Scientific Research Publishing

Food and Nutrition Sciences, 2018, 9, 1066-1077
http://www.scirp.org/journal/fns
ISSN Online: 2157-9458
ISSN Print: 2157-944X

Antioxidant Activities of Okra Protein Concentrate and Isolate after Enzymatic Hydrolysis

Abdulkareem A. Kareem[1], Khalidah A. Shakir[2], Marie K. Walsh[3]

[1]Department of Food Science, College of Agriculture, Tikrit University, Tikrit, Iraq
[2]Department of Food Science, College of Agriculture, University of Baghdad, Baghdad, Iraq
[3]Department of Nutrition, Dietetics and Food Sciences, Utah State University, Logan, UT, USA
Email: marie.walsh@usu.edu

How to cite this paper: Kareem, A.A., Shakir, K.A. and Walsh, M.K. (2018) Antioxidant Activities of Okra Protein Concentrate and Isolate after Enzymatic Hydrolysis. *Food and Nutrition Sciences*, **9**, 1066-1077.
https://doi.org/10.4236/fns.2018.99078

Received: August 20, 2018
Accepted: September 14, 2018
Published: September 17, 2018

Abstract

Three different proteolytic enzymes (pepsin, trypsin and papain) were used to prepare okra seed protein concentrate and okra seed protein isolate hydrolysates. The hydrolysates were assayed for antioxidant properties using radical scavenging, reducing power and metal chelating assays. The highest degree of hydrolysis (after 360 min) for okra protein isolates was 35.20%, 35.21% and 10.53% for pepsin, papain and trypsin respectively. The highest degree of hydrolysis (after 360 min) for okra protein concentrates was 26.8%, 28.59% and 6.47% for pepsin, papain and trypsin respectively. Pepsin hydrolysates showed higher metal chelating activity and radical scavenging activity than trypsin and papain hydrolysates. Trypsin hydrolysates showed the lowest antioxidant activities, which may be due to the low degree of hydrolysis. In general, for antioxidant activity, there was an increase in activity with an increase in the degree of hydrolysis. Similar antioxidant activity was found in both the okra protein isolate and concentrate hydrolysates except for metal chelating activity which was higher in okra protein isolate hydrolysates. This may be due to the higher ash concentration in the concentrates (9.4% in concentrates vs. 2.6% in isolates). In this study, pepsin hydrolysates with a final DH of 35.2% showed higher reducing power and metal chelating activity than trypsin and papain hydrolysates. Okra protein hydrolysates were found to have varying levels of antioxidant activity, which was dependent on the specificity of the protease and proportional to the degree of hydrolysis achieved.

Keywords

Okra Protein, Enzymatic Hydrolysis, Antioxidant Properties

1. Introduction

Antioxidants are compounds that can inhibit, delay, or control the oxidation process caused by reactive oxygen species (hydroxyls, superoxide anion radicals, hydrogen peroxide and singlet oxygen) [1]. Oxidation via a free radical mediated chain reaction can be initiated by heat, light, ionizing radiation, metal ions, metallo-proteins or pro-oxidative enzymes [1] [2]. Oxidation can occur in unsaturated lipids and leads to rancidity and off flavors in foods. Additionally there are health benefits associated with consumption of antioxidants. Antioxidants have been associated with controlling oxidative stress. Oxidative stress can lead to cancer, coronary heart disease, obesity, type 2 diabetes and hypertension [1] [2].

Free radicals are controlled by compounds that can donate or transfer hydrogen to the radical or free radical acceptors and are generally phenolic compounds such as vitamin E, beta hydroxyltoluene (BHT), butylated hydroxyanisole (BHA) and flavonoids. Controlling prooxidants (iron and copper) is generally done with chelators by preventing metal redox cycling, occupation of metal coordination sties and the formation of insoluble metal complexes. Metal chelators added to foods include citric and phosphoric acid and polyphosphates [1]. Additionally, reducing agents can reduce peroxides and other oxidants through redox reactions and are referred to as oxygen scavengers or secondary antioxidants [1] [2].

Increasing the oxidative stability of foods includes controlling free radicals, prooxidants and oxidation intermediates. Antioxidants can occur naturally and be found in plants and animals or be synthesized chemically. Naturally occurring antioxidants (tocopherols, flavonoids, and polyphenols) are found in spices, herbs, fruits, vegetables, seeds, and oils. Synthetic antioxidants, such as BHT and BHA have been used in foods but with consumers demanding a "clean label" the use and identification of natural antioxidants is rising [2].

