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Research Article

The Effect of Improper Packaging on Moisture and Fatty Acid Composition in Frozen Bluefin Tuna (*Thynnus Thynnus*)

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ABSTRACT

This article investigates the effect of improper packaging and freezer burn on the moisture content and fatty acid profile of frozen bluefin tuna. Improper packaging caused serious freezer burn on the surface of a bluefin tuna slice during frozen storage. The moisture content of the surface affected from freezer burn and an inner part of the tuna slice was analysed. Visual examination showed that the surface of the tuna slices were dried and different from normal flesh colour. Moisture content of the frozen tuna slices dropped significantly on the surface compared to the inner part unaffected by freezer burn. Direct methylation method was successfully achieved on the sample without any lipid extraction. Separation of fatty acid methyl esters of the bluefin tuna was successfully achieved by using GC-FID 100 m column in 65 minutes. Significant changes were observed in saturated and polyunsaturated fattyacids, whereas monounsaturated fatty acids remained the same. The level of polyunsaturated fatty acids reduced by half on the surface of the flesh compared to the inner part. Among the PUFA, n3 and n6 fatty acids were greatly reduced, but more intense in n3 fatty acids.

Keywords: Bluefin tuna, lipid oxidation, fatty acid, moisture, improper packaging

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INTRODUCTION

The Atlantic (or Northern) bluefin tuna (*Thunnus thynnus*, ABT) is one of the tuna species with the greatest commercial interest (Zohar et al., 2016). Storage condition of tuna is important for both commercial interest and nutritional quality. Frozen storage is generally chosen to extend the shelf life of ABT (Martinez et al., 2022). Normally, when tuna is caught, it is bled immediately to minimize deterioration in the muscle, and to obtain high quality raw material for further processing. Tuna fish is frozen at much lower temperatures than other seafood to reduce freezing time due to its size. It must be frozen as quickly as possible and conserved at temperatures under −50°C (C. Chow et al., 1989). Some processors operate freezers for tuna up to as low as -70°C to freeze the whole tuna. Correct storage temperatures are important in order to prevent the deterioration of marine product quality. Various researchers have showed the chemical changes in fish from different freezing storage periods and temperatures. During shelf-life lipid and hemoglobin oxidations, protein coagulation and color changes are the main alteration (Ayala et al., 2005; Chow et al., 2004; Tanaka et al., 2016; Torrieri et al., 2011). These changes were also related to freezing time. During slow freezing, conformation of large extracellular ice crystals creates higher injury to the cells than the small intracellular ice crystals that form in quick freezing (Badii & Howell, 2002; García et al., 1999). The loss of color during storage is also slower in quickly frozen tuna than in gradually frozen tuna (Bito, 1968). The formation of large ice crystals during freezing reduces the quality of fish, for instance, rupturing the membrane

structure. For this reason, it is important to carefully manage marine products in terms of both freezing speed and temperature.

Marine products contain more unsaturated lipids than other organisms and the oxidation of these unsaturated lipids results in deterioration of texture, appearance, flavor, and consistency. Especially, polyunsaturated fatty acids (PUFA) are prone to oxidation and formation into lipid hydro peroxides (L-OOHs) during the early stages of storage. L-OOHs generate a variety of volatile compounds, such as ketones, alcohols, and aldehydes (Frankel, 1984) These products cause the rancid odor in rotten fish (Swoboda & Peers, 1977). Moreover, the L-OOHs alter other unoxidized lipids, proteins (Lenz et al., 1990), carbohydrates (Ravussin et al., 1986) and nucleic acids (Lewis et al., 1986). Differently, the modification in the meat color is correlated to the amount of oxidized myoglobin (Kannan et al., 2001). The amount of the myoglobin content to hemoglobin content in the meat influences the color of the fish (Richards & Hultin, 2003). Changes in fish color depend on various components, such as light exposure, storage temperature, redox potential, muscle pH, and susceptibility to lipid oxidation. The accumulation of hydro peroxide during the initial phase of lipid oxidation differs between fish species and between ordinary and dark muscles (Sohn et al., 2005).

Frozen fish that has been improperly packaged is a severe issue during the frozen storage. Over time, the unfavourable moisture loss and the presence of air cause the oxidation of the fish lipids, especially the long-chain unsaturated fatty acids. The oxidation of these nutritionally important unsaturated fatty acids is the major problem in frozen fatty fish. Moreover, undesirable flavours and colours changes occur as a result of the oxidative deterioration. The aim of the study is to determine the impact of improper packaging on the moisture loss and the severity of the long chain unsaturated fatty acid loss of frozen bluefin tuna.

