PREPRATION OF SESAME SEED PROTEIN ISOLATE AND STUDYING THE EFFECT OF ENZYMTIC HYDROLYSIS IN ANTIOXIDANT ACTIVITIES

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ABSTACT

Sesame protein isolate (SPI) was prepared from dehulled, defatted sesame protein concentrate (SPC) using alkaline extraction and isoelectric precipitation. The optimum conditions for SPI preparation were "pH 12, sample to solvent ratio 1:30 (w/v), extraction temperature 60 ^{0}C and extraction time 60 minutes". The protein recovery using the conditions above was 63.83% with 90.05% of protein content and low oil content (0.05%). The optimum conditions for protein isolate hydrolysate preparation (depending on antioxidant activity) was achieved using trypsin (2000 U/gm) for 2 hours hydrolysis followed by hydrolysis with pepsin (1000 U/gm) for one hour at 37C^{0}, which gave bitterness-free hydrolysate. The antioxidant activates for selected hydrolysates (free of bitterness) ranged from (23.95-49.22%) and (0.078-1.07) nm in Hydroxyl radical scavenging activity and metal chelating agent test respectively.

Key word: protein concentrate, chemical compositions, synergist effect, DPPH.

***Part of M.Sc. thesis for the 1st author.**

مجلة العلوم الزراعية العراقية -2019 :50(2):713-720 كاظم وشاكر

تحضير معزول بروتين بذور السمسم ودراسة تأثير متحللاته الإنزيمية في القابلية المضادة للأكسدة

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المستخلص:

تم تحضير معزول بروتين بذور السمسم من مركز بروتين بذور السمسم مزالة القشور والدهن باستخدام الاستخلاص القاعدي والترسيب عند نقطة التعادل الكهربائي. وجد بان الظروف المثلى لتحضير معزول بروتين بذور السمسم كانت عند "الرقم الهيدروجيني 12 ونسبة مزج (مركز: الماء) 1: 30 (و/ح) ودرجة حرارة الاستخلاص 60 م^{0} ووقت الاستخلاص 60 دقيقة". إذ بلغت كفاءة استخلاص البروتين ونسبة البروتين عند هذه الظروف 63.83% و90.05% على التوالي، وانخفضت نسبة الدهن إلى 0.05%. وكانت الظروف المثلى لتحضير متحلل معزول بروتين بذور السمسم بالاعتماد على الخاصية المضادة للأكسدة هي باستخدام أنزيم trypsin بواقع 2000 وحدة/غم لمدة ساعتين يتبعها إضافة أنزيم pepsin بواقع 1000 وحدة/غم لمدة ساعة ودرجة حرارة 37 م^{0} لكلا الأنزيمين، مع ملاحظة غياب الطعم المر في هذا المتحلل. وتراوحت القابلية المضادة للأكسدة للمتحللات المنتخبة والتي لم يظهر فيها الطعم المر بين (23.95–49.22%) و(0.078–1.07) نانومتر اعتمادا فحص على قابلية كبح الجذور الحرة والقوة الاختزالية على التوالي.

الكلمات المفتاحية: مركز بروتيني، التركيب الكيميائي، الفعل التأزري، DPPH.

