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Promoting Soybean Protein Functional Properties By Enzymatic Hydrolysis:Characterization, Antioxidant Activity, Functional Properties And Application

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Abstract

Fortification of ready-made food is preferred by most consumers with nutrient-enriched foods. High protein value like soybean is of great value, since soy can replace dairy proteins and meat. Therefore, this study aims to produce soybean protein hydrolysate using papain to improve its functional properties and to formulate fortified biscuits with soybean protein hydrolysate. Soybean protein isolate was prepared and hydrolyzed with papain. It was characterized by electrophoresis (SDS-PAGE) and degree of hydrolysis. Its functional properties and antioxidant activity were determined and fortified biscuits was formulated at levels (5,10 and 15%). Results of hydrolysate electrophoresis revealed the presence of low-molecular weight peptides (10.5, 13, 13.5, 24.5, 25.5, 27 kDa). The Degree of hydrolysis as α–amino groups was 29.9%. The detected hydrolysate antioxidant activity and functional properties were better than those of soybean flour and isolate. Both of water holding capacity (WHC), oil binding capacity (OBC) were decreased after hydrolysis of protein while, the emulsifying capacity (EC) of soybean was improved from 1.97±0.03 to 2.28±0.04 ml oil/g. Solubility index (SI) was increased to reach 56.9%. Foaming capacity was improved by increasing it from 0.93±0.01 to 5.93±0.07ml/g. The fortified biscuits recorded a good palatability at levels of 5 and 10% for all sensory characteristics. In conclusion, enzymatic hydrolysis of soybean protein improved its functional properties and produced bioactive peptides. Thus, the production of biscuits fortified by 10% soybean protein hydrolysate as a functional food is recommended.

Keywords: Soybean protein, enzymatic hydrolysis, papain, functional properties, bioactive peptides, fortified biscuits.

1. Introduction

There is no doubt that food consumption habits like increasing ready meals' intake as well as increasing the intake of tasty foods rather than healthy foods is nowadays constitute a big problem as it rises the incidence of many diseases like obesity, cardiovascular diseases, metabolic syndrome, diabetes and dyslipidemia as well as many other diseases [1, 2, 3, 4]. Consumers resort to eating ready-made fast food, which lacks many nutrients, in addition to being unhealthy and containing many harmful substances such as high fat content, trans fat and high salt or sugar content [5]. These problems could be solved by enriching diets with nutrient-enriched foods that are easy-to-cook with high protein and nutrient value [6].

Recently, it was found that the higher the consumption of plant products, the lower the risk for some chronic diseases. Soybean has been known as a rich source of vegetable protein and polyphenols [7]. Soy proteins are utilized in many foods widely as nutritional ingredients due to its high nutritive value, good digestibility properties and balanced amino acid composition. In food industry, soy proteins constitute a major protein source replacing dairy and meat proteins and used as a functional ingredient due to its emulsifying, gelling and stabilizing capacity [8]. Also, it could be utilized as a food preservative to extend some products' shelf-life such as meat products [9]. However, functionality of the isolates of soy protein is relatively low owing to the higher denaturation degree as well as the native high molecular weight and highly compact structure associating this protein leading to poor foaming properties [10]. Nowadays, current research is focusing on how to improve the foaming characteristics of soy protein [11].

Soybean protein hydrolysates play an important role in food industry as a result of improving functionality of soy protein such as the elevation of gelling, solubility, water-holding and emulsifying properties as well as the generation of bioactive peptides by protein partial proteolysis with less secondary structure and reduced molecular size and higher physiological activities. This leading to creating new food

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applications due to their beneficial health effects such as antioxidant, anti-inflammatory, immunomodulatory effects, antihypertensive, anticancer, hypocholesterolemic, anti-obesity, as well as lowering the risk for many chronic diseases [12]. Karami & Akbari-adergani [13] mentioned that there was a direct link between the structure of protein hydrolysate and their functional activity due to more efficient and faster intestinal absorption in human.

Also, bioactive peptides are considered as a part of the original parent protein sequence that are found normally in the inactive form however, they can be activated by either food processing or gastrointestinal digestion or enzymatic processing or fermentation or germination [13, 14]. Enzymatic hydrolysis is the method of choice to produce physiologically active peptides. Although proteases are good for this purpose, yet, their use is associated with many challenges as the high cost and the undesirable taste of bitterness [15]. On the other hand, the method of chemical hydrolysis (using HCl) is an effective and simple way to produce low molecular weight peptides, but its use is accompanied by the generation of toxic chloride compounds such as 1,3- dichloropropan-2-ol and 3-monochloropropane-1,2-diol [16]. Production of bioactive peptides by either of the previously mentioned methods potentiates their health benefits [7]. The length of the bioactive peptides is about two to thirty amino acids and they are absorbed through the human intestine into the blood stream to perform beneficial and health promotional impacts in target tissues [17].

