



Determination of Some Quality Criteria of Cold Stored Marinated Anchovy under Vacuum and Modified Atmosphere Conditions

Uğur Günşen^{1*}, Ali Özcan², Ali Aydın³

¹ Balıkesir University, Bandırma Vocational High School, Department of Food Technology, 10200, Bandırma, Balıkesir, Turkey.

² The Central Institute of Food Control and Research, 16036, Hurriyet, Bursa, Turkey.

³ Istanbul University, Veterinary Faculty, Department of Food Hygiene and Tech., 34320, Avcilar, Istanbul, Turkey

* Corresponding Author: Tel.: +90.266 7149302; Fax: +90.266 7149304;
E-mail: ugurgunsen@hotmail.com

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Abstract

This study was performed to determine the effects of vacuum packaging (VP) and modified-atmosphere packaging (MAP) by different gas mixtures (70% CO₂ / 30% N₂ = M1, 50% CO₂ / 50% N₂ = M2) on the potential quality index, shelf-life and histamine formation in marinated anchovies during the storage at 2±2°C. Results showed that thiobarbituric acid (TBA) values marinated anchovies under M1 and M2 exceeded the limit given by Turkish Food Codex and the sensoric qualities were reduced from “first quality” to “not consumed” on month 11 of storage at 2±2°C as compared to vacuum packaging (7th month). The levels of TVB-N, histamine and microbial load in the samples from both of the packaging methods were under the limits given in Turkish Food Codex. It was concluded that TBA values depending on the level of fatty oxidation and sensory evaluation determined the shelf life of marinated anchovies under vacuum and MAP conditions during storage at 2±2°C. Marinated anchovies under MAP conditions were edible until the 11th month of storage. The shelf-lives of marinated anchovies could be extended 4 months by MAP, as compared to vacuum packaging (7th month), and the two different gas mixture (M1 and M2) in MAP had same effect on taking to this shelf lifes.

Keywords: Marination, packaging, histamine, shelf-life.

Modifiye Atmosfer Koşulları ve Vakum Altında Soğuk Depolanan Marine Hamsinin Bazı Kalite Kriterlerinin Belirlenmesi

Özet

Bu çalışmada, vakum paketleme (VP) ve farklı gaz kombinasyonu (%70 CO₂ / %30 N₂ = M1, %50 CO₂ / %50 N₂ = M2) kullanılarak uygulanan modifiye atmosfer paketleme (MAP) tekniğinin, 2±2°C’de depolanan marine hamsilerin potansiyel kalite indeksi, raf ömrü ve histamin düzeyleri üzerine etkileri incelendi. Sonuçlar, VP ile karşılaştırıldığında (7. ayda), 2±2°C’de depolanan M1 ve M2 şartlarındaki marine hamsilerin tiyobarbiturik asid (TBA) değerlerinin depolanmanın 11. ayında Türk Gıda Kodeksi’nde verilen limitleri aştığını, duyu kalitelerinin ise “birinci kalite”den “tüketilemez” düzeye indiğini gösterdi. Marine hamsi örneklerinin TVB-N değerleri, histamin düzeyleri ve mikrobiyel yüklerinin her iki paketleme durumunda da Türk Gıda Kodeksi’nde verilen limitlerin altında kaldığı belirlendi. Soğukta depolanan marine hamsilerin raf ömrünün tespit edilmesinde, yağ oksidasyonu düzeylerine bağlı olarak elde edilen TBA ve duyu analiz sonuçlarının belirleyici olduğu, MAP tekniği ile paketlenmiş soğukta depolanan marine hamsilerin raf ömrünün vakum paketli şekline göre 4 ay daha uzayarak 7 aydan 11 aya ulaştığı ve bu sürenin elde edilmesinde iki farklı gaz karışımının aynı etkiye sahip olduğu sonucuna varıldı.

Anahtar Kelimeler: Marinasyon, paketleme, histamin, raf ömrü.

Introduction

In general, fish have a limited shelf-life in comparison with meat products (veal, lamb, pork, poultry) as a result of the high postmortem pH in the flesh (usually >6.0), the presence of large amounts of non-protein nitrogen (NPN), the high content of polyunsaturated fatty acids (PUFA) and the presence of autolytic enzymes (Gram and Huss, 1996;

Sivertsvik *et al.*, 2002). Spoilage reactions can be inhibited by traditional processing and preservation procedures (Huss *et al.*, 1997; Gould, 1996).

