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Functional properties of soy protein isolate of crude and enzymatically hydrolysed at different times

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Abstract

The partial hydrolysis may be used to modify the functional properties of proteins that are added to food and beverage formulations. The present study aimed to hydrolysing the soy protein isolate using *Flavourzyme®* and compares their functional properties (foaming capacity, foaming stability, solubility, water capacity absorption and oil-holding capacity) with the same functional properties of the crude protein. The hydrolysis reaction was conducted for 240 minutes to determine the degree of hydrolysis over time. The samples used for the analysis of functional properties were obtained in 30 minutes of hydrolysis (Degree of Hydrolysis of 36.5%) and 60 minutes of hydrolysis (Degree of Hydrolysis of 51%). To capacity foaming, were obtained results of up to 63.3% however the hydrolysis did not significantly alter this property. The foaming stability increased to 16% protein hydrolysate. The solubility of hydrolysed protein increased 46% compared to in non-hydrolysed protein. The water capacity absorption and oil-holding capacity decreased for the samples hydrolysed compared to non-hydrolysed.

Introduction

Soy is considered a staple food for the world population, for its nutritional quality, ease of cultivation, low cost and high production. Among the cereals and other species of legumes, soy is the one that contains the highest protein content. The soy contains about 40% of its constitution in protein, the canola contains between 20% and 30% and other cereals between of 8% to 15% (Friedeck *et al.*, 2003; Fischer, 2006).

The direct conversion of soy protein in foods in western countries is small, compared to the 95% of the soybeans that are intended to the produce oil and bran used for animal feed. However, because of population growth, demand for soy protein has increased, although the foods of animal origin are still the main source of dietary protein. This demand has stimulated the food industry to use plant protein in their formulations (Liu, 1997; Rodrigues *et al.*, 2003), being that their functional properties are factors that can affect the technological process food. The functional properties are defined as physical and chemical properties that affect the protein contained in food systems during processing, storage, preparation and consumption. As examples can cite the texture, solubility, viscosity, water holding capacity, oil-holding capacity, syneresis, among other properties (deMan, 1999).

The study of the functional properties it is essentially important in determining the potential application of soy protein in products, allowing also defining the use of the proteins as substitute or supplementing in traditional foods in the form of crude protein or protein hydrolysates (Silva, 2007). The enzymatic modification of proteins using prepared of the proteolytic enzymes selected to hydrolysed specific peptide bonds, is widely used in the food industry (Mulally *et al.*, 1994). The partial hydrolysis of proteins or limited proteolysis of the protein with the use of proteolytic enzymes is one strategy to improve the functional properties such as solubility, dispersibility, foaming and emulsification (Fennema, 2000).

As a result of the hydrolysis of proteins, in addition to altering the functional properties due to modifying of the molecule, also occurs the producing small peptides and amino acids for use in diet foods or food flavoring agent (Roman and Sgarbieri, 2005) and also in food processing, providing great benefits such as: improved digestibility, changes in sensory quality (like the texture or taste), improved of the antioxidant capacity and reduced of the allergenic compounds (Tavano, 2013).

The enzymatic hydrolysis compared to chemical hydrolysis has many advantages because it allows the control of the reaction by improving the properties of the final product. Furthermore, the

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enzymatic hydrolysis process is simple, efficient and involves mild conditions that do not destroy the proteins retrieved by other chemical reactions. Normally, enzymatic processes avoid side reactions and preserve the nutritional value of the protein source (Maldonado *et al.*, 1998; Fonkwe and Singh, 2005). Protein hydrolysates have been successfully tested for incorporation into different food systems such as cereal products, fish and meat products, desserts, crackers, soups and sauces (Fennema, 2000; Chalamaiah *et al*., 2012). The main function of hydrolysed proteins is the use in products for people with difficulty digesting (Fennema, 2000) and who are allergic to crude protein.

The present study aimed obtaining hydrolysed of soy protein isolate (SPI) using a proteolytic enzyme and comparison the functional properties of the hydrolysate at different hydrolysis times with the properties of non-hydrolysed protein (crude). The functional properties which were determined were the ability of foaming capacity, foaming stability, solubility, water capacity absorption and oil-holding capacity.

