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## Effect of Dietary Supplementation of Bioactive Peptides on Antioxidant Potential of Broiler Breast Meat and Physicochemical Characteristics of Nuggets

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**Abstract** Poultry meat is generally exposed to quality deterioration due to lipid oxidation during storage. Oxidative stability of meat can be increased by feed supplementation. Aim of the current study was to investigate the effect of dietary supplementation of fish waste derived bioactive peptides on antioxidant potential of broiler breast meat and physico-chemical characteristics and quality parameters of nuggets prepared from breast meat. 180 broiler birds (six groups of 30 birds) were purchased. Each group was given different concentrations of bioactive peptides i.e. 0, 50, 100, 150, 200, and 250 mg/kg feed. After completion of six weeks birds were slaughtered and breast meat was stored at  $-18^{\circ}\text{C}$  for six months. Nuggets were prepared and stored at  $-18^{\circ}\text{C}$  for 45 days. Meat samples were analyzed for antioxidant activity [total phenolic contents (TPC), DPPH<sup>\*</sup> scavenging activity, and ferric reducing antioxidant power] and lipid oxidation assay at regular intervals of 1, 2, 3, 4, 5, and 6 months while nuggets were analyzed for quality (pH, color, texture and water holding capacity) parameters after regular interval of 15 days. A significant ( $p<0.05$ ) effect of feed supplementation was observed on antioxidant status such as TPC, DPPH<sup>\*</sup> scavenging activity, and FRAP of broiler breast meat. Dietary interventions of bioactive peptides significantly ( $p<0.05$ ) delayed lipid oxidation of breast meat than control. All the quality parameters were also significantly affected due to dietary bioactive peptides and storage duration. Thus, dietary interventions of bioactive peptides can increase the antioxidant and shelf stability of broiler breast meat and nuggets.

**Keywords** broiler meat, antioxidant potential, bioactive peptides, dietary supplementation, nuggets

### Introduction

Poultry meat and poultry products are popular and widely consumed worldwide because of their high nutritive value, comparatively low production cost and less cooking time. Therefore, it has become integral part of human diet all around the

globe (Huang and Ahn, 2018). Poultry sector in Pakistan has gained massive success in the previous couple of decades. The production of poultry meat was 1,391 metric tons in Pakistan during 2017–2018, an 8% increase in poultry meat production was recorded as compared to previous year (Government of Pakistan, 2019).

Coronary heart diseases accounts 17.5 million deaths global each year, while 25% casualties are connected to cardiovascular diseases (CVDs) yearly in Pakistan (Zubair et al., 2018). Red meat has been reported to increase the risk of CVDs causing dyslipidemia. Most of the risks are associated with the high saturated fatty acid content and cholesterol of red meat. This led to the suggestions for lowering the consumption of red meat and preferring white meat (Mente et al., 2009). Moreover, oxidation of the meat lipids and cholesterol have been linked with the atherogenesis (Guardiola et al., 1996; Kubow 1993). In order to avoid these diseases, consumers prefer white meat over red meat due to its nutritional value but chicken meat is more at risk of oxidative rancidity owing to high proportion of polyunsaturated fatty acids content (Luna et al., 2010). One of the primary reasons of quality degradation of poultry meat products is lipid oxidation (Buckley et al., 1995).

The oxidation of lipid substances in animals is caused by disproportion between pro-oxidants and antioxidants in the animal's defense system. The damage of lipids in animals can be increased within the immediate post slaughter duration and especially it occurs at some stage in handling, processing, storage, and transportation. The most effective and viable method for enhancing oxidative balance in chicken meat is the enrichment of muscles with the antioxidant substances through feed additives which will be helpful to postpone or lessen oxidative rancidity and generation of free radical within the resultant muscles. In order to control the oxidation of poultry meat, different natural (alpha tocopherol, alpha lipoic acid, and bioactive peptides) and artificial antioxidant compounds are broadly used in feed to solve the oxidation issues in muscle foods and its products (Ismail et al., 2013). The antioxidants from biological origin quench the free radicals, via stopping chain reactions, chelation of metallic ions, preventing the generation of free radicals, degradation of the peroxides and reducing the localized oxygen concentration (Brannan, 2007).

Fish protein hydrolysate is an excellent source of biologically active proteins (Je et al., 2005). Numerous marine species like Alaska Pollack (Je et al., 2005), herring (Sathivel et al., 2013), horse mackerel (*Magalopsis cordla*) have extensively been utilized for manufacturing of bioactive peptide containing fish protein hydrolysate. Antioxidant activity of biologically active proteins have been reported against lipid oxidation in number of lipid systems. Bioactive peptides from food sources also exhibit beneficial health impacts such as antihypertensive potential and hypocholesterolemic effect (Lee and Hur, 2017).

Different scientists investigated the antioxidant capacity and lipid stability of broiler meat by incorporation of natural antioxidant substances in broiler diet. The results described by Auwal et al. (2017) inferred that the bioactive peptides in fish protein hydrolysate from stone fish have potential to chelate ferric ions. DPPH<sup>•</sup> scavenging potential of fish protein hydrolysate from fresh water carp was reported by Elavarasan et al. (2014). Protein hydrolysate containing bioactive peptides fractions of blue mussel showed high ABTS scavenging potential (Wang et al., 2008). Higher TPC of broiler meat given different feed treatments supplemented with natural bioactive compounds was observed by Qwele et al. (2013). Positive impact of dietary antioxidant substances on FRAP of broiler meat was mentioned by Parveen et al. (2015). Higher DPPH<sup>•</sup> scavenging potential of broiler meat fed on natural antioxidant substances was observed than broiler group fed on basal diet (Fasseas et al., 2008; Yasin et al., 2012). Improved ABTS scavenging potential of chicken breast muscles fed on antioxidant additive was noticed (Azizah et al., 2010).

Availability of natural antioxidant substances in biological systems can also limit the production of malondialdehyde (MDA). The production of MDA in broiler meat can be reduced by inclusion of natural antioxidant substances in broiler feed. Lower TBARS value of meat enriched with natural antioxidant substances was noticed (Muthukumar et al., 2012). Bioactive

peptides have the potential to donate hydrogen from amino acids to break the oxidation chain reaction. Biologically active proteins with antioxidant capacity react with hydroperoxides and convert them into stable compounds thus enhancing the shelf stability of broiler meat (Jang et al., 2008). Lower peroxide value in broiler meat fed on natural antioxidant enriched diet was observed by Khan et al. (2015).

Chicken nuggets are prepared from ground chicken meat and seasons by frying at 190°C until a crisp and golden color developed. Chicken industrial processed products such as nuggets are becoming popular in Pakistan. Consumer acceptance of nuggets is dependent upon color, flavor and texture of the product. Lipid oxidation in chicken-based products can spoil color flavor and texture of the products (Tanwar et al., 2017). Enrichment of antioxidants can improve the lipid stability of chicken products. The nuggets from meat with antioxidant supplemented diet had lower TBARS values as compared to those prepared by chicken meat without antioxidants. The outcome of this research indicated that supplementation of broiler feed with antioxidants can reduce the chances of lipid oxidation in meat products (O'Sullivan et al., 2004). The current study was depicted under the objective of ameliorating the antioxidant status and lipid stability of broiler meat and physico-chemical characteristics of broiler meat nuggets during storage through the intervention of biologically active proteins derived from fish waste in broiler feed.

