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The effect of thawing methods on the quality of eels (*Anguilla anguilla*)

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ABSTRACT

Physical (colour), chemical (pH, total volatile basic nitrogen (TVB-N), thiobarbituric acid values (TBA)) and microbiological (total aerobic mesophylic bacteria, salmonella, coliform, yeast and mould counts) analyses were carried out on thawed European eel (*Anguilla anguilla*). Different thawing treatments were used (in a refrigerator, in water, in air at ambient temperature and in a microwave oven). The results obtained were compared statistically with those of fresh fish. pH, TBA and a^* values of thawed samples usually decreased significantly ($P < 0.05$) when compared to the fresh control. *Salmonella* was not detected in any of the samples. Coliform and mould counts of fresh control and thawed samples were <1 CFU/g. Total aerobic mesophylic bacteria count of all thawed fish decreased. However, the yeast count of the refrigerator–thawed samples increased. The lowest total aerobic mesophylic bacteria and yeast counts were determined in water–thawed samples. Water thawing is therefore suitable for frozen eel.

1. Introduction

European eel (*Anguilla anguilla*) is a very important commercial species in Turkey. 158 ton of eel were caught in 2003 (Anonymus, 2003). Most of these eels are exported to other countries. Nowadays, fish is kept frozen for relatively long periods before being consumed. Frozen storage is an important preservation method for sea-foods. Its effectiveness stems from internal dehydration or immobilization of water and lowering of temperature. However, meat and fish may undergo quality losses such as protein denaturation, colour deterioration, weight decrease, oxidation of lipids and textural changes due to the freezing and thawing processes (Foegeding, Lanier, & Hultin, 1996). The extent of quality loss depends on careful prefreezing preparation, control of the freezing rate, storage conditions and thawing conditions (Giddings & Hill, 1978). During thawing, foods can be damaged by chemical, physical and microbiological changes. The freezing and thawing processes can have a profound effect on muscle physicochemical characteristics (Wagner & Anon, 1985).

The thawing rate during conventional thawing processes is controlled by two main parameters outside the product: the surface heat transfer coefficient and the surrounding ambient temperature of the sample. This ambient temperature is supposed to remain below 15 °C during thawing to prevent the development of a microbial flora (Chourot, Lemaire, Cornier, & Le Bail, 1996). There are many commercial methods for thawing fish. Water thawing is suitable for a short-time batch or continuous process immediately prior to boning and gives a net weight gain which partially offsets the previous weight loss during cooling and freezing. Air thawing is suitable for a batch process with a cycle time between 8 h and 60 h, which determines the air temperature, required in the range 2–18 °C (35–65 °F). A high relative humidity is required to minimize weight loss (Vanichseni, Haughey, & Nottingham, 1972). The satisfactory techniques for thawing large portions of animal tissue include thawing in a refrigerator and microwave (Karel & Lund, 2003).

Numerous papers have been published on effects of freezing–thawing on sensory, chemical and physical quality of fish muscle (Boonsomrej, Chaiwanichsiri, Tantratian, Suzuki, & Takai, 2007; Kilinc & Çakli, 2004; Rouillé, Lebail, Ramaswamy, & Leclerc, 2002; Schubring, Meyer, Schlüter, Boguslawski, & Knorr, 2003; Sigurgisladottir, Ingvarsdottir, Torrissen, Cardinal, & Hafsteinsson, 2000; Turhan, Ustun, & Bank, 2006). However, there are a few papers on the effects of thawing methods on the physical, chemical and microbiological quality of frozen fish (Karaçam, Kutlu, Pulat, & Boran, 1999; Lin, Hung, & Park, 2000). It is important to determine the physical, chemical and microbiological quality of frozen–thawed fish in order to evaluate the possible risks of fish consumption for humans.

In the present study, pH, TVB-N, TBA, colour values and microorganism counts have been determined in fresh and thawed eels (*A. anguilla*). To evaluate the possible effect of thawing methods on physical, chemical and microbiological quality of European eel, the values obtained in the frozen–thawed samples were compared with the values found in the same fish while they were fresh.

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2. Materials and methods

2.1. Fish samples

Eels *A. Anguilla* (average length: 35–45 cm) used in this study were obtained from the fish bazaar in Hatay, Turkey. They were kept iced in boxes and transported to the laboratory. Eels were gutted, washed with tap water, filleted and cut up into pieces. Fish fillets were then divided into three groups (2 kg fish each). The first group was unfrozen. The other two groups (two replicates) were quickly frozen in polyethylene bags and then stored at −18 °C for five days.

2.2. Thawing process

Eels were thawed in a refrigerator (+4 °C for 4 h), in water (14 °C for 1.5 h, in polyethylene bags), in air at ambient temperature (15 °C for 2 h) and microwave oven (for 5 min). Fresh and frozen–thawed samples were homogenized in a stainless-steel meat mincer and blender and each group was analysed in the same way.