Materials and Method

The experimental analyzes were performed at the Bioprocess Laboratory, Microbiology Laboratory and Food Chemistry Laboratory in Department of Food Engineering - State University of Santa Catarina. As substrate was used soy protein isolate with 90% protein (Solae) and the enzyme used for the hydrolysis reactions was *Flavourzyme*® 1000L, an exopeptidase with an activity of 1 LAPU/g. One LAPU (Leucine aminopeptidase unit) is the amount of enzyme which hydrolyses 1 micromol leucine-ρ-nitroanilide per minute. All experiments were performed in triplicate.

Preparation of protein hydrolysates

The dispersions were prepared in Erlenmeyer flasks of 250 mL using a 5% ratio of substrate and 8% of the mass of the enzyme on the substrate mass in sodium phosphate buffer 0.1 M pH 7. The Erlenmeyer flasks were placed on shaker (Solab) at a temperature of 55°C (temperature of use for the enzyme recommended by the manufacturer) and 50 rpm agitation, where they remained for a total time of 240 minutes and samples taken every 30 minutes After the hydrolysis time, the samples were heated at 75°C for 10 minutes for denaturation and consequent inactivation of the enzyme. The reactional medium was centrifuged in a centrifuge (Quimis) for 10 minutes and 3000 rpm and the supernatant obtained used to determine the Degree of Hydrolysis (DH).

Estimation of degree of hydrolysis (DH)

After centrifugation and removal of supernatant, the DH was expressed according to the ratio of the amount of protein (mg/mL) by the Biuret method. The method consists in determining the protein concentration of this sample by reaction with the Biuret reagent, and later reading the absorbance at 540 nm, then obtaining the amount of protein obtained by the linear equation of the calibration curve using bovine serum albumin as standard. To obtain the values of protein hydrolysates after centrifugation, 2 mL of supernatant was transferred to a test tube to which was added 8 mL of Biuret reagent. The test tubes remained at rest for 30 minutes at room temperature and after this time, samples were read in spectrophotometer (Biospectro) 540 nm (Macedo *et al.*, 2005), being DH calculated using the values of protein in the supernatant before and after hydrolysis.

Foaming capacity and foaming stability

Foaming capacity and foaming stability of SPI, crude and hydrolysed, were determined according to the method of Liu *et al.* (2010). Protein solutions (20 mL of 0.5%), prepared in a buffer (0.02M sodium phosphate and 0.01M citric acid, pH 3.0–11.0), were homogenised using homogenizer (Philips Walita) for 2 minutes. The whipped sample was immediately transferred into a 25 mL cylinder and the total volume was read after 30 seconds. The foaming capacity (FC) was calculated according to the equation 1:

$$\text{Foaming capacity}\,(\%) = \left(\frac{A-B}{B}\right) x\,100 \quad \text{(equation 1)}$$

where A is the volume after whipping (mL); B is the volume before whipping (mL).

The whipped sample was allowed to stand at 20ºC for 3 minutes and the volume of whipped sample was then recorded. Foaming stability was calculated according to the equation 2:

$$\text{Foaming stability}\,(\%) = \left(\frac{A-B}{B}\right) x\,100 \quad \text{(equation 2)}$$

where A is the volume after standing (mL); B is the volume before whipping (mL).

Solubility

To determine protein solubility, 1,33 g of SPI, crude and hydrolysed, were dispersed in a buffer solution (0.02M sodium phosphate and 0.01M citric acid), in pH 3.0–11.0. The mixtures were stirred at room temperature for 30 minutes and centrifuged

at 3000 rpm for 15 minutes. Protein contents in the supernatants were determined using the Biuret method. Total protein content in SPI samples was determined after solubilisation of the samples in 0.5M NaOH (Liu *et al*., 2010, modified). Protein solubility was calculated according to Equation 3:

$$\text{Solubility (\%)} = \left(\frac{A}{B}\right) x\ 100 \quad \text{(Equation 3)}$$

where A is protein content in supernatant and B is total protein content in sample.