## Materials and Methods

All chemicals needed for this study were procured from Sigma Aldrich (St. Louis, MO, USA). Broilers chicks of 45±10 g body weight (180 birds) were obtained from Olympia chicks Faisalabad, Pakistan. Beds for broiler chicks were prepared by using fine saw dust. Potassium permanganate and formalin of Sigma Aldrich (USA) were used to disinfect the broiler pens in the poultry shed were purchased from the scientific store of Faisalabad. The initial weight of broiler chicks was recorded and distributed in 18 pens each pen containing 10 birds.

### Preparation of biologically active protein hydrolysate

A chemical method was used for the extraction of soluble fish proteins as described by Arnesen et al. (2007). 2 kg of the minced rahu fish waste including head, tail, skin and fins was mixed with 2 L of water, the pH of mixture was maintained at 11 with 62 mL of 3 molar sodium hydroxide solution. In first step sample mixture was distributed in equal volume to centrifuge tubes and centrifugation of sample was performed at 4,000×g for 15 min at temperature of 4°C. Afterwards supernatant was cumulated and pellet was dissolved in 2 L of distilled H<sub>2</sub>O and 15 mL of 3 molar NaOH solution was used to maintain the pH at 11. The second step of protein extraction was conducted for 1 hour and the sample was centrifuged. Again, supernatant was cumulated and pellet was dispersed in 2 L of water, 145 mL of hydrochloric acid was used to adjust the pH up to 2. The pellet was again dispersed in 2L of distilled H<sub>2</sub>O and the pH was regulated to 2 using 3 molar HCl solution (145 mL) before centrifugation for 15 minutes. All these supernatants from the three extractions were stocked altogether. pH of the resultant solution was balanced at 7 via addition of 3 molar NaOH solution. The samples were placed at 25°C to precipitate for 15 minutes and the protein fraction was extracted by centrifugation at 5,000×g for 60 min at 4°C.

Protein hydrolysis was performed in water bath shaker at 150 rpm using alcalase enzyme under optimized conditions to get maximum degree of hydrolysis at temperature 46.37°C, time of hydrolysis 116.91 minutes, [E]/[S] ratio 1.97% and pH of 7.99. After completion of hydrolysis process sample was heated at 100°C for 10 minutes to discontinue the reaction. Centrifugation of sample was performed at 4°C and 3,025×g for 40 min, the hydrolysates consisting of the antioxidant

peptides were collected as the supernatant then lyophilized and stored at  $-40^{\circ}\text{C}$  prior to analysis.

### Antioxidant activity of bioactive peptides

Antioxidant activity of bioactive peptides was determined by DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity and hydroxyl radical scavenging activity.

#### 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay

DPPH radical scavenging activity of protein hydrolysate was estimated by the procedure explained by Hwang et al. (2013). A 0.25 mM DPPH solution was prepared in methanol 100  $\mu\text{L}$  of 1% sample was added with 100  $\mu\text{L}$  of DPPH solution and allowed to rest at  $25^{\circ}\text{C}$  in dark for half an hour and absorbance of the sample mixture was estimated at 517 nm. Percentage DPPH· scavenging activity of the fish protein hydrolysate was calculated by applying following equation:

$$\text{DPPH}\cdot \text{ scavenging activity (\%)} = \frac{[1 - (A_s - A_b)] \times 100}{\text{Blank's absorbance}}$$

$A_b$  = Absorbance of blank

$A_s$  = Absorbance of sample

#### Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity was quantified by the procedure given by Wang et al. (2008). 1 mL of ample solution was added with the 1 mL of 750  $\mu\text{M}$  1,10-phenanthroline solution. pH of this mixture was adjusted up to 7.4 by adding 0.2 molar sodium phosphate buffer. After that 1 mL of 750  $\mu\text{M}$  ferrous sulfate solution was added and reaction was initiated by mixing the 1 mL of  $\text{H}_2\text{O}_2$ . Mixture is heated at  $37^{\circ}\text{C}$  for 60 minutes in water bath. Spectrophotometric absorption was estimated at wavelength of 536 nm. Glutathione reductase was utilized as control and hydroxyl radical scavenging activity was quantified by the given equation.

$$\text{OH scavenging activity (\%)} = \frac{A_s - A_o \times 100}{A_c - A_o}$$

Where,

$A_s$  = Sample's absorbance

$A_o$  = Absorbance of the solution in which sample was replaced by equivalent vol. of  $\text{dH}_2\text{O}$

$A_c$  = Absorbance of the solution in which sample and  $\text{H}_2\text{O}_2$  replaced by equivalent vol. of  $\text{dH}_2\text{O}$

### Experimental plan

Different levels of biologically active proteins prepared from fish waste were added in broiler feed according to the plan described in Table 1. The birds were distributed into six groups each group with 3 replicates and each replicate was comprised of ten birds, 180 birds were purchased for experiment. The nutritional composition of broiler basal diet is depicted in Table 2. The nutritional composition of the basal diet consisted of crude protein 21.13%, Lysine 1.10% and methionine 0.52%. The calculated metabolized energy for 1 kg of basal diet was 2,903 kcal.

**Table 1. Composition of broiler basal feed**

Ingredients	Quantity (g/kg)
Yellow corn	490
Rice polishing	56
Cotton seed meal	22
Rice broken	20.7
Canola meal	20
Corn gluten	23
Soybean meal	150
Fish meal	66
Sun flower meal	124
L-Lysine	1.50
DL-Methionine	0.80
Dicalcium phosphate	12
Limestone	12
Premix	2

**Table 2. Treatment plan for different levels of bioactive peptides in broiler feed**

Treatment number	Peptides (mg/kg feed)
T <sub>1</sub>	0
T <sub>2</sub>	50
T <sub>3</sub>	100
T <sub>4</sub>	150
T <sub>5</sub>	200
T <sub>6</sub>	250

### Experimental site management

Research trial was carried out in student research area at Poultry Research Center, University of Agriculture Faisalabad. The research room was thoroughly white washed. The room was disinfected with the potassium permanganate and formalin before the start of the trial. Saw dust (2-inch layer) was used to prepare the pen beds. Eighteen pens each of twelve square feet were prepared. All the feeders and drinkers were washed properly and disinfected with aqueous solution of potassium permanganate. During first week of trial temperature of experimental room was maintained at 34°C, which was lowered by 5°C till it reached to 24°C. Proper light and ventilation was maintained during experimental trial. The fresh water and feed was provided to bird's *ad-libitum*.

### Bird's vaccination

A sugar solution of 50 g per 5 L was provided to broiler chicks within first 2 hours of birds' allocation in pens. To prevent the bacterial infection birds were provided with the Cotrium-50 solution (one gram per five liters of water) at the age of two

days. Chicks were treated with ND vaccine to prevent the New Castle Disease at age of 3 days.

