Enzymatic Protein Hydrolysates of Common Carp Fish: II. Antioxidant Activity

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ABSTRACT

Common carp fish were hydrolysed using papain, bacterial protease, and bovine protease. The antioxidant activity of the three hydrolysates were determined. Partial hydrolysates with different degrees of hydrolysis and peptide fractions were also examined for their antioxidant activity. Then, the hydrolysate prepared using papain enzyme was chosen for further investigation. It was added to a meat model system capable to inhibit lipid oxidation, and its Hunter Lab. colour was tested. Moreover, the effects of three drying methods on the antioxidant activity and some functional properties of the papain hydrolysate were examined. Data revealed that the three fish hydrolysates possessed antioxidant activities which were boosted to about 98% by addition of 0.5% BHT. Partial hydrolysates exhibited antioxidant activities which increased with the increase in degree of hydrolysis. The highest antioxidant activity (73.2 %) was achieved with 35.2 % degree of hydrolysis for papain hydrolysate. Results also showed that the smaller the molecular weight of the peptide fraction, the higher its antioxidant activity. Addition of papain hydrolysate to meat model system delayed oxidation. Freeze drying of papain hydrolystate maintained their antioxidant activity and functional properties.

Key words: common carp, enzymatic hydrolysis, proteases, functional properties, molecular weight, antioxidant activity, freeze drying.

INTRODUCTION

Functional food is any food or food ingredient which imparts health benefits to humans other than their nutritional ones. Antioxidants that combat free radicals (FR), reactive oxygen species (ROS) and reactive nitrogen species (RNS) are among the important functional food ingredients. Those harmful species FR, ROS and RNS are being constantly formed in the human body and have been implicated in the pathology of human disease (Auroma & Cuppett, 1997). In the normal healthy state of the humans, the endogenous antioxidants are enough to combat those harmful species. But in cases of illness, aging, depression and others, the endogenous antioxidants are not enough, hence began the thought of the intake of antioxidants as prophylactic agents (Halliwell & Auroma, 1997). Antioxidants are also very important to the food industry because they are increasingly used to improve stability of food. Lipid peroxidation contributes to the development of off flavour, off colour and poor texture, and may also reduce nutritive value and generate potentially toxic products (Thiansilikul *et al.*, 2007).

Protein hydrolysates from many animal and plant sources, individual peptides and amino acids have been found to possess antioxidative properties (Marcuse, 1962, Decker & Crum. 1993). The antioxidant activity of protein hydrolysates from Capelin and Harp seal have been examined and found to possess antioxidant activity (Shahidi & Amarowicz, 1996, Amarowicz & Shahidi, 1997). This was followed by studying four peptide fractions separated by gel filtration of Capelin protein hydrolysates. One of the peptides exhibited a strong antioxidant activity; two peptides were weak antioxidants and the fourth exhibited pro-oxidant activity. Other authors reported that the antioxidative activity of hydrolysates and peptides isolated from whole fish and some by- products of the fish industry are usually discarded as waste (Sathivel *et al.*, 2003, Rajapakse *et al.*, 2005, Thiansilikul *et al.*, 2007).

Amino acids and peptides are typical chelating agents frequently present in foods and found in abundance in protein hydrolysates. However, amino acids may exert a pro-oxidant activity in the absence of metals or under certain other conditions such as when present in aqueous media (Krishma

& Prabhakav, 1994). The activity of specific amino acids as an antioxidant or a pro-oxidant is dependant on certain conditions such as pH, concentration, and relative humidity (Marcuse, 1962, Chen *et al.*, 1995). Antioxidant activity of amino acids or short chain peptides is caused by the reaction of amino or sulphur groups with lipid hydroperoxides resulting in the formation of imines, sulphides, thiosulphinates and sulphoxides (Pokorny & Korczak, 2001, Flaczyk, *et al.*, 2003). Antioxidative peptides are involved not only in singlet, oxygen and free radical scavenging, but also in metal chelation (Egorov *et al.*, 1992). Histidine containing peptides exhibit metal chelating ability as well as lipid trapping potential through their imidazole ring (Uchida & Kawakishi, 1992, Murase *et al.*, 1993, Wu *et al.*, 2003). Generally, higher levels of free amino acids, anserine, carnosine and other peptides are generated in the hydrolysates using proteases, compared with those using autolysis (Wu *et al.*, 2003).

Bioactive peptides derived from various fish protein hydrolysates have shown numerous bioactivities besides their antioxidant properties such as antihypertensive, antithrombotic, immunomodulatory (Kim & Mendis, 2006), hypocholestrolemic effects (Wergedahl *et al.*, 2004), and antiproliferative activity (Picot *et al.*, 2006).

The present work aimed to invest a value adding of Common Carp fish found in abundance in Egypt and not desirably palatable because of its high bone content. This goal was achieved through the use of several proteases to prepare protein hydrolysates from this fish. Partial hydrolysis was carried out to obtain hydrolysate fractions with different degrees of hydrolysis and the hydrolysates were further fractionated by gel filtration and peptides were tested for their antioxidant activities. Different drying methods for the papain hydrolysate were investigated to reveal their effects on the antioxidant activity.