There have been many articles describing the antioxidant properties of protein hydrolysates from plant and animal sources [3]-[8]. The antioxidant properties are dependent on the characteristics of the native protein (amino acid composition), the purification of the protein to produce concentrates or isolates and degree of hydrolysis (molecular weight of peptides) [6] [7] [8]. Additionally, the effectiveness of antioxidants is influences by their concentration, the temperature, the physical state of the system and type of oxidation substrate [2].

The evaluation of the total antioxidant capacity (TAC) can be used to determine the antioxidant properties of food ingredients, generally in lipid free systems [1]. Methods to assess the TAC include the DPPH method, which is a radical scavenging assay. DPPH (2,2-diphenyl-1-picrylhydrazl) is a dark colored organic compound with an absorption band at about 517 nm. DPPH is known as a radical scavenger and upon reaction with radicals; the compound becomes colorless or pale yellow with a decrease in absorption at 517 nm. The absorbance change is related to antioxidant activity. The potassium ferricyanide reducing power (PFRAP) method is also called the reducing power method (RP) method

involves the compound with antioxidant activity to react with potassium ferricyanide to form potassium ferrocyanide which then reacts with ferric trichloride giving ferric ferrocyanide which has an absorbance at 700 nm which relates to antioxidant activity. Transition metals can stimulate lipid oxidation and metal chelators can be used to inhibit this reaction. The metal chelation capacity of a compound is determined by measuring the chelating activity of antioxidants to ferrous ion (ferrozine). A loss of absorbance at 562 nm after the addition of an antioxidant represents the formation of metal-antioxidant complex [1] [2].

The total okra production worldwide is estimated at six million tones per year. It is mainly grown in India, Nigeria, Sudan, Pakistan, Ghana, Egypt, Benin, Saudi Arabia, Mexico and Cameroon with the largest production in India followed by Nigeria and Sudan [9]. The okra seed meal contains greater than 50% good quality protein, comparable to protein obtained from soy and cottonseed [10]. The okra seed meal may be a good source for food fortification to enrich foods that are consumed in the tropic and temperate regions of the world. Our previous research investigated the use of okra seeds (*Abelmoschus esculentus*) for the preparation of defatted okra powder (DOP), okra protein concentrate (OPC) and okra protein isolate (OPI) [11]. Additional research [12] investigated the amino acid composition and functional properties (water absorption, fat absorption, foam capacity and emulsion activity of DOP, OPC and OPI). This research investigated the antioxidant activity of okra seed protein (OPC and OPI) hydrolyzed by either pepsin, trypsin or papain. The protein hydrolysates were tested for free radical scavenging activity (DPHH assay), reducing power (RP assay) and metal chelating activity (ferrozine assay) to determine the relationship between the type of protease and degree of hydrolysis to antioxidant activity. This is the first report on the antioxidant properties of okra seed protein hydrolysates.

2. Materials and Methods

2.1. Preparation of Protein Hydrolysates

Okra seeds (*Abelmoschus esculentus* variety) were purchased from a local market in Baghdad, Iraq. The enzymes pepsin (4500 U/g), trypsin (9500 U/g) and papain (8500 U/g) were purchased from Sigma Aldrich, USA. All chemicals used were of analytical grade.

Okra protein isolate (OPI) and concentrate (OPC) were prepared according to Kareem and Shaker [11] from defatted okra powder. Briefly, defatted okra powder was prepared by removal of the seed peel then grinding the seed meal. The ground meal was defatted with ethanol to produce OPC which contained 72.1% protein, 0.86% fat, 9.94% ash and 12.37% carbohydrate. OPI was prepared from OPC by precipitation with HCl at pH 4 resulting in a composition of 91.2% protein, 0.32% fat, 2.67% ash and 3.2% carbohydrate.

Enzymatic hydrolysis of OPI and OPC was conducted as described by Fan *et al.* [3] and named OPIH and OPCH. Five grams of protein sample was dissolved in distilled water then brought to 100 mL with Glycine-HCl buffer (0.5 M, pH

2). Pepsin was added resulting in an enzyme:protein ratio of 1:50 (g/g). The sample was placed in a shaking incubator at 37˚C for 360 min. Aliquots, 10 mL, were removed at times 0, 15, 30, 60, 90, 120, 180, 240, 300, and 360 min. Aliquots were heated to 90˚C for 5 min to stop the enzymatic reaction. For trypsin hydrolysis, 5 g of protein sample was dissolved in distilled water and brought to 100 mL with sodium phosphate buffer (0.05 M, pH 7.5) and trypsin was added resulting in an enzyme:protein ratio of 1:100 (g/g) and incubated in a shaking incubator at 45˚C for 360 min. Aliquots were removed at the 10 time points and the reaction stopped as described above for pepsin. For papain hydrolysis, 5 g of protein sample was dissolved in distilled water and brought to 100 mL with sodium phosphate buffer (0.05 M pH 6.0) and papain was added for an enzyme:protein ratio of 1:100 (g/g). Aliquots were removed at the 10 time points and the reaction stopped as described above for pepsin. Each hydrolysis reaction was replicated and the aliquots at each time point were pooled.

