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Influence of Enzymatic Hydrolysis on the Nutritional, Functional and Antioxidant Properties of Protein Hydrolysates Prepared from Seinat (*Cucumis melo* var. *tibish*) Seeds

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Abstract The effect of enzymatic hydrolysis time on nutritional, functional properties and antioxidant activities of seinat seed protein isolates were studied. Hydrolysates of seinat seed protein isolates (HSSPIs) were prepared at different time (30–180 min), using two food-grade proteases. Trypsin was applied in the first hydrolysis stage followed by Pepsin. The hydrolysis time showed significant differences (p<0.05) on nutritional parameters such as essential amino acid score, essential amino acid index, biological value and predicted protein efficiency ratios. All the functional properties studied decreased as hydrolysis time increases. Antioxidant activities; (DPPH radical scavenging, ABTS radical scavenging and ferrous chelating) of HSSPIs increased with increase in hydrolysis time. Large amounts of small-sized peptides (3,000–5,000 Da) were observed with decreased as hydrolysis time increased (2.07–1.28%). The results in this study indicated that increase in hydrolysis time has positively affected nutritional and antioxidant properties of HSSPIs, but had a negative impact on the functional properties studied.

Keywords: *seinat seed, protein hydrolysis nutritional parameters, functional properties, antioxidant activities*

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1. Introduction

Seinat (*Cucumis melo* var. *tibish*) is a type of melon that belongs to the Cucurbitaceae family. It is grown mostly in Sudan but is not well-known in neighboring countries and is cultivated for its edible seed. As reported by El-Tahir and Pitrat [1] there are mainly five types of melons that are grown in Sudan. The melons have different morphological characteristics and they also differ in their use and each of these types has a specific local name. The seinat cultivar could be considered a type of *Cucumis melo*. The seinat seeds are roasted and eaten. Other well-known and more investigated oilseed proteins such as pumpkin and melon seeds are utilized directly as snacks after salting and roasting mostly in Arabian countries [2]. These seeds possess the potential for functional properties which could expand their use in the development of a wide variety of food products, since seinat seed, like, pumpkin seed can provide energy, protein, minerals and fatty acids required for human health [3]. Seinat seed contains crude oil content of 31.1%, moisture content of 4.2%, 24.7% fiber, 28.5% protein, 4.3% ash and 6.9% content of total sugars. Seinat seed is an excellent source of edible oils, comprising the main fatty acids namely: linoleic acid 61.10%, oleic acid 18.75%, palmitic acid 10.37%, and stearic acid 9.18% [4]. Enzymatic hydrolysis has been reported to increase solubility, modify foaming, emulsifying and gelation properties as well as producing bioactive peptides from certain proteins. There are a large range of seeds that have been used as protein sources for antioxidant hydrolysates, some of which include, soybean, peanut, rapeseed, flaxseed and sesame seed [5]. Plant proteins from seeds such as seinat which are not well known also have the potential of producing improved functional properties and increased bioactivity through enzymatic hydrolysis.

The objective of this research was to evaluate the nutritional and functional properties of seinat seed protein hydrolysates prepared at different time intervals from 30-180 min using two food-grade proteases (trypsin and pepsin). Changes in molecular weight distribution and amino acid composition during hydrolysis were also investigated to assess their relation with antioxidant activities. Diphenyl-1-picryhydradzyl (DPPH) radical scavenging activity, ABTS radical scavenging activity, ferrous chelating activity, and reducing power assays were used to evaluate antioxidant activities.

2. Materials and Methods

2.1. Raw Material and Chemicals

Dried seinat fruits were brought from a local farm in (Wad Medani City, Gezira State, Sudan), in June of 2012 after harvesting, and transported to the Food Processing and Ingredients laboratory in Jiangnan University, China. The seeds were removed manually from the fruits and were kept dry at room temperature in desiccators.

2.2. Chemicals

DPPH (2,2-diphenyl-1-picrylhydrazyl) was purchased from Sigma Chemical Co. (St. Louis, MO, USA), trypsin (2,500 units/mg) was purchased from Sinopharm Chemical Reagent Co-Ltd (Shanghai city, China), pepsin (3,000 units/g) was purchased from Shanghai Heng Yuan Biotechnology Co., Ltd., Shanghai, China. All other chemicals and reagents were of the highest grade commercially available.

2.3. Preparation of Defatted Seinat Seed Flour and Protein Isolates

The defatted seinat seed flour was carried out as previously reported by Azhari et al. [6]. After that, the defatted flour was suspended in water in the ratio of 1:10 (w/v). The pH of the slurry was adjusted to pH 10 with 0.1 N NaOH, and agitated for 1 h with an overhead mechanical stirrer (IKA® RW 20 digital, UK-plug, Staufen city, Germany) at 400 rpm. The pH was periodically monitored and, adjusted back to pH 10 where necessary. The slurry was centrifuged at 1,600× *g* for 30 min. The supernatant was decanted. The residue was suspended again in water and the extraction procedure was repeated. Both supernatants were mixed and the pH was lowered to 4.5 with constant agitation. The precipitate was separated by centrifugation under the same conditions as described above, followed by re-suspension in water and pH adjustment to 6.89 with NaOH solution (0.1 N). The precipitate was dried using a lab-scale freeze-dryer (Floor-model Freeze Dryer, serial No. 050639219 A, Labconco Co., Kansas, USA). The dried seinat seed protein isolate powder was packed in polyethylene bags and stored in refrigerator at 4°C until use.

2.4. Enzymatic Hydrolysis of Seinat Seed Protein Isolate

Trypsin and pepsin were used in the hydrolysis process which was carried out according to the method by Kong and Xiong [7]. The freeze-dried seinat seed protein fractions were dissolved at 1% (w/v) protein in distilled water adjusted to pH 8 using 0.1N NaOH. Trypsin solution was prepared in distilled water adjusted to pH 8 using 0.1N NaOH to get 0.1% (w/v) solution. The protein solutions were mixed with pepsin solution at enzyme/substrate ratio of 1:100 (w/w). The mixtures were incubated at 37° C for 3 h. The trypsin was inactivated by adjusting the pH to 7 using 0.3N HCl and then 0.1% (w/v) pepsin solution was added at the same enzyme/substrate ratio and further incubated at 37° C at time 30-180 min. Then, the protein solutions were boiled in a water bath at 95°C for 15 min to inactivate the enzymes and then centrifuged at 10,000 ×*g* at 4°C for 30 min (Sigma 4–15C centrifuge digital, Sigma Chemical Co. St. Louis, MO, USA). The supernatants were freeze-dried to obtain protein hydrolysate powders, which were then kept at -18°C until further use.