MATERIALS AND METHODS

Materials

Fish: Fresh Common Carp fish (*Cyprinus carpo* L.) of average weight 1.43 kg and length 50 cm were obtained from aquaculture Abbassa-Abou Hammad, Sharkeiah, Egypt. The fish was headed gutted, skinned, and then minced. The minced fish was soaked in 2% sodium bicarbonate for 1 hr to ease the defatting process then the minced fish was defatted using n-hexane until the oil did not exceed 1%.

Proteolytic enzymes: Papain, Bovine and Bacterial Protease were obtained from Sigma Chemicals Co. (St. Louis. Missouri, USA). Papain, (Papaya Latex, MW 21 kDa) product of Srilanka, bovine protease of pancreas, (MW 27 kDa) product of Germany and bacterial Protease [(*Bacillus Licheniformis*), MW 27 kDa)], product of Denmark.

Methods:

Preparation of protein hydrolysates

The basic hydrolysis experiment was carried out using the minced fish with the three enzymes under the optimum conditions for 3 hr as mentioned by Mohamed *et al.* (2009). After decolourization and filtration, aliquots were taken for antioxidant activity determination.

The partial hydrolysis of fish protein was carried out as in the basic hydrolysis experiment, using the three enzymes under the optimum conditions previously determined (Mohamed *et al.*, 2009). Fourty ml aliquots were withdrawn from the bulk hydrolysate at 30 min intervals. The enzymes were immediately inactivated and partial hydrolysates were charcoal treated then filtered. Formol titration was carried out on the filtrate to determine the degree of hydrolysis (DH). The antioxidant activity of the filtrate was also determined.

$$\text{Degree of Hydrolysis (\%)} = \frac{(B_1 - B_2) \times 14 \times 100}{SW \times TN}$$

Where:

B_1= ml alkali consumed by control at zero time

B_2= ml alkali consumed by sample at certain time

SW= weight of sample in g.

TN= % total nitrogen in sample.

Fractionation of protein hydrolysates to peptides

The molecular weight distribution of the three fish hydrolysates was carried out by gel chromatography on Sephadex G-100 (this size was used to avoid the bitter taste of the low molecular weight peptides) in a glass column, diameter to height ratio of 1:18 that would give a bed volume of 210-260 ml according to Fox & Tarassuk (1968). The first 10 ml eluted from each sample were collected from the column. This volume was considered as the void volume (Vo). The rate of elution was maintained at 5 ml / 7 sec. The effluent was monitored at 280 nm by a UV detector connected to a recorder.

Egg albumin of 4200 MW, hemoglobin of 17,000 MW and insulin of 6,500 MW were used as standard proteins. Fractions of 10 ml each were collected and analyzed for the effect of the enzymatic hydrolysis on the molecular weight, according to the method of Colowick & Kaplan (1995). A control sample of non hydrolysed fish was run on the Sephadex column in the same manner described before for the enzymatically hydrolyzed samples. Peptide fractions with different molecular weights were tested for their antioxidant activities.

Determination of antioxidant activity:

In vitro determination

Antioxidant activities of protein hydrolysates and standard BHT (Sigma Chemical Co.) were determined according to the β-carotene bleaching method following a modification of the procedure described by Velioglu *et al.* (1998). For a typical assay, 1 ml of β-carotene (Sigma) solution, 0.2 mg /ml in chloroform was added to round bottom flask (50 ml) containing 0.02 ml linoleic acid (Sigma) and 0.2 ml of Tween 20 (Sigma). Each mixture was then dosed with 0.2 ml of 80% MeOH (as control) or BHT in 80% Me OH (as standard) or corresponding hydrolysate. After evaporating to dryness under vacuum at room temperature, oxygenated distilled water (50 ml) was added and the mixture was shaken to form a liposome solution. The samples were then subjected to thermal autoxidation at 50°C for 2 hr. The absorbance of the solution at 470 nm was monitored on a spectro UV-vis rs spectrophotometer (Labomed inc.) at 10 min intervals. Antioxidant activity was interpreted by plotting absorbance versus time. Antioxidant activity was calculated according to Al-Shaikhan *et al.* (1995).

Degradation rate (sample) = ln (a/b) x 1/t

Where ln = natural log; a = initial absorbance at time 0; b = absorbance at 10, 20, 30 min; t = time (min).

