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Chemical quality and sensory attributes of marinated Pacific saury (*Cololabis saira*) during vacuum-packaged storage at 4 °C

Kh.I. Sallam [a,*,1], A.M. Ahmed [b], M.M. Elgazzar [a], E.A. Eldaly [c]

[a] *Department of Food Hygiene and Control, Faculty of Veterinary Medicine, Mansoura University, Mansoura, Egypt*
[b] *Department of Food Hygiene and Control, Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt*
[c] *Food Control Department, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt*

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Abstract

This study was carried out to evaluate the chemical changes and sensory attributes of Pacific saury (*Cololabis saira*), brined (12% NaCl brine solution) or marinated (12% NaCl + 2% acetic acid; or 12% NaCl + 3% acetic acid solutions) followed by vacuum-packaging and storage at 4 °C for 90 days. The chemical analysis revealed a significant reduction in the pH value, total volatile bases nitrogen (TVBN), and trimethylamine (TMA) contents in marinated versus brined fillets. Lipid oxidation, as indicated by the 2-thiobarbituric acid (TBA) values, was significantly delayed in marinated fillets in comparison with the brined fillets. The growth rate of psychrotrophic bacteria was significantly ($P < 0.05$) reduced in marinated versus brined fillets. No significant differences were detected for the sensory attributes between the two marinating conditions although the overall acceptability was significantly higher in marinated versus brined fish. Both conditions of the marinating process resulted in an extension of the shelf life of the product to more than 90 days versus only 60 days for the control brined fillets. The study concluded that marination of Pacific saury can delay the undesirable chemical changes, retard lipid oxidation, improve the sensory attributes and extend the shelf life of the product during refrigerated storage.

Keywords: Marinated Pacific saury; Psychrotrophic count; TVB-N; TMA; TBA; Lipid oxidation; Sensory attributes; Shelf life

1. Introduction

Fish constitute a major part of animal protein consumption in many parts of the world. Globally, around 100 million tons of fish are landed annually but only about 70 million tons are utilized as human food (Huss, Reilly, & Ben Embarek, 2000). Approximately 27% of this amount is consumed as fresh fish while the remainder is processed using almost every known food preservation techniques, e.g. freezing, salting, drying, smoking, or canning (Huss et al., 2000).

The term "marinades" or "marinated fish" is used to define fish products which consist of fresh, frozen or salted fish or portions of fish processed by treatment with an edible organic acid, usually acetic acid, and salt and put into brines, sauces, or oil (Meyer, 1965). Marinated fish are typically consumed as ready-to-eat products with no heat treatment (Gram & Huss, 1996). Marinades are semi-preserves; 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 retard 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) keep for a long time.

* Corresponding author. Tel.: + 81 11 857 8935; fax: + 81 11 857 8980.
E-mail address: sallam.kh.i@aist.go.jp (Kh.I. Sallam).
[1] Present address: Proteolysis and Protein Turnover Research Group, Institute of Genome-Based Biofactory, National Institute of Advanced Industrial Sciences and Technology (AIST), 2-17-2-1 Tsukisamu-Higashi, Toyohira-ku, Sapporo 062-8517, Japan.

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Pacific saury (*Cololabis saira*) is a member of the family Scomberesocidae. This fish is also known by the name mackerel pike or skipper, while its name in Japanese is sanma. Pacific saury is a highly commercial species of economic importance in the parts of the world where they are found. They are desired by Taiwanese and Japanese fisherman as they are easy to clean and delicious.

Pacific saury is a fat-rich fish. In addition to the high protein content of its flesh, it is also rich in unsaturated fatty acids, such as omega-3 fatty acids. Omega-3 fatty acids provide health benefits for human not found in other foods. They have been reported to reduce the probability of developing blood clots that lead to heart attacks and stroke and improve blood circulation. Omega-3 fatty acids may be particularly beneficial for overweight people with hypertension who are on weight loss diets. The American Diabetes Association (ADA) and the American Heart Association (AHA) advocate eating fatty fish as a safe effective way to obtain the heart health benefits of omega-3 fatty acids. Eating fatty fish regularly is an important strategy for improving health in diabetes (Nettleton, 1995).