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INTRODUCTION

sesame seed (*sesamum indicum L.*) is an important oil seed crop in the world (19). It's grown in tropical and subtropical regions where temperate regions between latitudes 40^0 north and south (27). In 2016, the world production of sesame seed was (6111548 tons), and the main production area was Africa (3295173 tons) and Asia (2630409 tons), about 53.9% and 43% of the total word production. The most production of sesame of the word is in Tanzania, Myanmar, India, China, Sudan, Nigeria, Ethiopia, Burkina Faso, South Sudan and Chad respectively. While the production of sesame seed (at 2016) in Iraq was (2344 tons) (10). Sesame seed and grains are used as food (preparation of sweets, confectionary and bakery products), medicine, cosmetics application and as dietary enhancement in different parts of the world (27, 30, 2). Traditionally, organic solvent or mechanical pressure were used for oil extraction from sesame seed (9). Sesame cake (which obtained after oil extraction) known a good source for protein (40-50%) with well-balanced amino acid composition and brilliant physiological importance (5). Beside oil and protein, sesame seed are good source for vitamins E, A, B complex and minerals such as calcium, phosphorus, iron, copper, magnesium, zinc, and potassium (31).Dehulling process is very important during the preparation of sesame seed protein isolate, because the presence of hulls that contains high amount of oxalic acid reduces its bioavaibility. Also the presences of hulls leads to reducing protein digestibility and impart a dark color to the cake (9). Sesame protein isolate can be prepared from whole or cake sesame seed using several extractions methods like alkaline, salt extraction and isoelectric precipitation (10,11,12) or aqueous enzymatic extraction (13,14). An enzymatic hydrolysis of food protein generally improve their functionality improved (solubility, emulsifying, foaming properties) and water and oil holding capacities (15). The protein (solubility, emulsifying and foaming) properties can be improved with a limited degree of hydrolysis, whereas excessive hydrolysis leads to loss of these functionalities (22). Many proteins from plants are attracting attention in scientific research fields, because of their importance as economical source for natural bioactive peptides as well as nutrition and other health benefit (12) . Depending on the amino acid sequence, the short peptides may show various activities such as antihypertensive, anti-cancer, anti-obesity, hypocholesterolemia, anti-thrombotic and antioxidant activity (7). Short peptides show a strong antioxidant activity in both model and in situ systems including radical scavenging, reducing and chelating metal ion activity (3). The present study aimed to investigate the optimum conditions for sesame protein isolate preparation and study the effect of enzymic hydrolysis on antioxidant activity (scavenging and reducing power of free radicals) of sesame protein isolate

MATERALS AND METHODS

Sesame seed were collected from sesame fields at AL_Latifyia, Iraq. Chemicals and solvents were obtained from Sigma-Aldrich (Germany), BDH (England), FLUKA (Switzerland), Chem Cruz (USA). Initially seed were cleaned to remove dirt, foreign matter, rot and then washed with water and dried in oven at 60^0C for 24h and packed in polyethylene bags. Then dehulling sesame seed was done by soaking in water at 4^0C for 24 hours followed by removing the hulls by ribbing in piece of gloves.

Preparation of defatted sesame flour and concentrate

Defatted sesame flour (DSF) was prepared according to the method of Poveda *et al* (29). Then the defatted sesame protein concentrate (SPC) was prepared by re-extracting protein from defatted sesame flour using ethanol 70% for 2 hours. The product grounded and passed through 125 mesh and kept in 4^0C for further analysis.

Preparation of sesame protein isolate

Sesame protein isolate (SPI) was prepared as described by Onsaard *et al* (28). SPC was mixed with distilled water (D.W) at different ratio (1:10 – 1:50 W/V) and the protein extraction carried out at different temperatures (30-70 C^0) for (15-90 min). The mixture was then centrifuged at 10000_xg for 20 min., and the collected supernatant pH was adjusted to 4.5 to precipitate protein. The precipitate washed, then re-suspended in a small amount

of water, neutralized to pH 7, freeze dried and kept at 4C for further analysis.

Proximate composition

Moisture, oil, protein, fiber and ash of whole sesame seed (WSS), dehulling sesame seed (DSS), SPC and SPI were determined according to AOAC [1] standard methods. The protein recovery was calculated using the following equation (3):

$$\mathrm{P.R}\ (\%) = \frac{\text{weight of SPI (gm)} \times \text{protein content of SPI (\%)}}{\text{weight of SPC(gm)} \times \text{protein content of SPC(\%)}} \times 100$$

Preparation of protein isolate hydrolysate

Papain-hydrolyzed protein isolate

Papain hydrolysis of SPI ($HSPI_1$) was carried out due to the method describe by Chatterjee *et al* (3) method. SPI was mixed with distilled water in the ratio of 1:20 (W/V), the mixture pH was adjusted to 10 and incubated at 50 ^{0}C for 1 hours with shaking. The reaction mixture pH re-adjusted to 8.0, hydrolysis conducted using papain (1000 U/gm isolate) at 37 ^{0}C for 7 hours in shaking incubator. The (DH %) was determined after 1 hour up to 7 hours, each time the enzyme was inactivated by heating the reaction mixture at 95 ^{0}C for 5 min then centrifuged at 5000$_X$g for 15 min and the supernatant was collected and stored at -20 ^{0}C for further analysis.

