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Functional Properties of Mixed Hydrocolloid Systems Formed by Hard-to-*Cook Phaseolus vulgaris* Protein Hydrolysates and Carboxymethylated Flamboyant (*Delonix regia*) Gum

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Abstract

In this work, the interactions and functional properties of the mixed hydrocolloid system (MHS) formed by the limited protein hydrolysate of hard-to-cook *Phaseolus vulgaris* (LHv) and carboxymethylated galactomannan from flamboyant seed (CGF) were studied at pH values of 3, 3.5 and 4. An LHv with a degree of hydrolysis of 8.3% and a CGF with a degree of substitution of 0.91 was obtained. By fluorometry, the highest interaction between LHv and CGF was observed at pH 3 and decreased with increasing pH; furthermore, the soluble nitrogen of MHS was higher than that of LHv and increased with pH. The MHS had a higher foaming capacity and stability than the LHv dispersion; it was also observed that the stability increased with increasing pH of the system, reaching a foaming capacity of up to 267% and a stability time of 35 min with the MHS at pH 4. On the other hand, the emulsifying activity index (EAI) of MHS and LHv increased with increasing pH, with MHS reaching 219% EAI; whereas, the emulsifying stability index (ESI) of MHS increased slightly with pH value. Based on the results obtained, the use of MHS in food industry products where foam control or emulsion generation and stabilization are required, could be considered.

Keywords

Hard-to-cook bean hydrolysate, Flamboyant gum, Hydrocolloid system, Functional properties

Abbreviations

MHS: Mixed hydrocolloid system; **LHv**: Limited protein hydrolysate of hard-to-cook *Phaseolus vulgaris*; **CGF**: Carboxymethylated galactomannan from flamboyant seed; **EAI**: Emulsifying activity index; **ESI**: emulsifying stability index; **PCv**: protein concentrates of hard-to-cook *P. vulgaris*; **Trp**: Tryptophan; **DH**: degree of hydrolysis; **MFG**: Modified flamboyant gum

Introduction

Proteins and polysaccharides are two hydrocolloids that are present in most food products and, due to their functional capabilities, are used to produce novel and attractive products [1]. The amphiphilic character of proteins makes them a versatile component in food matrices as they have functional properties such as solubility, gelation, emulsifying capacity, and foam formation among others; these properties depend mainly on the primary structure of the protein and its molecular weight [2], so their modification can improve their functional properties. One form of modification is enzymatic hydrolysis, hydrolysates with