Antioxidant activity (AOA) was expressed as % inhibition relative to the control using:

$$AOA\ \% = \frac{\text{Degradation rate (control)} - \text{Degradation of sample} \times 100}{\text{Degradation rate (control)}}$$

In meat model system:

The effect of papain hydrolysate on the oxidative stability of lipids in meat model system was carried out according to Lee *et al.* (1998). Fourty ml water were added to 160 g minced meat (fresh beef) and mixed well with the hydrolysates (concentrations 200, 500, and 1000 ppm) or Butylated Hydroxy Toluene (BHT) (concentrations 50 and 100 ppm) and cooked well, in a water bath at 75°C for 40 min, cooled, divided into 4 lots and stored for 9 days at 4°C. The surface colour changes of the meat were estimated with a Hunter Lab. Labscan Spectrophotomrter (Hunter Associates Labs. Reston Va., USA). Redness (a*), yellowness (b*) and luminescence (L*) of meat were obtained using a setting of D65 (daylight 65 light angle), as described by Smith & Avarez (1988). The samples were analyzed for thiobarbituric acid reactive substances (TBARS) at days 0, 3, 6, and 9 according to Vyncke (1970).

Drying and functional evaluation of papain hydrolysate:

The Papain hydrolysates were dried by three methods, namely oven in a draft air at 60°C, freeze dryer (Edwards Modulo), and mini spray dryer Büchi (Egorov *et al.*, 1992). Nitrogen solubility (NS %) was determined according to Lyman, *et al.* (1953), Emulsifying Capacity (EC) was determined as reported by Shahidi *et al.* (1995). Wettability (WA), flowability (FA), and thermostability (TS) were determined as described by Taha & Ibrahim (2002).

RESULTS AND DISCUSSION

Antioxidant activity (AOA):

Protein hydrolysates

The antioxidant activities of the three protein hydrolysates prepared from filleted minced Common Carp fish using the three enzymes Pa., Bac. P. and Bov. P. are presented in Table (1). The AOA were measured using the β-carotene/ linoleate model system after 120 min. It can be clearly seen that the three prepared fish hydrolysates possess moderate / good antioxidative properties with more or less the same values. Perhaps the closeness in AOA values is attributed to the fact that the three enzymes have broad specificity. The results of the effect of adding BHT (at levels of 0.2 and 0.5 %) to the hydrolysates indicate that the hydrolysates work in synergy with BHT to boost the AOA of the hydrolysates. The increase in BHT concentration increases the AOA. The addition of BHT to the protein hydrolysates made their AOA approach that of BHT at both levels (Table 1).

Table 1: Antioxidant activity of Pa, Bac.P and Bov.P fish protein hydrolysates with/ or without the addition of BHT

Hydrolysate	Antioxidant activity
Pa. hydrolysate	62.02 ± 0.09
Bac.P. hydrolysate	59.69 ± 0.13
Bov.P. hydrlysate	60.83 ±0.14
Pa. hydrolysate + 0.2% BHT	88.93 ± 0.21
Bac.P. hydrolysate + 0.2% BHT	85.81 ± 0.16
Bov.P. hydrolysate + 0.2% BHT	89.23 ± 0.18
Pa. hydrolysate +0.5% BHT	96.63 ± 0.09
Bac.P. hydrolysate + 0.5% BHT	94.28 ± 0.18
Bov.P. hydrlysate + 0.5% BHT	98.56 ± 0.22
0.2% BHT	89.96 ± 0.16
0.5%BHT	98.23 ± 0.25

Pa. = Papain Bac.P. = Bacterial protease
Bov.P. = Bovine protease
Results are mean values of three replicates ± standard deviation

The AOA of fish protein hydrolysates have been reported by several authors (Shahidi & Amarowicz, 1996, Sathivel *et al.*, 2003, Jun *et al.*, 2004, Je *et al.*, 2005a, Thiansilikul, *et al.*, 2007). The synergistic effect of Capelin protein hydrolysate (CPH) with synthetic antioxidants (BHT, BHA, TBHQ) in a β- carotene-linoleate model system was studied (Amarowicz *et al.*, 1999). The results revealed that CPH and synthetic antioxidants inhibited oxidation of linoleic acid effectively. A synergistic effect was observed only for CPH and TBHQ when incubation time was 60, 90, and 120 min. Alaska Pollack frame protein hydrolysate was reported to have both antioxidants activity and a synergistic effect with α-tocopherol using linoleic acid in water /alcohol system (Je *et al.*, 2005a). It is worth mentioning that while hydrolysates may serve as antioxidant in emulsion systems, their synergism with synthetic antioxidants depends on the chemical nature of the compound involved. Furthermore, it should be noted that results from one system cannot be extrapolated to other systems, especially when dealing with bulk oils (Amarowicz *et al.*, 1999). Since the three fish protein hydrolysates under study exhibited AOA, so, further study is needed for the AOA of partially hydrolysed fish, as well as the AOA of peptide fractions from the hydrolysates fractionated by gel filtration.

