

Effects of multiple freezing and refrigerator thawing cycles on the quality changes of sea bass (*Dicentrarchus labrax*)

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Abstract

The freezing-thawing effect on the meat quality of whole, gutted and fillets of sea bass (*Dicentrarchus labrax*) were investigated. It was aimed to investigate the changes in the meat quality of whole, gutted and fillets of sea bass in multiple frozen ($-18\pm 2^{\circ}\text{C}$) and thawed cycles in refrigerated conditions ($4\pm 2^{\circ}\text{C}$). The meat quality assessment of the sea bass groups (whole, gutted and fillets) subjected to multiple freeze and thaw cycles was performed by monitoring sensory quality, chemical analysis such as pH, Total Volatile Basic Nitrogen (TVB-N), trimethylamine nitrogen (TMA-N), thiobarbituric acid (TBA), crude protein and lipid content. The length of the thawing process caused quality changes such as dryness of the skin and undesirable odor formation. Thus the sea bass groups reached unacceptable levels after the 5th freeze/thaw process. According to the sensorial evaluation, no significant differences ($P>0.05$) were found in general acceptability values among the sea bass groups thawed under refrigerator conditions, but there was a significant difference in pH, TVB-N, TBA and crude protein ($P<0.05$) results. Significant differences ($P<0.05$) in TMA-N concentrations and crude fat were observed between the whole and gutted samples and also between the whole and fillet samples.

Keywords :Sea bass, *Dicentrarchus labrax*, Storage, Freeze-Thaw cycles, Chemical changes, Sensory assesment.

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Introduction

Frozen storage is an important preservation method for fish and seafood. Quality deterioration is seen during freezing and frozen storage due to the osmotic removal of water, denaturation of protein and mechanical damage (Thyholt and Isaksson, 1997). The main purpose of freezing seafood is protect the initial sensory and chemical properties of fresh products (Ekinçi and Yapar, 2004). Shelf-life and the quality of frozen fish depend on the handling conditions of the fish, the frozen amount of product, packing, freeze-thaw abuse, temperature of storage, temperature fluctuations and sustainability (Turan et al., 2006). Quick freeze and thaw processes are widely used at home and restaurants (Mol et al., 2004). Applying inaccurate freezing, storage and thawing process applied on foods generally cause microbiological, chemical and physical deteriorations (Bulduk, 2002).

Mostly there are four basic methods to thaw frozen foods; in refrigerator, in microwave oven, in water and at room temperature (Baygar et al., 2004). Most common and widely preferred method is thawing in refrigerator. Generally after thawing, the excess amount of food may be put in the freezer again. When thawing to food once more, quality changes occur and can affect people's health seriously (Hallier et al., 2007). Especially at restaurant and fish markets, these freeze and thaw cycles may be repeated several times. It is very important to determine the quality changes that occur during multiple freeze-thawing treatments. The changes induce by the freezing-thawing cycle are mainly due to three

phenomena that are often closely related; mechanical damage, denaturation of muscle proteins and loss of water-holding capacity (Hallier et al., 2007).

It is important to determine the fish quality because of the increasing demands in international markets for fish and the growing aquaculture industry. Fish is being transported long distances and means of evaluating freshness are required to predict their shelf-life (Connell, 1975). Temperature fluctuation generally occurs during transportation, storage or consumption. This directly contributes to the biochemical and physico-chemical changes of the muscle system (Benjakul and Bauer, 2001). Although there are a large number of publications on the effects of frozen storage on fish quality, little has been reported about the effect of freeze-thaw cycles on the meat quality (Baygar et al., 2004; Mol et al., 2004; Turan et al., 2006). Quality loss occurs in frozen products at homes, restaurants and fish outlets due to the irregularities at storage conditions, nonobservance of sanity rules, overloading the freezer, power outages, keeping the freezer door open for a long time, etc. (Baygar et al., 2004). Such quality loss during frozen storage of seafood is attributed to myosin denaturation, as well as cross-linking and aggregation of myofibrillar proteins. Generally, freezing and subsequent thawing process cause denaturing of fish muscle proteins (Sikorski et al., 1976).

