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RESEARCH ARTICLE

Obtainment and physical-chemical and functional characterization of a lupin (*Lupinus mutabilis Sweet*) protein hydrolyzate

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Abstract

A lupin protein isolate was used as substrate for the sequential enzymatic hydrolysis with papain and Flavourzyme® nutritious degree. The electrophoretic analysis revealed the presence of 4 predominant bands with molecular weights between 21 and 7.0 kDa. With the extensive hydrolysis, the dispersibility index of the protein isolate increased from 98 to 100%, the solubility increased to 93.3 % value with pH 4.5. The pH and protein concentration influenced positively on the capacity of foam formation, reaching 460% of volume increase with pH 10. However the formed foams were more unstable than those from the protein isolate. The proteolysis did not affect drastically to viscoelastic properties of the protein.

Introduction

The enzymatic or chemical modifications have been used to improve the functional properties of proteins. The first ones, generally involve the use of proteolytic enzymes to hydrolyze specific peptide bonds and its use presents certain advantages relative to the chemical methods, among them it is mentioned: its specificity, effectiveness to low concentrations and in moderate conditions and its general security make unnecessary to remove them from the final product (Ludwig *et al*., 1995)

The enzymatic hydrolysis originates smaller peptides and with less secondary structure, which contributes to improve certain functional properties as the solubility in a wide pH range, significant characteristic for fortification of acid drinks with protein (Jones & Tung, 1983). The use of enzymes to modify the functional properties is promising the food industry.

Hydrolyzed proteins have a wide application, in the elaboration of chopped and ground meats, mix for pastries, creams for coffee, milk, mayonnaise, seasonings for salad, frozen desserts and in the pharmaceutical field, for formulation of special diets for people with allergies to proteins or who are unable to digest and absorb them.

MATERIALS AND METHODS

Materials

Protein isolate from lupine seed (*Lupinus mutabilis* Sweet)

Papain 3.4.22.2 (Fluka.Bio.Chemika) and Flavourzyme® 3.4.1.2. (Novo Industries) were used for the enzymatic modification of protein isolate.

Bovine serum albumin (BSA) Nº. 23209 and reagents for the determination of proteins. Folin & Ciocalteaus´s Phenol, F-9252 and Bovine Hemoglobin H-2625 (Sigma)

Methods

Obtainment of an enzymatic hydrolyzate from lupine protein isolate

The hydrolysis of protein was carried out in a reactor which has a capacity of 4 liters. It was thermostated at 50°C, provided with a magnetic stirrer and equipped with a pH electrode. The isolated was dispersed in water, (1:20 w/v), the pH suspension was adjusted to 7.0 with NaOH 1N. An enzyme/substrate relation of 0.32 UA/g substrate was used for papain and 167 LAPU/g substrate for the Flavourzyme®.

A sequential enzymatic hydrolysis was carried, adding first papain, it was maintained to pH 7.0, 50°C during 60 minutes. Then Flavourzyme® was added, maintaining the same reaction conditions as papain and it was allowed that the exopeptidase acts for 90 minutes. After this time, any residual enzymatic activity was destroyed by heating the solutions for 10 minutes. The product was centrifuged to 5000 rpm (8 392 x gr) during 30 minutes, the supernatan containing soluble peptides was frozen and freeze-dried. The advance of the hydrolysis was determined quantifying the content of soluble protein and calculating the degree of hydrolysis (DH) of the samples extracted at different times of reaction (0, 1, 3, 5, 10, 20, 30, 40, 50, 60, 65, 70, 80, 90, 100, 110, 120, 150 min.), (Cheftel *et al*, 1989).

Degree of Hydrolysis (DH)

The degree of hydrolysis was determined using the method described by Kim *et al.*, (1990) and measuring the soluble protein of the samples treated with 10% of trichloroacetic acid (TCA).

Physicochemical Characterization

Proximate analyses: A.O.A.C methods (1984).
Alkaloids: Method described by Von Baer *et al*., (1979)
Total starch: According to the enzymatic method of Holm *et al*., (1986)
Amino acids: Following the method described by Shimadzu Corporation, (1993).
In vitro protein digestibility: Method of Hsu *et al*., (1997)
Molecular weight distribution: According to the method of Weber and Orborn, (1969).