At the age of 10 days chicks were treated with the infectious bursal disease (IBD) vaccine to prevent the wild cough. At the age of 2 weeks birds were treated with the vaccine to prevent the Gamboro Disease. Birds were vaccinated with ND at the age of 22 days and with Lasota vaccine at age of 28 to prevent the New Castle Disease.

### **Slaughtering of the broiler birds**

After the completion of experimental trial, three birds from each treatment were picked and slaughtered by following the Halal Ethical guidelines for further analysis. The broiler breast was separated and packaged in polythene zip bags. The meat samples were put under freezing storage at  $-18^{\circ}\text{C}$  for further analysis.

### **Antioxidant potential of broiler meat**

#### **Sample preparation**

The meat samples from breast muscles (5 g) were homogenized using the 20% glycerol and phosphate buffer (pH 7.4) with homogenizer. After giving the proper rest time in ice cold water mixture was filtered by muslin cloth to make it free from connective tissues.

#### **Total phenolic contents (TPC)**

TPC in broiler meat was estimated using the technique explained by Senevirathne et al. (2006). The 100  $\mu\text{L}$  prepared broiler meat sample was added to 2.5 mL distilled water, 500  $\mu\text{L}$  of 95% ethanol and 250  $\mu\text{L}$  of Folin-Ciocalteu reagent (50%). After that above mixture was given rest time for 5 minutes, then 500  $\mu\text{L}$  of  $\text{Na}_2\text{CO}_3$  (5%) was added to this solution. The final mixture was swirled in vortex meter and placed in dark for 1 hour. The sample absorbance was estimated at 725 nm through spectrophotometer. TPC of broiler meat samples were quantified as Gallic acid equivalent (mg of gallic acid/100 g of meat sample).

#### **2,2-Diphenyl-1-picrihydrzyl (DPPH) assay**

DPPH scavenging capacity of broiler meat sample was evaluated using the approach explained by Hwang et al. (2013). A 0.25 mM solution of DPPH was developed in methanol. 100  $\mu\text{L}$  of 1% sample was added with 100  $\mu\text{L}$  of DPPH solution and kept at  $25^{\circ}\text{C}$  in dark for half an hour and sample absorbance was taken at 517 nm via spectrophotometer. Percentage DPPH-scavenging activity of the broiler meat was calculated by applying given equation:

$$\text{DPPH} \cdot \text{scavenging activity (\%)} = \frac{[1 - (A_s - A_b)] \times 100}{\text{Blank's absorbance}}$$

$A_b$  = Blank absorbance

$A_s$  = Sample absorbance

#### **Ferric reducing antioxidant power (FRAP)**

FRAP in broiler meat was measured by adopting the technique explained by Oyaizu (1986). A homogenized meat sample of 200  $\mu\text{L}$  was added to 500  $\mu\text{L}$  of 0.2 molar sodium phosphate buffer to adjust the pH at 6.6. Resultant solution was kept in water bath at  $50^{\circ}\text{C}$  for 20 min. The centrifugation of the sample mixture was performed at  $2,200 \times g$  for 10 min after adding

with the 2.5 mL 10% trichloroacetic acid. 100  $\mu$ L of ferric chloride solution (0.1%) and 500  $\mu$ L of distilled H<sub>2</sub>O was added to supernatant. The spectrophotometric absorbance of the sample was estimated at 700 nm. The FRAP was calculated as  $\mu$ mol/Fe<sup>2+</sup>/g meat.

### Extent of lipid oxidation in broiler meat

#### Peroxide value (POV)

POV of breast meat was estimated by method outlined by the Sallam (2007). A meat sample of 5 g was placed in glass stoppered flask of 250 mL and heated in water bath for 3 minutes at 60°C to melt the fat. A 30 mL (3:2 v/v) mixture of acetic acid and chloroform solution was added to sample and homogenized thoroughly. The resulting mixture was filtered, 0.5 mL of KI solution was added to filtrate and stored in dark place for 1 hour. This mixture containing starch solution as indicator was titrated against the 0.3 N sodium thiosulfate solution. Peroxide value was calculated as milliequivalent per kilogram (meq/kg) of meat.

$$\text{Peroxide value (meq/kg)} = \frac{\text{Vol. of titration used} \times \text{Normality of Na}_2\text{S}_2\text{O}_3}{\text{Weight of sample in kg}}$$

#### TBARS assay

Thiobarbituric acid reactive species (TBARS) of homogenized breast meat samples were analyzed by adopting the technique outlined by Asghar et al. (1989). The peroxidation reaction was commenced by addition of 0.1 molar FeSO<sub>4</sub> 7·H<sub>2</sub>O (700  $\mu$ L) and 0.1 molar hydrogen peroxide (200  $\mu$ L) in 7 mL of homogenized meat sample in a flask and heated in a water bath up to 37°C. After each 30 minutes 1 mL of aliquot was removed from reaction flask for the period of 2 hours and added to the solution containing 0.4% TBA, 10% trichloroacetic acid and HCl of 0.25N. The resultant mixture was kept in water bath for 15 min, cooled and centrifuged. Supernatant was collected and spectrophotometric absorbance was estimated at 532 nm. The amount of lipid oxidation products was quantified by following the given formula. TBARS was calculated as mg MDA/kg of meat.

$$\text{TBA value} = \frac{(A_s - A_b) \times \text{Sample volume}}{0.000156 \times 1,000/\text{mL}}$$

Where,

A<sub>s</sub> = Sample's absorbance

A<sub>b</sub> = Blank's absorbance

#### Product development (nuggets)

Nuggets were prepared from breast meat of broiler from all treatments by following the standard recipe as 250 g broiler breast meat, 60 g fine flour, 5 g garlic paste 50 g onion paste, 10 g salt, 40 g bread crumbs, one egg and oil for frying. All ingredients were mixed with minced broiler meat and mixed thoroughly. After that mixture was formed in to discs of 12 mm thickness and 30 mm diameter. When meat nuggets were prepared, these were coated with corn flour then dipped in egg and again coated with bread crumbs. Nuggets were fried in cooking oil at 180°C till dark brown color was developed. Total 150 nuggets 25 from each treatment group were prepared. After cooling at room temperature nuggets were packed in polythene

zip bags and stored at freezing temperature  $-18^{\circ}\text{C}$  for further analysis.

### **Physico-chemical and quality characteristics of broiler nuggets**

#### **pH measurements**

The pH of nuggets prepared from broiler breast muscles was estimated utilizing the methodology explained by Turienzo et al. (2011). Before pH determination calibration of pH meter was performed using buffer solutions of pH 4, 7, and 10. A 10 g of the sample was homogenized thoroughly in distilled  $\text{H}_2\text{O}$ . The pH of nuggets from each treatment sample was estimated by dipping the probe of pH meter in meat sample.