Trypsin-hydrolyzed protein isolate

Trypsin hydrolysis of SPI ($HSPI_2$) was carried out according to Liu and Chiang (22) method with some modification. SPI was mixed with distilled water in the ratio of 1:20 (W/V), the mixture pH was adjusted to 10 and incubated at 50 ^{0}C for 1 hours with shaking. The pH mixture re-adjusted to 8.0, and then the hydrolysis was carried out using trypsin (2000 U/gm isolate) at 37 ^{0}C for 7 hours in shaking incubator. Aliquot of hydrolysate was taken every one hour. The enzyme was inactivated as described in papain hydrolyzed isolate preparation.

Pepsin-hydrolyzed protein isolate

Pepsin hydrolysis of SPI ($HSPI_3$) was carried out due to the method described by Chatterjee *et al* (3). SPI was dissolved in 0.1N HCl (1:20 W/V) and placed in shaking water bath for 1 hour at 50C^0, then the pH of the reaction mixture adjusted to 2 and pepsin solution (2000 U/gm isolate) was added. The hydrolysis conducted for 7 hours at 37C. Aliquot of the hydrolysate was taken every one hour and the enzyme inactivated by adjusting the pH to 7 and cooled in ice pack the resulted hydrolysate centrifuged at 5000 $_x$g for 15 min and the collected supernatant stored at -20C^0 for further analysis.

Synergistic effect of enzymes in hydrolysis of protein isolate

SPI hydrolysis conditions under synergistic function of papain, pepsin and trypsin is described in Table 1. SPI first treated separately with papain, trypsin and pepsin (as described previously). After certain time of hydrolysis (as mentioned in Table 1), the pH re-adjusted to the optimum pH of selected enzyme and the hydrolysis continued by selected enzyme for two or one hour. Aliquot of hydrolysate was taken every one hour. The enzyme was inactivated by heating the reaction mixture at 95 ^{0}C for 5 min, each hydrolysate was centrifuged at 5000$_X$g for 15 min and then supernatant was collected and stored at -20 ^{0}C for further analysis.

Table1. SPI hydrolysis conditions under Synergistic function of papain, pepsin and trypsin

Sample key	pH	Enzyme (u/gm)	Hydrolysis time (hour)	pH	Enzyme (u/gm)	Hydrolysis time (no bitterness)
($HSPI_4$)	8	Trypsin (2000)	2	8	Papain (1000)	2
($HSPI_5$)	2	Pepsin (2000)	2	8	Papain (1000)	1
($HSPI_6$)	8	Trypsin (2000)	2	2	Pepsin (1000)	1
($HSPI_7$)	8	Trypsin (1000) + Papain (1000)	1	2	Pepsin (1000)	1

Determination of degree of hydrolysis (DH %): The degree of hydrolysis determined according to Liu and Chiang (22) method, which included 0.250 ml of each hydrolysate mixed with 2ml phosphate buffer (0.2M, pH8.2), 2ml of 1% SDS and 2mL of 0.1% TNBS. The reaction mixture was incubated in shaking water bath at 50 °C for 60 min in dark place. The reaction was terminated by adding 4mL of HCl (0.1 N) and kept at room temperature for 15 min before reading the absorbance against water at 340 nm. L-leucine solution (5-55mM) was used for standard curve preparation. The following question used to calculate (DH %).

$$\mathbf{DH\ (\%)} = [(L_t - L_0)/(L_{MAX} - L_0)] \times 100$$

Where:

L_t is the amount of the specific amino acid at time.

L_0 is the amount of the specific amino acid at time= zero

L_{MAX} is the maximum amount of the specific amino acid in the substrate obtained after hydrolysis using 6 N HCl at 120 ^{0}C for 24 hours.

Anti-oxidant activity of SPI hydrolysate

Hydroxyl radical scavenging activity

The DPPH radical scavenging was carried out according to Laohakunjit *et al* (19) method with some modification. One milliter of DPPH (0.1mM in 95 % ethanol) was added to 1ml of sample solution (3 mg/mL) and to BHT solution (0.1mg/ml ethanol) in test tubes; the mixture was mixed vigorously, placed in dark please for 30 min at room temperature and centrifuged at 10000 $_x$g for 5 min. The resultant color was measured at 517nm using spectrophotometer. The scavenging activity was calculated as follow:

Radical scavenging activity=$[C-(B-A)/C]\times 100$

Where:

C(control) is the absorbance value of 1 mL of distilled water + 1 mL of 0.1-mM DPPH.

B(blank) is the absorbance value of 1 mL of sample solution + 1 mL of 95% ethanol.