a degree of hydrolysis of less than 10% (generally known as limited hydrolyzed) generally improve the original functional properties of the protein due to the exposure of hydrophobic sections present in the original molecule and the increase of ionizable groups [3]. Studies have been conducted on the enzymatic modification of proteins in order to increase their functional properties [4-6]; Daliri et al. [7] hydrolyzed the quinoa protein concentrate using the enzyme pancreatin, obtaining an improvement in solubility and emulsifying capacity, in addition, the foaming capacity was increased from 20 to 100% approximately. One source of protein consumed in the Americas is the common bean (*Phaseolus vulgaris* L.); however, when exposed to inadequate storage conditions it can harden, diminishing its quality and losing commercial value. Therefore, it has been proposed to use hard-to-cook *Phaseolus vulgaris* beans to obtain protein hydrolysates with functional properties. Guzmán-Méndez et al. [8] obtained a limited hydrolysate of *Phaseolus vulgaris* with higher emulsifying, foaming, and nitrogen solubility capacity than the protein concentrate. On the other hand, the presence of polysaccharides in food products can change their rheological properties due to their interaction with water and their high molecular weight, being used as thickening agents, gellants, and stabilizers of emulsions and foams [9]. However, some natural polysaccharides are limited in their use due to their low solubility so, to overcome this limitation, their molecular structure can be modified by carboxymethylation [10]. Galactomannans are a type of polysaccharide widely used for the rheological properties of their aqueous dispersions, they have a main chain of mannose linked by β-(1-4) bonds of which there are galactose residues linked by α-(1-6) bonds [11]. An unconventional and underutilized source of galactomannan is the seed of the flamboyant tree (*D. regia*) which has a molecular weight of $4.86x10^5$ Da, a galactose to mannose ratio of 1:3.48, and a water solubility of 50% [12]. Due to the low solubility of this galactomannan, Pacheco-Aguirre et al. [13] modified the flamboyant galactomannan by carboxymethylation, thereby improving the solubility, reaching a value of approximately 90%. Systems where there are gums and proteins, like the flamboyant, can be called mixed hydrocolloid systems (MHS), where the interactions that occur between proteins, protein hydrolysates, and polysaccharides are a key factor in the development and processing of novel products since they influence the functional properties of foods [14]; these interactions can be electrostatic due to the charges that the protein chains and polysaccharides may present at a given pH, or they can also occur due to hydrogen bonding and hydrophobic interactions. Polysaccharide-protein interactions can result in the formation of a single phase of soluble aggregates (coacervates) or a segregative one with the soluble components distributed in the medium; two phases can also be obtained due to the insolubility of the coacervates or the immiscibility between proteins and polysaccharides [15]. Corzo-Rios et al. [16] studied the interactions of protein hydrolysates from *P. lunatus* with carboxymethylated flamboyant gum (CFG) at different pH values and protein/gum ratios by capillary electrophoresis, observing that there is an interaction between these components from low protein/gum ratios. For this reason, the functional properties of mixed hydrocolloid systems formed by protein hydrolysates of hard-to-cook *Phaseolus vulgaris* and modified flamboyant (*Delonix regia)* seed gum were evaluated.

Materials and Methods

Hard-to-cook *P. vulgaris* flour

15 kg of common beans (*P. vulgaris*) were obtained from the grain and seed supply center in the city of Mérida, México. Beans free of defects were selected, cleaned, and stored hermetically until use. The *P. vulgaris* beans presented a hardness of 15N and a cooking time of 70 min, superior to the texture and time established by the Mexican standard for fresh beans (5 N and 55 min, respectively). The grains were crushed in a mechanical disc mill (Grinding Mill, model 4E) to separate the tegument by friction. The material was then passed through screens with 4.76 mm and 2.38 mm diameter holes, respectively. The material retained on the screens was processed in a fluidized bed machine to separate the tegument. The grinding and tegument separation cycle was repeated two more times until the biggest amount possible was separated. Finally, fine grinding was performed with a Cyclotec 1093 mill (Tecator, Sweden) to obtain flour with a particle size < 250 μ m.

Delonix regia flour

Whole and dried pods were randomly collected from trees in the city of Mérida, México. These were opened and the seeds in good physical condition and larger than 1 cm in length were selected. 10 kg of seeds were obtained and stored hermetically at room temperature until processing. The endosperm flour was obtained by an adaptation of the method by Pacheco-Aguirre et al. [13] . 4 kg of seeds were hydrated in a 1:5 ratio (w/v) with distilled water at 70°C for 10 h. Subsequently, they were subjected to wet milling in a Cemotec 1090 (Tecator, Sweden), so that the rupture of the seeds released the endosperm. The peel and germ were removed by 3 washes with distilled water. The endosperm was dried at 55 °C in an air-circulating oven (Imperial V) for 24 h. Finally, it was milled in a Thomas Wiley equipment (model no. 4) until the flour reached a particle size of less than 833 μm (20 mesh). The product obtained was stored in hermetically sealed plastic bottles.