Fish, however, are perishable food commodities which generally spoil faster than do other muscle foods. The spoilage of fish is a complex process in which physical, chemical and microbiological mechanisms are implicated. Enzymatic and chemical reactions are usually responsible for the initial loss of freshness, whereas microbial activity is responsible for the overt spoilage which thereby establishes product shelf life (Gram, 1995; Gram & Huss, 1996).

Microbiological, biochemical, and sensory methods have been used to assess the quality of fish and marine products during handling and storage. The appeal of biochemical and chemical methods for evaluation of seafood quality is related to the ability to set quantitative standards (Huss, 1995). Biochemical and chemical indicators have been used to replace the time-consuming microbiological methods. Such objective methods should, however, correlate with sensory quality evaluations and the chemical compounds to be measured should increase or decrease with the level of microbial spoilage or autolysis (Huss, 1995).

The objective of the present work was to evaluate the chemical quality, and sensory attributes, as well as to determine the shelf lives of marinated and brined Pacific saury during vacuum-packaged storage at a refrigerated temperature of 4 °C.

2. Materials and methods

2.1. Sampling, preparation and marination of fish

A total of 55 kg of fresh ice-chilled Pacific saury (*Cololabis saira*) was purchased, within 24 h post harvesting, from a local seafood market in Sapporo, Hokkaido, Japan. Fish were transported to the laboratory in polystyrene boxes with a suitable quantity of flaked ice within 1 h of purchasing. The average length and body weight of the sampled fish were 32 ± 1.5 cm and 158 ± 7 g, respectively. Within 2 h of arrival, fish were washed, headed, gutted, skinned and filleted into two sides. The preparation process was carried out in a cold room at a temperature of 4 °C. Saury fillets were then divided into three batches (~15 kg each) and prepared for marination. The first batch was marinated by immersing the fish fillets in a pre-chilled (4 °C) solution containing a combination of 2% acetic acid (v/v) and 12% NaCl (w/v), with a final pH of 2.14; the second batch was marinated in a pre-chilled solution containing 3% (v/v) acetic acid and 12% (w/v) salt, with a final pH of 2.05. The third batch was only brined in 12% NaCl (w/v at 4 °C; pH 6.32) without the addition of acetic acid. Sodium chloride and acetic acid used for the marination process were of high quality (Wako Pure Chemical Industries Ltd., Osaka, Japan). Marinating and brining processes were completed after 72 h at 4 °C. The fish to solution ratio was 1:1.5, and the mixture was stirred at 3 h intervals. After marination, fish fillets were removed from the marinating or brining solutions and left to drain on sterile stainless steel wire mesh for 30 min. Five fillets from each treatment were vacuum-packaged in polyethylene bags, labelled, and stored at 4 °C for 90 d. At 10 day predetermined time intervals, three randomly chosen packages were taken from each batch to be analyzed for chemical changes, sensory attributes as well as the psychrotrophic population count. Some fresh raw fillets were kept untreated to be subjected to the different analyses on the first day of the experiment.

2.2. Psychrotrophic bacterial population

For the determination of total psychrotrophic count (PTC), 25 g of fish samples were aseptically removed from the package and homogenized for 1 min in a stomacher (Stomacher 400 Lab Blender; Seward Medical, London, UK) containing 225 ml of pre-chilled sterile peptone-physiological saline solution (0.1% peptone + 0.85% NaCl) (Katayama Chemical, Osaka, Japan). After recovery for 20 min at room temperature, further decimal serial dilutions were prepared from this homogenate in the same chilled sterile diluent. PTC was determined by inoculating 0.1 ml of the sample homogenate, at selected dilutions, onto duplicate sterile plates of pre-poured and dried Standard Method Agar (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan), using the surface spread technique, then the plates were incubated at 7 °C for 10 days (Cousin, Jay, & Vasavada, 1992).

2.3. Chemical analyses

2.3.1. Compositional analysis

Before vacuum-packaging and storage at the refrigerating temperature, composite flesh samples (comprising dorsal, abdominal and caudal regions) of the marinated fish fillets were analyzed for moisture, protein, fat and ash contents, according to the methods of AOAC International (1999). The compositional analysis was also conducted

for the raw un-marinated fish. Analyses were conducted in triplicate, and all reagents were of analytical grade.

