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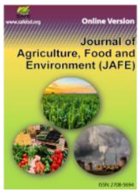

Original Article

Physico-chemical, microbiological and organoleptic assessment for the stored frozen products of tuna

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A B S T R A C T

The current study aimed to evaluate the physico-chemical, microbiological and organoleptic characteristics of proposed tuna (*Thunnus thynnus*) products during the storage under freezing conditions. Tuna products of burger, nuggets and fingers were prepared and stored at -18°C for six months. The above-mentioned characteristics were measured every two weeks as a time interval during the storage period. The moisture content significantly decreased over the storage time (3.11±0.83 g every two weeks). As a result, at the end of storage time (6 weeks), the loosing ratios in moisture content for burger, nuggets and fingers were 15.87, 16.03 and 15.84 (%), respectively. Heavy metals content in tuna was arranged in a descending order as iron> copper> lead> cadmium> mercury, with values being 27.7, 6.30, 2.035, 1.214, and 0.547 mg/kg fresh weight, respectively. There were no significant differences observed among the levels of the toxic metals (Hg, Pb and Cd) in raw tuna meat and its products. The results of microbiological analysis revealed that the total bacterial count and numbers of *Salmonella sp*. and *E. coli* were decreased over time under freezing condition. Except for firmness and gumminess, the texture characteristics of tuna products showed no significant changes during the storage period. The results concluded the stability and safety of tuna burger, nuggets and fingers under freezing conditions besides the high acceptability for these products between the consumers.

Introduction

Tuna is one of the most consumed fish worldwide and it is marketed as chilled, frozen, smoked, canned, breaded and battered, salted and dried forms (Murthy *et al*., 2014). Tuna meat has numerous health benefits due to its high-quality protein content, low saturated fatty acids and high content of omega-3(n-3) fatty acids which known to support human health (Rodriguez-Mendivil *et al*., 2019). Omega-3 fatty acids have known to reduce cholesterol levels, which is known by the hypocholesterolic effect (anti-atheriosclerosis), consequently reduces the incidence of heart diseases and stroke (Patterson, 2002; Daviglus *et al*., 2002; Ikem and Egiebor, 2005). Additionally, tuna meat is a very rich source of vitamins, A, B12, and D, and essential minerals such as calcium, phosphorus, iron, iodine, and fluorine (Ismail, 2005). As a result, it is highly recommended to consume tuna meat and its products to acquire the above-mentioned health benefits. In Egypt, canned salted tuna meat is the most commonly consumed form of tuna products. However, other meat based products; such as burger, sausages, nuggets, hot dogs and salami, are basically depended on cattle and poultry meat. Therefore, the current study focused on the assessment of the physico-chemical, microbiological and organoleptic characteristics of tuna meat and its proposed products at zero time (fresh) and during the storage under freezing condition prior to be introduced to the Egyptian market as new products. Consequently, these evaluation data of the nutritive value, microbial load and safety, texture stability, and other quality characteristics will draw the consumer's attention and encourage him to increase his consumption form these fish based healthy products.

Materials and Methods

1. Materials

1.1. Tuna fish

Bluefin tuna (*Thunnus thynnus*) was captured from Burullus Center in Kafr El-Sheikh Governorate, Egypt.

1.2. Commercial fish burger, nuggets and fingers

These fish products were purchased from a local market at Mansoura city, Egypt.

1.3. Vegetables

Garlic, red pepper, shallots, and yellow pepper were purchased from a local vegetable market at Mansoura city.

1.4. Other ingredients

Breadcrumbs, butter, olive oil, wheat flour, vinegar, corn flour, salt, egg, cumin, white pepper and black pepper were also purchased from a local market at Mansoura city.

2. Methods

2.1. Preparation of tuna products

Tuna samples were immediately transferred to the laboratory, carefully washed under the tap water, cut into small pieces, and minced prior to be manufactured into products.