Concentrates (PCv) and hydrolyzed (LHv) protein of hard-to-cook *P. vulgaris*

The method reported by Guzmán-Méndez et al. [8] was used with some modifications. Batches of 1 kg of flour were processed by dispersion in distilled water at a 1:10 (w/v) ratio and the pH was adjusted to 11 with NaOH 1N. The dispersion was kept under constant stirring for 1 h at 400 rpm. Subsequently, the suspension was sequentially filtered through 80 (173 μm) and 100 (147 μm) mesh to separate the fibers from the liquid fraction containing starch and protein. The filtered dispersion was allowed to settle to decant the starch from the soluble protein, which was separated from the settled starch, adjusted with 1N HCl to its isoelectric point (4.5) to precipitate the protein, and passed through a centrifuge (mistral 3000i) at 1317 x g for 25 min. The supernatant was discarded and the precipitate was dried at -47 °C and $13x10^{-3}$ mbar

in a freeze dryer (Labconco). To obtain the hydrolysates of hard-to-cook *P. Vulgaris*, the method of Megías et al. [17] was followed with some modifications. The enzyme Pancreatin® (SIGMA P-3292) was used to obtain a limited hydrolysate (LHv). For Pancreatin® treatment, a 5% (w/v) protein concentrate suspension was prepared and hydrolyzed in a 1:10 (v/v) enzyme-substrate ratio at 37 °C and pH 7 for 15 min. The reaction was stopped by heating at 85 °C for 20 min. The degree of hydrolysis (DH) or the number of hydrolyzed peptide bonds was determined following the ortho-phthalaldehyde (OPA) technique proposed by Nielsen et al. [18].

Flamboyant gum extraction and modification

The extraction of the native flamboyant gum was done by an adaptation of the method by Betancur-Ancona et al. [19]. The process consisted of preparing a suspension of 1 kg of endosperm flour in water at a ratio of 1:30 (w/v). The suspension was heated at 50 °C with constant stirring for 30 min and passed through 40 (351 µm) and 100 (147 µm) screens to separate the fibrous particles from the native gum of flamboyant. The obtained sample was precipitated with 95% (v/v) ethanol and dried at 55 °C for 24 h in an air circulation oven. It was then ground in a Thomas Wiley machine to a particle size of 173 µm (80 mesh). The gum was chemically modified by carboxymethylation following Betancur-Ancona et al. [19]. The degree of substitution was 0.91 and was determined according to the method proposed by Pacheco-Aguirre et al. [13] and is given by the amount of acid consumed by the substituted carboxymethyl groups.

Preparation of mixed hydrocolloid systems (MHS)

It was conducted based on adaptations made to the methods reported by Weinbreck et al. [20] and Espinosa-Andrews et al. [21]. Dispersions of 1% (w/w) protein hydrolysate and carboxymethylated gum were prepared in deionized water. They were gently stirred for 2 h at room temperature and then kept stirred for 12 h at 5 °C. The dispersions were diluted in deionized water and mixed to a final volume with a total concentration of 0.1% at a protein:polysaccharide ratio of 3:1 (w/w). The mixtures were acidified or alkalinized using HCl or NaOH 0.1N respectively, until reaching pH 3, 3.5, and 4, since it has been shown that this pH range is where most electrostatic complex formation takes place [20]. The ionic strength was adjusted using 10 mM NaCl and samples were taken for each of the established measurements.

Intrinsic fluorescence of tryptophan (Trp)

Intrinsic fluorescence measurements of Trp were performed according to the method reported by Perez et al. [22] in a spectrofluorometer. The hydrophobic fluorescent probe 1-anilino-8-naphthalenesulfonate (ANS) was added to the MHS dispersions. A filter was required to attenuate the light intensity and an excitation wavelength of 295 nm was used to avoid excitation of Tyr residues. The emission spectrum was run from 310 to 450 nm. For excitation and emission, a slit of 10 nm was set. Phosphate buffer pH 7 was used as a blank to correct the background fluorescence of the spectra obtained. The correlation that exists between the fluorescence intensity in relation to the emission wavelength is how the interaction between the protein and the polysaccharide is determined.

Functional properties

The hydrolysate limited (LHv) and mixed hydrocolloid systems had their functional properties determined as described below:

Nitrogen solubility

It was evaluated according to the method proposed by Were et al. [23]. A 0.5% (w/v) suspension of LHv was prepared with 25 mL of distilled water, the pH was adjusted (3, 3.5, and 4) with 0.1 N NaOH or 0.1 N HCl. It was kept in agitation for 30 min. Subsequently, it was centrifuged at 4320 x g for 30 min and 20 mL were taken for the determination of the nitrogen content by the Kjeldahl method [24]. Nitrogen solubility was expressed as the percentage of solubilized nitrogen in relation to the nitrogen content in the sample.

