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Effect of Walnut Protein Hydrolysate on Antioxidant Properties

*[1]Abdulkareem Abdulrazzaq Kareem
[1]*Food Science Department, College of Agriculture, Tikrit University, Iraq*

Abstract: *The enzymatic hydrolysate of walnut proteins Isolate was prepared by incubation with three different proteases, including pepsin, papain, and trypsin, Allhydrolysates showed different degrees of hydrolysis (DH), papain enzyme showed a higher hydrolytic activity followed by pepsin hydrolysate, Then trypsin enzyme that shows lowest. The hydrolysates were found to possess excellent antioxidant properties , papain exhibited a higher 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity 16.7% at 120 minute , the lowest level by trypsin hydrolysate 4.3% at 15 minute , pepsin exhibited a higher Fe2+ ion-chelating activity for hydrolysate walnut protein isolate 55.6% at 120 minutes while the lowest for trypsin 20.4 % at 15 minutes, higher reducing power was 0.31 nm for walnut protein isolate hydrolysate by pepsin at 180 minutes while lowest absorbance was 0.14 nm for walnut protein isolate hydrolysate by trypsin at 15 minutes .*

Keywords*: walnut protein isolate., walnut protein hydrolysates. Degree of hydrolysis ,Antioxidant capacity*

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I. Introduction

Walnuts (Juglansregia L.) are widely distributed all over the world, and they are common in China. On a global basis, walnuts rank second behind almonds in tree nut production. In 2010, global production of walnuts was 1,500,000 t. China leads the world production of walnuts, followed by the US. In 2010, China accounted for 33.33% of global walnut production. Moreover, walnut is not only an agricultural commodity, but its leaves, barks, stems, pericarps, fruits, flowers and are all applied for different medicinal uses in China. These fruits are receiving increasing interest as a healthy foodstuff because their regular consumption has been reported to decrease the risk of coronary heart disease [1][2][3][4]. Antioxidant peptides are a kind of bioactive peptides which demonstrate strong antioxidant capacity. They are considered as a potential source to control various oxidative processes in the human body as well as in food[5] . Walnut protein hydrolysates (WPHs), normally obtained by hydrolyzing defatted walnut meal or walnut protein isolates extracted from the defatted walnut meal, usually incorporate antioxidant peptide chains as well as POHs. Both of them are potent food-driven antioxidants that have received significant attention due to their remarkable antioxidant capacity and the related clinical implications.[6][7]Many studies showed that the bioactive peptides that are produced as a result of enzymatic hydrolysis of nut proteins had better functions than proteins,[8] Therefore, much attention has been paid to producing antioxidant peptides from food proteins. Antioxidant peptides can be produced using protease hydrolysis and fermentation methods[9]. Current Study aimed to hydrolysate walnut protein isolate with three type of enzyme (pepsin, trypsin, and papain) , select the best enzyme with high antioxidant properties.

II. Material And Methods

2.1. Materials

Walnut purchased from local market in Tikrit province, pepsin , papain trypsin was obtained from Sigma Aldrich, USA DPPH (1,1-diphenyl-2-picryl hydrazyl) were prod- ucts of Sigma Chem. (St. Louis, MO, USA) , All other chemicals and reagents used were of analytical grade.

2.2. Preparing Of Walnut Protein Isolates (WPI)

Dehulling Walnut was carried out manually, cleaned to remove all foreign matter, production of Walnut Protein Isolate (WPI). Walnuts were ground and defatted with petroleum ether. The defatted flour was dried in the drying oven overnight at 50 ◦C. Then, the defatted four was dispersed in NaOH solution (pH 9.0) at ratio 1:15 (w/v) and extracted by stirring for 1 h at 45 ◦C. After pH adjustment to 4.5, the precipitate obtained by centrifugation at 4000 ×g for 20 min was lyophilized and stored in plastic bags at −20 ◦C. [10]

2.3. Preparation Of Antioxidant WPI Hydrolysates

The hydrolysis conditions such as different proteases, substrate concentration, initial pH, hydrolysis temperature and incubation time were optimized to produce antioxidant peptides. The initial hydrolysis

conditions were as follows: amount of protease, 2.5%; concentration of substrate (WPI), 5%; hydrolysis time, 4 h; temperature and pH were set up according to the optimal conditions of each enzyme , Pepsin (pH 3.0 at 37 _C), Papain (pH 6.0 at 55 _C), Trypsin (pH 8.0 at 37 _C). Enzymatic hydrolysis was carried out in a temperature-control shaker at a speed of200 rpm. Then the proteases were terminated after boiling for 10 min. The hydrolysate supernatant (WPI hydrolysate) was separated by centrifugation at 5000 rpm for 15 min and then concentrated in a rotary evaporator for further use.[11]