Water capacity absorption

Water capacity absorption was determined using the centrifugation method (Wang *et al.*, 2006, modified). Duplicate samples (1.5 g) of crude and hydrolysate SPI were dissolved with 9 mL of water in centrifuge tubes and dispersed with a shaker for 30 seconds. The dispersion was allowed to stand at room temperature for 10 minutes, and it was then centrifuged at 2300 rpm for 35 minutes. The supernatant was removed and the centrifuge tubes was placed in an inclined (angle of 15° to 20°) in an oven at 50°C with air circulation for 25 minutes. The centrifuge tubes was cooled in a desiccator and weighed. The Water capacity absorption was calculated for 100 g of sample.

Oil-holding capacity

Oil-holding capacity was measured as the volume of edible oil held by 0.5 g of material. The sample 0.5 g was added to 10 ml of soybean oil in a centrifuge tube, and mixed for 30 seconds in shaker (100 rpm) in duplicate. The oil dispersion was centrifuged at 3000 rpm for 30 minutes. The volume of oil separated from the hydrolysate and crude SPI was measured and oil-holding capacity was calculated as the mL of oil absorbed per gram of protein sample (Wasswa *et al*., 2007).

Statistical analysis

Data were analyzed using the Statistica 10.0 (Statsoft Inc.). Significant differences ($p < 0.05$) between means were identified using Tukey procedures. All the experiments were carried out in triplicate.

Results

Degree of Hydrolysis (DH) over time

The profile obtained for the degree of hydrolysis (%) of SPI, which samples were with crude every 30 minutes in 240 minutes is shown in Figure 1 (the experimental conditions were of 55°C of temperature and 50 rpm of agitation). The DH is defined as the percentage of disruption of peptide bonds of a protein (Silva, 2009). In 30 minutes the reaction, was obtained a value of DH of 36.50 ± 5.22 % and at 60 minutes obtained value of the DH 51.00 ± 2.59 %, statistically different ($p < 0.05$) by Tukey test. From of the 60 minutes of the hydrolysis, the values of DH (%) showed no significant difference between together ($p > 0.05$) until the time of 240 minutes. So for the next step was used the hydrolysates obtained in 30 and 60 minutes, whose values of the DH (%) showed statistical differences.

Functional properties

For crude SPI, foaming capacity was higher for the hydrolysates, except hydrolysed for 60 minutes at pH 5 and hydrolysates at pH 11. At pH 3, no foaming for crude SPI, however, presented to the hydrolysates, with the highest values at pH 11. There is a tendency to increase the foaming capacity with the pH variation of acid to alkaline.

Table 1 shows the mean values obtained for the foaming capacity (%) of crude and hydrolysed SPI for 30 and 60 minutes, at different pH values. For crude SPI, it is verified in Table 1, that there is a statistically significant difference ($p < 0.05$) for the results of foaming capacity for the pH 3, 8 and 11. For the hydrolysate in 30 minutes, the statistical difference ($p < 0.05$) occurs between pH 3, 5 and 11 and the hydrolysed in 60 minutes occurs statistical difference ($p < 0.05$) the results of the foaming capacity in pH 3-5 in relation to other values of pH.

In relation to the same pH, comparing between samples, it is noted that for pH 3, foaming capacity the SPI hydrolysed for 60 minutes differs from the other samples ($p < 0.05$). To pH 7, the foaming capacity of the protein hydrolyzate of 30 minutes differs from the other samples ($p < 0.05$). For other pH, there is no difference. Enzymatic hydrolysis did not alter the stability of the foaming being similar results for crude protein and the hydrolysates. Table 2 shows the mean values obtained for the foaming stability (%) of crude and hydrolysed SPI for 30 and 60 minutes, at different pH values.

For crude SPI, there was statistical difference ($p < 0.05$) for the results of foaming stability at pH 5, 8 and 11. For the hydrolysate in 30 minutes, occurs statistical difference ($p < 0.05$) between pH 3, 5 and 11. For hydrolysed in 60 minutes, occurs statistical difference ($p < 0.05$) to the results of the foaming stability pH 7 and 8 compared to other pH values. In relation to the same pH, comparing between samples, it is observed that for pH 3, the foaming stability of the SPI hydrolysed for 60 minutes differs from the

