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Fish Burger Quality Treated by Pomegranate Peels Powder During Cold Storage.

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ABSTRACT

The Kapreeta fish is rejected in the Egyptian market due to its dark bloody flesh and the presence of many blood vessels. To overcome this problem, this study carried out a trial to utilize Kapreeta fish in manufacturing fish burgers to provide a desirable and acceptable product for Egyptian consumers. Four fish burger samples were prepared by the addition of 0.0, 0.5, 1.0, and 1.5 % of pomegranate peels powder. The control and treated samples were stored at 4±1°C for 12 days and were analyzed afterwards every 3 days. Moisture, protein, and pH values were significantly decreased ($P \leq 0.05$) with the increase in the duration of the cold storage period. While, a significant increase ($P \leq 0.05$) was recorded in lipid, ash, and carbohydrate with increasing the storage period. After 12 days of storage, The values of cooking loss, TVB-N, TMA-N, and TBA showed a significant increase ($P \leq 0.05$). Sensory results detected a gradual decrease in flavor and overall acceptability for all samples. The obtained data revealed that control samples (T1) had the lowest scores from the start to the end of cold storage, while the fish burger samples (T4), which contained 1.5% pomegranate peel powder, had a higher score compared to all samples.

INTRODUCTION

Fish products are very important for human nutrition. Nowadays, as parallel to the increasing business tempo, the consumption of food as “heat and eat” has become important. Hence, food sector has become one of the most important sectors. . Frozen technology is important for the preservation of productswith respect to long storage, among which seafood is considered. Several studies conducted on fish fingers of different species, have determined the chemical composition, quality parameters, sensory evaluation and microbiological load **(Sehgal & Sehgal, 2002; Çakli *et al.*, 2005; Tokur *et al.*, 2006)**. Application of plant extracts in fish products as natural antioxidants have been attempted by different researchers. By-products of food processing contain valuable substances, such as fibres, pigments, sugars, organic acids, flavours, antimicrobial and antioxidant substances. Fish processing industry is widely spread and quite varied in terms of types of operations, scales of production and outputs. The current fish treatment technology requires to introduce new products based on fish mince which are acceptable,

nutritious and stable. Fish in the diet not only develops the nourishment quality but also results in the excess of meal consumption. Thus, fish products, as seasoning in ready-to eat form,have good properties **(Reddy *et al*., 2012)**. Fish burgers are common satisfactory fast foods and have been widely distributed in the world food market; therefore, the quality of this product has won the interest of many researchers **(Tokur *et al*., 2004, 2006; Al-Bulushi *et al*., 2005; Hassab Alla *et al*., 2009)**. Burgers from catla fish (*Catla catla*) were assessed for proximate, chemical, microbiological and sensory quality changes over 17 days of refrigerated storage at 4 (±1)°C (**Vanitha *et al*., 2015)**. The present study was carried out to utilize kapreeta fish in manufacturing fish burger supplemented with pomegranate peels powder as a natural antioxidant and evaluate the quality properties during cold storage.

MATERIALS AND METHODS

Preparation of pomegranate peel powder

Mature and healthy pomegranate (*Punica granatum)* fruits were washed, cut manually, and the peel of pomegranate was excised and dried in an air-oven dryer (DHG-9140A; Yiheng Instrument Co., Ltd., Shanghai, China) at 50±1°C for 16h. The dried peel was ground using moulinex peeler, passed through a 40-mesh sieve and packed in polyethylene bags and then stored at -18°C until further usage **(Qin *et al*., 2013)**.

Preparation of fish burger samples

Fish burgers were processed from the samples of fresh Kapreeta fish. The commercial style dressing as described in the study of **Francisco *et a1*. (1972)** was followed including the removal of head, viscera, skin and backbone. Fish fillets were minced after removing the traces of blood from flesh fillets and cleaned with tap water. After being strained, fish flesh was mixed according to the recipe presented in Table (1) **(Chandrasekhar & Mohite, 1978)**. Some modifications were added, such as evaluating the effect of pomegranate peel powder as a natural preservative, preparing dried pomegranate peels powder and using itwith ratios of 0.0, 0.5, 1.0 and 1.5 % of the total fish burger recipe. Every portion was mixed continuously for another five minutes to ensure equal distribution of spices and salt. After mixing, the meat batch was transferred to a Hollymatic Patty maker. For burger patties, each of which hadweight of 75 grams. The control and the treated samples were then packed in polyethylene bags, chilled in a refrigerator at 4°C and stored for 12 days. During the refrigeration storage, the control and the treated samples were examined on day 0, 3, 6, 9, 12 for physical, chemical and microbiological deteriorative criteria.

Table 1. Ingredients and recipe of spices mixture used in burger preparation.