2.4. Degree of hydrolysis (DH)

Determination of α-Amino Acid and Degree of Hydrolysis(DH), According to [12] Properly diluted samples (125 µL) were mixedthoroughly with 2.0 mL of 0.2125 M phosphate buffer, pH 8.2,followed by the addition of 1.0 mL of 0.01% 2,4,6-Trinitrobenzenesulfonic acid (TNBS) solution.The mixtures were then placed in a water bath at 50 °C for30 min in the dark. The reaction was terminated by adding2.0 mL of 0.1 M sodium sulfite. The mixtures were cooleddown at ambient temperature for 15 min. The absorbance wasmeasured at 420 nm and α -amino acid was expressed in termsof L-leucine.Figure 1. The DH was defined as follows:

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

Where$L_t$corresponded to the amount of R-amino acid releasedat time *t*. ,L_0 was the amount of R-amino acid in originalWPI. L_{MAX} was the maximum amount of R-amino acid inWPI obtained after acid hydrolysis.

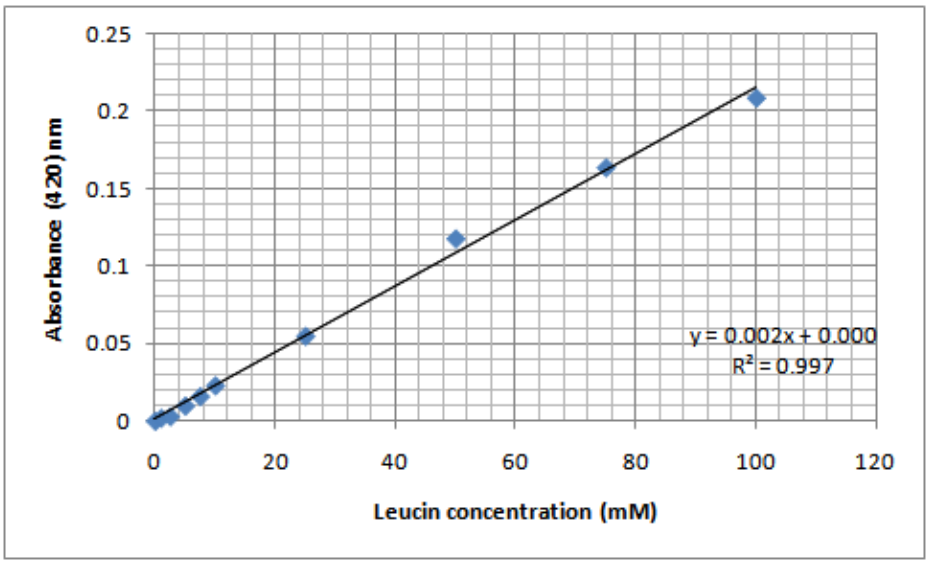

Figure **1 Standard curve of L-Leucine**

2.5. Antioxidant activity

2.5.1(1, 1-Diphenyl-2-picrylhydrazyl) (DPPH) Radical Scavenging Activity Assay.

The radical scavenging activity was determined according to [13]. To the diluted sample (1.5 mL), 1.5 mL of 0.15-mM DPPH in 95% ethanol was added. The mixture was then mixed vigorously and allowed to stand at room temperature in the dark for 30 min. The absorbance of the resultant solution was read at 517 nm using a UV-1601 spectrophotometer (Shimadzu, Kyoto, Japan). The blank was pre- pared in the same manner except that distilled water was used instead of the sample. The scavenging effect was calculated as follows:

Radical scavenging activity % = [(B-A)/B] ×100

Where A is $A_{517}$of sample and B is $A_{517}$of the blank.

2.5.2. Fe2+ Ion-Chelating Activity

Chelation of Fe2+ was measured using the method of [14] . A 0.5mL sample aliquot was mixed with 1mL of 20 µM $FeCl_2$ and 1mL of 0.5 mMferrozine. The reaction mixture was allowed to stand for 20min at 25^oC. The absorbance was read at 562 nm. The metal ion chelating activity was calculated as:Metal ion chelating activity (%) = [1− (As−A_0)/Ac] ×100A blank (A_0) was prepared in the same mannerexcept that distilled water was used instead of a sample (As). EDTA was used as positive control (Ac).