Functional properties

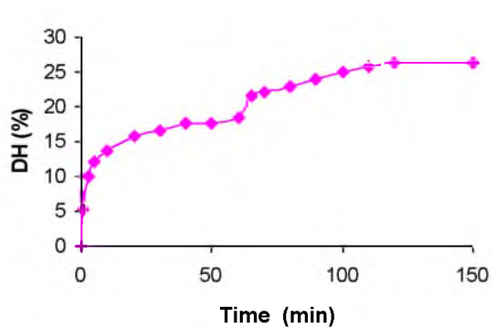
Protein dispersibility Index (P.D.I): 46-24, A.A.C.C. method (1984).
Protein solubility: 46-23, A.A.C.C. method (1984).
Water and oil -Holding Capacity: Method of Naczk *et al*., (1985)
Emulsion capacity and Stability: Method of Sathe and Salunke, (1981)
Foaming capacity and stability: Method of Chau *et al*., (1977).
Apparent viscosity: It was determined in a MA 02072 digital viscosimeter Brookfield Engineering with the axis UL adapter.

RESULTS AND DISCUSSION

Obtainment of an enzymatic hydrolyzate from lupin protein isolate

A 0.32 UA/g enzyme/substrate ratio for papain and 167 LAPU/g substrate for Flavourzyme® were determined, then it was carried out an enzymatic hydrolysis in sequence, adding first papain and second Flavourzyme®. In the Figure 1, the progressive hydrolysis of lupine isolate, to pH 7.0 y 50°C is shown.

Figure 1. Hydrolysis of lupin protein isolate by sequential action of papain and Flavourzyme®



Throught the hydrolytic action of papain, the following degrees of hydrolysis were obtained: 5.30, 9.94, 12.07, 13.57, 15.66, 16.69, 17.69, 17.73 and 18.31%, in the following reaction times: 1, 3, 5, 10, 20, 30, 40, 50, and 60 minutes. From this time on, the Flavourzime® was added, it catalyzed the protein hydrolysis, allowing to reach 21.64, 22.00, 23.00, 24.00, 25.12, 25.9, 26.26 and 26.27% degrees of hydrolysis at 65, 70, 80, 90, 100, 110, 120, and 150 minutes of reaction, respectively.

Physicochemical Characterization

Proximal analysis

The hydrolytic process did not affect to total protein content of the isolate, nevertheless the other components (starch, sugars, alkaloids, crude fiber and fat) experienced a decrease (Table 1), that could have its origin in the temperature to which the proteolysis was carried out, contributing to the best sugar extraction which were removed by centrifugation as undesirable constituents, together with starch and fiber.

Table 1. Protein Hydroyzate proximal composition in comparison with lupin protein isolate*

Component	Percentage (dry weight basis)	
	Isolate	Hydrolyzate
Moisture	2.0 ± 0.04	2.0 ± 0.10
Total protein (N x 6.25)	99.3 ± 0.47	99.3 ± 0.33
Crude fiber	0.4 ± 0.05	0.2 ± 0.02
	0.4 ± 0.20	0.3 ± 0.15
Fat	0.5 ± 0.05	5.9 ± 0.06
Ash	0.2 ± 0.15	0.07 ± 0.06
Total starch (as glucose)	0.1 ± 0.10	0.02 ± 0.01
Total sugars (as glucose	0.02 ± 0.02	n.d.
Alkaloids (as lupanine)		

Data are means of three determinations ± S.D.
n.d. non detectable

Amino acids profile

The enzymatic hydrolysis did not affect the isolate protein and amino acids content, however a low concentration of sulfured amino acids was determined (Table 2).

In vitro protein digestibility

The protein isolate presented a 85.9% digestibility, with the enzymatic treatment, this slight decrease in the hydrolyzed digestibility could have been originated in the processing of the protein (enzymes thermal inactivation, freeze-dry, etc). According to Fukushima, (1980), the thermal treatments can induce the alteration of some amino acids bonds, increasing the digestion time; equally they can propitiate the formation of new amino acids bonds, which can not be attacked for the digestive enzymes.