Ingredient	%	Spices mixture 2.00%	
Fish meat	75.00	Coriander	5.00
Fat	9.00	Cubeb	2.00
Starch	8.00	Cumin	23.00
Sodium bicarbonate	0.40	Black pepper	42.00
Salt	2.30	Red pepper	1.00
Onion	2.50	Cardamon	2.00
Sodium polyphosphate	0.30	cloves	2.00
Garlic	0.50	Ginger	5.00
Spices mixture	2.00	All spices	18.00

Physiochemical and chemical analysis

Moisture, crud protein, fat and ash contents of Kapreeta fish burger were determined as described by **A.O.A.C. (2012)**. The pH value was measured following the method of **Egbert *et al*. (1992)**. Total volatile basic nitrogen (TVB-N) was determined according to the method of **Pearson (1976)**. Trim ethylamine-nitrogen (TMA-N) was assayed as described in **A.O.A.C. (2012)**. Thiobarbituric acid (TBA) was determined using spectrophotometric ally according to the procedure of **Tarladgis *et al.* (1960)**.

Microbiological examination

The total viable bacterial count (TVBC) and total spore forming bacterial count were determined using nutrient agar medium **(Oxoid, 2006)**. Yeasts and molds counts were determined in accordance to **ISO 21527-2 (2008)**.

Sensory evaluation

The sensory evaluation of fried fish burger samples were evaluated by 10 panelists from National Institute of Oceanography and Fisheries, El-Kanater El-Khiria branch. A 9 point hedonic scale was employed in this sensory analysis as described by **Teeny and Miyaauchi (1972)**.

Statistical analysis

Data were expressed as the mean values of three replicates. Standard deviations were statistically analyzed by performing analysis of variance technique (ANOVA), using the statistical analysis system according to **SAS (2008)**. Differences among means were compared using Duncan's multiple range test **(Duncan, 1955)** at significant level of 95% ($P \leq 0.05$).

RESULTS AND DISCUSSION

The proximate chemical composition was determined with respect to different fish burger samples prepared by the addition of 0.0, 0.5, 1.0 and 1.5 % pomegranate peels powder and stored at 4±1°C for 12 days. The moisture content of fish burger control samples (T1), prepared without pomegranate peel powder was 60.08 %. While, it showed

values of 60.07, 60.24 and 60.59 % for fish burger samples prepared with the addition of 0.5, 1.0 and 1.5 % of pomegranate peel powder at the beginning of the storage period, respectively. As the storage period increased, the moisture content of different prepared fish burger samples was significantly decreased ($p \leq 0.05$). It was noticed that, the control fish burger sample showed the highest decline in moisture content reaching a value of 52.48 % compared to 54.05, 54.70 and 54.92% for the other prepared fish burger samples at the end of the storage period. A significant decrease ($p \leq 0.05$) in moisture content of prepared fish burger samples could be noticed as a result of the addition of pomegranate peel powder.

The loss of the moisture content of fish burger during the cold storage may be due to the decrease of protein solubility, and subsequently the reduction of water holding capacity of fish burger samples. These results coincide with those of **Habbal (2000)** and **Gomma (2005)**. Moreover, results showed that, the addition of different ratios of pomegranate peels powder limited the reduction in moisture content and improved water holding capacity.

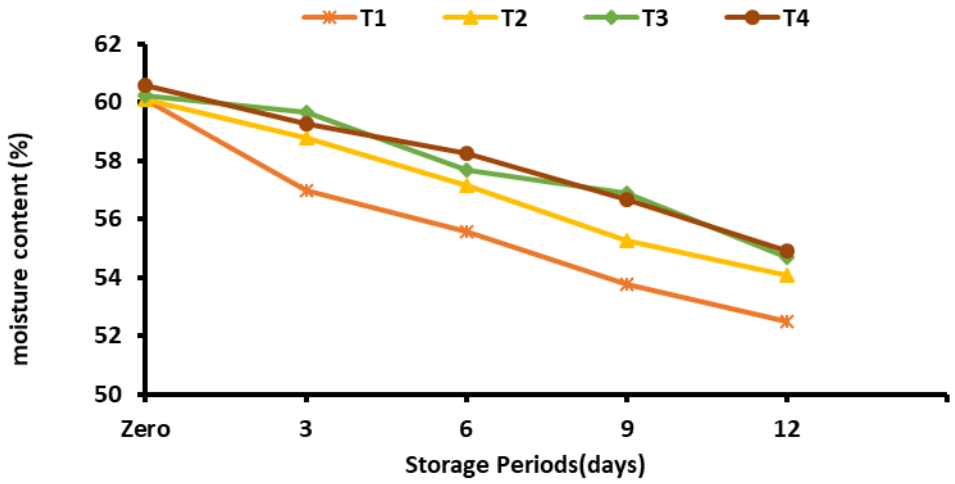

Fig. 1. Moisture content (%) of fish burger samples during cold storage at 4±1ºC

As the storage period increased, the protein content of different prepared fish burger samples decreased significantly ($p \leq 0.05$). It was observed that, the control samples (T1) recorded the highest decline of protein content, with a value of 14.24% compared to 15.17, 15.56 and 16.08 % for prepared fish burger which contain 0.5, 1.0 and 1.5 % of pomegranate peel powder, respectively, at the end of the storage period. The reduction in protein content of the prepared fish burger samples during the storage period could be attributed to the loss of soluble and volatile amino compounds associated with protein as a result of loss in water content of fish burger samples. In conclusion, fish burger prepared with pomegranate peel powder T3 and T4 showed higher protein content compared to the control samples after 12 days of cold storage.