2.3.2. Salt and acetic acid contents

NaCl and acetic acid contents were determined in marinated flesh of Pacific saury, before packaging and storage. NaCl content in fish muscle was calculated from the amount of chlorine by boiling in HNO_3 with excess of $AgNO_3$, followed by titration with NH_4SCN (AOAC International, 1999). The salt content was expressed as the percentage of NaCl in the water phase of fish flesh. The acetic acid content was determined in perchloric acid extracts of marinated saury, as described by Truelstrup Hansen, Gill, and Huss (1995). Acetic acid content was analyzed by high performance liquid chromatography (D-2000 Elite type HPLC System; HITACHI High-Technologies Corporation, Tokyo, Japan) using a BioRad Aminex HPX-87H ion exclusion column (300 × 7.8 mm; BioRad, Hercules, CA, USA) and a UV–vis detector set at 210 nm. Identification and quantification were done on the basis of relative retention times of authentic standards.

2.3.3. pH measurement

Ten grams of each sample were blended with 20 ml of distilled water in a blender for 30 s and the pH value of fish homogenate was measured by a digital pH-meter (HM-5S; TOA Electric Industrial Co. Ltd., Tokyo, Japan) standardized at pH 4 and 7.

2.3.4. Total volatile bases nitrogen (TVB-N) and trimethylamine (TMA)

Fish extracts for determination of total volatile bases nitrogen (TVB-N) and trimethylamine (TMA) were prepared by homogenizing 100 g of fish sample with 200 ml of 7.5% (v/v) aqueous trichloroacetic acid (TCA) solution in a laboratory homogenizer for 1 min at high speed. The homogenate was centrifuged at 3000 rpm for 5 min and the supernatant liquid was then filtered through Whatman No. 1 filter paper. TVB-N was measured by steam-distillation of the TCA-fish extract, using the modified method of Malle and Tao (1987). Twenty-five millilitres of the filtrate were loaded into a Kjeldahl-type distillation tube, followed by 5 ml of 10% (w/v) aqueous NaOH solution. Steam-distillation was performed using a vertical steam distillation unit, and the distillate was received into a beaker containing 15 ml of 4% (v/v) aqueous boric acid solution up to a final volume of 50 ml. The titration was allowed to run against aqueous 0.05 M sulphuric acid solution, using an automatic titrator (DL 25 Titrator, Mettler-Toledo AG, Greifensee, Switzerland) equipped with stirrer and pH electrode. The same experimental procedure as for TVB-N was used for the TMA measurement (Malle & Poumeyrol, 1989). The only difference was the addition of 20 ml of 35% (v/v) formaldehyde to the distillation tube to block the primary and secondary amines, whilst leaving only the tertiary amines to react. The amounts of TVB-N and TMA were calculated from the volume of 0.05 M sulphuric acid used for titration and the results were expressed in mg nitrogen/100 g of sample.

2.3.5. Assessment of lipid oxidation

The 2-thiobarbituric acid (TBA) assay, as an index for lipid oxidation, was carried out according to the procedure of Schmedes and Holmer (1989). Fish sample (10 g) was mixed with 25 ml of 20% trichloroacetic acid (w/v) and homogenized in a blender for 30 s. After filtration, 2 ml of the filtrate were added to 2 ml of 0.02 M aqueous TBA in a test tube. The test tubes were incubated at room temperature in the dark for 20 h; then the absorbance was measured at 532 nm by using a UV–VIS spectrophotometer (model UV-1200, Shimadzu, Japan). TBA value was expressed as mg malonaldehyde (MA) per kg of fish sample.

2.4. Sensory attributes of marinated fish

The sensory attributes of the brined and marinated fish were evaluated by a panel of 15 semi-trained panellists on each day of sampling. Fish samples from the different treatments were individually presented in covered small porcelain dishes to each panellist. The judges were not informed about the experimental approach and the samples were blind-coded with 3-digit random numbers. An eight-point hedonic scoring scale was employed for evaluation of the appearance (8 = extremely acceptable to 1 = extremely unacceptable), colour (8 = faint pink to 1 = deep brown), texture (8 = firm and consistent to 1 = extremely soft or extremely hard), odour (8 = characteristic saury odour to 1 = extreme off-odour), rancidity (8 = no rancidity to 1 = extremely rancid), juiciness and tenderness (8 = extremely juicy/tender to 1 = extremely dry/tough), sour and salty taste (8 = not sour/salty to 1 = extremely sour/salty), as well as flavour and aftertaste (8 = characteristic saury flavour to 1 = extreme off-flavour). A nine-point hedonic scale (9 = like extremely; 8 = like very much; 7 = like moderately; 6 = like slightly; 5 = neither like nor dislike; 4 = dislike slightly; 3 = dislike moderately; 2 = dislike very much; 1 = dislike extremely) was used for evaluation of the overall acceptability. Saury samples receiving overall scores of more than 5 were considered acceptable, while a score between 4 and 5 was considered as the borderline of acceptability. The sensory attributes of brined samples were not evaluated after day 60 of storage due to their higher microbial counts, while marinated samples were evaluated until the end of the storage period (day 90).

