EFFECT OF MODIFIED ATMOSPHERE AND VACUUM PACKAGING CONDITIONS ON SELECTED CHEMICAL AND PHYSICO-CHEMICAL PARAMETERS OF MARINATED AND SALTED ATLANTIC MACKEREL (*Scomber scombrus*)

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ABSTRACT: Chemical and physico-chemical parameters of marinated and salted Atlantic mackerel (*Scomber Scombrus*), with emphasis on the quality and safety parameters in modified atmosphere packaging (MAP) and vacuum packaging (VP), were investigated. Quality assessment of mackerel stored in MAP (40% CO_2 +60% N_2) and VP for up to 50 days at 4±1 °C was done by the monitoring of pH value, total volatile basic nitrogen (TVB-N), thiobarbituric acid (TBA) and histamine. The pH value of fish meat was significantly lower in the marinated samples. The highest concentration of TVB-N was recorded in the salted mackerel stored under VP whereas the lowest TVB-N in the marinated mackerel stored under MAP conditions. The formation of TBA increased with the time of storage and was the lowest in the marinated mackerel stored in MAP. The concentration of histamine increased during storage and its level reached over 10 mg/100 g for the salted mackerel stored under VP conditions. The marinated mackerel stored and extended shelf life at 4±1 °C compared to that packaged in VP according to physico-chemical analysis.

Key words: Atlantic mackerel, histamine, pH value, TBA, TVB-N

INTRODUCTION

Fish is one of the most perishable food products and the shelf life of such products is limited in the presence of air by the chemical effects of atmospheric oxygen and the growth of aerobic spoilage microorganisms (Ivanović et al., 2014). Modification of the atmosphere within the package by decreasing the oxygen concentration, while increasing the content of carbon dioxide and/or nitrogen has been shown to significantly prolong the shelf life of perishable food products at chill temperatures (Ivanović et al., 2015; Özoğul et al., 2004). Modified atmosphere packaging (MAP) and vacuum-packaging (VP), along with refrigeration temperature, have become increasingly popular preservation techniques, which have brought major changes in storage, distribution and marketing of raw and processed products. MAP and VP systems could provide further improvement in seafood shelf life, organoleptic quality, and product range (Koral et al., 2010).

Marinades are salty-sour unpasteurized products, produced with adequate technological methods, with brine, acetic acid, sauce or oil, etc. (Stamatis and Arkoudelos, 2007; Siverstvik et al., 2007). Marinated products, in most cases, require an additional, usually, heat treatment (Sandhya, 2010), but under certain conditions (depending on the duration of marinating, amount of salt, acid and type of fish) marinated products can be "ready-to- eat" products (Mendes and Goncalvez, 2008; Rivas et al., 2008). The inhibitory effect of marinade depends on the concentration of acid and salt. Marinated fish at the refrigeration temperature (4-6 °C) can be stored for up to several months, depending on the concentration of salts and acids (Babić et al., 2013; Cadun et al., 2005).

Thiobarbituric acid (TBA) content (Tozawa et al., 1971) and total volatile basic nitrogen (TVB-N) (Antonacopoulos and Wilfried, 1989) have been proposed as indicators of fish quality deterioration (Luong et al., 1992). Levels of biogenic amines can also be useful in estimating freshness or spoilage degree of fish since their formation is associated with bacterial spoilage (Mackie et al., 1997).

Fish containing relatively high concentrations of histamine can cause poisoning or allergic reactions when consumed by some individuals. Histamine is produced by microbial decarboxylation of the amino acid histidine (Cabrer et al., 2002). The importance of estimating the concentration of histamine in fish and fish products is related to its impact on human health and food quality. Histamine formation in MAP has been found to be lower than in the air (Özoğul et al., 2002; Watts and Brown, 1982).

The effects of modified atmosphere packaging and vacuum conditions on fish have been reviewed extensively but little information is available on the storage of Atlantic mackerel under modified atmosphere and vacuum packing conditions. Therefore, the main objective of this study was to investigate the effects of modified atmosphere and vacuum on the quality and safety of the Atlantic mackerel. Quality attributes were assessed by different methods, including physico-chemical evaluation.