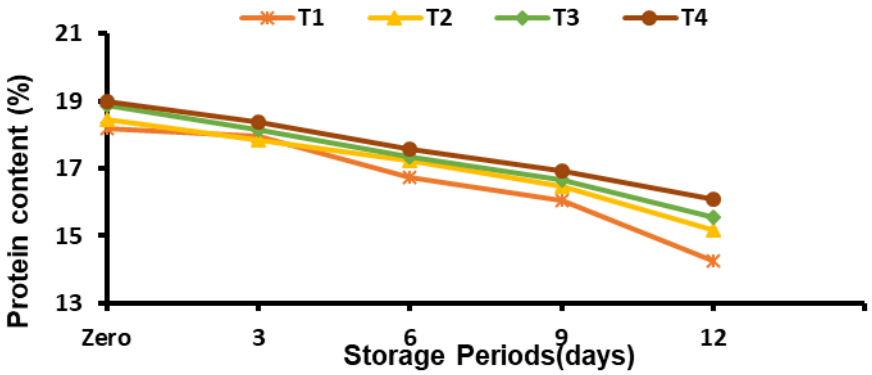

Fig. 2. Protein content (%) of fish burger samples during cold storage at 4±1ºC.y;

Fat content of prepared fish burger samples at the beginning of storage period was not significantly affected by the addition of different concentrations of pomegranate peel powder as its values was around 8.38 to 9.17 %. At the same time, as the storage period increased, the fat content of different prepared fish burger samples showed significant increase ($p \leq 0.05$). This trend of fat content for stored fish burger samples may be explained by the autolysis of lipoprotein to protein and lipid which led to increase the ether extract (fat content). Since the values of fat content were 10.48, 10.26, 9.81and 9.33% for prepared fish burger samples containing 0.0, 0.5, 1.0 and 1.5 % of pomegranate peel powder; respectively. These results showed a good accordance with those obtained by **lhm *et al.* (1992)** and **Taşkaya, *et al.*, (2003).**

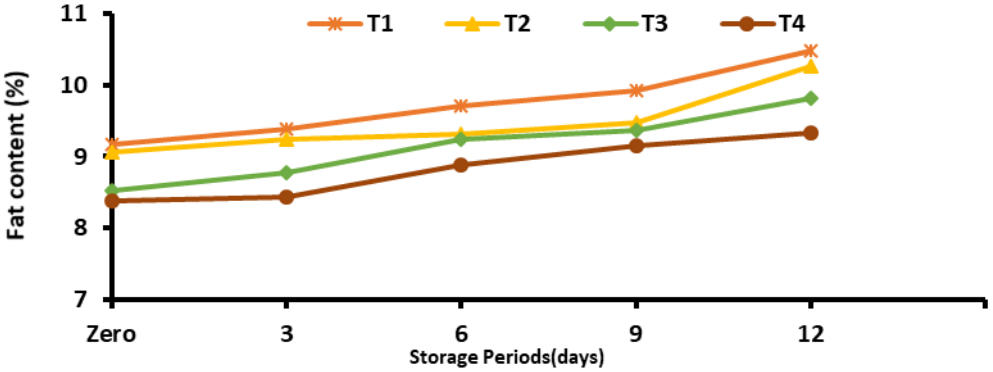

Fig. 3. Fat content (%) of fish burger samples during cold storage at 4±1ºC

Ash content of the prepared fish burger samples at the beginning of the storage period was significantly affected by the addition of different concentrations of pomegranate peel powder as its values were around 2.24 to 2.59 %. As the storage period increased, the ash content of different prepared fish burger samples showed a significant increase ($p \leq 0.05$). It is worth mentioning that, the values of ash content were 5.23, 5.37, 4.60 and 4.37% for the prepared fish burger samples containing 0.0, 0.5, 1.0 and 1.5 % of pomegranate peel powder, respectively.

