Quality changes in reared, hot-smoked meagre (*Argyrosomus regius* Asso, 1801) during chill storage at 4 ± 1 °C
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1 Introduction

Smoking techniques have been used for centuries as a method for fish and meat preservation (Hattula et al., 2001). The advantages of exposing food to smoke are enhanced flavor, more preserved food and prevention of infestation of insects. Wood smoke consists of highly complex compound as organic acids, alcohols, ammonia, carbon dioxide, phenols, esters, furans, hydrocarbon, benzene, toluene, thymol (Martin, 1994). Salting, drying and heating process also take place in smoking technology. Finally, vacuum packaging can prolong shelf life of smoked seafood. The production and consumption of smoked fish is high in Northern European Countries.

Smoked fish species are generally salmon, cod, eel, trout, mackerel, carp, herring, whiting, flounder, caviar, hake and some crustaceans. Some fish are hot smoked while others are cold smoked. This depends on the countries where the process is carried out and the climate (Çaklı, 2007). Some aquacultured species have been used for smoking technology except for meagre.

Total production of meagre was reported to be 4112 tons in 2009 (Grigorakis, 2017). In Turkey, production of cultured meagre was 2463 tons in 2016 (Turkstat, 2018). Meagre has a high potential in seafood industry due to its growth performance and good adaptation to captivity, good fecundity, high fillet quality, meat quality and taste (Saavedra et al., 2017). Meagre is being farmed in Italy, France, Spain, Greece, Turkey, Egypt, Croatia, Portugal and Malta (Monfort, 2010). The main markets for farmed meagre are Italy, Spain, Portugal and Israel (Kružić et al., 2016) and it has a wide harvest and product processing (Dias et al., 2014), good processing yield (Monfort, 2010) and it is a potential species for aquaculture (Duncan et al., 2013). Meagre reaches commercial size in a short period and can be processed into portions (Monfort, 2010). Also the nutritional value is high (Monfort, 2010; Bilgin et al., 2016). The aim of the current study was to investigate changes in quality and nutritional parameters of hot smoked meagre during chill storage.

2 Material and methods

2.1 Sample preparation

In this study, 51 fresh meagre (*Argyrosomus regius*) with an average length of 35.75 ± 1.74 cm and a weight of 382.13 ± 55.93 g were used. The fifty-one fish were randomly selected for study. The fish were obtained from a special marine fish production facility located in Gerende Bay in İzmir province. After harvesting, the fish were placed in special styrofoam boxes, plastic bags filled with ice were placed on them, and the strapless box was securely closed and sent to the Food Laboratory of the Eğirdir Fisheries Faculty. The fish samples were washed with ice cold water. Each fish was weighed on a sensitive scale (Shimadzu BX420H, Japan) measuring to 0.001 g, and their lengths were measured with a length-measuring board. The internal organs of the fish were carefully removed and their scales were cleaned.
Duplicate sampling was carried out each sampling day for all analyses. Sampling for analysis was done once a week for smoked products and every two days for fresh samples.

2.2 Smoking process

Forty meagre samples were used for the smoking process and divided into two groups: the control and hot smoked (HS). The hot smoking method reported by Gulyavuz & Unlusayın (1999) was used by modifying the self-combustion method in an electrostatic smokestack cabinet. First of all, the fish were kept in a 20% (w/v) brine solution (fish/brine solution ratio 1:1) for 90 minutes. After salting, the fish were washed lightly with cold water to remove salts from the surface of the fish. Excess water was drained from the fish for 15 minutes at 20 °C. Oak sawdust was used in the smoking process, and the temperature was gradually increased. For the first 30 minutes, a temperature of 30 °C was applied and then increased up to 50 °C, 60 °C and 70 °C. The maximum temperature reached was 85 ± 2 °C. The temperature of the center of the fish reached 65 °C during the smoking process. Afterwards, the fish samples were cooled at room temperature for 30 minutes and then cut into skinless fillets in the food laboratory. Fresh (control) and smoked skinless fillets were packaged with a vacuum machine (Abant Makine Sanayi, Turkey). The samples were then stored at + 4 ± 1 °C until the end of the shelf life.

