 https://doi.org/10.1051/e3sconf/202453201005

Hydrolyzed *Pisum sativum* as a protein source for baked products

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Abstract. The present investigation was based on the extraction of proteins from Pisum sativum pea flour assisted by hydrolysis, to obtain a protein hydrolyzate for incorporation into a baked product. With a descriptive level study and experimental methodology, three operating conditions were established: solute/solvent ratio, stirring time and hydrogen potential. Next, the hydrolyzate was carried out in two stages: basic, for the solubilization of proteins and acid, for the precipitation of the protein paste. As a result, the hydrolyzate was obtained with a protein level close to 80% that notably exceeded the protein level of 14.7% reported in Pisum sativum flour. Then, the implementation of the protein hydrolyzate was evaluated by preparing three types of bread: bread 1 with 0% hydrolyzate, bread 2 with 15% hydrolysate and bread 3 with 20% hydrolyzate. In short, it was detected that the baked product bread 2, presented better quality indicators, bromatological properties and amino acid profile. In turn, the research was able to specify that the optimal percentage for nutritional development is 15% hydrolyzate substitution, however, to achieve or develop better bakery quality characteristics, it is recommended that future research operate in the range of 10% - 14%.

1 Introduction

Nowadays, interest in the use of *Pisum sativum* (PS) peas has been renewed to provide added value to food products, which generates an advantage from a nutritional point of view, regarding proteins, crude fiber, minerals, vitamins and antioxidant substances. Likewise, it stands out as an economic and viable alternative due to the protein percentage, being an easily obtained raw material that is not used in the food sector [1,2]. Likewise, it has been detected that bread products on the market show a deficit in protein quality and nutritional value and are not complete foods due to the lack of essential amino acids [3].

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There is research that shows how flours obtained from cereals generally have low protein levels and a lack of lysine [4], while PS pea flours contain a higher level of protein that borders 26% and are considered an alternative protein source [5].

Thus, obtaining protein hydrolysates has become a proposal presented in recent years through different types of extractions [6-9,2,10,11]. However, PS peas contain low levels of methionine, and some studies show that the protein has a biological value based on its amino acid composition [12]. While, Urrutia [6] states that in bread products made with soy flour and/or pea flour; the latter presents greater adverse effects than soy flour concerning the physical and sensory properties of the baked products, therefore, the use of pea flour was detected up to 18% replacement.

In this sense, the objective of this research is to evaluate the effect of using protein hydrolyzate to produce a baked product that allows for enhancement of the physical and nutritional characteristics of molded breads, formulated with PS hydrolysates at three substitutions levels 0%, 15% and 20.

2 Materials and methods

2.1 Sample preparation

Pisum sativum (PS) pea flour was obtained from the process and quality control laboratory of the Prodicereal S.A. industry, where dehydrated PS grains are inactivated by immersion in water at the same grain temperature for 2 min, then they are dried by forced circulation with air at less than 48 °C, then grinding is carried out in a Maquinova model MMQ40 hammer mill until an average particle diameter of between 30 μm was achieved.

2.2 PS pea hydrolysates obtention

A completely randomized experimental design was performed during protein extraction. The hydrolysis of PS flour was carried out in two stages: first stage, a suspension was prepared with 10 g of PS flour in 100 mL of distilled water (ratio 1:10), and the pH was adjusted to 8.0 with 1 N Merck sodium hydroxide, for protein solubilization. Then it was stirred for 60 min using a magnetic stirrer. The suspension was placed in the YINGTAI centrifuge, model TGL16 for 20 min at 8,000 rpm, recovering the supernatants. In the second stage, the insoluble residues were re-suspended with distilled water at a ratio of 1:5, then the pH was adjusted to 10.0 with 1 N Merck sodium hydroxide by stirring mechanically for 10 min. Then, the residues were centrifuged at a speed of 8,000 rpm recovering the supernatant to discard the insoluble material. Next, acid precipitation was carried out with supernatants from the basic extraction, adjusting the pH to 4.0 with a 2 N Titrisol-HCl solution. Then, the mixture was shaken for 20 min and centrifuged at 12,000 rpm until obtain a protein paste, which will be subjected to dehydration.

2.3 Preparation of bread premixes

The preparation of three premixes (1, 2, 3) was carried out using different proportions of substitution of wheat flour for PS pea hydrolyzate: bread 1 (0% hydrolyzate – 100% fortified flour); bread 2 (15% hydrolyzed – 85% fortified flour); (20% hydrolyzed – 80% fortified flour). Table 1 shows the estimated formulation and calculation basis to produce molded bread, the purpose of which is to elucidate the impact of protein hydrolyzate for supplementation of a baked product.

