimization.

Results

- **Selection of proteolytic enzyme and feasibility of using an activating agent.**

The Table 1 shows that neutrase and papain caused the same solubility and degree of hydrolysis in the soy-maize protein. Neutrase was selected for being cheaper. The use of cysteine depicted no significant effect ($p>0.05$) on the solubility and degree of hydrolysis.

Table 1. Solid content in the supernatant and Free Alfa Amino Nitrogen (FAN).

Concentration	Cysteine	Solid content in the supernatant (%)			Free Alfa Amino Nitrogen (mg/g)		
		Neutrase	Papain	Mixture	Neutrase	Papain	Mixture
0	0	2.61±0.03 ^{a}	2.28±0.03 ^{b}	2.77±0.02 ^{a}	1.63±0.11 ^{a}	1.24±0.16 ^{a}	1.89±0.37 ^{a}
	W	3.02±0.19 ^{ab}	2.25±0.07 ^{b}	2.95±0.05 ^{a}	1.63±0.13 ^{a}	1.31±0.18 ^{a}	1.25±0.41 ^{a}
0.064	0	3.56±0.24 ^{cd}	2.83±0.14 ^{a}	3.35±0.08 ^{b}	2.78±0.13 ^{b}	1.98±0.09 ^{b}	2.52±0.61 ^{b}
	W	3.18±0.01 ^{bc}	2.24±0.03 ^{b}	3.28±0.08 ^{b}	2.86±0.09 ^{b}	1.61±0.28 ^{b}	2.06±0.16 ^{b}
0.5	0	3.68±0.16 ^{d}	2.76±0.03 ^{a}	3.67±0.01 ^{d}	5.21±0.18 ^{c}	3.08±0.16 ^{c}	6.61±0.18 ^{cd}
	W	3.06±0.07 ^{ab}	2.58±0.05 ^{a}	2.55±0.15 ^{ab}	5.27±0.05 ^{c}	2.84±0.08 ^{b}	6.81±0.05 ^{c}
1	0	3.20±0.13 ^{bcd}	2.82±0.05 ^{a}	3.20±0.13 ^{b}	5.81±0.55 ^{c}	2.80±0.17 ^{b}	6.27±0.18 ^{c}
	W	3.01±0.38 ^{ab}	2.82±0.06 ^{ab}	3.23±0.02 ^{b}	5.62±0.14 ^{c}	2.70±0.03 ^{ab}	5.57±0.38 ^{c}

Values within a column followed by the different letters are significantly different ($p < 0.05$).

Through the optimization of hydrolysis time and enzyme concentration was determined that to 0.45% of neutrase and 30 minutes; soy-maize protein reached the highest solubility.

- **Optimization of enzyme concentration and hydrolysis time.**

After 10 hours of hydrolysis, solubility and content of FAN increased in 18.54 and 9.5%, respectively (Figures 2, 3 & 4). In economic terms, this increase is not significant for industry. Therefore, was confirmed that the soy-maize protein had a higher amount of solids in the supernatant and FAN content neutrase with 0.45% to 30 minutes of hydrolysis.

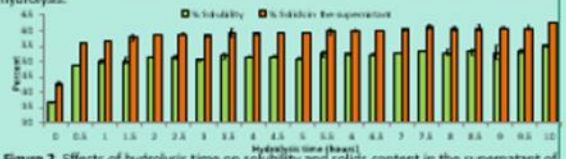


Figure 2. Effects of hydrolysis time on solubility and solids content in the supernatant of the soy protein.

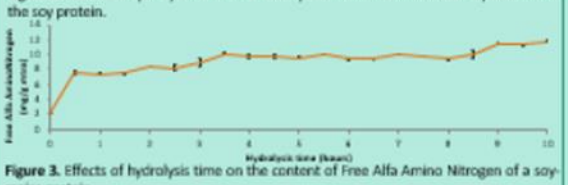


Figure 3. Effects of hydrolysis time on the content of Free Alfa Amino Nitrogen of a soy-maize protein.
The electrophoresis profile at different times allowed verify the increase of the degree of hydrolysis of soy-maize protein (Figure 4).