MATERIAL AND METHODS

Raw material, sampling and processing

Frozen Atlantic mackerel (Scomber scom*brus*) was used for the experiment. Fish was taken from the local fish company (Squadra.rs), commercial size, weight between 350 and 400 g and processed in a common way for the fish processing plant. They were immediately eviscerated and divided into four groups. The first group was treated with brine (10% of salt), and was packed under a vacuum, the second group was treated with brine (10% of salt) and packed under the modified atmosphere (40% CO_2 / 60% N_2). The third group was marinated in a solution containing 10% salt and 0.5% of acetic acid and was packed in a vacuum. The fourth group was marinated with solution containing 10% of salt and 0.5% of acetic acid and was packed in the modified atmosphere (40% CO_2 / 60% N_2). The marinating process lasted 24 hours. All samples were stored under the same conditions, at the temperature of 4±1 °C. For the packaging of samples, a packing machine "Variovac" (Variovac Primus, Zarrentin, Germany) was used. The samples were packed in OPA/EVOH/PE (oriented polyamide/ethylene vinyl alcohol/polyethylene foil (Dynopack, POLI-MOON (Kristiansand, Norway), characterized with low permeability to gas. The degree of permeability to O₂ was 3.2 cm^3/m^2 / day at 23 °C, to N_2 1 cm^3/m^2 / day at 23 °C, to CO_2 14 cm^3/m^2 / day at 23 °C, and to water vapor 15 g/m² / day at 38 °C. The gas / sample ratio in the package was 2:1. Six fish were removed from each batch for each sampling day but data were obtained using three samples (3 x 2) for chemical analysis (two fish minced for each sampling).

Analysis

Samples were stored at 4 °C and examined at day(s) 0, 10, 20, 30, 40 and 50. Moisture content was determined by oven drying of 5 g of fish muscle at 105 °C until a constant weight was obtained (AOAC, 1995, Method 985.14). Results were expressed as g water/100 g muscle. Ash

content was determined by the AOAC (1980) method 7.009. The crude fat content was determined using a solvent extractor Velp SER 148/6 (Velp Scientifica, Milano, Italy) with petroleum ether (130 °C) and protein content was determined by AOAC (1980) method 2.507. The method according to Mohr was used to determine salt content (NaCl) in fish muscle as described in Keskin (1982). Chemical analysis of Atlantic mackerel (moisture, ash, salt, fat and protein content) were performed at the beginning of the experiment (0 day). pH value was measured with pH Meter "Testo 205" (Testo AG, Lenzkirch, Germany).

The method of Lucke and Geidel was used to determine TVB-N (Total Volatile Basic Nitrogen) content as described by Inal (1992). TBA (thiobarbituric acid) values, expressed in mg of malonaldehyde/kg, were estimated by using the method of Tarladgis et al. (1960) which is described by Smith et al. (1992) and Varlik et al. (1993).

Histamine quantification in fish muscle was carried out by the colorimetric method of Patange et al. (2005), based on the interaction between the imidazole ring and p- phenyldiazonium sulfonate. The concentration of histamine in the sample was obtained from the standard curve and expressed as mg per 100 g of wet weight (mg/100 g W.W).

Statistical Analysis

The obtained data were analyzed by analysis of variance (ANOVA) and when significant differences were found, comparisons among means were carried out by using theTukey test (P<0.01; P<0.05). Statistical analysis of the results was performed using software GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego, California USA, www.graphpad.com.

RESULTS AND DISCUSSION

Table 1 shows the moisture, protein, lipid, ash and salt contents of raw, salted and marinated Atlantic mackerel. Differences between moisture, lipid, ash and salt contents of raw, salted and marinated Atlantic mackerel were found to be significant (P <0.01; P <0.05), but the differences between protein content were not found to be significant (P >0.05). Baltić and Teodorović (1997) reported that the chemical composition of Atlantic mackerel was 74.5% water, 20.7% protein and 3.4% lipid. Also, Mbarki et al. (2009) reported in their experiment that moisture was 71.78%, ash 2.26%, protein 21.38% and fat 4.13%. The variation of the Atlantic mackerel chemical composition is closely related to nutrition, living area, fish size, catching season, sexual variations as well as other environmental conditions (Baltić and Teodorović, 1997).

