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EVALUATION OF ANTI-OXIDANT AND ANTI-BACTERIAL FUNCTIONALITIES OF COMMON CARP (*CYPRINUS CARPIO*) ENZYMIC ELASTIN HYDROLYSATE

Kadhim A. M.*[1], Shakir K. A.*[2]
*[1]Scientific Research Commission, Baghdad, Iraq
*[2]Department of Food Science- College of Agricultural Engineering Science. / University of Baghdad

ABSTRACT

This study was amid to assess the radical scavenging activity, reducing power and total antioxidant capacity of elastin enzymatic hydrolysate (EEH) which prepared by treating common carp bulbus elastin extract with elastase for 10 hours at 40 C°. Aliquots of reaction solution were taken every 2 hours to point out the optimum degree of hydrolysis based on the highest antioxidant value. The hydrolysate which obtained after 8 hours hydrolysis was chosen to be evaluated for improving minced beef meat shelf-life based on the bacterial contents and lipid oxidation under refrigerated storage (4 C°/ 10 days). This study included 6 groups: group C without EEH addition, groups EEH1, EEH2 and EEH3 with 50, 100 and 150 mg EEH/ 100 gm meat, in addition to group A (100 mg ascorbic acid/ 100 gm meat) and group B (100 ppm of BHT) for comparison. The antioxidant activity of elastin hydrolysate was proportional to the time of hydrolysis, where the highest radical scavenging activity (RSA) and total antioxidant capacity (TAC) were achieved after eight hours of hydrolysis (21.44 % and 205.41 mg equivalent ASC/ 100 ml respectively). While the highest value for reducing power test was after ten hours of hydrolysis (0.422 nm). The results indicate that the concentration of elastin hydrolysate was an effective factor in reducing the peroxide value (PV), Thiobarbituric acid (TBA) and total volatile nitrogen (TVN) during storage period. Meanwhile, the pH value was increased with increasing the concentration of elastin hydrolysate, while the free fatty acid (FFA) values varied during storage when the EEH3 group was recorded the least FFA value. The bacterial contents of minced meat were declined with increasing the concentration of EEH, where EEH3 group recorded the lowest count of TPC, S. aureus and Salmonella as compared to A, B and C groups. In conclusion, the fish enzymatic elastin hydrolysate is a promising natural antioxidant/ antibacterial as a good alternative for synthetic compound.

Key words: meat spoilage, microbial count, minced meat, oxidation, shelf life.
***Part of Ph.D. Dissertation for the 1st author.**

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INTRODUCTION

Meat is a nutritious protein-rich food that is highly perishable and has a short life ranging 5-7 days for refrigerated storage and 21 days for vacuum-packed storage (Crowley et al., 2010; Olaoye and Ntuen, 2011), which it is affected by bacterial contamination and oxidation during processing and in storage conditions (Al-Salmany and AL-Rubeii, 2020). Minced meat is used frequently in the food industry and private households, which concept a highly perishable, microbiological activities, and physiological and chemical changes product unless preservation methods