A(sample) is the absorbance value of 1 mL of sample solution + 1 mL of 0.1-mM DPPH

Reducing power activity

Reducing power test was carried out according to method described by Li *et al* (21). A series of sample solutions (3 mg/mL) were prepared. Aliquot of 1mL was mixed with 2.5mL phosphate buffer (0.2M, pH 6.6) and 2.5mL of (1%) potassium ferricyanide. The mixtures were mixed vigorously and incubated in a water bath at 50 °C for 20 min. Subsequently, 2.5mL of TCA (10%) was added to the mixture and centrifuged at 3000$_X$g for 10 min., 2.5 mL of supernatant was mixed with 2.5mL of distilled water and then mixed with 0.3mL ferric chloride (0.1%). The absorbance of the resulting solution was measured at 700nm. An increase in absorbance of the mixture indicates an increase in the reducing power as measured by the reduction of ferric ions. BHT (3mg/ml) was used as comparison sample. The collected data were statistically analyzed using analysis of variance (ANOVA). Differences between treatment means were compared using Least Significant Difference (LSD) $\leq$ 0.05 probability level.

RESULTS AND DISCUSSION

Chemical compositions

The chemical compositions of whole sesame seed (WSS), dehulled sesame seed (DSS), defatted sesame protein concentrate (SPC) and SPI are illustrated in Table 2. The protein content in SPC was (55.54%) higher than that in WSS and DSS (22.32% and 25.16%) respectively. Similar results were reported by Inyang and Iduh (13), who found that protein content increased from 24.1% in dehulled sesame seed to 59.7% in defatted sesame flour. Onsaard *et al* (28) reported that the high protein content in defatted sesame seed could be consider as a potential source that could be used in protein concentrates preparation. After oil extraction, the protein content in SPC increased to 55.54% and this result is similar that reported by Chatterjee *et al* (3). SPI prepared from SPC were found to be of improved quality with protein content of 90.05% and protein recovery 63.83%. The isolation process helped to recover more than 50% of the crude proteins from SPC. The SPI also retained little amount of oil, fiber and ash.

Table 2. Chemical composition of WSS, DSS, DSF and SPI

constituents	WSS	DSS	SPC	SPI
Moisture	3.33 ±0.02 d	3.73 ± 0.01 c	4.8 ± 0.13 b	7.1 ± 0.1 a
Oil	46.97 ± 0.07 b	49.36 ± 0.31 a	1.3 ± 0.2 c	0.05 ± 0.005 c
Protein[b]	22.32 ± 0.2 d	25.16 ± 0.13 c	55.54 ± 0.16 b	90.05 ± 0.05 a
Fiber	10.77 ± 0.32 a	8.98 ± 0.37 b	3.62 ± 0.15 c	0.75 ± 0.1 d
Ash	11.52 ± 0.01 a	7.82 ± 0.03 b	7.70 ± 0.11 c	2.4 ± 0.01 d

[a] values are from duplicate

[b] 6.25 was used as the nitrogen conversion factor

WSS: whole sesame seed, DSS: dehulled sesame seed, SPC: defatted sesame protein concentrate and SPI: sesame protein isolate

Optimum conditions for preparation of sesame protein isolate

Effect of pH on protein isolate recovery :

Fig.1 shows the protein recovery at different pH values (8-13) when DSF mixed with distilled water (D.W) 1:10 (W/V) and incubated at 30 C^0/45 minutes. The highest protein recovery (43.36%) obtained at pH 12, while the lowest (17.36%) protein recovery noticed at pH 8, and there were significant difference ($p \leq 0.05$) among all pH values. Naji (25) found that pH 10 was optimum pH for sesame protein isolate preparation. The result of this study agree with Essa *et al.* (9) findings who stated that the protein recovery of SPI increased as pH value increased due to the changes in net charge of protein, which enhance protein solubility.