Marinades are semi-preserved; the preserving principal is the combination of acetic acid and salt. The inhibitory effects of these substances on bacteria and enzymes increase with concentration. The aim is not only to hold up the action of bacteria and enzymes, but also to tenderize or to change the taste,

textural and structural properties of raw material, resulting in a product with a characteristic flavour and an extended but limited shelf-life. Marinades stored at cooler temperatures (4-6°C) kept for a long time (Clucas and Ward, 1991; Gökoğlu et al., 2004).

The packaging of food products in high-barrier materials and changing the gaseous environment to slow down respiration rates is a process called modified-atmosphere packaging (MAP) (Koski, 1988). Vacuum packaging (VP) is also a type of MAP system because air is removed from a pack and not replaced. VP is normally placed in a pack of low oxygen permeability, air is evacuated and the package sealed (Church, 1998). An evacuated VP pack collapses around the product so that the pressure inside is a little less than atmospheric. The gaseous atmosphere of VP is likely to change throughout storage; hence the pack atmosphere is modified indirectly (Özoğul and Özoğul, 2006).

MAP in combination with chill storage is an ever increasing food preservation method used for a great variety of foods (fresh meat, poultry, fish, sausages, cheese, bakery products, coffee, dry seeds, dry soup, fruits, vegetables) (Goulas and Kontominas, 2006). Though MAP technology has a potential seafood safety risk related to *Clostridium botulinum* type E, consumer preferences for usefulness in handling, good visual display, attractive and safe packaging, hygiene and extension of acceptable shelf-life of seafood products have continued to increase (Özoğul and Özoğul, 2006).

Fish muscle is capable of supporting the bacterial formation of a wide variety of amine compounds that come from the decarboxylation of amino acids. Biogenic amine formation in fish and fish products depends on the amino acid content of fish, the presence of bacterial biogenic amine decarboxylases and favourable environmental conditions (Brink et al., 1990; Shalaby, 1996; Silla-Santos, 1996). Certain types of bacteria produce decarboxylase enzymes, which act on free amino acids in the fish muscles during spoilage. Biogenic amine formation in seafood is important as histamine, and possibly other biogenic amines, are responsible for scombrototoxic fish poisoning (Taylor, 1986). Furthermore, biogenic amines, have been used as chemical indicators of seafood quality (Jorgensen et al., 2000).

Fish are different from other MAP products in terms of their biological structure and chemical composition since they are extremely perishable and generally spoil faster than other muscle foods. Sensory methods, microbiological, chemical and biochemical methods have been used to assess the quality of fish and marine products during handling and storage (Huss, 1995).

The effects of MAP on seafood have been reviewed extensively (Boknaes et al., 2002; Cann et al., 1983; Chen et al., 2003; Davis, 1993; Fagan et al., 2004; Farber, 1991; Goulas, 2007; Goulas and

Kontominas, 2006; Goulas and Kontominas, M.G., 2007; Masniyom et al., 2002; Metin et al., 2002; Özoğul et al., 2000; Özoğul et al., 2004; Reddy et al., 1997; Pastoriza et al., 1998); Sivertsvik et al., 2002; Stammen et al., 1990) but limited information exists regarding the some quality criteria and histamine production in cold stored marinated anchovies under VP and MAP conditions. This study was performed to determine the effects of VP and MAP by different gas mixtures on the potential quality index, shelf-life and histamine formation in marinated anchovies during the storage at 2±2°C.

Materials and Methods

Marination, Packaging and Storage of Anchovies

In research, fifty kilograms of fresh anchovies (*Engraulis encrasicolus*), used as research material were purchased in the morning from the wholesale fish market in Bandırma, Balıkesir, Turkey (3 km from point of processes). The average size and weight of each fish were 11.288±0.042 cm and 9.352±0.073 g, respectively. They were immediately gutted, their heads and bones were removed and the fish were filleted. Then they were placed in brine for marination. The marination process was performed in 4% acetic acid and 10% salt at 4°C for 10 h.

After that, marinated products were divided into three groups. First group were vacuum packed (VPed) in polyethylene bags. The remaining two groups were packed under MAP conditions by using two different food grade gas mixture, (Oslo, Norway), 70% CO₂ / 30% N₂ = M1, in second group, and 50% CO₂ / 50% N₂ = M2, in third group, were introduced into the package, Sudpac PVC + PE as first sheet and Oriente Poliamid PE + EVC antifog material as second sheet onto non-barrier polypropilene dishes on a automatic packaging machine (INAUEN Maschinen AG, CH-9199 Type VC999). The final gas/sample ratio was about 2:1 (v/w) for MAP conditions. Packages of VPed and modified atmosphere packaged (MAPed) (M1 and M2) marinated anchovies were stored at 2±2°C.