2.5.3. Reducing power

The reducing power of the hydrolysates was measured according to [15]. A Sample (2 ml) was added to 2 ml of 0.2 M phosphate buffer (pH 6.6) and 2 ml of 1% potassium ferricyanide. The mixture was incubated

at 50 C for 20 min. Then 2 ml of 10% TCA was added to the reaction mixture. A volume of 2 ml from each incubated mixture was mixed with 2 ml of distilled water and 0.4 ml of 0.1% ferric chloride in the test tube. After a 10 min reaction, the absorbance of the resulting solution was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power.

2.6. Statistical Analysis

Statistical analysis was performed by SPSS for one-way ANOVA and the significant differences were analyzed by the Duncan range test ($p < 0.05$). All Data were expressed as mean ± standard deviation.

III. Result And Discussion

Determination of α-Amino Acid and Degree of Hydrolysis (DH).

The DH is an important element that highly related to the hydrolysis process yield [16]. Figure 2 shows all the curves have a rapid hydrolysis for the first 1 h. At the same level of the enzyme($P < 0.05$), papain hydrolysateshowed a higher hydrolytic activityfollowed by pepsin hydrolysate. Then trypsin hydrolysate.that show lowest hydrolysis progress, Upon further incubation (120 min), the rate of enzymatic hydrolysis decreased for papain, While further incubation (105 min) the rate of enzymatic hydrolysis decreased for pepsin, whereas further incubation (90 min) for trypsin, the rate of enzymatic hydrolysis decreased for pepsin. , then the enzymatic reaction reached the steady-state phase when no apparent hydrolysis took place and no change in DH percentage($P < 0.05$), This indicated that maximum cleavage of peptides occurred within the first hour of hydrolysis for all enzyme These results agreed with [17] when they use different enzyme to Hydrolysis of Defatted Sesame, and with [18] they find hydrolysis of silver carp protein with Alcalase or Flavourzyme proceeded at a high rate during the initial 15 min and then slowed down thereafter,and agree with [19][20].

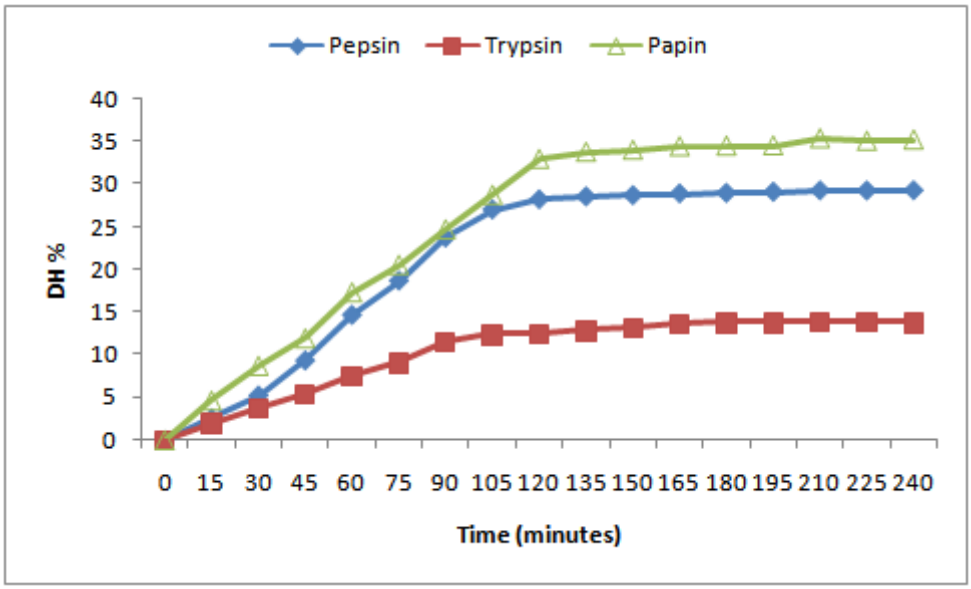

Figure 2 Hydrolysis curves of walnut protein isolate treated with different enzymes: pepsin, trypsin and papain