The degree of hydrolysis (DH) of each protein sample at each time point with each enzyme was determined according to Benjakul and Morrissey [13]. Briefly, OPCH and OPIH, 0.125 mL (or 10 mg sample) was added to 2 mL sodium phosphate buffer (0.2125 M, pH 8.2) and 1 mL of 2,4,6-trinitrobenzenesulfonic acid (TNBS) (0.1% solution in water) were added. Samples were placed in a water bath at 50˚C for 30 min in the dark. Adding 2.0 mL of 0.1 M sodium sulfite terminated the reactions. The mixtures were cooled to room temperature for 15 min. The absorbance was measured at 420 nm and alpha-amino acid was expressed in terms of L-leucine. The DH was determined using the following equation:

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

L_t corresponds to the amount of amino acid liberated at time *t*. L_0 was the amount of amino acid in the original samples. L_{max} was the total amount of amino acid in the original OPC and OPI after acid hydrolysis. Lmax was determined by mixing 50 mg protein from OPC or OPI with 4.5 mL of 6 N HCl. Tubes were flushed with nitrogen gas and sealed in a screw-cap tube. The hydrolysis was run at 100˚C for 24 hrs., then filtered with Whatman paper No. 1 and neutralized with 6 N NaOH before amino acid determination as described above [13].

2.2. Antioxidant Activity

The radical scavenging activity of OPC and OPI hydrolysates was determined using the DPPH assay as described by Thiansilakul *et al.* [14]. A sample of 1.5 mL of hydrolysate (50 mg sample) was mixed with 1.5 mL DPPH (0.15 mM in 95% ethanol) and held for 30 min at room temperature in the dark. A control was prepared with 1.5 mL of distilled water and 1.5 mL DPPH. The absorbance at 517 nm was recorded and compared to unhydrolyzed OPC or OPI dissolved in distilled water. The scavenging activity was calculated using the following equation where A_{517} is the absorbance at 517 nm of the sample and B_{517} is the absor-

bance at 517 nm of the control.

$$\text{Radical scavenging activity (\%)} = [(B_{517} - A_{517})/B_{517}] \times 100$$

The reducing power of OPIH and OPCH was conducted according to Wu *et al.* [15]. A sample (1 mL or 0.05 mg) was added to 1 mL phosphate buffer (0.2 M, pH 6.6) and 1 mL 1% potassium ferrocyanide. This was incubated at 50˚C for 20 min then 1 mL of a 10% solution of tricloroacetic acid was added along with 1 mL of distilled water and 0.2 mL of 1% $FeCl_3$. A control sample was prepared using distilled water in place of the protein hydrolysates. After 10 min at room temperature, the absorbance at 700 nm was recorded.

The metal chelating activity of OPCH and OPIH was determined according to Boyer and McCleary [16]. Aliquots, 4.7 mL (240 mg sample) was combined with 0.1 mL of $FeCl_2$ (2 mM) and 0.2 mL of 5 mM ferrozine solution. The control samples were 4.7 mL of distilled water in place of the protein hydrolysates. The samples were kept at room temperature for 20 min then the absorbance at 562 nm was recorded. The following equation was used to determine chelating activity with B_{562} as the absorbance at 562 nm of the control and A_{562} as the absorbance of the hydrolysate samples at 562 nm.

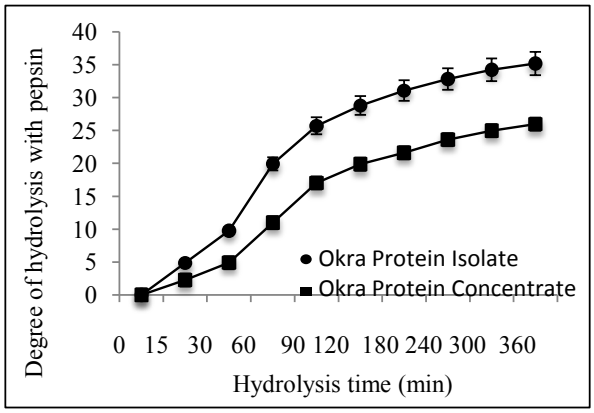
$$\text{Chelating activity (\%)} = [(B_{562} - A_{562})/B_{562}] \times 100$$

All antioxidant activity assays were conducted in triplicate and averaged and the data was normalized by dividing the activity by the amount of protein in the OPCH and OPIH samples used.