To measure the effect of protein-polysaccharide interactions on the solubility of the hydrocolloid systems, these were prepared at 1% (w/w) in a 3:1 ratio at pH 3, 3.5, and 4 according to the methodology proposed by Morr et al. [25]. The dispersions were adjusted to the desired pH using 0.1 N HCl or NaOH. The solubility percentage was obtained by dividing the soluble protein content by the total protein content (x 100).

Foaming capacity and stability

These measurements on MHS and LHv dispersions at different pH values (3, 3.5, and 4) were performed from 30 mL at a biopolymer concentration of 0.5% (w/v). The dispersion was placed in an acrylic container adapted to a column of the same material containing a scale for measuring the volume of foam formed by bubbling nitrogen at a constant flow rate (10 cm^3/s) for 10 s. Foaming capacity was measured at 0 min and it was expressed as the percentage increase in volume after 10 s of bubbling; stability was recorded for 60 min using a multimeter, which recorded changes in the conductivity of the drained liquid.

Emulsifying activity and stability index

The technique reported by Pearce and Kinsella [26] was used with some modifications. To form the emulsion, 1% (w/v) solutions of LHv and MHS were prepared at pH values of 3, 3.5, and 4, to each of which "Maceite®" corn oil (10 mL) was added. Once the mixtures were prepared and the pH adjusted, they were homogenized at 2,000 rpm for 1 min in Stirpak equipment (model 50002-30) to produce an emulsion. Aliquots (50 µL) were taken from the bottom of the tube and diluted in 5 ml of 0.1% (w/v) sodium dodecyl sulfate (SDS). The mixture was vortexed to obtain a uniform mixture and immediately placed in a 1 cm quartz cell, absorbance was measured at 0 and 10 min after homogenization at 500 nm in a spectrophotometer. The absorbance of the SDS served as blank, while the absorbance at time 0 (A_0) was used to determine the emulsifying activity index (EAI) of the protein while the absorbance at 10 min (A_{10}) was used to determine the emulsion stability index (ESI) by using the following equations:

$$EAI\ (m^2/g) = \frac{2 \times 2.303 \times A_0}{0.25 \times protein\ weight\ (g)}$$

$$ESI\ (\%) = \frac{A_0 \times A_{10}}{A_{10}} \times 100$$

Statistical analysis

The results of the analyses were expressed as average values. The data obtained were statistically analyzed by one-way analysis of variance (ANOVA). Differences between means were identified by applying Duncan's multiple range method using the Statgraphics Plus 5.1 software package [27].

Results and Discussions

Degree of Hydrolysis (DH) of the protein hydrolysates of *P. vulgaris*

The *P. vulgaris* hydrolysate obtained with the Pancreatin® enzyme with a hydrolysis time of 15 min presented a DH of 8.32%, so it can be classified as a limited hydrolysate. These hydrolysates, such as LHv, can improve their techno-functional properties due to the change in their molecular structure, as well as by exposing some hydrophobic sections without presenting bitter tastes like extensive hydrolysates [2]. Hydrolysis rates of 5 and 12.34% have been reported for hard-to-cook *P. vulgaris* using Pancreatin® enzyme at 15 and 90 min respectively; in these, emulsifying and foaming capacities, as well as nitrogen solubility, were improved compared to the protein concentrate [28]. The differences in the degrees of hydrolysis obtained with those reported by Betancur-Ancona et al. [28] may be due to the specificity of each enzyme, as well as to the various factors that could influence the reaction such as substrate concentration, enzyme concentration, variations in pH and/or temperature as well as the composition of amino acids present in each concentrate as reported by Vioque et al. [3].