Table 1.

Chemical composition of Atlantic mackerel (mean ± standard deviation)

Parameters (%)	Raw fish	Salted mackerel	Marinated mackerel			
Protein content	20.05±0.94	19.94±0.81	19.95±0.90			
Lipid content	9.56 ^{AB} ±0.41	10.40 ^{AC} ±0.38	11.40 ^{BC} ±0.56			
Moisture content	69.17 ^{Aa} ±1.25	66.24 ^a ±2.18	65.13 ^A ±1.84			
Ash content	1.12 ^{AB} ±0.05	2.92 ^{AC} ±0.09	3.46 ^{BC} ±0.08			
Salt content	0.10 ^{AB} ±0.05	1.80 ^{AC} ±0.011	2.34 ^{BC} ±0.13			
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Legend: Within a row, means with a common superscript letters are significantly different: $^{A-C}$ (P <0.01) and a (P <0.05)

Table 2.

pH value of packaged Atlantic mackerel (mean ± standard deviation)

	Days of storage ($\overline{X} \pm SD$)					
Group	0	10 th	20 th	30 th	40 th	50 th
	6.14 ^{AB} ±0.04	5.56 ^{aAB} ±0.05	5.33 ^{AB} ±0.05	5.16 ^{ABC} ±0.03	5.09 ^{ABC} ±0.02	4.80 ^{ABC} ±0.07
II	6.14 ^{CD} ±0.04	5.66 ^{aCD} ±0.07	5.42 ^{CD} ±0.03	5.29 ^{ADE} ±0.05	5.30 ^{ADE} ±0.04	5.00 ^{ADE} ±0.09
111	5.09 ^{AC} ±0.06	4.81 ^{ACb} ±0.05	4.59 ^{ACE} ±0.08	4.50 ^{BD} ±0.06	4.50 ^{BD} ±0.06	4,47 ^{BD} ±0.04
IV	5.09 ^{BD} ±0.06	4.72 ^{BDb} ±0.03	4.44 ^{BDE} ±0.05	4.43 ^{CE} ±0.05	4.50 ^{CE} ±0.11	4.43 ^{CE} ±0.04
l agand · M	lithin column m	eans with a com	mon superscript la	etters are significa	ntly different ^{. A-D}	(P < 0.01) and ^{a, b}

Legend: Within column, means with a common superscript letters are significantly different: ^{A-D} (P < 0.01) and ^{a, b} (P < 0.05)

Group I: salted mackerel-vacuum; Group II: salted mackerel – MAP; Group III: marinated mackerel-vacuum; Group IV: marinated mackerel – MAP

The results of average pH value in the samples of Atlantic mackerel, packaged in modified atmosphere and under vacuum conditions, are shown in Table 2. The initial pH value of muscle was 6.14±0.04 in salted and 5.09±0.06 in marinated mackerel.

During the storage period the pH value decreased, but pH value is not only criteria of spoilage (Kilinc and Cakli, 2005). After the marinating process, the pH values decreased sharply from 5.09 ±0.06 to 4.47±0.04 (VP) and 4.43±0.04 (MAP) for marinated mackerel. A significant differrence (*P* <0.01; *P* <0.05) was found in pH value between some groups (Table 2). Aksu et al. (1997) reported that pH value in anchovy marinated with 2% and 4% acetic acid increased from 4.25 and 4.18 to 4.53 and 4.31, respectively. In mackerel, red muscle is well developed and contains more glycogen than white muscle (Shimizu et al., 1992). It appears that the breakdown of glycogen to lactic acid of fish after capture, especially in mackerel, makes pH lower (Sainclivier, 1983). Poligne and Collignan (2000) determined that the pH value of fish increased from 3.90 to 4.21 after 2 days of storage, and then remained constant until the end of storage (50th day). Kilinc and Cakli (2004) found that pH value of marinated sardine samples packaged in MAP significantly decreased with prolonged storage. Some literature data indicate that pH value increases during storage when fish is not treated (marinated, salted) (Losada et al., 2003). Small increases in pH values have been reported for Atlantic mackerel stored in slurry ice (Banks et al., 1980). The reported data indicate that pH values of the MAP and vacuum packed samples were significantly lower (P < 0.05) through-out the storage period. For MAP-stored fish it is well known that carbon dioxide can be absorbed into fish muscle surface. acidifying it via the formation of carbonic acid (Losada et al., 2003).