MATERIAL AND METHODS

Bluefin tuna fillet was purchased from the fish market in Konya. The total weight of the tuna was 70 kg. The fish were sliced at about 2 cm thicknesses and wrapped with cling film, frozen with a home type freezer at -24°C and then stored at -18°C for a year. Visual examination of the fish slices showed some freezer burn on the surface of the slices. The freezer-burned surface of the slice and its underneath, which seemed to be free from freezer burn, was gently cut in a flake-like manner with a sharp knife while still frozen.

Moisture analysis

The moisture content of the frozen bluefin tuna was determined according to oven drying method (Official Methods of Analysis, AOAC, 2000). The drying process was done by holding the sample at 105°C for eight hours and a constant weight was obtained. Dried samples were cooled in a desiccator for 30 minutes. The moisture content was calculated based on the weight loss after drying.

Preparation of fatty acid methyl esters

Fatty acid methyl esters of the samples were prepared according to Joseph & Ackman (1992) with a minor modification. Approximately 1 g of the fish flesh (instead of fish oil) was cut finely while it was frozen and then transferred into a screw capped glass tube and 2 ml of 0.5 M methanolic NaOH was added, and then capped tightly. Tubes were heated up at boiling temperature for 7 minutes, and then cooled down to room temperature. After addition of 1.5 ml methanolic BF_3 (%14), the tubes were capped tightly and then heated for a further 5 minutes. Tubes were cooled to room temperature and fatty acid methyl esters were extracted with 2 ml iso-octane using a vortex mixer. After phase separation, the upper phase was taken into a 2 ml amber vial via a glass Pasteur pipette, and then immediately injected into GC-FID (Shimadzu GC-2025) to determine fatty acid composition.

Separation of fatty acids methyl esters

Fatty acid methyl esters were separated with Shimadzu Gas Chromatography (GC-2025) equipped with auto injector (AOC-20I). The injection temperature was maintained at 230° C and detector (FID) temperature was set to 250°C Column specification and column temperature program as follows: Teknokroma TR-CN100, TR-882192 100% biscyanopropyl polysiloxane, column length 100 m, id 0.25 mm and film thickness 20 mm. Initial column temperature was hold at 45°C for 3 minutes, then raised to 225°C with a ramp rate 5°C per minute and held at this temperature for 26 minutes. Fatty acid methyl esters were separated in 65 minutes.

Injection volume 1 µL Injection mode split 1:25 total flow 68.3 mL, column flow 1.28 mL/min. purge flow 3 mL/min. Identification of fatty acid methyl esters was done comparing their retention time with standards of fatty acid methyl ester (Restek Food Industry FAME Mix 37 and PUFA Mix No 3 Supelco-47085u). The results were expressed as a percentage area of individual fatty acid.

Statistical analysis

All biochemical analyses were done in triplicate and presented as mean values ± SD, after controlling for the normality and homogeneity of the data. The statistical analysis was performed with SPSS 27 package program by using Student's t test. The significance level of the values were determined at 95% level where $p<0.05$ was considered to be significantly different.

RESULTS AND DISCUSSION

Moisture content of the frozen tuna slice is shown on Table 1. It was found that the moisture level of the fish slice changed from 42.6% on the surface to the inner part with a level of 59%. This result indicates that significant moisture loss occurred on the surface of the frozen tuna slice compared to inner part. Moisture content of bluefin tuna may vary in the body part depending on their lipid content. Loss of moisture in the slice has an undesirable effect on the sensory and texture properties of the flesh. It

Table 1. Moisture content of frozen Bluefin tuna.

Tuna Slice	Moisture (%)	Ref
Surface	42.64±0.98*	61.1[a] ; 62.9[b];59.9[c]; 61[d]
Inner part	59.17±1.46*	

*significance level at 0.05; (Öksüz, 2017[a]; Parisi et al., 2007[b]; Roy et al., 2010[c]; Topic Popovic et al., 2012[d])

becomes dry and takes a cotton-like texture. Even further dryness may occur when they are cooked. Therefore, it is crucial to prevent moisture loss during frozen storage in order to maintain the quality of the flesh. Moisture content of fresh Bluefin tuna ordinary muscle was reported to be 61.1%, having a level of 20.3 % lipid (Oksuz, 2017). Similarly, bluefin tuna moisture content was stated by different researchers as 58.96 to 60.08% in female and male bluefin tuna (Parisi et al., 2007). Our findings in moisture content of inner part of the bluefin tuna slices are close to the literature values. However, the most prominent moisture loss was observed on the surface compared to the moisture content of the inner part of the slice and stated in the literature. This value was almost 16.5 % lower than inner part of the tuna slices. Moisture loss results in undesirable changes to the flesh quality, and the loss of moisture on the surface causes degradation and dryness in the flesh texture, reducing sensory characteristics.