Degree of substitution of the native gum of *D. regia*

A higher degree of carboxymethylation can increase the solubility of the polysaccharide, due to the number of carboxymethyl groups replacing hydroxyl groups, decreasing intra-molecular interactions by hydrogen bond and steric repulsion. When the native gum of flamboyant was subjected to chemical modification by carboxymethylation, a degree of substitution of 0.91 was achieved, which value corresponds to 30% carboxymethyl substitution. This was higher than the degrees of substitution of 0.63 (21%) and 0.33 (11%) reported by Pacheco-Aguirre et al. [13], who worked with gum of the same species. The value obtained in this study was similar to values reported for the carboxymethylation of cellulose and sesbania gum whose degree of substitution was 0.95 (31.60%) [29]. In modified pachyman gums under this same method, degrees of substitution from 0.44 to 0.88 were achieved [30].

Intrinsic Trp fluorometry

Tryptophan (Trp) is the most studied intrinsic fluorophore inside proteins structure, the fluorescence of this amino acid is highly sensitive to the local conditions of the protein environment [22]. Protein-polysaccharide interactions can lead to perturbations or modifications in the fluorescence parameters of Trp, such as when a lower amount of the ANS probe bound to the chromophore results in a decrease in fluorescence intensity according to Jung et al. [31]. Therefore, the analysis of the latter can be used to determine the nature and origin of the interactions between these biopolymers as referred to in different studies carried out by Klassen y Nickerson [32].

The interaction between hydrolysates and polysaccharides in MHS is shown in figure 1. In which it can be observed that MHS at pH 3, presented a lower fluorescence intensity of Trp in relation to pH 4, this could be attributed to the formation of complexes at a protein:gum ratio of 3:1 at this pH, preventing the probe from binding to the chromophore.

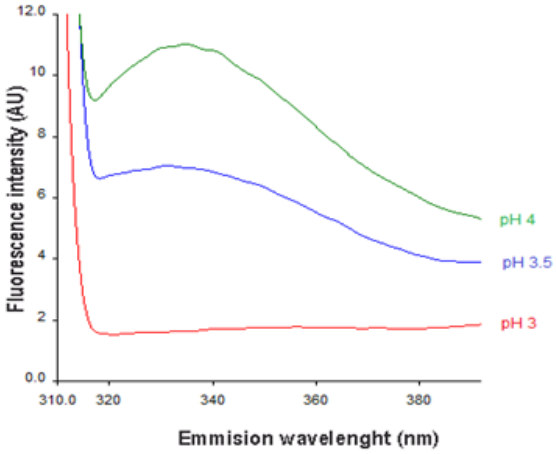

Figure 1: Intrinsic intensity of Trp in mixed hydrocolloid systems of limited hydrolysate of hard-to-cook bean (LHv) and modified flamboyant gum (MFG) at pH 3, 3.5, and 4.

Electrostatic attractions between biopolymers occur when they have opposite net charges. Likewise, the formation of soluble protein-polysaccharide complexes takes place at pH values above the pI [33]. In addition, the presence of Na^+ and Cl^- microions at low concentrations (10 mM) favored the electrostatic affinity between the hydrolysates and MFG resulting in the formation of complexes in both systems. Electrostatic interactions play a very important role during the formation of complexes between biopolymers. Weinbreck et al. [20] suggest that the number of protein molecules available per polysaccharide chain will be important in the formation of electrostatic complexes, and there will be specific conditions of pH, protein-polysaccharide ratio, and ionic strength in order to achieve electro neutrality of the complex. Gallegos Tintoré et al. [34] report that a mixed hydrocolloid system of extensive *Phaseolus vulgaris* hydrolysates with modified flamboyant gum at a ratio of 5:1 required a higher number of peptides with lower molecular weight (3.70-30. 44 kDa) compared to that of the larger and more branched flamboyant anionic polysaccharide, since as there were a greater number of patches with a net positive charge on the protein molecule, electrostatic complexes were formed at pH close to the pI of the protein. Pérez et al. [22] conducted studies on mixed systems based on whey protein and polysaccharides measuring tryptophan fluorescence and observed that the intensity of the fluorophore decreased significantly. The decrease in fluorescence intensity in this study demonstrated the existence of significant interactions between the biopolymers in the aqueous phase at pH 3, 3.5, more than pH 4.