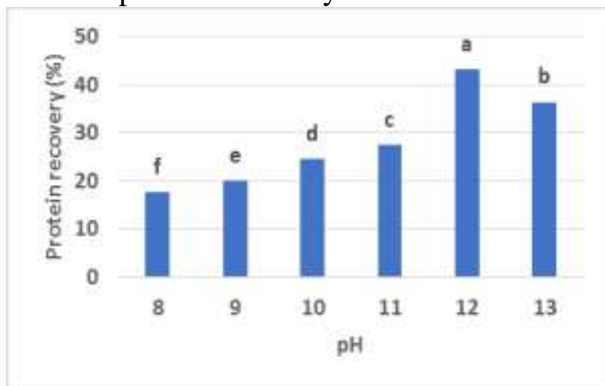

Fig. 1. Effect of pH on protein recovery in alkaline extraction of protein from DSF

Effect of solid to liquid ratio on protein isolate recovery

The effect of solid to solvent ratio on protein recovery in SPI preparation is shown in Fig.2. Different mixing ratio (1:10 – 1:50) (W/V) at pH 12 and incubation time 30 C^0/45 minutes were applied, the highest (53.02%) protein recovery was achieved at 1:30 w/v solid to solvent ratio, whereas the lowest protein recovery (42.92%) was achieved at 1:10 w/v. There was non-significant difference between mixing ratio 1: 20 and 1: 40, also between 1: 30 and 1: 50. Khedr and Mohamed (17) found similar results that 1: 30 w/v is the best mixing ratio for sesame protein isolate preparation. Di *et al.* (8) noticed that 1:10 (w/v) mixing ratio was not efficient for protein extraction from oats. This could be attributed to the high viscosity of the extraction mixture and insufficiency of the solvent which consequently hinders the protein solubility.

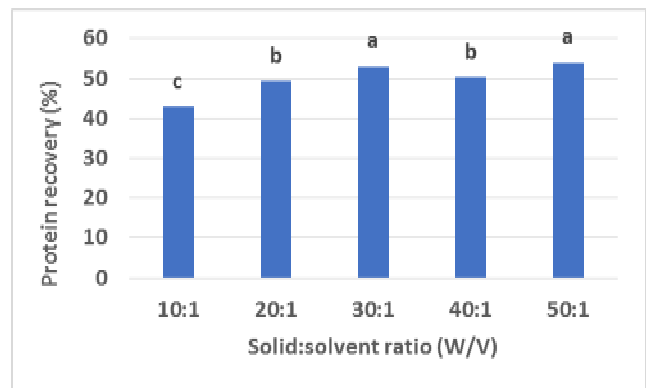

Fig. 2. Effect of sample: solvent mixing ratio on protein recovery in SPI

Effect of extraction temperature on protein isolate recovery

Fig.3 illustrate the effect of extraction temperatures (30-70 ^{0}C) at pH 12, mixing ratio 1:30 (W/V) for 60 minutes on protein recovery of SPI. The highest protein recovery (63.83%) observed at 60 ^{0}C, whereas the lowest protein recovery (60.21%) resulted at 30 ^{0}C and there was non-significant deference between 60, 70 minutes and 30, 40, 50 minutes. These results disagreed with Naji (25) and agreed with Essa *et al* (9), who reported that protein recovery increased as extraction temperature increased, and that was attributed to improving of the solid solubility and reducing the extraction solution viscosity. Whereas Kain *et al* (14) reported that the peanut protein isolate yield decreased as the extraction temperature increased beyond 70 ^{0}C, they suggested that could be due to thermal degradation of the protein.

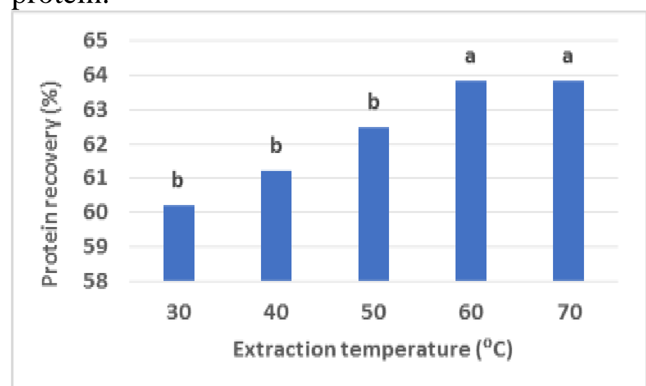

Fig. 3. Protein recovery of SPI at various extraction temperature

Effect of extraction time on protein isolate recovery

Fig.4 shows protein recovery through different extraction time (15-90 minutes) at pH 12, mixing ratio 1:30 (W/V) and incubation temperature 60 ^{0}C. The highest protein

recovery (60.35%) recorded after 60 minute and there was non-significant difference among extraction time 60, 75 and 90 minutes. These results agreed with Kain *et al*, (14) who reported that the protein yield from peanut increased with increasing extraction time to 60 minutes, after that the protein recovery started to drop down. This result also agreed with Liu *et al* (23) findings for soy protein isolate preparation.