#### **Water-holding capacity (WHC)**

WHC of the breast nuggets was determined by filter press method as mentioned by Wierbicki and Deatherage (1958). One-gram sample of nuggets was kept between 18 pieces of filter paper (11 cm diameter). A pressure of 2.81 kg was applied for 5 minutes. A loss of weight after pressing the sample was estimated and termed as expressed juice. Total moisture content of the nugget sample was calculated by AOAC (2002) method. The water holding capacity of nuggets was determined by following equation.

$$\text{WHC (\%)} = \frac{[1 - (\text{Expressed juice})] \times 100}{\text{Total moisture content}}$$

#### **Color measurements**

Color of the nuggets was determined by a tristimulus colorimeter (Color Test Meter II) at uniform storage intervals (0, 15, 30, and 45 days) as outlined by (El-Gasim et al., 2000). Colorimeter was calibrated using standard for darkness and lightness (54 CTn for dark and 151 CTn for light). Sample was placed under photocell of colorimeter to determine the color transmittance number (CTn).

#### **Texture analysis**

The shear force for broiler nuggets was measured by texture analyzer (Model TX-XT2, stable microsystem, surrey, UK) using the method given by Piga et al. (2005).

#### **Statistical analysis**

All experiments were performed in triplicate. Statistical analysis of the data obtained from all experiments was performed through completely randomized design using the “Statistic 8.1” statistics software package. Moreover two-way analysis of variance (ANOVA) was performed to check the level of significance for all parameters. Mean comparisons for variable parameters were performed using Tukey’s test.

## **Result and Discussion**

### **Antioxidant activity of bioactive peptides**

The free (DPPH) radical scavenging activity and hydroxyl radical scavenging activity in fish bioactive peptides was

recorded 51.39% and 54.61% respectively. Current results for DPPH scavenging activity of fish bioactive peptides are in line with the findings of Jeevitha et al. (2014). The capacity of rahu fish protein hydrolysate to scavenge DPPH<sup>\*</sup> could be due to their ability to stabilize these free radicals in reaction medium and to terminate the propagation of free radicals (Kong and Xiong, 2006). This effect might also be due to the specific amino acid composition and amino acid sequence of bioactive peptides. Hydrophobic amino acids have the ability to chelate free radicals and can trap lipids by forming physical barriers around the fat droplets which inhibits the free radical chain reactions. Zarei et al. (2012) have earlier reported the increase in DPPH<sup>\*</sup> scavenging activity with the increase in protein hydrolysis time. The findings related to hydroxyl radical scavenging activity of presents study are in support to the results obtained by Bernardi et al. (2016) and Jeevitha et al. (2014) based on fish protein hydrolysates. Bioactive peptides consisting of hydrophobic amino acid residues, including His, Cys and Met have hydroxyl radical scavenging capacity (Hernandez-Ledesma et al., 2005), while the presence of aromatic amino acids (Tyr, Trp, and Phe) give the peptides higher potential to donate electrons (Girgih et al., 2015).

### Antioxidant potential of broiler breast meat

#### Total phenolic contents (TPC) of broiler breast meat

TPC revealed about the antioxidant potential of muscle tissues; higher TPC present in meat indicate the higher free radical scavenging potential. Phenolic compounds present in meat enhance the quality, shelf stability and acceptability of meat products as antioxidants and flavor compounds. These phenolic compounds have the potential to react with the hydroxyl and lipid radicals and stabilize them. The results of TPC of broiler breast meat provided with different concentrations of biologically active proteins in feed are displayed in Table 3. The supplementation of the biologically active proteins in broiler feed improved the TPC of broiler breast meat with momentous ( $p < 0.05$ ) difference among samples from birds fed on different levels of biologically active proteins. The broiler birds fed on highest level of bioactive peptides ( $T_6 = 250$  mg/kg of feed) showed highest TPC 161.2 mg GAE/100 g in breast meat tissues at 0 day of storage which decreased during storage period to 153.1 mg GAE/100 g of meat at 180<sup>th</sup> day of storage. The lowest TPC 111.7 mg GAE/100 g in broiler breast meat were observed in  $T_1$  at 0 day which further decreased to 104.2 mg GAE/100 g meat. A 30% increase in TPC values was observed in broiler breast meat by adding bioactive peptides in broiler feed at concentration of 250 mg/kg of feed. These

**Table 3. Total phenolic contents of broiler breast meat during freezing storage at  $-18^{\circ}\text{C}$  for 180 days**

Storage days	Total phenolic contents (mg GAE/100 g meat)					
	$T_1$	$T_2$	$T_3$	$T_4$	$T_5$	$T_6$
0	111.7±0.05 <sup>i</sup>	121.3±0.06 <sup>a</sup>	128.7±0.03 <sup>U</sup>	136.4±0.06 <sup>O</sup>	147.3±0.03 <sup>I</sup>	161.2±0.05 <sup>A</sup>
30	110.3±0.05 <sup>j</sup>	120.2±0.05 <sup>c</sup>	127.1±0.03 <sup>W</sup>	135.4±0.03 <sup>P</sup>	146.3±0.08 <sup>J</sup>	160.1±0.09 <sup>B</sup>
60	108.8±0.05 <sup>k</sup>	119.2±0.06 <sup>d</sup>	126.2±0.07 <sup>X</sup>	133.8±0.06 <sup>Q</sup>	145.2±0.03 <sup>K</sup>	158.1±0.03 <sup>C</sup>
90	107.1±0.05 <sup>l</sup>	118.1±0.03 <sup>e</sup>	125.1±0.03 <sup>Y</sup>	132.2±0.06 <sup>R</sup>	144.3±0.07 <sup>L</sup>	157.2±0.03 <sup>D</sup>
120	105.4±0.03 <sup>m</sup>	117.2±0.05 <sup>f</sup>	123.4±0.03 <sup>Z</sup>	131.1±0.02 <sup>S</sup>	142.3±0.02 <sup>M</sup>	155.6±0.05 <sup>E</sup>
150	104.1±0.05 <sup>n</sup>	115.6±0.04 <sup>h</sup>	121.4±0.03 <sup>a</sup>	129.7±0.02 <sup>T</sup>	141.2±0.03 <sup>N</sup>	154.3±0.07 <sup>F</sup>
180	104.28±0.04 <sup>n</sup>	116.4±0.07 <sup>g</sup>	120.56±0.03 <sup>b</sup>	128.52±0.03 <sup>V</sup>	139.50±0.02 <sup>O</sup>	153.15±0.04 <sup>G</sup>

$T_1$ , basal feed (control);  $T_2$ , 50 mg bioactive peptides/kg of feed;  $T_3$ , 100 mg bioactive peptides/kg of feed;  $T_4$ , 150 mg bioactive peptides/kg of feed;  $T_5$ , 200 mg bioactive peptides/kg of feed,  $T_6$ , 250 mg bioactive peptides/kg of feed.

Mean values with different superscripts differ significantly.

All values expressed as mean±SD (n=3).

findings are in consistent with findings mentioned by Selim et al. (2015). Antioxidant potential of TPC was reported by Chanda and Dave (2009). The potential of phenolic compounds to scavenge free radicals was also described by Jyotsna Mishra et al. (2007). Basmacioglu et al. (2003) also reported the increased level of TPC in chicken meat fed on antioxidant enriched diet.