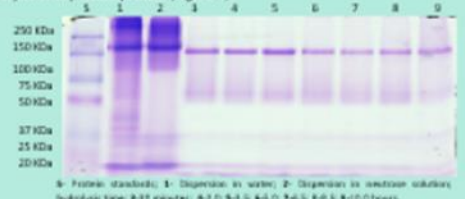


Figure 4. Electrophoretic profile of samples obtained at different hydrolysis time.

Conclusions

- The use of neutrase or papain produces the same degree of hydrolysis in the soy-maize protein. Neutrase was selected for being cheaper than papain .
- Cysteine showed no synergistic effect on enzymatic hydrolysis.
- Incorporating 1% of enzyme did not produce higher solids content in the supernatant as expected.
- The hydrolysis time of 10 hours increased the solubility and content of Free Alfa Amino Nitrogen in 18.54 and 9.5%, respectively compared with initial sample.
- Nevertheless, the highest percentage of solids in the supernatant and FAN content was reached practically after 30 minutes of enzymatic reaction.
- For this reason the recommended hydrolysis parameters are: 0.45% (w/v) of neutrase and 30 minutes of hydrolysis time.
- The soy-maize protein with more solubility may be used greater extent as an ingredient in food formulation.

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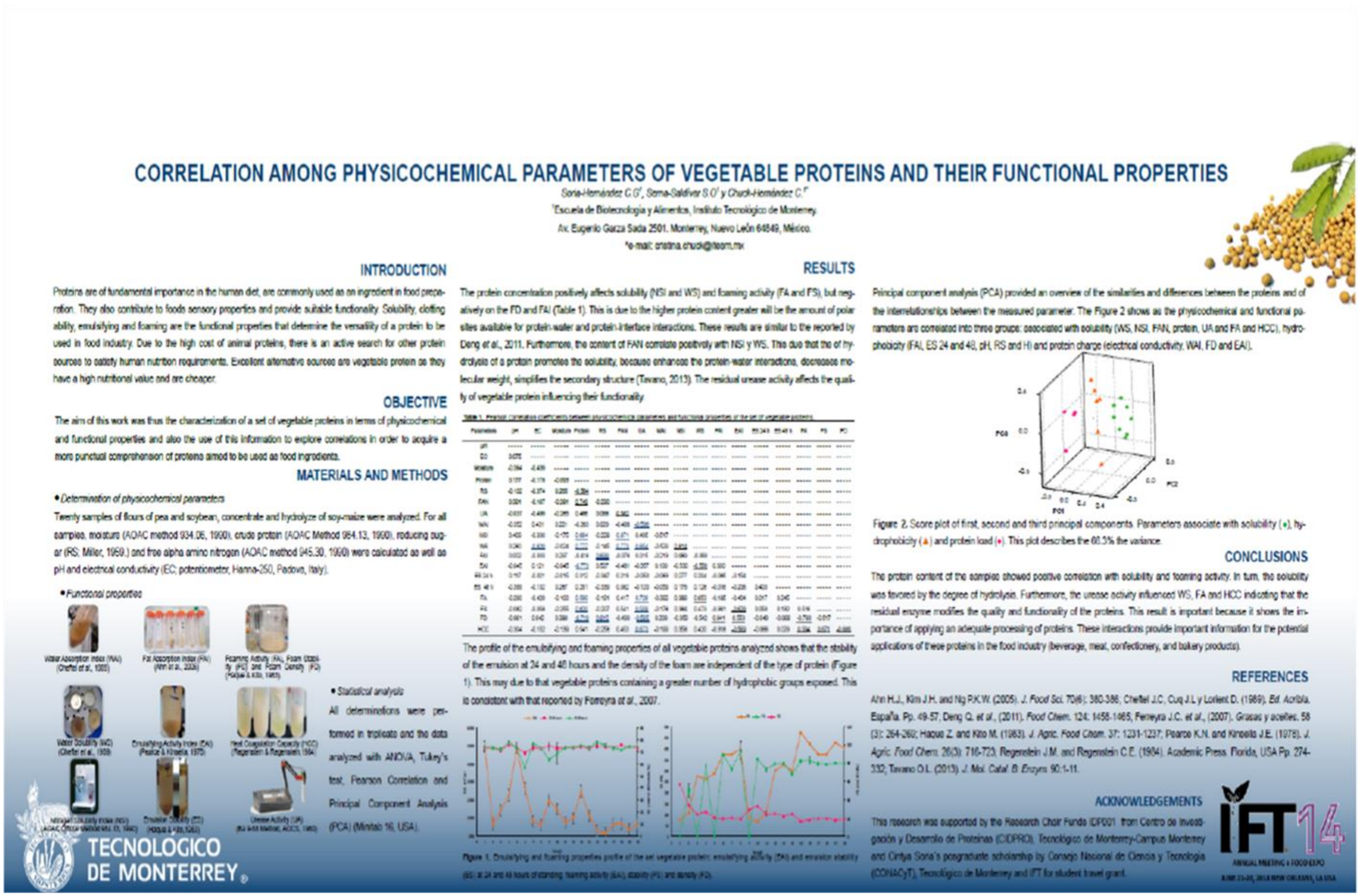
CORRELATION AMONG PHYSICOCHEMICAL PARAMETERS OF VEGETABLE PROTEINS AND THEIR FUNCTIONAL PROPERTIES
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INTRODUCTION
Proteins are of fundamental importance in the human diet, are commonly used as an ingredient in food preparation. They also contribute to foods sensory properties and provide suitable functionality. Solubility, clotting ability, emulsifying and foaming are the functional properties that determine the versatility of a protein to be used in food industry. Due to the high cost of animal proteins, there is an active search for other protein sources to satisfy human nutrition requirements. Excellent alternative sources are vegetable protein as they have a high nutritional value and are cheaper.
OBJECTIVE
The aim of this work was thus the characterization of a set of vegetable proteins in terms of physicochemical and functional properties and also the use of this information to explore correlations in order to acquire a more punctual comprehension of proteins aimed to be used as food ingredients.
MATERIALS AND METHODS
• Determination of physicochemical parameters
Twenty samples of flours of pea and soybean, concentrate and hydrolyze of soy-maize were analyzed. For all samples, moisture (AOAC method 934.06, 1990), crude protein (AOAC Method 984.13, 1990), reducing sugar (RS; Miller, 1959.) and free alpha amino nitrogen (AOAC method 945.30, 1990) were calculated as well as pH and electrical conductivity (EC; potentiometer, Hanna-250, Padova, Italy).
• Functional properties
• Statistical analysis