The European Union directive on fish hygiene specifies that if the organoleptic examination reveals any doubt as to the freshness of the fish, inspectors must use TVB-N as a chemical check (EU 91/493/EEC, 1991). TVB-N as an indicator of freshness is a measurement of the amount of basic volatile compounds recovered by distilling fish muscle, or extracts of fish muscle, under alkaline conditions (Howgate, 2010). In literature, different TVB-N values were suggested for the freshness of fish and fish products; however, maximum limit 35 mg/100 g is generally acceptable (Connell, 1990; Lopez-Caballero et al., 2000; Kim et al., 2002). TVB-N values increased significantly at all experimental groups (P < 0.05) (Table 3). TVB-N values of samples stored at 4±1 °C were just over the maximum value at the 50th day, 38.49±0.38 mg/100 g (I group). Therefore, packaging in MAP conditions provided improvement in terms of quality relating to TVB-N values. The more rapid increase of TVB-N at higher microbial numbers indicated the stage of substantial spoilage of the fish.

Fraser and Sumar (1998) indicated that bacterial catabolism of amino acids in fish muscle results in the accumulation of ammonia and other volatile bases. The statistical analysis of the TVB-N showed significant differences (P < 0.01) between the TVB-N values at different stages of storage of fish in VP and MAP. As for many fish species, the formation of TVB-N increased with time. During marinating, TVB-N content can be reduced due to the action of acids and salts which release nitric components from the fish, which migrate into the marinade (Günsen et al., 2011). Also, Kilinc and Cakli (2005) determined a considerable decrease in TVB-N, from 10.3 to 6.5 mg/100 g, after marinating sardine fillets in a solution containing 7% acetic acid and 14% salt. During storage, a significant increase in TVN-B value was noticed. A similar pattern of increase in TVB-N values during refrigerated storage has been reported in marinated sardines (Gökoglu et al., 2004; Banks et al., 1980) and brined chub mackerel (Goulas et al., 2005). On the other hand, Pons-Sanchez-Cascado (2005) reported that TVB-N levels in marinated fish in vinegar remained constant during two weeks of the marinating process, and throughout the storage period of 3 months under refrigerated vacuum-packed storage, as well.

One of the other quality parameters is known as TBA and maximum allowed level is accepted as 8 mg malonal-dehyde/kg (Schormüller, 1969). Salted fish and marinated fish contained 0.20 and 0.10 mg malonaldehyde/kg, respectively (table 4). The values increased significantly in all samples (P < 0.01; P < 0.05) although samples were within acceptable levels for this parameters. The content of malondialdehyde in vacuum packaging was higher than in samples packaged in MAP (Table 4).

Sallam et al. (2007) found that malondialdehyde values of Pacific saury in 2% and 3% acetic acid were 1.88 and 1.61 mg MDA/kg, respectively, at the end of the storage period (90 days), reaching the maximum level after 70 days. Cadun et al. (2005) reported a malondialdehyde value of 6.50 mg MDA/kg in marinated shrimp, after 40 days of storage.

Biogenic amine content in fish can be used to estimate freshness and degree of spoilage. These amines are found at low levels in fresh fish and their presence is associated with bacterial spoilage. The amount and type of amine formed depends on fish species, microbial flora and several parameters which influence bacterial growth during storage, such as temperature, packaging and antimicrobial agents Erkan and Özden (2008).The results for histamine content of the packed Atlantic mackerel samples during fifty days of storage are shown in Table 5.

In the present study, the content of histamine, the causative agent for fish poisoning, was 4.51±0.22 mg/100 g (I, II groups) and 4.55±0.21 mg/100 g (III, IV groups) at initial day (0 day).

Table 3.