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are used (Olaoye and Ntuen, 2011; Østerlie and Lerfall, 2005). So, it is important to make meat safe for consumers in terms of stability, transportation and storage (Hassanien et al., 2019). The meat mincing process is likely to contribute to the higher levels of contamination in minced meat as compared to carcasses, where more microorganisms are added to the exposed surfaces of tissue (Tassew et al., 2010) Leading to a major public health hazard and economic loss in terms of food poisoning and meat spoilage (Sallam et al., 2004). In addition to microbial concepts, lipid oxidation is a major problem during storage in minced meat products where the risk of spoilage increases with slicing (Aksu, 2007), because of the increase in surface area of meat exposed to air after micing (Naveena et al., 2008) leading to the deterioration of nutritional quality, color stability and flavor of minced meat (Nikolić et al., 2009). Therefore, many studies added synthetic antioxidant/ antibacterial agents to meat and meat products to reduce fat oxidization and microbial growth like butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), ascorbic acid and nitrate salt in lamb patties, minced pork, beef patties minced chicken breasts during refrigerated and/ or frozen storage (Zahid et al., 2019). However, adding these agents led to concern consumers about the safety due to their instability and have a negative effect on health. Thereby this concern has led to arouse a great interest in avoiding purchasing products containing synthetic additives and preferring foods with natural additives (Al-Zubaidi et al., 2021; Sallam et al., 2004). For example, BHA could potentially disrupt endocrine glands, body weight gain, delayed sexual maturation, slower sperm motility and female breast cancer (Nguyen et al., 2023). So, there is high interest in using natural antioxidant antimicrobial agents to prolongation the shelf life of meat during storage such as a-tocopherol, grape seed extract, olive leaves, thyme, Garlic oil, and lemon grass oil, bioactive peptides, enzymic collagen hydrolysate (Abbas and Shakir, 2021; Aksu, 2007; Amany et al., 2010; Amin and Edris, 2017). Besides collagen and polysaccharides, Elastin is a major extracellular protein component of animal tissue like humans, livestock, fish and birds which provides elasticity to tissues that experience repeated stretching and recoiling like blood vessels, skin, lungs, cartilage and arteries (Jenkins et al., 2021; Shiratsuchi et al., 2013). It is prepared commercially from bovine ligaments, however bovine spongiform encephalopathy (BSE) has led to concern regarding using elastin of bovine origin (Nakaba et al., 2006). Thus, many authors studying more social acceptability and free from BSE from natural sources like fish (Nakaba et al., 2006; Shiratsuchi et al., 2013) and poultry (Kamaruzaman and Yusop, 2021; Nadalian et al., 2019; Yusop et al., 2016). Also, elastin was used to prepare bioactive peptides from poultry skin and skipjack tuna that shown antioxidant properties and beneficial effects on human health (Nadalian et al., 2019; Shiratsuchi et al., 2013). In addition to improving taste, flavor, and texture, reducing the percentage of fat and salt in products, packaging techniques, and improving the pathogen detection system (AL-Ghanimi and AL-Rubeii, 2020; Sallam et al., 2004). The present study aimed to evaluate the antioxidant and antibacterial activities of elastin hydrolysate, prepared by treating elastin from the bulbus arteriosus of common carp with partially purified pancreatic catfish elastase, as natural additives in elongation the shelf-life of minced meat at 4 C° over 10 days of storage by determining the pH value, peroxide value (PV), thiobarbituric acid (TBA), free fatty acid (FFA), total volatile nitrogen (TVN) and bacterial content.

MATERIALS AND METHODS

Preparation of elastin hydrolysate

Bulbus arteriosus of common carp was collected from local fisheries to extract and purify elastin. Five methods were studied for optimization the extraction condition. The optimum method to purify elastin was chosen based on amino acid analysis, SDS-PAGE electrophoresis, scanning electron microscopy (SEM) and sulfhydryl content. High elastin powder (HEP) was prepared by removing the

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soluble protein as reported by (Kamaruzaman and Yusop, 2021) with some modifications. Bulbus arteriosus from common carp was thawed and washed with distilled water then suspended in 1 M of NaCl at a mixing ratio 1: 10 (w: v), then homogenate using a blender at high speed and homogenize using a homogenizer at 15000 ×g for 5 minutes, then held for two hours at 4 C° and the precipitate was collected by centrifugation at 11000 ×g for 20 minutes and this process was repeated more than one time, then defatted by (Lansing et al., 1952) method. The (HEP) was macerated in ethanol for one hour and in acetone for another hour at a mixing ratio of 1: 20 (w: v) with a stirrer at room temperature. The supernatant was removed by centrifugation at 11000 ×g for 20 min. then left to dry in a vacuum oven and stored at -18 C° for further analysis. Daamen et al., (2007) method with some modifications was used to prepare purified elastin. A solution of 4 M of urea, pH 7.2 containing 1 % β-mercaptoethanol was mixed with HEP at a 1:10 (w: v) mixing ratio. held for 24 h at 4 C°. Then diluted up to four-fold with distilled water and the precipitate was collected by centrifugation at 11000 ×g for 20 minutes. Followed by an autoclave at 121 C° for 15 minutes and washed with distilled water four times, centrifuged after each washing step then lyophilized and stored at -18 C° for further analysis. Elastin hydrolysate was prepared using partially purified pancreatic catfish elastase isolated as described previously in (Kadhim and Shakir, 2024). 2% of Partially purified elastase in 0.1 M Tris-HCl buffer, pH 8 was mixed with elastase to substrate ratio 1: 50 (w: v) and incubated at 40 C° for eight hours and the samples were taken every two hours. The enzyme was inactivated by heating at 100 C° for 2 min. The resulting hydrolysate was then rapidly cooled and centrifuged at 10000 ×g for 20 minutes at 4 C°. Then the supernatant was collected and lyophilized for further analysis.

Determination of the degree of hydrolysis

The degree of hydrolysis (DH %) of elastin hydrolysate was determined as described in (Laohakunjit et al., 2017) with some modifications where all hydrolysate diluted to a concentration of 2.5×10^{-3} amino equivalent/L with distilled water. A 2 ml of 0.2125 M phosphate buffer pH 8.2 was added to 0.250 ml of diluted hydrolysate in a test tube then 2 mL of 0.1 % 2,4,6 trinitro benzene sulfonic acid (TNBS) was added and incubated in a shaking water bath at 50 C° for 60 minutes in a dark place. The reaction was terminated by adding 4 mL of 0.1 M HCl and kept at room temperature for 15 min before reading the absorbance against water at 340 nm. A 5-55 mM of L-leucine solution was used for standard curve preparation. The following question is used to calculate the degree of hydrolysis:

$$\mathbf{DH}\ (\%) = \frac{\boldsymbol{Lt - L0}}{\boldsymbol{Lmax - L0}} \times \mathbf{100}$$

Where:

Lt: the specific amino acid at the time.