2.3 Proximate composition analysis

In all samples, moisture analysis was performed with an automatic moisture determination device (AND MX-50, Japan). The amount of protein was determined using a protein pre-burning unit (Velp UD-20, Italy) and a fully automated protein distillation unit (Velp UDK 142, Italy), according to the Kjeldahl method (N×6,25) (Method No. 940.25) (Association of Official Analytical Chemists, 2000a). The fat content was determined according to Lovell (1975) and the ash (inorganic matter) was determined according to Lovell (1981).

2.4 Fatty acid analysis

Fatty acids were measured by the Çukurova University (CU) Directorate of Experimental and Observational Student Research and Application Center using a gas chromatographic method. The fatty acid composition was analyzed using a Perkin Elmer Canus 500 (GC) equipped with an SGE column (30 m, 0.32 mm ID BPX20, 0.25 μm, USA). The column temperature was held at 140 °C for 5 minutes, raised to 200°C at a rate of 4°C/min and then up to 220 °C at 1 °C/min. The injection temperature was 220 °C and the flame ionization detector (FID) was set at 280°C. The sample size was 2 μl and the carrier gas was checked at 16 psi. The split ratio was 1:100. Fatty acid methyl esters, 2M KOH in methanol and n-heptane, were prepared from extracted lipid using the transmethylation method. Two milliliters of heptane with 4 ml of 2M KOH was added to 10 mg of an extracted fat sample. It was then vortexed for 2 minutes at room temperature and centrifuged for 10 minutes at 4000 rpm before the heptane layer was tested using gas chromatography (GC) analysis (Ichihara at al., 1996). Fatty acids were classified by comparing the time of arrival in the fatty acid methyl esters (FAME) mixture consisting of the standard 37 compounds. The results were expressed as % ± Standard error.

2.5 Quality analysis

A pH analysis of the fish was made according to Varlik et al. (2007), with a pH meter (Hanna HI 221, Romania). Thiobarbituric acid (TBA) analysis expressed in mg of malondialdehyde (MDA)/kg was performed using the method of Erkan & Oezden (2008). Accordingly, 2 g homogenized fish meat was weighed and placed in a 50 mL tube. Then, 100 microliters of BHT (1g/L) and 25 mL of TCA (50g/L) were added to the mixture and homogenized for 5 minutes in a homogenizer (Heidolph Dixa 900 Germany) at high speed. The mixture was filtered through filter paper (Whatman No 1). Then, 2 mL of the filtrate was transferred to a glass tube, and 2 mL of freshly prepared TBA reagent (Malondialdehyde bis-diethyl acetal, Merck Germany) was added. The tubes were kept in water bath at 75-80 °C (Memmert WB 22, Germany) for 40 minutes to complete reaction. Same process was applied for blind and standards. After cooling the tubes, absorbance was measured on a spectrophotometer (T80 + UV/VIS Spectrometer PG) at 532 nm wave length. TBA value was calculated with absorbance and standard equation of regression curve.

A Total Volatile Basic Nitrogen (TVB-N) analyse was carried out according to Nicholas (1992). Accordingly, 25 g of fish meat was homogenized for 30 sec with 50 mL of 7.5% TCA (trichloroacetic acid). The mixture was transferred to a centrifuge tube and centrifuged for 20 min at 4,000 rpm in a refrigerated centrifuge. The upper liquid phase was taken in glass bottles and kept in the refrigerator. Then, 15 mL of this liquid was taken and 4 mL 10% NaOH was added and transferred to the distillation unit. Subsequently, 10 mL of distilled water was added and 15 mL of 4% boric acid and indicator were added to the distillate vessel. For distillation process steam distillation unit (Velp Scientifica UDK 142 Italy) was used. After distillation, 50 mL of distillate was taken and titrated with 0.25N HCI. This value was expressed as mg/100g.