Molecular weight distribution

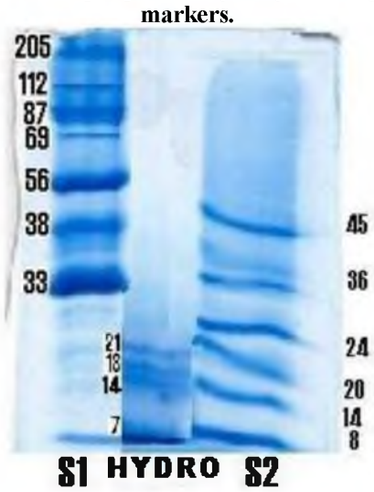
The electrophoretic analyses of protein hydrolyzate, revealed the presence of 4 proteic species (Figure 2) with apparent molecular weight between 21 and 7.0 kDa, which, due to the enzymatic proteolyses were of smaller size that the characteristic species of protein isolate. These results show that the lupin protein hydrolyzate, can be used as a nutrient for feeding individuals unable to digest the macromolecules and also in infantile hipoallergics formulations.

Table 2. Amino acids composition of lupin protein hydrolyzate and isolate (g/16 g N)*

Amino acid	Hydrolyzate	Isolate
Aspartic acid	12.0 ± 0.11	12.2 ± 0.15
Threonine	3.9 ± 0.08	4.0 ± 0.10
Serine	5.8 ± 0.10	5.9 ± 0.12
Glutamic acid	23.4 ± 0.07	23.4 ± 0.62
Proline	3.9 ± 0.05	3.9 ± 0.13
Glyicine	4.2 ± 0.10	4.4 ± 0.50
Alanine	3.3 ± 0.09	3.1 ± 0.09
Cysteine	1.0 ± 0.11	1.0 ± 0.26
Valine	3.2 ± 0.07	3.3 ± 0.04
Methionine	0.5 ± 0.10	0.5 ± 0.10
Isoleucine	5.1 ± 0.10	5.2 ± 0.11
Leucine	8.5 ± 0.08	8.7 ± 0.19
Tyrosine	4.3 ± 0.06	4.0 ± 0.12
Phenylalanine	3.3 ± 0.11	3.3 ± 0.14
Histidine	2.8 ± 0.05	2.8 ± 0.17
Lysine	5.3 ± 0.10	5.1 ± 0.09
Arginine	11.0 ± 0.11	11.2 ± 0.41
Cystine	0.3 ± 0.08	0.3 ± 0.10
Tryptophan	0.7 ± 0.12	0.7 ± 0.20

* mean of triplicate determinations ± S.D.

Figure 2. Molecular weight distribution of lupin protein hydrolyzate. High (S1) and low (S2) molecular weigh markers.



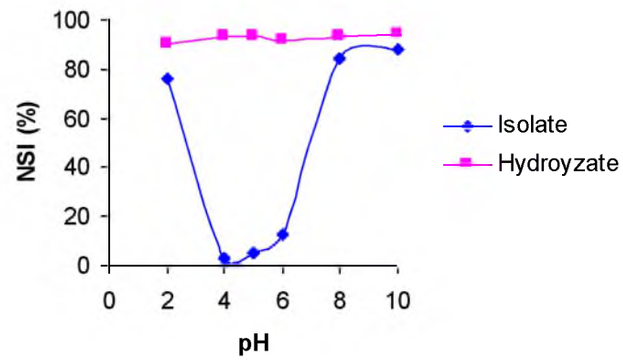
Functional properties

- **Dispersibility index (PDI)**

By means of enzymatic hydrolysis, a 100% PDI was reached. According to Mutilangi *et al.*, (1996), this result is due to the low molecular weight peptides, that are generated by action of the proteases. Also it is due to the higher exposure of the charged and polar places of these, to the aqueous environment that increases the afinity of proteins for the water molecules, favoring its dispersibility in it; this is an important property in the juices preparation, infantile formulations, powdered nutritious drinks, etc.