2.2. Preparation of tuna burger

Tuna burger was prepared based on the method of Bochi *et al*. (2008) with some modifications. The fresh minced fish meat (200g) was transferred to a mixing bowl containing wheat flour (35g), corn flour (5g), salt and white pepper (3g for each). The burger mixture was blended very well and was formed in a layer with a 1 cm of thickness, then kept in a deep-freezer set at -18ºC for 1 hour. After that, the layer was cut into equals squares and immersed in whiskered eggs followed by the coverage with seasoned breadcrumbs. Tuna burger was collocated in a cork dish then covered with polyethylene and stored frozen at -18ºC.

2.3. Preparation of tuna nuggets

The nuggets formulation was done based on Ulfah *et al*. (2016) with modifications. The fresh minced tuna meat (200g) was transferred to the mixing bowl. Wheat flour (35g), corn flour (5g), salt (3g), white pepper (1.5g) and cumin (2g) were added to the fish meat and the mixture was well blended. The nuggets mixture was straightened to a 1 cm of thickness, and then was kept in a freezer set at -18ºC for about 1 hour. Following that, the nuggets layer was cut into equal small squares and immersed in whiskered eggs then covered by seasoned breadcrumbs. Fish nuggets were collocated in a cork dish then covered with polyethylene and stored frozen at -18ºC.

2.4. Preparation of tuna fingers

The formulation was based on Cakli *et al*. (2005) with modifications. Cleaned fresh fish meat (240g) turned into the mixing bowl. Wheat flour (35g), corn flour (5g), salt (3g), white pepper (1.5g), olive oil (10g), vinegar (5g) and black pepper (2g) added to the tuna meat. Tuna mixture was straightened with 1 cm thickness, and then was cut into equal small fingers which were put in a whiskered egg then put in seasoned breadcrumbs. Tuna fingers were collocated in a cork dish then covered with polyethylene and stored in a freezer set at -18ºC.

2.5. Physico-chemical properties

The pH values were measured as described by Jackson (1967). While acidity values were determined as citric acid by titration with 0.1 N sodium hydroxide after adding a few drops of phenolphthalein as an indicator according to AOAC (2000). Water holding capacity (WHC) and plasticity (in cm2) were determined as described by Russo *et al*. (1999) and Traynham *et al*. (2007).

2.6. Gross chemical composition of tuna samples

Moisture, ash, crude protein, crude fiber and total crude fats were determined as described by (AOAC, 2000). While total carbohydrates were determined by difference as follows: % carbohydrates = 100 - (% moisture + % protein + % fat + % ash) (Gul and Safdar, 2009).

2.7. Mineral’s content

Heavy metals in tuna meat and its products were determined according to Sneddon *et al*. (2006). Briefly, 50g sample was digested in a microwave digestion system using 5 mL of HNO_3 (65%) and 2 mL of H_2O_2 (30%). Metal concentrations were determined, in the digested samples, using inductively coupled plasma-optical emission spectrometer (ICP-OES).

2.8. Microbiological assay

Total bacterial count (TBC) was determined using nutrient agar according to the method described by Ragab (1997). *Salmonella sp*. was determined on *Salmonella Shigella* Agar (SS Agar) modified oxoid according to Bryan (1991). *E. coli* determined on Macconkey agar according to Unluturk and Turantas (1996).

2.9. Sensory evaluation of fish products

The sensory attributes of tuna burger, nuggets, and fingers were estimated using a panel taste by 20 persons at the Food Industries department, Faculty of Agriculture, Mansoura University. The evaluation parameters included appearance, color, aroma, texture, taste, and overall acceptability according to Rohall *et al*. (2009).

2.10. Statistical analysis:

Statistical analysis was performed using the statistical software package CoStat, (2005).