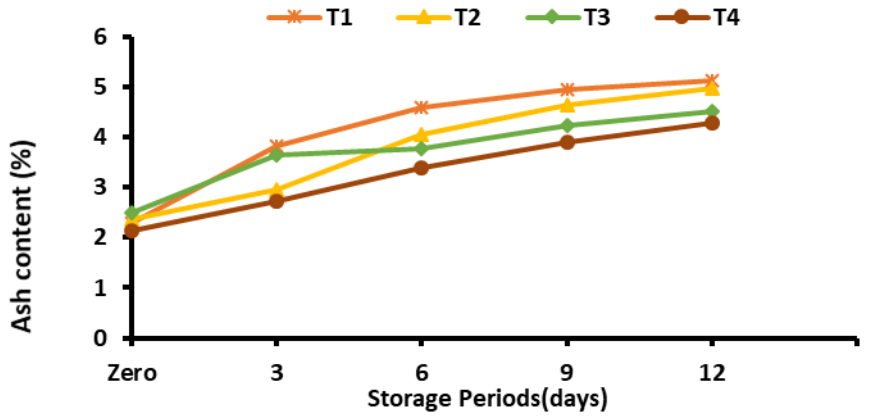

Fig. 4. Ash content (%) of fish burger samples during cold storage at 4±1ºC

Carbohydrate content of fish burger samples containing different concentrations of pomegranate peel powder were calculated by the difference of moisture content, protein content, fat content and ash content. The addition of pomegranate peel powder during the preparation of fish burger led to a significant increase ($p \leq 0.05$) in total carbohydrate values. Their values were 10.19, 9.96, 9.76 and 9.82 % for the prepared fish burger samples containing 0.0, 0.5, 1.0 and 1.5 % of pomegranate peel powder at the beginning storage period, respectively. At the same time, as the storage period increased, the values of total carbohydrates increased significantly.

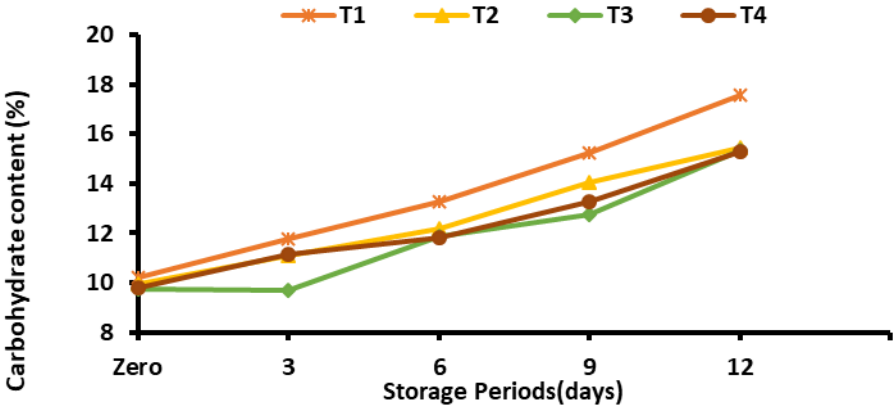

Fig. 5. Carbohydrate content (%) of fish burger samples during cold storage at 4±1ºC

The cooking loss and the cooking yield (%) of different prepared fish burger containing (0.0, 0.5, 1.0 and 1.5 %) pomegranate peel powder were evaluated. The cooking loss was studied for the relation between this factor and fish tenderness, and the effect of cold storage period at 4±1°C for 12 days on these parameters. The cooking loss of fish burger samples increased from 9.20, 8.09, 7.87and 6.30% at zero time to record 18.32, 17.52, 16.77and 14.59% after 12 day of cold storage regarding T1, T2, T3 and T4, respectively. The cooking loss was significantly increased ($p \leq 0.05$) as cold storage period progressed. The cooking yield of fish burger samples decreased from 90.80, 91.91, 92.13

and 93.70% at zero time to 81.68, 82.48, 85.41and 83.41% after 12 days of cold storage with respect to T1, T2, T3 and T4, respectively. All samples of fish burger showed a remarkable significant decrease ($p \leq 0.05$) in cooking yield during cold storage at 4±1°C for 12 days. Such behavior could be explained by the damage in starch and dietary fibre granules present in fish burger and different concentration of pomegranate peel powder upon cold and cooking processing, reducing capability of starch and dietary fibres in pomegranate peel powder to absorb high amount of water and swelling during cooking causing slightly losses of moisture to the heating media. These results concur withthose of **Naveena *et al.* (2008)**, **Devatkal *et al.* (2010)**, **Qin *et al.* (2013)** and **Cao *et al.* (2016)**.