Nitrogen solubility

Through the soluble protein value contained in the aqueous phase, information can be obtained about the aggregates formed in the MHS depending on the pH of the medium

since, depending on the conditions in which the biopolymers and the solvent are found, the solubility of the mixed system could be increased or decreased. The solubility presented by MHS (Figure 2) at pH values 3.5 and 4 was higher than LHv. The behavior observed in this pH range may be due to the electrostatic charges present in both biopolymers, which contributed to the formation of insoluble complexes when at pH close to isoelectric point (IP) and, as pH increases, these charges are modified resulting in an increase in solubility. The solubility behavior coincides with that obtained in the intrinsic fluorometry of Trp, which could mean that the greater amount of interactions at pH 3 cause some chains of the hydrolysates to form insoluble complexes with the polysaccharide, decreasing the amount of free protein in the solution.

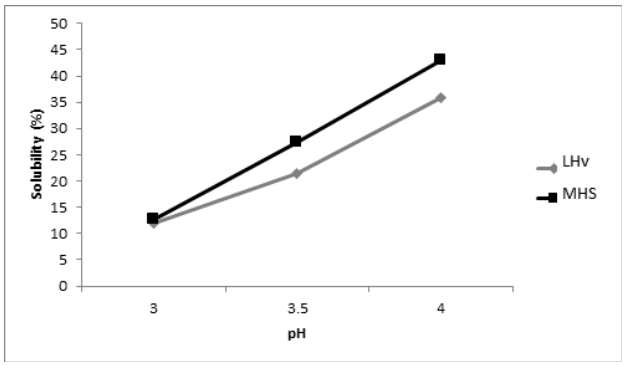

Figure 2: Nitrogen solubility of the limited hydrolysate of hard-to-cook *P. vulgaris* (LHv) and the mixed hydrocolloid system (MHS) at pH 3, 3.5 and 4.

The force that possibly induced an aggregation around the isoelectric point in LHv and MHS is probably due to a combination of hydrophobic attractions, van der Waals attractions, and some electrostatic attractions between the positive groups of the protein and the negative groups of the polysaccharide. Likewise, Liu et al. [35] report that at pH 3 and 4 large complexes were formed by electrostatic attraction between the biopolymers, reducing solubility. Similarly, in mixed systems formed by pea and gum arabic concentrates, they observed that the interactions of the biopolymers were optimal and neutralization of charges occurred, thus reducing solubility.

Foaming capacity and stability

The foaming capacity of the mixed systems (Figure 3) was higher than the percentages obtained by the hydrolysate, being statistically different ($p<0.05$). Foam formation is probably based on the combined adsorption of free protein and protein-gum complexes at the interface [35]. The electrostatic interactions of the biopolymers produced conformational changes that strongly assisted the viscoelastic film for better integration at the interface. The negative charges of the modified flamboyant gum are more numerous at pH 4 than at pH 3, therefore, more binding sites will be available for the positively charged protein molecules. An increase in the surface hydrophobicity of the complexes formed (due to the polysaccharide and the conformational changes induced in the bond) may result in increased adsorption at the air-water interface.

Figure 4 shows the stability of the foam obtained by the hydrolysate and the mixed system by measuring the conductivity in relation to the time evaluated (60 min). The MHS at the pH values evaluated presented higher stability compared to the hydrolysate since they had a low conductivity, very close to zero, and a longer time in which the conductivity increased, which indicates the loss of foam at the same level between tests. The low initial conductivity because the mixture solution was completely transformed into foam and therefore exerted a resistance due to air bubbles. Similarly, it was observed that the incorporation of modified flamboyant gum in MHS improved foam stability as opposed to when only the hydrolysate was present. This could be because the modified gum increases the viscosity of the solution, thus forming a more effective physical barrier that stops the tendency of the discontinuous gas phase to form a continuous phase by approximation and formation of bubbles that cause the liquid to drain, which is observed with the increasing time for the increase in conductivity. At pH 3.5 and 4, the films formed by the complex are likely to have exhibited low gas permeability with increased foam stabilization properties as suggested by Schmitt et al. [36].