2.5. Statistical analysis

On each sampling occasion, three samples were selected from each treatment batch to be subjected to the different analyses. All measurements were carried out in triplicate. Data were subjected to analysis of variance (ANOVA) using the General Linear Models procedure of the

Statistical Analysis System software of SAS Institute (SAS, 1990). Differences among the mean values of the various treatments and storage periods were determined by the least significant difference (LSD) test, and the significance was defined either at $P < 0.05$ or at $P < 0.01$.

3. Results and discussion

Acetic acid is generally recognized as safe (GRAS). The application of vinegar as a food preservative is a traditional method of preventing spoilage. Vinegar is an effective acidulant that causing depression of pH below the growth range of many bacteria (Jay, 2000). It exists in acid foods, largely in the undissociated form (pK 4.75), which can penetrate through the cell membrane of microorganisms where it can acidify the cytoplasm and denature proteins (Baird-Parker, 1980; Eklund, 1983). Salting has been used for centuries as a method of fish preservation. Sodium chloride (NaCl) is added to foods for its effects on sensory, functional and preservation properties. NaCl inhibits microbial growth by restriction of the available water (i.e. lowers a_w) in the meat and fish products. However, its pro-oxidant activity is reported to accelerate the development of lipid oxidation in marinated and salted fatty fish products (Aubourg & Ugliano, 2002; Goulas & Kontominas, 2005; Kilinc & Cakli, 2004).

3.1. Psychrotrophic bacterial count

Psychrotrophic bacterial count (PTC) in raw Pacific saury used in this study was 3.95 $\log_{10}$ CFU/g. The initial PTC is significantly ($P < 0.05$) reduced after the marinating process. Reduction rates of 1.55- and 1.7-logs were achieved for fish marinated with 2% acetic acid and 3% acetic acid, respectively in comparison with the raw untreated samples. The brining process did not induce a significant reduction in the initial PTC although it resulted in 0.6-log lower than that of the raw fillets. Kilinc and Cakli (2004) determined a marked inhibition in the growth of the psychrotrophic count, which decreased from 4.88 $\log_{10}$ CFU/g in raw sardine to <10 CFU/g after marination in 7% acetic acid and 14% NaCl solution. Likewise, Hernandez-Herrero, Roig-Sagues, Lopez-Sabater, Rodriguez-Jerez, and Mora-Ventura (2002) reported a reduction rate of more than 1.2-logs in the PTC in dry-salted anchovies (fish to salt ratio about 1:4) after one week during ripening in plastic cans at 20 °C.

For both marinated and brined samples, the storage time had a significant effect on the PTC, which tended to increase as the time increased. By storage day 40 and afterwards, significantly ($P < 0.05$) lower counts were detected for the marinated than for brined samples (Fig. 1). By day 60, brined fillets showed a high PTC of 6.92 $\log_{10}$ CFU/g, which is very close to the maximal permissible limit of 7 $\log_{10}$ CFU/g for the bacterial count in fish (ICMSF, 1986), indicating a microbiological shelf life of about 2 months under vacuum storage at 4 °C.