Results and Discussion

1. Effect of freezing on the physio-chemical properties of raw tuna meat and its processed products

Data in Table (1) revealed that values of the pH, acidity, WHC and plasticity of raw tuna meat were 6.43, 1.23, 4.75 ($cm^{2/}$ 0.3g) and 3.15, respectively. These results were in agreement with Nakazawa *et al*. (2020) who found that raw tuna pH located in the range of 6.2-6.9. Acidity values of tuna products was slightly higher than raw tuna meat which could be attributed to the ingredients, such as spices and herbs, used in the formation of tuna products as reported by previous studies (El-Shawaf, 1990; Darwish *et al*., 2012). Also, the pH values of tuna products slightly decreased, over the time of storage, in the stored tuna products. The slight decrease in pH of the products could be attributed to the partial release of amino acids and carbonyls due to the protein denaturation (Leygonie *et al*., 2012; Aziz *et al*., 2020). Similarly, Darwish *et al*. (2012) stated that some of the existing oxygen inside the packaging may trigger fat oxidation, therefore resulting in lowering the pH values. Moreover, previous studies have reported that the releasing and losing of water from tuna products, during freezing, may cause an increase the solutes concentration, resulting in a pH decrease of thawed tuna or meat processed products (Leygonie *et al*., 2012; Wei *et al*., 2017).

Table 1. Physio-chemical properties of raw tuna meat and its processed products stored under freezing

Products	Storage period (month)	pH	Acidity	WHC $cm^{2/}$ 0.3g	Plasticity
Raw meat	0	6.43	1.23	4.75	3.15
Burger	0	6.58	0.91	4.72	4.36
	2	6.17	1.05	4.65	4.28
	4	5.95	1.19	4.30	4.26
	6	5.46	1.28	4.15	4.22
Nuggets	0	6.53	0.98	4.62	4.83
	2	6.09	1.13	4.55	4.62
	4	6.02	1.31	4.42	4.55
	6	5.57	1.39	4.25	4.14
Fingers	0	6.65	1.05	4.61	4.03
	2	6.26	1.21	4.56	3.38
	4	6.15	1.42	4.45	2.98
	6	5.72	1.47	4.40	2.89

Furthermore, the obtained results of physio-chemical properties also indicated that WHC and plasticity values for burger, nuggets and fingers slightly decreased through the storage time. The loss of WHC and plasticity in tuna products could be attributed to the protein denaturation and losses in protein solubility gradually through the freezing storage (Hegazy, 2004; Darwish *et al*., 2012).

2. Effect of freezing on the chemical composition (g/100g DM) of tuna processed products as compared to raw tuna meat

The chemical composition of fish is of great importance in terms of its nutritional value, quality, and safety as a human food. Table (2) showed the chemical composition of raw tuna meat and its products, through the storage periods. The results were presented on dry weight basis for the fair comparison. The moisture content of raw tuna meat was 60.85%. While the chemical composition of raw tuna meat (g/100g wet basis) was found to be as follows; 23.94g protein, 9.6g fat, 1.43g carbohydrates, 0.51g crude fiber and 4.81g ash. The low-fat content of raw tuna makes it a good choice for the preparation of fish-based products. These results agreed with Aberoumand & Fazeli (2019) and Sardenne *et al*. (2020) who reported that raw tuna meat contained 23.1-25.2 (g) protein, 10.5g fat and 1g carbohydrates.

Table 2. Chemical composition (g/100g DM) of raw tuna meat and tuna processed products stored under freezing

Tuna products	Storage period (month)	Moisture (%)	Dry matter	Crude Protein (g)	Total Fat (g)	Ash (g)	T. carbohydrates (g)	Crude fiber (g)
Raw meat	0	60.85	39.15	61.14	24.5	10.7	3.7	3.4
Burger	0	58.15	41.85	54.26	25.16	8.29	7.50	4.22
	2	55.92	44.08	46.91	28.74	9.48	14.45	4.83
	4	51.66	48.34	37.13	29.20	10.61	23.04	9.53
	6	48.92	51.08	32.55	31.08	11.88	24.47	9.69
Nuggets	0	58.68	41.32	51.96	26.35	7.45	14.23	4.91
	2	56.41	43.59	46.08	29.93	8.87	15.09	5.66
	4	52.43	47.57	36.64	30.94	9.48	22.72	8.57
	6	49.27	50.73	31.71	31.95	11.23	25.09	10.27
Fingers	0	59.27	40.73	57.15	24.72	9.30	8.81	3.09
	2	57.13	42.87	49.42	28.22	10.84	11.49	4.41
	4	53.09	46.91	39.69	29.09	12.15	19.03	6.61
	6	49.88	50.12	34.41	30.72	12.74	22.11	8.39