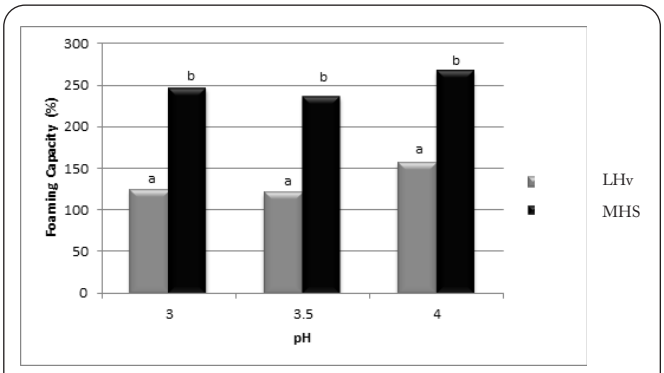

Figure 3: Foaming capacity of limited hydrolysate of hard-to-cook *P. vulgaris* (LHv) and mixed hydrocolloid system (MHS) at pH 3, 3.5 and 4. [a-b] Different letters at the same pH indicate significant statistical difference ($p<0.05$).

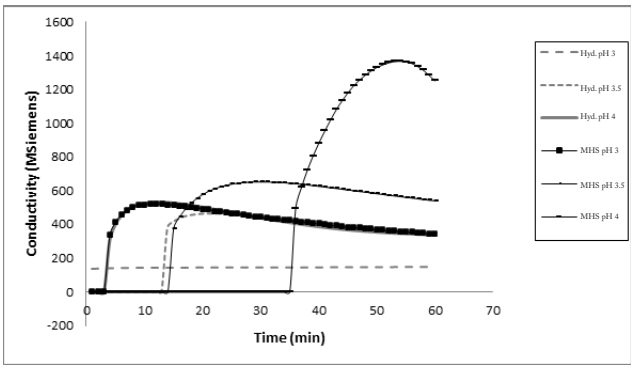

Figure 4: Foaming stability of limited hydrolysate of hard-to-cook *P. vulgaris* (LHv) and mixed hydrocolloid system (MHS) at pH 3, 3.5 and 4.

Ganzevles et al. [37] reported that the formation of protein-polysaccharide complexes can be used to modify the adsorption kinetics of proteins at the air-water interface, but also modifies the microstructure of the adsorbed layer and controls its stability. Liu et al. [35] reported that the electrostatic interactions of the biopolymers help to strengthen the viscoelastic film at the interface, induce conformational changes in the protein for better integration at the interface and increase the viscous phase.

Emulsifying activity and stability index

The emulsifying activity index (EAI) and emulsifying stability index (ESI) were determined for MHS in the pH ranges of 3, 3.5, and 4. Mahmoud et al. [38] point out that the molecular weight of the polypeptides is of importance, since the optimum molecular size or chain length to provide significant emulsification properties is approximately 20 amino acid residues. The interactions at pH 3.5 and 4 (Figure 5) presented a better EAI (144.5 and 218.8%) as opposed to those formed at pH 3 (81.66%). The high rate of emulsifying activity by the interactions at 3, 3.5 and 4 was related to the ability of the biopolymers to rapidly adsorb at the interface and subsequently acquire the appropriate conformational rearrangement to form the viscoelastic film to support the oil droplets. However, the ESI was reduced over the time evaluated. In investigations conducted by Liu et al. [35] on the elastic behavior of pea-gum arabic globulin films formed at the interface, it was observed that it was higher near the isoelectric point, due to the organization of the protein in the complex. Ducel et al. [39] reported an increase for the pea globulin-gum arabic complex in interphase adsorption relative to globulin alone, as a result of a decrease in interfacial tension, due to the increased hydrophobic nature of the complex formed.