On the freeze burned flesh surface, lipid concentration increases on dry weight basis, and lipid become more available to atmospheric oxygen to initiate oxidation. Therefore, PUFA in the fish flesh are oxidised rapidly, which lowers the nutritional quality of fish lipid. Oxidation of lipid is considered to be a major problem in fatty fish, such as tuna, mackerel and sardine. Polyunsaturated fatty acids were highly affected from the lipid oxidation in fish muscle during the frozen storage. As a consequence, fish muscle becomes rancid, inedible and loses its nutritional value.

Oxidation of PUFAs is a multistep process that occurs after exposure to atmospheric oxygen during the production stage. At the initial step of oxidation, formation of peroxides and dienes occurs and with the aid of oxygen in the cold store, secondary oxidation products such as carbonyl and aldehydes are produced. These secondary oxidation products results in an undesirable odour and colour changes (Mason & Sherratt, 2017). In our personal experience, a yellowish colour formation was observed in the oil rich mackerel skin around the belly in prolonged frozen storage. Prolonged exposure to oxygen produces secondary oxidation products carbonyl and aldehyde (Albert et al., 2013).

Lipid oxidation may be retarded by rapid freezing along with proper packaging and preventing fluctuation in cold store temperature.

The distribution of fatty acid composition of bluefin tuna muscles are presented in Table 2 and 3. Total saturated fatty acids (SFA) on the oxidised surface of the slice compromised more than half of (54.5%) the total lipid. However, the inner part of the slice contained only 31.5% of total saturates of lipid. Among the saturated fatty acids, palmitic acid (C18:0) was the most prominent fatty acid followed by stearic acid (C16:0), and myristic acid (C14:0). The monounsaturated fatty acid (MUFA) contents of oxidised surface and inner part were similar. Nevertheless, the level of polyunsaturated fatty acid (PUFA) in the inner part (37.0%) was significantly higher than the oxidised surface (17.9%). PUFAs have multiple unsaturated bonds, and are therefore more susceptible to oxidation (Tao, 2015). Hydrolysis of lipids during storage is one of the causes of lipid oxidation in fish meat (Tanaka et al., 2016). Lipid oxidation causes quality losses, production of unpalatable flavour and odour, shortening of shelf life, loss of nutritional quality, and possible production of unhealthy molecules (malondialdehyde) (Secci & Parisi, 2016). Past studies have shown the positive effects of n-3 fatty acids in fish meat and fish oil on health. Especially eicosapentaenoic acid (C20:5 n-3, EPA) and docosahexaenoic acid (22:6 n-3, DHA) are crucial because synthesis of EPA and DHA in mammalians is slow and show significant genetic variation (Nowicki et al., 2020). Therefore, regular consumption of fish and seafood is important for adequate intake of EPA and DHA. In this study ratio of total n-3, EPA and DHA in oxidised part was significantly two times lower than inner part (Table 2 and Table 3). Although the bluefin tuna slice was stored at -24 ^{0}C, oxidation on the surface caused loss of PUFA.

Moreover, 4-hydroxy-2-alkenals are the main aldehyde substances produced during the peroxidation of PUFAs. During peroxidation of n-3 PUFAs, 4-hydroxy-2-hexenal (HHE) is formed (Yamada et al., 2004). Pathological conditions in relation to HHE in humans and mammals have been reported by previous researchers. HHE is a toxic end-product of lipid peroxidation and is shown to be involved in the pathogenesis of several degenerative dis-

Table 2. Fatty acid composition (in percentage) of oxidised surface and unoxidized part in Bluefin tuna.