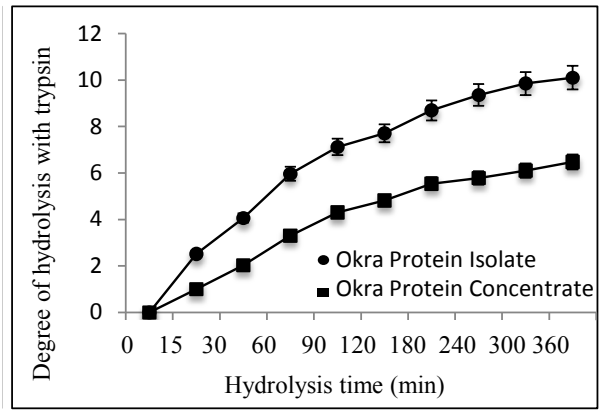
3. Results and Discussion

3.1. Okra Protein Hydrolysis

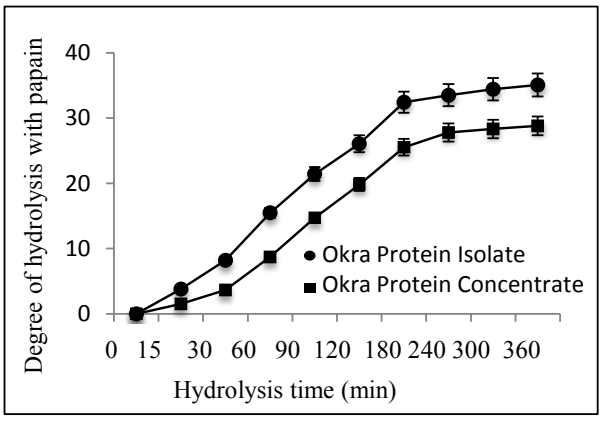
Okra seed protein was purified into a concentrate which contained 72.1% protein and an isolate which contained 91.2% protein. The protein samples were hydrolyzed with three different proteases, pepsin, trypsin, and papain under optimal conditions at times from 0 to 360 min (15 hrs.). **Figure 1** shows the results of the DH for each hydrolysate. For pepsin hydrolysis, (**Figure 1(a)**) the hydrolysis rate increased rapidly in a linearly fashion until 120 min (DH of 29.28%), then there was a slow increase in DH reaching a maximum of 35.20% at 360 min for OPI. For OPC, a similar trend was observed with a DH of 19.89% at 120 min and a maximum of 26.28% at 360 min. For OPI, 83% of the total hydrolysis was completed at 120 min and for the OPC, 76.5% of the total hydrolysis was completed by 120 min. For hydrolysis with trypsin (**Figure 1(c)**), at 120 min the DH of OPI was 7.68% (73% of the total hydrolysis) and the DH for OPC was 4.85% (75% of the total hydrolysis). The maximum DH values for OPI and OPC were 10.53% and 6.47% respectively. For hydrolysis with papain (**Figure 1(c)**), at 180 min the DH of OPI was 32.84% (93% of the total hydrolysis) and the DH for OPC was 25.86% (90.5% of the total hydrolysis). The maximum DH values for OPI and OPC were 35.21% and 28.59% respectively. Hydrolysis with pepsin and papain were similar with DH values for OPI at 35%. The least effective protease



(a)



(b)



(c)

Figure 1. Degree of hydrolysis of okra protein isolate and concentrate with pepsin, trypsin, and papain. Degree of hydrolysis is a percentage. Black circles are the okra protein isolates and black squares are the okra protein isolates. Error bars show the standard deviation.

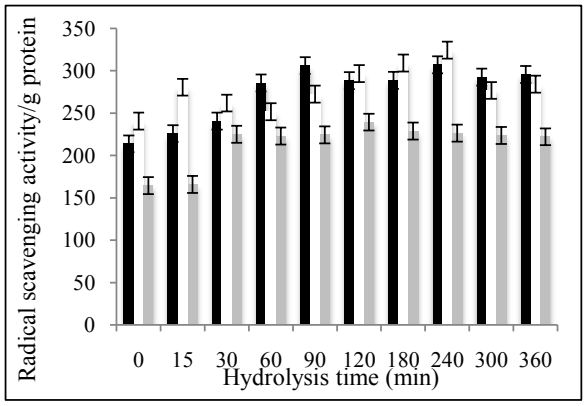
was trypsin. We are unclear why the DH for the OPC was consistently lower than that of the isolate but we believe it may be due to inhibitory compounds present in the OPC which were removed during the preparation of the OPI.