#### DPPH' scavenging activity of broiler breast meat

Results regarding DPPH of broiler breast meat fed on different concentrations of bioactive peptides in feed are displayed in Table 4. The supplementation of the bioactive peptides in broiler feed improved the DPPH' scavenging potential of broiler breast meat with substantial ( $p<0.05$ ) difference among samples from birds fed on various levels of bioactive peptides. The DPPH scavenging activity of broiler breast meat was momentarily ( $p<0.05$ ) decreased in all treated groups during storage duration of 180 days.

The maximum DPPH' scavenging activity was noticed in T<sub>6</sub> at 0 day (85.1%) which decreased to 78.4% at 180<sup>th</sup> day of storage. The DPPH' scavenging activity of T<sub>1</sub> at 0 day was witnessed 57.1% which further decreased to 51.2% at 180<sup>th</sup> day of storage. It has been observed that supplementation of broiler feed with bioactive peptides had witnessed to be effectual in improving the antioxidant capacity of broiler breast meat. The dietary addition of bioactive peptides in broiler feed improved the antioxidant content of muscles and enhanced the shelf life by maintaining lipid stability and quality of meat through scavenging the pro-oxidant substances. Mechanism elaborates that substances with antioxidant potential inactivate the free radicals produced in muscles and enhance oxidation stability of meat. Bioactive peptides quench the free radicals and oxygen reactive species subsequently inhibit the oxidative damage in biological systems (Kim et al., 2001). Higher DPPH' scavenging activity indicated the higher antioxidant content present in broiler meat which have the potential to donate one proton to form a stable DPPH<sub>2</sub> compound thus scavenging the free radicals (Prior et al., 2005). These findings are in close harmony with the findings observed by Yasin et al. (2012). Likewise, Fasseas et al. (2008) reported the higher free radical scavenging potential of broiler meat fed on natural antioxidant substances than boilers fed on basal diet. Easssawy et al. (2016) also reported the improved DPPH' scavenging potential of broiler breast meat through quinoa seed extract as dietary antioxidant substances. Jung et al. (2010) also found positive effect of dietary natural antioxidant substances on broiler breast meat.

**Table 4.** DPPH radical scavenging activity of broiler breast meat during freezing storage at  $-18^{\circ}\text{C}$  for 180 days

Storage days	DPPH radical scavenging activity (%)					
	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>	T <sub>5</sub>	T <sub>6</sub>
0	57.1±0.05 <sup>U</sup>	66.9±0.05 <sup>P</sup>	75.4±0.05 <sup>K</sup>	79.3±0.04 <sup>FG</sup>	83.2±0.04 <sup>BC</sup>	85.1±0.05 <sup>A</sup>
30	56.6±0.05 <sup>U</sup>	66.2±0.07 <sup>PQ</sup>	75.2±0.05 <sup>KL</sup>	78.2±0.05 <sup>G-I</sup>	81.4±0.04 <sup>DE</sup>	84.1±0.04 <sup>AB</sup>
60	55.1±0.06 <sup>V</sup>	65.2±0.05 <sup>QR</sup>	73.0±0.05 <sup>MN</sup>	77.1±0.04 <sup>IJ</sup>	81.3±0.04 <sup>DE</sup>	83.3±0.06 <sup>BC</sup>
90	54.8±0.04 <sup>V</sup>	64.3±0.06 <sup>RS</sup>	73.2±0.04 <sup>MN</sup>	76.3±0.03 <sup>JK</sup>	80.4±0.06 <sup>EF</sup>	82.2±0.05 <sup>CD</sup>
120	53.0±0.04 <sup>W</sup>	64.1±0.03 <sup>RS</sup>	71.1±0.05 <sup>O</sup>	75.2±0.05 <sup>KL</sup>	79.2±0.05 <sup>FG</sup>	80.7±0.04 <sup>E</sup>
150	52.3±0.04 <sup>WX</sup>	63.1±0.05 <sup>S</sup>	70.28±0.04 <sup>O</sup>	73.9±0.05 <sup>LM</sup>	77.6±0.03 <sup>H-J</sup>	79.1±0.03 <sup>FG</sup>
180	51.2±0.05 <sup>X</sup>	61.7±0.04 <sup>T</sup>	70.0±0.03 <sup>O</sup>	72.6±0.04 <sup>N</sup>	77.6±0.05 <sup>H-J</sup>	78.7±0.05 <sup>GH</sup>

T<sub>1</sub>, basal feed (control); T<sub>2</sub>, 50 mg bioactive peptides/kg of feed; T<sub>3</sub>, 100 mg bioactive peptides/kg of feed; T<sub>4</sub>, 150 mg bioactive peptides/kg of feed; T<sub>5</sub>, 200 mg bioactive peptides/kg of feed, T<sub>6</sub>, 250 mg bioactive peptides/kg of feed.

Mean values with different superscripts differ significantly.

All values expressed as mean±SD (n=3).

### FRAP of broiler breast meat

The results of FRAP of broiler breast meat from birds given different concentrations of biologically active proteins in feed are displayed in Table 5. The supplementation of the bioactive peptides in broiler feed improved the FRAP of broiler breast meat with significant ( $p<0.05$ ) difference among samples from birds fed on various levels of biologically active proteins. The FRAP was significantly ( $p<0.05$ ) lower in all treated groups during storage duration of 180 days. Highest ( $682.6 \mu\text{mol}/\text{Fe}^{2+}/\text{g}$  meat) was noticed in  $T_6$  at 0 day which gradually decreased during storage period ( $665.3 \mu\text{mol}/\text{Fe}^{2+}/\text{g}$  meat) at 180<sup>th</sup> day of storage. The lowest FRAP value ( $541 \mu\text{mol}/\text{Fe}^{2+}/\text{g}$  meat) was noticed in  $T_1$  at 0 day which decreased further to ( $527 \mu\text{mol}/\text{Fe}^{2+}/\text{g}$  meat) at 180<sup>th</sup> day of storage. The antioxidant content present in broiler muscles tend to reduce ferric ion ( $\text{Fe}^{3+}$ ) to ferrous ion ( $\text{Fe}^{2+}$ ) by donating one electron. Results of higher metal chelating potential also indicated the inhibition of tissue damage due to oxidation in animal body. Higher FRAP values in broiler meat fed on  $T_6$  (250 mg bioactive peptides/kg of feed) was owing to the antioxidant capacity of ingredients used in broiler diet. The current findings were comparable to the results discussed in previous studies. Higher metal ion reducing potential was noticed in meat enriched with natural antioxidant substances in comparison with control treatment (Banerjee et al., 2012). Moreover, Amarowicz et al. (2005) mentioned the direct relationship between FRAP and antioxidant activity by chelating the free radicals and discontinuing the oxidation chain reactions. Parveen et al. (2015) elucidated the impact of flax seed and alpha-tocopherol acetate on FRAP of broiler leg meat and found significant effect of dietary enrichment through natural antioxidant sources.