Analytic Procedures

Triplicate homogenized samples were taken for analyses after 1, 4, 7 days, and monthly after packaging. Results were recorded as the arithmetic means of the values.

Physical and Chemical Analyses

pH values, salt (%) (Ludorff and Meyer, 1973), thiobarbituric acid (TBA) as mg malonaldehyde/kg (Varlık et al., 1993), total volatile base-nitrogen (TVB-N) as mg N/100 g (Schormüller, 1968) of VPed and MAPed marinated anchovies were determined.

Determination of Histamine Level

Histamine were extracted from the samples using perchloric acid (Merck 100519) and analyzed by HPLC, Hewlett Packard Series 1100, in a Spherisorb ODS2 column (10 µm., 200 x 4.6 mm) with a Diode Array Detector at 254 nm after derivatization with dansyl chloride (Sigma D-2625, Sigma-Aldrich, St. Louis, MO, USA) (Şenöz et al., 2000). HPLC flowrate was 1.0 ml/min, the injection volume was 20 µl, and column temperature was 40°C. This method was accredited according to ISO/EN 17025 and during the method validation procedures, the limit of detection was determined as 2.81 mg/kg, the rate of recovery % was ranged to 83.92 and 98.96% and linearity was found to be $R^2 = 0.9997$ by using five different levels of calibration standarts, 0, 0.1, 1, 2, 3 mg/kg histamine dihydrochloride (Sigma-Aldrich H7250, Sigma-Aldrich, St. Louis, MO, USA) (Figure 1).

Microbiological Analyses

Twentyfive grams of marinated anchovies were aseptically weighed and transferred into 225 ml of sterile buffered peptone water (Oxoid CM509) and homogenized by Stomacher 400 Lab Blender (Seward Medical, UK) for 2 min. After the decimal dilutions prepared, Total Aerobic Mesophilic Bacteria (TMAB) and Psychrotrophic Bacteria (PB) Counts (Plate Count Agar (PCA) Oxoid CM463), Total Coliform Bacteria (TCB) Count (Violet Red Bile Agar (VRBA) Oxoid CM107), *Escherichia coli* (*E. coli*) (Tryptone Bile X-glucuronide (TBX) Medium Oxoid CM945), *Salmonella* spp. (Rappaport Vassiliadis (RVS) Broth Oxoid CM0866, Selenite Cystine Broth Oxoid CM0699, Brilliant Green Phenol Red Agar Oxoid CM0263, Salmonella-Shigella (SS) Agar Oxoid CM0099, Xylose Lysine Deoxycholate (XLD) Agar Oxoid CM0419), *Listeria monocytogenes* (*L. monocytogenes*) (Listeria Enrichment Broth Oxoid CM897, Listeria Selective Enrichment Suppl. SR141,

Listeria Selective Agar Oxoid CM856, Listeria Selective Supplement SR140), *Staphylococcus aureus* (*S. aureus*) (Baird-Parker Agar (BP) Oxoid CM 275 + SR054C), *Vibrio parahaemolyticus* (*V. parahaemolyticus*) and *Vibrio cholerae* (*V. cholerae*) (Thiosulfate Citrate Bile Sucrose (TCBS) Agar Oxoid CM0333), and *Lactobacillus* spp. (Man Rogosa Sharpe Agar (MRS) Oxoid CM361), and Sulphite Reducing Anaerobes (SRA) (Sulphite Polymyxine Sulphadiazine (SPS) Agar Merck 1.10235) were determined (FDA, 2000; USDA/FSIS, 2000; ISO 2001).

Sensory Analyses

Sensory evaluations of VPed and MAPed marinated anchovies were performed as described by Schormüller (1968) and were conducted by five previously trained panellists, who were asked to evaluate appearance, odour, flavour and texture by using a form. According to the scoring table, a total score of sensory attributes of 15 means first quality, scores from 14.9 to 13 indicate second quality, scores from 12.9 to 11.0 indicate third quality and scores from 10.9 to 6.0 indicate fourth quality and a score of 6 or less corresponds to spoiled products.