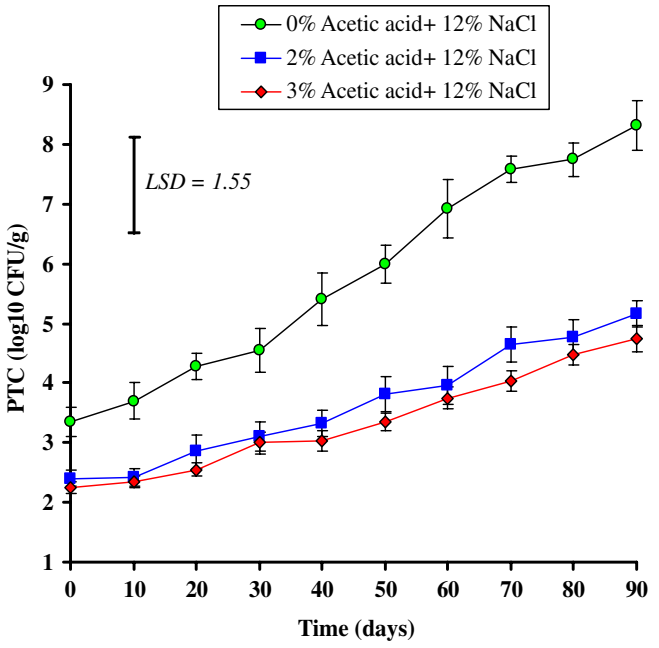

Fig. 1. Changes in the psychrotrophic bacterial counts (PTC) in brined and marinated Pacific saury fillets during vacuum-packaged storage at 4 °C. Values represent means ± SE of three samples; LSD is defined at $P < 0.05$.

By the end of the storage period (day 90), brined fillets exhibited a much higher PTC of 8.31 $\log_{10}$ CFU/g, while the fillet samples marinated with 2% or 3% acetic acid exhibited much lower counts of 5.16 and 4.75 $\log_{10}$ CFU/g, respectively indicating a shelf life of more than 3 months for the marinated products under the same storage condition.

A significant increase in psychrotrophic count with time has also been reported for anchovies brined in 14% NaCl solution and stored at 4 °C for 150 days (Karaçam, Kutlu, & Köse, 2002) as well as for vacuum-packaged "gravad" rainbow trout stored at 3 °C, during which the psychrotrophic bacteria increased from an initial count of 2.3–3.1 $\log_{10}$ CFU/g to 6.88–7.84 $\log_{10}$ CFU/g at the point of spoilage after 27 days (Lyhs et al., 2001). In contrast, Hernandez-Herrero et al., 2002, reported a gradual decrease in the count of psychrotrophic bacteria during a 9-week ripening process of dry-salted anchovies kept in plastic cans at 20 °C, while Kilinc and Cakli (2005) detected a very low count of <10 CFU/g for the psychrotrophic bacteria in marinated sardine throughout a storage period of 6 months in tomato sauce at 4 °C. The discrepancy in the psychrotrophic bacterial populations among the different studies could be attributed to the differences in salt and acetic acid concentrations, the time taken for the brining or marination processes, the initial microbial population, and the storage condition.

3.2. Chemical quality

Many chemical methods have been suggested as indices of deterioration of fish quality during storage. Chemical

tests usually measure the amounts of breakdown products derived from enzymatic, bacterial or oxidative activities. The assay of some of these substances usually provides useful data for the evaluation of fish freshness or quality. In the present work, the potential chemical quality indicators assessed to determine the chemical changes in marinated and brined Pacific saury fillets during cold storage at 4 °C were pH, TBA value, TMA and TVB-N contents.

3.2.1. Proximate composition, salt, and acetic acid content

The chemical composition of fish muscle varies greatly from one species to another and even among the individuals within the same species. Such variation depends on age, size, sex, environment and season (Huss, 1995; Silva & Chamul, 2000). In fact, the variation in the chemical composition of fish is closely related to feed intake, migratory swimming and sexual changes in connection with spawning. Processors have a direct interest in the chemical composition of fish, needing to know the nature of the raw material before the different manufacturing techniques can be correctly applied (Murray & Burt, 1969).

The compositional analysis of raw, brined, and marinated Pacific saury fillets is shown in Table 1. No significant differences were detected in the protein and fat contents between the raw fillets and the treated (brined or marinated) samples ($P > 0.05$); however significant differences ($P < 0.05$) between the raw and treated fillets were detected for the moisture, ash and NaCl contents. The brining and marination processes decreased the moisture contents and increased the other components analyzed in comparison with the control raw samples. Moreover, a significant difference ($P < 0.05$) in acetic acid content was noticed between fillets marinated in 2% acetic acid and that marinated with 3% acetic acid.