2.5. Determination the Degree of Hydrolysis (DH)

The DH was defined as the percentage ratio of the number of peptide bonds broken to the total number of peptide bonds in the substrate studied. DH was determined according to the method described by Adler-Nissen [8].

2.6. Amino Acid Analysis

The lyophilized hydrolysates were digested with HCl (6 M) at 110°C for 24 h under nitrogen atmosphere. Reversed phase high performance liquid chromatography (RP-HPLC) analysis was carried out in an Agilent 1100 series (Agilent Technologies, Palo Alto, CA, USA) assembly system after precolumn derivatization with o-phthaldialdehyde (OPA). Each sample (1 µl) was injected on a Zorbax 80 A C18 column (5 µm 4.0 × 250 mm, Agilent Technologies, Palo Alto, CA, USA) at 40°C with detection at 338 and 262 nm. Mobile phase A was 7.35 mmol/l sodium acetate/triethylamine/tetrahydrofuran (500:0.12:2.5, v/v/v), adjusted to pH 7.2 with acetic acid, while mobile phase B (pH 7.2) was 7.35 mmol/l sodium acetate/methanol/acetonitrile (1:2:2, v/v/v). The amino acid composition was expressed as g of amino acid per 100 g of protein.

2.7. Determination of Molecular Weight Distribution

Seinat seed protein hydrolysate fractions were analyzed for molecular weight distribution according to the procedure described by Yang et al., [9] with a little modification. A Waters TM 600E Advanced Protein Purification System (Waters Corporation, Milford, MA, USA) was used. The hydrolysates were loaded onto TSK gel G2000 SWXL column (7.8 i.d. × 300 mm, Tosoh, Tokyo, Japan), eluted with 45% (v/v) acetonitrile containing 0.1% (v/v) trifluoroacetic acid at a flow rate of 0.5 mL/ min and monitored at 220 nm. A molecular weight calibration curve was obtained from the following standards from Sigma: cytochrome C (12,500 Da), aprotinin (6500 Da), bacitracin (1450 Da), tetrapeptide GGYR (451 Da), and tripeptide GGG (189 Da). Results were processed using Millennium 32 Version 3.05 software (Waters Corporation, Milford, MA 01757, USA).

2.8. Determination of Nutritional Parameters

Nutritional features of enzymatic hydrolysates of seinat seed protein isolates (HSSPIs) were determined on the basis of their amino acid profiles. Essential amino acid score (EAA score) was calculated using the FAO/WHO [10] reference pattern, according to the suggested pre-school child (2–5 years) requirement. Essential amino acid index (EAAI) was calculated by using the amino acid composition of the whole egg protein as a standard. The predicted protein efficiency ratio (P–PER) calculated

according to the equations developed by Adeyeye [11]. Biological values (BV) were calculated according to Oser [12].

$$EAA\ \ score = \frac{g\ of\ \ EAA\ in\ 100g\ of\ \ test\ sample}{g\ of\ \ EAA\ in\ 100g\ \ FAO/WHO\ ref.\ pattern}$$

$$EAAI = \sqrt{\frac{[Lys \times Thr \times Val \times Met \times Ile \times Phe \times His \times Tryp]a*}{[Lys \times Thr \times Val \times Met \times Ile \times Phe \times His \times Tryp]b*}}$$

Where a* represents the content of amino acids in test sample, and b*, the content of the same amino acids in standard protein (%) [Egg], respectively.

$$BV = 1.09 \times EAAI = 11.7$$

$$P - PER = -0.468 + 0.545(Leu) - 0.105(Tyr).$$

2.9. Determination of Functional Properties

2.9.1. Protein Solubility

Protein solubility of HSSPIs was determined at pH 8.0. The hydrolysates (50 mg) were dissolved in distilled water and adjusted to pH 8.0 with 0.1 N NaOH solutions. The final volume of the solution was 20 mL. The protein solution was centrifuged at 5000 ×*g* for 15 min. The protein in the supernatant was determined by using the Kjeldahl method. Protein solubility was expressed as percentage on the basis of protein present in the protein isolate.

2.9.2 Water Absorption Capacity (WAC) and Oil-Absorption Capacity (FAC)

WAC and FAC of were determined according to the method reported by Ogunwolu et al. [13]. WAC was determined by dissolving 0.5 g of the samples with 10 mL of distilled water, the samples were then vortexed for 30 s. The dispersions were allowed to stand at room temperature (22°C) for 30 min then centrifuged at 1,800 ×*g* for 20 min. The supernatant was filtered and the volume retrieved was accurately measured. The difference between initial volumes of distilled water added to the protein samples and the volume retrieved was recorded as the volume (mL) of water absorbed per g of protein sample. FAC was determined by using 2 g of protein stirred with 20 mL of sunflower oil for 30 min and centrifuged at 13,600 *g* for 15 min. The volume of supernatant was measured. The FAC was expressed as the volume (mL) of oil retained by 100 g of proteins.

2.9.3 Emulsifying Capacity (EC)

The EC was determined by the method reported by Vioque et al.[14], with a slight modification. In brief, 20 mL of 7% (w/v) protein suspension was homogenized with a homogenizer (FA 25 model, Fluko, Shanghai, China) at 8000 rpm for 30 s. 10 mL of sunflower oil was added, and the mixture was homogenized for another 30 s. Then, 25 mL of sunflower oil was added again, and the mixture was homogenized for 90 s. The emulsion was centrifuged at 1100 ×*g* for 5 min. EC percentage was calculated as the volume of the emulsified layer divided by the volume of the emulsion before centrifugation.