Statistical Analyses

Statistical comparison based on 6 samples for each treatment for each specific storage time. Analysis of variance (ANOVA) was performed with Minitab 14 (Minitab Inc., US) using General Linear Model with Tukey's HSD test at level $P < 0.05$ (95%). The analyses determined the main effects of the experimental variables (time and packaging method) on the responses.

Results and Discussion

Initial quality of raw materials, considering their freshness, microbiological load and physical damage,

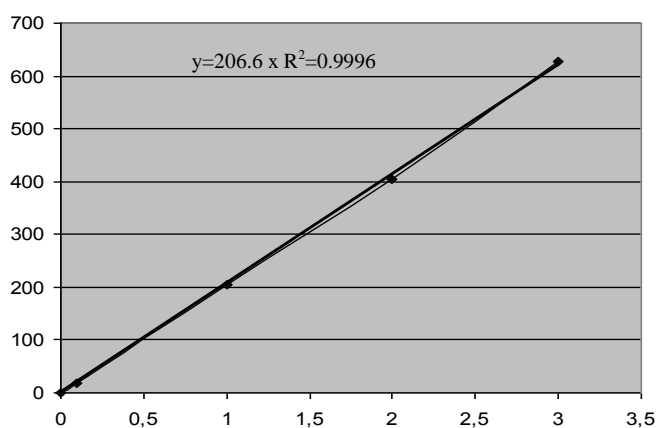


Figure 1. Linearity, obtained using five different levels of histamine standart.

Table 1. Physical and chemical quality changes and the levels of histamine of VPed and MAPed marinated anchovies during storage period