3.2.2. Changes in pH value

An important intrinsic factor related to fish flesh is the very high *post-mortem* pH (>6.0). Most fish contain only very little carbohydrate (<0.5%) in the muscle tissue and only small amounts of lactic acid are produced *post-mortem* (Gram & Huss, 1996). During storage, however, decomposition of nitrogenous compounds leads to an increase in pH in the fish flesh, which may be partly attributed to the production of alkaline compounds. Such increase in the pH indicates the bacterial growth, loss of quality and possible spoilage. Since pH influences the susceptibility to microbial growth, acidification, by the addition of acids, is used in the preservation of many foods, including fish products.

The pH of the raw Pacific saury fillets used in this study was 6.32. Brining resulted in a small but significant reduction ($P < 0.05$) of the initial pH, while marination with either 2% or 3% acetic acid resulted in a sharp decrease ($P < 0.01$) in the initial pH by about 2 units (Table 2). This finding is consistent with those reported for anchovies (*Engraulis encrasicholus*) and sardine (*Sardina pilchardus*), in which a sharp decrease in the pH, by about 2–2.5 units, was found after their marination with different concentrations of acetic acid and salt solutions (Gökoğlu, Cengiz, & Yerlikaya, 2004; Kilinc & Cakli, 2005; Sen & Temelli, 2003). A significant difference ($P < 0.05$) in the pH value was detected throughout the storage period between fillets marinated in 2% and those marinated in 3% acetic acid solutions. Similar differences in the pH have been reported between sardines marinated in 2% and in 4% acetic acid solutions during a storage period of 150 days at 4 °C (Gökoğlu et al., 2004).

The pH of both brined and marinated fillets in the present study significantly increased ($P < 0.01$) by the end of the storage period; however, such increase in the pH was more in the brined fillets (0.51 unit) than in the marinated fillets (~0.2 unit). A significant increase in the pH values with the increase of the storage time has also been recognized in brined chub mackerel (Goulas & Kontominas, 2005), marinated anchovies (Poligne & Collignan, 2000) and marinated sardine (Kilinc & Cakli, 2005) during their storage at the refrigerated temperature. It has been reported that, during the storage of marinades, heterofermentative lactic acid bacteria can grow and degrade the amino acids with the formation of carbon dioxide and other decarboxylation products, which bind acetic acid and raise the pH of marinades (Shenderyuk & Bykowski, 1990).

3.2.3. Total volatile bases nitrogen

Total volatile bases nitrogen (TVB-N) is a general term which includes the measurement of trimethylamine (TMA), dimethylamine (DMA), ammonia, and other volatile basic nitrogenous compounds associated with seafood spoilage (Huss, 1995; Malle & Poumeyrol, 1989). Both TVB-N

Table 1
Chemical composition, salt and acetic acid content (% on a wet weight basis) of raw, brined, and marinated Pacific saury at day 0 of storage

Product	Moisture	Protein	Lipid	Ash	NaCl	Acetic acid
Raw Pacific saury	66.18 ± 1.87^{x}	21.3 ± 1.12^{x}	10.74 ± 0.84^{x}	2.24 ± 0.29^{x}	0.26 ± 0.03^{x}	–
Brined Pacific saury (0% acetic acid + 12% NaCl)	64.32 ± 1.55^{y}	21.8 ± 1.18^{x}	11.05 ± 0.93^{x}	3.32 ± 0.22^{y}	4.27 ± 0.09^{y}	–
Marinated Pacific saury (2% acetic acid + 12% NaCl)	62.15 ± 1.23^{yz}	22.6 ± 1.13^{x}	11.43 ± 1.13^{x}	4.49 ± 0.19^{z}	4.61 ± 0.07^{z}	1.06 ± 0.06^{x}
Marinated Pacific saury (3% acetic acid + 12% NaCl)	61.39 ± 1.45^{z}	22.9 ± 1.39^{x}	11.81 ± 1.27^{x}	4.56 ± 0.31^{z}	4.65 ± 0.11^{z}	1.19 ± 0.04^{y}

$^{x–z}$ Means ± SE in the same column followed by different superscript are significantly different ($P < 0.05$).