Partial hydrolysate fractions

Table (2) shows the degree of hydrolysis (DH %) at different time intervals during hydrolysis. The data revealed that DH steadily increases with the time of hydrolysis, Pa. hydrolysate reached the highest DH (35.2 %) after 3 hr, whereas the DH of the Bac. P. and Bov. P. were 17.4 and 19.5 % DH, respectively, after 3hr. Figures 1, 2, and 3 illustrate the AOA of hydrolysate fractions (with different DH %) which were separated from Pa, Bac. P., and Bov. P. hydrolysates, respectively. It was obvious from the aforementioned figures that the AOA of the fractions increases with increasing DH. This is probably attributed to the formation of more amino acids and smaller peptides possess high AOA (Marcuse 1962, Decker & Crum 1993). The difference in the DH % and AOA in the three hydrolysates are probably due to differences in enzyme specificities toward the protein substrate. The results are in agreement with the results of Kawashima *et al.* (1979), Chen, *et al.* (1995) and Thiansilikul *et al.*, (2007). Figures 1-3, also indicate that the elongation in time of hydrolysis was not directly proportional to the increase in AOA. This result is in agreement

Table 2 : Degree of hydrolysis (DH%) of partially hydrolysed fish protein fractions for 3 hr, using 3 different enzymes

Hydrolysate	Time of hydrolysis (min)	DH%
Pa. hydrolysate	30	24.8 ± 0.31
	60	27.4 ± 0.28
	90	29.5 ± 0.18
	120	31.7 ± 0.19
	150	33.5 ± 0.20
	180	35.2 ±0.22
Bac.P. hydrolysate	30	10.9 ± 0.15
	60	11.7 ± 0.22
	90	12.7 ± 0.23
	120	14.2 ± 0.19
	150	15.1 ± 0.17
	180	17.4 ± 0.30
Bov.P. hydrolysate	30	12.3 ± 0.31
	60	14.6 ± 0.29
	90	15.2 ± 0.33
	120	16.9 ± 0.31
	150	17.4 ± 0.26
	180	19.5 ± 0.37

Pa = Papain
Bac.P =Bacterial protease
Bov.P= Bovine protease
Results are mean values of three replicates ± standard deviation

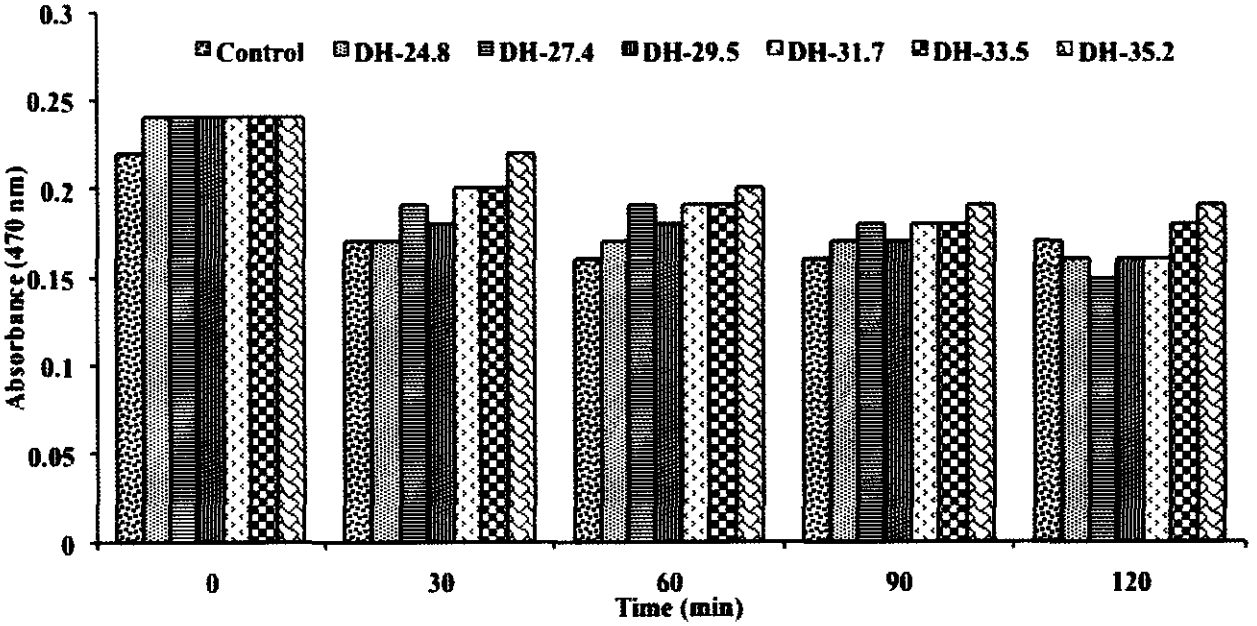

Fig. 1: Antioxidant activity of partially hydrolysed fish protein at different degree of hydrolysis using papain