### Extent of lipid oxidation of broiler meat

#### Peroxide value (POV) of broiler breast meat

Oxidation occurs at the position of unsaturated bonds in fatty acids. A number of oxidation compounds such as hydroperoxides and free radical are produced during oxidation of unsaturated fatty acids. Quantification of these first stage oxidation products (peroxides and hydro-peroxides) is known as peroxide value. The results of POV of broiler breast meat fed on different concentrations of bioactive peptides are displayed in Table 6. The supplementation of the biologically active proteins broiler feed reduced the POV of broiler breast meat with considerable ( $p<0.05$ ) difference among samples from birds fed on various levels of bioactive peptides. The POV content was significantly ( $p<0.05$ ) increased in all treated groups during

**Table 5. Ferric reducing antioxidant power of broiler breast meat during freezing storage at  $-18^\circ\text{C}$  for 180 days**

Storage days	Ferric reducing antioxidant power ( $\mu\text{mol}/\text{Fe}^{2+}\cdot\text{g}$ meat)					
	$T_1$	$T_2$	$T_3$	$T_4$	$T_5$	$T_6$
0	$541\pm 1.5^W$	$651.3\pm 0.5^{L-O}$	$663\pm 1^{F-H}$	$663\pm 1^{F-H}$	$675.67\pm 0.5^{BC}$	$682.6\pm 1.5^A$
30	$539\pm 1^{WX}$	$645\pm 1^{P-R}$	$662\pm 0.5^{G-I}$	$662\pm 1^{G-I}$	$672\pm 1^{CD}$	$679.3\pm 0.5^{AB}$
60	$537\pm 0.5^{W-Y}$	$641\pm 1^{Q-T}$	$660\pm 0.5^{GH}$	$660\pm 0.5^{G-J}$	$669\pm 0.05^{DE}$	$676.6\pm 1.1^{A-C}$
90	$535\pm 1^{X-Z}$	$639\pm 0.5^{R-T}$	$655\pm 1^{J-M}$	$655\pm 2^{J-M}$	$665\pm 1.5^{E-G}$	$674\pm 1.5^{B-D}$
120	$532\pm 1^{Y-a}$	$635\pm 2^{T-V}$	$653\pm 2^{K-M}$	$652\pm 0.5^{K-N}$	$665\pm 1^{E-G}$	$671.6\pm 3.2^{C-D}$
150	$529.6\pm 1.5^{Z-a}$	$633\pm 2.6^{UV}$	$660\pm 2.5^{G-J}$	$660\pm 2.6^{G-J}$	$660\pm 2.6^{G-J}$	$669\pm 4.3^{D-F}$
180	$527\pm 1^a$	$630.6\pm 1.5^V$	$653\pm 4.3^{K-M}$	$653.6\pm 3.7^{K-M}$	$653.6\pm 1.5^{K-M}$	$665.3\pm 2^{E-G}$

$T_1$ , basal feed (control);  $T_2$ , 50 mg bioactive peptides/kg of feed;  $T_3$ , 100 mg bioactive peptides/kg of feed;  $T_4$ , 150 mg bioactive peptides/kg of feed;  $T_5$ , 200 mg bioactive peptides/kg of feed,  $T_6$ , 250 mg bioactive peptides/kg of feed.

Mean values with different superscripts differ significantly.

All values expressed as mean $\pm$ SD (n=3).

**Table 6. Peroxide value of broiler breast meat during freezing storage at  $-18^{\circ}\text{C}$  for 180 days**

Storage days	Peroxide value (meq/kg of meat)					
	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>	T <sub>5</sub>	T <sub>6</sub>
0	0.82±0.02 <sup>d</sup>	0.79±0.02 <sup>de</sup>	0.77±0.02 <sup>d-f</sup>	0.74±0.02 <sup>d-f</sup>	0.71±0.01 <sup>ef</sup>	0.68±0.02 <sup>f</sup>
30	1.85±0.04 <sup>X</sup>	1.62±0.02 <sup>Y</sup>	1.49±0.02 <sup>a</sup>	1.36±0.02 <sup>b</sup>	1.20±0.02 <sup>c</sup>	1.11±0.02 <sup>c</sup>
60	2.57±0.04 <sup>T</sup>	2.32±0.02 <sup>U</sup>	2.18±0.02 <sup>V</sup>	2.01±0.03 <sup>W</sup>	1.84±0.02 <sup>X</sup>	1.52±0.02 <sup>Za</sup>
90	3.46±0.03 <sup>P</sup>	3.25±0.02 <sup>Q</sup>	3.04±0.02 <sup>R</sup>	2.86±0.04 <sup>S</sup>	1.92±0.02 <sup>WX</sup>	1.60±0.02 <sup>YZ</sup>
120	4.55±0.03 <sup>K</sup>	4.42±0.02 <sup>L</sup>	4.27±0.04 <sup>M</sup>	3.82±0.02 <sup>N</sup>	3.67±0.03 <sup>O</sup>	3.34±0.02 <sup>Q</sup>
150	5.74±0.02 <sup>G</sup>	5.42±0.02 <sup>H</sup>	5.14±0.02 <sup>I</sup>	4.8±0.02 <sup>IJ</sup>	4.64±0.02 <sup>K</sup>	4.55±0.03 <sup>K</sup>
180	7.21±0.04 <sup>A</sup>	6.79±0.02 <sup>B</sup>	6.57±0.03 <sup>C</sup>	6.31±0.02 <sup>D</sup>	6.07±0.05 <sup>E</sup>	5.93±0.01 <sup>F</sup>

T<sub>1</sub>, basal feed (control); T<sub>2</sub>, 50 mg bioactive peptides/kg of feed; T<sub>3</sub>, 100 mg bioactive peptides/kg of feed; T<sub>4</sub>, 150 mg bioactive peptides/kg of feed; T<sub>5</sub>, 200 mg bioactive peptides/kg of feed; T<sub>6</sub>, 250 mg bioactive peptides/kg of feed.

Mean values with different superscripts differ significantly.

All values expressed as mean±SD (n=3).

storage duration of 180 days. Lowest peroxide value (0.68 meq/kg meat) was observed in T<sub>6</sub> at 0 day which gradually increased during storage period (5.68 meq/kg meat) at 180<sup>th</sup> day of storage. The highest POV value (0.82 meq/kg meat) was noticed in T<sub>1</sub> at 0 day which further increased to (7.21 meq/kg meat) at 180<sup>th</sup> day of storage. Decrease in POV values of broiler breast meat was observed through supplementation of broiler feed as compared to control diet. Current studies are supported by Khan et al. (2015) who reported lower POV in broiler meat fed on natural antioxidant enriched diet. These findings are also corroborated by outcomes of Mohamed et al. (2008) who found that antioxidant enrichment of broiler meat reduced POV value while POV increased with storage duration. Kastoe (2013) and Parveen et al. (2015) examined the efficacy of linseed meal enrichment in broiler diet on antioxidant status of broiler meat and found significant difference of treatments on reducing peroxide value of broiler muscles.