Trimethylamine (TMA) analyses was performed according to Association of Official Analytical Chemists (2000b) methods and Varlik et al. (2007). Accordingly, 10 g fish meat was homogenized with 90 mL of 10% TCA for 3 minutes and filtered, 4 mL filtrate was taken and 1 mL of 20% formaldehyde, 10 mL 99% toluene and 3 mL of 50% KOH were added. The mixture was stirred and rested for 10 minutes. The same process was done for the blind sample. Then, 5 mL of upper toluene phase was taken to another tube and 5 mL of 0.02% picric acid was added and mixed. The absorbance of the samples was measured immediately against the blank at 410 wave length spectrophotometer (T80 + UV/VIS Spectrometer PG). This value was given as mg/100g.

In addition, a microbiological analysis was performed according to Varlik et al. (1993). In aseptic conditions, 25 g of fish meat was weighed with the help of sterile pens, scalpel and scissors, and then diluted 10⁻¹ and homogenized for 2 to 3 minutes in a pre-sterilized blender after adding 225 mL of buffered peptone water (Merck 7228). Sterilized and buffered peptone
water was used to dilute it by $10^{-6}$, and duplicate samples were produced from each dilution using the pour plate method. Plaques containing 30 to 300 active colonies were counted on a petri dish. Microbial counts were carried out in duplicate and expressed as log cfu/g. Plate Count Agar (Merck 5463) was used for the total mesophilic aerobic bacteria count (TMAB). Petri plates were incubated at 30 ± 1 °C for 72 hours after the plating, and the colonies formed at the end of the period were counted. Plate Count Agar (Merck 5463) was used for the total psychrophilic aerobic bacteria count (TPAB). Petri plates were counted after 10 to 14 days of incubation at 4 ± 1°C. Yeast Extract Glucose Chloramphenicol Agar (Merck 1.16000) was used as the medium. Plates were counted after incubation at 22 ± 1 °C for 3 to 5 days.

### 2.6 Sensory analysis

The smoked fish were assessed on the basis of appearance, odor, taste, texture and color parameters. The 10 panelists evaluated the overall acceptability of the samples using a 10-point descriptive scale. According to scale, 10 to 9 is perfect, 8 to 7 is good, 6 to 5 is medium, 4 to 3 is the limit for acceptability/ unacceptability and < 3 is unacceptable (Altug & Elmacı, 2005).

### 2.7 Statistical analysis

The data obtained from the study were subjected to analysis of variance (One way Anova) using an SPSS 15.00 Windows software program and the mean values of significant variance sources that are sensory, chemical and microbiologic parameters were selected and compared with the Duncan Multiple Comparison test (P<0.05) (Ozdamar, 2001). Results were given as mean ± standard error (SE).

### 3 Results and discussion

#### 3.1 Chemical components

The chemical composition of the fresh meagre was found to contain 79.28% moisture, 17.28% protein, 2.19% fat and 1.25% ash. Similar results were found in the nutrient composition of the same species (fresh) (Hernandez et al., 2009; Grigorakis et al., 2011). The low fat content in fish meat is an important quality parameter. This is very attractive for the consumer (Poli et al., 2003). Poli et al. (2003) reported that the meat quality of meagre was high. In this study 66.13% moisture, 26.83% protein, 3.32% fat and 3.72% ash were detected in the smoked meagre. Bilgin et al. (2001), reported that the amount of moisture decreased as a result of the hot smoking process on C. gariepinus. In the study conducted with Atlantic salmon, moisture samples at 68% (raw) were found to fall to 64.9% in the smoked samples (Holland et al., 1991). It was also emphasized by the different investigators that moisture content decreases in the fish meat under the effect of salt and heat after the hot smoking process (Unal, 1995; Sigurgisladottir et al., 2000).

Protein increase was determined to be due to salt addition in the smoked meagre. The percentage of protein in fresh samples of Atlantic salmon rose from 18.4% to 25.4% in smoked samples (Holland et al., 1991). Similar increases were seen in hot smoked C. gariepinus (Bilgin et al., 2001) and rainbow trout (Kolsarci & Ozkaya, 1998). The fat content of the meagre increased from 2.19% to 3.32%. The amount of ash was 1.25% in fresh fish and 3.72% in hot smoked fish. It has been reported that this increase in hot smoked mackerel results from the applied salting and heat treatment (Goulas & Kontominas, 2005). This has also been reported by Bilgin et al. (2001), and Salam & Khalafall (1993) with the hot smoking process. The results of all these studies are similar to our findings.