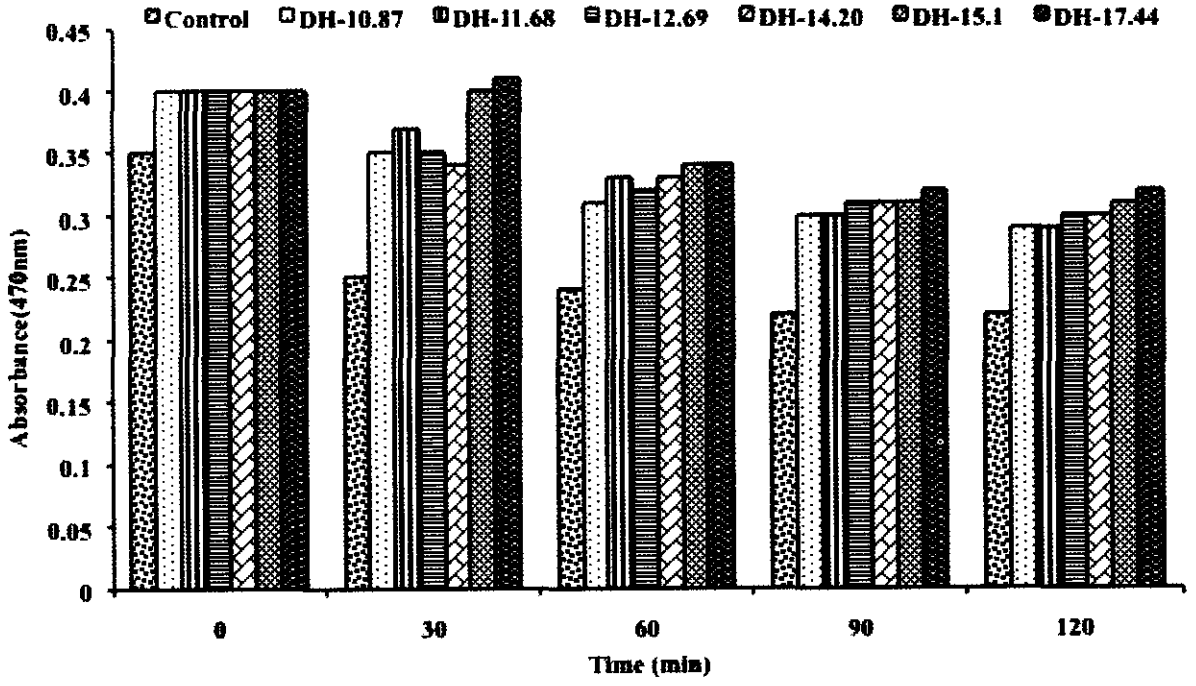

Fig. 2: Antioxidant activity of partially hydrolysed fish protein at different degree of hydrolysis using bacterial protease

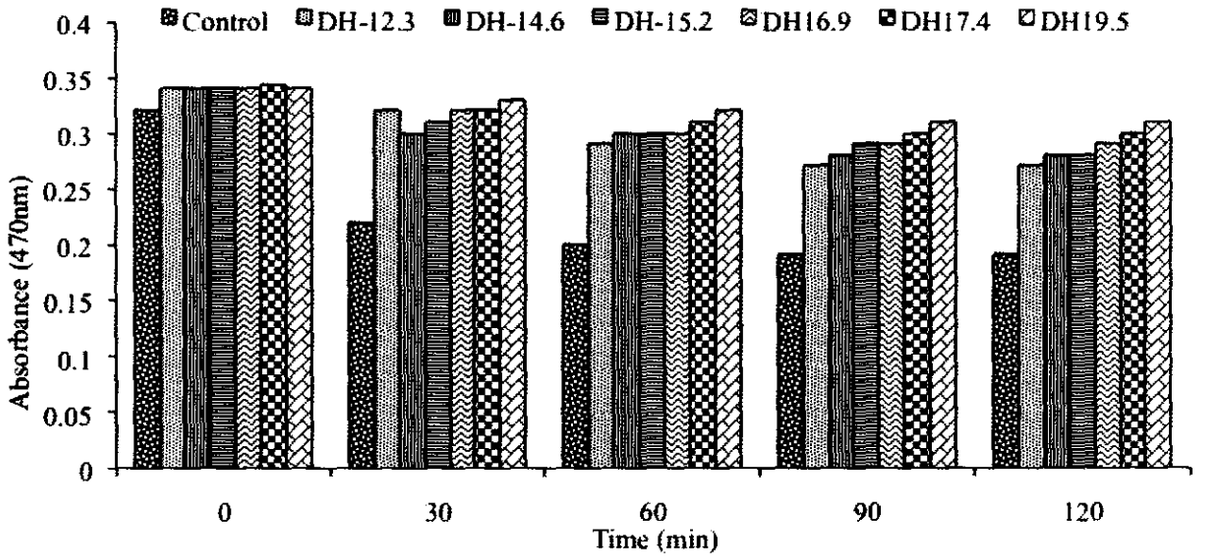

Fig. 3: Antioxidant activity of partially hydrolysed fish protein at different degree of hydrolysis using bovine protease

with the work of Jao & Ko, (2002), who found that when tuna cooking juice was hydrolysed at 37°C for 6 hr, the DH kept increasing during the 6 hr, yet the DPPH (2,2-diphenyl-1-picryhydrazyl) radical scavenging activity, which indicates AOA, showed maximum activity after 2.5 hr, then it decreased.

