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Quality Changes and Shelf-Life of Cultured and Wild Hot-Smoked Mediterranean Horse Mackerel (*Trachurus mediterraneus*, Steindachner, 1868) at Frozen Storage (-20±2°C)

Serkan Koral[1,]*, Bekir Tufan[2], Nadir Başçınar[2], Sevim Köse[2]

[1] Recep Tayyip Erdoğan University, Faculty of Fisheries, Rize, Turkey
[2] Karadeniz Technical University, Faculty of Marine Sciences, 61530 Çamburnu, Trabzon, Turkey.

* Corresponding Author: Tel.: +90.464 2233385; Fax: +90.464 2234118;
E-mail: serkankoral@gmail.com

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Abstract

Smoking is a traditional preserving method used for both fish and meat products around the world. Smoked fish products have wide acceptance today due to their accustomed taste and aroma as well as their extended shelf-life. In this study, we aimed to determine quality changes and shelf-life of wild hot-smoked Mediterranean horse mackerel (*Trachurus mediterraneus*, Steindachner, 1868) during frozen storage (-20±2°C) in comparison with its cultured counterparts. After gutting, cleaning and draining the samples, they were salted in 10% brine solution for 1 h and hot smoked within the same day. The samples were placed in styrofoam plates covered with a stretched film and frozen at -40°C for 24 h. Then, all samples were stored at -20±2°C until spoilage. Chemical, microbiological and sensory analyses were performed monthly to investigate their quality changes and the shelf stability of the products. The results of total volatile basic-nitrogen, thiobarbituric acid and trimethylamine were obtained within the acceptable levels. Histamine values were found well below the permitted limits set by Food and Drug Administration and European Union. Sensory results showed that both storage groups (wild and cultured) had 7 months of shelf-life. This study showed that hot-smoked wild and cultured horse mackerel can be stored at -20±2°C for 7 months. Culturing did not make any significant differences (P<0.05) in sensory quality despite of differences in chemical and microbiological changes between wild and cultured groups during storage. On the other hand, better consumer acceptance was observed for cultured samples indicating the advantage of culturing this species for a better market value. We also observed that frozen storage can retard the formation of biogenic amine contents and therefore, it is suggested to apply for such products to avoid histamine health risk.

Keywords: Mariculture, horse mackerel, hot-smoked, frozen storage, shelf-life, biogenic amines.

Sıcak Tütsülenmiş Kültür ve Doğal İstavritin (Trachurus mediterraneus, Steindachner, 1868) Donmuş Muhafaza (-20±2°C) Koşullarındaki Kalite Değişimleri ve Raf Ömrü

Özet

Tütsüleme dünyada hem balık hem de et ürünlerinde kullanılan geleneksel bir koruma yöntemidir. Günümüzde tütsülenmiş balıklar kendilerine özgü tat ve aromanın yanı sıra uzun raf ömrüne sahip olmalarından dolayı da çokça rağbet görmektedirler. Bu çalışmada, doğal ve kültüre edilmiş istavrit balıklarının sıcak tütsüleme yöntemiyle tütsülendikten sonra donmuş muhafaza (-20±2°C) koşullarında depolanmaları esnasındaki kalite değişimleri ve buna bağlı olarak raf ömürlerinin karşılaştırmalı olarak tespit edilmesi amaçlanmıştır. Balıkların iç organları temizlenip yıkanmış, daha sonra %10'luk tuz salamurası ile 1 saat boyunca bekletildikten sonra sıcak tütsüleme işlemi uygulanmıştır. Örnekler strafor tabaklara dizilip üzerleri streç film ile kaplanmış ve -40°C'de 24 saat şoklandıktan sonra donmuş muhafaza koşullarında (-20±2°C) depolanmışlardır. Ürünlerdeki kalite değişimine bağlı raf ömrünün tespiti için aylık olarak kimyasal, mikrobiyolojik ve duyusal analizler yapılmıştır. Toplam Uçucu Bazik Azot (TVB-N), Tiyobarbitürik Asit (TBA) ve Trimetilamin (TMA) analizi sonuçlarına göre her iki grup da kabul edilebilir sınırlar içerisinde kalmıştır. Her iki grubun da histamin miktarları hem Amerikan Gıda ve İlaç Dairesi hem de Avrupa Birliği kuruluşlarının izin verdiği değerlerin altında bulunmuştur. Duyusal analiz sonuçlarına göre her iki grubun da 7 aylık raf ömrüne sahip olduğu tespit edilmiştir. Bu çalışma, sıcak tütsülenmiş doğal ve kültür istavrit balıklarının donmuş muhafaza koşullarında 7 ay süreyle depolanabileceğini göstermiştir. Kimyasal ve mikrobiyolojik kalite parametrelerinde her iki grupta da istatistiki olarak önemli değişimler gözlenmesine rağmen (P<0,05), duyusal değerlendirmede önemli bir farka rastlanmamıştır. Bununla beraber, kültür örneklerinin tüketici beğenisinin daha iyi olması bu türün kültüre edilmesinin daha iyi pazar değeri olacağına işaret edebilir. Ayrıca bu çalışmada biyojenik amin gelişiminin yavaşladığı gözlemlendiği için, bu tür örneklerde donmuş depolama sayesinde histaminden kaynaklı sağlık riski azalacaktır.

Anahtar Kelimeler: Kafes yetiştiriciliği, istavrit, sıcak tütsüleme, donmuş muhafaza, raf ömrü, biyojenik aminler

Introduction

Marine foods have recently attracted more attention from consumers as sources of nutritional components that have positive benefits in human health and nutrition (Simopoulos, 1997). Food and Agricultural Organization of United Nations (FAO, 2012a) reported that demand for fish for human consumption, particularly marine fatty fish, has been increasing over the years. However, a major source of omega-3 fatty acids is becoming scarcer as the most captured fisheries become fully-exploited and overexploited (FAO, 2012b; Gjerde *et al*., 2013). This increasing demand and the dwindling of wild fish stocks require further development of the aquaculture sector in order to sustain the supply of nutritional products rich in fatty acids and other valuable nutritional substances for the human diet (Lenas *et al*., 2011). Therefore, mariculture is emerging as a sustainable and alternative way to increase production of fish rich in omega-3 fatty acids.