L0: is the amount of the specific amino acid at time zero.

Lmax: is the maximum amount of the specific amino acid in the substrate obtained after hydrolysis using 6 N HCl at 120 C° for 24 hours.

Assessment of the antioxidant activities

Free radicals scavenging activity (RSA):

The DPPH radical scavenging of elastin hydrolysate was carried out according to the (Laohakunjit et al., 2017) method. one milliliter of DPPH (0.1 mM in 95 % ethanol) was added to 100 µL of sample solution and 900 µL of distilled water in test tubes; the mixture was mixed vigorously, placed in the dark place for 30 min at room temperature. The resultant color was measured at 517 nm using a spectrophotometer. The scavenging activity was calculated using the following equation as described in (Laohakunjit et al., 2017):

$$\boldsymbol{RSA}\ (\%) = \frac{\boldsymbol{C - (A - B)}}{\boldsymbol{C}} \times \mathbf{100}$$

Where:

A: (sample) is the absorbance value of 0.1 ml of sample solution + 0.9 ml of distilled water +1 mL of 0.1 mM DPPH.

B: (blank) is the absorbance value of 0.1 ml of sample solution + 0.9 ml of distilled water + 1 mL of 95 % ethanol.

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

Reducing power activity

The reducing power test was carried out according to the method described by (Zhang et al., 2018) with some modifications. An Aliquot of 750 µL of sample solutions was mixed with 2.5 mL of 0.2 M phosphate buffer, pH 6.6 and 2.5 mL of 1 % potassium ferricyanide. The mixtures were mixed vigorously and incubated in a water bath at 50 C° for 20 minutes. Subsequently, 2.5 mL of 10 % trichloroacetic acid (TCA) was added to the mixture and centrifuged at 10000 ×g for 10 minutes. Then 2.5 mL of the supernatant was transferred to an empty tube and mixed with 2.5 mL of distilled water and 0.5 mL of 0.1 % (w: v) ferric chloride. After standing at room temperature for 10 min, the absorbance was measured at 700 nm. An increase in absorbance of the mixture indicates an increase in reducing power as measured by the reduction of ferric ions.

Total antioxidant capacity

Total antioxidant capacity (TAC) was determined by (Louaileche et al., 2015) method with some modifications. TAC is based on the reduction of molybdenum (Mo^{+4}) in acidic pH to form a green complex of phosphate Mo V. A 1 ml of phosphomolybdic solution (28 mM sodium phosphate, 0.6 M sulfuric acid and 4 mM ammonium molybdate) was added to the test tube containing 187.5 µL of sample solution. Then incubated at 95 C° for 90 minutes and cooled at room temperature, the absorbance was measured at 695 nm. A 20-300 µg/ml of ascorbic acid was used as standard. The antioxidant capacity was expressed as mg of ascorbic acid equivalent per 100 gm dry matter (mg equivalent ASC/1 ml sample)

Preparation of minced meat

Beef from the leg muscle of a calf, age one and a half years old, besides fat, were purchased from the local market in Baghdad. An 1800 gm of meat with 200 gm of fat was minced using a mincing device (Gosonic, China 1800 w Max. The minced meat and fat samples were mixed well and divided into six portions (75 gm each) in duplicate. For control group (C) without any additives comparison groups (B) with 100 ppm Butylated Hydroxy Toluene (Ahmed, 2024; Alimentarius, 1995), 100 mg/100 gm Ascorbic acid group (A) (Alimentarius, 1995; Nariya et al., 2013) and enzymic elastin hydrolysate group (EEH1, EEH2 and EEH3) with 50, 100 and 150 mg/100 gm meat respectively.

pH value

pH value was measured due to (Sallam et al., 2004) method. A sample of 10 gm of meat products was mixed with 50 ml of distilled water using a blender for one minute then the pH value was measured by a portable pH meter (HI98103; HANNA Instruments Inc. ROMANIA).