Parameters	pH		Salt (%)		TBA (mg/malonaldehyde/kg)		TVB-N (mg N / 100 g)		Histamine (mg / kg)	
	VP	MAP M1 M2	VP	MAP M1 M2	VP	MAP M1 M2	VP	MAP M1 M2	VP	MAP M1 M2
Fresh (Day 0)	6.03±0.01		0.19±0.01		0.02±0.01		9.7±0.01		9.18±0.04	
Day 1	4.18±0.02 ^{a*}	4.18±0.02 ^a	4.03±0.005 ^a	4.03±0.005 ^a	1.04±0.02 ^a	1.04±0.02 ^a	8.24±0.025 ^a	8.24±0.025 ^a	5.52±0.035 ^a	5.52±0.035 ^a
Day 4	4.18±0.02 ^a	4.18±0.02 ^a	4.05±0.011 ^a	4.04±0.012 ^a	1.05±0.01 ^a	1.05±0.02 ^a	8.29±0.01 ^a	8.28±0.02 ^a	5.56±0.03 ^a	5.58±0.06 ^a
Day 7	4.19±0.02 ^a	4.19±0.02 ^a	4.05±0.011 ^a	4.05±0.018 ^a	1.05±0.01 ^a	1.05±0.01 ^a	8.32±0.02 ^a	8.37±0.01 ^a	5.64±0.035 ^a	5.64±0.054 ^a
Month 1	4.22±0.015 ^b	4.20±0.02 ^{ab} 4.21±0.02 ^{ab}	4.21±0.005 ^b	4.06±0.006 ^a 4.17±0.05 ^b	1.64±0.01 ^{ab}	1.05±0.01 ^a 1.46±0.025 ^{ab}	9.14±0.02 ^b	8.35±0.02 ^a 9.82±0.025 ^{ab}	5.97±0.05 ^a	5.72±0.035 ^a 7.60±0.0 ^{ab}
Month 2	4.23±0.01 ^b	4.22±0.02 ^b 4.23±0.02 ^b	4.35±0.011 ^{cd}	4.18±0.049 ^b 4.26±0.015 ^c	2.14±0.005 ^b	1.49±0.026 ^{ab} 2.32±0.015 ^b	10.09±0.025 ^c	10.27±0.01 ^b 10.3±0.015 ^b	6.39±0.055 ^a	7.50±0.0 ^{ab} 7.66±0.025 ^{ab}
Month 3	4.25±0.02 ^{bc}	4.21±0.015 ^b 4.22±0.015 ^b	4.12±0.005 ^{ab}	4.23±0.025 ^{bc} 4.28±0.014 ^c	2.54±0.005 ^{bc}	2.68±0.02 ^{bc} 2.63±0.02 ^{bc}	11.08±0.02 ^d	11.32±0.02 ^{bc} 11.29±0.01 ^{bc}	8.92±0.08 ^{ab}	9.17±0.05 ^b 9.19±0.025 ^b
Month 4	4.26±0.02 ^{bc}	4.18±0.02 ^b 4.19±0.015 ^{bc}	4.06±0.011 ^a	4.14±0.05 ^b 4.17±0.032 ^b	3.18±0.01 ^c	3.27±0.015 ^c 3.32±0.02 ^c	11.94±0.02 ^{de}	12.15±0.02 ^c 12.19±0.02 ^c	12.85±0.079 ^{bc}	11.48±0.075 ^{bc} 11.80±0.045 ^{bc}
Month 5	4.28±0.015 ^c	4.17±0.02 ^{bc} 4.17±0.02 ^c	3.85±0.015 ^{de}	4.15±0.045 ^b 4.15±0.045 ^b	3.92±0.02 ^d	4.24±0.035 ^d 4.37±0.03 ^d	12.74±0.03 ^{ef}	13.01±0.01 ^{cd} 13.14±0.02 ^{cd}	17.69±0.066 ^{cd}	14.37±0.04 ^c 14.18±0.03 ^c
Month 6	4.33±0.005 ^d	4.16±0.01 ^{bc} 4.16±0.02 ^{bc}	3.72±0.011 ^{de}	4.09±0.04 ^a 4.07±0.05 ^a	5.19±0.02 ^e	4.82±0.03 ^{de} 4.76±0.03 ^{de}	13.86±0.025 ^{fg}	14.21±0.02 ^d 14.27±0.01 ^d	24.39±0.14 ^e	19.00±0.03 ^d 19.27±0.03 ^d
Month 7	4.36±0.01 ^{de}	4.15±0.01 ^c 4.14±0.015 ^c	3.62±0.025 ^e	3.92±0.03 ^a 3.95±0.03 ^a	6.74±0.02 ^e	5.29±0.03 ^e 5.32±0.03 ^e	15.78±0.02 ^h	15.18±0.015 ^{de} 15.21±0.026 ^{de}	37.42±0.09 ^{gh}	23.08±0.07 ^{de} 23.19±0.05 ^{de}
Month 8	-	4.13±0.015 ^{bc} 4.11±0.015 ^d	-	3.88±0.06 ^{cd} 3.86±0.04 ^{cd}	-	5.92±0.05 ^f 5.97±0.03 ^f	-	15.89±0.024 ^{de} 15.94±0.025 ^e	-	29.23±0.05 ^f 30.32±0.1 ^f
Month 9	-	4.1±0.02 ^{cd} 4.03±0.015 ^{de}	-	3.79±0.058 ^d 3.81±0.045 ^d	-	6.42±0.03 ^{fg} 6.51±0.02 ^{fg}	-	17.09±0.01 ^{ef} 17.21±0.015 ^{ef}	-	38.54±0.29 ^{gh} 38.56±0.3 ^{gh}
Month 10	-	4.04±0.01 ^d 3.88±0.02 ^c	-	3.68±0.065 ^{de} 3.7±0.062 ^{de}	-	6.95±0.003 ^h 6.91±0.003 ^h	-	19.64±0.015 ^{fg} 19.57±0.02 ^{fg}	-	43.61±0.41 ^{hi} 45.32±0.15 ^h
Month 11	-	3.91±0.015 ^e	-	3.51±0.06 ^{ef} 3.54±0.054 ^{ef}	-	7.74±0.003 ⁱ 7.82±0.003 ⁱ	-	22.47±0.025 ⁱ 23.01±0.02 ^{ji}	-	46.89±0.24 ^h 47.24±0.05 ^{hi}

* Means with different upper case superscripts in the column are significantly different by ANOVA.

is an important factor which influences the quality of end product (Fuselli *et al.*, 1994). In research, the results of physical, chemical, and microbiological analyses and the histamine level of fresh anchovy samples, used as research material were shown in Table 1 and Table 2. All the results were in agreement to the maximum limits given by Turkish Manual of Seafood Quality Control Limits (Anonymous, 2008).

In marinated products, pH value should not be more than 4.8 (Rehbein and Oehlenschläger, 1996). The average pH level of fresh anchovy samples was 6.03 ± 0.01 on day 0. The average pH level in the samples of marinated anchovy was 4.18 ± 0.02 on day 1 and increased to an average 4.36 ± 0.01 in VPed marinated anchovy samples on the 7th month of storage at $2 \pm 2^\circ\text{C}$ (Table 1). There were significant differences between the increased average values as depended on the storage period, ($P < 0.05$). The average pH level in the samples of VPed marinated anchovy were in agreement with those reported by Aksu *et al.* (1997) and Dokuzlu (1997) for marinated anchovies stored in cold condition.