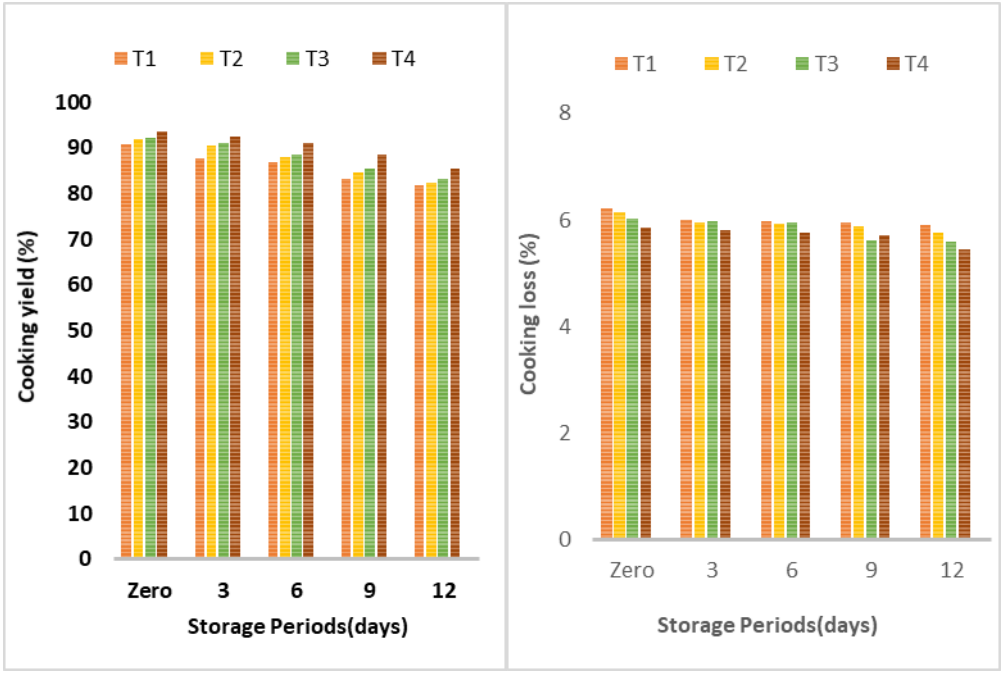

Fig. 6. Cooking loss and cooking yield content (%) of fish burger samples during cold storage at 4±1°C

Significant differences ($p \leq 0.05$) were observed in pH values of different prepared fish burger containing 1 and 1.5 % of pomegranate peel powder. During the storage period, the pH values of the studied samples decreased, with no significant effect ($p \geq 0.05$) on fish burger samples containing different ratios of pomegranate peel powder. On the contrary, the pH of the control (T1) fish burger sample, T2, T3 and T4 decreased during the storage period without significant effect ($p \geq 0.05$). The slight decrease in pH values during cold storage period in different burger samples might be attributed to the breakdown of glycogen with the formation of lactic acid. These results agree with the findings of **Devatkal *et al.* (2010)**, **Erkan *et al.* (2011)**, **Jasour *et al.* (2011)** and **Qin *et al.* (2013)**.

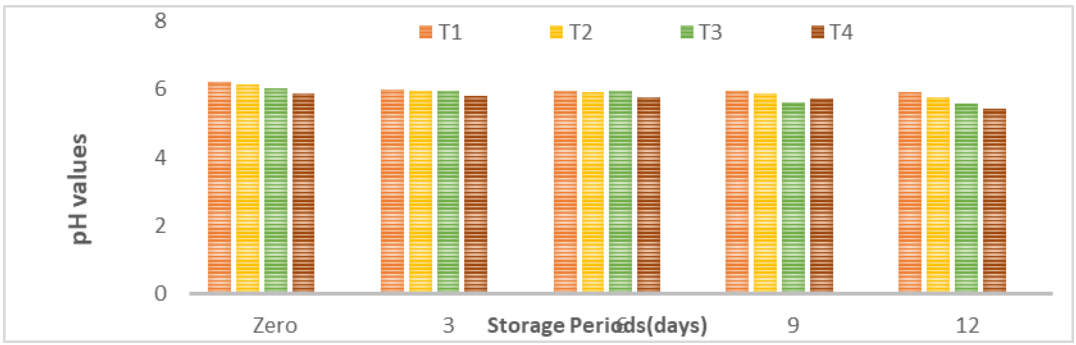

Fig. 7. The pH values of fish burger samples during cold storage at 4±1ºC

TBA values of different fish burger samples prepared by the addition of 0.0, 0.5, 1.0 and 1.5 % pomegranate peel powder and stored at 4±1 °C for 12 days are given in Fig. (8). The positive effect of addition of pomegranate peel powder as a natural antioxidant was noticed with significant differences ($p \leq 0.05$) in TBA values of prepared fish burger samples containing 1 and 1.5% pomegranate peel powder in comparison to the control fish burger (T1) prepared without the addition of pomegranate peel powder. The TBA values of sample T2 showed a decrease until day 6, then they increased at the end of the storage period. TBA values were 1.33, 1.28, 1.23 and 1.19 mg of malonadehyde/kg sample for the prepared fish burger samples, respectively, at the beginning of the storage period and reached 3.36, 1.46, 0.96 and 0.89 mg, respectively, at the end of the storage period (after 12 days of storage at 4±1°C). Therefore, pomegranate peel powder could be used as a natural antioxidant for preventing lipid oxidation in meat and fish products. This result could be correlated to the presence of phenolic compounds in pomegranate peel powder. The afore-mentioned results are in agreement with the results of **Naveena *et al.* (2008)**, **Devatkal *et al.* (2010)** and **El-Gharably and Ashoush (2011)**. They reported that pomegranate peel powder improved the storage stability of meat products, especially at cold storage by reducing the rate of lipid oxidation expressed as TBA values.