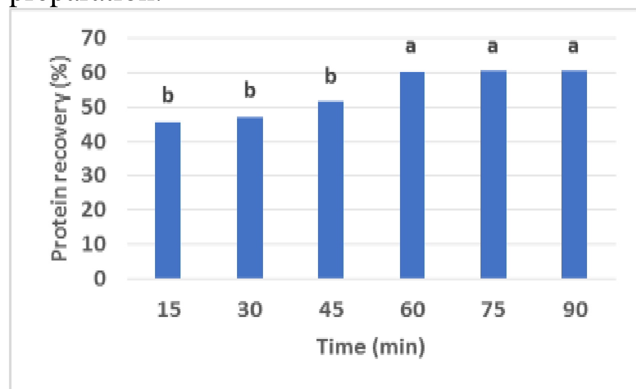

Fig. 4. Protein recovery of SPI at various extraction time

Hydrolysis of sesame protein isolate

The degree of hydrolysis for hydrolyzed SPI by different proteolytic enzyme presented in Fig.5. It has been noticed that degree of hydrolysis increased with time of hydrolysis and it ranged from 15.57-36.34%. ($HSPI_3$) had highest (DH %) followed by ($HSPI_7$) and ($HSPI_4$) while the lowest (DH %) noticed by ($HSPI_1$) and ($HSPI_6$) after 7 hours. There were no bitterness taste noticed in ($HSPI_2$), ($HSPI_5$), ($HSPI_6$) and ($HSPI_7$) after 3 hour of hydrolysis, moreover ($HSPI_1$), ($HSPI_4$) and ($HSPI_3$) were bitterness-free after 5, 4, 2 hour respectively. Hence, these products were selected for antioxidant activity assay. Lovšin-Kukman *et al* (24) suggested that the main reason for bitterness of soy protein hydrolysate treated by alcalase was hydrophobic bitter peptides of relative molecular weight less than 1000 Da.. Cho *et al.* (4) mentioned that the bitterness increased as the peptide molecular weight decreased to 3000Da and 2000Da respectively. On other hand, Kodera and Nio (18) reported that there was no direct relationship between molecular size of the peptides and bitterness.

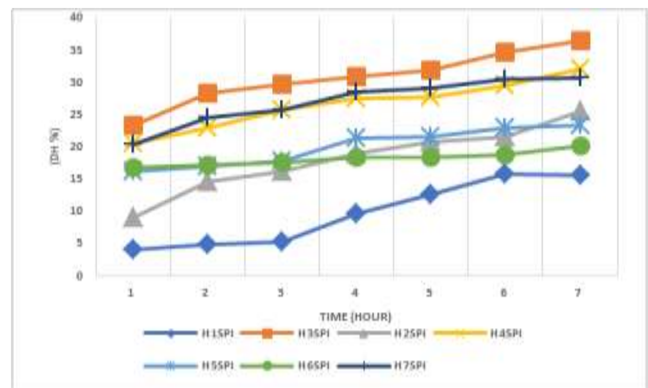

Fig. 5. Degree of hydrolysis as time function

Antioxidant activity of the hydrolysate

Fig.6 shows the antioxidant activities of selected hydrolysate using DPPH and reducing power. The anti oxidant of ($HSPI_6$) in both systems show the highest (49.22%, 1.07 respectively) antioxidant activity in both system while the lowest antioxidant activity was ($HSPI_5$) in DPPH system (23.95%) and ($HSPI_2$) in reducing power (0.078) nm. There were no-significant difference between ($HSPI_6$), ($HSPI_7$) and between ($HSPI_4$), ($HSPI_5$) in radical scavenging activity, also there was no-significant difference between ($HSPI_6$) and BHT, between ($HSPI_7$), ($HSPI_4$) and between ($HSPI_2$), ($HSPI_3$) in reducing power ability. This could be attribute to the variations in peptide size and structure due to significant influence of (DH %) on the antioxidant activity of peptides during hydrolysis. Liu (23), Sun (32) reported that the radical scavenging activity of the hydrolysates is related to amino acid sequences of peptides, which depends on protease specificities. While increasing reducing power could be attribute to increasing side chain of liberated amino acids during hydrolysis, thus providing additional amino acids which donate protons and electrons to maintain a relatively high redox potential (33). In this study, radical scavenging activity and reducing power was higher than those reported by Liu and Chiang (22) for bromelain treated sesame seed protein and by Nwachukwu and Aluko (26) in Flaxseed protein hydrolysates, but lower than those studied by Liu and Chiang (22) for trypsin treated sesame protein isolate.