Thiobarbituric acid value

2- Thiobarbituric acid (TBA) was measured due to (Sallam et al., 2004). Minced meat (10 gm) was homogenized using a blender with 25 ml of trichloroacetic acid (TCA) solution (200 gm/ L of TCA in 135 ml/L phosphoric acid solution) for 30 seconds. After the filtration using Whatman filter paper No.1, then 2 ml of filtrate was added to a test tube containing 2 ml of TBA solution (3 gm/ L) and kept at room temperature in the dark for 20 hours. Then the absorbance was measured at 532 nm by spectrophotometer. TBA value was expressed as mg malonaldehyde per kg of minced meat:

$$\boldsymbol{TBA\ value}\ (\frac{\boldsymbol{mg}}{\boldsymbol{Kg}}) = \frac{\textbf{As} - \textbf{Ab}}{\textbf{weight of sample (gm)}}$$

Where:

As: the absorbance of the sample

Ab: the absorbance of the blank

Free fatty acid value

Free fatty acid (FFA) was measured by the method described in (Aksu, 2007) with some modifications. 10 gm of minced meat was added to a test tube containing 30 ml of chloroform and 0.5 gm sodium sulfate then homogenized using a blender and left to settle at room temperature for 5 minutes. The mixture was filtrated using No. 1 Whatman filter paper, 2 drops of 1% of phenolphthalein were added to 25 ml of the filtrate and titrated with 0.01 N of potassium hydroxide solution at 40 C°. The result was expressed as gm of Olic acid/100 gm of fat.

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$$FFA = \frac{\text{Akali Volume} \times \text{N} \times 28.2}{\text{sample weight (gm)}}$$

Total volatile nitrogen

The Total Volatile Nitrogen (TVN) was measured using the Kjeldahl method as described in (Jinadasa, 2014) with some modifications. 10 gm of minced meat was mixed with 20 ml of 7.5 % TCA in a blender for 2 minutes and filtrated using No.1 Whatman filter paper. The filtrate was placed in the Kjeldahl distillation system and 30 ml of 10 % NaOH and 100 ml of distilled water were added, the steam distillate was collected in a flask containing 25 ml of 4 % H_3BO_3. The steam distillation procedure was continued until the volume reached 100 ml and titrated against standard 0.025 N of H_2SO_4 to the endpoint indicated by pH metter. The following equation was used to calculate the TVN as mg/ 100gm:

$$TVN\ (\frac{mg}{100gm}) = \frac{\text{Acid Volume} \times \text{N} \times 14 \times 300}{25\ \text{ml}}$$

Bacterial analysis

The microbiological analysis was performed on minced meat due to (Djenane et al., 2012) methods with some modifications. Ten grams of minced meat was suspended in 90 ml of tetrathionate broth and incubated for 30 minutes at 37 °C. Then total plate count (TBC), *S. aureus* and *Salmonella* tests were conducted at 0, 3, 7 and 10 days of refrigerated storage at 4 °C using Nutrient agar, Mannitol salt agar for *S. aureus* and XLD agar for *Salmonella*. The plates were incubated at 37 °C for 24-48 hours, colonies were counted and results were expressed as colony forming unit (CFU)/ gm of minced meat.

Statistical analysis

The collected data were statistically analyzed by Minitab software 17th version using analysis of variance (ANOVA) as mentioned in (Lesik, 2018). Differences between treatment means were compared using the Least Significant Differences (LSD) P≤ 0.05 probability level.

RESULTS AND DISCUSSION

The degree of elastin hydrolysate using elastase at 40 C°, pH 8 for 10 hours was presented in Figure 1. It has been noticed that the degrees of hydrolysis were increased with time, where increased rapidly through the first hours of hydrolysis then increased slowly to reach 65.53 % after 10 hours of hydrolysis. The increase of DH % with time could be attributed to the elastase function on minced meat protein. These findings were in agreement with (Yusop et al., 2016), who reported that the DH % of Spent hen and Broiler water-soluble elastin was rapid increase was observed until 12 hour using elastase and alcalase individually, after that slightly decreased and remained constant until the end of the hydrolysis.