Determination of DPPH Radical Scavenging Activity DPPHDPPH Radical Scavenging Activity DPPH radical scavenging assay is quick, convenient, and efficient in portending the antioxidant activities of protein hydrolysates, their fractions, and purified peptides ,Therefore, the relatively stable DPPH radical has been widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors and thus to evaluate the antioxidant activity. [21] ,DPPH is a stable free radical with an absorbance maximum at 517 nm in methanol, and the relatively stable DPPH radical has been widely used to evaluate the ability of some substances to act as free radical scavengers or hydrogen donors [22]. Figure 3 show The higher level of the DPPH radical scavenging activity was found for papain hydrolysate at 120 minutes compared with all enzymatic treatment($P < 0.05$), whereas The lowest level of the DPPH radical scavenging activity was found for trypsin hydrolysate at 15 minutes in the samples used in the test ($P < 0.05$), different higher level of the DPPH radical scavenging activity depended on different peptide released from enzymatic hydrolysis , The scavenging effect for the hydrolysates increased slightly in the first 120 minutes and remained unchanged during the outspread period of hydrolysis. The result revealed that the walnut isolate hydrolysates possibly contained substances which were electron donors and could react with free radicals to convert them to more stable products and

terminate the radical chain reaction. This result agrees with [23] who showed an increase in DPPH radical scavenging activity with increased DH in porcine collagen hydrolysatesbut was in contrast with[24]they report on increases in the DH significantly reduced the activity. The DH dependence of the radical scavenging activity of PPH was consistent with that of yellow stripe trevally protein hydrolysate.

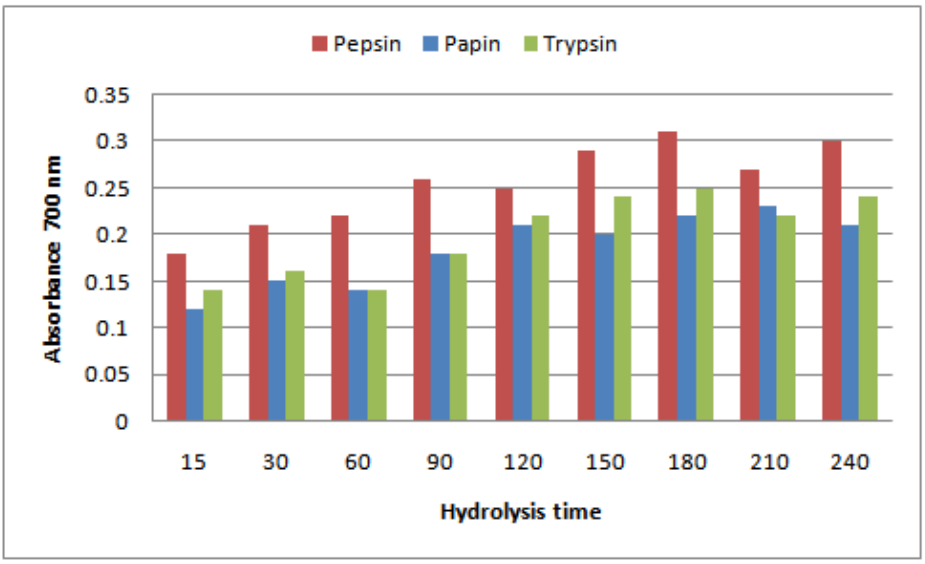

Figure 5 Reducing power of walnut protein isolate treated with different enzymes: pepsin, trypsin and papain

1. Fe2+ Ion-Chelating Activity

Transition metal ions, such as Fe2+ and Cu2+ can catalyze the generation of reactive oxygen species which accelerates lipid oxidation. Fe2+ can also catalyze the Haber-Weiss reaction and induce superoxide anions to form more hazardous hydroxyl radicals. These hydroxyl radicals react with adjacent biomolecules to cause severe tissue damage[25], Fig. 4 show the Fe2+ ion-chelating activity for hydrolysate walnut protein isolate by pepsin,papain, and trypsin , the result indicate contrast in result among the enzymes , highest percentage of the Fe2+ ion-chelating activity was report for hydrolysate walnut protein isolate by pepsin at 120 minute ($P < 0.05$), while the lowest for trypsin at 15 minutes ($P < 0.05$), result also shows the Fe2+ ion-chelating activity was increased with hydrolysates increased slightly in the first 120 minutes and remained unchanged during the outspread period of hydrolysis. It thus suggests that higher degree of cleavage of peptide bonds render a hydrolysate with higher metal chelating activities. A similar observation was also reported by[26] for protein hydrolysate of Peanut [24]for protein hydrolysate of yellow stripe trevally,and [13]for round scad muscle protein hydrolysate.