As a result of fatty acid analysis, the polyunsaturated fatty acid (PUFA) value increased from 35.53% (control) to 36.11% (day 1 HS) and the monounsaturated fatty acid (MUFA) value increased from 25.4% (control) to 29.23% (day 1 HS). The most evident decrease rate by day 56 of storage was determined to be the PUFA content. The difference in the fatty acid contents of fresh (control) and hot smoked samples was significant (P<0.05) (Table 1). Significant (P<0.05) decreases due to storage in the MUFA and PUFA contents of meagre were found (Table 1). Masniyom (2011), stated that fish muscles contain PUFA at high levels and that these compounds may undergo oxidation during processing and storage. The Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA) ratio was 18.53% in fresh fish and 17.82% (day 1) in hot smoked meagre in this study. The American Heart Association (AHA) recommends taking 500 mg of EPA and DHA per day (Kris-Etherton et al., 2002). Ribeiro et al. (2013), reported an EPA and DHA ratio of about 19% in meagre sausages, which is a good amount of the daily EPA and DHA requirement (420 grams of EPA and DHA with 150g of sausage). The results of the analyses in this study reveal that the resultant EPA and DHA value is close to this value (17% to 18%) and therefore can meet the daily EPA and DHA requirement.

Grigorakis et al. (2011), investigated lipid quality and fillet yield in cultivated meagre. Similar results were found by them, except for MUFA. Linoleic acid was found to have the highest value among the n-6, with n-6 fatty acids being less than n-3 in their study. A similar result was found in this study (Table 2). The same results were obtained in studies conducted with aquaculture fish such as sea bream, sea bass and Dentex dentex (Grigorakis, 2007; Erkan & Ozden, 2008). The reason for the high level of linoleic acid in cultivated species is attributed to the vegetable oils in the diet. In natural species such as anchovy, silver, wild sea bream and mullet, this fatty acid is reported to be at a very low ratio (Grigorakis et al., 2011).

#### 3.2 Chemical quality changes

As a result of the pH analyses, it was determined that the value was close to neutral (Table 2 to 3). Huss (1995) reported that the near-neutral pH of fresh fish may be reduced in pH due to anaerobic fermentation of glucose or glycogen. In this study, the pH value was found to be consistent with the information given by Huss (1995). The changes on all days in the pH value of smoked meagre samples, except between days 35 to 42, were significant (P<0.05). Kolsarci & Ozkaya (1998) reported that the initial pH of rainbow trout (O. mykiss) was 6.12 (in fresh fish) and 6.47 on day 48 of storage at 4 ± 1 °C (for hot smoked fish). The pH value of the hot smoked rainbow trout was initially...
Table 1. Changes in fatty acids of fresh (control) and smoked stored meagre according to storage days (%).

<table>
<thead>
<tr>
<th>Days</th>
<th>pH</th>
<th>Control 1st day</th>
<th>Control 7th day</th>
<th>Control 14th day</th>
<th>Control 21st day</th>
<th>Control 35th day</th>
<th>Control 42nd day</th>
<th>Control 49th day</th>
<th>Control 56th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>HS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C12-0</td>
<td></td>
<td>1.21 ± 0.02</td>
<td>0.33 ± 0.04a</td>
<td>0.02 ± 0.00b</td>
<td>0.02 ± 0.00</td>
<td>0.02 ± 0.00</td>
<td>0.02 ± 0.00</td>
<td>0.02 ± 0.00</td>
<td>0.02 ± 0.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14.00 ± 0.13</td>
<td>18.50 ± 0.13</td>
<td>19.58 ± 0.22</td>
<td>17.48 ± 0.39</td>
<td>6.63 ± 0.13</td>
<td>2.84 ± 0.04</td>
<td>13.11 ± 0.53</td>
<td>3.11 ± 0.18</td>
</tr>
<tr>
<td></td>
<td></td>
<td>18.30 ± 0.13</td>
<td>0.07 ± 0.005a</td>
<td>19.58 ± 0.22</td>
<td>17.48 ± 0.39</td>
<td>6.63 ± 0.13</td>
<td>2.84 ± 0.04</td>
<td>13.11 ± 0.53</td>
<td>3.11 ± 0.18</td>
</tr>
</tbody>
</table>

Table 2. Quality changes of fresh fish during storage in the refrigerator conditions (4 ± 1°C).