2.3. Physical and chemical analysis

Colour measurements were performed on fresh and thawed samples according to Schubring (1999) using a tristumulus colorimeter CR 300 (MINOLTA). In the CIE Lab system, L^* denotes lightness on a 0 to 100 scale from black to white; a^* (+) red or (−) green; and b^* (+) yellow or (−) blue. pH value was measured as described by Lima dos Santos, James, and Teutscher (1981), by using a digital pH meter (HANNA). Thiobarbituric acid (TBA, mg malonaldehyde/kg) and total volatile basic nitrogen (TVB-N, mg N/100 g) values were determined, as described by Tarladgis, Watts, Younathan, and Dugan (1960) and Antonacopoulas and Vyncke (1989), respectively. Chemical analyses were carried out in triplicate.

2.4. Microbiological analysis

For all microbiological counts, 10 g of sample were taken and transferred into 90 ml 0.1% peptone water and homogenized. From the 10^{-1} dilution, other decimal dilutions were prepared. Total aerobic mesophylic bacteria count was determined by using the pour plate method. Plate count agar (Merck) was used as medium. Plates were incubated at 30 °C for 48 h. Potato dextrose agar (Merck) was used as the medium for total mould–yeast counts. Plates were incubated at 25 °C for 72 h. Violet red bile agar was used for the coliform bacteria count. The samples were incubated at 37 °C for 24 h and the red precipitation zones that occured during incubation were counted. For *salmonella* sp., 25 g of sample were taken and transferred into peptone water (Merck) and homogenized and incubated at 37 °C for 24 h, then the samples were incubated in a xyloselysine deoxycholate medium and the typical colonies were evaluated (Halkman, 2005).

2.5. Statistically analysis

Analysis of variance was used to evaluate the analysis data and significant differences among means were determined by one-way analysis of variance (ANOVA) and Duncan's multiple range test ($P = 0.05$) (SPSS 13.0 for windows).

3. Results and discussion

The values of pH, TVB-N and TBA with means ± standard deviation are given in Table 1. pH value does not offer a certain criterion of spoilage. It has to be supported by other chemical and sensory and microbiological analyses. The pH value of fresh fish is often between 6.0 and 6.5 (Ludorf & Meyer, 1973; Schormüller, 1968; Varlık, Uğur, Gökoğlu, & Gün, 1993). In this study, the pH value of fresh fish was found to be 6.23. After the freezing-thawing process, the pH value of fish was significantly ($P < 0.05$) decreased when compared to the fresh control. However, significant differences ($P < 0.05$) between pH values of thawed fish were not found. The pH values of both fresh and thawed samples were generally low and not exceeding 6.5. It has been reported that the pH value of refrigerator thawed cod was 6.9 (Bøknaes, Østerberg, Sørensen, Nielsen, & Dalgaard, 2002). This value was higher than the pH value of our samples.

The quality of the frozen samples was also determined from the TVB-N values. The TVB-N value is affected by species, catching season and region, age and sex of fish. A level of 35 mg/100 g has been considered the upper limit, above which fishery products are considered unfit for human consumption (Ludorf & Meyer, 1973; Schormüller, 1968). The TVB-N value of fresh material was found to be 12.47 mg/100 g. TVB-N values of all frozen–thawed samples were between 11.53 mg/100 g and 12.52 mg/100 g. Similarly, it has been reported that the TVB-N values of shrimp thawed in a microwave oven and in a refrigerator were between 10.2 mg and 14.6 mg N/100 g (Boonsomrej et al. (2007). According to variance analysis, no significant differences were found between TVB-N values of fresh and thawed fish. The decrease in TVB-N value of air thawed fish (11.53 mg N/100 g) was only significant ($P < 0.05$) when compared to the fresh control. For mackerel thawed under air, water and refrigerator, similar TVB-N values were reported (Karaçam, et al., 1999).

Table 2
Colour analysis of fresh and frozen–thawed eel

Thawing medium	L^*	a^*	b^*
Fresh	46.30	0.93[b]	−1.73
Air	45.07	0.50[a]	−1.20
Water	43.76	1.02[b]	−2.35
Microwave	46.53	0.46[a]	−2.62
Refrigerator	46.86	0.71[ab]	−2.37

[a] Values are shown as mean $n = 5$.
[b] Within the column values with different letters are significantly different ($P < 0.05$), values without letters are not significantly different ($P > 0.05$).

Table 1
pH, TVB-N and TBA values of fresh and frozen–thawed eel

Thawing medium	pH	TVB-N (mg N/100)	TBA (mg malonaldehyde/kg)
Fresh	6.23 ± 0.01[b]	12.47 ± 0.34[b]	1.10 ± 0.03[c]
Air	6.00 ± 0.20[a]	11.53 ± 0.11[a]	0.54 ± 0.01[a]
Water	5.90 ± 0.10[a]	12.38 ± 0.55[b]	0.61 ± 0.04[ab]
Microwave	6.03 ± 0.06[a]	12.36 ± 0.54[b]	1.04 ± 0.20[c]
Refrigerator	5.83 ± 0.06[a]	12.52 ± 0.26[b]	0.72 ± 0.02[b]

[a] Values are shown as mean ± standard deviation of triplicates, $n = 3$.
[b] Within the column values with different letters are significantly different ($P < 0.05$).