Mediterranean horse mackerel (*Trachurus mediterraneus,* Steindachner, 1868) is the second most important commercial species in the Black Sea (TUIK, 2014). This is a semi-pelagic carnivore species and has a schooling habit, feeds on other fishes, (especially sardines, anchovies and small crustaceans) (Fish Base, URL-1). It is most commonly found at a depth of 20-200 meters (Šantič *et al*., 2003). Although world capture of Mediterranean horse mackerel trended upwards until 1990, the production began to decline since then, though with some fluctuation. In 2011, total world production was 206.724 tons, only 3.3% of which was contributed by Turkey (FAO, 2013). In 2012, Turkish production was recorded as 24.625 tons, approximately 86% of which came from Black Sea region (TUIK, 2014).

In our project study (TAGEM-10/AR-GE/19), the possibility of culturing Mediterranean horse mackerel (*T. mediterraneus*) in Turkish waters of Black Sea was demonstrated (Boran *et al*., 2013). Later, we also investigated the effect of culturing on hot-smoked quality of this species at refrigerated temperatures in comparison to its wild counterparts (Koral *et al*., 2015). The results showed that although there was a significant difference ($P<0.05$) in proximate composition between cultured and wild horse mackerel, culturing this species did not affect its storage quality parameters after smoking at refrigerated conditions. We observed three weeks of shelf-life for hot smoked horse mackerel at chilled conditions.

Nowadays, frozen smoked fish products are commonly sold commercially around the world in seafood market. Various hot and cold smoked fish products from both wild (anchovy, bonito and herring) and cultured fish (trout and salmon) species are also produced and marketed in Turkey. Some of these products are exported to Europe and USA (Köse, 2010; Köse *et al*., 2010). However, frozen fish products can be prone to quality losses such as protein denaturation, color deterioration, weight decrease, oxidation of lipids and textural changes due to the freezing and storage conditions (Foegeding *et al*., 1996). Shelf life and the quality of frozen seafood depend on the handling conditions, amount and the type of products, packing method, freeze-thaw abuse and temperature of storage and temperature fluctuations (Turan *et al*., 2004). There is no existing study on the quality changes and the shelf-life of hot-smoked cultured horse mackerel during frozen storage. Therefore, this study was aimed to compare the effect of culturing on the quality changes of hot-smoked Mediterranean horse mackerel (*T. mediterraneus*) during frozen storage.

Materials and Methods

Experimental Design

Wild Mediterranean horse mackerel (*T. mediterraneus*) samples were purchased from the fish market in Rize, Turkey in December 2012. This study was carried out in parallel to our previous study on hot-smoked horse mackerel stored at refrigerated storage (Koral *et al*., 2015). Therefore, the properties of the raw materials, the processing method and yield results are the same as described in Koral *et al*. (2015). The samples were divided into two groups as wild horse mackerel (WHM) and cultured horse mackerel (CHM). In total, 440 fish weighing 18.8 kg were used for both groups in the study. The ages of fish were between 1+ and 2 years old. Each group was processed separately as described in our previous study (Koral *et al*., 2015) and in Figure 1. After smoking and packaging, products were frozen at -40 °C for 24 h and stored at -20±2°C.

Chemicals and Reagents

All chemicals and solvents used were analytical and chromatographic grade, respectively. Putrescine dihydrochloride (Cat.no: P7505, ≥%98), dansyl chloride (Cat.no: D2625-5G, ≥%99.0) and trimethylamine hydrochloride (Cat.no: T7, 267-1, ≥%99) were purchased from Sigma-Aldrich (Switzerland). 2-phenylethylamine hydrochloride (Cat.no:77905, ≥%99), histamine dihydrochloride (Cat.no: 53300, ≥%99), cadaverine dihydrochloride (Cat.no: 33220, ≥%99) tyramine hydrochloride (Cat.no: 93820, ≥%97), spermidine trihydrochloride (Cat.no: 85580, ≥%99.0), spermine tetrahydrochloride (Cat.no: 85607, ≥%99.5), triptamine hydrochloride (Cat.no: 93650, ≥%99), bromocresol purple (Cat.no: 17490), HPLC grade acetonitrile (Cat.no: 1.0030.2500, ≥%99.8,) and HPLC grade ultra-pure water (Cat.no: 1.15333.2500) were obtained from Fluka (Germany and Switzerland). Ammonium acetate (Cat.no: 0103-500G, %100) was provided