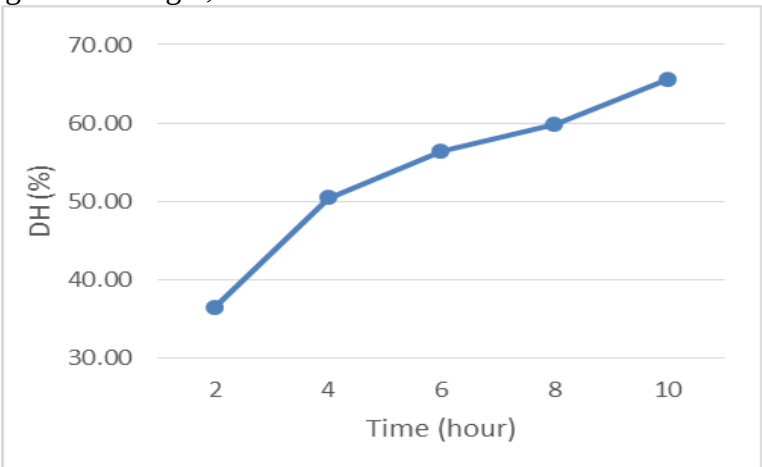

Figure 1. Degree of fish elastin hydrolysis during 10 hours at pH 8 and 37 C°

Table 1 illustrate the radical scavenging activity (RSA), reducing power and total antioxidant capacity (TAC) of enzymic elastin hydrolysate at different time. The RSA and TAC increased from 17.48 % and 150.71 mg equivalent ASC/ 100 ml after two hours to reach the highest RSA and TAC after eight hours of hydrolysis (21.44 % and 205.41 mg equivalent ASC/ 100 ml respectively), then the antioxidant activities were decreased after 10

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hours, reaching to 19.87 % and 145.04 mg equivalent ASC/ 100 ml respectively. The increasing of RSA % and TAC with hydrolysis times could be explained to that the side chain of liberated amino acids increased with time of hydrolysis. Thus providing additional amino acids that donate protons and electrons to maintain a relatively high redox potential. Figure 1 also shows the reducing power activity of elastin hydrolysate. The reducing power of enzymic elastin hydrolysate was increased with time of hydrolysis and reached 0.283, 0.303, 3.339, 0.347 and 0.422 nm after 2, 4, 6, 8 and 10 hours of hydrolysis respectively. Similar results were noticed by (Oliveira et al., 2014), who reported that the reducing power of soy protein isolate hydrolyzed with a novel protease preparation from *Chryseobacterium* sp. kr6 increased with time of hydrolysis due to release of peptides with low molecular mass, increased number of ionizable groups, and exposure of hidden hydrophobic groups, which are related to antioxidant activities depending on the degree of hydrolysis, bitterness and antioxidant activities: the elastin hydrolysate prepared by partially purified elastase at 2:100 (W elastin: V 0.1 N Tris-HCl buffer, pH 8) mixing ratio at 40 C° for 8 hours was chosen as the optimum reaction condition for further experiments.

Table 1. The antioxidant activities of elastin enzymic hydrolysate at different times

Time (hrs)	RSA (%)	Reducing power (Abs. at 700 nm)	TAC (mg equivalent ASC/ 100 ml)
2	17.48	0.283	150.71
4	19.78	0.303	157.90
6	21.44	0.339	153.32
8	21.44	0.347	205.41
10	19.87	0.422	145.04

RSA represent radical scavenging activity and TAC represent total antioxidant capacity

Minced meat is highly spoiled by lipid oxidation and microbial growth due to high-fat content and high water activity leading to unacceptable sensory attributes, meat spoilage and foodborne diseases (Amin and Edris, 2017). So many authors extended the shelf life of minced meat by reducing lipid oxidation and preventing bacterial growth by using natural antioxidant agents (Nasri et al., 2013; Østerlie and Lerfall, 2005; Papuc et al., 2018; Verma and Sahoo, 2000). In this study, the hydrolysate which was obtained after 8 hours hydrolysis was chosen to be evaluated for elongation minced beef meat shelf-life based on the bacterial contents and lipid oxidation under refrigerated storage (4 °C) for 10 days. The variation in pH values of the experimental samples (A, B, C, EEH1, EEH2 and EEH3) during the refrigerated storage for 0, 3, 7, and 10 days were represented in table 2. The pH value of minced meat was 5.6 at zero time, then the pH increased after 3 and 7 days of storage to reach a range of pH from 5.7-6.2 and 6-7.2 respectively. After 10 days of storage, the pH values also increased and the odour and texture of C and EEH3 groups were changed due to the formation of alkaline compounds from the degradation of protein by Gram-negative bacteria such as Pseudomonas, Moraxella and Acinetobacter (Verma and Sahoo, 2000; Zhang et al., 2020). These findings agreed with (Aksu, 2007) finding when they studied the effect of a-tocopherol on a cooked meat product named kavurma and disagreed with (Abbas and Shakir, 2021) who reported that pH values of minced meat treated with fish collagen hydrolysate declined through the storage period. The same table revealed that the pH of minced meat was inconsistently increased with increasing the concentration of elastin hydrolysate and this could be due to the pH of elastin hydrolysate (Amin and Edris, 2017). Regarding the used dose of elastin hydrolysate, it was observed that the meat samples pH increased with increasing the concentration of elastin hydrolysate. The statistical analysis shows that increasing the concentration of EEH leads to a significant increase in pH values after 7 and 10 days of storage periods compared to other groups after 10 days of storage periods.