2.9.4. Foaming Capacity (FC) and Foaming Stability (FS)

To determined FC of HSSPIs, 25 mL of 1.5% (w/v) protein suspension was homogenized at 8000 rpm for 5 min using a homogenizer (FA 25 model, Fluko, Shanghai, China). The FC was expressed, in percent, as the foam volume divided by the total volume. The FS was expressed as the percentage of foam remaining after 15, 30, 45 and 60 min at room temperature (25°C).

2.9.5. Emulsifying Activity Index (EAI) and Emulsion Stability Index (ESI)

Emulsifying properties of HSSPIs were determined according to the method reported by Klompong et al.[15], with some modifications. Sunflower oil (10 ml) and 30 ml of 1% protein solution were mixed and the pH was adjusted to 7 using 0.1N NaOH. The mixture was homogenized using a homogenizer (FA 25 model, Fluko, Shanghai, China) at a speed of 20,000 rpm for 1 min. Aliquots of the emulsion (50 µl) were pipetted from the bottom of the container at 0 and 10 min after homogenization and mixed with 5 ml of 0.1% SDS solution. The absorbance of the diluted solution was measured at 500 nm using a spectrophotometer (UVmini-1240; Shimadzu, Kyoto, Japan). The absorbance measured immediately (A_0) and 10 min (A_{10}) after emulsion formation were used to calculate the EAI and ES as follows:

$$EAI\left(m^2/g\right) = \frac{\left(2 \times 2.303 \times A_{500nm}\right)}{F \times \text{Protein weight}\left(g\right)}$$

Where, F is the oil volume fraction of 0.25. The ES percentage was calculated as:

$$ES(\%) = \frac{A_0 - A_{10}}{A_0} \times 100\%.$$

2.9.6. Bulk Density

Bulk density of HSSPIs was determined according to Ogunwolu et al [13]. A calibrated plastic centrifuge tube was weighed, protein samples were filled to 25 mL and the tubes were tapped to eliminate the spaces between the particles, the final volume was taken as the volume of the sample. The tube was weighed again. The bulk density of the HSSPIs were calculated from the difference in weight and expressed as (g/ml).

2.10. Determination of Antioxidant Properties

The antioxidant properties of HSSPIs were determined according to the following methods: DPPH radical scavenging activity, ABTS radical scavenging activity and reducing power assay by Zeng et al. [16] ferrous chelating activity, Kong and Xiong [7].

2.11. Statistical Analysis

All experiments were conducted at least in triplicate. One-way analysis of variance (ANOVA) was used to determine significant differences between means and Duncan's test was used to perform multiple comparisons between means using SPSS 16.0 software (SPSS Inc. Chicago, IL, USA). The significance level was defined as $p<0.05$.

3. Results and Discussion

3.1. Degree of Hydrolysis (DH) of Seinat Protein Hydrolysates

As shown in Figure 1, the DH values of HSSPIs showed a steady increased over 180 min period of incubation with slowing hydrolysis rate as the hydrolysis time increased. The same trend of DH versus incubation time was also observed for pumpkin (*Cucurbita moschata*) protein meal [17] and peanut [18]. In this study, the low range of DH values (11.27 – 28.23%) obtained for HSSPIs when compared with other hydrolysates: 19 - 87 % for palm kernel cake protein hydrolysates produced using different enzymes [19]; 26.8 to 44.7% (with Alcalase) and 23.84 to 43.14% (with papain) for raw herring press cake, may be due to the type and concentration of enzymes used.

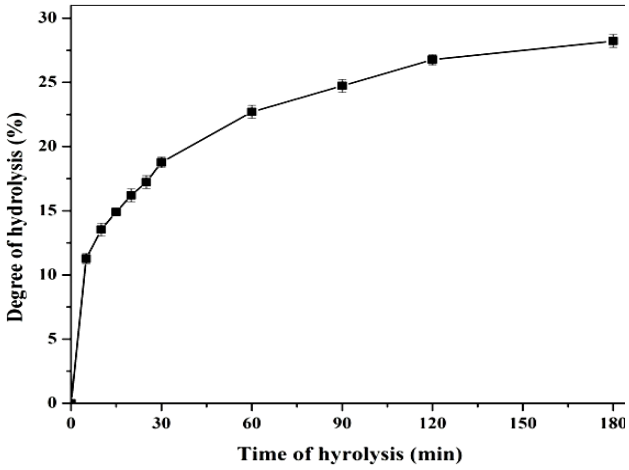

Figure 1. Kinetic curve of DH of HSSPIs (Results are presented as means ± standard deviations (n = 3)

This reduction in the reaction rate which leads to lower DH values may be explained by the formation of reaction products, the decrease in concentration of peptide bonds available for hydrolysis, enzyme inhibition and/or enzyme deactivation [20]. Alkaline proteases such as alcalase exhibit higher activities than neutral or acid ones such as pepsin. In addition, the differences in DH values could also be further explained on the basis of differences in protein composition [21].

3.2. Amino Acid Composition of Seinat Protein Hydrolysates

The results for amino acid content of the HSSPIs have been given in Table 1. The hydrolysis time did not have a clear effect on percentage quantity for most amino acids of seinat seed protein isolates in the hydrolysis process. These results are supported by in agreement with those reported by Kong and Xiong [7], who indicated that hydrolysis does not change the amino acid composition of soluble fractions of protein hydrolysates. The amino acid composition of seinat seed flour, according to a study carried out by Azhari et al. [6], showed that alanine acid was the highest amount of amino acid (9.78%), followed by proline (9.24%) and 8.80% isolucine. In this study, HSSPIs contained arginine (9.14–9.52%), cysteine (5.91–8.36%), aspartic acid (6.30–7.96%), and leucine (4.29–5.32%) of the total amino acids, respectively. Tryptophan was not detected in HSSPIs, it was, however present in seinat seed protein isolate which was the raw material. The results show that the contents of lysine and methionine were relatively low compared to other amino acids. HSSPIs also showed lower amounts of total hydrophobic amino acids (alanine, isoleucine, leucine, methionine, phenylalanine, proline, and valine) compared to the isolate (37.67).