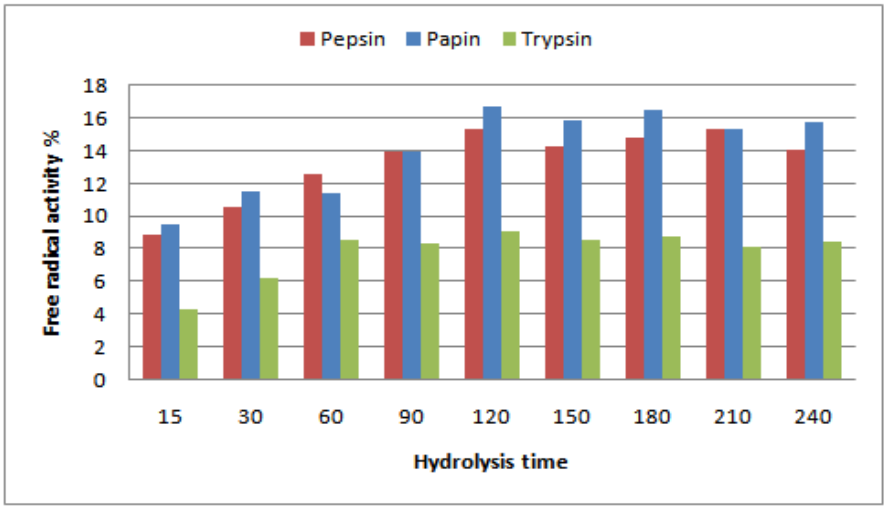

Figure 3 DPPH radical-scavenging activity of walnut protein isolate treated with different enzymes: pepsin, trypsin, and papain

Reducing power

The reducing capacity of a given compound may serve as a significant indicator of its potential antioxidant activity. An electron-donating reducing agent is able to donate an electron to a free radical. As a result, the radical is neutralized and the reduced species subsequently acquires a proton from the solution[27].Fig.5 shows the reducing powerofhydrolysate walnut protein isolate by pepsin,papain, and trypsin, the result indicates contrast in result among the enzymes, highest absorbance was 0.31 for walnut protein isolate hydrolysate by pepsin at 180 minutes ($P < 0.05$), while lowest absorbance was 0.14 for walnut protein isolate hydrolysate by trypsin at 15 minutes ($P < 0.05$).the result also shows the reducing power of the hydrolysatesincrease when hydrolysis was performed using different enzymes and was positively related to the DH.The result agreed with[13]who report that increased reducing the power of round scad protein hydrolysate prepared using Flavourzyme with increasing DH up to 60%, whereas those of round scad protein hydrolysate prepared using Alcalase increased when DH increased up to 40%. Disagree with[28]reported that Decrease in the reducing power with the increase in the DH has been reported for hydrolysate prepared using Alcalase from buckwheat.