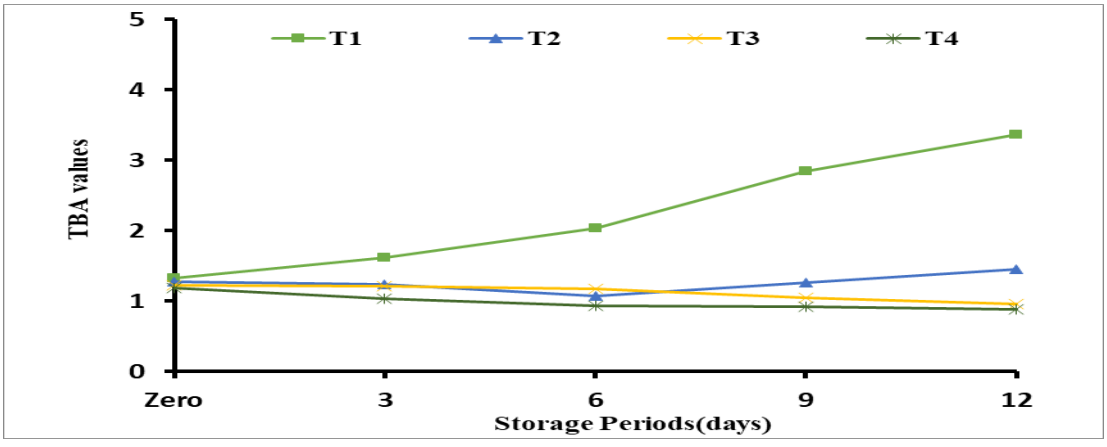

Fig. 8. TBA values of fish burger samples during cold storage at 4±1ºC

Total volatile basic nitrogen (TVB-N) is a useful parameter to detect spoilage in fresh and lightly preserved seafood **(Dalgaard, 2000)**. It is known as a product of bacterial spoilage and endogenous enzymes action; its content is often used as an index to assess the keeping quality and shelf life of products **(EEC, 1995)**. Remarkably, a level of 35 to 40 mg TVB-N/100 g of fish muscle is usually regarded as spoiled **(Lakshmanan, 2000).**

The total volatile basic nitrogen content of fish burger samples was determined at several time intervals during the cold storage for 12 days and the results were expressed in terms of mg TVB-N/100g sample on wet weight basis. The obtained data in Fig. (9) show that, TVB-N content was gradually and significantly increased ($p \leq 0.05$) during the storage of the studied samples. The TVB-N values of fish burger samples increased from 16.94, 16.46, 14.93 and 14.64 mg/100g at zero time to 35.42, 31.33, 28.19 and 25.42 mg/100g after 12 day of cold storage for T1, T2, T3 and T4, respectively.

These results indicate the significant ($p \leq 0.05$) positive effect of the addition of pomegranate peel powder on the inhibition of microbial growth, especially proteolytic microorganisms that cause the breakdown of protein resulting in volatile nitrogen compounds. The increase in TVB-N during the cold storage of burger products may be attributed to the breakdown of nitrogenous substances by microbial activity.

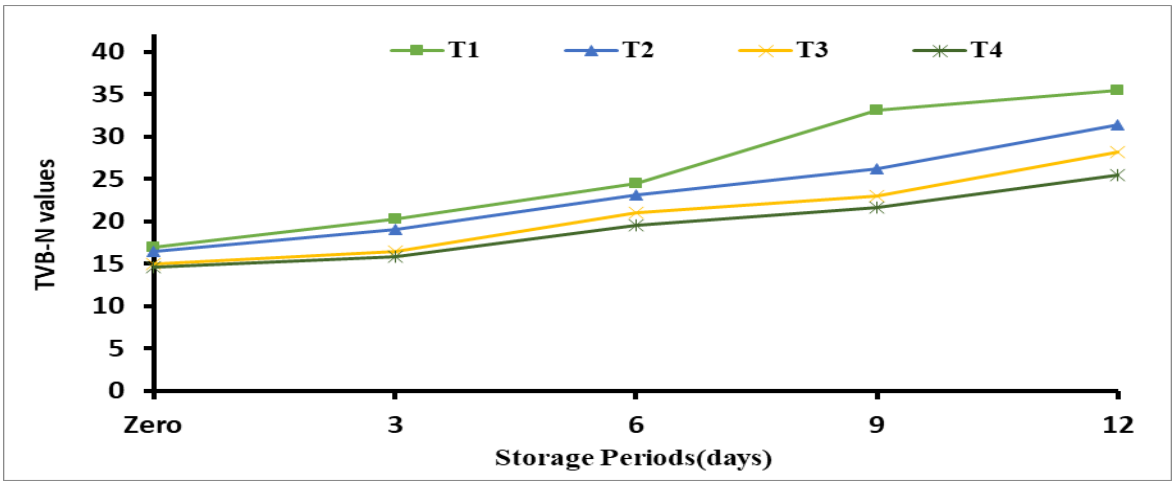

Fig. 9. Total volatile basic nitrogen values of fish burger samples during cold storage at 4±1ºC

The obtained data in Fig. (10) indicate that, TMA-N content was gradually with no significant increase ($p \geq 0.05$) during the storage of different prepared fish burger samples. The TMA-N values of fish burger samples increased from 3.33, 2.68, 2.39 and 2.09mg/100g at zero time to 9.69, 7.86, 6.82 and 6.48 mg/100g after 12 day of cold storage in T1, T2, T3 and T4, respectively.