Ktari *et al.* [4] investigated the hydrolysis of cuttlefish protein with a variety of

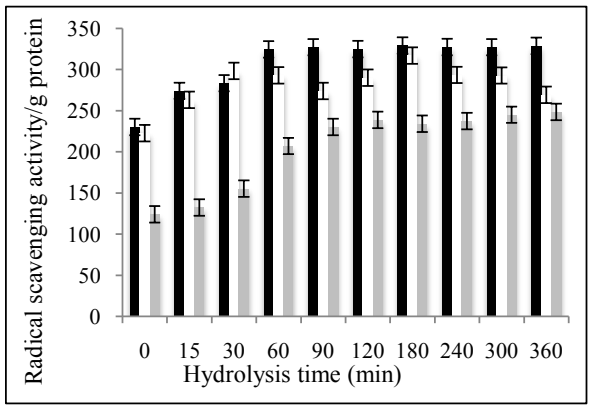
proteases including trypsin, chymotrypsin and proteases from various *Bacillus* species. For each protease, there was rapid hydrolysis up to 200 min, but slowed to negligible increases in DH after that. One of the least effective protease was trypsin which reached only a 4% DH. Yet in a study by Fan *et al.* [3] who hydrolyzed tilapia protein with various enzymes including pepsin, trypsin and papain, the trypsin hydrolysates showed the highest DH values. The DH values in this study [3] for pepsin, trypsin and papain were 5.3%, 15.1% and 9.5% respectively. Pena-Ramos and Xiong [5] showed that the DH of soy protein isolate with papain was almost double that of the pepsin hydrolysate. These studies indicating that the DH value is related to the specificity of the protease and will vary with each protein substrate used.

3.2. Antioxidant Activity

The radical scavenging activity (RSA) of okra protein concentrate hydrolysate (OPCH) and okra protein isolate hydrolysate (OPIH) using the DPPH assay over hydrolysis time for each protease is shown in **Figure 2**. On average the RSA



(a)



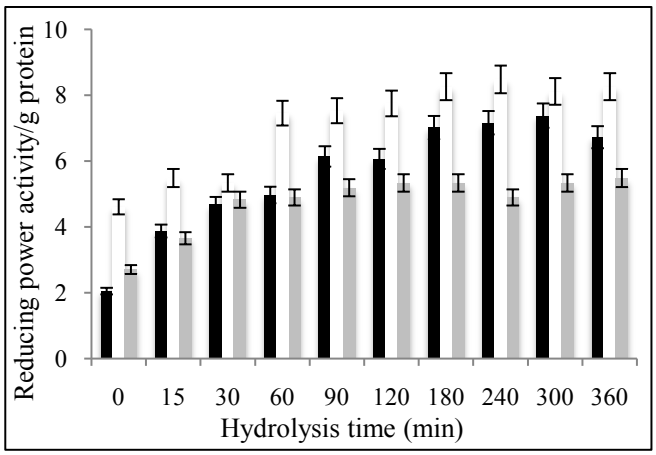
(b)

Figure 2. Radical scavenging activity of protein hydrolysates over time. (a) Hydrolyzed okra protein concentrate. (b) Hydrolyzed okra protein concentrate. Black bars are papain hydrolysates, white bars are pepsin hydrolysates and grey bars are trypsin hydrolysates. Error bars show the standard deviation.