Regarding the chemical composition of tuna product as compared to the raw tuna meat, it was clearly observing that ratios of the crude protein and total fat contents in tuna products were lower than the raw meat due to the addition of other ingredients to make tuna products. Concerning the changes in the chemical composition of the frozen products through the storage time, it was clearly finding that the moisture content significantly decreased over the storage time. Where the moisture content decreased in tuna products (burger, nuggets, and fingers) by the mean value of 3.11±0.83(%) every 2 weeks. As a result, at the end of storage time (6 weeks), the loosing ratios in moisture content for burger, nuggets and fingers were 15.87, 16.03 and 15.84 (%), respectively. Notably, the dry matter ratios of tuna products were consequently increased theoretically because of the reduction of moisture content during storage as confirmed by Aberoumand and Fazeli (2019).

3. Minerals content in raw tuna meat and its processed products

Presented data in Table (3) revealed that tuna meat and its products may be considered as a good source of minerals due to the presence of major minerals such as K, Mg and Fe. In particular, the raw tuna meat contained 49.6, 358.4, 81.3, 33.9 and 296.5 (mg/100g) respectively for Na, K, Mg, Ca, P and Cu. The nutritive value of tuna was determined according to the content of major minerals in relation to the recommended daily allowances (RDA) for an adult man.

With regard to tuna products, it was clearly noting that the above-mentioned essential minerals in both nuggets and fingers were very close. Meanwhile levels of the same minerals in burger samples were higher than nuggets and fingers. As well, no significant differences were observed among the levels of the toxic metals (Hg, Pb and Cd) in raw tuna meat and its products. Tuna pollution with toxic metals and their relationship to human health makes it questionable as a safe food for humans. In this regard, the detected amounts of Hg, Pb and Cd in tuna meat and its products located in the ranges of 0.547-0.598, 2.035-2.316 and 1.214-1.265 (mg/kg FW), respectively. Orescanin *et al*. (2006) found a comparable average of Cd in fresh tuna samples from Croatia being 2-7 (mg/kg FW). On the other hand, our results were higher than those of Renieri *et al*. (2014) who reported 0.19, 0.09 and 0.16 (mg/kg FW) for Hg, Pb and Cd in bluefin tuna from the Mediterranean Sea. As well, lower levels of Pb and Cd were reported, in Egyptian tuna samples, by Hussein and Khaled (2014) being 1.135 and 0.347 mg/kg wet weight. Our levels of Pb and Cd were higher than the MRLs set by the EU (EC No 1881/2006) however, Hg concentration located within the permissible limits. Tamele *et al*. (2020) reported that concentrations of Cd and Pb in fish samples from the African Red Sea adjacent countries, such as Egypt and Sudan, were higher than the permitted limit by FAOUN/EU regulations (>1 and 1.5 mg/Kg, respectively).

Table 3. Minerals content in raw tuna meat and its processed products

Tuna	mg/100g								mg/kg	
Product	**Na**	**K**	**Mg**	**Ca**	**P**	**Fe**	**Cu**	**Hg**	**Pb**	**Cd**
Raw meat	49.6	358.4	81.3	33.9	296.5	2.77	0.63	0.547	2.035	1.214
Burger	85.4	427.3	105.2	53.6	340.1	2.13	0.41	0.598	2.316	1.265
Nuggets	71.9	391.2	90.7	46.3	319.5	1.84	0.26	0.566	2.280	1.239
Fingers	78.3	408.3	97.6	49.8	327.6	1.96	0.32	0.582	2.297	1.251