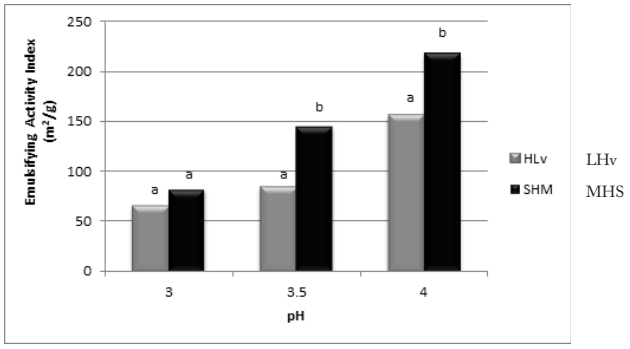

Figure 5: Emulsifying activity index of limited hydrolysate of hard-to-cook *P. vulgaris* (LHv) and mixed hydrocolloid system (MHS) at pH 3, 3.5 and 4. [a-b] Different letters at the same pH indicate significant statistical difference ($p<0.05$).

Regarding the emulsion stability in the mixed hydrocolloid systems (Figure 6), it was observed that it was dependent on the pH range of 3, 3.5, and 4, probably due to a low surface charge in the complexes formed. In addition, the steric stabilization and the viscosity of the continuous phase could inhibit the coalescence of neighboring oil droplets.

The emulsion stability in the mixed hydrocolloid system could be associated with protein-protein aggregates and with the protein-gum complex which allows an increase in stability under acidic pH conditions since depending on the differences in the structure presented by both biopolymers at the interface will influence the emulsion stability. Increased stability of emulsions using biopolymers has also been reported for bean concentrate and carrageenan, carboxymethylcellulose, and potato concentrate [40] and pea globulin and gum arabic [39]. The high emulsion stability percentages obtained in this study for MHS (LHv:MFG) suggest that the interaction between these two biopolymers could be exploited in the processing of acidic foods.

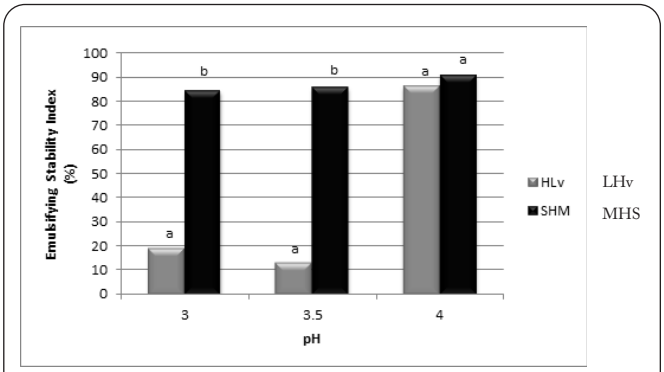

Figure 6: Emulsifying stability index of the protein hydrolysate (LHv) and mixed hydrocolloid system (MHS). [a-b] Different letters at the same pH indicate significant statistical difference ($p<0.05$).

Conclusions

The enzymatic modification caused the DH achieved with the Pancreatin® enzyme (8.32%) to positively influence the functional properties of the hard-to-cook *Phaseolus vulgaris* protein hydrolysate since the polypeptides and peptides generated favored the hydrophilic-hydrophobic balance, which resulted in an increase in nitrogen solubility, foaming properties, and emulsifying properties. The MHS (LHv:MFG) at pH 3 showed higher interaction than the MHS formed at pH 3.5 and 4 at low ionic strength (10 mM) in a 3:1 ratio of biopolymers.

The MHS (LHv:MFG) resulted better than hydrolysates in solubility, foaming, and emulsifying capacities, however foaming capacity was better than emulsifying capacity. These results demonstrate that protein hydrolysates from hard-to-cook *P. Vulgaris* are a good source of peptides with bioactivity and functionality whose interaction with modified flamboyant (*D. Regia*) gum form mixed hydrocolloid systems that improve these properties and could be incorporated into food systems.

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Conflict of Interest

The authors have declared that no competing interest exist.

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