Table 1. Total amino acid composition (g/100 g) of HSSPIs prepared with different hydrolysis times

Essential amino acid	Time of hydrolysis (min)						
	Isolate	30	60	90	120	180	FAO/WHO
Histidine (His)	2.21±0.13	1.19±0.19	1.69±0.06	1.77±0.11	1.80±0.08	2.06±0.05	1.90
Threonine (Thr)	2.87±0.09	2.01±0.20	2.19±0.08	2.28±0.09	2.30±0.09	2.64±0.04	3.40
Valine (Val)	4.42±0.08	2.85±0.26	2.99±0.10	3.22±0.06	3.19±0.07	3.63±0.03	3.50
Methionine (Met)	2.47±0.14	1.35±0.21	1.57±0.12	1.63±0.10	1.64±0.08	1.96±0.06	2.50b
Phenylalanine (Phe)	5.30±0.07	3.47±0.22	3.56±0.10	3.79±0.03	3.75±0.07	4.39±0.02	6.30c
Isoleucine (Ile)	3.40±0.11	2.54±0.23	2.82±0.08	2.98±0.08	3.01±0.11	3.36±0.08	2.80
Leucine (Leu)	6.27±0.10	4.34±0.20	4.29±0.09	4.60±0.05	4.60±0.10	5.32±0.03	6.60
Lysine (Lys)	2.76±0.09	1.31±0.19	1.48±0.14	1.51±0.08	1.57±0.12	1.78±0.12	5.80
Tryptophan (Try)	-	-	-	-	-	-	-
Non-essential amino acid							
Tyrosine (Tyr)	2.86±0.09	1.74±0.19	2.09±0.10	2.22±0.09	2.25±0.08	2.51±0.03	
Cysteine (Cys)	5.91±0.06	6.40±0.18	7.92±0.05	6.89±0.04	7.40±0.10	8.36±0.04	
Aspartic acid (Asp)	6.72±0.08	6.30±0.10	6.67±0.17	6.69±0.07	6.79±0.06	7.96±0.02	
Glutamic acid (Glu)	14.48±0.06	12.10±0.12	11.40±0.10	12.20±0.12	12.00±0.10	14.60±0.13	
Serine (Ser)	3.93±0.04	3.13±0.13	2.82±0.08	2.86±0.08	2.98±0.13	3.42±0.03	
Glycine (Gly)	3.86± 0.11	3.64±0.06	3.15±0.12	3.98±0.11	4.08±0.10	4.48±0.03	
Arginine (Arg)	12.42± 0.13	9.52±0.13	9.14±0.10	9.65±0.05	9.68±0.11	9.24±0.06	

Values represent the means±standard deviation of triplicates.
[a] Suggested profile of daily essential amino acid requirements for pre-school children (2–5 years), FAO/WHO, (1991).
[b] Methionine + cysteine.
[c] Phenylalanine + tyrosine.
(-): Not detected.

This lowering in the content of the mentioned amino acids can be useful for the treatment of congenital illness such as phenylketonuria or tyrosinamie, in which diets low in these amino acids are recommended [22].

3.3 Molecular Weight Distribution (MWD) of Seinat Protein Hydrolysates

The results of MWD of HSSPIs are presented in Table 2. In all the samples a large amount of small-sized peptides (3,000–5,000 Da) were decreased at the end of hydrolysis, and some differences in percentage content of other peptide fractions were found. During the incubation process, peptides of high molecular size (>10,000 Da) gradually decomposed into small-sized peptides (0.34%–0.24%) as the hydrolysis time increased (30–180 min). MWD of HSSPIs also showed that the amount of peptide fractions from >180–3,000 Da were high compared to others. This trend of decrease in the quantity of peptide fractions was similar to the findings reported in by Pownall, Udenigwe and Aluko [23], who found the decrease in the quantity of peptide fractions in the rang (39.97–7.91%) for pea seed (*Pisum sativum* L.) protein hydrolysate.

Table 2. Molecular weight distribution of HSSPIs prepared with different hydrolysis times

Percentage content of each peptide fraction (Da)	Time of hydrolysis (min)				
	30	60	90	120	180
<180	5.59±0.01	6.03±0.21	5.84±0.15	5.94±0.11	5.68±0.14
180~500	43.44±0.10	44.58±0.09	46.67±0.10	42.90±0.07	48.36±0.08
500~1,000	28.79±0.08	29.32±0.13	29.51±0.13	30.60±0.15	29.79±0.08
1,000~2,000	13.88±0.02	12.49±0.05	11.47±0.08	13.24±0.13	10.72±0.06
2,000~3,000	3.41±0.09	2.69±0.09	2.50±0.09	3.01±0.08	2.38±0.12
3,000~5,000	2.07±0.04	1.70±0.08	1.41±0.08	1.37±0.19	1.28±0.15
5,000~10,000	2.48±0.11	2.88±0.02	2.28±0.11	2.60±0.09	1.55±0.12
>10,000	0.34±0.05	0.31±0.06	0.32±0.05	0.34±0.06	0.24±0.13

Values represent the means ± standard deviation of triplicates.

3.4. Nutritional Values of Seinat Protein Hydrolysates

Nutritional parameters of HSSPIs were computed and results are reported in Table 3. Lysine was found to be the first limiting amino acid. Baldwin [24] reported that essential amino acids such as methionine, isoleucine, theronine, lysine, leucine, valine and tryptophan were the limiting amino acids in many oilseed proteins. The essential amino acids score (EAA Score) for HSSPIs were calculated with comparison to the FAO pattern (FAO/WHO, 1991) based on the suggested pre-school children (2–5 years) requirement. The EAA scores were significantly different (p<0.05) and ranged from 0.83 to 1.09 (Table 3). These results indicate that the differences in the EAA scores were due to the differences in essential amino acid composition among the samples. Chemical scores for essential amino acids (EAA Score) of HSSPIs increased with hydrolysis time from 30–180 min in the range (51.59–72.54%). The EAAI method can be useful as a rapid tool to evaluate food formulations for protein quality. However, it does not account for differences in nutritional quality due to various processing methods or certain chemical reactions [25].