It is well documented that increasing pH values during storage may be attributed to the production of basic compounds such as ammonia, dimethylamine, trimethylamine as well as other biogenic amines as a result of fish spoilage bacterial action (Goulas and Kontominas, 2007).

As it is seen in Table 1, in contrast, the average pH level of 4.18 ± 0.02 in the samples of MAPed marinated anchovy on day 1 was decreased to an average 3.88 ± 0.02 in the samples of M1 and 3.91 ± 0.015 in the samples of M2 on the 11th month of storage at $2 \pm 2^\circ\text{C}$. This is probably due to both, to dissolution of CO_2 in the marinated anchovies which is associated with increased carbonic acid production (Sivertsvik *et al.*, 2004) and to the lower production of basic compounds in the samples as a result of the preservative effect of CO_2 (Sivertsvik *et al.*, 2002; Farber, 1991). There were statistical importance between the decreased average pH values as depended on the storage period ($P < 0.05$). In addition, no statistical importance were found between the average pH values in the samples of M1 and M2 obtained from each analysis time of storage period.

The average salt level in fresh anchovy samples was found to be $0.19 \pm 0.01\%$ on day 0. The average salt level in the samples of VPed marinated anchovy was $4.03 \pm 0.005\%$ on day 1 and increased to an average $4.35 \pm 0.011\%$ on the 2nd month of storage and decreased to an average $3.62 \pm 0.025\%$ on the 7th month of storage at $2 \pm 2^\circ\text{C}$ (Table 1). This finding was in agreement with those reported by Dokuzlu (1997) for VPed marinated anchovy stored in cold condition. There were statistical importance between the decreased average salt levels as depended on the storage period ($P < 0.05$).

In MAPed marinated anchovy samples, the level of salt % were increased to an average $4.26 \pm 0.015\%$ in the samples of M1 and $4.28 \pm 0.014\%$ in the samples

of M2 on the 2nd month of storage ($P < 0.05$), and decreased to an average $3.51 \pm 0.06\%$ in the samples of M1 and $3.54 \pm 0.054\%$ in the samples of M2 on the 11th month of storage at $2 \pm 2^\circ\text{C}$ (Table 1) ($P < 0.05$). Pastoriza *et al.* (1998) reported that a delay in chemical, microbiological and sensorial alterations was found and the exudation were reduced markedly in comparison with MAP, when sodium chloride was combined with MAP, this was also reflected by a significantly higher water binding capacity of NaCl-dipped MAP-stored samples. In our study, the exudation in MAPed marinated anchovies was extremely less than the exudation in VPed marinated anchovies TBA analysis is an important quality index, indicating fat oxidation. Oxidative rancidity is a complex spoilage, and especially occurs in fatty fishes (Connell, 1980). In perfect quality material, TBA value should be less than 3 mg malonaldehyde/kg and, in good quality material, TBA value should not be more than 5 mg malonaldehyde/kg. Consumption limits are from 7-8 mg malonaldehyde/kg (Schormüller, 1968; Schormüller, 1969). Nonetheless, Tarladgis *et al.* (1960) reported that rancidity was occurred when TBA value exceeded to 4 mg malonaldehyde/kg.

The average TBA value of fresh anchovy samples was 0.02 ± 0.01 mg malonaldehyde/kg on day 0. The average TBA level of VPed and MAPed marinated anchovy samples was 1.04 ± 0.02 mg malonaldehyde/kg on day 1 and increased to an average 6.74 ± 0.02 mg malonaldehyde/kg in the samples of VPed marinated anchovy on the 7th month, and to an average 7.74 ± 0.003 mg malonaldehyde/kg in the samples of M1 and 7.82 ± 0.003 mg malonaldehyde/kg in the samples of M2 on the 11th month of storage at $2 \pm 2^\circ\text{C}$ (Table 1). There were statistical importance between the monthly TBA values in the samples of VPed and MAPed marinated anchovy as depended on the storage period ($P < 0.05$). Nonetheless, there were more significant differences in TBA values of VPed and MAPed (M1 and M2) marinated anchovy samples on month 6th and month 8th than the other months, respectively. No statistical importance were between the average TBA values in the samples of M1 and M2 obtained from each analysis time of storage period.