All determinations were performed in triplicate and the data analyzed with ANOVA, Tukey's test, Pearson Correlation and Principal Component Analysis (PCA) (Minitab 16, USA).
RESULTS
The protein concentration positively affects solubility (NSI and WS) and foaming activity (FA and FS), but negatively on the FD and FAI (Table 1). This is due to the higher protein content greater will be the amount of polar sites available for protein-water and protein-interface interactions. These results are similar to the reported by Deng et al., 2011. Furthermore, the content of FAN correlate positively with NSI y WS. This due that the of hydrolysis of a protein promotes the solubility, because enhances the protein-water interactions, decreases molecular weight, simplifies the secondary structure (Tavano, 2013). The residual urease activity affects the quality of vegetable protein influencing their functionality.
The profile of the emulsifying and foaming properties of all vegetable proteins analyzed shows that the stability of the emulsion at 24 and 48 hours and the density of the foam are independent of the type of protein (Figure 1). This may due to that vegetable proteins containing a greater number of hydrophobic groups exposed. This is consistent with that reported by Ferreyra et al., 2007.
Principal component analysis (PCA) provided an overview of the similarities and differences between the proteins and of the interrelationships between the measured parameter. The Figure 2 shows as the physicochemical and functional parameters are correlated into three groups: associated with solubility (WS, NSI, FAN, protein, UA and FA and HCC), hydrophobicity (FAI, ES 24 and 48, pH, RS and H) and protein charge (electrical conductivity, WAI, FD and EAI).
PC3
PC2
PC1
Figure 2. Score plot of first, second and third principal components. Parameters associate with solubility (•), hydrophobicity (▲) and protein load (•). This plot describes the 68.3% the variance.
CONCLUSIONS
The protein content of the samples showed positive correlation with solubility and foaming activity. In turn, the solubility was favored by the degree of hydrolysis. Furthermore, the urease activity influenced WS, FA and HCC indicating that the residual enzyme modifies the quality and functionality of the proteins. This result is important because it shows the importance of applying an adequate processing of proteins. These interactions provide important information for the potential applications of these proteins in the food industry (beverage, meat, confectionery, and bakery products).
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Effect of thermal treatment on the susceptibility to enzymatic hydrolysis of a Soy-Maize protein