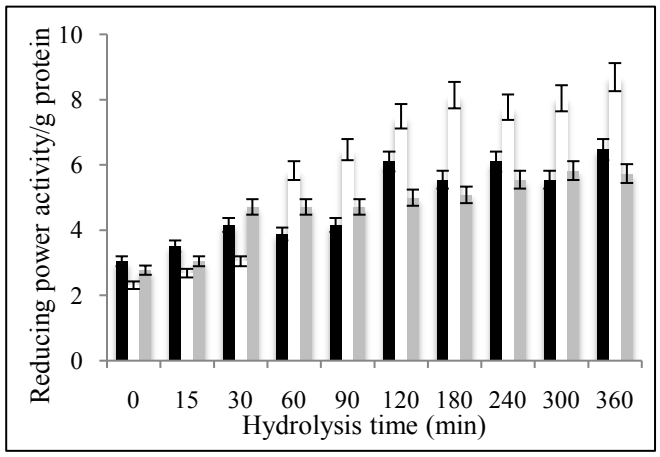
of hydrolysates prepared using papain and pepsin were the highest in both the OPCH and OPIH. This may be due to the DH of the hydrolysates, which were similar in both papain and pepsin and the lowest with trypsin. Although on average, the DH of the trypsin hydrolysates were about 3.5 times lower than those of papain and pepsin, the RSA values for trypsin are approximately half of the values for papain and trypsin initially and 1.5 times lower with longer hydrolysis times. Therefore, the peptides released during the trypsin hydrolysis have higher RSA activities than those released with pepsin and papain. In general, there is a slight increase in RSA activity with an increase in hydrolysis time, which is most noticeable in the trypsin hydrolysates. On average, considering the data was normalized for the protein concentration, there are little differences between the RSA values of the isolate and concentrate hydrolysates even though the concentrates had lower DH values. The only exception is the initial RSA values for trypsin hydrolysates which are lower in the concentrate samples compared to the isolates. Hydrolysate samples after times 120 - 180 min show RSA activities that are similar. For each protease, at times greater than 120 - 180 min, there were negligible increases in DH, therefore as mentioned above, the RSA activity of the hydrolysates is related to the DH values.

The reducing power (RP) of OPCH and OPIH over hydrolysis time for each protease is shown in **Figure 3**. Overall, the RP of the pepsin hydrolysates were higher than for the papain and trypsin hydrolysates. Trypsin RP values were similar to the values for papain, which is not expected since the DH for trypsin was approximately 3.5 times lower than for papain. Again, as stated above for RSA activity, the peptides released by trypsin have higher RP activities than those released by papain. In general, there is an increase in RP activity with an increase in time until 60 min for OPIH and 120 min for OPCH which is expected since at times after these, there were negligible increases in DH in the hydrolysates. As stated above for RSA, the RP activity of hydrolysates increases with DH. The RP values of the OPIH and OPCH are similar at the higher DH values. The only differences in RP values are at times less than 120 min, where the RP values for the concentrates are lower, presumably due to the lower DH values of these samples compared to the isolates.

The metal chelating activity (MCA) of the OPCH and OPIH samples is shown in **Figure 4**. In **Figure 4(b)**, for OPCH, the MCA values for trypsin are consistently lower than those for papain and pepsin. This may be due to the lower DH in the trypsin hydrolysates. Additionally, the MCA values for trypsin are similar over the time period while the MCA values for papain and pepsin show a slight increase up to 30 min, which is related to the DH. The MCA values in **Figure 4(a)** for OPIH show a different trend, the pepsin samples have higher MCA values compared to the papain and trypsin values, which are similar to each other. This is not expected considering the results for OPCH and we do not have an explanation. In general the MCA values for pepsin increase up to 120 min, then remain steady which is related to the DH. There are slight increases in the MCA



(a)

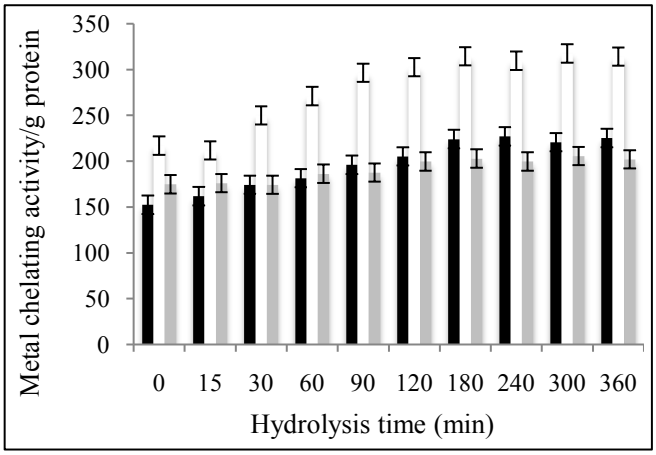


(b)

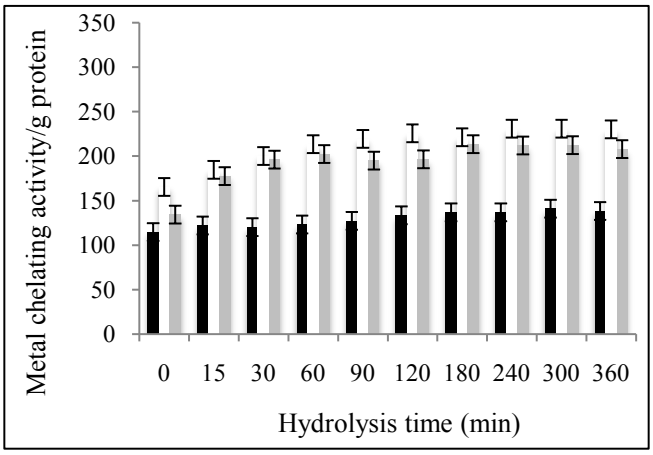
Figure 3. Reducing power activity of hydrolysates over time (as absorbance at 700 nm). (a) Hydrolyzed okra protein concentrate. (b) Hydrolyzed okra protein concentrate. Black bars are papain hydrolysates, white bars are pepsin hydrolysates and grey bars are trypsin hydrolysates. Error bars show the standard deviation.