It is worth mentioning that the degree of hydrolysis must be put in consideration, to achieve the functional properties that are desirable and expected from hydrolysates of soy protein, the hydrolysis must be carried out under conditions strictly controlled to obtain the specified degree of hydrolysis which is generally low [18]. Vioque et al. [19] mentioned that hydrolysates can be classified into three major groups depending on their degree of hydrolysis (DH) which determine their application: 1. broad DH hydrolysates which are commonly used as special dietary supplements for medical and nutritional use), 2. various DHs hydrolysates that are used generally as flavorings), 3. low DH hydrolysates with improved functional features**.**

Recently, owing to the progress that was achieved in food industry in the field of soy protein hydrolysis based on the new and innovative biotechnological methods, new applications have been found for soy protein hydrolysate with greater functional and nutritional benefits.

Therefore, the aim of this study is to carry out soy protein hydrolysis using papain to improve its functional and nutritional properties as the bioactive peptides with their antioxidant properties are formed. Then, to characterize the obtained hydrolyzed protein and to evaluate its antioxidant and functional properties. Finally, to produce biscuits with good palatability and high nutritional value by fortifying biscuits with the obtained soy protein hydrolysate as a functional food.

2. Materials and Methods

Soybean (*Glycine max*) was purchased from Agricultural Research Centre, Giza, Egypt. Crude papain used for protein hydrolysis was obtained from Technolab, India. Most chemicals were purchased from Sigma-Aldrich Company (St. Louis, MO, USA).

2. *I. Soybean protein isolate and hydrolysate*

2.1.1. Soybean protein isolates (SPI)

Soybean was milled into fine powder, then defatted by Sohxlet apparatus using hexane as a solvent. Isolate of soy protein was prepared as described by the method of Wang et al. [20].

2.1.2. Enzymatic hydrolysis (soybean protein isolate hydrolysate SPIH)

Crud papain was used for hydrolysis of soy protein isolate (SPI) as following: ten g of SPI were mixed with 300 mg of enzyme in 300 ml d. w. at pH 8.5 and a temperature of 38 ^{o}C. Hydrolysis lasted for 36 hours then stopped by heat shock at 100 ^{o}C for 7 min to inactivate the enzyme. Finally,it was centrifuged at 4000 rpm for 20 min, and supernatant was separated and dried by freeze dryer and stored at – 20 ^{o}C.

2.2. Protein characterization

2.2.1. Electrophoresis (SDS-PAGE)

Gel electrophoresis; sodium dodecyl sulfate-polyacrylamide was done using stacking 40 g /L and separating 120 g /L acrylamide gels. Tris-HCl as 0.375 mol/L with pH 8.8, 1 g /L SDS separating gel; Tris-HCl as 0.025 mol/L, glycine as 0.192 mol/L and SDS 1 g /L, running buffer pH 8.3, and Tris-HCl 0.125 mol/L, pH 6.8, glycerol 200 ml/L, SDS 10 g /L in addition to bromophenol blue as 0.5 g /L sample buffer. Brilliant Blue Coomassie Stain was used for staining. The system of image analysis; Gel Doc 1000 (Bio-Rad, Richmond, CA, U.S.A.), analyzed with the software of Molecular Analyst which is Bio-Rad for determination of polypeptides' molecular weights as a function of the bands relative intensity.

2.2.2. Degree of hydrolysis

Free amino groups determination was carried out according to the method of Adler-Nissen [21] using 0.1% trinitrobenzenesulfonic acid (TNBS). The absorbance was measured at 340 nm using the spectrophotometer (Unicam UV 300 Thermo N.J., USA). L-leucine 1.5 mM was used as the standard. The degree of hydrolysis or DH was then calculated as this equation:

$$\% \text{ DH} = \frac{\text{Free amino acids}}{\text{Total peptide bonds group}} \quad \text{X } 100$$

2.2.3. Estimation of peptide content

Peptide content was estimated by method of the O-phthaldialdehyde as described by Zhu et al. [22]. Calculation of the peptide content based on of the standard curve that

was constructed by the use of the reduced form of L-glutathione as a standard.