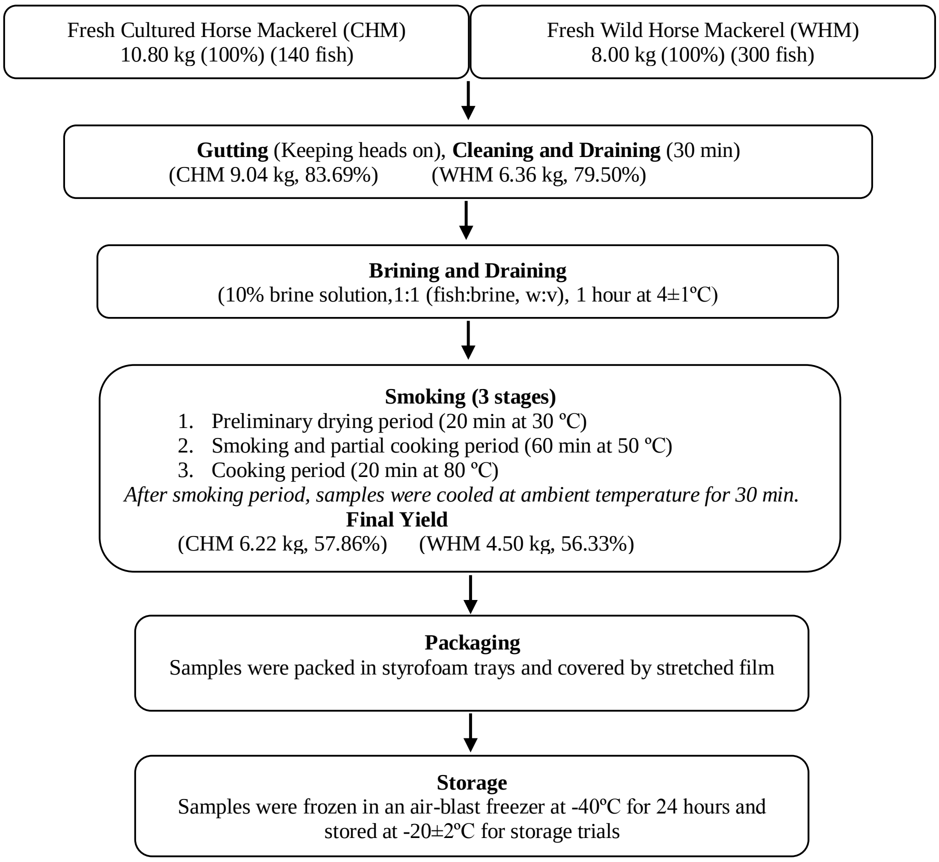

Figure 1. Yield and processing flow diagram of hot-smoked wild and cultured horse mackerel
CHM: Cultured horse mackerel, WHM: Wild horse mackerel

from Amresco (ABD), trichloroacetic acid (Cat.no: 411527, ≥%99), acetone (Cat.no: 412102, ≥%99.8), formaldehyde (Cat.no: 7040, %37) were supplied from Carlo Erba (Italy). NaOH (Cat.no: 480507, %99-100), hydrochloric acid (Cat.no: 07102, %37) and potassium chromate (Cat.no: 12249, ≥%99) were purchased from Riedel-de Haen (Chez Republic and Germany). Perchloric acid (Cat.no: 1.00518.2500), ammonia solution (Cat.no: 1.105422.2500, %25), sodium bicarbonate (Cat.no: 1.06329.1000), boric acid (Cat.no: 1.00160.1000, %99.5-100), potassium carbonate (Cat.no: 1.04428.1000, ≥%99), toluene (Cat.no: 108389), picric acid (Cat.no: 1.00621.0500, ≥%99), thiobarbituric acid (Cat.no: 1.0880.0025), magnesium oxide (Cat.no: 105862), calcium carbonate (Cat.no: 1.04928.1000, ≥%99), silver nitrate (Cat.no: 1.01512.0025), plate count agar (Cat.no: 1.05463.0500), agar agar (Cat.no: 1.01613.0500), L -Histidine monohydrochloride (Cat.no: 1.04350.0100), yeast extract (Cat.no: 1.0325.0500) and NaCl (Cat.no: 1.06404.1000) were obtained from Merck (Germany).

Analysis

Age was determined according to the procedure of Macer, (1968; 1972) and Marecos, (1986). Moisture content was determined by oven drying of 5g of fish muscle at 105°C until a constant weight was obtained (AOAC 1995, Method 985.14). The method of Lücke and Geidel (1935) was used to determine total volatile basic-nitrogen (TVB-N) content as described by Goulas and Kontominas (2005). Thiobarbituric acid (TBA) values, expressed in mg malonaldehyde (MA)/kg, were estimated by using the method of Tarladgis *et al*. (1960) described by Smith *et al*. (1992). The method of Boland and Paige (1971) was used for trimethylamine (TMA) analysis. Biogenic amines were analyzed using high performance liquid chromatography (HPLC) method according to Köse *et al*. (2011) as modified from Eerola *et al*. (1993). HPLC equipment was Shimadzu Prominence LC-20 AT series (Japan) HPLC with autosampler (SIL20AC, Shimadzu, Japan), Diode Array Detector (SPD-M20A, Shimadzu, Japan) and Intertsil column (GL Sciences, ODS-3, 5 µm, 4.6x250 mm). All chemical analyses were carried out in triplicate. Total viable counts (TVC) were counted according to Köse *et al*. (2001). Total aerobic viable psychrotrophic and mesophilic bacteria were counted using plate count agar incubated at 4°C for 8 days for psychrophilic microorganisms and at 37°C for 24-48 h for mesophilic microorganisms. Histamine-forming bacteria (HFB) were determined according to

Yoshinaga and Frank (1982) using a modified Niven's medium (Niven *et al*., 1981) by adjusting the pH to 6.5. Microbial counts were carried out in duplicate and expressed as log cfu/g. Sensory analyses were performed by using modified methods of Amerina *et al*. (1965) and Archer (2010). Smoked fish samples were assessed on the basis of appearance, odor, taste and texture characteristics. Eight trained panelists judged the overall acceptability of the samples using ten point descriptive scale. According to the scale, 10–9 is excellent, 8–7:good, 6–5: medium, 4: the limit for acceptable⁄unacceptable and <4: unacceptable.

Statistical Analysis

The data obtained were analyzed by one way analysis of variance (ANOVA) and when significant differences were found, comparisons among means were carried out by using a Tukey and Mann Whitney U test (data not provided in the normality of assumptions) (P<0.05) under the program called JMP 5.0.1 (SAS Institute. Inc. USA) and SPSS18.0 (SPSS Inc., Chicago, IL) (Sokal and Rohlf, 1987).

Results

Moisture contents of fresh cultured and wild horse mackerel samples were 68.7 and 75.3%, respectively (Figure 2). The results showed significant differences (P<0.05) in moisture contents of fresh and hot-smoked samples. The levels decreased significantly to 63.6 % and 64.1 % in the same respect (P<0.05). Although there were significant difference (P<0.05) in the moisture contents between cultured and wild sample groups within the same months the changes were not found significant (P>0.05) throughout the storage for both groups as shown in Figure 2. Lower values were attributed to the cultured hot-smoked mackerel.

Table 1 represents sensory results (appearance+ texture, odour and taste). Sensory values significantly decreased throughout frozen storage (P<0.05). Although there were significant differences (P<0.05) in the values between cultured and wild sample groups the shelf-life of both sample groups were 7 months. At the end of 8th month of frozen storage, sour and bitterness in taste were accompanied by the off odours. The main reasons that panelists liked cultured samples were the size and appearance of the fish. Also according to sensory results panelists appreciated cultured samples much more than wild samples in terms of taste.