Table 1. Means obtained for the foaming capacity (%) of the crude soy protein isolate and hydrolysed for 30 and 60 minutes

pH	Foaming capacity (%) of the soy protein isolate[a]		
	Crude protein	Hydrolysed in 30 minutes	Hydrolysed in 60 minutes
3	$0.00^{aA} \pm 0.01$	$5.00^{aA} \pm 0.01$	$20.00^{aB} \pm 5.00$
5	$12.50^{abA} \pm 6.61$	$28.33^{bB} \pm 2.89$	$9.33^{aA} \pm 4.91$
7	$25.00^{abA} \pm 10.00$	$43.33^{bcA} \pm 7.64$	$36.67^{bA} \pm 7.64$
8	$31.67^{bA} \pm 7.64$	$35.00^{bA} \pm 10.00$	$42.50^{bA} \pm 6.61$
11	$63.33^{cA} \pm 15.28$	$58.33^{cA} \pm 7.64$	$51.67^{bA} \pm 2.89$

[a]Means ± standard deviation followed by different lowercase letters, in the column, are different at the 95% level of confidence between pH. Means followed by different capital letters, in the line, are different at the 95% level of confidence between samples (for the same pH).

Table 2. Means obtained for the foaming stability (%) of the crude soy protein isolate and hydrolysed for 30 and 60 minutes

pH	Foaming stability (%) of the soy protein isolate[a]		
	Crude protein	Hydrolysed in 30 minutes	Hydrolysed in 60 minutes
3	$0.00^{aA} \pm 0.00$	$5.00^{aA} \pm 0.01$	$18.33^{bB} \pm 7.22$
5	$5.00^{aA} \pm 0.01$	$21.67^{bB} \pm 5.77$	$5.00^{aA} \pm 2.50$
7	$19.17^{abA} \pm 6.29$	$35.00^{bcB} \pm 5.00$	$30.00^{cAB} \pm 5.00$
8	$26.67^{b} \pm 7.64$	$31.67^{b} \pm 7.64$	$30.83^{c} \pm 1.44$
11	$50.00^{cA} \pm 13.23$	$47.50^{cA} \pm 4.33$	$42.50^{dA} \pm 2.50$

[a]Means ± standard deviation followed by different lowercase letters, in the column, are different at the 95% level of confidence between pH. Means followed by different capital letters, in the line, are different at the 95% level of confidence between samples (for the same pH).

Table 3. Means obtained for the solubility (%) of the crude soy protein isolate and hydrolysed for 30 and 60 minutes

pH	Solubility (%) of the soy protein isolate[a]		
	Crude protein	Hydrolysed in 30 minutes	Hydrolysed in 60 minutes
3	$49.59^{aA} \pm 3.50$	$57.74^{aA} \pm 4.71$	$73.80^{aB} \pm 4.77$
5	$25.75^{bA} \pm 1.01$	$71.63^{bcB} \pm 5.92$	$69.59^{abB} \pm 2.17$
7	$27.95^{bA} \pm 2.58$	$73.34^{cB} \pm 6.03$	$70.22^{abB} \pm 7.00$
8	$29.26^{bA} \pm 1.64$	$39.31^{dB} \pm 2.69$	$67.56^{abC} \pm 4.17$
11	$28.57^{bA} \pm 1.77$	$59.58^{abB} \pm 4.09$	$59.15^{bB} \pm 3.15$

[a]Means ± standard deviation followed by different lowercase letters, in the column, are different at the 95% level of confidence between pH. Means followed by different capital letters, in the line, are different at the 95% level of confidence between samples (for the same pH).

other samples ($p < 0.05$). For pH 5, the foaming stability of the protein hydrolyzate of 30 minutes differs from the other samples ($p < 0.05$). For pH 7, the foaming stability of the crude SPI differs from the other samples ($p < 0.05$). For other pH, there is no difference.

Table 3 shows the mean values obtained for the solubility (%) of crude and hydrolysed SPI for 30 and 60 minutes, at different pH values. Through statistical analysis, it was found that for crude SPI occurs significant difference ($p < 0.05$) between the results obtained for the solubility for samples in pH 3 and the other values of the pH. For the hydrolysate for 30 minutes, the solubility values for pH 3, pH 5 and pH 8 are statistically different ($p < 0.05$) but, the solubility values for obtained at pH 3 and pH 11 are statistically equal ($p > 0.05$) and the solubility values for obtained at pH 5 and pH 7 are statistically equal ($p > 0.05$).