2.2.4. Determination of the average peptide chain length

The average length of the hydrolysate peptide chain or PCL was evaluated as described by Netto and Galeazzi [23] by using TNBS reaction for the estimation of the free amino group, then the PCL was calculated by the equation:

PCL (peptide chain length) =100/DH

where, DH is the degree of hydrolysis

2.3. Measurement of antioxidant activity of peptides

2.3.1. Determination of DPPH radical scavenging activity

The impact of each of soybean flour, isolate and hydrolysate on DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical was determined based on the procedures described by Aboelsoued et al. [24]. The absorbance of the obtained color was measured at 517nm. DPPH scavenging capacity was measured as following:

$$\text{Scavenging activity (\%)} = \frac{Ac - As}{Ac} \times 100$$

where each of Ac and As are the control and the sample absorbance at 517nm of, respectively.

2.3.2. Determination of ferric reducing power (FRAP) assay

The assay of FRAP of de Moraes Barros et al. [25] depends on the phenolics ability to reduce the Fe^{+3} into the Fe^{+2}. The absorbance of the obtained color was measured at 593 nm by a spectrophotometer. The obtained results were then expressed as mg Trolox equivalents/g sample.

2.3.3. Determination of reducing power (RP)

Each of soybean flour, isolate and hydrolysate reducing power was measured as described previously by Oyaizu [26]. The absorbance of the obtained color was measured at 700 nm against the blank.

2.4. Determination of soybean flour & hydrolysate functional properties

The functional properties that were evaluated were: capacity of water holding (WHC), protein solubility, capacity of oil binding (OBC), foaming capacity in addition to emulsifying capacity (EC)

2.4.1. Protein solubility

It was performed according to Ghribi et al. [27]. Calculation of the soluble protein content as gram soluble protein for each 100 g sample depended on their original weights.

2.4.2. Water holding capacity

It was performed as described by Heywood et al. [28]. Water holding capacity was then calculated as:

WHC = (W2 - W1)/ W0

where, W0 denotes for the dry sample weight in gram while, W1 is weight of the tube + the dry sample (g), and W2is the weight of the tube + the sediment (g). Result was expressed as grams of water / grams of protein.

2.4.3. Oil binding capacity

It was performed according to Heywood et al. [28]. The capacity of fat absorption as milliliter of oil for each gram of protein was calculated as following:

FAC = (V1– V2)/W0.

2.4.4. Emulsifying capacity

It was evaluated by the classic method that was described by Pearce and Kinsella [29]. The total volume of the added oil was recorded for calculation of the emulsifying capacity or EC as volume in mL of emulsified oil for each gram of sample**.**

2.4.5. Foaming capacity

It was determined by the classic method of Lin et al. [30]. Foaming capacity (FC) has been calculated as following:

FC = VF/VI.

2.5. Fortification of biscuits with soybean protein hydrolysate as a source of bioactive peptides

2.5.1. Preparation of biscuits dough and baking

Biscuits have been prepared as described by the method of Mahmoud et al. [31]., The fortified biscuits with soybean protein hydrolysate (SPH) flour have been prepared by the same formula with replacing the wheat flour by three levels of SPH (5, 10 and 15 %) in the same way like the control sample of biscuits without SPH.

2.5.2. Analysis of the formulated biscuits

2.5.2.1. Baking quality of the formulated biscuits

The diameter (D) and thickness (T) of biscuit samples were measured as described in the AACC [32] method.

1.5.2.2. Physical characteristics of the formulated biscuits

2.5.2.2.1. Measurement of color

Color was measured according to Mahmoud et al. [33] for the baked biscuits. Hunter L, a and b parameters were determined using a Spectro- colorimeter (Tristimulus Color Machine) with the CIE lab color scale (Hunter, Lab Scan XE - Reston VA, USA) in the reflection mode. The difference in color (Δ E*) was calculated according to Palou et al., [34] as follows:

$\Delta E^* = (\Delta a^2 + \Delta b^2 + \Delta L^2)^{1/2}$.

where, a = a-a° , b = b-b° and L = L-L°

2.5.2.2.2. Biscuits sensory evaluation

The biscuits fortified with either 5, 10 and 15 % soybean protein hydrolysate (SPH) were evaluated including color, flavor, texture, taste, after taste, and overall acceptability by a trained 10-member panelists selected from the staff members of the department of Food Science and Technology (DFST), National Research Centre (NRC), Egypt according to Mahmoud et al. [35]. A ten-point hedonic scale was used to evaluate biscuits (1-dislike extremely to 10=like extremely). This rating scale was also applied for the other parameters as described by Larmond [36].