Figure 3 demonstrates the chemical quality changes of smoked horse mackerel samples stored at -20±2°C. The samples were analyzed until the products spoilt according to sensory analysis. TVB-N values of cultured and wild hot-smoked horse mackerel samples were observed as 13.16 mg/100g and 14.26 mg/100 g, respectively. Then, TVB-N values increased significantly for during storage at -20±2°C (P<0.05). Significant differences (P<0.05) were also obtained between two groups within the same months. TMA values of cultured and wild hot-smoked horse mackerel samples significantly increased starting from 3rd month during the storage in both groups (P<0.05). The values were found 2.26 mg/100 g and 2.21 mg/100 g, respectively at the end of storage. The values of TBA for both groups increased significantly during storage (P<0.05). The highest value was found as 4.02 mg MA/kg for CHM group in the 8th month. Smoke compounds might have had antioxidative effect to prevent lipid from oxidation that may explain low TBA values in the study. TBA values were significantly higher (P<0.05) for CHM in comparison with WHM after 2nd month of frozen storage

Table 2 shows the results of biogenic amine

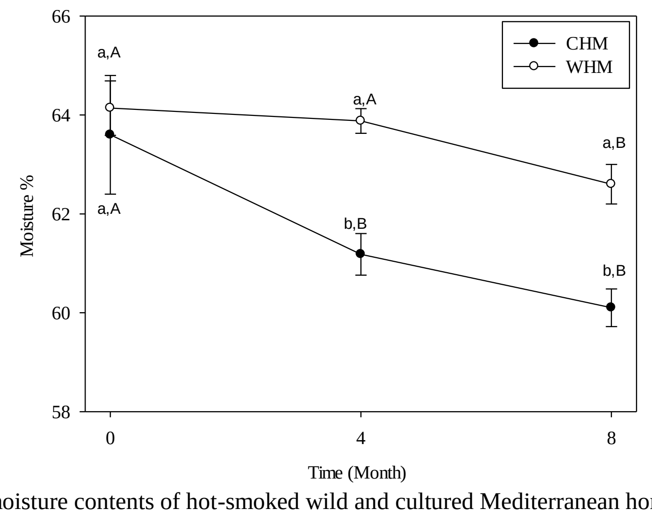

Figure 2. The changes of moisture contents of hot-smoked wild and cultured Mediterranean horse mackerel during frozen storage (-20±2°C).

CHM: Cultured Mediterranean horse mackerel, WHM: Wild Mediterranean horse mackerel. Different uppercase letters (A,B) represent significant differences before and after processing and also amongst the months. Different lowercase letters (a,b) represents differences amongst cultured and wild samples within the same month.

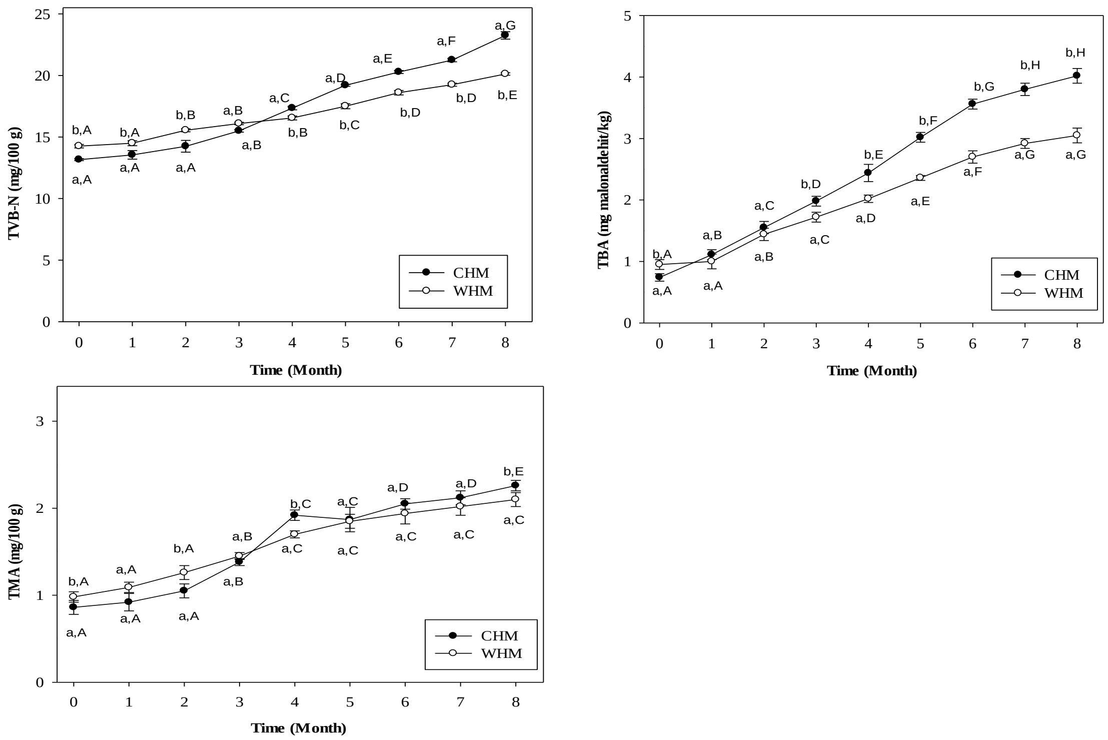

Figure 3. The changes in the values of chemical quality parameters for cultured and wild hot-smoked horse mackerel samples during frozen storage (-20±2°C).

n=3, TVB-N: Total Volatile Basic Nitrogen, TBA: Thiobarbutiruc acid, TMA: Trimethylamine, Different small letters (a,b) represent significant difference among groups (P<0.05). Different capital letters (A,B,C,…) represent significant difference amongst different months within the same group during storage (P<0.05). CHM: Smoked cultured horse mackerel, WHM: Smoked wild horse mackerel