For a same pH value, comparing between samples, was statistical difference ($p < 0.05$) to the solubility values in pH 5, 7 and 11 to the crude SPI and hydrolysates. For the samples of the pH 3, the solubility of the crude SPI are different ($p < 0.05$) of solubility of hydrolysed for 60 minutes. For pH 8, a significant difference ($p < 0.05$) between the values of the three samples, with the larger value for the sample hydrolysed for 60 minutes.

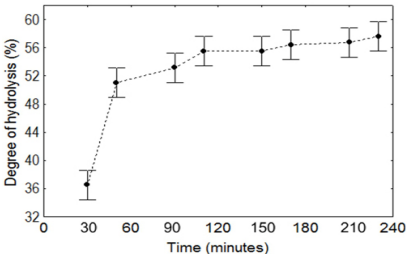

Figure 1. Degree of hydrolysis (%) for isolated soy protein over time using enzyme *Flavourzyme®*, temperature of 55°C and agitation of 50 rpm.

The enzymatic hydrolysis decreased the water capacity absorption of the SPI. For the hydrolyzate 30 minutes, the water capacity absorption was 291.21% and for the hydrolyzate 60 minutes, the Water capacity absorption was 315.85%, values statistically equal ($p > 0.05$). There was a reduction of almost 50% in Water capacity absorption compared to crude protein isolate which showed 633.15%. The crude SPI showed oil-holding capacity of 2.4 mL/g sample, SPI hydrolysed in 30 minutes showed oil-holding capacity of 1.67 mL oil/g sample, while the SPI hydrolysed in 60 minutes showed oil-holding capacity of 1 mL oil/g sample. The samples of the crude SPI and SPI hydrolysed in 60 minutes presented significant difference ($p < 0.05$). It is found that there was a decrease in this property to enzymatic hydrolysis.

Discussion

The *Flavourzyme®* enzyme is a complex of endo and exopeptidases, with greater exopeptidase activity. It operates at the extremes of the protein chain, releasing final amino acids. Its efficacy in processing the rheological characteristics of the protein is limited due to relatively small changes in chain length. It is known that this enzyme hydrolyses peptide bonds containing mainly hydrophobic residues on the carboxyl side (Adler-Nissen, 1986; Hrčková *et al.*, 2001; Santos *et al*., 2010), which may explain the constant profile after 60 minutes of hydrolysis.

Candido and Sgarbieri (2003), after testing different enzymes, opted to *Flavourzyme®* to hydrolyse myofibrillar proteins of Nile Tilapia (*Oreochromis oreochromus*). They observed that the rate of hydrolysis was higher in the first 30 minutes, but decay significantly after this incubation time. This decline could be explained by the following phenomena: reduction of peptides available for hydrolysis, enzyme inhibition by potential reaction products formed by the partial denaturation or the enzyme. Hrčková *et al*. (2001) used a 5% dispersion of degreased soy flour at temperature of 40°C and pH 8. After 8 hours of reaction they found a DH of 39.5%

to *Flavourzyme*®, a DH of 33.3% to *Novozyme*® and a DH of 35.10% to alcalase. They also observed that during the hydrolysis using *Flavourzyme*®, the increase of free amino acids was more significant.

Zavareze *et al.* (2009) evaluated the functionality of protein hydrolysates of Cabrinha using alcalase, *Flavourzyme*® and *Novozym*®. As in the present study, found that the higher amount of protein was hydrolysed in the first 30 minutes of reaction. For alcalase and *Novozym*®, observed an increase in DH (%) after 45 minutes of reaction, however for the enzyme *Flavourzyme*®, the DH (%) was not changed after 30 minutes of hydrolysis, behavior next this study, which value did not change after 60 minutes of hydrolysis. In Table 1 it is seen that there has been a decrease in the foaming capacity in the sample hydrolysed for 60 min, determined at pH 5. At pHs distant from the isoelectric point, the foaming capacity of a protein is satisfactory (Fennema, 2000). The isoelectric point of soy protein is in pH 5.0, according Takeiti *et al.* (2004). For foaming capacity a concentration of higher soluble fraction is necessary, and the hydrolysis may have contributed to the exposure of hydrophobic group, which explains the decrease in the value of the foaming capacity for the sample hydrolysed in 30 minutes for the sample hydrolysed 60 minutes, at pH 5.0. It is observed to influence the isoelectric point of the foaming capacity, since for all sample, the lowest means for foaming capacity are for pH 3 and pH 5.