values for papain and trypsin, but not as dramatic as seen for pepsin. In general the MCA values for OPIH are much higher than those for OPCH. This may be due to the difference in the ash content between the two. The ash in the OPC is 9.4% and 2.6% in the OPI. With a higher amount of minerals in the OPC sample, the lower MCA values for the OPCH samples are due to the excess minerals. In general, the carboxyl and amino groups in proteins and the acidic and basic amino acids groups in amino acids play important roles in metal chelation and an increase in DH generally results in an increase in MCA activity due to the increase number of carboxyl and amino groups [7].

Jamdar *et al.* [6] reported that peanut protein hydrolysate (using alcalase) showed that with an increase in DH, there was an increase in RSC, MCA, and ACE inhibitory activity while RP decreased. Ajibola *et al.* [7], showed the effect of peptide size on the antioxidant properties of yam bean seed treated with alcalase. There was an increase in RSA and RP with samples containing peptides less



(a)



(b)

Figure 4. Metal chelating activity of protein hydrolysates over time. (a) Hydrolyzed okra protein concentrate. (b) Hydrolyzed okra protein concentrate. Black bars are papain hydrolysates, white bars are pepsin hydrolysates and grey bars are trypsin hydrolysates. Error bars show the standard deviation.

than 3 kDa which is in agreement with results reported by Girgih *et al.* [8]. This might explain the trypsin samples that result in more active antioxidant activity than expected based on the low DH. Trypsin hydrolysis may have resulted in more peptides less than 3 kDa and free amino acids which have more RSA activity. But, they [7] did find that MCA was not influenced by the size of the hydrolysates in this yam protein system. They showed that small peptide size and high level of hydrophobicity are important for RP and DPPH activity.

In general, the DH is related to antioxidant properties with higher DH values resulting in higher antioxidant values. Fan *et al.* [3] hydrolyzed tilapia protein with pepsin, trypsin and papain and the trypsin hydrolysate showed the highest DH (15.1%) and antioxidant activity as measured with RSA, superoxide anion scavenging, hydrogen peroxide scavenging, and the hydroxyl radical scavenging assays. Hydrolysates obtained with papain (DH of 9.5%) showed higher antioxidant activity than those obtained from pepsin hydrolysis (DH of 5.3%) but both

were lower than the trypsin hydrolysates. While the study by Fan *et al.* [3] showed that a high degree of hydrolysis is related to a high DH, this is not always true. Ktari *et al.* [4] hydrolyzed cuttlefish protein with a variety of enzymes. Chymosin hydrolysates had higher DH values than those produced by trypsin (about 2 times higher DH with chymosin) yet the RSA values of trypsin hydrolysates were significantly higher than chymosin produced hydrolysates, while the RP activity of both were similar. Ktari *et al.* [4] stated that peptides generated during hydrolysis by different proteases have different antioxidant capacities.

Therefore, the antioxidant activity of protein hydrolysates is related to the protease used which results in a variety of peptides of different molecular weights and hydrophobicities. Low molecular weight fractions and free amino acids in protein hydrolysates generally contain more antioxidant activities depending on the assay used to test the the activity. Generally low molecular weight peptides result in high RCA activity, but not always metal chelating activity [3] [7]. In this study, pepsin hydrolysates with a final DH of 35.2% showed higher RP and MCA than trypsin and papain hydrolysates.

Acknowledgements

This project was partially supported by the Utah State University Utah Agricultural Experiment Station and approved as journal paper number 9124.

Conflicts of Interest

The authors declare no conflicts of interest.

References

[1] Pisoschi, A.M. and Negulescu, G. (2011) Methods for Total Antioxidant Activity Determination: A Review. *Biochemistry & Analytical Biochemistry*, **1**, 106.