### TBARS of broiler breast meat

TBA reactive substances are produced due to oxidation of lipids as result of oxidative stress and comprised of aldehydes, ketones and hydroperoxides. TBARS analysis results are presented in MDA is a compound that produced by degradation of lipid peroxides of polyunsaturated fatty acids. Results regarding TBARS of breast meat from broilers fed on different concentrations of bioactive peptides are displayed in Table 7. The dietary supplementation of bioactive peptides substantially ( $p<0.05$ ) reduced the TBARS of broiler breast meat. A momentous ( $p<0.05$ ) difference among samples from birds fed on various levels of bioactive peptides was noticed. The TBARS content was momentously ( $p<0.05$ ) increased in all treated groups during storage duration of 180 days. Lowest TBARS value (0.13 mg MDA/kg meat) was observed in T<sub>6</sub> at 0 day which gradually increased during storage period (4.56 mg MDA/kg) at 180<sup>th</sup> day of storage. The highest TBARS value (0.21 mg MDA/kg meat) was observed in T<sub>1</sub> at 0 day which further increased to (6.66 mg MDA/kg meat) at 180<sup>th</sup> day of storage. Decrease in TBARS values of broiler breast meat was observed through bioactive peptide supplementation of broiler feed as compared to control diet. The higher values obtained with the TBA test have been attributed to several other lipid oxidation products such as alkenals, alkadienals, other aldehydes, and ketones. During storage of meat products deterioration occurs due to the rancidity resulting from oxidation of triglycerides at double bond site. It has been evident from the previous studies that the oxidation of meat lipids during storage is responsible for huge losses (Sohaib et al., 2017). The results of the instant study are supported by results reported by Selim et al. (2015). Comparable outcomes were reported by Naveena et al. (2008)

**Table 7. Thiobarbituric acid reactive substances of broiler breast meat during freezing storage at  $-18^{\circ}\text{C}$  for 180 days**

Storage days	Thiobarbituric acid reactive substances (mg MDA/kg of meat)					
	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>	T <sub>5</sub>	T <sub>6</sub>
0	0.21±0.01 <sup>U</sup>	0.20±0.01 <sup>U</sup>	0.18±0.01 <sup>U</sup>	0.17±0.01 <sup>U</sup>	0.15±0.01 <sup>U</sup>	0.13±0.01 <sup>U</sup>
30	2.35±1.7 <sup>O-R</sup>	2.29±0.1 <sup>O-S</sup>	2.08±0.06 <sup>Q-T</sup>	1.64±0.04 <sup>R-T</sup>	1.37±0.1 <sup>ST</sup>	1.22±0.05 <sup>T</sup>
60	3.60±0.1 <sup>J-N</sup>	3.34±0.04 <sup>L-N</sup>	3.08±0.07 <sup>M-P</sup>	2.70±0.05 <sup>N-Q</sup>	2.20±0.08 <sup>P-S</sup>	2.05±0.02 <sup>Q-T</sup>
90	4.44±0.04 <sup>E-K</sup>	4.15±0.01 <sup>H-L</sup>	4.02±0.1 <sup>I-M</sup>	3.51±0.2 <sup>K-N</sup>	3.20±0.1 <sup>M-O</sup>	2.95±0.05 <sup>N-Q</sup>
120	5.27±0.3 <sup>B-E</sup>	5.04±0.2 <sup>C-H</sup>	4.94±0.2 <sup>C-I</sup>	4.64±0.08 <sup>D-I</sup>	4.45±0.07 <sup>E-J</sup>	4.26±0.02 <sup>G-L</sup>
150	5.55±0.05 <sup>B-D</sup>	5.37±0.04 <sup>B-E</sup>	5.14±0.04 <sup>B-G</sup>	4.57±0.1 <sup>E-I</sup>	4.29±0.04 <sup>F-K</sup>	4.01±0.6 <sup>L-M</sup>
180	6.66±0.03 <sup>A</sup>	6.06±0.01 <sup>AB</sup>	5.84±0.03 <sup>A-C</sup>	5.35±0.05 <sup>B-E</sup>	5.22±0.03 <sup>B-F</sup>	4.56±0.07 <sup>E-I</sup>

T<sub>1</sub>, basal feed (control); T<sub>2</sub>, 50 mg bioactive peptides/kg of feed; T<sub>3</sub>, 100 mg bioactive peptides/kg of feed; T<sub>4</sub>, 150 mg bioactive peptides/kg of feed; T<sub>5</sub>, 200 mg bioactive peptides/kg of feed; T<sub>6</sub>, 250 mg bioactive peptides/kg of feed.

Mean values with different superscripts differ significantly.

All values expressed as mean±SD (n=3).

who found the positive effect of natural antioxidant substances enrichment in reducing the lipid oxidation of broiler meat. Bioactive peptides have the potential to donate hydrogen from amino acids to break the oxidation chain reaction. Young et al. (2003) reported the decreased MDA production in broiler meat fed on diet supplemented with the ascorbic acid and alpha-tocopherol. Jiang et al. (2009) reported the positive impact of dietary soy isoflavone in reducing the MDA production in broiler meat. Zahid et al. (2018) found the positive impact of incorporation of natural antioxidant substances on oxidation stability of beef patties during cold storage.

### Physico-chemical and quality characteristics of broiler nuggets

The mean values with reference to different physico-chemical parameters such as pH, WHC, color and texture are illustrated in Table 8. The outcomes demonstrated that different feed treatments had substantial effect ( $p<0.01$ ) on pH, color, texture and water holding capacity of broiler breast nuggets. All physico-chemical characteristics of broiler nuggets also affected substantially ( $p<0.01$ ) during storage duration. The combined effect of feed treatments and storage duration also had momentous effect ( $p<0.01$ ) on pH, color, WHC of broiler breast nuggets. The combined effect of feed treatments and storage period did not show any momentous effect ( $p>0.05$ ) on values of shear force for broiler breast nuggets.

Highest pH of breast nuggets at 0 day (6.22) was observed in T<sub>6</sub> which gradually increased to 6.53 during storage period of 45 days. The lowest pH at 0 day (5.72) was observed in T<sub>1</sub>, which further increased to 5.94 at 45<sup>th</sup> day of storage. pH is an important parameter to determine the shelf life, freshness and desirability of poultry meat products. The WHC and yield of meat is also affected by pH of meat. During storage of meat microbial population increased which might be responsible for production of volatile bases by protein breakdown which raise the pH of broiler meat. This situation can be improved with improving antioxidant status of broiler muscles because oxidation of meat is restricted in presence of antioxidant substances. Outcomes of instant research are supported by the results reported by Jang et al. (2008) who described that higher pH in breast meat of broiler fed on plant extract enriched diet was observed as compared to basal diet. Current results are also comparable to the outcomes of Kumar and Tanwar (2011) who found increment in pH during storage period. The increment in pH value might be due to the development of volatile basis by degradation of proteins during storage. Jiang et al. (2009) also found the increment in pH value through dietary supplementation of broiler feed by soy isoflavone as source of natural antioxidant compounds. Edens (2001) also reported the increase in pH value of broiler meat through dietary supplementation