It was reported that there was a correlation between the TBA value and sensory evaluation in many researches (Barnett and Nelson, 1991; Ramanathan and Das, 1992). In our study, results of TBA analysis were parallel to sensory analysis. The TBA level of 5 mg malonaldehyde/kg, as described good quality material was exceeded on the 6th month and on the 7th month of storage period in the samples of VPed and MAPed (M1 and M2) marinated anchovy, respectively. Much more rancidity was experienced by the panellists on the 7th month of storage than on the 6th month in the samples of VPed marinated anchovy and on the 11th month of storage than on the 10th month in the samples of MAPed (M1

and M2) marinated anchovy. According to the TBA results, VPed and MAPed (M1 and M2) marinated anchovies could not be consumed on the 7th and 11th months of storage at 2±2°C, respectively, since fat oxidation had intensely progressed. Under MAP conditions, the shelf-life of marinated anchovies was extended up to 4 months for two different gas mixture conditions (M1 and M2) as compared to VP condition.

TVB-N value is a quality index for unprocessed fishery products indicative of fish spoilage as a result of metabolic activity of fish spoilage bacteria and endogenous enzymes action (Connell, 1990; EC Directive 95/149/EC). A level of 35 mg/100 g has been considered as an upper limit above which fishery products are considered to be unfit for human consumption (Ludorf and Meyer, 1973; Schormüller, 1968). In marine fish, TVB-N values of 15-20 mg N/100 g show good quality, whereas TVB-N values of 50 mg N/100 g show poor quality (Connell, 1980). However, upper levels from TVB-N values of 28 mg N/100 g have been reported as “unacceptable” in processed fish, according to the Turkish Manual of Seafood Quality Control Limits (Anonymous, 2008).

In our study, the average TVB-N value in fresh anchovy samples was found to be 6.03±0.01 mg N/100 g on day 0. The average TVB-N level in the samples of VPed and MAPed marinated anchovy was 8.24±0.025 mg N/100 g on day 1 and increased to an average 15.78±0.02 mg N/100 g in the samples of VPed marinated anchovy on the 7th month of storage at 2±2°C (Table 1). This findings were in agreement with those reported by Dokuzlu (1997), Aksu *et al.* (1997), Cascado *et al.* (2005) and Olgunoğlu (2007). In MAPed marinated anchovy samples, the average TVB-N level were reached to an average 22.47±0.025 mg N/100 g in the samples of M1 and 23.01±0.02 mg N/100 g in the samples of M2 on the 11th month of storage at 2±2°C (Table 1). This findings were in agreement with those reported by Metin *et al.* (2002)

There were statistical importance between the monthly TVB-N values in VPed and MAPed (M1 and M2) marinated anchovy samples as depended on the storage period (P<0.05). In addition, there were more significant differences in TVB-N values of VPed and MAPed (M1 and M2) marinated anchovy samples on month 6th and month 9th than the other months, respectively. No statistical importance were found between the average of TVB-N values in the samples of M1 and M2 obtained from each analysis time of storage period.

According to TVB-N values, marinated anchovies under VP and MAP conditions were rated “good quality” as described by Connell (1980) and under the unacceptable limit according to the Turkish Manual of Seafood Quality Control Limits (Anonymous, 2008).

In our study, the average histamine level in fresh anchovy samples was 9.18±0.04 mg/kg on day 0. The average histamine level in the samples of VPed and

MAPed marinated anchovy were found to be 5.52±0.035 mg/kg on day 1 and increased to an average 37.42±0.09 mg/kg in the samples of VPed marinated anchovy on the 7th month of storage at 2±2°C (Table 1) (P<0.05), and to an average 57.89±0.24 mg/kg in the samples of M1 and 58.24±0.15 mg/kg in the samples of M2 on the 11th month of storage at 2±2°C (Table 1) (P<0.05). No statistical importance were between the average levels of histamine in the samples of M1 and M2, obtained from each analysis time of storage period.

The average level of histamine in the samples of VPed marinated anchovy was in agreement with those reported by Olgunoğlu, (2007). Özoğul *et al.* (2002) reported that histamin levels were 19.68±5.30 mg/100 g on 16 day and 28.42±4.45 mg/100 g on 16 day in herring stored under VP and MAP, respectively and that the lowest histamine level was obtained from herring stored under modified atmosphere packaging, which suggested that CO₂ inhibits the growth of psychrotrophic, aerobic, and gram-negative bacteria with histidine decarboxylase activity. In another study performed by Özoğul *et al.* (2004), it was reported that amounts of histamine in sardines (*Sardina pilchardus*) increased during the storage and reached 14.0±1.2 mg/100 g for VP and 10.5±1.2 mg/100 g for MAP.