4. Effect of storage under freezing on microbiological assay of tuna products

The results showed that the total bacterial count (TBC) in raw tuna meat was 9x10^{6} cfu/g, while all the investigated tuna products had no detected TBC (Table 4). Also, from the same table, it could be revealed that *Salmonella sp*. count was not detected in raw tuna meat, but it was observed at zero time in all tuna products. In contrast, *Salmonella sp*. was not detected in all the storage period of tuna fingers, the contents were decreased from 10x10^{3} to 8x10^{2} cfu/g at zero and six months of storage, respectively. Our results were in accordance with those reported by Gandotra *et al*. (2012) who noted that freezing of fish muscles slows down the bacterial growth as well as decreases the biochemical decomposition of fish muscle.

Table 4. Microbiological assay of tuna products stored under freezing

Tuna products	Storage period (month)	T.B.C.*	*Salmonella sp.*	*E. coli*
Raw meat	0	9x 10^{6}	Nil	Nil
Burger	0	Nil	2x10^{2}	5x10^{3}
	2	Nil	Nil	22x10^{2}
	4	Nil	Nil	17x10^{2}
	6	Nil	Nil	10x10^{2}
Nuggets	0	Nil	2x10^{2}	4x10^{3}
	2	Nil	Nil	3x10^{2}
	4	Nil	Nil	Nil
	6	Nil	Nil	Nil
Fingers	0	Nil	10x10^{3}	20x10^{2}
	2	Nil	21x10^{2}	15x10^{2}
	4	Nil	11x10^{-2}	Nil
	6	Nil	8x10^{2}	Nil

*T.B.C.: Total bacterial count

According to *E. coli* essay, there is no detection of *E. coli* in raw tuna meat as well as at four- and six-months of freezing storage, for both tuna nuggets and fingers. In tuna burger, *E. coli* was observed at all the storage periods: at zero-time, *E. coli* count was 5x10^{3} cfu/g, while it was decreased until it reached 10x10^{2} cfu/g after six months of freezing storage. Additionally, tuna nuggets and fingers had high *E. coli* at zero time and after two months of freezing storage. The reason behind these results may be due to the additives (vegetable and other ingredients) which used in the preparation of fish products as previously confirmed by Yogesh *et al*. (2013).

5. Effect of freezing on the texture profile of tuna products stored under freezing

Texture profile analysis is a commonly used technique in industry for the evaluation of food textural behavior, as it can give an indication of sensory properties (Burey *et al.*, 2009). Table (5) showed the texture profile analysis of tuna products during the storage periods under freezing. At zero time, the texture characteristics for burger, nuggets and fingers were (12.750, 0.525, 6.689, 4.234, 0.633 and 0.435), (14.530, 0.564, 8.192, 5.016, 0.612 and 0.359) and (7.150, 0.632, 4.521, 3.010, 0.666 and 0.401) respectively for firmness, cohesiveness, gumminess, chewiness, springiness, and resilience, respectively. These results indicated that tuna nuggets had the highest firmness and gumminess as compared to other products at zero time.

Cohesiveness indicates the ability of the product to hold its ingredients. The highest cohesiveness values were recorded for tuna fingers (0.632, .0.634, 0.632 and 0.607) followed by tuna nuggets (0.564, 0.569, 0.498 and 0.525), while tuna burger represented the lowest values (0.525, 0.509, 0.500 and 0.499). These results agreed with Potter and Townshend (1973) who stated that the starch content in nuggets is gelatinized during the perfect frying and the resulted texture is then hard and elastic.

Overall results indicated that, except for firmness and gumminess, no significant reduction in values of texture profiles was found in the tested products from zero to six months of storage under freezing. However, for firmness and gumminess in all products, their values at zero and two months were very close which means no marked changes have existed.