2.6. Statistical analysis

Results have been analyzed statistically by the computerized program SPSS (version 20) for windows. The one-way

ANOVA test was used for data analysis. Data have been represented by mean ± SD. Significance level of 0.05 was considered otherwise were not significant.

3. Results and discussion

3.1. Characterization of hydrolyzed protein

In order to know the extent of the enzymatic hydrolysis of soy protein, a characterization of the resulting protein hydrolysate was done by SDS-PAGE followed by determination of the degree of hydrolysis, peptide chain length and peptide content as follows:

3.1.1. Electrophoresis (SDS-PAGE)

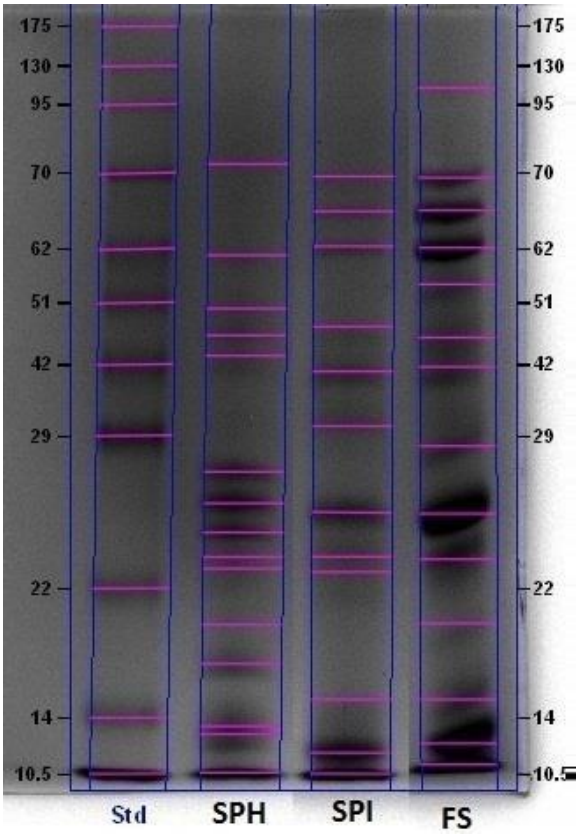

Fig. (1): SDS PAGE profile of standard peptides (Std), soybean protein hydrolysate (SPH), soy protein isolate (SPI) and soybean flour (FS) expressed as K Dal.

Figure (1) shows the soy flour SDS–PAGE (SF), protein isolate (SPI), protein hydrolysate (SPH). The main subunits of protein which can be clearly identified in soybean flour are: 7S of β-conglycinin which consists of subunits of α and β and 11S of glycinin which consists of the acidic and basic subunits (A) and (B), respectively. Soybean flour (lane 4) showed bands ranged from 11, 12.3, 15, 25.2, 62, 66, 70 and 108 KDa with a percentage of 20%, 31%, 2.3%, 18.6%, 9.1%, 8.7%, 5.4% and 1.4%, respectively. After processing of soybean flour into soybean protein isolate, most of the subunits of 7S and 11S in soybean protein isolate (lane 3) were broken down as a result of acid treatment during the preparation processes into low-molecular weight peptides of about10.5,12, 15, 25.5, 40.7 and 62.4 kDa, with the percentage of 36%, 42%, 2% 12%, 1.6% and 1.3%, respectively. However, by enzymatic hydrolysis of the protein isolate with papain enzyme, the protein 7S subunits degraded relatively more than the 11S subunits of protein giving rise to low-molecular weight peptides of 10.5, 13, 13.5, 24.5, 25.5, 27 kDa with the percentage of 39%, 7.5%,6.3%, 12.5%, 15% and 10.5%, respectively, or amino acids (lane 2). The obtained data indicates that the enzyme used in the present study results in protein degradation with exo- peptidase activity which results in free amino acids and smaller molecular weight peptides with more bioactivity as evidenced from the functional properties. Jara et al. [37] found relatively similar results when enzymatic hydrolysis was done to the soybean protein isolate using peptidases prepared from the extract of Maclur apromifera fruits.