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Table 2. Effect of elastin hydrolysate, BHT and ascorbic acid on minced meat pH for 10 days storage at 4 °C

Time (days)	Control	EEH1	EEH2	EEH3	BHT	Ascorbic acid
0				5.6		
3	6	5.7	5.8	6.2	5.8	5.7
7	6.7	6	6.4	7.2*	6.4	6.1
10	6.5*	6.3	6.6	7.6*	6.8	6.4

EEH1, EHH2 and EHH3 represent the minced meat incorporated with (50, 100 and 150 mg/ 100 gm meat) elastin hydrolysate. The capital letters represent the differences among columns (storage period), while the small letters represent the differences among groups in the same row (treatment).
*** Change in odour and texture**

The TBA values of C, EHH1, EEH2, EEH3, A and B meat groups were presented in Table 3. TBA values were reduced in all treated minced meat samples during storage as compared with control, additionally the concentration of elastin hydrolysate had a positive effect on reducing the TBA values. These findings agreed with (Haider et al., 2023) finding who reported that the TBA value of beef treated with alcoholic extract of common Fig leaves declined during refrigerated storage due to the reduction of lipid oxidation. The TBA value increased during the storage period in all meat groups, it was increased from 0.02 in 0 days to 0.48 after 10 days of storage. TBA values for the control and treated groups can be arranged in descending order as follows: C > EEH1 > EEH2 > EEH3 > B > A. The TBA values were used to evaluate the lipid oxidation in meat during storage, where the acceptability limit of TBA Value was no more than 1 Verma and Sahoo, 2000).

Table 3. Effect of elastin hydrolysate, BHT and ascorbic acid on TBA value (mg malonaldehyde/ kg) of minced meat during storage period at 4 °C

Time (days)	Control	EEH1	EEH2	EEH3	BHT	Ascorbic acid
0				0.02		
3	0.05	0.07	0.06	0.04	0.04	0.03
7	0.31	0.20	0.12	0.07	0.07	0.03
10	0.48	0.23	0.19	0.13	0.12	0.09

EEH1, EHH2 and EHH3 represent the minced meat treated with (50, 100 and 150 mg/ 100 gm meat) elastin hydrolysate. The capital letters represent the differences among columns (storage period), while the small letters represent the differences among groups in the same row (treatment).

Table 4 displayed the free fatty acid values (%) of untreated minced meat (control) and treated with elastin hydrolysate, BHT and ascorbic acid. Free fatty acid normally liberated from the decomposition of lipids through the storage period in refrigerated or frozen meat (Smith et al., 1980). FFA (%) values were vacillated during refrigeration for each treatment, it varied from 0.21 % at the sample preparation time to 0.05-0.08 % by the end of 10 days of the storage period. After 3 days of refrigeration, FFA values decreased for EEH3, A and B groups. The lowest FFA value was found in the B group followed by EEH3 group (0.08 %) and A group (0.11 %) after 7 days of the storage period, the FFA of minced meats was decreased in C, EEH1 and EEH2 group to reach 0.06, 0.07, 0.07 and 0.06 % respectively. Whilst the FFA value was increased in group B (0.11 %) and A (0.23 %) and this could be attributed to enzymatic hydrolysis of esterified lipid (lipolysis). After 10 days of storage, the FFA values declined to a range of 0.04-0.1 % in all samples. These findings agreed with (Aksu, 2007), who reported that the amount of FFA value varied during storage depending on the hydrolytic activity of the lipases, the microbial metabolic processes and the oxidative reactions that work on the FFA released by the lipolysis. Also, Toldra, (1998) reported that lipids and phospholipids are hydrolyzed by lipase and phospholipase to release free fatty acid that oxidized to peroxides, leading to a reduction of FFA value during the storage period . The same table shows that the EEH3 group was superior in reducing FFA value formation as compared to C, EEH1 and EEH2 especially

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after 3 days of storage period, so elastin hydrolysate could be a good natural alternative for the synthetic antioxidant agent. Similarly, (Abbas and Shakir, 2021) noticed that addition of fish collagen enzymic hydrolysate to minced meat resulted in a reduction of FFA.

Table 4. Effect of elastin hydrolysate, BHT and ascorbic acid on free fatty acid (%) in minced meat during storage period at 4 °C

Time (days)	Control	EEH1	EEH2	EEH3	BHT	Ascorbic acid
0				0.21		
3	0.19	0.23	0.21	0.08	0.04	0.11
7	0.06	0.07	0.07	0.06	0.11	0.23
10	0.05	0.04	0.10	0.04	0.08	0.08

EEH1, EHH2 and EHH3 represent the minced meat samples treated with (50, 100 and 150 mg/ 100 gm meat) elastin hydrolysate. The capital letters represent the differences among columns (storage period), while the small letters represent the differences among groups in the same row (treatment).