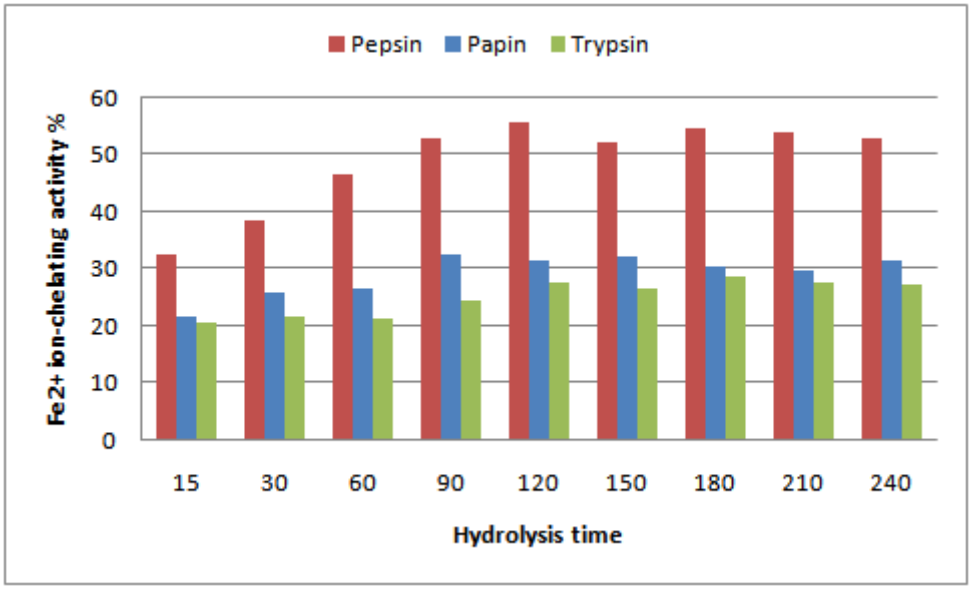

Figure 4 Fe2+ ion-chelating activity of walnut protein isolate treated with different enzymes: pepsin, trypsin and papain

IV. Conclusion

The enzymatic hydrolysate of walnut proteins Isolates with different enzyme show different degree of hydrolysis, thathydrolysates were found to possess excellent antioxidant properties.The higher level of the DPPH radical scavenging activity was found for papain hydrolysate at 120 minutes compared with all enzymatic treatment

References

[1]. [X.-Y. Mao and Y.-F. Hua, "Chemical composition, molecular weight distribution, secondary structure and effect of NaCl on functional properties of walnut (Juglans regia L) protein isolates and concentrates," *J. Food Sci. Technol.*, vol. 51, no. August, pp. 1473–1482, 2012.

[2]. R. Blomhoff, M. H. Carlsen, L. F. Andersen, and D. R. Jacobs, "Health benefits of nuts: Potential role of antioxidants," *Br. J. Nutr.*, vol. 96, no. SUPPL. 2, pp. 52–60, 2006.

[3]. L. Davis *et al.*, "The effects of high walnut and cashew nut diets on the antioxidant status of subjects with metabolic syndrome," *Eur. J. Nutr.*, vol. 46, no. 3, pp. 155–164, 2007.

[4]. C. M. Albert, J. M. Gaziano, W. C. Willet, J. E. Manson, and C. H. Hennekens, "Nut consumption and decreased risk of sudden cardiac death in the physicians' health study," *Arch. Intern. Med.*, vol. 162, pp. 1382–1387, 2002.

[5]. S. Su, Y. Wan, S. Guo, C. Zhang, T. Zhang, and M. Liang, "Effect of peptide-phenolic interaction on the antioxidant capacity of walnut protein hydrolysates," *Int. J. Food Sci. Technol.*, pp. 1–8, 2017.

[6]. M. Gu *et al.*, "Identification of antioxidant peptides released from defatted walnut (Juglans Sigillata Dode) meal proteins with pancreatin," *LWT - Food Sci. Technol.*, vol. 60, no. 1, pp. 213–220, 2015.

[7]. R. Jahanbani *et al.*, "Antioxidant and Anticancer Activities of Walnut (Juglans regia L.) Protein Hydrolysates Using Different Proteases," *Plant Foods Hum. Nutr.*, vol. 71, no. 4, pp. 402–409, 2016.

[8]. X. Wang, H. Chen, S. Li, J. Zhou, and J. Xu, "Physico-chemical properties, antioxidant activities and antihypertensive effects of walnut protein and its hydrolysate," *J. Sci. Food Agric.*, vol. 96, no. 7, pp. 2579–2587, 2016.

[9]. R. Di Bernardini *et al.*, "Antioxidant and antimicrobial peptidic hydrolysates from muscle protein sources and by-products," *Food Chem.*, vol. 124, no. 4, pp. 1296–1307, 2011.