3.1.2. Degree of hydrolysis, peptide content and peptide chain length (PCL)

TNBS (Trinitrobenzosulfonic acid) reacting substances; α–amino groups, NH2 groups, resulting from enzymic hydrolysis were measured. The percentage of the degree of hydrolysis (DH) was 29.9% giving a chain length of peptide (PCL) of 3.34 residues of amino acids and a percentage of peptide content as glutathione was 55.98%. It is clear that hydrolysis of soybean proteins resulted in production of a mixture of mediate hydrolysis and low chain length peptides, which makes them suitable to be used as food supplement with improvement of their functional properties as mentioned by Vioque et al. [19]. They reported that protein hydrolysis gives rise to three main categories depending on the DH which are either broad DH hydrolysates that can be used for medical and nutritional use or various DHs hydrolysates used for flavoring or low DH hydrolysates that can be used for production of functional foods as in the present study.

3.2. Antioxidant Activities

The antioxidant activity of each of soybean flour, isolate and hydrolysate was determined using three different methods (Fig. 2): DPPH radical scavenging activity, TPTZ or FRAP (ferric reducing power) and the reducing power activity (peroxyl radical activity). Results showed that the differences are significant ($p<0.05$) among soybean flour, protein isolate and protein hydrolysate. The hydrolysate gave the highest antioxidant activities compared to unhydrolyzed protein, while soybean protein isolate gave less antioxidant activities. This means that, the enzymatic hydrolysis of soybean protein by papain enzyme had led to breakdown of the soybean protein into short chain length peptides and amino acids with more bioactivity compared to the soybean flour. The main effect generated by the hydrolysis of proteins is the significant increase in the ionic charge/molecular mass ratio. These results are in the same line of the results obtained by Samaei et al. [38]. On the other hand, soybean isolate recorded a lower value for antioxidant activity compared to either the soybean hydrolysate or the soybean flour. This may be explained on the basis of the loss of most polyphenolic content of soybean isolates during the acidic treatment of the soybean flour.

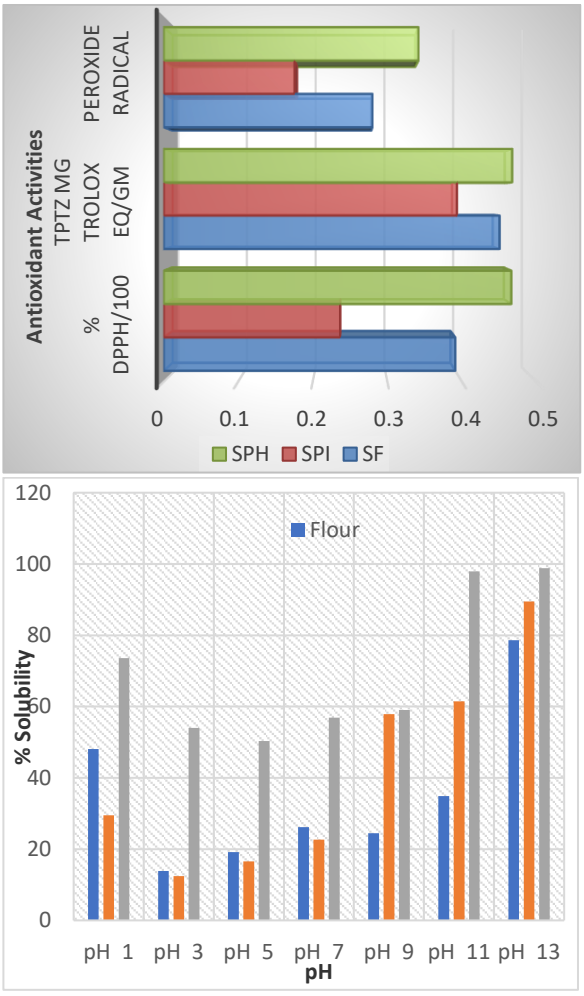

Fig. 2: Antioxidant activities and solubility index (SI) as gram soluble protein per 100 g flour of both soybean flour (SF), soybean protein capacity, ferric reducing power and peroxide radical.

It is worth mentioning that some of the soybean flour antioxidant activity is attributed to its polyphenolic content as mentioned previously by Mahmoud et al. [7]. These may be due to the existing isoflavones in soybean flour which were washed out during protein isolate preparation, however the hydrolysate gave more antioxidant activities. Devi et al., [39] demonstrated that a well-established correlation is found between antioxidant activity of each of soybean and soy products and their total phenolic content (TPC) and total isoflavones (TI) contents.

3.3. Functional properties

These functional properties are depending on the protein chemical structure. Polysaccharides and proteins arethe most important thickening and food-gelling agents, as well as stabilizers for emulsions and foams that serve the keystructural functions in food systems. Thus, processing affectsdramatically the food products physico-chemical properties leading to modifying the functional properties [40].