- **Solubility**

The solubility profile of the hydrolyzed protein was improved through the whole pH scale (Figure 3), reaching a maximum of 93.3% at pH 4.5. This result can be attributed to the formation of smaller peptides and to the higher exposure of the amino and carboxyl ionizables groups, which improve the likeness of the protein for the water molecules. These results agree with those obtained for soybean protein hydrolyzates (Bernardi Don *et al.*, 1991).



- **Water and oil-holding Capacity**

The hydrolyzed protein showed a smaller water-holding capacity (0.54 g water/g protein) and a bigger oil-holding capacity (2 g oil/g protein) compared to the isolated (0.85 g water/g protein and 1.24 g oil/g protein). The treatment with papain and Flavourzyme®, possibly originated the disruption of the protein network, so it was responsible for the imbibition and holding of water, determining a decrease in the liquid-holding capacity. The increase in the oil holding capacity of hydrolyzed compared with the isolate, suggests a higher exposure of the hydrophobic groups, which react strongly with lipids.

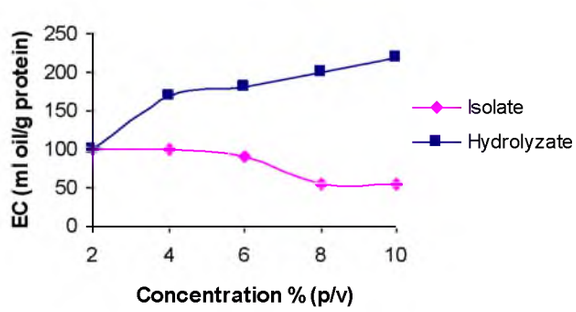
- **Emulsion capacity (EC) and emulsion stability (EE)**

The highest emulsion capacity (220 ml oil/g protein), for 2% protein hydrolyzate suspensions, was obtained at pH 10.0. A 26.3% hydrolysis degree, determined a small increment of the emulsion capacity compared with the isolate, this could be attributed to a better balance between the hydrophilic and lypophylic groups as well as to the higher exposure of the hydrophofic animo acid residuals, that was induced by the proteolytic disintegration of protein, Kim *et al.*, (1990).

The protein hydrolyzate and isolate showed a good emulsion capacity to pH far from the isoelectric point (pH 4.5). At a 2% concentration, the thickness and the viscosity of protein hydrolyzate and also the isolated adsorbed in the interface, were seemingly insufficient to stabilize the emulsions at pH 4.0, as a consequence the volume of the oil separated after the centrifugation, at this pH was higher. The emulsion stability improved above the isoelectric point.

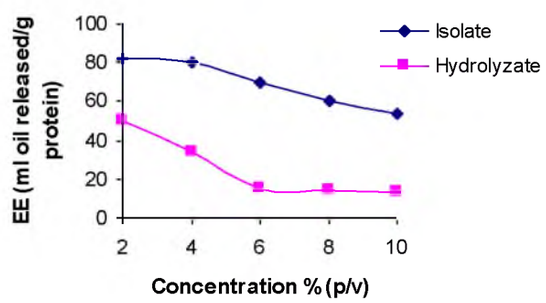
Opposite to what happens with the protein isolate, the protein hydrolyzate emulsion capacity at pH 4.0, was increased with the concentration (Figures 4). Hettiarachchy and Kalapathy, (1997) indicate that the presence of a higher quantity of hydrolyzed protein in the oil/water interface, favors the formation of interfacial films and also the emulsion stability.

Figure 4. Effect of the concentration on the emulsion capacity (EC) of lupin protein isolate and hydrolyzate



Concentrations of protein hydrolyzate above 6%, had a positive effect (smaller quantity of oil released) on the superficial load of the oil drops, contributing to a higher stability of the emulsions (Figure 5).