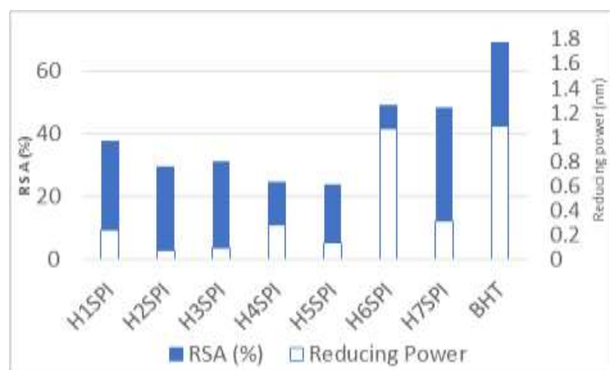

Fig. 6. Hydroxyl radical scavenging activity and reducing power for sesame protein hydrolysates

REFRENCES

1. A.O.A.C. 1995. Official methods of analysis.16th ed. Washington, DC: Association of Official Analytical Chemists

2. Amoo, S.O.; A.O.M. Okorogbona; C.P. DuPlooy and S.L. Venter. 2017. Sesamum indicum. In Medicinal Spices and Vegetables from Africa pp: 549-579.

3. Chatterjee, R.; T.K. Dey; M. Ghosh and P. Dhar. 2015. Enzymatic modification of sesame seed protein, sourced from waste resource for nutraceutical application. Food and Bioproducts Processing, 94: 70-81.

4. Cho, M.J.; N. Unklesbay; F.H. Hsieh and A.D. Clarke 2004. Hydrophobicity of bitter peptides from soy protein hydrolysates. Journal of Agricultural and Food Chemistry, 52(19): 5895-5901

5. Das, R. and C. Bhattacharjee. 2015. Processing Sesame Seeds and Bioactive Fractions. In Processing and Impact on Active Components in Food, pp: 385-394

6. Das, R.; C. Bhattacherjee and S. Ghosh. 2009. Effects of operating parameters and nature of fouling behavior in ultrafiltration of sesame protein hydrolysate. Desalination, 237(1-3): 268-276

7. Das, R.; A. Dutta and C. Bhattacharjee. 2012. Preparation of sesame peptide and evaluation of antibacterial activity on typical pathogens. Food Chemistry, 131(4): 1504-1509.

8. Di, A.H.; N. Ji and H.Z. Liu. 2006. Study on the extraction of separate protein of oats. Food science, 27(12): 439-441

9. Essa, Y.R.; R.A. Elhady; H. Kassab and A. Ghazi. 2015. Isolation and charcteristizaten of protein isolated from sesame seeds (*Sesamum indicum*) cake. Weber Agricultural Research and Management, 1(1): 1-9

10. FAOSTAT. 2018. FAO Statistics Division 2018; Sesame Seed. In: Food and Agriculture Organization of The United Nation

11. Fasuan, T.O.; T.O. Omobuwajo and S.O. Gbadamosi. 2018. Optimization of simultaneous recovery of oil and protein from sesame (*Sesamum indicum*) seed. Journal of Food Processing and Preservation, 42(1), 13341

12. Garc´ a. M.; C.P. Puchalska; C. Esteve and M.L. Marina. 2013.Vegetable foods: a cheap source of proteins and peptides with antihypertensive, antioxidant, and other less occurrence bioactivities. Talanta 106: 328–349

13. Inyang, U.E. and A.O. Iduh. 1996. Influence of pH and salt concentration on protein solubility, emulsifying and foaming properties of sesame protein concentrate. Journal of the American Oil Chemists' Society, 73(12): 1663-1667

14. Kain, R.J.; Z. Chen; T.S. Sonda and J.C. Abu-Kpawoh. 2009. Study on the effect of control variables on the extraction of peanut protein isolates from peanut meal (*Arachis hypogaea* L.). American Journal of Food Technology, 4(1): 47-55

15. Kanu, P.J.; J.B. Kanu; E.H. Sandy; J.B. Kandeh; P.M. Mornya and Z. Huiming. 2009. Optimization of enzymatic hydrolysis of defatted sesame flour by different proteases and their effect on the functional properties of the resulting protein hydrolysate. Am. J. Food Technol, 4(6): 226-240

16. Khalid, E.K.; E.E. Babiker and A.E. Tinay. 2003. Solubility and functional properties of sesame seed proteins as influenced by pH and/or salt concentration. Food Chemistry, 82(3): 361-366.