The biological values (BV) of the HSSPIs were in the range of 44.53 to 67.36% (Table 3). The predicted protein efficiency ratio (P-PER) of HSSPIs after incubation of 180 min was 1.65. Friedman [26] reported that protein efficiency ratio (PER) below 1.5 implies a protein of low or poor quality; whereas PER between 1.5 and 2.0 indicates an intermediate protein quality; and PER above 2.0 means protein of good to high quality. Therefore according to Friedman's [26] ranking of protein quality, HSSPIs could be considered of intermediate quality. Based on the equation used, HSSPIs P-PER values were variable and significantly different (p<0.05). From the results of nutritional characteristics of HSSPIs, hydrolysis time, generally affects nutritional properties positively.

Table 3. Functional properties of HSSPIs prepared with different hydrolysis time

Functional properties	Time of hydrolysis (min)				
	30	60	90	120	180
Solubility at pH 8 (%)	81.60± 0.36a	78.83± 0.76b	77.90 ± 0.31c	75.39± 0.53d	74.83 ± 0.29d
Water absorption capacity (mL/g)	3.72 ± 0.08a	3.65 ± 0.07ab	3.61 ± 0.10ab	3.53 ± 0.13ab	3.46 ± 0.12b
Fat absorption capacity (mL/g)	2.09 ± 0.06a	2.03 ± 0.09ab	1.96 ± 0.13ab	1.92 ± 0.07ab	1.89 ± 0.13b
Emulsifying activity (%)	40.33 ± 0.58a	36.43 ±0.40b	34.17 ± 0.29c	31.93± 0.12d	29.20 ± 0.34e
Foaming capacity (%)	46.17 ± 0.76a	42.47± 0.84b	39.46 ± 0.50c	37.90± 0.36d	35.96 ± 0.15e

Values represent the means ± standard deviation of triplicates.

3.5. Functional Properties of Seinat Protein Hydrolysates

The results of the influence of hydrolysis time on the protein solubility, water/oil absorption capacities, emulsifying activity and foaming capacity are given in Table 4. Protein solubility is considered one of the most important properties of protein hydrolysates because a low solubility may cause an unattractive appearance and a sandy mouth feel of the final product [27]. The solubility of HSSPIs was in the range of 81–64%, after 30 min. the solubility percentage was higher (p<0.05) than that the 60 and 90 min hydrolysis time interval samples, while no significant differences were observed in the 120 and 180 min samples. Water absorption capacity of HSSPIs decreased with increase in hydrolysis time (3.72–3.46 mL/g); fat absorption capacity also showed a similar trend (2.09–1.98 mL/g). The protein subunit structure generally dissociates on heating or under extreme alkali conditions, and any possible factors this may affect these groups and may cause changes in the water and oil absorption capacities [28].

Table 4. Nutritional evaluation of HSSPIs prepared with different hydrolysis times

	Time of hydrolysis (min)				
	30	60	90	120	180
Limiting AC*	Lysine (Lys) (23.94±0.04)b	Lysine (Lys) (26.97±0.03)ab	Lysine (Lys) (27.52±0.01)ab	Lysine (Lys) (28.55±0.02)ab	Lysine (Lys) (32.36±0.02)a
EAA score[a]	0.83±0.06c	0.93±0.03b	0.94±0.02b	0.96±0.03b	1.09±0.02a
EAAI%[c]	51.59±1.61c	59.33±2.74bc	61.59±2.21b	62.52±2.47b	72.54±1.63a
BV%[d]	44.53±1.60c	52.97±2.99bc	55.44±2.14b	56.45±2.69b	67.36±1.77a
P-PERe	1.31±0.07bc	1.26±0.03c	1.38±0.02 b	1.38±0.04b	1.65±0.01a
Σ EAA[f]	19.08±1.69c	20.58±0.79bc	21.79±0.60 b	21.84±0.72b	25.05±0.46a
Σ NEAAg	39.21±0.64c	40.74±0.92bc	41.37±0.77b	42.43±0.85b	46.44±0.49a
Percentage of amino acid with different characteristics[h]					
Basic	12.02±0.37c	12.30±0.31bc	12.93±0.23ab	13.04±0.30ab	13.08±0.19a
Acidic	7.52±0.22b	7.80±0.27b	7.91±0.19b	8.00±0.15b	9.43±0.15a
Hydrophobic	21.84±1.05c	23.06±0.69bc	24.06±0.54b	24.20±0.65b	27.59±0.37a
polar	16.93±0.49c	18.16±0.43b	18.24±0.39 b	19.04±0.47b	21.40±0.17a

Values represent the means ± standard deviation of triplicates.

Emulsifying activity index (EAI) of HSSPIs also decreased with increasing hydrolysis time in the in the range of 40.33–29.20%. The foaming capacity of HSSPIs were significantly different (p<0.05). Figure 2 shows both emulsifying activity index (EAI) and emulsifying stability index (ESI) at pH 7.0 (Figure 2a and b).