Table 2
Effects of brining and marinating treatments on the pH of Pacific saury fillets during vacuum-packaged storage at 4 °C

Storage time (days)	Brined fillets (0% acetic acid + 12% NaCl)	Marinated fillets (2% acetic acid + 12% NaCl)	Marinated fillets (3% acetic acid + 12% NaCl)
0	$6.07 \pm 0.04^{ab;z}$	$4.37 \pm 0.05^{a;y}$	$4.26 \pm 0.03^{b;x}$
10	$6.03 \pm 0.03^{a;z}$	$4.39 \pm 0.03^{a;y}$	$4.31 \pm 0.04^{bc;x}$
20	$6.11 \pm 0.03^{bc;z}$	$4.44 \pm 0.06^{ab;y}$	$4.23 \pm 0.02^{ab;x}$
30	$6.17 \pm 0.05^{bc;z}$	$4.48 \pm 0.04^{bc;y}$	$4.17 \pm 0.03^{a;x}$
40	$6.21 \pm 0.04^{c;z}$	$4.54 \pm 0.03^{c;y}$	$4.25 \pm 0.02^{b;x}$
50	$7.33 \pm 0.02^{d;z}$	$4.49 \pm 0.04^{bc;y}$	$4.31 \pm 0.03^{bc;x}$
60	$6.37 \pm 0.03^{d;z}$	$4.45 \pm 0.02^{ab;y}$	$4.29 \pm 0.02^{bc;x}$
70	$6.36 \pm 0.04^{d;z}$	$4.51 \pm 0.02^{bc;y}$	$4.36 \pm 0.01^{cd;x}$
80	$6.47 \pm 0.02^{e;z}$	$4.49 \pm 0.03^{bc;y}$	$4.39 \pm 0.04^{d;x}$
90	$6.58 \pm 0.01^{f;z}$	$4.56 \pm 0.03^{c;y}$	$4.47 \pm 0.02^{e;x}$

[a–f] Means ± SE in the same column followed by different superscripts are significantly different.
[x–z] Means ± SE in the same row followed by different superscripts are significantly different.

and TMA are the traditional chemical means most widely used for evaluation of the degree of spoilage in seafood.

The initial TVB-N in raw Pacific saury was 9.12 mg/100 g fish. Brining or marinating processes did not produce a significant reduction in the TVB-N values and only a minor decrease (0.89–1.25) was recognized in the initial TVB-N after the treatment process. Conversely, Kilinc and Cakli (2004) determined a considerable decrease in TVB-N, from 10.3 to 6.5, after the marinating process of sardine fillets in a solution containing 7% acetic acid and 14% salt. During storage, however, a slight increase (<4 mg/100 g fish) in the TVB-N value was noticed by the days, 30, 40 and 50 for saury fillets marinated in 0%, 2%, and 3% acetic acid, respectively, during which their TVB-N values were below 12 mg/100 g (Fig. 2) while, by the end of the storage period, a significant increase ($P < 0.05$) in such values to relatively high levels of 34.6, 24.1 and 20.5 mg/100 g were detected for fillets marinated with 0%, 2% and 3% acetic acid, respectively and, although the brined fillets (0% acetic acid) almost reached the maximal permissible level of 35 mg TVB-N/100 g fish flesh specified by the EC guidelines (Commission Decision 95/149/EC, 1995), the marinated fillets were still below this limit by >10 mg TVB-N/100, indicating the significant effect of acetic acid in the reduction of chemical changes in marinated fish. The slight increase in TVB during the first stage of storage may be initiated by autolytic degradation of nucleotides and free amino acids while the larger increase in TVB-N during the late stage of storage is most likely caused by a combination of microbiological and autolytic activities and the complete microbial reduction of TMAO to TMA. Since TVB-N is produced mainly by bacterial decomposition of fish flesh, the higher microbial counts of the brined fillets, detected by day 60 and afterwards during storage, could explain the higher TVB-N values in brined samples.

A comparable pattern of the increase in TVB-N has been reported in marinated sardine (Gökoğlu et al., 2004; Kilinc & Cakli, 2005), brined chub mackerel (Goulas & Kontominas, 2005), and brined anchovies (Karaçam et al., 2002) during refrigerated storage. On the other hand, Pons-Sánchez-Cascado, Vidal-Carou, Mariné-Font, and Veciana-Nogués (2005) reported that TVB-N levels in anchovies marinated in vinegar remained constant (<10 mg/100 g) during a 2-week marinating process and throughout a storage period of 3 months under refrigerated vacuum-packed storage. Conversely, a much higher TVB-N level (60.5 mg/100 g) was reported by day 42 in vacuum-packaged, salted sea bream stored under refrigeration at 4 °C (Chouliara, Savvaidis, Panagiotakis, & Kontominas, 2004).