Peptide fractions

Peptide fractions having molecular weight (MW) from 3000 to 72000 were chosen from MW distribution curves (Mohamed *et al.*, 2009) and their AOA were determined. Figure (4) shows that the AOA of chosen peptide fractions from the three hydrolysates having MW of about 72000 had AOA of 10.5, 12.32, and 19.11 % for Pa, Bac.P., and Bov.P., respectively. The highest AOA % was attained with the smallest MW peptide fractions. It reached 70.98 % for Pa.hydrolysate, whereas 68.22 % and 69.81 % were obtained from peptide fractions of Bac.P. hydrolysates (MW 3000) and Bov.P hydrolysates (MW 2500), respectively. Perhaps the higher AOA of the smaller peptide fractions is attributed to the presence of higher concentration of amino acids having high AOA. This result is confirmed by several authors (Kawashima *et al.*, 1979, Chen *et al.*, 1995, Je *et al.*, 2005 a, b, Jung *et al.*, 2005).

In meat systems

This experiment was carried out to confirm the AOA of the fish hydrolysates. Papain hydrolysate was only used in this experiment due to the effectiveness, availability and cheapness of Pa. enzyme. Table (3) demonstrates the changes in thiobarbituric acid reactive substances (TBARS) values during storage of cooked meat with Pa. hydrolysate added at levels of 200, 500, 1000 ppm and cooked meat containing 50 and 100 ppm BHT, for comparison. The results revealed that the addition of the Pa. hydrolysate at the different levels delayed the oxidation process of fat during storage. The power of delaying the fat oxidation in the meat was found to be proportional to the concentration of the Pa. hydrolysate in the cooked meat samples. After 9 days of storage, the TBARS values was 22.41 mg malonaldehyde / Kg sample in control sample, while values for meat with added Pa. hydrolysate at 200, 500, 1000 ppm levels, reached 14.45, 10.65, 6.21 mg malonaldehyde / Kg sample, respectively. Addition of BHT to the meat at 50 and 100 ppm levels indicated 13.63 and 4.57 mg malonaldehyde/ Kg sample, respectively at day 9. These results show the efficacy of the antioxidative power of the Pa. hydrolysate. Addition of Pa. hydrolysate at 1000 ppm to the meat competed well with the addition of 100 ppm BHT. The inhibition percentage of TBARS formation for Pa. hydrolysate (1000 ppm) and BHT (100 ppm) were 72.5 and 79.6 % inhibition, respectevily. Shahidi *et al.*, (1995) reported that incorporated Capelin protein hydrolysate up to 3 % in meat model system, showed an increase of 4% in cooking yield and inhibition of oxidation (determined by TBARS test) by 17.7-60.4 %.

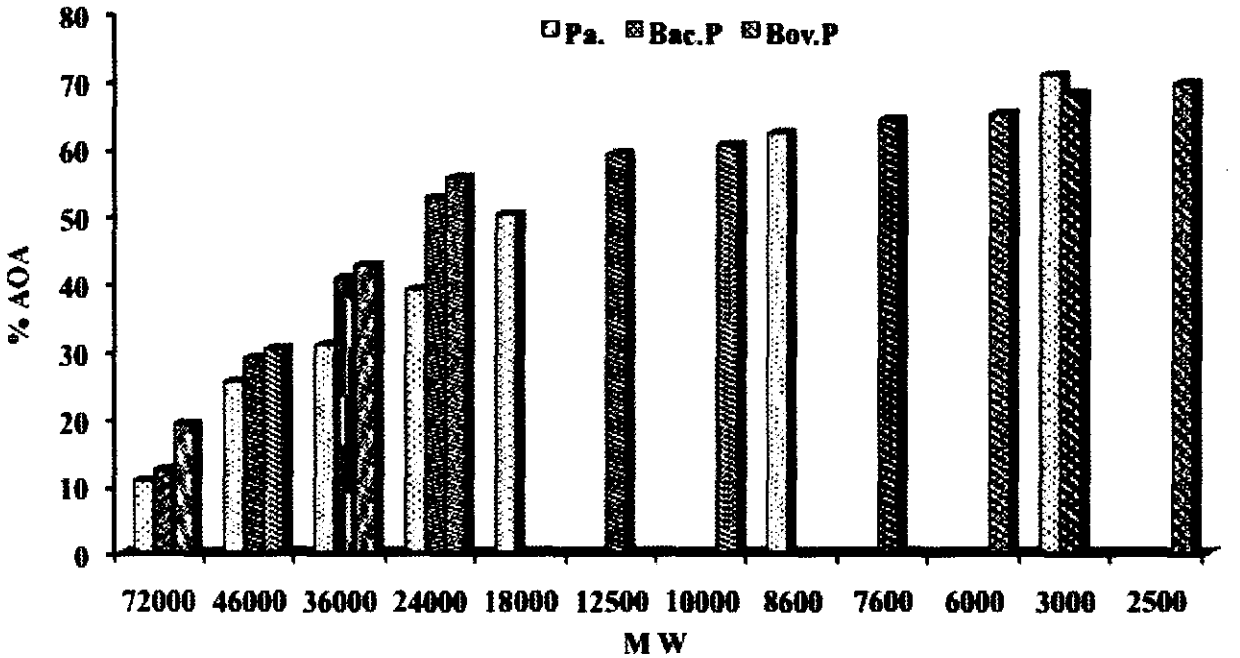

Fig. 4 : Antioxidant activity % (AOA) of peptide fractions from Pa, Bac. P and Bov. P hydrolysates