TVB-N values of packaged Atlantic mackerel (mg/100 g)

•	Days of storage ($\overline{\mathrm{X}}$ ± SD)					
Group	0.	10 th	20 th	30 th	40 th	50 th
I	10.15±0.40	14.11 ^{AB} ±0.59	18.71 ^{ABC} ±0.23	25.43 ^{ABC} ±0.34	31.28 ^{ABC} ±0.40	38.49 ^{ABC} ±0.38
II	10.15±0.40	12.37 ^{ACD} ±0.29	17.10 ^{ADE} ±0.15		26.25 ^{ADE} ±0.39	32.26 ^{ADE} ±0.36
111	10.15±0.40	13.55 ^{CE} ±0.26	16.61 ^{BDF} ±0.29	20.43 ^{BDF} ±0.34	24.47 ^{BDF} ±0.33	30.03 ^{BDF} ±0.59
IV	10.15±0.40	11.54 ^{BDE} ±0.22	13.19 ^{CEF} ±0.26	18.69 ^{CEF} ±0.36	22.31 ^{CEF} ±0.30	27.43 ^{CEF} ±0.35
Legend.	Within column	means with a comm	on superscript let	ters are significal	ntly different ^{. A-D}	(P < 0.01) · means

Legend: Within column, means with a common superscript letters are significantly different: ^{A-D} (P <0.01); means without a superscript letters no significantly different

Group I: salted mackerel-vacuum; Group II: salted mackerel – MAP; Group III: marinated mackerel-vacuum; Group IV: marinated mackerel – MAP

Table 4.

TBA in the packaged Atlantic mackerel (mg malonaldehyde/kg)

				, 0/			
	Days of storage $\overline{\mathbf{X}}$ ± SD						
Group	0	10 th	20 th	30 th	40 th	50 th	
	0.20±0.01	0.84 ^{AB} ±0.06	1.30 ^{ABC} ±0.02	1.67 ^{ABC} ±0.04	1.80 ^{ABC} ±0.04	2.10 ^{ABC} ±0.07	
II	0.20±0.01	0.79 ^C ±0.01	1.21 ^{ADE} ±0.02	1.50 ^{AD} ±0.04	1.70 ^{Aa} ±0.03	1.89 ^{Aa} ±0.02	
111	0.19±0.01	0.73 ^{AD} ±0.03	1.04 ^{BDa} ±0.06	1.45 ^{BE} ±0.04	1.65 ^{BD} ±0.04	1.80 ^{BD} ±0.06	
IV	0.19±0.01	0.70 ^{BCD} ±0.01	0.96 ^{CEa} ±0.05	1.35 ^{CDE} ±0.04	1.58 ^{CaD} ±0.03	1.69 ^{CaD} ±0.06	
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Legend: Within column, means with a common superscript letters are significantly different: ^{A-D} (P < 0.01) and ^{a, b} (P < 0.05): means without a superscript letters no significantly different

Group I: salted mackerel-vacuum; Group II: salted mackerel – MAP; Group III: marinated mackerel-vacuum; Group IV: marinated mackerel – MAP

Table 5.

Histamine content in the packaged Atlantic mackerel (mg/100 g)

-	Days of storage \overline{X} ± SD					
Group	0	10 th	20 th	30 th	40 th	50 th
I	4.51±0.22	6.57 ^{ABC} ±0.27	7.34 ^{ABC} ±0.29	8.26 ^{ABC} ±0.12	9.38 ^{ABC} ±0.17	10.20 ^{ABC} ±0.11
II	4.51±0.22	6.14 ^{AD} ±0.12	6.63 ^{ADE} ±0.10	6.96 ^{AD} ±0.05	7.77 ^{ADE} ±0.14	8.35 ^{ADE} ±0.10
111	4.55±0.21	5.54 ^{BDE} ±0.24	6.61 ^{₿₽₽} ±0.12	6.87 ^{8E} ±0.08	7.14 ^{₿₽₽} ±0.16	7.74 ^{BDE} ±0.12
IV	4.55±0.21	5.41 ^{℃⊧} ±0.11	5.69 ^{CEF} ±0.09	6.21 ^{CDE} ±0.1	6.40 ^{CEF} ±0.09	6.91 ^{CEF} ±0.05

Legend: Within column, means with a common superscript letters are significantly different: ^{A-D} (P <0.01): means without a superscript letters no significantly different