Table 3
Microorganisms counts of fresh and frozen–thawed eel (cfu/g)

Microbiological analyses	Thawing medium				
	Fresh	Air	Water	Microwave	Refrigerator
Aerob mesophylic bacteria count	5.7×10^5	4.1×10^5	1.2×10^5	4.0×10^5	2.6×10^5
Salmonella count	Nd	Nd	Nd	Nd	Nd
Koliform bacteria count	<1	<1	<1	<1	<1
Yeast count	5.4×10^3	2.2×10^3	2.2×10^3	3.2×10^3	6.0×10^3
Mould count	<1	<1	<1	<1	<1

Nd-not detectable.
Values are shown as mean of triplicate, $n = 3$.

The TBA value is a good indicator of the quality of chilled or frozen fish (Tarladgis et al., 1960; Vareltzis, Zetou, & Tsiaras, 1988). The extent of lipid oxidation is reported as the TBA value and is expressed as milligrams of malonaldehyde (MA) equivalents per kilogram sample or as micromoles MA equivalents per gram sample (Shahidi & Wanasundara, 2002). The fish may be consumed up to level of 8 mg malonaldahyde/kg in the TBA value (Schormüller, 1969). The TBA value of fresh fish was found as 1.10 mg malonaldahyde/kg. The TBA value of thawed fish decreased when compared to the fresh control. This decrease was significant ($P < 0.05$) except for the microwave thawed samples. In our opinion, this decrease may be related to transformation of secondary products of lipid oxidation. It was reported that secondary products of lipid oxidation, such as malonaldehyde, 2-alkenals and 2,4-alkadienals, react with the TBA reagent. The exact mechanism of their reaction with the TBA reagent is not well understood (Shahidi & Wanasundara, 2002). Secondary products of lipid oxidation that react with the TBA reagent can be transformed into other chemical substances. Hence, TBA value may be increased or decreased (Regenstein & Regenstein, 1991). The final quality of thawed seafood will depend not only on the thawing process but also on factors such as frozen storage conditions and the length of time that it has been frozen, packaging, product form and product type (Anonymus, 2000). The highest TBA value was found in microwave–thawed samples at 1.04 mg malonaldahyde/kg. In another investigation, similar results were noted and it was reported that it was probably due to the fact that high energy generated under the microwave thawing might activate lipid oxidation (Boonsomrej et al., 2007). Similarly to our finding, Karaçam, et al. (1999) reported that the highest TBA value of thawed mackerel was found in the refrigerator–thawed samples when compared to the air and water thawed samples.

One of the most obvious quality changes caused by thawing was in colour. Colour changes were measured on both the fresh and frozen–thawed samples (shown in Table 2). Significant differences between L^* (lightness) and b^* (blueness) values of fresh and thawed fish were not found. The a^* value of thawed fish decreased when compared to the fresh control. This decrease was significant ($P < 0.05$) except for the water thawed samples. The highest a^* value was found in the water thawed samples as 1.02; while the lowest value was determined in microwave thawed samples as 0.46. Significant differences ($P < 0.05$) between two values were found. No other studies previously were found about colour changes of frozen–thawed fish.

Results of microbiological analyses are given in Table 3. Total aerobic mesophylic bacteria count, coliform count, yeast and mould counts of fresh material were 5.7×10^5 cfu/g, <1, 5.4×10^3 cfu/g and <1, respectively. *Salmonella* was not detectable in all samples. Total aerobic mesophylic bacteria counts decreased in the thawed eel. The highest total aerobic mesophylic bacteria count was found in air the thawed samples as 4.1×10^5 cfu/g. The lowest total aerobic mesophylic bacteria count was found in water thawed samples as 1.2×10^5 cfu/g, because microorganism devolvement from air is restricted with thawing in water. Coliform bacteria and mould amounts of all samples found were <1 cfu/g. Yeast count of the thawed samples under air, water and microwave was reduced. However, the yeast count of the refrigerator–thawed samples increased when compared to the fresh control. The lowest yeast count was found thawed samples under air and water as 2.2×10^3 cfu/g.

4. Conclusion

Thawing process had a significant effect on the physical, chemical and microbiological quality of frozen eel. The a^* value of water thawed samples was similar to the fresh control group. The lowest total aerobic mesophylic bacteria count and yeast count were determined in water thawed samples. Therefore, the water thawing method was most suitable for frozen eel. This method is economic and prevents weight loss. For the prevention of nutrient loss, samples in water must be in polyethylene bags. We believe that the results of the present study can be of considerable practical importance.

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