Table 3 : Effect of addition Pa. hydrolysate on the formation of TBARS*of lipids in cooked meat stored at 4°C

Treatment	Storage period (days)			
	0	3	6	9
Control	1.55 ± 0.19	3.61 ± 0.26	11.39 ± 0.33	22.41 ± 0.20
Pa.hydrol.(200 ppm)	1.62 ± 0.31	2.78 ± 0.18	5.86 ± 0.29	14.45 ± 0.42
Pa. hydrol.(500ppm)	1.62 ± 0.38	2.65 ± 0.19	4.93 ± 0.23	10.65 ± 0.22
Pa.hydrol.(1000ppm)	1.65 ± 0.17	2.55 ± 0.21	4.01 ± 0.37	6.21 ± 0.37
BHT (50 ppm)	1.46 ± 0.28	3.52 ± 0.09	7.36 ±0.41	13.63 ± 0.39
BHT (100ppm)	1.46 ± 0.36	2.54 ± 0.26	3.33 ± 0.13	4.57 ± 0.21

Pa. hydrol.= Papain hydrolysate BHT= Butlated Hydroxy Toluene
TBARS = Thiobarbituric acid reactive substances in mg malonaldehyde / Kg sample
Results are mean values of three replicates ± standard deviation

Colour changes in model meat system:

Table (4) shows the colour changes that took place in cooked meat stored at 4°C for 9 days when Pa hydrolysate and BHT were added at different concentration levels. Hunter a* colour indicates the redness of meat. The results demonstrated that the addition of antioxidants (Pa. hydrolysate and BHT) delayed the fading of red colour a* of meat. Colour of the control sample declined from 5.6 at zero time to 2.4 after storage for 9 days, whereas addition of Pa. hydrolysate at levels of 200 ppm, 500 ppm and 1000 ppm, exhibited a red colour of 3.4, 3.6, and 4.0, respectively. The BHT (50 ppm and 100 ppm) demonstrated red colour at day 9 to be 4.1 and 4.4, respectively. The increase in the concentration of the antioxidants was accompanied by the delay in fading the red colour of meat. Hunter L* indicates the luminescence or lightness of the meat. When antioxidants are added with different concentrations, the data in Table (4) show relative increment, but the L* values of the samples were less than the control at 6 and 9 days of storage. This means that meat samples were protected from becoming opaque by the addition of tested antioxants. Hunter colour b* indicates the yellowness of meat. Also, the results in Table (4) show that the control and the samples containing different concentrations of Pa. hydrolysate followed the same pattern where

Table 4: Changes in Hunter colour (a*, L* and b*) during storage of cooked meat containing different concentrations of Pa. hydrolysate and BHT

Treatment	Storage (days)											
	0			3			6			9		
	a*	L*	b*	a*	L*	b*	a*	L*	b*	a*	L*	b*
Control	5.6±0.2	58.5±0.1	18.6±0.1	4.3±0.1	58.2±0.1	19.1±0.1	3.7±0.1	63.2±0.2	19.2±0.2	2.4±0.1	66.4±0.4	17.6±0.1
Pa. hydrol. (200ppm)	5.6±0.3	59.7±0.3	18.1±0.2	4.1±0.2	59.6±1.2	17.8±0.1	3.4±0.1	61.6±0.8	18.3±0.3	3.4±0.1	66.9±0.3	16.8±0.3
Pa. hydrol. (500ppm)	5.7±0.1	60.6±0.1	17.1±0.1	4.5±0.1	59.1±0.3	17.3±0.2	3.7±0.3	59.9±0.3	17.3±0.4	3.6±0.2	62.1±0.6	16.3±0.2
Pa.Hydrol. (1000ppm)	5.8±0.3	59.0±0.3	17.1±0.1	4.8±0.1	55.9±0.1	17.5±0.1	4.6±0.2	56.5±0.4	17.1±0.2	4.±0.1	58.8±0.4	16.1±0.1
BHT (50ppm)	5.7±0.01	59.6±0.2	17.8±0.2	4.7±0.1	59.8±0.2	18.4±0.1	4.3±0.1	61.5±0.6	19.1±0.3	4.1±0.1	62.8±0.5	19.1±0.2
BHT (100ppm)	5.8±0.1	60.3±0.1	18.1±0.3	4.8±0.2	60.7±0.4	18.4±0.2	4.7±0.2	61.9±0.7	18.9±0.3	4.4±0.3	60.8±0.7	19.01±0.1