In this study, it was aimed to determine the sensory and chemical quality of whole, gutted and scaleless fillets of sea bass that were treated with multiple freeze and thaw cycles in the

refrigerator. One of the main purposes of this study is to point out the quality changes of such kinds of seafood that subjected to multiple freeze and thaw cycles which affected the consumer health directly.

Material and methods

Raw fish, sampling and processing

The farmed sea bass (*Dicentrarchus labrax*) (the total weight of samples: 20 kg and average weight: 350 ± 10 g) were purchased from Mugla (Kilic Fisheries Co., Turkey) between September 2008 and March 2009. Fish were supplied in foam boxes as three groups; whole, with scale (350 ± 10 g), whole, gutted and scaleless (300 ± 10 g) and scaleless fillets (100 ± 10 g). Six fish from each group, whole, gutted (scaleless) and scaleless fillets, were put into foam plates, sealed with plastic wrap (Özgülmak, Turkey) and stored in -18 ± 2 °C freezer (Uğur, Turkey).

Analysis

Sensory and chemical analyses were carried out initially and analyses were repeated after each thawing processes once every 15 days. Six thawing periods were applied. The frozen samples were thawed by placing the foam plates in the refrigerator (4 ± 2 °C). All samples of fish groups were taken into the refrigerator one day before analyses (Fish groups were kept in the refrigerator as 24.28 ± 0.01 h for whole samples, 23.18 ± 0.01 h for gutted and scaleless and 22 ± 0.01 h for scaleless fillets). The thawing process was carried on until the middle point temperature decreased to 4 ± 2 °C (The temperature at the center of thawed samples measured by the Precision Temperature Measuring

Instrument, P 300 W). For each analyses, all groups were taken from the freezer and thawed in the refrigerator. For each thawing period, sufficient amount of fish were taken for analyses and the rest were put into the freezer again. The thawed samples were placed on ice during handling or analysis. Prior to analysis, the fillets were chopped to homogeneity (The experiments were done in duplicate).

Sensory Analysis

Sensory analyses were conducted according to the Aubourg (2001) by 6 panelists. According to the scale, points of 3-4 were evaluated as “best quality”, points between 2-3 were evaluated as “good quality”, points between 1-2 were evaluated as “moderate quality” and points lower than 1 were evaluated as “not acceptable”.

Nutritional analysis

The nutritional composition of sea bass was determined as crude protein (N x 6.25) and crude lipid, using the methods of AOAC (1998).

Chemical analysis

For pH analyses, Inolab WTW pH meter was used. 10 g of fish samples were weighed, diluted 1:1 and homogenized. The prob of the pH meter was dipped into the solution and the pH values recorded according to the AOAC (1995). Total Volatile Basic Nitrogen (TVB-N) analysis were determined according to the Varlık et al. (1993). Volatile bases were separated by steam distillation of homogenized samples, those separated bases were collected in 0.1 N HCl and titrated back with 0.1 N NaOH. TVB-N was calculated and expressed as mg/100 g sample. The Trimethylamine Nitrogen (TMA-N)

content of sample was determined according to the method of Schormüller (1968) and expressed as mg TMA-N per 100 g fish muscle. Samples were extracted with trichloroacetic acid (7.5%). Bases in the extract were fixed with formaldehyde and after adding picric acid, the absorbance was measured at 410 nm. Thiobarbituric Acid (TBA) analysis was calculated and expressed as mg malonaldehyde/kg fish sample according to the Tarladgis et al. (1960). HCl were added to the fish samples and processed at condenser. TBA solution prepared with 90% glacial acetic acid was added to the distilled solution and was left in a water bath. The absorbance was determined by a spectrophotometer at 538 nm.

SPSS 14 for Windows was used to test the differences between mean values of the different analysed parameters. Differences between means were analyzed by one-way analysis of variance (ANOVA) followed by the Tukey multiple comparison test,

when a significant difference was detected between the days of storage ($P < 0.05$).

Results

The average of all sensory analysis results evaluated during the thawing at refrigerated conditions are shown in Table 1. After the initial thawing at refrigerated conditions general average results of whole, gutted and fillet samples by panellist are 3.60 ± 0.02 , 3.50 ± 0.03 and 3.50 ± 0.04 respectively; after the sixth thawing which is last one 0.92 ± 0.06 , 1.01 ± 0.04 and 0.96 ± 0.06 was evaluated. While the general acceptability results of sensory analysis evaluated statistically there are no significantly differences identified between the fish groups ($P > 0.05$). When general acceptability levels of sensory properties of sea bass samples after the 6th thawing were considered, it was determined as 1.01 for gutted sea bass.