The enzymatic hydrolysis generally increases the foaming capacity, which occurred to the other samples analyzed in this study. This is due to increased molecular flexibility and greater exposure of hydrophobic groups. However, the extensive hydrolysis affects the foamability because the low molecular weight peptides cannot form a cohesive film at the interface (Fennema, 2000; Damodaran *et al.*, 2010). Hrčková *et al.* (2001) tested the functional properties of soybean bran hydrolysed by three different proteases. The hydrolysate using *Flavourzyme*® arrived at 70% of the foaming capacity, superior value to those found in the present study for all tested pH value. Fontanari *et al.* (2007), characterized isolated guava seed protein, obtained the highest foaming capacity when the sample was subjected to alkaline pH and lower values when the sample was subjected to acid pH (near the isoelectric point). This behavior is similar to the present study results.

In Table 2, the sample hydrolysed for 60 minutes, there is a lower foaming stability to pH 5. This is appropriate for this sample because shown the lower foaming capacity. It is found also that the crude sample, in pH 7, differs statistically from the hydrolyzed samples, which did not occur for the foaming capacity. The foaming stability is related to the ability to remain stable front of gravitational or mechanical efforts, unlike the foaming capacity, which is characterized as the interfacial area that can be created by protein (Fennema, 2000). For this sample, the ability to remain stable in the crude sample can be justified by the exposure of hydrophobic groups, which, in larger quantity in the soluble fraction, maintains stable foam.

The foam stability is important for foods such as ice cream, Chantilly, mousses and marshmallow, with respect to shelf-life and appearance of the product, and must be maintained when subject to process variations such as heating, mixing and cutting (Foegeding *et al.*, 2006). In general, proteins that exhibit low foaming capacity show good stability and vice versa. At pH different from the isoelectric point of the protein, the foamability is usually satisfactory, but the foam stability is low (Damodaran *et al*., 2010). The foam may become stable in the presence of salts and the stability of the soy protein foam increases with increasing NaCl concentration. The addition of sugars usually harms the foamability. The foam stability is also increased by large protein concentrations, since this increases the viscosity and facilitates the formation of a cohesive multilayer protein film at the interface (Fenema, 2000).

The larger foaming stability of a protein can be found in pH of the isoelectric point. This is due to the fact that intermolecular electrostatic attractions produced in these pH values are great and increasing the thickness and stiffness of the protein adsorbed at the air/water interface, resulting in greater stability (Ordoñez, 2005; Damodaran *et al*., 2010). According to the results of this study, it is possible to verify that none of the samples (crude SPI and hydrolysed SPI) showed large foaming stability at pH 5 which is the isoelectric point of the protein under study. Silva (2007) performed treatment acid with salt of polyphosphate to modify commercial protein isolates and did not observe good foam stability at pH 5. In pH 3 and 4, the behavior of the foam stability curve was similar to the isolate protein and modified.

It can be seen in Table 3, there was an increase in the solubility of hydrolyzed samples. This result may be due to the decrease in molecular size of the protein creating small peptides and unfolding the protein molecule leading to the exposition of more polar and ionizable groups on the protein surface, which could improve the ability of the protein molecule to form hydrogen bonds with water, thereby augmenting solubility (Centenaro *et al*., 2009; Castro and Sato,

2014).

The solubility of a protein is the thermodynamic manifestation of the balance between interactions protein-protein and protein-solvent and is characterized by its balance of hydrophilicity/hydrophobicity. Thus, their amino acid composition affects its solubility. However, the characteristics of hydrophobicity and hydrophilicity of the protein surface are the most important factors that affecting their solubility characteristics, because it is the protein surface that comes into contact with water that is around you (Damodaran et al., 2010). According Santos *et al.* (2010), the hydrolysed soy protein with alcalase and *Flavourzyme*® originated in greater amounts the amino acids: threonine (Thr), lysine (Lys), tryptophan (Trp), leucine (Leu), aspartic acid (Asp), arginine (Arg), proline (Pro), glutamic acid (Glu) and serine (Ser). Amino acids with aliphatic chains (Ala, Ile, Leu, Met, Val and Pro) and aromatic (Phe, Trp and Tyr) are hydrophobic and therefore exhibit limited solubility in water (Fennema, 2000). The amino acids present in the soy hydrolyzate of Santos *et al.* (2010), only Trp, Leu and Pro are present, which may explain the increased water solubility of the hydrolysed SPI compared to the SPI crude, in this study.