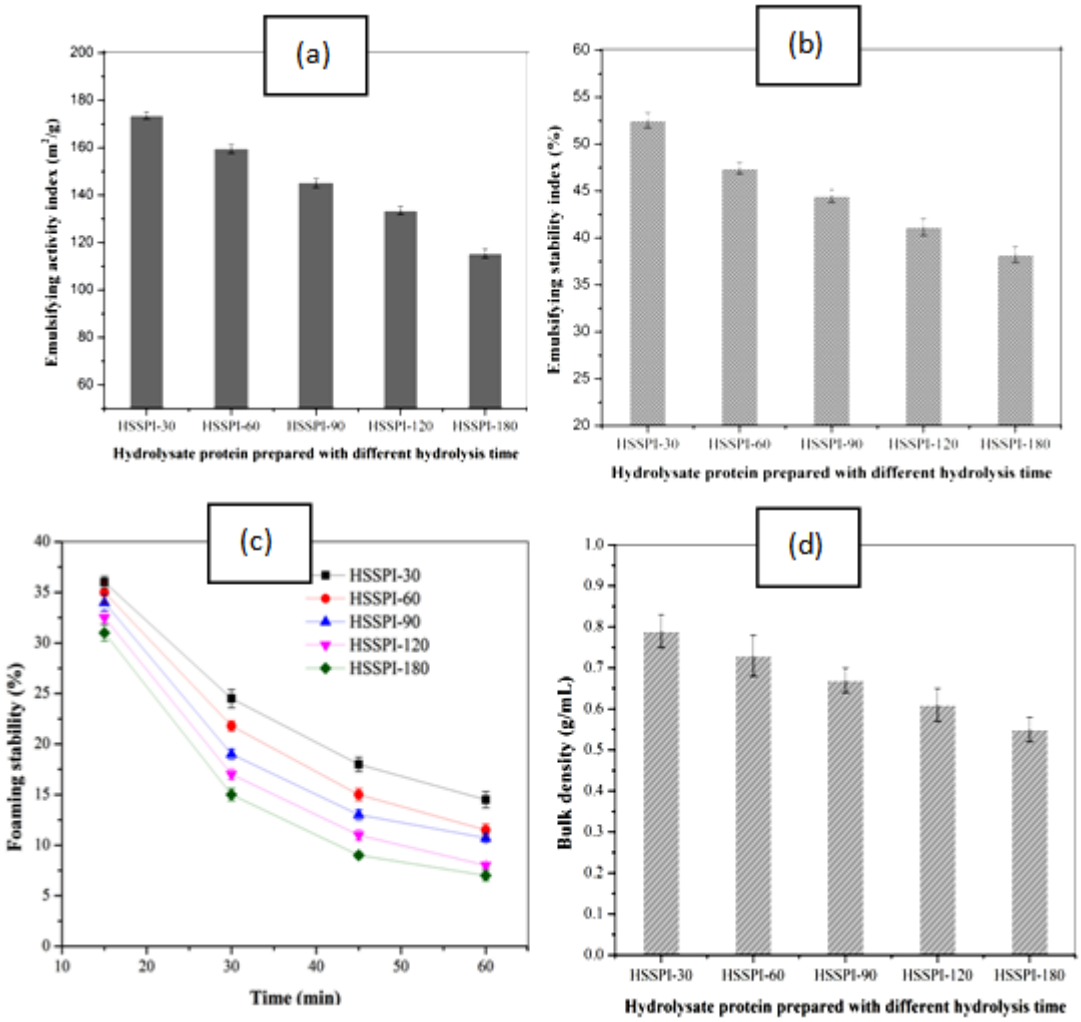

Figure 2. Functional properties of HSSPIs (at pH 7.0) prepared with different hydrolysis time: (a) Emulsifying activity index (EAI); (b) Emulsifying stability index (ESI); (c) Foam stability; (d) bulk density. (Results are presented as means ± standard deviations (n = 3)

EAI and ESI of HSSPIs decreased (p<0.05) with increase in hydrolysis time. This trend was expected because as hydrolysis time increased, the peptides of higher molecular size gradually decomposed into small-sized peptides, which are less effective in stabilizing emulsions. improvements in emulsifying property upon very limited hydrolysis could be attributed to exposure of the hydrophobic protein interior which enhances adsorption at the interface, forming a cohesive interfacial film, with the hydrophobic residues interacting with oil and hydrophilic residues with water [29]. Thus, the peptides with low molecular weight may not be amphiphilic enough to exhibit good emulsifying properties.

The foaming stability results of HSSPIs are presented Figure 2c. In this study, HSSPIs showed the highest foaming stability; 36.47% after 15 min. of hydrolysis; although smaller peptides are able to incorporate air into the solution, they do not have enough strength to give stable foam. Figure 2d shows that the bulk density of HSSPIs also decreased with increase in hydrolysis time (0.79–0.55 g/mL) and the results were significantly different (p<0.05). These results are also show increase in hydrolysis time had a negative effect on functional characteristics therefore; limited protein hydrolysis may improve its functional It can be concluded that, limited protein hydrolysis may improve its functional properties. So, a compromise must be reached between hydrolysis time and functional properties [30].

3.6 Antioxidant Activity of Seinat Protein Hydrolysates

Antioxidant properties of HSSPIs such as DPPH radical scavenging activity, reducing power assay (absorbance at 700 nm), IC_{50} of ABTS radical scavenging activity and $Fe2^{+}$ chelating activity are shown in Figure 3.

The DPPH scavenging activity measures the ability of the antioxidant compound to donate electrons or hydrogen, thereby converting the radical to a more stable species [23]. The percentage of DPPH radical scavenging activity of HSSPIs, at various concentrations (1-5 mg/mL) increased with an increase in hydrolysis time (58.83–78.0%) at a concentration 5 mg/mL, after 180 min (Figure 3a), and the IC_{50} was in the range of 2.49–1.81 mg/mL and these results were significantly different (p<0.05). Therefore, among all HSSPIs values, the highest DPPH radical scavenging activity for the HSSPIs was found at the end of 180 min hydrolysis time period. This trend is similar to that reported by Li *et al.* [35] who found the highest DPPH activity at the last degree of hydrolysis (15%) for chickpea protein hydrolysate. The inhibitory concentration of 50% (IC50) of DPPH radical activity was found to be higher than that of buckwheat (*Fagopyrum esculentum* Moench) protein hydrolysates (0.56–0.94 mg/mL) which was reported by Tang et al [31].