The means of all chemical composition analysis results of the fish groups thawed at refrigerator are shown on Table 2. The mean crude protein and lipid content results of fresh samples were calculated as $19.69 \pm 0.27\%$ and $8.54 \pm 0.12\%$, respectively. After the first thawing period in refrigerator, the mean crude protein and the mean lipid results of the whole, gutted and fillets were found as $19.53 \pm 0.37\%$, $19.33 \pm 0.20\%$, $19.44 \pm 0.16\%$ and $7.66 \pm 0.38\%$, $8.83 \pm 0.22\%$, $8.45 \pm 0.06\%$, respectively. After the 6th (final) thawing period, the mean crude protein and the lipid content analysis results were found as $18.57 \pm 0.34\%$, $18.49 \pm 0.001\%$, $19.21 \pm 0.06\%$ and $7.45 \pm 0.69\%$, $8.19 \pm 0.35\%$, $6.62 \pm 0.22\%$, respectively. Crude protein values of sea bass groups that were thawed in refrigerator environment dropped during thawing period. After the 6th thawing, minimum protein ratio was measured in whole and gutted samples and maximum ratio was measured as 19.21% in fillet samples. Statistically, difference between crude protein values of fish groups (whole, gutted and fillet) detected as notable ($P < 0.05$) in our study. Lipid content of sea bass groups that were thawed in refrigerator environment dropped in a balanced way during thawing period. Initially lipid was 8.75%. After 6th thawing

the minimal lipid value was 6.62% in fillet samples also the maximal value was 8.19% in gutted samples. According to the statistical difference between fish groups there are significant differences between gutted with whole fish and fillet fish ($P < 0.05$). On the other hand there was no significant difference between whole and fillet fish ($P > 0.05$)

The mean pH, TVB-N, TMA-N and TBA results of fresh sample was calculated as 6.48 ± 0.002 , 18.85 ± 0.10 mg/100 g, 3.16 ± 0.00 mg/100 g and 0.43 ± 0.01 mg malonaldehyde/kg, respectively. After the first thawing period in refrigerator, the mean pH, TVB-N, TMA-N and TBA results of the whole, gutted and fillets were found 6.46 ± 0.002 , 6.45 ± 0.001 , 6.45 ± 0.002 ; 17.19 ± 0.41 , 17.90 ± 0.26 , 18.22 ± 0.11 mg/100g; 3.24 ± 0.06 , 3.24 ± 0.02 , 3.16 ± 0.03 mg/100g and 0.42 ± 0.01 , 0.47 ± 0.01 and 0.50 ± 0.01 mg malonaldehyde/kg fish sample, respectively. After the 6th (final) thawing period, the mean pH, TVB-N, TMA-N and TBA results of the whole, gutted and fillets were found to be 6.59 ± 0.003 , 6.60 ± 0.002 , 6.55 ± 0.002 ; 21.51 ± 0.01 , 22.42 ± 0.34 , 22.17 ± 0.20 mg/100g; 3.67 ± 0.01 , 3.64 ± 0.01 , 3.59 ± 0.03 mg/100g and 0.68 ± 0.02 , 1.14 ± 0.03 , 0.84 ± 0.03 mg malonaldehyde/kg fish sample, respectively.

Table 2: Chemical changes of sea bass (*Dicentrarchus labrax*) multiple freezing and thawing cycles in refrigerator conditions (4±2 °C)