The total volatile nitrogen (TVN) of A, B, C, EEH1, EEH2 and EEH3 groups were presented in Table 5. The TVN for control minced meat (C) increased rapidly from 8.43 mg/ 100 g at zero day to reach 28.91 mg/ 100 g after 10 days of storage period and this could be attributed to the degradation of meat protein by microbial and natural existed proteolytic enzymes which resulted accumulation of the free nitrogen in minced meat during storage (Haider et al., 2023). Whereas group A showed the lowest TVN value among the rest, reaching 19.9, 18.68.18.32 mg/ 100 g after 3, 7 and 10 of the storage periods. A similar result was noticed by (Haider et al., 2023), who reported that essential oil of Cestrum Nocturnum flowers and leaf extract of F. carioca was effective in reducing the TVN value in beef meat during the refrigerated storage . Also, the results showed that treating minced meat with elastin hydrolysate had significant effects on TVN values compared to C and B groups during the storage period, were reached (18.3, 18.21 and 26.22), (16.01, 16.77 and 25.87) and (16.01, 14.95 and 23.25) for EEH1, EEH2 and EEH3 groups respectively after 3, 7 and 10 day of storage period, so these compounds inhibited bacteria activities and lead to consequently decreased TVN value (Amany et al., 2010).

Table 5. Effect of elastin hydrolysate, BHT and ascorbic acid on total volatile nitrogen (mg/ 100 g) of minced meat during storage period at 4 °C

Time (days)	Control	EEH1	EEH2	EEH3	BHT	Ascorbic acid
0				8.43		
3	17.76	18.30	16.01	14.13	16.14	19.90
7	21.62	18.21	16.77	14.95	19.65	18.68
10	28.91	26.22	25.87	23.25	28.73	18.32

EEH1, EHH2 and EHH3 represent the minced meat treated with (50, 100 and 150 mg/ 100 gm meat) elastin hydrolysate The capital letters represent the differences among columns (storage period), while the small letters represent the differences among groups in the same row (treatment).

Effect of different concentrations of fish elastin hydrolysate, synthetic antioxidant (BHT) and ascorbic acid on the total plate count (TPC), *S. aureus* and *Salmonella* of minced beef meat stored at 4 °C for 10 days presented in Table 6. Results showed a progressive increase in bacterial counts during the storage period. In the control group (C), the initial TPC of the minced meat was 3.7×10^3 and increased to 9.52×10^6, 2×10^7 and 5×10^8 after 3, 7 and 10 days of storage period. The result of *Salmonella* was illustrated for positive and negative growth in the same table. A negative result for *Salmonella* was shown only in C, EEH1 and EHH2 groups after 7 and 10 days of storage periods. The bacterial growth was decreased with increasing the concentration of enzymic elastin hydrolysate from 50 – 150 mg/ 100 gm EEH. These findings agreed with (Badawy et al., 2019), finding who reported that E. coli and S. typhimurium growth was reduced with increasing the silver content in the products when they studied the antibacterial activity of chitosan silver nanoparticles in the preservation of minced meat . The results also indicated that the addition of enzymic elastin hydrolysate, BHT and ascorbic acid resulted in

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a reduction in all counts of studied bacteria compared to the C group and the highest reduction was observed in EEH3 group for TBA and *Salmonella* growth while A group for *S. aureus* growth which was reached 7×10^5 and 2×10^2 after 10 days of storage period respectively and this could be attributed to the function of elastin hydrolysate in lowering the ability of bacteria to attach to surfaces and embed themselves in mucous membranes which leads to an acceleration of cell membrane permeability (Naveena et al., 2013; Zarzosa-Moreno et al., 2020). In contrast, Abbas and Shakir, (2021) reported that the fish collagen hydrolysates lacked antibacterial activity against Gram positive and Gram-negative bacteria when they studied the effect of Catfish collagen hydrolysate in beef minced meat samples stored at 4 °C for 10 days.

Table 6. Effect of EEH, BHT and ascorbic acid on TPC, S. aureus and Salmonella counts (CFU/ gm) grown in minced meat samples

Isolates	Time (days)	Control	EEH1	EEH2	EEH3	BHT	Ascorbic acid
TPC	0				3.7×10^3		
	3	9.52×10^6	9.5×10^5	7.7×10^5	5.4×10^5	2.12×10^6	2×10^5
	7	2×10^7	2×10^6	9×10^5	3×10^5	5×10^6	8×10^5
	10	9×10^6	3×10^5	7×10^4	4×10^2	3×10^3	2×10^2
S. aureus	0				0		
	3	7×10	1×10	3×10	0	0	0
	7	4×10	2×10	2×10	2×10	1×10	2×10
	10	9×10^6	3×10^5	7×10^4	4×10^2	3×10^3	2×10^2
Salmonella	0				-		
	3	-	-	-	-	-	-
	7	+	+	+	-	-	-
	10	+	+	+	-	-	-