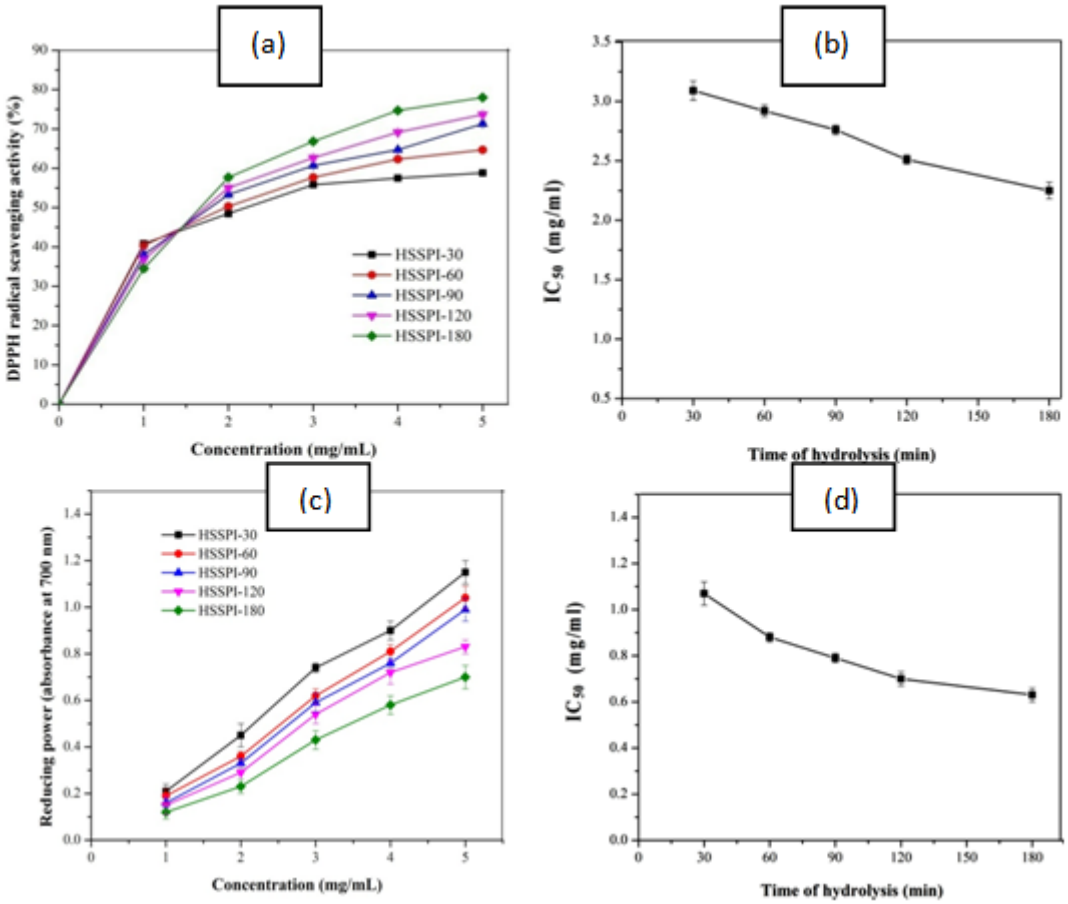

Figure 3. Antioxidant activities of HSSPIs prepared with different hydrolysis time: (a) DPPH radical scavenging activity; (b) ABTS radical scavenging activity; (c) Fe^{2+} chelating activity; (d) reducing power (Results are presented as means ± standard deviations (n = 3)

The ABTS radical scavenging activity, at different concentrations (1–5 mg/mL) was used to measure the in vitro antioxidant activity of HSSPIs (Figure 3b). The IC_{50} value of HSSPIs after 30 min was 3.09 mg/mL which was higher (p<0.05) than what was found at the end of hydrolysis (2.25 mg/mL), these results show that, the IC_{50} value of HSSPIs decreased with increase in hydrolysis time. The highest percentage of activity at hydrolysis time 180 min was 71.13%. This is the same trend that Taha et al. [32], reported for Sunflower Protein Isolate, in which the antioxidant activity increased with increase in hydrolysis time.

Ferrous chelating activity at various concentrations (0.1–2.0 mg/mL), was also used to evaluate antioxidant activity of HSSPIs (Figure 3c). The highest percentage of activity was found at a concentration 2.0 mg/mL after 180 min (71.13%), while IC_{50} value of HSSPIs was in the range of 1.07–0.63 mg/mL. The transition metal ion, Fe^{2+} possess the ability to move single electrons by virtue of which it can allow the formation and propagation of many

radical reactions, even starting with relatively non-reactive radicals [33].

The reducing power of a compound is used to assess its ability to donate electrons and may serve as an important indicator of its potential antioxidant activity [34]. The reducing power of HSSPIs increased with an increase in concentration from 1–5 mg/mL.

There are different studies have indicated that antioxidant activity and reducing power are related [23,35]. The highest absorbance (1.15) was at concentration of 5 mg/mL after 30 min of hydrolysis, while at the concentration 1, 2, 3 and 4 mg/mL the absorbance was; 0.12–0.21, 0.23–0.45, 0.43–0.74 and 0.58–0.90, respectively (Figure 3d). Therefore, HSSPIs can be considered as potent antioxidants and also hydrolysis time period of HSSPIs has a positive effect on antioxidant properties.

4. Conclusion

This study revealed that HSSPIs were affected by the hydrolysis time in term of their nutritional quality, functional and antioxidant properties., small-sized peptides are produced, which leads to a decrease in the functional properties of HSSPIs and an inverse effect on antioxidant activities. Thus, in order to obtain HSSPIs with both strong antioxidant activity and improved functional properties, a controlled hydrolysis is necessary. The HSSPIs managed to achieve the normal requirements of all the essential amino acids except for lysine. Enzymatic hydrolysis of seinat seed protein isolate has shown to be a suitable route for improving nutritional and antioxidant properties although the type of enzyme used for hydrolysis is also a determining factor in this improvement. Therefore, these results suggest the possible use of HSSPIs as nutrient supplements and functional ingredients in food systems, but further research still needs to be done in real food systems.

References

[1] I. M. El Tahir and M. Pitrat, *Cucurbit Genetics*, 1999.

[2] T. A. El-Adawy and K. M. Taha, *Food Chemistry*, 2001, 74, 47-54.

[3] D.-B. Yuan, X.-Q. Yang, C.-H. Tang, Z.-X. Zheng, I. Ahmad and S.-W. Yin, *Food research international*, 2009, 42, 700-706.