Thawed Time	WHOLE (WITH SCALE)						WHOLE (GUTTED AND SCALELESS)						SCALELESS FILLETS					
	pH	TVB-N	TBA	TMA-N	Crude Protein	Lipid	pH	TVB-N	TBA	TMA-N	Crude Protein	Lipid	pH	TVB-N	TBA	TMA-N	Crude Protein	Lipid
Flesh	6.48±0.002 ^a	18.85±0.10 ^a	0.43±0.01 ^a	3.16±0.00 ^a	19.69±0.27 ^a	8.54±0.12 ^a	6.48±0.002 ^a	18.85±0.10 ^a	0.43±0.01 ^a	3.16±0.00 ^a	19.69±0.27 ^a	8.54±0.12 ^a	6.48±0.002 ^a	18.85±0.10 ^a	0.43±0.01 ^a	3.16±0.00 ^a	19.69±0.27 ^a	8.54±0.12 ^a
1 st	6.46±0.002 ^a	17.19±0.41 ^a	0.42±0.01 ^a	3.24±0.06 ^a	19.53±0.37 ^a	7.66±0.38 ^a	6.45±0.001 ^a	17.90±0.26 ^a	0.47±0.01 ^a	3.24±0.02 ^a	19.33±0.20 ^a	8.83±0.22 ^a	6.45±0.002 ^a	18.22±0.11 ^a	0.50±0.01 ^a	3.16±0.03 ^a	19.44±0.16 ^a	8.45±0.06 ^a
2 nd	6.47±0.000 ^a	17.91±0.23 ^a	0.44±0.00 ^a	3.42±0.01 ^{ab}	19.64±0.12 ^a	7.96±0.62 ^a	6.48±0.002 ^a	19.74±0.18 ^a	0.49±0.02 ^a	3.40±0.04 ^a	19.45±0.04 ^a	8.19±0.20 ^a	6.46±0.001 ^a	19.77±0.41 ^a	0.49±0.01 ^a	3.39±0.03 ^a	19.42±0.12 ^a	6.78±0.19 ^a
3 rd	6.55±0.001 ^a	18.65±0.19 ^a	0.53±0.02 ^a	3.20±0.03 ^a	19.28±0.05 ^a	7.08±0.32 ^a	6.53±0.005 ^a	18.80±0.10 ^a	0.63±0.04 ^{ab}	3.17±0.01 ^a	19.36±0.06 ^a	8.47±0.32 ^a	6.49±0.004 ^a	19.18±0.64 ^a	0.66±0.02 ^{ab}	3.11±0.10 ^a	19.44±0.34 ^a	7.49±0.35 ^a
4 th	6.54±0.002 ^a	18.58±0.21 ^a	0.61±0.04 ^a	3.07±0.02 ^a	19.12±0.03 ^a	8.16±0.12 ^a	6.58±0.003 ^a	21.49±0.47 ^{ab}	0.90±0.06 ^b	3.12±0.06 ^a	19.16±0.40 ^a	7.22±0.11 ^a	6.56±0.003 ^a	20.88±0.68 ^{ab}	0.76±0.00 ^b	3.07±0.01 ^a	19.26±0.50 ^a	7.78±0.39 ^a
5 th	6.51±0.001 ^a	20.74±0.19 ^a	0.55±0.00 ^a	3.74±0.04 ^b	19.17±0.11 ^a	7.49±0.21 ^a	6.49±0.002 ^a	22.01±0.33 ^b	0.91±0.02 ^b	3.81±0.04 ^a	18.75±0.07 ^a	7.76±0.15 ^a	6.49±0.002 ^a	22.00±0.03 ^b	0.73±0.01 ^b	3.73±0.12 ^a	19.16±0.37 ^a	6.55±0.20 ^a
6 th	6.59±0.003 ^a	21.51±0.01 ^a	0.68±0.02 ^a	3.67±0.01 ^b	18.57±0.34 ^a	7.45±0.69 ^a	6.60±0.002 ^a	22.42±0.34 ^b	1.14±0.03 ^b	3.64±0.01 ^a	18.49±0.01 ^a	8.19±0.35 ^a	6.55±0.002 ^a	22.17±0.20 ^b	0.84±0.03 ^b	3.59±0.03 ^a	19.21±0.06 ^a	6.67±0.22 ^a

*Mean values± standard deviation (n=6)

Mean values in the same column followed by different superscripts are significantly different (P<0.05).