<table>
<thead>
<tr>
<th>Days</th>
<th>pH</th>
<th>TBA (mgMDA/kg)</th>
<th>TVB-N (mg/100g)</th>
<th>TMA (mg/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td></td>
<td>6.60 ± 0.01c</td>
<td>0.07 ± 0.005c</td>
<td>15.04 ± 0.37c</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>6.29 ± 0.02c</td>
<td>0.077 ± 0.03c</td>
<td>16.13 ± 0.15c</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>6.25 ± 0.03c</td>
<td>0.25 ± 0.05c</td>
<td>17.23 ± 0.37c</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td>6.25 ± 0.01b</td>
<td>0.44 ± 0.07c</td>
<td>19.90 ± 0.22c</td>
</tr>
</tbody>
</table>

Table 3. Quality changes of smoked fish during storage in the refrigerator conditions (4 ± 1°C).

<table>
<thead>
<tr>
<th>Days</th>
<th>pH</th>
<th>TBA (mgMDA/kg)</th>
<th>TVB-N (mg/100g)</th>
<th>TMA (mg/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td></td>
<td>6.32 ± 0.01a</td>
<td>0.67 ± 0.00c</td>
<td>19.58 ± 0.22c</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td>6.29 ± 0.02b</td>
<td>0.29 ± 0.10c</td>
<td>22.02 ± 0.30c</td>
</tr>
<tr>
<td>14</td>
<td></td>
<td>6.30 ± 0.03bc</td>
<td>0.53 ± 0.03cd</td>
<td>22.52 ± 0.08c</td>
</tr>
<tr>
<td>21</td>
<td></td>
<td>6.25 ± 0.02bc</td>
<td>0.86 ± 0.12bc</td>
<td>23.19 ± 0.15c</td>
</tr>
<tr>
<td>28</td>
<td></td>
<td>6.23 ± 0.01c</td>
<td>0.89 ± 0.13bc</td>
<td>26.97 ± 0.77c</td>
</tr>
<tr>
<td>35</td>
<td></td>
<td>6.14 ± 0.01i</td>
<td>0.91 ± 0.13bc</td>
<td>30.16 ± 0.22c</td>
</tr>
<tr>
<td>42</td>
<td></td>
<td>6.16 ± 0.02d</td>
<td>0.97 ± 0.11ab</td>
<td>31.43 ± 0.17c</td>
</tr>
<tr>
<td>49</td>
<td></td>
<td>6.32 ± 0.01a</td>
<td>1.08 ± 0.14a</td>
<td>35.97 ± 0.22b</td>
</tr>
<tr>
<td>56</td>
<td></td>
<td>6.23 ± 0.02e</td>
<td>1.12 ± 0.20a</td>
<td>39.50 ± 0.37c</td>
</tr>
</tbody>
</table>

The TBA value relates to the questionable taste and odor development in fish meat at 1 to 2 mg of MDA/kg. Because TBA is a product of lipid oxidation, it causes an unwanted taste and smell (Connell, 1995). In this study, in fresh fish, the TBA value was 0.07 mg of MDA/kg on day 0, increasing to 0.44 mg of MDA/kg by the end of day 7. Hernandez et al. (2009) investigated the quality changes during the storage of meagre fillets in ice and, similar to our study, found that TBA values were 0.10 at day 0 and 0.86 mg of MDA/kg at day 7. The TBA value on day 0 was calculated to be 0.67 mg for the smoked meagre and the TBA value was increased by the hot smoking process. At the end of day 56, the TBA value increased to 1.12 mg of MDA/kg, but the limit values were not exceeded in terms of these values (Table 3). Similar results were reported by Goulas & Kontominas (2005) in smoked chub mackerel. An increase in TBA value due to storage was also determined by Koral et al. (2016) for hot smoked horse mackerel.