Taking into consideration the solubility as a specific property to the use of SPI in foods or beverage, it turns out that the hydrolysates demonstrates a potential applications due the solubility values obtained are higher than the solubility values obtained for crude SPI. This behavior is characterized as an advantage, because the hydrolysed protein, in the time and the conditions tested, can be used in juices, ready-made soups, beverage and other foods that incorporation of hydrolysed is feasible nutritionally and/or technologically. It is noticed that the crude protein showed higher solubility in acidic pH, suggesting some restriction on their use.

Takeiti *et al*. (2004) reported that soybean protein has low solubility at pH 5 (region of isoelectric point), increasing in the pH above and below this value. The occurrence of low solubility region close to the isoeletric point is, mainly, due to the lack of electrostatic repulsion, which promoted precipitation and aggregation via hydrophobic interactions (Damodaran, 1996). In this study, the low solubility of crude protein, compared to hydrolysates occurs in a wide range of pH (5-11). The SPI contains many hydrophobic amino acid residues, and it is physically impossible to achieve a conformation that conceals of the residues the water (Fennema, 2000; Damodaran *et al.*, 2010). During hydrolysis, these residues are broken taking increasing the solubility. Zhao *et al.* (2011) assessed the functional properties of peanut protein isolate that has been enzymatically hydrolysed. They observed a large increase in solubility after enzymatic hydrolysis. Klompong *et al.* (2007) state that enzymatic hydrolysis of fish protein favors its solubility.

As the results presented in this study, Wong and Kitts (2003) found an inverse correlation between the solubility and water capacity absorption to an isolated soy protein. According to the authors, the formation of a stable partially denatured matrix of the protein during the drying process involves reducing the protein surface exposed to water. Roman and Sgarbieri (2005) analyzed the effect of enzymatic hydrolysis on bovine casein coagulated by chymosin, and found that in the sample does not hydrolyzed the water capacity absorption was significantly higher that hydrolyzate with DH 35.85% and much lower in lower DH 5.7%. This shows that water capacity absorption depends on both the size of the molecule and the balance of hydrophilic and hydrophobic groups.

Equality to the oil-holding capacity for crude and hydrolysed SPI in 30 minutes can be justified by unfolding of the protein structure and the exposure of more hydrophobic groups, allowing for physical entrapment of oil (Achouri *et al*., 1998). Wasswa *et al.* (2007) and Zavareze *et al.* (2009) obtained values oil-holding capacity of 2.4 to 2.8 mL oil/g sample of fish protein hydrolysates. Silva *et al.* (2012) obtained 3.0 mL/g to unhydrolyzed protein isolate rice.

Conclusion

It was observed that the functional properties of crude soy protein isolate are different from the properties of the hydrolysates soy protein isolate (SPI). In 30 minutes of hydrolysis with the enzyme *Flavourzyme*®, the degree of hydrolysis increases considerably, however, after 60 minutes, of hydrolysis the degree of hydrolysis is linear and constant. The solubility did not differ statistically between the two protein hydrolysates (30 and 60 minutes) even among different pH, however there is a significant difference between the solubility of crude SPI and hydrolysates. For the foaming capacity, there was no difference between crude SPI and hydrolysates, only between pH. The enzymatic hydrolysis led to reduced water absorption capacity and also in oil-holding capacity. The hydrolysates obtained in this study could be used in foods such as ice cream, due to increase of foaming capacity and good stability of the foaming obtained for most samples. Due to increase in solubility, a possible application would be in liquid products, such as beverages for athletes and yoghurts.

Due to the reduced capacity of water absorption and Oil-holding capacity, the use in emulsified and gelled foods such as mayonnaise and gelatins, may not provide good results.

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