17. Khedr, M. A. and S. S. Mohamed. 2004. Ternary diagram of extract proteins / solvent systems: Sesame, soybean and lupine proteins. Grasas y Aceites, 55 (3).

18. Kodera, T. and N. Nio. 2006. Identification of an angiotensin I-converting enzyme inhibitory peptides from protein hydrolysates by a soybean protease and the antihypertensive effects of hydrolysates in 4 spontaneously hypertensive model rats. Journal of food science, 71(3).

19. Laohakunjit, N.; O. Kerdchoechuen; R. Kaprasob and F.B. Matta. 2017. Volatile flavor, antioxidant activity and physicochemical properties of enzymatic defatted sesame hydrolysate. Journal of Food Processing and Preservation, 41(4).
20. Latif, S. and F. Anwar. 2011. Aqueous enzymatic sesame oil and protein extraction. Food chemistry, 125(2): 679-684.
21. Li, X.; J. Deng; S. Shen; T. Li; M. Yuan; R. Yang and C. Ding. 2015. Antioxidant activities and functional properties of enzymatic protein hydrolysates from defatted Camellia oleifera seed cake. Journal of Food Science and Technology, 52(9): 5681-5690
22. Liu, B.L. and P.S. Chiang. 2008. Production of hydrolysate with antioxidative activity and functional properties by enzymatic hydrolysis of defatted sesame (*Sesamum indicum* L.). International Journal of Applied Science and Engineering, 6(2): 73-83
23. Liu, M.M.; B. Qi; Z.X. Liu; J.S. Zhan; K. Zhan and G.Q. Zhao. 2017. Optimization of low-abundance protein extraction and abundant protein removal from defatted soybean meal. Journal of Zhejiang University-science b, 18(10): 878-885
24. Lovšin-Kukman, I.; M. Zelenik-Blatnik and V. Abram. 1996. Bitterness intensity of soybean protein hydrolysates—chemical and organoleptic characterization. Zeitschrift für Lebensmittel- Untersuchung und Forsch-ung, 203(3): 272-276
25. Naji E.Z. 2016. Optimum conditions for the extraction of sesame seed (*sesamum indicum*) proteins and study some of its functional properties. J. Food and Dairy Sci., Mansoura Univ., 7(10): 427 – 433
26. Nwachukwu, I.D. and R.E. Aluko. 2018. Antioxidant properties of flaxseed protein hydrolysates: influence of hydrolytic enzyme concentration and peptide size. Journal of the American Oil Chemists' Society. DOI 10.1002/aocs.12042
27. Onsaard, E. 2012. Sesame proteins. International Food Research Journal. 19(4): 1287-1295
28. Onsaard, E.; P. Pomsamud and P. Audtum. 2010. Functional properties of sesame protein concentrates from sesame cake. Asian Journal of Food and Agro-Industry, 3(4): 420-431
29. Poveda, T.; R. Vilcacundo; C. Carpio and W. Carrillo. 2016. Analysis of sesame proteins isolate (*sesamum indicum* L) with water and salt treatment. Asian Journal of Pharmaceutical and Clinical Research, 9(3): 404-407
30. Shivhare, N. and N. Satsangee. 2012. Wonders of Sesame: Nutraceutical Uses and Health Benefits. In Chemistry of Phyto potentials: Health, Energy and Environmental Perspectives. Springer Berlin Heidelberg. pp: 63-68
31. Singharaj, S. and E. Onsaard. 2015. Production and characteristic of sesame proteins. Journal of Food Science and Agricultural Technology (JFAT), 1: 188-192
32. Sun, Q.; H. Shen and Y. Luo. 2011. Antioxidant activity of hydrolysates and peptide fractions derived from porcine hemoglobin. Journal of Food Science and Technology, 48(1): 53-60
33. Zhang, Q.; X. Tong; B. Qi; Z. Wang; Y. Li; X. Sui and L. Jiang. 2018. Changes in antioxidant activity of Alcalase-hydrolyzed soybean hydrolysate under simulated gastrointestinal digestion and transepithelial transport. Journal of Functional Foods, 42: 298-305.