**Table 8.** Physico-chemical characteristics of nuggets during freezing storage at  $-18^{\circ}\text{C}$  for 45 days

	Storage days	Treatments					
		T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>	T <sub>5</sub>	T <sub>6</sub>
pH	0	5.72±0.02	5.83±0.02	5.94±0.02	5.95±0.02	6.04±0.02	6.22±0.04
	15	5.79±0.02	5.89±0.01	6.04±0.02	6.09±0.01	6.14±0.01	6.33±0.03
	30	5.85±0.01	5.94±0.01	5.76±0.5	6.16±0.01	6.21±0.02	6.39±0.02
	45	5.94±0.03	5.96±0.01	6.12±0.03	6.18±0.01	6.26±0.02	6.53±0.02
Water holding capacity (%)	0	80.90±0.5	81.93±0.1	83.20±0.4	84.56±0.2	85.20±0.1	86.23±0.1
	15	80.23±0.3	81.23±0.3	82.30±0.4	83.20±0.2	83.76±0.9	85.50±0.1
	30	78.26±0.1	79.26±0.1	80.467±0.5	81.53±0.2	82.33±0.4	83.53±0.1
	45	74.23±0.1	75.33±0.1	76.40±0.4	77.10±0.1	78.20±0.6	79.53±0.1
Color (CTn)	0	104.3±3	111±1	118.3±2.5	119±2	123.6±2	130.6±1.5
	15	102.3±1.15	104.6±1.15	111.0±1	114±1	115.6±0.5	118.3±1.5
	30	100.0±1	101.0±1	103.0±1	107±1	109.0±1	109.67±1.5
	45	100.0±1	101.0±2	103.0±1.15	106.0±1.73	109.0±1	112.6±1.15
Texture	0	1,143±1	1,135±1	1,131±1	1,126±2	1,118±1.5	1,114±2.5
	15	1,147±2	1,142±2	1,139±1	1,129±1	1,124±1	1,118±1
	30	1,155±1	1,149±2	1,144±2	1,138±1	1,133±1	1,125±2
	45	1,157±1	1,151±1	1,147±1	1,142±1	1,133±1	1,129±1

T<sub>1</sub>, basal feed (control); T<sub>2</sub>, 50 mg bioactive peptides/kg of feed; T<sub>3</sub>, 100 mg bioactive peptides/kg of feed; T<sub>4</sub>, 150 mg bioactive peptides/kg of feed; T<sub>5</sub>, 200 mg bioactive peptides/kg of feed; T<sub>6</sub>, 250 mg bioactive peptides/kg of feed.

Mean values with different superscripts differ significantly.

All values expressed as mean±SD.

of organic selenium.

Highest color value of breast nuggets at 0 day (130 CTn) was observed in T<sub>6</sub> which gradually decreased during storage period (112 CTn) at 45<sup>th</sup> day of storage. The lowest color value 104 CTn at 0 day was observed in T<sub>1</sub>, which further decreased to 100 CTn at 45<sup>th</sup> day of storage. Current study indicated that color values increased with increasing the bioactive peptides supplementation level in feed. The higher color values indicated the red color of nuggets while lower color values indicated brown color of meat products (Arshad et al., 2016). The outcomes of the current investigation are in corroboration with the results of Chandralekha et al. (2012) who described that use of pomegranate based natural antioxidant supplementation in chicken meat balls had positive impact on color of meat product. Same findings were mentioned by Kala et al. (2007) in chicken patties. The color values of stored chicken nuggets decreased during storage which can be ascribed to moisture loss and oxidation of meat proteins. Jiang et al. (2009) reported that dietary soy isoflavone significantly improved the color of broiler meat. Kim et al. (2019) reported that dietary interventions of humic acid and blueberry powder in animal diet significantly improved the color values of meat.

Lowest values for shear force (N) of nuggets from broiler breast at 0 day (1,114) was observed in T<sub>6</sub> which gradually increased during storage period (1,129) at 45<sup>th</sup> day of storage. The highest shear force value at 0 day (1,143) was recorded in T<sub>1</sub>, which further increased to (1,157) at 45<sup>th</sup> day of storage. The shear force required for broiler nuggets fed on supplemented feed was lower than control groups. This decrease can be ascribed to the denaturation of desmin proteins which restrict the removal of moisture from intra and extra myofibrillar space. Bioactive peptides supplementation can delay the oxidation

which can reduce the drip loss improving the texture of broiler meat products. Mean values regarding shear force for breast nuggets stored on freezing temperature increased which can be ascribed to aggregation and cross linking of myofibrillar proteins as oxidation prevails during this duration. The current findings are also validated by results of Moran et al. (2012) who described that the mean values of shear force decreased due to antioxidant supplementation of broiler meat. Current findings are also substantiated by results of Ruiz-Ramirez et al. (2005) who found that texture of meat products become harder and toughness of product increased during storage.

Highest WHC of breast nuggets at 0 day (86.23%) was observed in T<sub>6</sub> which gradually decreased to 79.53% during storage period of 45 days. The lowest WHC at 0 day (80.90%) was observed in T<sub>1</sub>, which further decreased to 74.23% during 45 days of storage. The WHC of nuggets prepared from meat of treated groups was higher than control group. The WHC increased during storage because texture of product become firm with the progress of storage duration ascribed to moisture loss. Rowe et al. (2004) described that antioxidant substances present in meat have impact on proteolysis of meat proteins. WHC of postmortem muscles was affected by pH change protein degradation which initiated with the action of  $\mu$ -calpain (Huff-Lonergan and Lonergan 2005). Jiang et al. (2009) reported the 17% increase in WHC of broiler meat fed on 40 mg soy isoflavone/kg of feed. Likewise, the outcomes of current investigation are also confirmed by the results of Ruiz-Ramirez et al. (2005) who described that texture of meat patties became harder and WHC was reduced with the course of time during storage duration. Delles (2013) also described the improvement in WHC of broiler breast meat during storage fed on tocopherol and selenium-based antioxidants.

## Conclusion

Dietary supplementation of bioactive peptides produced from fish waste demonstrated favorable impact on reducing lipid oxidation than control treatment. Among different treatments the group fed on highest level of bioactive peptides i.e. 250 mg/kg feed, showed higher TPC, DPPH, FRAP values and significantly lower peroxide value and MDA content during freeze storage of 180 days. Likewise, dietary interventions of bioactive peptides in broiler feed also improved quality parameters (pH, color, texture, WHC) of broiler nuggets. The results demonstrated that application of dietary bioactive peptides can be potential substitutes to commercial antioxidant substances, for enhancing the shelf-stability of poultry meat during frozen storage. In future the development of strategies to use natural dietary antioxidant substances in animal feed requires more attention of researchers.

## Conflicts of Interest

The authors declare no potential conflict of interest.

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## Author Contributions

Conceptualization: Aslam S, Shukat R, Khan MI, Shahid M. Data curation: Aslam S, Shukat R, Shahid M. Formal analysis:

Aslam S, Shukat R, Khan MI. Methodology: Aslam S, Shukat R. Software: Khan MI. Validation: Khan MI, Shahid M. Investigation: Aslam S, Shukat R, Khan MI, Shahid M. Writing - original draft: Aslam S, Shukat R. Writing - review & editing: Aslam S, Shukat R, Khan MI, Shahid M.

## Ethics Approval

The research has been approved by Institutional Animal Care and Use Committee (IACUC) of University of Agriculture, Faisalabad (UAF-IRB-3774).

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