[4] Azhari S., Xu Y. S., Jiang Q. X., Xia W. S., "Physicochemical properties and chemical composition of Seinat *(Cucumis melo* var. *tibish*) seed oil and its antioxidant activity", Grasas Y Aceites, 65, 1-8, 2014.

[5] Ajibola C. F., Fashakin J. B., Fagbemi T. N., Aluko R. E., "Effect of peptide size on antioxidant properties of African yam bean seed (Sphenostylis stenocarpa) protein hydrolysate fractions", International Journal of Molecular Sciences, 12, 6685-6702, 2011.

[6] Siddeeg A., Xu Y., Jiang Q., Xia, W., "Physicochemical and functional properties of flour and protein isolates extracted from seinat (*Cucumis melo* var. *tibish*) seeds", Food Science and Biotechnology, 23, 345-353, 2014.

[7] Kong B., Xiong Y.L., "Antioxidant activity of zein hydrolysates in a liposome system and the possible mode of action", Journal of Agricultural and Food Chemistry, 54 (16): 6059-6068, 2006.

[8] J. Adler-Nissen, *Enzymic hydrolysis of food proteins*, Elsevier Applied Science Publishers, 1986.

[9] Yang B., Yang H., Li J., Li Z., Jiang Y., "Amino acid composition, molecular weight distribution and antioxidant activity of protein hydrolysates of soy sauce lees", Food Chemistry, 124, 551-555, 2011.

[10] W. H. Organization, *Energy and protein requirements: report of a joint FAO/WHO/UNU expert consultation*, WHO, 1991.

[11] E. Adeyeye, *African Journal of Food, Agriculture, Nutrition and Development*, 2011, 11.

[12] B. L. Oser, *Protein and amino acid nutrition*, 1959, 281.

[13] S. O. Ogunwolu, F. O. Henshaw, H.-P. Mock, A. Santros and S. O. Awonorin, *Food Chemistry*, 2009, 115, 852-858.

[14] J. Vioque, R. Sánchez-Vioque, A. Clemente, J. Pedroche and F. Millán, *Journal of the American Oil Chemists' Society*, 2000, 77, 447-450.

[15] V. Klompong, S. Benjakul, D. Kantachote and F. Shahidi, *Food Chemistry*, 2007, 102, 1317-1327.

[16] L.-B. Zeng, Z.-R. Zhang, Z.-H. Luo and J.-X. Zhu, *Food Chemistry*, 2011, 125, 456-463.

[17] V. Muhamyankaka, C. Shoemaker, M. Nalwoga and X. Zhang, *International Food Research Journal*, 2013, 20, 2227-2240.

[18] Dong X., Zhao M., Shi J., Yang B., Li J., Luo D., Jiang G., Jiang, Y., "Effects of combined high-pressure homogenization and enzymatic treatment on extraction yield, hydrolysis and function properties of peanut proteins", Innovative Food Science and Emerging Technologies, 12 (4): 478-483, 2011.

[19] Zarei M., Ebrahimpour A., Abdul-Hamid A., Anwar F., Saari N.,"Production of defatted palm kernel cake protein hydrolysate as a valuable source of natural antioxidants", International Journal of Molecular Sciences, 13(7): 8097-8111, 2012.

[20] Guerard F., Guimas L., Binet A, "Production of tuna waste hydrolysates by a commercial neutral protease preparation", Journal of Molecular Catalysis B: Enzymatic 19-20: 489-498, 2002.N. T. HOYLE and J. MERRITT, Journal of Food Science, 1994, 59, 76-79.

[21] Hoyle N.T., Merritt J.H., "Quality of fish protein hydrolysates from herring (*Clupea harengus*)", Journal of Food Science, 59 (1): 76-79, 1994.

[22] T. L. Pownall, C. C. Udenigwe and R. E. Aluko, *Journal of Agricultural and Food Chemistry*, 2010, 58, 4712-4718.

[23] Pownall T. L., Udenigwe C. C., Aluko R. E., "Amino acid composition and antioxidant properties of pea seed (*Pisum sativum* L.) enzymatic protein hydrolysate fractions", Journal of Agricultural and Food Chemistry, 58, 4712-4718, 2010.

[24] Baldwin A.R., "World conference on Emerging Technologies in the fats and Oils Industries" In American Oil Chemists' Society (Eds), pp. 51-56. Illinois: Champaign, 1986.

[25] S. S. Nielsen, *Introduction to the chemical analysis of foods*, Jones and Bartlett Boston, 1994.

[26] Friedman M., "Nutritional value of proteins from different food sources. A review", Journal of Agricultural and Food Chemistry, 44 (1): 6-29, 1996.

[27] Taha F.S., Ibrahim, M.A., "Effect of degree of hydrolysis on the functional properties of some oilseed proteins", Grasas y Aceites, 53 (3): 273-281, 2002.

[28] S. E. M. Ortiz and M. C. An, *Journal of the American Oil Chemists' Society*, 2000, 77, 1293-1301.

[29] A. Mahajan, S. Bhardwaj and S. Dua, *Journal of Agricultural and Food Chemistry*, 1999, 47, 3093-3098.

[30] F. Taha and M. Ibrahim, *Grasas y Aceites*, 2002, 53, 273-281.

[31] Jamdar S.N., Rajalakshmi V., Pednekar M.D., Juan F., Yardi V., Sharma A., "Influence of degree of hydrolysis on functional properties, antioxidant.

[32] F. S. Taha, S. S. Mohamed, S. M. Wagdy and G. F. Mohamed, *World Applied Sciences Journal*, 2013, 21, 651-658.

[33] A. M. AbouL-Enein, F. K. El-Baz, G. S. El-Baroty, A. Youssef and H. H. Abd El-Baky, *J. Med. Sci*, 2003, 3, 87-98.

[34] S. Meir, J. Kanner, B. Akiri and S. Philosoph-Hadas, *Journal of Agricultural and Food Chemistry*, 1995, 43, 1813-1819.

[35] Y. Li, B. Jiang, T. Zhang, W. Mu and J. Liu, *Food Chemistry*, 2008, 106, 444-450.