Trimethylamine (TMA) resulting from the bacterial reduction of Trimethylamine oxide (TMA-O) is associated with the fishy odor of spoiling seafood **(Ozogul *et al.*, 2006)**. The acceptable limit of TMA value for fish and fish products is stated as 5-10

mg/100 g **(Huidobro *et al.*, 2002; Taşkaya *et al.*, 2003)**. While, TMA-N limits of 10-15 mg/100 g were determined in the study of **Connell (1990)**. The obtained results of **Özer *et al.* (2012)** showed that TMA-N of burger did not exceed the legal limit of 8 mg/100g for muscle **(FAO, 1986)** during 56 days under vacuum cold storage conditions at 4ºC and did not show any significant variations ($p>0.05$).

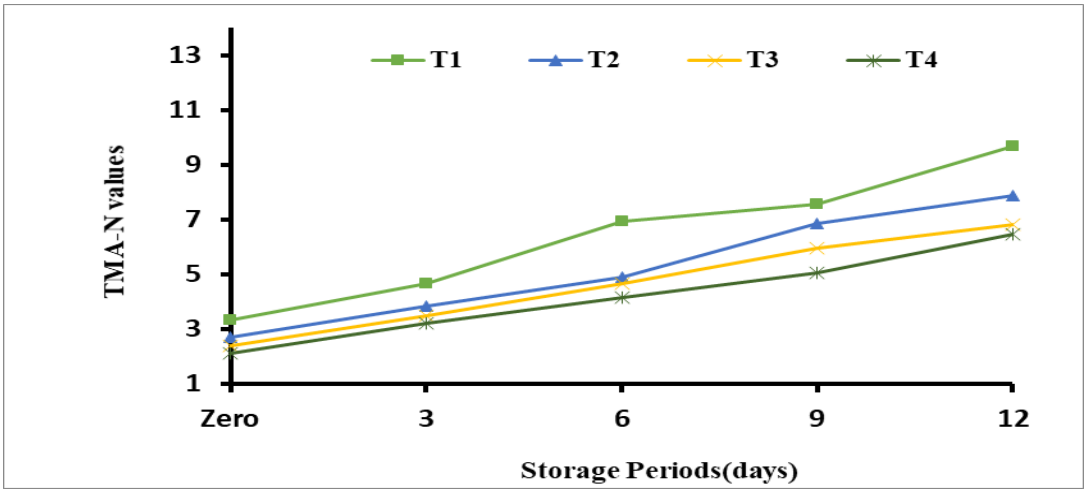

Fig. 10. Trimethylamine (TMA) values of fish burger samples during cold storage at 4±1ºC

Fish burger samples were analyzed for total bacterial counts, yeast & mold counts and spore forming bacterial counts (Table 2). Total plate count values of different prepared fish burger ranged from 4.11 to 4.46 log cfu/g at the beginning of the storage period, while a gradual increase could be noted in the total plate count of the control fish burger samples, reaching 5.48 log cfu/g at the end of the storage period.

On the other hand, the obtained data revealed that, the other prepared fish burger samples which contained different ratios of pomegranate peel powder 0.5, 1.0 and 1.5 % showed a progressive reduction in the total bacterial count over the time of the storage period; where, the total plate counts reached 3.81, 3.48 and 3.32 log cfu/g, respectively. These results could be due to the antimicrobial effect of pomegranate peel powder, especially when the concentration of pomegranate peel powder was increased. The afore-mentioned results were also observed for all other tested microbial criteria, where counts of yeast and mold and spore forming bacteria were reduced during the storage period for all the prepared fish burger containing different concentrations of pomegranate peel powder in comparison with the control fish sample prepared without the addition of pomegranate peel powder. The microbial counts were gradually decreased as the storage period increased. The observed results match with those of **Al- Zoreky (2009)**, **Kanatt *et al.* (2010)** and **Agourram *et al.* (2013)**, who evaluated the antimicrobial characteristics of pomegranate peel and found that pomegranate peel have an inhibition effect against Gram positive and Gram negative bacteria.

Table 2. Microbiological analysis of kapreeta fish burger samples during cold storage at 4±1ºC

Storage Period (day)	Total plate counts (log cfu/g)			
	T1	T2	T3	T4
Zero	4.46	4.34	4.22	4.11
3	4.51	4.25	4.12	3.93
6	4.71	4.06	3.89	3.66
9	5.22	3.94	3.61	3.45
12	5.48	3.81	3.48	3.32
Yeast and mold counts (log cfu/g)				
Zero	2.57	2.67	2.39	2.15
3	2.61	2.62	2.26	1.95
6	2.68	2.57	2.05	1.77
9	3.06	2.46	1.93	1.65
12	3.22	2.42	1.78	1.45
Spore forming bacterial counts (log cfu/g)				
Zero	1.64	1.62	1.58	1.61
3	1.65	1.58	1.51	1.43
6	1.78	1.54	1.39	1.28
9	1.91	1.43	1.33	1.17
12	2.01	1.34	1.17	0.98

Sensory evaluation is an important indicator of potential consumer preferences. In spite of its shortcomings, it will remain the most serious quality assessment technique for meat products and other foods

Sensory qualities, color, tenderness, juiciness, flavor and overall acceptability of prepared fish burger samples containing different ratios of pomegranate peel powder were evaluated and the results are presented in Fig. (11). It could be noticed that, the addition of pomegranate peel powder had no significant effect ($p \geq 0.05$) on improving the sensory qualities of the prepared fish burger product, but their acceptability was increased during storage. In general, as concentration of pomegranate peel powder increased, the acceptability of prepared fish burger samples was also increased.