Hydrolysis of soybean protein by papain led to reducing the molecular size which may has an effect on the hydration properties due to increasing the surface area**.**

3.3.1. Water Holding Capacity (WHC)

Significant differences ($p < 0.05$) were found for processed samples compared to flour (Table 1). Preparation of proteinisolate, decreased water holding capacity to 1.26 ± 0.01 gmwater/ gm, while hydrolysis of protein increased water holding capacity of sample from 2.17±0.01 to 2.72±0.01. This decrease in WHC value could be attributed to the formation of more small peptides and amino acids which have fewer WHC than bigger peptides. Peighambardoust et al, [41] reported that low molecular weight peptides show more WHC than high molecular weight as a result oftheir smaller peptides chain length with increasing hydrophilic properties. This finding indicates that the WHC of protein may be reduced by excessive enzyme hydrolysis. In a native protein

Table (1): Functional properties of soybean flour, isolate and hydrolysate.

	WHC	OHC	Foaming	Emulsifying
Flour	2.17±0.01 [b]	1.05±0.02 [a]	0.93±0.01 [a]	1.97±0.03 [a]
Protein Isolate	1.26±0.01 [a]	1.18±0.03 [a]	2.53±0.01 [b]	1.95±0.03 [a]
Protein Hydrolysate	2.72±0.01 [c]	1.46±0.02 [b]	5.93±0.07 [c]	2.28±0.04 [b]

WHC: Water holding capacity (grams of water per gram of protein) and OHC: Oil holding capacity (milliliter of oil per gramof protein)

molecule, groups that are hydrophobic are buried in the folded structure core. After partial hydrolysis of protein, some of these buried hydrophobic groups are exposed to the surface, leading to in incrementof WHC.

3.3.2. Oil Binding capacity (OBC)

Generally, processing of soybean protein resulting in an increase in the OBC for all samples in comparison to flour. Table (1) display the effect of protein isolation and hydrolysis on oil binding capacity (OBC)(g oil / g samples). A significant difference ($P \leq 0.05$) occurred as aresult of protein processing. Oil binding capacity was increased (not significant) for soy protein isolate to 1.18±0.03g oil / g, but after hydrolysis with enzyme, it increased to1.46±0.02 g oil / g, compared to soy flour which was 1.05±0.02 g oil / g sample.

3.3.3. Foaming capacities (FC)

The impact of soy protein enzymatic hydrolysis on its foaming capacity was evaluated. Hydrolysis of soybean protein was found to improve foaming capacity by increasing it from 0.93±0.01 ml/g in soy flour to 2.53±0.01ml/g in protein isolate, however it reached 5.93±0.07 ml/g by papain enzymatic

hydrolysis (Table 1). These results were in accordance with those obtained by Rwubatse et al. [42]. The significant (P< 0.05) improvement of this functional property could be due to both of the degree of hydrolysis and molecular weight as seen previously. However, increasing the degree of hydrolysis leads to decreasing molecular weight.However, foaming capacity dependes on the flexible protein molecules which could decrease surface tension but. globular protein that is highly ordered is difficult to surface denaturation. The hydrolysis occurred in protein molecules may be the result of the dissociation of glycinin into subunits AB and also to their denaturation as suggested by Panizzolo and Añón [8]. Soybean flour had low foaming capacity which can be explained by its low reactivity as a result of being complicated protein and diverse structural either in primary, secondary, tertiary or quaternary form [43].

3.3.4. Emulsifying capacity

Enzyme modification of soybean improved significantly (P< 0.05) the emulsifying activity index from 1.97±0.03 to 2.28±0.04 ml of oil/gm for soy flour and protein hydrolysate. There was no significant improvement between emulsifying properties of flour and protein isolate which was 1.93±0.0 ml of oil/gm (Table 1). This may be attributed to the reduced surface hydrophobicity for protein isolate. After the partial hydrolysis, the function and structure of the protein were altered. Data obtained concerning degree of hydrolysis or molecular weight reflected that the protein hydrolysate of a smallermolecular size and a higher solubility may facilitate the spread and diffusion at oil–water interfaces. The interaction between lipids and proteins were enhanced by the exposed hydrophobic groups [44].

Hence, degree of hydrolysis as well as molecular size and solubility, rather than water retention capacity, may be the main factors for the increased peptide emulsifying activity with smaller size as evidenced by peptide chain length (3.34 amino acid residues and a percentage of peptide content as glutathione 55.98%).