Table 1. Formulation of breads 1, 2 and 3

Ingredients	Bread 1 (0% PS pea hydrolyzate)	Bread 2 (15% PS pea hydrolyzate)	Bread 3 (20% PS pea hydrolyzate)
Fortified flour (g)	500	425	400
Sugar (g)	28	28	28
Salt (g)	10	10	10
Water (15 ºC)	250	280	340
Margarine (g)	18	18	18
Yeast (g)	7	7	7
Egg (units)	1	1	1
Milk powder (g)	5	5	5
Protein hydrolyzate (g)	0	75	100

2.4 Direct baking method

The preparation of the baked product consisted of five elementary stages:

Stage 1: The different ingredients were mixed for 20 min at 50 rpm using a mixer (SKYMSEN) with a single arm with a spiral design (pig's tail). So, an elastic paste was obtained. Gluten formation and residence time prevent the breaking of bonds, ensuring the construction of the so-called viscoelastic network.

Stage 2: the dough fragment obtained in the first stage was kneaded manually, then formed into a ball and left to rest for 10 minutes to recover.

Stage 3: the dough fragment was rolled out with a rolling pin, until a bar-shaped structure was obtained. From there, a fraction was separated to arrange the 300 g mold and the 500 g mold.

Stage 4: Fermentation gives rise to the dough rising until reaching mold height. At the same time, CO_2 is generated, which, when captured by the dough, sponges up, giving the bread its main characteristics. At this stage, the three pre-mixes (A, B, C) were left to rest for 60 min.

Stage 5: The cooking time was programmed according to the mold: 15 min for 300 g molds and 20 min for 500 g molds, both assisted by an Electric Oven with a temperature ramp upto 185 ºC.

It should be noted that three constant operating conditions were maintained; magnetic stirring time, hydrogen potential (pH) and solute/solvent ratio, so that, at the end of the process, the three types of bread were received with two different molds: 300 g mold and 500 g mold. In addition, the amount of necessary ingredients and residence time were established for the different stages of the method, with the purpose that the addition of concentrate is the only determining factor of the process.

2.5 Proximate analysis

A proximate analysis was realized by triplicate at the pea flour *Pisum sativum*, the protein concentrate obtained by chemical hydrolysis and the baked products, with the purpose of comparing the macro components presents, using the VDLUFA [13] procedure based on the Weende method [14,15], to identify five parameters; moisture, ash, fiber, fat and total protein content.

2.6 Amino-acid profile

The amino acid profile was determined by triplicate using High Performance Liquid Chromatography (HPLC) according to method 982.30 of the AOAC 2006.

3 Results and discussion

3.1 PS flour characterization

Once the Pisum sativum (PS) pea flour was obtained, the proximate analysis of PS was carried out (Table 2). PS flour showed 14,7 % of protein, that is close to that determined in the study reported by Vallejos [2].

Table 2. Proximate analysis of PS pea flour

Sample	Parameters	Method	Content %
Pisum sativum pea flour	Humidity	INSP-LAB-SOP-005/AOAC 19th 942.05	11,16
	Fat	AOAC 20th 920.85/920.39 C	3,39
	Ash	INSP-LAB-SOP-005/AOAC 19th 942.05	1,76
	Fiber	AOAC 20th 978.10	0,53
	Protein	AOAC 20th 979.09	14,7

3.2 Characterization of PS hydrolyzate

In this work, the protein content of PS hydrolizate was determined by proximate analysis, the value obtained was 79,14%, higher than PS pea flour (14,70%). Thus, the chemical hydrolysis extraction method allows increasing the percentage of protein from PS protein hydrolysate (Table 3). According to Jaimes et al. [5], a percentage protein content between 65% to 90% is called protein concentrate. Therefore, the PS protein hydrolysate could be used as an ingredient for bakery products, providing a significant protein content.

Table 3. Proximate analysis of PS pea hydrolyzate.

Sample Parameters	Method	Content (%)
Humidity	INSP-LAB-SOP-001/AOAC 19th 930.15	7,49
Protein Fat	AOAC 20th 920.85/920.39 C	2,09
hydrolyzate Ash	INSP-LAB-SOP-005/AOAC 19th 942.05	4,5
Fiber	AOAC 20th 978.10	0,37
Protein	AOAC 20th 979.09	79,14

3.3 Characterization of baked goods

The Figure 1 shows the three types of bread for each manufacturing mold: 500 g mold and 300 g mold.

Fig. 1. White bread: bread 1 (0% PS pea hydrolyzed); bread 2 (15% PS pea hydrolyzed; bread 3 (20% PS pea hydrolyzed).

To determine the optimal characteristics of both protein and baked product, parameters shown in the Table 4 were determined. Regarding the protein and fat content, when comparing bread 2 (15% hydrolyzed PS) and bread 3 (20% hydrolyzed PS), no significant differences were observed, but significant differences were observed with bread without the addition of PS hydrolysate, which had a lower value in the case of protein content. An increase in moisture content was observed in breads 2 and 3 compared to bread 1.