Compound Name	Oxidised surface	Inner part	t	p
C14:0	9.53±0.95	4.62±0.09	8.933	0.001*
C16:0	32.94±4.25	18.62±0.64	5.773	0.004*
C17:0	1.15±0.17	0.69±0.02	4.531	0.011
C18:0	10.31±1.63	7.06±0.10	3.448	0.074
C20:0	0.61±0.07	0.55±0.02	1.488	0.211
C22:0	0.22±0.03	0.24±0.02	-.941	0.400
C14:1 n5	1.05±0.13	0.55±0.04	6.203	0.003
C16:1 n7	8.35±0.82	6.84±0.17	3.138	0.035
C17:1 n7	0.30±0.01	0.63±0.01	-44.505	<0.001*
C18:1 n9c	10.87±0.79	11.24±0.34	-.745	0.498
C18:1 n7c	4.04±0.23	3.93±0.11	.776	0.481
C20:1 n9	1.39±0.09	1.57±0.11	-2.335	0.080
C20:3 n3	0.53±0.02	0.54±0.03	-.658	0.547
C18:2 n6	1.83±0.45	2.50±0.00	-2.560	0.125
C20:2 n6	0.30±0.05	1.41±0.01	-37.904	<0.001*
C20:3 n6	0.22±0.03	0.24±0.02	-0.941	0.400
C20:4 n6	0.65±0.20	1.36±0.10	-5.549	0.005*
C22:4 n6	0.27±0.10	0.56±0.04	-4.735	0.009*
C22:5 n6	0.51±0.03	0.56±0.01	-3.785	0.019
C18:3 n3	0.63±0.14	0.90±0.03	-3.232	0.032
C20:4 n3	0.49±0.09	0.71±0.02	-4.027	0.016
C20:5 n3	6.69±2.45	13.53±0.13	-4.824	0.040
C22:5 n3	0.92±0.29	2.14±0.02	-7.257	0.018
C22:6 n3	5.42±1.84	13.10±1.42	-5.719	0.005*

*significance level at 0.01

Table 3. Total SFA, MUFA and PUFA content of oxidised surface and unoxidized part surface in Bluefin tuna.

Fatty acid	Oxidised surface	Inner part	t	p
Tot SFA (%)	54.53±7.06	31.54±0.88	5.600	0.005*
Tot MUFA (%)	26.52±1.77	25.31±0.79	1.079	0.341
Tot. PUFA (%)	17.94±5.60	37.01±1.53	-5.691	0.005*
n3	14.16±4.82	30.38±1.49	-5.572	0.005*
n6	3.78±0.78	6.63±0.04	-6.292	0.024
n6:n3	0.28±0.04	0.22±0.01	2.688	0.055
EPA:DHA	1.23±0.03	1.04±0.11	2.981	0.041

*significance level at 0.01

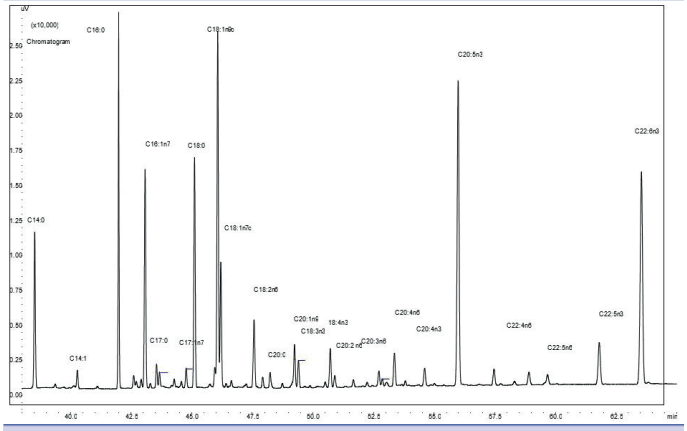


Figure 1. Fatty acid profile of bluefin tuna unaffected from freezer burn.

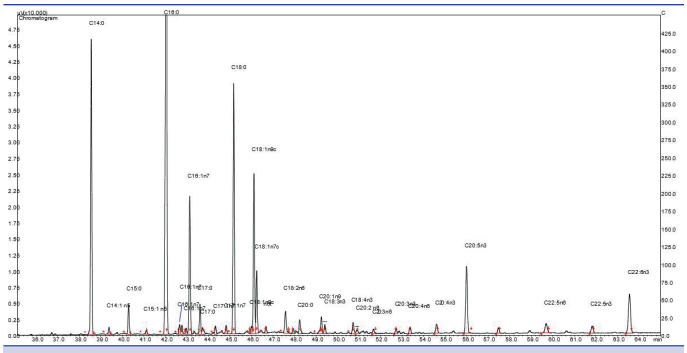


Figure 2. Fatty acid profile of bluefin tuna affected from freezer burn.

eases (Leonarduzzi et al., 2012; Long & Picklo, 2010; Yamada et al., 2004). Therefore, the level of HHE could be a useful indicator for quality assessment of marine products. In literature, various studies have shown several agents inhibiting lipid oxidation. Salt, plants extracts, chitosan, chitooligosaccharide, bacteriocins, antimicrobial and antioxidant peptides, and essential oils were commonly used (Kaewprachu et al., 2017; Mariutti & Bragagnolo,