Table 5. Texture profile analysis of tuna products stored under freezing

Products	Storage period (month)	Firmness	Cohesiveness	Gumminess	Chewiness	Springiness	Resilience
Burger	0	12.750	0.525	6.689	4.234	0.633	0.435
	2	11.830	0.509	6.020	3.766	0.626	0.441
	4	10.670	0.500	5.336	3.304	0.619	0.438
	6	9.930	0.499	4.951	3.046	0.615	0.411
Nuggets	0	14.530	0.564	8.192	5.016	0.612	0.359
	2	12.850	0.569	7.317	4.274	0.584	0.356
	4	11.730	0.498	5.836	3.348	0.574	0.488
	6	10.990	0.525	5.769	3.039	0.527	0.384
Fingers	0	7.150	0.632	4.521	3.010	0.666	0.401
	2	6.700	0.634	4.249	2.876	0.677	0.435
	4	6.210	0.632	3.925	2.801	0.714	0.521
	6	5.760	0.607	3.499	2.488	0.711	0.533

6. Sensory evaluation of tuna products in comparison with some commercial fish products

Results of sensory evaluation for tuna products as compared to the commercial products of fish burger, nuggets and fingers were shown in Table (6). The commercial or market products represented the control samples. Regarding burger samples, except for the taste scores, no significant differences were observed between tuna burger and the control sample in appearance, color, aroma, and texture. While the taste score of the control sample was slightly higher than that of tuna burger sample.

Regarding the sensory evaluation scores of tuna nuggets, it was found that, except for the appearance score, scores of color, texture and taste were slightly lower than those of the control sample. It was worth mentioning that there were no significant differences between our tuna fingers and the control fingers.

Table 6. Sensory evaluation of tuna products in comparison with some commercial fish products

Samples	Appearance (20)	Color (20)	Aroma (20)	Texture (20)	Taste (20)	Over all acceptability (100)
B1	18.90 ± 0.55^{b}	19.05 ± 0.68^{a}	18.95 ± 0.68^{a}	19.10 ± 0.71^{a}	18.95 ± 0.75^{b}	94.95 ± 2.03^{b}
B2	19.15 ± 0.67^{ab}	19.30 ± 0.57^{a}	19.35 ± 0.67^{a}	19.45 ± 0.51^{a}	19.50 ± 0.68^{a}	96.75 ± 2.38^{a}
N1	18.90 ± 0.85^{a}	18.90 ± 0.85^{b}	18.75 ± 0.71^{b}	18.80 ± 0.76^{b}	19.10 ± 0.71^{b}	94.45 ± 3.23^{b}
N2	19.25 ± 0.55^{a}	19.40 ± 0.50^{a}	19.45 ± 0.60^{a}	19.65 ± 0.48^{a}	19.85 ± 0.36^{a}	97.60 ± 1.76^{a}
F1	19.60 ± 0.68^{a}	19.75 ± 0.55^{a}	19.50 ± 0.60^{a}	19.35 ± 0.67^{a}	19.55 ± 0.60^{a}	97.75 ± 2.33^{a}
F2	19.90 ± 0.30^{a}	19.80 ± 0.41^{a}	19.70 ± 0.57^{a}	19.70 ± 0.57^{a}	19.65 ± 0.58^{a}	98.75 ± 1.77^{a}

B: Burger N: Nuggets F: Finger 1- Tuna 2- Control

Generally, records of our proposed products were very close, sometimes equal to those of the control samples. These findings were clearly observed from the overall acceptability scores for tuna burger, nuggets and fingers which were 94.95±2.03, 94.45±3.23 and 97.75±2.33, respectively. These results concluded that tuna products of burger, nuggets and fingers were of high acceptability between the consumers.

Conclusion

Evaluation results of the introduced tuna products proved good records in their quality, safety, and acceptability properties when freshly prepared. Moreover, the results also indicated a good stability in the frozen tuna products concerning the physico-chemical and organoleptic properties over 6 months of storage with a slight decrease in the moisture content. In terms of food safety, the introduced tuna products were safe chemically and biologically, where levels of toxic metals located within the recommended safe limits and the total bacterial count significantly decreased under the freezing conditions. Finally, the organoleptic results confirmed that the introduced tuna products were of high acceptability between the consumers. As a result, we highly recommend the commercial production of these tuna products which will have a good marketing.

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