The obtained data revealed that, the control samples (T1) had the lowest scores from the start till the end of the cold storage. While, the fish burger samples (T4), which contained 1.5% pomegranate peel powder had a higher score than all sample.

The application of different concentrations of pomegranate peel powder improved the cooking characteristics, e.g. cooking loss and cooking yield. At the same time, the utilization of the investigated pomegranate peel powder could be useful to achieve high stability of fish burger during refrigeration storage without any negative effects on the sensory characteristics of the product.

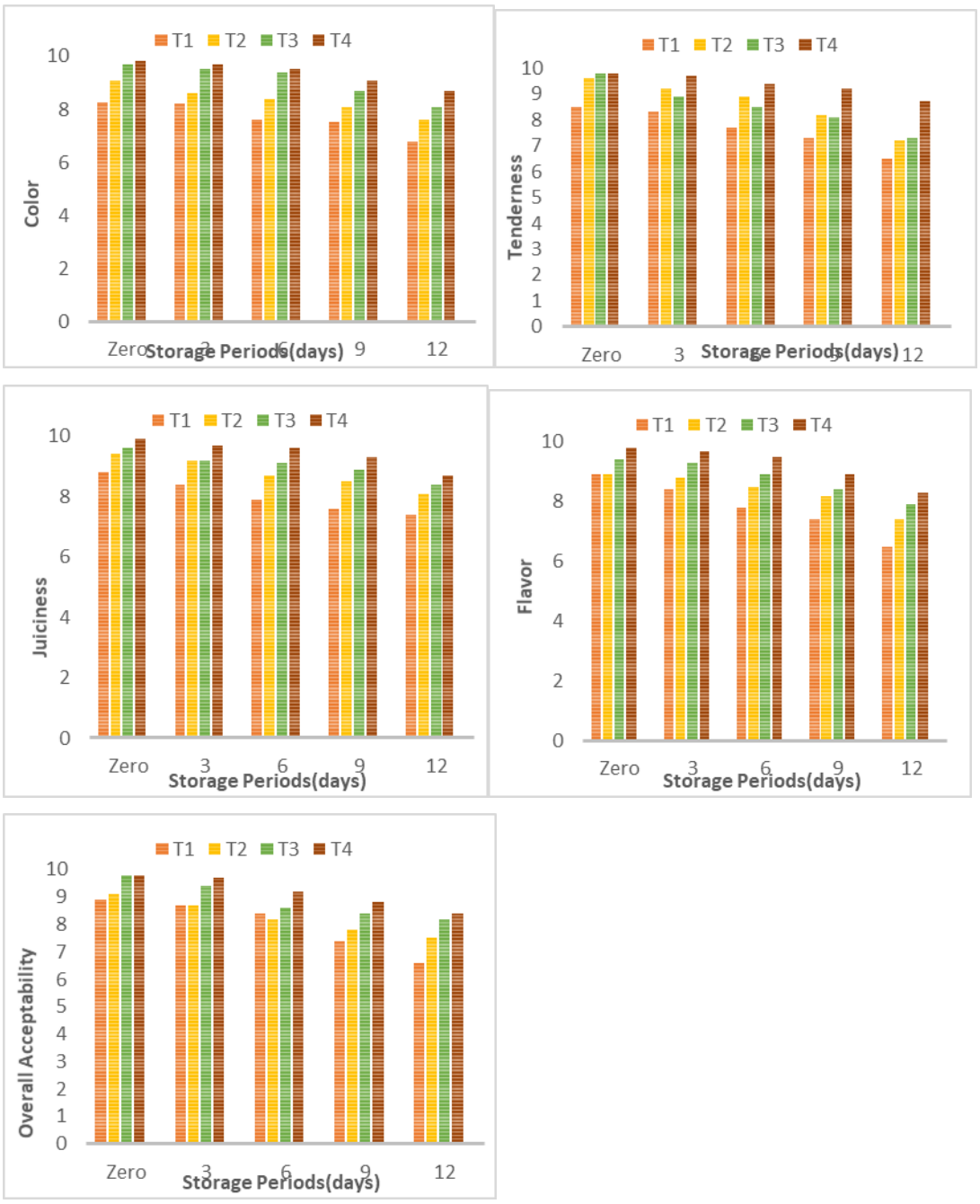

Fig. (11). Sensory qualities values of fish burger samples during cold storage at 4±1ºC

CONCLUSION

The obtained data revealed that, control samples (T1) had the lowest scores from the start to the end of the cold storage, while the fish burger samples (T4) which contained 1.5% pomegranate peel powder had a higher score than all samples. The application of different concentrations of pomegranate peel powder improved the cooking characteristics, e.g. cooking loss and cooking yield. At the same time, the utilization of

the investigated pomegranate peel powder could be useful to achieve high stability of fish burger during refrigeration storage without any negative effects on the sensory characteristics of the product.

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