EEH1, EHH2 and EHH3 represent the minced meat treated with (50, 100 and 150 mg/ 100 gm meat) elastin hydrolysate

CONCLUSION

Fish waste concept a promising source for elastin**.** Where the Bulbus arteriosus of common Carp is a reliable source for elastin extraction using the sequential solution reducing, chaotropic agents and autoclave treatment is the best procedure for elastin extraction**.** The purified fish elastase has a high affinity toward elastin. Also, the antioxidant activities of the hydrolysate increased with the increase in the degree of hydrolysis**.** When applied to minced beef during refrigerated storage, elastin enzymatic hydrolysate (EEH) effectively reduced lipid oxidation, limited protein degradation, and decreased bacterial growth in a concentration-dependent manner. Consequently, fish-derived elastin hydrolysate represents a promising, multi-functional natural alternative for the food industry to enhance the shelf-life, safety, and quality of meat products during storage.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHOR/S DECLARATION

We confirm that all Figures and Tables in the manuscript are original to us. Additionally, any Figures and images that do not belong to us have been incorporated with the required permissions for re-publication, which are included with the manuscript.

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تقييم الخواص المضادة للأكسدة والمضادة للبكتريا لمتحللات الانزيمية لايلاستين الكارب العادي (*cyprinus carpio*)

احمد محمد كاظم[1]، خالدة عبدالرحمن شاكر[2]

[1]هيئة البحث العلمي، بغداد، العراق.

[2]قسم علوم الاغذية، كلية علوم الهندسة الزراعية، جامعة بغداد، العراق.

المستخلص

هدفت هذه الدراسة الى تقييم قابلية كبح الجذور الحرة والقوة الاختزالية والقابلية المضادة للأكسدة لمتحللات الايلاستين الانزيمية المستحصلة من معاملة مستخلص ايلاستين bulbus اسماك الكارب العادي بأنزيم الايلاستيز لمدة 10 ساعات عند درجة حرارة 40 م°. تم اخذ نماذج من المتحلل الانزيمي لفترات زمنية بفارق 2 ساعة لغرض تحديد أفضل درجة تحلل استنادا الى اختبار الفعالية المضادة للأكسدة. تم اختيار المتحلل المستحصل بعد 8 ساعات من بدء التحلل الانزيمي لتقييم تأثير متحلل الايلاستين الانزيمي المنتخب على المحتوى الميكروبي واكسدة الدهون للحم البقر المفروم عند ظروف الخزن المبرد 4 م° ولمدة 10 أيام. تضمنت هذه الدراسة ستة مجاميع: المجموعة C (بدون إضافة) والمجموعات EEH1 وEEH2 وEEH3 بإضافة 50 و100 و150 ملغم متحلل الايلاستين/ 100 غم لحم على التوالي، فضلاً عن المجموعة A (100 ملغم حامض الأسكوربك / 100 غم لحم) والمجموعة B (100 جزء بالمليون من BHT) للمقارنة. أظهرت النتائج زيادة الخصائص المضادة للأكسدة مع زيادة وقت التحلل، اذ وصلت الى اعل قيمة لكل من قابلية كبح الجذور الحرة والسعة المضادة للأكسدة بعد ثماني ساعات من التحلل (21.44 % و205.41 ملغم حامض الاسكوربك/ 100 مل على التوالي) بينما القوة الاختزالية كانت بعد عشر ساعات من التحلل (0.422 نانومتر). اشارت النتائج الى ان متحللات الايلاستين كانت تملك عامل مؤثر في خفض كل من الرقم البيروكسيدي (PV) وحامض ثايوباربتيورك (TBA) والمواد النايتروجينية المتطايرة (TVN) خلال مدة الخزن. وعلى العكس ازدادت قيم الرقم الهيدروجيني مع زيادة تركيز متحللات الايلاستين، وكانت قيمة الاحماض الدهنية الحرة (FFA) متذبذبة خلال مدة الخزن وكانت المجموعة EEH3 الأفضل في خفض قيمة FFA. اما النمو الميكروبي فقد انخفض مع زيادة تركيز EEH، اذ أظهرت المجموعة EEH3 أدنى عد بكتيري لكل من TBC و S. aureus وSalmonella بالمقارنة مع كل من المجموعات A وB وC. نستنتج مما ورد ان متحللات ايلاستين السمك الانزيمية تعد مضادات اكسدة وميكروبية طبيعية واعدة وبديل جيد عن المركبات الصناعية.

الكلمات المفتاحية: فساد اللحوم، العد الميكروبي، اللحم المثروم، الاكسدة، العمر الخزني.

***جزء من اطروحة دكتوراه للباحث الأول.**