Table 4. Quality indicators of different formulations of bread with or without PS hydrolyzed

	Bread 1 (0% PS pea hydrolyzed)	Bread 2 (15% PS pea hydrolyzed)	Bread with 20% hydrolyzed PS
Protein (%)	15,61 ± 0,04[a]	20,51 ± 0,04[b]	20,92 ± 0,04[b]
Fiber (%)	0,07 ± 0,01[a]	0,08 ± 0,01[a]	0,04 ± 0,01[b]
Fat (%)	2,92 ± 0,11[a]	3,62% ± 0,09[b]	3,89% ± 0,10[b]
Humidity (%)	35,58 ± 0,23[a]	37,16% ± 0,25[b]	37,35% ± 0,24[b]
Ash (%)	2,00 ± 0,15[a]	2,50% ± 0,13[b]	2,00% ± 0,14[a]
Slice height (cm)	8,5 ± 0,09[a]	6,2 cm ± 0,08[b]	5,9 cm ± 0,07[b]
Weight (g)	275,2 ± 0,08[a]	281,6 g ± 0,07[b]	285,4 g ± 0,08[b]
Specific Volumen (mL/g)	4,42 ± 0,1[a]	3,26 mL/g ± 0,1[b]	3,20 mL/g ± 0,1[b]
Shape and symmetry	Symmetric shape	Asymmetric shape	Asymmetric shape
Bark color	Golden	Brown	Brown
Crumb color	White	Beige	Yellow

Mean and standard deviations for n=3 are reported
The same letter as superscript of data in a given row means non-significant differences ($p < 0,05$)

Furthermore, in Figure 2 the slice cut clearly presents the structure (fineness of pores with wall thickness) and physical characteristics of the three breads.

Fig. 2. Slices of white bread obtained by three formulations.

The bread 1, has open and thin-walled crumbs, bread 2 with a 15% concentrate has closed and thick-walled crumbs, as does bread 3 with a 20% concentrate. Likewise, when evaluating the crust of the breads, standard bread 1 has a smooth surface, while breads 2 and 3 maintain rough surfaces due to the gluten being weakened by the addition of concentrate. In terms of texture, bread 2 and 3 have not been affected by the substitution of concentrate and they do not lose their characteristic smell either. Regarding the flavor of the crumb for bread 2, it remains sensorially standard, while bread 3 is considered caked with slight astringency, causing the bite to exert greater pressure. Based on the above, two breads with optimal characteristics for both protein and baking.

An amino acid characterization of bread 1 and bread 2 was determined to detect the presence of fifteen amino acids within which eight essentials for human nutrition were examined and verification of the supplementation of the baked product called white bread [16].

Table 5. Amino-acid characterization of bread 1 and bread 2.

Aminoacid	Bread 1 (0% PS hydrolyzate)	Bread 2 (15% PS hydrolyzate)
Aspartic acid	0,36	0,57
Glutamic acid	2	1,2
Serine	0,41	0,05
Histidine*	0,13	0,41
Threonine*	0,17	0,21
Glicine	0,2	0,28
Arginine	0,3	0,45
Alanine	0,27	0,28
Tyrosine	0,18	0,27
Valine*	0,3	0,34
Methionine*	0,12	0,15
Phenylalanine*	0,3	0,32
Isoleucine*	0,27	0,27
Leucine*	0,43	0,46
Lisine*	0,17	0,47

Note: (*) indicates the essential amino acids for human nutrition. The values of amino acids are expressed in g AA/ 100g wet base sample.

The amino acid profile determined eight essential amino acids for nutritional supplementation. An increase in protein of around 5% was reported in the bread 2, relatively small in magnitude, for the 15% substitution level. On the other hand, the essential amino acids histidine and lysine content increases from 0.17 to 0.47 g/100 g wet base sample, and 0.17 to 0.47 g/100 g wet base for the bread with 15% PS pea hydrolyzate.

4 Conclusions

The present study has shown that obtaining protein concentrate from Pisum sativum pea flour assisted by chemical hydrolysis reaches a yield of 11%. Furthermore, the experimental design has allowed the protein hydrolyzate to increase the protein percentage to around 65% compared to the PS flour.

The volume of the bread was reduced to the extent that the level of substitution of the PS protein hydrolyzate in the formulation was increased, due to the relationship ofthe effect produced by a decrease in the amount of gluten in the mixture. For its part, the characterization of the baked product determined optimal bromatological properties in white bread 2, which reached 20.51% protein and its bakery quality characteristics, for example: shape/symmetry, crust color, texture of the crumb and bite pressure allowed it to be considered a product enriched in proteins and suitable for human consumption.

Subsequently, when evaluating the baked product; sliced bread, the partial replacement of flour with protein concentrate, affected the quality of bread-making proteins (glutenin and gliadin). Therefore, the following points must be taken into account at the time of preparation: kneading time for the formation of gluten and viscoelastic network, standard bread contains 12% bread-making proteins, and it does not exceed 20% replacement of flour with hydrolyzate. Based on the above, it was possible to detect that bread 1 visibly presented better baking characteristics. Bread 2 with 15% hydrolyzate needed longer kneading time for the formation of gluten and viscoelastic network since the bread-making proteins are reduced to 10.2%. Finally, bread 3 did not develop normally due to the increase in 20% hydrolyzate, which reduced the bread-making proteins to 9.6%, which generated ruptureswith CO_2 leaks, causing cracks, fissures and deformations in the loaf structure of the bread. Consequently, the research was able to specify that the optimal percentage for nutritional supplementation is 15% of hydrolyzate, however, to achieve or develop better bakery quality characteristics, it is recommended that future research operate in the range of 10% - 14% with hydrolyzate protein.

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