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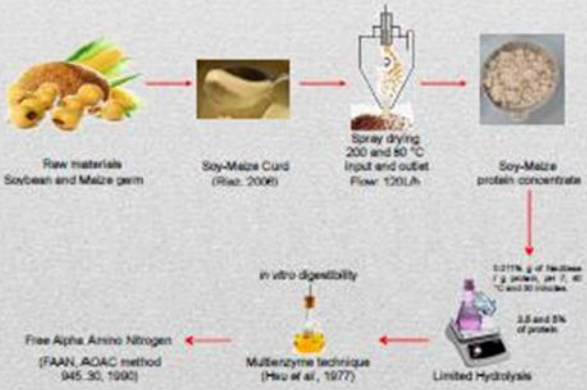
Introduction

Vegetable proteins represent an alternative of high nutritional value and low cost. The nutritional value of Soy-Maize protein is similar to casein. However, during its processing is subject to a drying heat treatment which causes the dissociation of its tertiary structure, denaturing of the subunits and exposure of the hydrophobic groups. These modifications affect susceptibility of proteins to various processes such as hydrolysis. Enzymatic hydrolysis is an effective form to improve different functional properties and increase the field of application of vegetable proteins in the food industry.

Objective

The objective of this study was to determine the effect of thermal treatment on the susceptibility of a Soy-Maize protein to hydrolysis with protease.

Methodology



Data by triplicate were statistically evaluated using an analysis of variance (Minitab 16, USA). The significant differences between means were determined by the Tukey's multiple comparison test at a 5% significance level.

Results

- Hydrolysis degree of Soy-Maize protein concentrate and curd.

Figure 1 shows that the thermal treatment reduced the hydrolysis degree and this is independent of protein concentration.

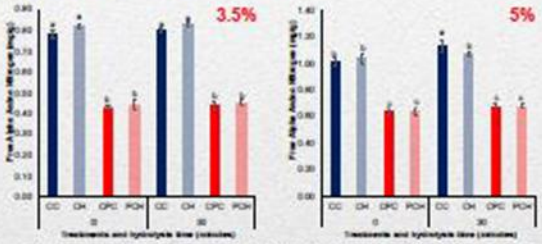


Figure 1. Limited Hydrolysis of Soy-Maize protein concentrate and curd to 3.5 and 5% of protein concentration. aCC: control curd; aCH: curd hydrolyzed; aCPC: Control protein concentrate; aPCH: Protein concentrate hydrolyzed.

- *In Vitro* Digestibility of Soy-Maize protein concentrate and curd.

In the Soy-Maize protein concentrate the spray drying increased 4.28% the digestibility and reducing the FAAN content, Figures 2 and 3 respectively.

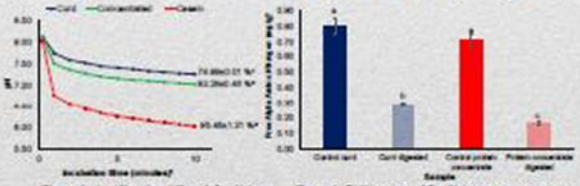


Figure 2. *In Vitro* digestibility of Soy-Maize protein concentrate and curd.

Figure 3. FAAN content of Soy-Maize protein concentrate and curd controls and digested.

Conclusions

- Spray drying causes an interaction in Soy-Maize curd proteins reducing the FAAN content in the concentrate, possibly by the formation of larger peptides.
- Hydrolysis degree of the Soy-Maize protein concentrate and curd after thermal treatment was independent of protein concentration (3.6 and 6%).
- Thermal treatment increased 4.28% protein digestibility of Soy-Maize protein concentrate, which showed adequate digestibility rates (83.28%). However, values were slightly lower compared to casein.
- The degree of hydrolysis did not affect the digestibility of the proteins. Digestibility promoted the formation of larger peptides as estimated with the FAAN test and as has been reported in previous works (Hsu *et al.*, 1977).

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