[10]. [N. Chen, H. Yang, Y. Sun, J. Niu, and S. Liu, "Purification and identification of antioxidant peptides from walnut (Juglans regia L.) protein hydrolysates," *Peptides*, vol. 38, no. 2, pp. 344–349, 2012.
[11]. J. Fan, J. He, Y. Zhuang, and L. Sun, "Purification and identification of antioxidant peptides from enzymatic hydrolysates of tilapia (oreochromis niloticus) frame protein," *Molecules*, vol. 17, no. 11, pp. 12836–12850, 2012.
[12]. S. Benjakul and M. T. Morrissey, "Protein Hydrolysates from Pacific Whiting Solid Wastes," *J. Agric. Food Chem.*, vol. 45, no. 9, pp. 3423–3430, 1997.
[13]. Y. Thiansilakul, S. Benjakul, and F. Shahidi, "From Round Scad Muscle Using Alcalase," vol. 31, no. 2007, pp. 266–287, 2006.
[14]. W. Wu, S. Zhao, C. Chen, F. Ge, D. Liu, and X. He, "Optimization of production conditions for antioxidant peptides from walnut protein meal using solid-state fermentation," *Food Sci. Biotechnol.*, vol. 23, no. 6, pp. 1941–1949, 2014.
[15]. H. Wu, H. Chen, and C. Shiau, "Free amino acids and peptides as related to antioxidant properties in protein hydrolysates of mackerel (Scomber austriasicus)," *Food Res. Int.*, vol. 36, pp. 949–957, 2003.
[16]. M. B. K. Foh, I. Amadou, B. M. Foh, M. T. Kamara, and W. Xia, "Functionality and antioxidant properties of tilapia (Oreochromis niloticus) as influenced by the degree of hydrolysis," *Int. J. Mol. Sci.*, vol. 11, no. 4, pp. 1851–1869, 2010.
[17]. B. Liu and P. Chiang, "Production of Hydrolysate with Antioxidative Activity and Functional Properties by Enzymatic Hydrolysis of Defatted Sesame (Sesamum indicum L .)," *Int. J.*, pp. 73–83, 2008.
[18]. S. Dong, M. Zeng, D. Wang, Z. Liu, Y. Zhao, and H. Yang, "Antioxidant and biochemical properties of protein hydrolysates prepared from Silver carp (Hypophthalmichthys molitrix)," *Food Chem.*, vol. 107, no. 4, pp. 1485–1493, 2008.
[19]. F. Bamdad, J. Wu, and L. Chen, "Effects of enzymatic hydrolysis on molecular structure and antioxidant activity of barley hordein," *J. Cereal Sci.*, vol. 54, no. 1, pp. 20–28, 2011.
[20]. S. Benjakul *et al.*, "Production of an extensive sunflower protein hydrolysate by sequential hydrolysis with endo- and exo-proteases.," *Int. J. Mol. Sci.*, vol. 12, no. 2, pp. 197–201, 2009.
[21]. C. F. Ajibola, J. B. Fashakin, T. N. Fagbemi, and R. E. Aluko, "Effect of peptide size on antioxidant properties of African yam bean seed (Sphenostylis stenocarpa) protein hydrolysate fractions," *Int. J. Mol. Sci.*, vol. 12, no. 10, pp. 6685–6702, 2011.
[22]. J. López *et al.*, "Effect of air temperature on drying kinetics, vitamin c, antioxidant activity, total phenolic content, non-enzymtic browning and firmness of blueberries variety óneil," *Food Bioprocess Technol.*, vol. 3, no. 5, pp. 772–777, 2010.
[23]. B. Li, F. Chen, X. Wang, B. Ji, and Y. Wu, "Isolation and identification of antioxidative peptides from porcine collagen hydrolysate by consecutive chromatography and electrospray ionization-mass spectrometry," *Food Chem.*, vol. 102, no. 4, pp. 1135–1143, 2007.
[24]. V. Klompong, S. Benjakul, D. Kantachote, and F. Shahidi, "Antioxidative activity and functional properties of protein hydrolysate of yellow stripe trevally (< i> Selaroides leptolepis</i>) as influenced by the degree of hydrolysis," *Food Chem.*, 2007.
[25]. Z. Xie, J. Huang, X. Xu, and Z. Jin, "Antioxidant activity of peptides isolated from alfalfa leaf protein hydrolysate," *Food Chem.*, vol. 111, no. 2, pp. 370–376, 2008.
[26]. [S. N. Jamdar, V. Rajalakshmi, M. D. Pednekar, F. Juan, V. Yardi, and A. Sharma, "Influence of degree of hydrolysis on functional properties, antioxidant activity and ACE inhibitory activity of peanut protein hydrolysate," *Food Chem.*, vol. 121, no. 1, pp. 178–184, 2010.
[27]. H. Wang, X. D. Gao, G. C. Zhou, L. Cai, and W. B. Yao, "In vitro and in vivo antioxidant activity of aqueous extract from Choerospondias axillaris fruit," *Food Chem.*, vol. 106, no. 3, pp. 888–895, 2008.
[28]. C. H. Tang, J. Peng, D. W. Zhen, and Z. Chen, "Physicochemical and antioxidant properties of buckwheat (Fagopyrum esculentum Moench) protein hydrolysates," *Food Chem.*, vol. 115, no. 2, pp. 672–678, 2009.

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