[2] Shahidi, F. and Zhong, Y. (2015) Measurement of Antioxidant Activity. *Journal of Functional Foods*, **18**, 757-781. https://doi.org/10.1016/j.jff.2015.01.047

[3] Fan, J., He, J., Zhuang, Y. and Sun, L. (2012) Purification and Identification of Antioxidant Peptides from Enzymatic Hydrolysates of Tilapia (*Oreochromis niloticus*) Frame Protein. *Molecules*, **17**, 12836-12850. https://doi.org/10.3390/molecules171112836

[4] Ktari, N., Fakhfakh, N., Balti, R. and Khaled H.B. (2013) Effect of Degree of Hydrolysis and Protease Type on the Antioxidant Activity of Protein Hydrolysates from Cuttlefish (*Sepia officinalis*) By-Products. *Journal of Aquatic Food Product Technology*, **22**, 436-448. https://doi.org/10.1080/10498850.2012.658961

[5] Pena-Ramos, E.A. and Xiong, Y.L. (2002) Antioxidant Activity of Soy Protein Hydrolysates in a Liposomal System. *Journal of Food Science*, **67**, 2952-2956. https://doi.org/10.1111/j.1365-2621.2002.tb08844.x

[6] Jamdar, S.N., Rajalakshmi, V., Pednekar, M.D., Juan, F., Yardi, V. and Sharma, A. (2010) Influence of Degree of Hydrolysis on Functional Properties, Antioxidant Activity and ACE Inhibitory Activity of Peanut Protein Hydrolysates. *Food Chemistry*, **121**, 178-184. https://doi.org/10.1016/j.foodchem.2009.12.027

[7] Ajibola, C.F., Fashakin, J.B., Fagbemi, T.N. and Aluko, R.E. (2011) Effect of Peptide

Size on Antioxidant Properties of African Yam Bean Seed (*Sphenostylis stenocarpa*) Protein. *International Journal of Molecular Sciences*, **12**, 6685-6702. https://doi.org/10.3390/ijms12106685

[8] Girgih, A.T., He, R., Hasan, F.M., Udenigwe, C.C., Gill, T.A. and Aluko, R.E. (2015) Evaluation of the *in Vitro* Antioxidant Properties of a Cod (*Gadus morhua*) Protein Hydrolysate and Peptide Fractions. *Food Chemistry*, **173**, 652-659. https://doi.org/10.1016/j.foodchem.2014.10.079

[9] Ngbede, S.O., Ibekwe, H.N., Okpara, S.C., Onyegbule, U.N. and Adejumo, L. (2014) An Overview of Okra Production, Processing Marketing Utilization and Constraints in Ayaragu in Ivo Local Government Area of Ebonyi State, Nigeria. *Greener Journal of Agricultural Sciences*, **4**, 136-143.

[10] Adelakun, O.E. and Oyelade, O.J. (2011) Potential Use of Okra Seed (Abelmoschusesculentus Moench) Flour for Food Fortification and Effects of Processing. In: Preedy, V.R., Watson, R.R. and Patel, V.B., Eds., *Flour and Breads and Their Fortification in Health and Disease Prevention*, Elserier Inc., New York, 205-212. https://doi.org/10.1016/B978-0-12-380886-8.10019-4

[11] Kareem, A.A. and Shakir, K.A. (2016) Studying the Factors Effecting the Production of Okra Protein Concentrate and Isolate and Their Thermal Properties. *The Iraqi Journal of Agricultural Sciences*, **47**, 1505-1513.

[12] Kareem, A.A. and Shakir, K.A. (2016) Study of the Functional and Nutritional Properties of Defatted Okra Powder and Okra Protein Isolate and Concentrate. *The Iraqi Journal of Agricultural Sciences*, **47**, 865-875.

[13] Benjakul, S. and Morrissey, M.T. (1997) Protein Hydrolysate from Pacific Whiteing Solid Wastes. *Journal of Agricultural Food Chemistry*, **45**, 3423-3430. https://doi.org/10.1021/jf970294g

[14] Thiansilakul, R., Benjakul, S. and Shahidi, F. (2007) Antioxidant Activity of Protein Hydrolysate from Round Scad Muscle Using Alcalase and Flavourzyme. *Journal of Food Boichemistry*, **31**, 266-287.

[15] Wu, H., Chen, H. and Shiau, C. (2003) Free Amino Acids and Peptides as Related to Antioxidant Properties in Protein Hydrolysates of Mackerel (*Scomber austriasicus*). *Food Research International*, **36**, 949-957. https://doi.org/10.1016/S0963-9969(03)00104-2

[16] Boyer, R. and McCleary, C.J. (1987) Superoxide Ion as a primary Reductant in Ascorbate-Mediated Ferritin Iron Release. *Free Radical Biology & Medicine*, **3**, 389-395. https://doi.org/10.1016/0891-5849(87)90017-7