Statistically, there was a significant difference ($P < 0.05$) among the groups according to the pH, TVB-N and TBA values. After the first thawing period in refrigerator, the pH results of the whole, gutted and fish fillets increased similarly during the thawing period. After the 6th (final) thawing period, the highest pH value was found as 6.60 for gutted fish samples. TVB-N values of the fish samples thawed in refrigerator decreased at the first thawing period although this values increased at the other periods. After the 6th (final) thawing period, the lowest TVB-N was found 21.51 mg/100 g fish sample for whole sea bass, the highest TVB-N detected for the gutted and fillets. TBA values of the samples thawed at refrigerator increased continuously during the thawing period. The lowest TBA value was calculated as 0.68 mg malonaldehyde/kg fish sample for whole sea bass, the highest value was calculated as 1.14 malonaldehyde/kg fish sample for gutted sea bass. TMA-N values of the fish samples thawed in refrigerator showed a well-balanced increase for whole, gutted and fish fillets during the thawing periods. Statistically, there was a significant difference ($P < 0.05$) between the gutted fish and the other groups, whole fish and fillets, but there was not a significant difference between the whole fish and fillets ($P > 0.05$).

Discussion

Özeren and Ersoy (2008) found that the smell content of eel (*Anguilla anguilla*) which they were thawed under refrigerator conditions were better than other thawing conditions. It is stated that thawing under refrigerator conditions is the best thawing method in terms of protecting the color quality of the fish, muscle structure and taste

quality. In the study on different methods of thawing frozen mussel, Günel (2005) found out that the thawing process did not affect the natural smell but when the color, tissue and appearance changes are considered, the most significant difference was in the samples thawed at room temperature. Baygar et al. (2004) stated that anchovies and blue fish thawed under refrigerator conditions lost their sensory freshness after the third thawing. Srivinasan et al. (1997) expresses that frozen freshwater shrimp exhibit losses in their physicochemical and tissue properties after the third thawing process. In the study where they thawed the frozen codfish in iced water (+4 °C) (Magnusson and Martlinsdottlr, 2001) state that fish frozen before rigor in the second month of storing are fresher than fish frozen after rigor. Fillets frozen and thawed before rigor exhibited higher values of freshness than fillets frozen after rigor. As a result of the study, it is indicated that fillets should be frozen before rigor. Hallier et al. (2007) indicated that fillets of european catfish became lighter and yellower after a freezing - thawing cycle due to the oxidation and degradation of pigments caused by mechanical damage. They also indicated that becoming less bright after a freezing thawing cycle is owing to an alteration of the optical properties of the muscle caused by muscle protein denaturation. Sensory analysis results of our study indicated that multiple freezing and thawing cycles cause quality changes such as decrease in protein content, moisture losses in fish flesh and eye, skin dryness and color changes in gills and affect the fish texture. The crude protein and lipid content in culture sea bass found by Alasalvar et al. (2002), Orban et al.

(2003), Beklevik et al. (2005), and Periago et al. (2005) were 23.37% and 6.66%, 20.70% and 5.20%, 19.58% and 9.36%, 19.75% and 1.22%, respectively. Beklevik et al. (2005) emphasized that crude protein and lipid contents of sea bass which they were stored at -18 °C changed by 19.31% and 3.58% in the month 9 of storing. In a study where they frozen sardine fillets and thawed them in microwave, refrigerator and on grill, Arias et al. (2003) they found that there was little reduction in the protein content of the samples thawed in refrigerator conditions and they underlined that the thawing process did not affect the protein content of the fish very much. Günel (2005) determined the protein value of frozen mussel during thawing in the refrigerator as 11.2%, its lipid as 2.99%. The pH value in culture sea bass found by Periago et al. (2005) and Orban et al. (2003) were 6.44 and 6.27, respectively. In another study where they exposed European eel to different thawing processes under refrigerator, water and environmental conditions and by using microwave, Ersoy et al. (2008) found the initial pH value as 6.23, TVB-N value as 12.47 mg/100g flesh of fish, and TBA value as 1.10 mg malonaldehyde/kg flesh of fish. Cakli et al. (2006) determined TMA-N value as 0.87 mg/100g, TVB-N value as 15 mg/100g; TBA value as 1.15 mg malonaldehyde/kg for gutted sea bream at the beginning of the storing period. Again, Cakli et al. (2007) found the initial TBA value as 0.259 mg malonaldehyde/kg, TVB-N value as 17.11 mg/100g flesh of fish, TMA-N value as 0.273 mg/100g flesh of fish for gilled and gutted sea bass stored in ice. Günel (2005) determined the pH value of frozen mussel