TVB-N is used to determine the quality of fresh and processed products. TVB-N values of "very good" up to 25 mg/100g, "good" up to 30 mg/100g and "marketable" up to 35 mg/100g are considered to be in the seafood quality classification (Connell, 1995). According to the European Union (EEC, 1995), the consumption limit value is 35 mg N/100g (Goulas & Kontominas, 2005). Several researchers declared different acceptability limit values for TVB-N. For example, López-Caballero et al. (2000), 25 to 30 mg N/100g and Kim et al. (2002), 25 to 30 mg N/100g. This has been reported to be due to product variability, specific


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3.3 Microbial quality changes

Masniyom (2011) reported that microbial growth is associated with environmental factors, transport, harvesting methods, habitat and storage. The initial microbial flora counts can vary depending on the region where the fish were caught, the season and the handling conditions (Careche et al., 2002). Initially, the TMAB value of 3.25 log cfu/g increased with storage. Bacterial counts during storage were statistically significant (P<0.05). It was determined that the TMAB numbers for fresh meagre increased to 7.87 log cfu/g on day 9. In the smoked samples, it was determined that the TMAB value was 2.55 log cfu/g on day 0 and 8.15 log cfu/g on day 49 of storage. The TPAB values for fresh meagre increased from 3.07 log cfu/g to 7.56 log cfu/g (in 9 days). The TPAB value was determined to be 2.60 log cfu/g on day 0 for the smoked meagre and 6.56 log cfu/g after day 42 (Table 4-5). In a study where two different (hot and cold) smoking techniques were applied to rainbow trout, it was determined that TMAB and TPAB values increased in both methods (Kolsarıcı & Özkan, 1998). This study also showed an increase due to storage for both groups of microorganisms.

Yeasts molds are not found in the flora of fish. It is reported that these microorganisms originate from soil, and they can be transmitted from the equipment used while fish are being caught (Oksuztepe et al., 2010). In our study, the number of yeast molds in fresh meagre was determined to be 2.53 log cfu/g on day 9, which was not detectable on days 3, 5 and 7 with fresh fish. This value was determined to be 2.46 log cfu/g at the end of day 56 for the smoked meagre. The microbiological degradation criterion is reported as a limit value of 6 to 7 log cfu/g for fresh fish and its products (International Commission on Microbiological Specifications for Foods, 1986; Çakılı, 2007; Çalikoğlu et al., 2018; Plahar et al. 1999). After the hot smoking process, TMAB presence in spoiling fish is due to the bacterial reduction of Trimethylamine oxide that is naturally present in the living tissue of many marine fish (Huss, 1995). The limit value for TMAB-N has been reported as 10 - 15 mg/100 g (Huss, 1988) while the European Union reports this value as 12 mg N/100 g (Goulas & Kontominas, 2005). The TMAB value increased from 3.59 ± 0.08 to 3.75 ± 0.08 on day 9. The TPAB value increased to 7.87 ± 0.13 on day 9. On the smoked samples, the limit value was exceeded.
4 Conclusion

Meagre has a high nutritional value in terms of fat quality and protein value. Meagre is rich in PUFA, showing the highest measured value in every sampling period compared to other reared marine fish. Identification of quality in terms of pH, TBA and TMA-N values for both the fresh and hot smoked samples do not exceed the limit values during storage. Meagre seems to be suitable material for hot smoking technology. Thus, this product could be used to provide a new and alternative opportunity for the consumption of meagre.

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References


ECC. (1995). 95/149/EC: commission decision of 8 March 1995 fixing the total volatile basic nitrogen (TVB-N) limit values for certain categories of fishery products and specifying the analysis methods to be used. Official Journal of European Communities, L97, 84-87.


Sigurgisladottir, S., Sigurdardottir, M. S., Torrissen, O., Vallet, J. L., & Hafsteinsson, H. (2000). Effects of different salting and smoking process on the microstructure, the texture and yield of Atlantic


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