a*=redness of meat L*= lightness of meat b*=yellowness of meat
Pa.hydrol. = Papain hydrolysate BHT = Butylated Hydroxy Toluene
Results are mean values of three replicates ± standard deviation

b* values either increased or remained the same and then at day 9, they decreased. On the other hand, meat samples containing BHT showed an increase in b* colour at day 9. Several authors reported that the addition of antioxidants from herbs, phytic acid, and sunflower hull ethanolic extract delayed lipid peroxidation and maintained colour in beef and fish meat systems (Sanchez-Escalante *et al.*, 2003, Park *et al.*, 2004, Mohamed & Taha, 2005).

Effect of drying methods on the antioxidant activity and functional properties of papain hydrolysate

Table (5) illustrates the effect of air oven drying, freeze drying and spray drying on the AOA and some functional properties of Pa. hydrolysate. The results clearly showed that freeze drying was preferable for drying because it maintained the antioxidants in the hydrolysate as well as the nitrogen solubility of the protein. The TS and EC values were very close for both freeze dried and spray dried hydrolysates. Values for wetability (WA) and flowability (FA) were the same. Although spray drying was the lowest cost option for food dehydration, yet the high temperature used during spray drying (130-200°C) probably causes damage to vitamins, antioxidants, colour and volatilization of flavour compounds (Desorby *et al.*, 1997). Several studies illustrate nutrient retention in freeze dried powders and whole fruits and vegetables (Ratti, 2001, Pilosof & Terebiznik, 2002).

CONCLUSION

Underutilized Common Carp fish can be value added by preparing enzymatic protein hydrolysates that can be used as nutritional ingredient for the elderly, in sport foods, instant foods due to their nutritional and functional properties, and as a functional food ingredient due to its antioxidative properties.

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Table 5: Effect of drying methods on the antioxidant activity and functional properties of papain protein hydrolysate

Properties \ Drying method	Air oven dryer	Freeze dryer	Spray dryer
Antioxidant activity% (AOA%)	54.32 ± 0.46	63.1± 0.36	52.8 ± 0.32
Nitrogen solubility % (NS%)	72.1 ± 0.24	92.64 ± 0.21	81.2 ± 0.19
Thermostability % (TS%)	31.56 ± 0.36	42.36 ± 0.35	44.84 ± 0.28
Emulsifying capacity % (EC%)	48.23 ± 0.13	50.25 ± 0.25	51.14 ± 0.16
Wettability (sec) (WA)	5	2	2
Flowability (sec) (FA)	25	20	20

Results are mean values of three replicates ± standard deviation

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بروتينات سمك المبروك المتحللة انزيميا
٢- النشاط المضاد للأكسدة

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تم اجراء تحلل لبروتين سمك المبروك العادى باستخدام ثلاثة انزيمات محللة للبروتين وهى البابين و البروتييز البكتيرى و البروتييز البقرى وتقدير النشاط المضاد للأكسدة لنواتج التحلل. وقد تم اختيارالبروتينات المحللة جزئيا والتى تحتوى على درجات مختلفة من الببتيدات ذات أوزان جزئية مختلفة و قدر نشاطها المضاد للأكسدة. اختير ناتج التحلل البروتينى الناتج باستخدام انزيم البابين لمعرفة مدى تأثيره على تثبيط أكسدة الدهون وتغير اللون فى نموذج اللحم. أيضا تم دراسة تأثير ثلاث طرق لتجفيف ناتج التحلل البروتينى باستخدام انزيم البابين على النشاط المضاد للأكسدة وبعض الخواص الوظيفية.

أوضحت النتائج أن نواتج التحلل البروتينية كانت لها قدرة عالية كمضادات للأكسدة حيث أنها قللت من الأكسدة بنسبة ٩٨٪ عند اضافة ٠,٥ ٪ إليها من مادة BHT مع ملاحظة أن هذه النواتج يزداد تأثيرها المضاد للأكسدة مع زيادة درجة التحلل. وقد أشارت النتائج الى أن نسبة ٣٥,٢ ٪ درجة تحلل باستخدام انزيم البابين قد أدت الى أعلى درجة نشاط مضاد للأكسدة مقداره ٧٣,٢ ٪. أظهرت النتائج أيضا أن الببتيدات ذات الأوزان الجزئية المنخفضة تميزت بنشاط عال كمضادات للأكسدة حيث أن اضافة ناتج التحلل البروتينى باستخدام انزيم البابين إلى نموذج اللحم قد أدى إلى الإقلال من عمليات أكسدة الدهون. وكانت عملية التجفيد هى الأفضل فى الحفاظ على النشاط المضاد للأكسدة والخواص الوظيفية لناتج التحلل البروتينى باستخدام انزيم البابين.