during thawing in the refrigerator as 6.66, its TVB-N as 7.09 mg/100g, TBA as 3.05 mg malonaldehyde/kg. Baygar et al. (2004) found pH levels of anchovies and blue fish after the third thawing process in the refrigerator as 6.42 and 6.35; their TVB-N as 34.60 and 32.18 mg/100g; and their TMA-N as 5.48 and 3.98 mg/100g. According to the results of the third thawing, which is the last thawing, it was seen that the fish was approached inedibility levels in terms of TVB-N. It is indicated than freezing and thawing more than once cause losses in the quality of flesh of fish. Ersoy et al. (2008) featured that there were insignificant differences during different thawing methods for eel, that statistically insignificant changes occurred and pH contents of the samples did not exceed 6.5. It was determined that TVB-N content of eel was 12.47 before freezing and changed between 11.53 and 12.52 mg/100g after thawing; and there was a reduction in TBA amount after thawing which might arise from the interaction of the products that occurred as a result of lipid oxidation. In another study where they examined the quality parameters of imported mackerel under different thawing conditions, Mol et al. (2004) found the pH value of fish taken from the main store and thawed as 6.09; its TVB-N as 17.44 mg/100g, TMA-N as 3.50 mg/100g, and its TBA as 13.30 mg malonaldehyde/kg; the pH value of fish taken from the interim store as 6.16; TVB-N value was 14.63 mg/100g; TMA-N as 3.25 mg/100g; and TBA value as 19.14 mg malonaldehyde/kg. As a result of the study, it was found that the thawing process does not have a significant impact on the pH value of fish. And there was not a significant

difference between thawed fish which taken from the main or the interim store, and this thawing conditions did not have much impact on the TMA-N content both in the examples taken from the main store and the interim store. After the study, it was stated that thawing under refrigerator conditions did not have much impact on fish quality and it was underlined that imported mackerel can be healthily consumed by thawing in the refrigerator. In a study where they thawed the shrimps and then were frozen under three different conditions, Boonsumrej et al. (2006) found the TBA level of shrimps thawed in the refrigerator slightly higher. Tironi et al. (2007) found that the damage on the muscle structure of sea bass caught natural environment while being frozen and thawed cause negative changes on color and protein solubility. Magnusson and Martinsdottir (1995) found that the TMA-N and TVB-N content of codfish (*Gadus morhua*) and amberfish (*Sebastes marinus*) during waiting in the refrigerator after being frozen for a long time and thawed formed slower than the ones waiting in ice without being frozen. Results of TMA measurements showed slower formation of TMA on fillets frozen prerigor than those frozen post rigor. Benjakul and Bauer (2001) indicated that freezing and thawing catfish cause instability in muscle structure and increase in lipid oxidation. The highest level of water loss was observed when the number of freezing and thawing increased. It was emphasized that crystals of ice which formed as a result of repeated thawing processes damaged cell membrane and organelle. In another study, it is stated that protein solubility of fish decrease to 60%

after the fifth cycle of freezing and thawing (Benjakul and Bauer, 2000). According to our study results, beginning levels resemble those of other studies in many instances. These differences may occur due to the type of fish, type and season of fishing, the environment where the fish lives, the processes applied to the fish and methods of analysis. It has seem that only the 5th thawing process of the sensory analysis performed in this study exceeded the limited values. The terms of sensorial bad products are not preferred whatever the other properties of them. Even though being one of the most quality change chemical analysis, TVB-N as a thawing process has values in limits. The samples which were thawed in refrigerator particularly have low values of TBA. When the results of the study have been examined, it has seen that skin got dried and smell changed because of thawing process has long termed in refrigerator. Even though after the fifth thawing process fishes are not acceptable sensorial, all frozen foods should be consumed once it thawed. This study suggests that consumers should be careful when they make frozen or thawed fish. They should do this according to their needs. Consumers should be careful about some physical properties of the frozen fish storage conditions; they should take care of hygienic implementations. The freshness of fishes when they were taken to depots and long term opening of freezer's doors are also significant factors which should be considered by consumers.

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