Table 1. Changes in sensory values of hot smoked wild and cultured horse mackerel samples stored at frozen conditions (-20±2°C)

Months	Sample Type	Appearance+Texture	Odour	Taste	Overall mean
0	CHM	$9.85\pm0.15^{a}_{A}$	$9.50\pm0.22^{a}_{A}$	$9.85\pm0.10^{a}_{A}$	$9.73\pm0.20^{a}_{A}$
	WHM	$9.40\pm0.10^{b}_{A}$	$9.00\pm0.14^{b}_{A}$	$9.10\pm0.18^{b}_{A}$	$9.17\pm0.22^{b}_{A}$
1	CHM	$9.70\pm0.10^{a}_{A}$	$9.40\pm0.15^{a}_{A}$	$9.45\pm0.12^{a}_{B}$	$9.52\pm0.30^{a}_{A}$
	WHM	$8.80\pm0.14^{b}_{B}$	$8.90\pm0.16^{b}_{A}$	$8.65\pm0.20^{b}_{B}$	$8.78\pm0.18^{b}_{A}$
2	CHM	$9.10\pm0.08^{a}_{B}$	$8.85\pm0.10^{a}_{B}$	$9.20\pm0.14^{a}_{C}$	$9.05\pm0.18^{a}_{B}$
	WHM	$8.20\pm0.16^{b}_{C}$	$8.05\pm0.12^{b}_{B}$	$7.90\pm0.06^{b}_{C}$	$8.05\pm0.16^{b}_{B}$
3	CHM	$8.80\pm0.20^{a}_{C}$	$8.70\pm0.22^{a}_{B}$	$8.50\pm0.10^{a}_{D}$	$8.68\pm0.24^{a}_{C}$
	WHM	$7.60\pm0.12^{b}_{D}$	$7.45\pm0.08^{b}_{C}$	$7.65\pm0.12^{b}_{D}$	$7.56\pm0.08^{b}_{C}$
4	CHM	$8.30\pm0.08^{a}_{D}$	$8.10\pm0.06^{a}_{C}$	$8.00\pm0.12^{a}_{E}$	$8.12\pm0.16^{a}_{D}$
	WHM	$7.10\pm0.18^{b}_{E}$	$6.80\pm0.06^{b}_{D}$	$6.90\pm0.08^{b}_{E}$	$6.92\pm0.14^{b}_{D}$
5	CHM	$7.50\pm0.10^{a}_{E}$	$7.50\pm0.12^{a}_{D}$	$7.10\pm0.08^{a}_{F}$	$7.36\pm0.23^{a}_{E}$
	WHM	$6.20\pm0.10^{b}_{F}$	$6.10\pm0.08^{b}_{E}$	$6.35\pm0.12^{b}_{F}$	$6.23\pm0.10^{b}_{E}$
6	CHM	$6.10\pm0.20^{a}_{F}$	$6.35\pm0.10^{a}_{E}$	$6.10\pm0.06^{a}_{G}$	$6.18\pm0.28^{a}_{F}$
	WHM	$5.00\pm0.12^{b}_{G}$	$5.20\pm0.06^{b}_{F}$	$5.15\pm0.12^{b}_{G}$	$5.12\pm0.12^{b}_{F}$
7	CHM	$5.40\pm0.10^{a}_{G}$	$5.80\pm0.12^{a}_{F}$	$5.45\pm0.08^{a}_{H}$	$5.55\pm0.20^{a}_{G}$
	WHM	$4.50\pm0.12^{b}_{H}$	$4.20\pm0.10^{b}_{G}$	$4.40\pm0.22^{b}_{H}$	$4.38\pm0.18^{b}_{G}$
8	CHM	$3.80\pm0.06^{a}_{H}$	$4.10\pm0.08^{a}_{G}$	$3.70\pm0.04^{a}_{I}$	$3.86\pm0.18^{a}_{H}$
	WHM	$2.95\pm0.10^{b}_{I}$	$2.90\pm0.12^{b}_{H}$	$2.65\pm0.08^{b}_{I}$	$2.82\pm0.16^{b}_{H}$

'±' SD. n=8 (Each data represents the mean value of scores obtained from 8 panelists).'4' is the limit for acceptability/unacceptability of the samples. CHM: Smoked cultured horse mackerel. WHM: Smoked wild horse mackerel.. Different superscript small letters (a,b,c,…) represents significant difference among groups (P<0.05). Different superscript capital letters (A,B,C,...) represents significant difference amongst different weeks within the same group during storage (P<0.05).

contents for the fresh and hot-smoked cultured and wild horse mackerel samples. Histamine levels of fresh cultured and wild horse mackerel samples were under the detection limit (<0.86 ppm). The detectable histamine levels started at each sample at the beginning of storage as 1.61 ppm and 2.19 ppm for WHM and CHM, respectively. However, these values in both groups were found under the detection level (<0.86 ppm) during storage. With the exception of spermidine, the changes of all biogenic amine contents were not significant throughout the storage, implying the effect of frozen storage to prevent biogenic amine formation and therefore, help to avoid health risk arise from these amines. The highest levels of cadaverine, putrescine, phenylethylamine, tryptamine, tyramine, spermidine and spermine were determined as 2.86 ppm, 1.40 ppm, 9.28 ppm, 16.68 ppm, 3.18 ppm, 21.49 ppm and 2.52 ppm, respectively.

Initial counts of mesophilic and psychrophilic TVC, and mesophilic and psychrophilic HFB were found as 1.98 log cfu/g and 2.12 log cfu/g, 1.86 log cfu/g and 2.17 log cfu/g, 1.70 log cfu/g and 1.86 log cfu/g, and 1.80 log cfu/g and 1.92 log cfu/g in wild and cultured horse mackerel samples, respectively. Although there were significant differences ($P<0.05$) between two groups within the same months during storage the values of mesophilic TVB did not change significantly ($P>0.05$) throughout the storage with some exceptions for cultured samples (Figure 4). However, the values of phsycrophilic TVC significantly increased during storage for both groups ($P<0.05$). However, the counts of mesophilic total HFB did not change significantly during storage with some exceptions although significant differences ($P<0.05$) were observed between cultured and wild sample groups within the same month.