Group I: salted mackerel-vacuum; Group II: salted mackerel – MAP; Group III: marinated mackerel-vacuum; Group IV: marinated mackerel – MAP

This value was higher than those found by Erkan and Özden (2008) for sardine (1.2 mg/100 g) (S. pilchardus). This may be due to different fish species and catching area. The legal limits for histamine set by the EU Council Directive 91/493/EEC (1991) to as less than 10 mg/100 g have been reached by the group I after 50th days. Ababouch et al. (1991) also reported that histamine reached toxic levels after 6-10 days in sardines (S. pilchardus) stored at 8 °C and on the ice. FDA (2011) confirmed the average con-centration of histamine in fish at 5 mg/100g for health assurance of products, and more than this level is undesirable. The European Union has suggested that the average concentration of histamine in fish should not be more than 10 mg of histamine per 100 g of fish muscle and it seems to be good for general health (Le-hane and Olley, 2000). As well as the SABS (South African Boreau of Standard) and AFSC (Australian Food Standard Code), respectively, 10 mg/100g and 20 mg/100g of histamine levels in fish muscle have been suggested as a limit. Thus, the results of this study are consistent with international references. Fresh fish usually have low amount of histamine in the limit of 0.1 mg/100g. Red meat fishes, such as sardine and mackerel have higher levels of histamine compared with white meat fishes like cod and Hamour.

Özoğul et al. (2008), reported that amounts of histamine in sardines (*Sardina pilchardus*) increased during storage and reached 14.0 \pm 1.2 mg per 100 g, in a vacuum, and 10.5 \pm 1.2 mg per 100 g, in MAP. Also, in this study the content of histamine increased during storage, and this phenomenon was more pronounced in samples packaged in a vacuum. Larger amount of histamine were determined in samples packaged in MAP with a lower content of CO₂ (Duyar and Eke, 2009). Similar results were reported by other authors (Siverstvik, 2007).

CONCLUSIONS

At the end of this study, while the differrences between pH value, TVB-N, TBA and histamine value were statistically significant between pre-packaging treatment with acids and salt and packaging conditions (VP and MAP). MAP could improve some quality aspects and increased the shelf-life of fish especially of the marinated fish as compared with the salted fish packaged under the same conditions.

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УТИЦАЈ ПАКОВАЊА У МОДИФИКОВАНОЈ АТМОСФЕРИ И ВАКУУМУ НА ОДАБРАНЕ ХЕМИЈСКЕ И ФИЗИЧКО-ХЕМИЈСКЕ ПАРАМЕТРЕ МАРИНИРАНЕ И СОЉЕНЕ СКУШЕ (Scomber scombrus)

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Сажетак: Циљ овог рада је био испитивање хемијских и физичко-хемијских параметара мариниране и сољене скуше (*Scomber scombrus*), са посебним освртом на параметре квалитета и безбедности скуше паковане у модификованој атмосфери (МАП) и вакуум паковању (ВП). Оцена квалитета скуше која је пакована у МАП (40% CO2 + 60% N2) и ВП у трајању до 50 дана на 4 ± 1 ° C вршена је праћењем промена рН вредности, укупног испарљивог азота (*TVB-N*), тиобарбитурне киселине (TBA) и садржаја хистамина. Вредност рН рибљег меса је знатно нижа у маринираним узорцима скуше. Највећа концентрација *TVB-N* је забележена код сољених узорака скуше који су паковани у вакууму, а најнижа концентрација *TVB-N* је забележена код сољених узорака рибе пакованих у МАП (маринирана скуша). Формирање ТВА повећава се са временом складиштења, а ова вредност је била најнижа у узорцима мариниране скуше паковане у МАП-у. Концентрација хистамина се повећава током складиштења, а његов садржај био је преко 10 mg/ 100 g само за сољену скушу паковану у вакууму. У односу на физичко-хемијске анализе узорака скуше, резултати овог испитивања показују да маринирана скуша пакована у МАП, има дужи рок употребе у односу на узорке скуше пакаване у вакууму.

Кључне речи: Скуша, хистамин, pH вредност, TBA, TVB-N

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