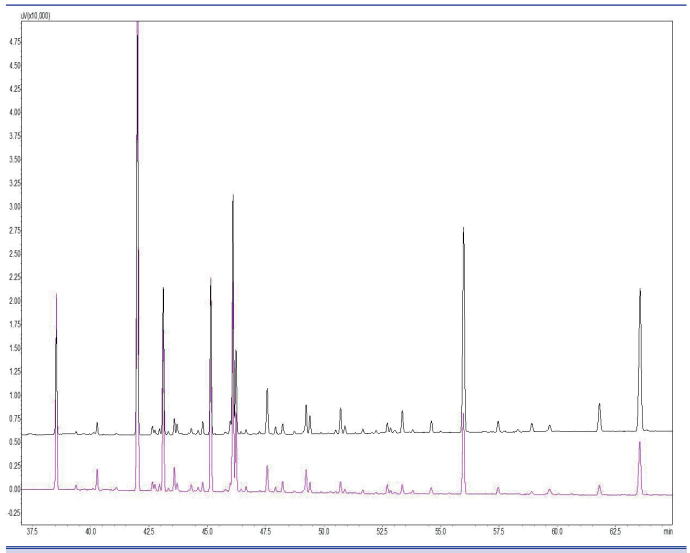


Figure 3. A comparative illustration of fatty acid profile of normal and freeze burned muscle.

2017; Olatunde & Benjakul, 2018). Lipid oxidation can be influenced by different factors, such as freezing temperature, storage temperature, fatty acid composition, pro-oxidants, myoglobin, pH, and oxygen consumption (Arab-Tehrany et al., 2012). If fish is frozen quickly, proper storage temperatures are maintained and thawed in the best manner, it can retain the same quality as when fresh (Cappeln et al., 1999).

Freezer burn manifests in whitish or yellow brown, dry, tree-like areas on the fish flesh, and has thus a major impact on the appearance and the sensory quality of the product (Pham & Mawson, 1997). High levels of water evaporation may also accelerate protein denaturation, resulting in a tough texture and lipid oxidation causing off-flavour production (Hyldig et al., 2012). Glazing is often used to protect the surface of both lean and fatty fish from oxidation and dehydration. The frozen product is either sprayed with or dipped in water, thereby, forming an 'ice cap' around the product. When cold storage is prolonged, it might be necessary to renew or reapply the glazing layer. Trials have shown that drip loss can be reduced by dipping the fish in a salt solution before freezing. However, this treatment has also been shown to accelerate the development of a rancid freezer taste due to the presence of cis-4-Heptenal formed by oxidation of n-3 fatty acids (Hyldig et al., 2012).

However, dietary LC n-3 PUFA are highly vulnerable to oxidation (Awada et al., 2012). Volatile secondary oxidation products formed as a result of the oxidation of PUFAs cause off-flavors (Let et al., 2005). Beyond sensory changes, the oxidation process can also result in the formation of substances that have negative effects on health. One of these potentially deleterious substances is 4-hydroxy-2-alkenals. It has been shown that the levels of serum 4-hydroxy-2-alkenals and inflammation biomarkers increase in rats fed with oxidized n-3 PUFA. It was emphasized that consumption of oxidized n-3 PUFA results in 4-HHE accumulation in blood and triggers oxidative stress and inflammation in the upper intestine (Awada et al., 2012).

Fish meat tends to oxidize easily due to its rich polyunsaturated fatty acid content. During storage oxidized lipids or secondary breakdown products interact with the proteins in fish meat. It causes insolubilization, polymerization, loss of enzymatic activity in protein and formation of lipid–protein complexes (Hematyar et al., 2019). It is crucial to prevent lipid oxidation in fish muscle in order to maintain protein quality. Nutritional loss in freezer burned fish may occur with other frozen meat and poultry products if similarly mishandled during transport and storage.

CONCLUSION

In summary, this research acknowledged that a tuna slice wrapped with cling film did not prevent moisture losses efficiently in extended storage. Therefore, it is advised that tuna slices stored in freezers should be vacuum-packaged when possible. In fish frozen without proper packaging, it was observed that the surface became freezer burned and took a yellowish dull colour. The fatty acid profile of the tuna slices showed that the level of omega3 PUFA's were significantly lower on the surface compared to inner part. Although the ratio of n3: n6 did not change significantly, the percentage of total n6 and n3 lowered by half in the freezer burn samples compared to their counterparts. Lipid oxidation in fish causes three problems: it reduces the nutritional value of lipid-containing fish, it increases the formation of off-flavors, and it gives rise to free radicals that may participate in the development of diseases like atherosclerosis. Moreover, the loss of nutritional quality and moisture are strictly connected with an economic loss. In order to avoid all these problems, it is strongly recommended to pack tuna slices in an air tight packaging materials such as vacuum packaging.

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