Discussion

Varying shelf life was reported by different studies on different smoked fish products. However, limited research exits on wild and cultured hot-smoked Mediterranean horse mackerel (*T. mediterraneus*) stored at frozen conditions. Cardinal *et al*. (2001) recommend moisture content below 65% for smoked fish. Our results showed slightly lower moisture contents than the suggested value. Goulas and Kontominos (2005), and Kolodziejska *et al*. (2002) also observed much lower levels for different smoked mackerel samples as 58.1% and 56.7%, respectively.

According to sensory results of this study showed that smoked wild and cultured horse mackerel (*T. mediterraneus*) air packed in styrofoam plates covered with a stretched film could be stored at -20±2°C for 7 months. Deng *et al*. (1974) reported 7 months of shelf-life at -12°C for smoked Spanish mackerel (*Scomberomorus maculatus*) in terms of sensory results.

TVB-N is one of the most widely used parameter to evaluate fish quality. It represents the sum of ammonia, dimethylamine, TMA and others basic nitrogenous compounds volatile under the analysis conditions. Varying levels of TVB-N have been suggested for different fish products to assess their freshness in literature (Connell, 1990; Huss, 1988). In freshly caught fish, TVB-N content is generally superior to 10 mg/100 g and does not exceed 15 mg/100 g. However, a level of 35 mg/100 g has been considered the upper limit, above which fishery products are considered unfit for human consumption (Ludorf and Meyer, 1973). European Union (EU) also set varying TVB-N limits as 25-35 mg/100 g for unprocessed fishery products shall be regarded as unfit for human consumption where organoleptic assessment has raised doubts as to their freshness (EU Directive, 2005b and 2008). However, Mediterranean horse mackerel (*T. mediterraneus*) or other horse mackerel species are not included in EU regulation. Therefore, TVB-N levels can be used only in support of sensory values. However, TVB-N levels of this study were within the acceptable quality set by EU at the end of 8^{th} month despite of unacceptable sensory values. Our previous study showed that TVB-N values of hot smoked cultured and wild horse mackerel products reached to unacceptable quality on the 4^{th} week at refrigerated storage (Koral *et al*., 2015). Magnusson and Martinsdottir (1995) also observed slower TVB-N formation in the cod fillets at the frozen conditions. Kaya and Erkoyuncu (1999) observed 35.5 mg/100 g TVB-N for smoked bonito stored at ambient temperature on the 4^{th} day while the levels were 32.9 mg/100 g for samples stored at refrigerator on the 13^{th} day. Therefore, this study demonstrates the advantage of frozen storage to improve the shelf life of hot smoked horse mackerel products. Trimethylamine is a pungent volatile amine often associated with the typical "fishy" odour of spoiling seafood. Its presence in spoiling fish is due to the bacterial reduction of trimethylamine oxide which is naturally present in the living tissue of many marine fish species. Although TMA is believed to be generated by the action of spoilage bacteria, the correlation with bacterial numbers is often not very good (Huss, 1995). A suggested acceptable level is reported as 12 mg/100 g (Goulas and Kontominos, 2005). The results showed that TMA values of both groups were within the acceptable range throughout the storage period. Goulas and Kontominos (2005) also found that vacuum packed smoked chum mackerel stored at 2.0±0.5°C had very low levels of TMA as 3.6 mg/100g on 30^{th} day. TBA is also used as quality parameters particularly related to lipid oxidation. Maximum allowed level of TBA is accepted as 8 mg MA/kg (Schormüller, 1969). The differences may be attributed to the high lipid contents of cultured fish. Deng *et al*. (2002) found that smoked Spanish mackerel (*Scomberomorus maculatus*) packed in thick polyethylene bags (1.5 mm) and stored at -12°C had high levels of TBA as 9 mg/kg in 7^{th} month.

Biogenic amines are biologically active compounds normally produced by decarboxylation of free amino acids and are present in a variety of foods, e.g. fish, fish products, meat, wine and cheese. They can cause pseudoallergic reactions due to their psychoactive and vasoactive properties such as nausea, headaches and vomiting in mild cases to intracerebral hemorrhage and even death in the severe

Table 2. Biogenic amine contents of hot-smoked cultured and wild horse mackerel samples during frozen storage (-20±2°C)