3.3.5. Solubility index (SI)

Enzyme modification significantly (P< 0.01) improved protein solubility (Fig. 2). Preparation of protein isolate resulted in decreased solubility from 26.2 to 22.7, while after enzyme hydrolysis, the solubility of protein increased to 56.9% at pH 7.0. The increment of protein solubility may be explained on the basis of peptides with smaller molecular weight that were produced by papain hydrolysis in addition. Also, protein molecules unfolding may be another explanation. Some polar amino acid groups which were buried within protein molecules in addition to the nonpolar groups of amino acids were exposed to the protein molecules' surface after unfolding. These polar amino acids that were exposed to the surface by unfolding, may bind to water molecules by formation of hydrogen may bind to water molecules by formation of hydrogen bonds as well as electrostatic interactions, giving rise to theincreased the hydrolysate protein solubility [45].

Protein solubility of SPI, as well as hydrolysate as a function of pH, were studied also (Fig.2). Hydrolysis elevated the soy protein solubility at all ranges of pH. At pH 1, 3, 5, 7, 9, 11 and 13, the solubility of protein increased from 29.5, 12.4, 16.6, 22.7, 57.9, 61.5 and 89.5% for unmodified SPI to 73.6, 54, 50.3, 56.9, 59, 97.9 and 98.8% for hydrolysate, respectively.

Slightly increased protein solubilities at pH 1, 3, 9,11 and 13 were observed with hydrolysate in comparison to protein isolate. At these values of pH, both unmodified SPI and hydrolysate have positive (pH 3) and negative (pH 9 or 11) electric charges, thus participating to solubility. Theimpact on protein solubility of exposure of hydrophilic groups and smaller peptides were minor in comparison with those of electric charges at pH 1, 3, 9, 11 and 13. However, at pH 5 and 7, of protein/peptide molecules electrostatic repulsions were less than those at pH 1, 3, 9, 11 and 13, so solubility of protein at those pH values couldrely on molecular size and the presence of hydrophilic groups. Hydrolysis using papain gives rise to smaller molecular weight peptides and exposure of more hydrophilic groups. Increased values for protein solubilities for the hydrolysate at both pH 5 and 7 were noticed compared to SPI.

3.4. Fortified biscuits with soybean protein hydrolysate (SPH)

3.4.1. Quality of baking of the prepared biscuits Physical characteristics as diameter and thickness ofprepared biscuits from different levels of soybean protein hydrolysate are illustrated in Table (2).

Results show that the diameter of biscuits recorded a significant ($P < 0.05$) gradual decrement while increasing the concentration of soybean protein hydrolysate. The highest value was observed for control sample S0 and S1 (5% soybean hydrolysate) (6.71 and 6.32 cm, respectively), whereas the least value was recorded for biscuits prepared with S3 (15 %) (5.43 cm). Bose and Shams-ud-Din [46] found similar results for biscuits fortified with chickpea.

Table (3) shows that the data of thickness of biscuit prepared form the flour containing (SPH) was varied significantly ($P < 0.05$) from the control sample. Thickness of the biscuits showed gradual increment as the level of (SPH) replacement. Thickness was increased with increasing amount of soybean protein hydrolysate and the highest value (0.61 cm) was recorded in S3 (15 %). Similarresults were reported in another study conducted by Adeolaand Ohizua [47] for the thickness of sweet potato and banana additives for biscuits samples. This increment was discovered as a result of diameter decrement by protein additives.

3.4.2. Color attribute of biscuits fortified with soybean protein hydrolysate

Color is one of the most important attributes because it could arouse individuals' appetite. It is one of the measurements used for controlling the baking process. Table (2) shows that there were significant differences ($P < 0.05$) that were found among color of all samples. It was noticed that the value of L^* decreased with increasing SPH content of biscuit. The values ranged from 78.7 to 63.31. On the other hand, the addition of the (SPH) led to increase either of the brightness b^* or the redness a^* of the biscuits. The a^* values increased from 8.95 to 10.95 and b^* value increased from 32.81 to 35.81. Damiana et al., [48] mentioned that redness and yellowness in biscuits is correlated to increased protein content. The changes that were noticed in the whiteness and yellowness of the biscuits as a result of adding the (SPH) is termed discoloration, which could be attributed to the baking period, that is, heat treatment [49]. The trend of ΔE* (color difference) was increased gradually when increasing (SPH) levels. The values recorded for ΔE* of (SPH) concentration of S3 (15 %) was 45.3 compared to the control sample which was 42.94.

Table 2. Physico-chemical properties and baking quality of biscuits formulated with different concentrations of soybean protein hydrolysate (SPH).