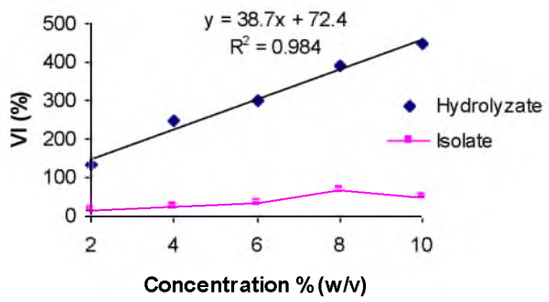
Figure 5. Effect of the concentration on the emulsion stability (EE) of lupin protein isolate and hydrolyzate



- **Foaming**

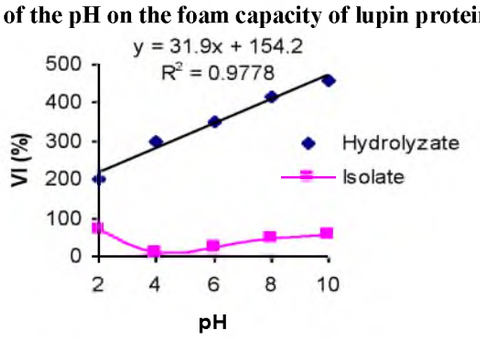
The foaming and stability from 2% protein hydrolyzate suspensions was increased according to the pH, reaching a volume increase of 460% to pH 10 (Figure 6), value that contrasts with the maximum foaming capacity of isolate (72%) to pH 2.0. These results showed the importance of a strong solubility of protein (r = 0.90) and a better orientation of the air/water interface to obtain a optimum foaming capacity (Figure 7).

Figure 6. Effect of the concentration on the foam capacity of lupin protein isolate and hydrolyzate



Equally, the increment in the concentration of hydrolyzed protein seems to be the cause of a favorable viscosity in the liquid phase and an appropriate thickness of the film adsorbed, reaching a 390% volume increase at 8% concentration .

Figure 7. Effect of the pH on the foam capacity of lupin protein isolate and hydrolyzate



At equal as with the protein isolate, the most stable foams were obtained from hydrolyzate suspensions at pH 2.0, however the late ones collapsed after 3 hours, while the isolate foams collapsed after 14 hours. Equally, concentrations of protein hydrolyzate in the order of 8 and 10% allowed to obtain foams that collapsed after 3 hours, while those of the protein isolate collapsed after 24 hours.

The reduction of the molecular size by proteolysis, was clearly favorable for the emulsifying and foaming properties of the lupin protein . It seems that the beneficial effects come from the surface hydrophobicity of soluble protein and its capacity to reduce the surface and interfacial tensions.

- Viscosity

The pH variations modified the viscosity of protein suspensions at 18 % concentration. The highest viscosity value was obtained at pH 10.0, this can be explained for the negative load that the protein acquires above the isoelectric point with a maximum unfolding and lengthening.

An viscosity increment was determined for suspensions at 14 % concentration, treated at 30, 40 and 50°C. At these concentration levels, the protein-protein interactions possibly are in enough number to show a viscoelastic behavior. The hydrolyzate viscosity was slightly lower than the isolated viscosity, especially at pH 2.0, the low water-holding capacity, for the isolate and the hydrolyzate, probably influenced the viscosimetric behavior of the two protein fluids. At a 20 % concentration and 50°C, a higher hydrolyzate viscosity was determined. Hettiarachchy and Kalapathy, (1997), mentioned that higher soluble protein concentrations, produce higher probability of intermoleculars contacts and it favors the viscosity.

CONCLUSIONS

The hydrolytic process did not affect the total protein content, it improved the protein isolate dispersibility from 98.2 to 100% and improved the solubility profile in a wide range of pH (2-10), but it lowered the stability of emulsions at pH 4.0 and of foams at pH 2.0. The decrease of molecular size by proteolysis, notably influenced on the solubility. The isolate viscosimetric behavior was not drastically affected by proteolysis, 30.7 ctp viscosity was obtained with a 20% hydrolyzate suspensions and 50°C.

For its high protein content, low carbohydrates content and smaller molecular size of species proteic characteristic, the hydrolyzate obtained is highly functional and soluble, useful for the preparation of fortified drinks and foods for people with medical diets, children population, sportsmen and third age groups.

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