Months	Sample groups	Biogenic amines (ppm)							
		Tryptamine	Phenyletylamine	Putrescine	Cadaverine	Histamine	Tyramine	Spermidine	Spermine
0	CHM	11.77±0.30$^{a}_{A}$	8.98±0.60$^{a}_{A}$	1.07±0.10$^{a}_{A}$	2.17±0.80$^{a}_{A}$	2.19±0.50$^{a}_{A}$	2.64±0.10$^{a}_{A}$	13.21±1.80$^{a}_{A}$	2.07±0.10$^{a}_{A}$
	WHM	16.22±1.30$^{b}_{A}$	9.10±0.40$^{a}_{A}$	1.10±0.10$^{a}_{A}$	2.18±0.30$^{a}_{A}$	1.61±0.80$^{b}_{A}$	3.11±0.30$^{b}_{A}$	17.02±1.40$^{b}_{A}$	1.72±0.50$^{a}_{A}$
1	CHM	12.05±0.50$^{a}_{A}$	8.48±0.50$^{a}_{A}$	1.12±0.16$^{a}_{A}$	2.28±0.08$^{a}_{A}$	1.42±0.18$^{a}_{B}$	2.38±0.08$^{a}_{A}$	14.22±0.46$^{a}_{B}$	1.86±0.16$^{a}_{A}$
	WHM	15.66±1.15$^{b}_{A}$	9.18±0.36$^{a}_{A}$	1.12±0.13$^{a}_{A}$	2.28±0.10$^{a}_{A}$	1.26±0.14$^{a}_{A}$	3.06±0.16$^{b}_{A}$	17.20±1.20$^{b}_{A}$	1.68±0.12$^{a}_{AB}$
2	CHM	12.20±0.62$^{a}_{A}$	8.60±0.30$^{a}_{A}$	1.28±0.20$^{a}_{A}$	2.36±0.12$^{a}_{A}$	<0.86*	2.12±0.16$^{a}_{A}$	15.68±0.86$^{a}_{B}$	2.05±0.08$^{a}_{A}$
	WHM	15.88±1.05$^{b}_{A}$	9.20±0.56$^{a}_{A}$	1.18±0.12$^{a}_{A}$	2.10±0.10$^{a}_{A}$	<0.86*	3.12±0.06$^{b}_{A}$	15.56±1.28$^{a}_{B}$	1.46±0.06$^{b}_{B}$
3	CHM	11.80±0.42$^{a}_{A}$	7.70±0.44$^{a}_{A}$	1.20±0.08$^{a}_{A}$	2.45±0.12$^{a}_{A}$	<0.86*	2.06±0.10$^{a}_{A}$	13.28±0.44$^{a}_{A}$	1.68±0.09$^{a}_{B}$
	WHM	16.20±1.74$^{b}_{A}$	8.90±0.60$^{a}_{A}$	1.28±0.26$^{a}_{A}$	2.42±0.14$^{a}_{A}$	<0.86*	2.88±0.08$^{b}_{A}$	18.20±0.36$^{b}_{A}$	1.52±0.12$^{a}_{B}$
4	CHM	11.98±0.38$^{a}_{A}$	7.86±0.40$^{a}_{A}$	1.32±0.20$^{a}_{A}$	2.48±0.08$^{a}_{A}$	<0.86*	1.88±0.10$^{a}_{B}$	14.56±0.52$^{a}_{B}$	1.58±0.06$^{a}_{B}$
	WHM	16.02±0.24$^{b}_{A}$	9.18±0.52$^{a}_{A}$	1.26±0.62$^{a}_{A}$	2.34±0.10$^{a}_{A}$	<0.86*	2.88±0.18$^{b}_{A}$	16.26±0.60$^{b}_{A}$	1.62±0.04$^{a}_{B}$
5	CHM	16.68±0.40$^{a}_{B}$	7.68±0.12$^{a}_{A}$	1.05±0.44$^{a}_{A}$	2.88±0.08$^{a}_{A}$	<0.86*	2.14±0.10$^{a}_{A}$	15.33±0.46$^{a}_{B}$	1.86±0.06$^{a}_{A}$
	WHM	15.90±0.55$^{a}_{A}$	9.28±0.28$^{b}_{A}$	1.32±0.38$^{a}_{A}$	2.48±0.12$^{a}_{A}$	<0.86*	3.10±0.16$^{b}_{A}$	14.60±0.58$^{a}_{B}$	1.44±0.08$^{b}_{B}$
6	CHM	11.80±0.32$^{a}_{A}$	8.28±0.52$^{a}_{A}$	1.40±0.24$^{a}_{A}$	2.60±0.10$^{a}_{A}$	<0.86*	2.06±0.10$^{a}_{A}$	16.20±0.38$^{a}_{B}$	1.72±0.12$^{a}_{B}$
	WHM	15.56±0.44$^{b}_{A}$	8.98±0.68$^{a}_{A}$	1.18±0.20$^{a}_{A}$	2.34±0.12$^{a}_{A}$	<0.86*	3.15±0.12$^{b}_{A}$	16.80±0.50$^{a}_{A}$	1.36±0.08$^{b}_{B}$
7	CHM	12.02±0.18$^{a}_{A}$	8.46±0.38$^{a}_{A}$	1.28±0.08$^{a}_{A}$	2.86±0.22$^{a}_{A}$	<0.86*	1.98±0.16$^{a}_{A}$	15.23±0.40$^{a}_{B}$	1.60±0.06$^{a}_{B}$
	WHM	16.12±1.20$^{a}_{A}$	9.28±0.46$^{a}_{A}$	1.26±0.16$^{a}_{A}$	2.55±0.14$^{a}_{A}$	<0.86*	2.90±0.10$^{b}_{A}$	18.30±0.34$^{b}_{A}$	1.40±0.04$^{b}_{B}$
8	CHM	12.20±0.48$^{a}_{A}$	8.12±0.20$^{a}_{A}$	1.26±0.18$^{a}_{A}$	2.70±0.40$^{a}_{A}$	<0.86*	2.06±0.16$^{a}_{A}$	14.20±0.18$^{a}_{B}$	1.48±0.09$^{a}_{C}$
	WHM	15.90±0.80$^{b}_{A}$	9.15±0.42$^{a}_{A}$	1.20±0.09$^{a}_{A}$	2.48±0.18$^{a}_{A}$	<0.86*	2.86±0.12$^{b}_{A}$	15.20±0.26$^{b}_{B}$	1.38±0.09$^{a}_{B}$

±SD, n=3,
*: The levels were under detection limit,
CHM: Cultured horse mackerel samples,
WHM: Wild horse mackerel samples. Different superscript letters (a,b,c) on the data represents significant differences amongst groups (P<0.05).
Different superscript capital letters (A,B,C.) represents significant difference amongst different months within the same group during storage (P<0.05).