In our study, the average histamine levels in marinated anchovy samples under VP and MAP conditions were under the unacceptable limits of 50 mg/kg and of 100 mg/kg reported by FDA and Turkish Manual of Seafood Quality Control Limits, respectively (FDA, 2001; Anonymous, 2008). In fresh fish, histamine-forming bacteria occurred at low concentration which was less than 0.1% of the total bacterial load isolated from stored fish (Lopez *et al.*, 1996). It was concluded that the initial quality of fish was important for marinade because of histamine which increased continuously. On the other hand, it was seen that the adequate acetic acid / salt ratio in marination was efficient directly on the microorganism growth.

The microbiological analyses results of VPed and MAPed (M1 and M2) marinated anchovy samples are seen in Table 2. The results of TMAB count indicated that MAP was more effective than VP for decreasing the microbial load in marinated anchovy samples stored at 2±2°C. CO₂ is the major bacteriostatic factor of MAP. CO₂ may extend the lag phase and/or generation time in the logarithmic phase of growth, thus inhibiting microbial growth. The effect increases as the temperature decreases, due to the increasing solubility participating of CO₂. This anti-microbial behaviour was reported for CO₂ content up to 30% (Chen *et al.*, 2003).

During storage period, TCB, *E. coli*, *Salmonella* spp., *L. monocytogenes*, *S. aureus*, PB, *Lactobacillus* spp. and SRA could not be detected in the samples of VPed and MAPed (M1 and M2) marinated anchovy. These results were probably due to both, the adequate

acetic acid / salt ratio in marination and to dissolution of CO₂ in MAPed marinated anchovies which is associated with increased carbonic acid production, and with reduced pH level. In addition, these results were in agreement with the maximum limits given by Turkish Manual of Seafood Quality Control Limits (Anonymous, 2008).

In a study performed by Fuselli *et al.* (1994) it was reported that *Staphylococcus* spp., coliform bacteria, *Enterobacteriaceae*, yeast and mould and *E. coli* could not be isolated in cold marinated anchovies (*Engraulis anchoita*) at the end of the storage. Dokuzlu (1997) reported that coliform bacteria was isolated in the level of 50 cfu/g in VPed marinated anchovies. In a study performed by Aksu *et al.* (1997), the counts of TMAB, TCB, *Staphylococcus-Micrococcus* and yeasts-moulds were reduced in anchovy marinades produced in different acid-salt concentrations. Similar results were also reported by Olgunoğlu (2007).

In MAP, the ratio between the volume of gas and volume of food product (G/P ratio) should usually be 2:1 or 3:1. This high G/P ratio is also necessary to prevent package collapse because of the CO₂ solubility in wet foods. In our study, G/P ratio was 2:1 and no package collapse occurred during storage period.

The appearance of the seafood product in VP/MAP is important because it influences the acceptability of the product to consumers. The appearance of a seafood product can be assessed visually, including pack collapse, production of drip, and discoloration. Organoleptic properties of the product can be assessed by colour, odour, flavour and texture (Özoğul and Özoğul, 2006).

In our study, when all sensory criteria were taken into consideration, VPed and MAPed (M1 and M2) marinated anchovy samples, determined as “first quality” in the beginning of the storage period, evaluated as “spoiled” and rejected by the panelists on the 7th and 11th months of storage at 2±2°C, respectively (Table 3, 4 and 5). Much more softness, brownish color, acidic odour and off-flavour were experienced by the panelists in the samples of VPed and MAPed (M1 and M2) marinated anchovy on the 7th and 11th months than on the 6th and 10th months of storage at 2±2°C, respectively.

Statistical comparisons between the results from sensoric changes during the storage period showed that there were significant differences for reducing values depended on the storage period (P<0.05). In addition, it was found that statistical importance between the values of appearance, odour, flavour and texture were higher in month 6th, and month 9th and month 10th than in the other months in the samples of VPed, and M1 and M2 marinated anchovy, respectively.

It is concluded that TBA values depending on the level of fat oxidation and sensory evaluation determined the shelf-life of VPed and MAPed

marinated anchovies during storage at 2±2°C. VPed and MAPed marinated anchovies were edible until the 7th and 11th months of storage, respectively. The shelf-life of marinated anchovies could be extended 4 months by MAP, as compared to VP, and two different gas mixture (M1 and M2) in MAP had same effect on taking to this shelf-life.

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