Treatments	Color				Baking quality	
	L^*	a^*	b^*	ΔE^*	Diameter (cm)	Thickness (cm)
S0 (Control)	78.70±2.5 ^{a}	9.14 ±1.4 ^{cd}	33.11 ±1.03 ^{c}	42.94 ±2.78 ^{d}	6.71±0.02 ^{a}	0.50±0.02 ^{c}
S1 (5 %)	71.40 ±2.3 ^{b}	9.36 ±1.01 ^{c}	34.53 ±3.2 ^{b}	43.79 ±2.9 ^{c}	6.32 ±0.02 ^{b}	0.55±0.02 ^{bc}
S2 (10 %)	67.45 ±3.4^{c}	10.81 ±0.78 ^{b}	35.89 ±3.15 ^{ab}	44.26 ±2.72 ^{b}	6.00±0.02 ^{bc}	0.59±0.02 ^{ab}
S3 (15 %)	63.31 ±2.1^{d}	10.95 ±0.89 ^{a}	36.1 ±2.0 ^{a}	45.30 ±2.80 ^{a}	5.43±0.02 ^{c}	0.61±0.02 ^{ab}

All values are means ± SD of triplicate determinations for each parameter.Means in the same column having different lettersare significant ($P<0.05$). L^* lightness a^* red/green coordinate b^* yellow /blue coordinate ΔE^* total color difference

Table 3. Evaluation of sensory attributes of biscuits prepared in different concentrations of soybean protein hydrolysate (SPH).

Treatments	Color	Taste	Flavor	Texture	Overall-acceptability
S0 (Control)	9.80 ±0.02 ^{c}	9.5 ±0.02 ^{c}	9.65 ±1.01 ^{c}	9.68 ±1.00 ^{c}	9.52 ±1.00 ^{c}
S1 (5 %)	9.62±0.03 ^{bc}	9.41± 0.03 ^{bc}	9.59 ±1.0 ^{c}	9.57 ±1.11 ^{c}	9.43 ±1.12 ^{bc}
S2 (10 %)	9.41±0.02 ^{b}	9.22 ±0.03 ^{b}	8.98 ±0.93 ^{b}	9.16 ±1.23 ^{b}	9.21±0.98 ^{b}
S3 (15 %)	8.22±0.03 ^{a}	8.23±0.02 ^{a}	8.35 ±0.9 ^{a}	8.68 ±0.95 ^{a}	8.34 ±1.30 ^{a}

Each value is expressed as mean ± standard deviation (SD) (n =10). Means within the same column having different lettersare significant ($P<0.05$).

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3.4.3. Effect of soybean hydrolysate as bioactive peptide source on biscuit sensory evaluation

Sensory attributes that are represented by taste, color, texture and flavor showed significant changes ($p < 0.05$) among the control and biscuit samples with soybean hydrolysate (SPH) additives with different concentrations (Table 3). Results showed that no significant differences were found between the control sample S0 and sample with 5% soybean additives S1 in all parameters. While, biscuits with S1 (5 %) SPH additives recorded the best score for overall-acceptability of all characteristics of the sensory evaluation which was 9.43 and followed by S2 (10 %) SPH which was 8.81 compared with S0 that corresponds to the control (9.52). Sample S2 with 10% SPH is close in score with S1 and control sample. However, there were no significant differences between them in either overall acceptability or color and taste. While, S3 15% soybean protein hydrolysate had slight differences as a result of some bitter astringent taste and the overall acceptability was less than other samples (8.34).

Soybean protein hydrolysate addition was proved to have high acceptability when compared to the control sample, with a gradient of admission. Thus, it could be concluded that fortification of biscuit with hydrolysate of soybean protein at 10% have a successful application as functional food with bioactive peptides.

4. Conclusion

Hydrolysis of soybean protein by papain leads to reductionof molecular size which affects the properties of hydrationas the surface area increased**.** Peptides of soybean protein produced by papain enzymatic hydrolysis had very good protein

solubility and emulsifying properties as well as high antioxidant activities. It can be used in biscuit preparation to get a better product with good functional properties and also contain small molecular weight bioactive peptides with antioxidant characteristics that are highly beneficial for health promotion.

Consent to participate and **consent to publish**

All authors participated equally in this work and all of them agree to publish this manuscript in Egyptian Journal of Chemistry. This manuscript is original research article and not published before or considered for publication anywhere else or found as a preprint in anywhere.

Conflict of interest

There isn't any conflict of interest.

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Availability of data and materials

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Ethical approval

Not applicable

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