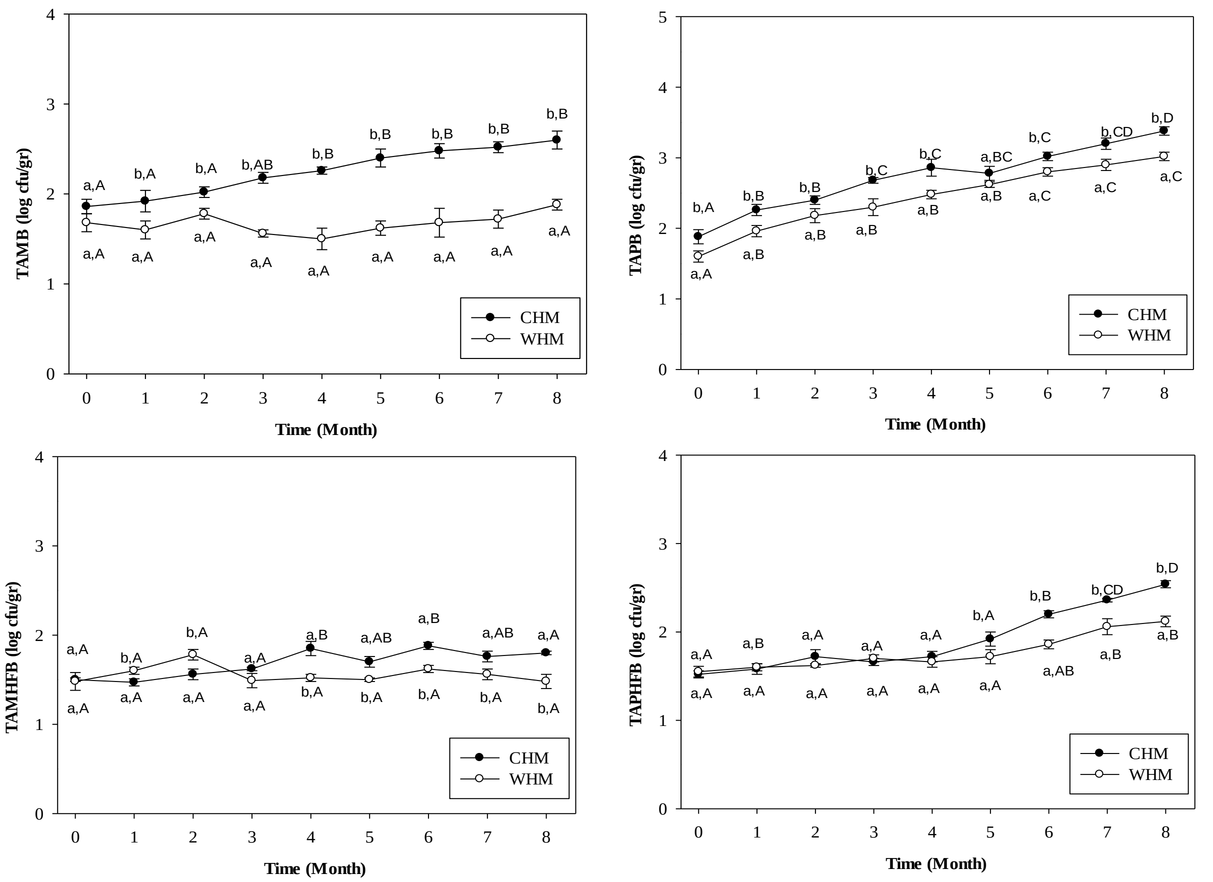

Figure 4. Microbial changes of cultured and wild hot-smoked horse mackerel samples during frozen storage (-20±2°C)
n=3, CHM: Smoked cultured horse mackerel samples, WHM: Smoked wild horse mackerel samples. TAMB: Total Aerobic Mesophilic Bacteria, TAPB: Total Aerobic Psychrophilic Bacteria, TAMHBF: Total Aerobic Mesophilic Histamine Forming Bacteria, TAPHBF: Total Aerobic Psychrophilic Histamine Forming Bacteria, Different small letters (a,b) represents significant difference among groups (P<0.05). Different capital letters (A,B) represents significant difference amongst different months within the same group during storage (P<0.05).

ones. Biogenic amines may also be considered as carcinogens because of their ability to react with nitrites to form potentially carcinogenic nitrosamines (Shalaby, 1997). Putrescine and cadaverine may potentiate the toxic effects of histamine and tyramine by inhibiting monoamine oxidase, diamine oxidase, and hydroxymethyl transferase (Bardócz *et al*., 1995; Spizzirri *et al*., 2013). The levels of cadaverine, putrescine and histamine were observed higher in our previous study (Koral *et al*., 2015) with hot smoked Mediterranean horse mackerel (*T. mediterraneus*) products at refrigerated storage. Therefore, the current study shows the advantage of using frozen storage over refrigerated storage to prevent formation of biogenic amines, particularly histamine. Only histamine level is regulated by various authorities for fish. The permitted level of histamine varies according to countries. However, legally permitted level of FDA is 50 ppm, while 100 ppm is allowed by EU or Turkish government (EU Directive, 2005a; Köse, 2010; FDA, 2011). Histamine levels obtained in this study were found well below the permitted levels set by various authorities.

Initial microorganism counts can vary depending on season and the region of fish caught, and also handling conditions (Careche *et al*., 2002). The recommended TVC limit for fish consumption is reported between 6 and 7 log cfu/g (ICMSF, 1992). TVB counts observed in this study did not exceed the recommended values throughout the storage period for all groups. Frozen storage is known to prevent bacteria formation (Rahman and Velez-Ruiz, 2007). Since bacterial growth will be prevent at -12°C as reported by Rahman and Velez-Ruiz (2007), the differences in the levels of phsycrophilic TVC can be attributed to uneven distribution of bacteria on different samples within the same group. Similar situation was also observed for phsycrophilic total HFB. Higher bacteria counts were observed for all groups in hot-smoked mackerel stored at refrigerated temperature in our previous study (Koral *et al*., 2015). Adeyemi *et al*. (2013) observed TVC as 3.48 log cfu/g for hot smoked *T. trachurus* by stored at room temperature on the 5th day. Daniel *et al*. (2013) and Marc *et al*. (2014) studied microbiological quality of smoked mackerel (*T. trachurus*) in markets of Benin. They reported high bacteria load which exceeded the recommended limit. In their study, high counts of thermo tolerant coliforms, yeasts and moulds were identified.

In conclusion, our study showed that although there were significant differences (P<0.05) in chemical, microbiological and sensory qualities of hot-smoked mackerel products between cultured and wild groups, the shelf life obtained for both groups were 7 months at frozen storage. However, better consumer acceptance was observed for cultured samples indicating the advantage of culturing of samples for a better market value. We also observed that frozen storage can retard the formation of biogenic amine contents and therefore, it is suggested to apply it for such products to avoid histamine health risk.

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