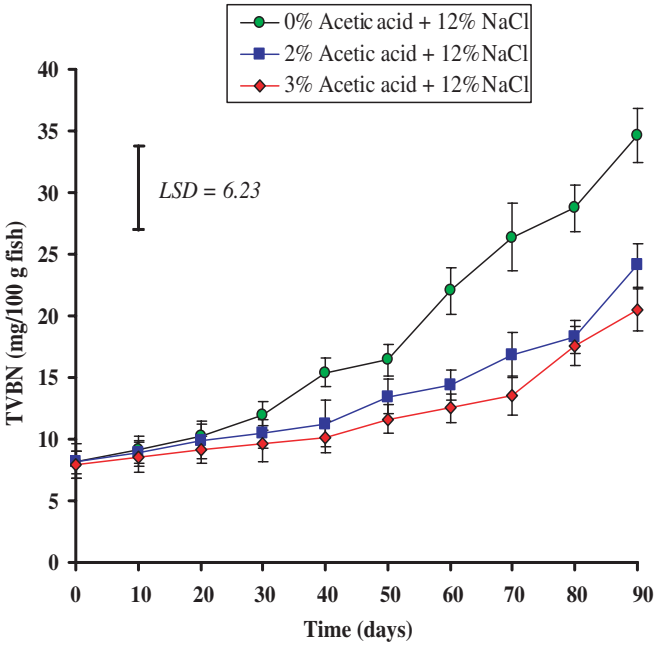

Fig. 2. Changes in the total volatile bases nitrogen (TVB-N) content in brined and marinated Pacific saury fillets during vacuum-packaged storage at 4 °C. Values represent means ± SE of three samples; LSD is defined at $P < 0.05$.

3.2.4. Trimethylamine content

Fresh fish naturally contain trimethylamine oxide (TMAO). TMAO is a tasteless non-protein nitrogen compound, which has an osmoregulating function, and its content varies with the fish species, environment, season, size

and age of fish (Huss, 1995; Koutsoumanis & Nychas, 1999; Özogul, Özogul, & Gökbulut, 2006). Certain numbers of naturally occurring well defined spoilage bacteria are able to reduce TMAO to TMA (Koutsoumanis & Nychas, 1999; Ólafsdóttir et al., 1997). In seafood spoilage, TMA particularly contributes to the characteristic ammonia-like off-odour and 'fishy' off-flavours (Gram & Huss, 1996; Ólafsdóttir et al., 1997).

The initial TMA level in raw fillets was 0.76 mg/100 g. After the marinating process, the initial TMA value remained almost unchanged (0.84, 0.69 and 0.72 mg/100 g for fillets marinated in 0%, 2% and 3% acetic acid, respectively) (Fig. 3). Kilinc and Cakli (2004) reported a slight increase in TMA level from 0.88 in raw sardine to 1.07 after marination in acetic acid (7%) and salt (14%) solution. For both brined and marinating fillets, storage time had a significant effect on the TMA values, which tended to increase as the storage time increased; however, during the first stage of storage, TMA increased very slowly and did not exceed the level of 2 mg/100 g by the days 30, 40 and 50 for fish fillets marinated with 0%, 2% and 3% acetic acid, respectively. By the end of the storage period, however, brined fillets presented a high level of 8.37, while fillets marinated in 2% and 3% acetic acid showed significantly lower TMA values of 5.52 and 4.47, respectively. The lower contents of TMA in the marinated samples than in the brined fillets, can be attributed to the inhibitory effect of the acetic acid on the microbial growth, including the TMAO-reducing organisms.

A similar pattern of the increase in TMA during refrigerated storage has also been reported for many seafood products, including marinated sardine (Gökoğlu et al., 2004; Kilinc & Cakli, 2005) vacuum-packaged, salted sea bream (Chouliara et al., 2004) and brined, non-smoked vacuum-packed chub mackerel (Goulas & Kontominas, 2005). In contrast, Pons-Sánchez-Cascado et al. (2005) claimed that the TMA level in the anchovies marinated in vinegar was low (<1 mg/100 g) and remained constant during vacuum-packed refrigerated storage for 3 months.

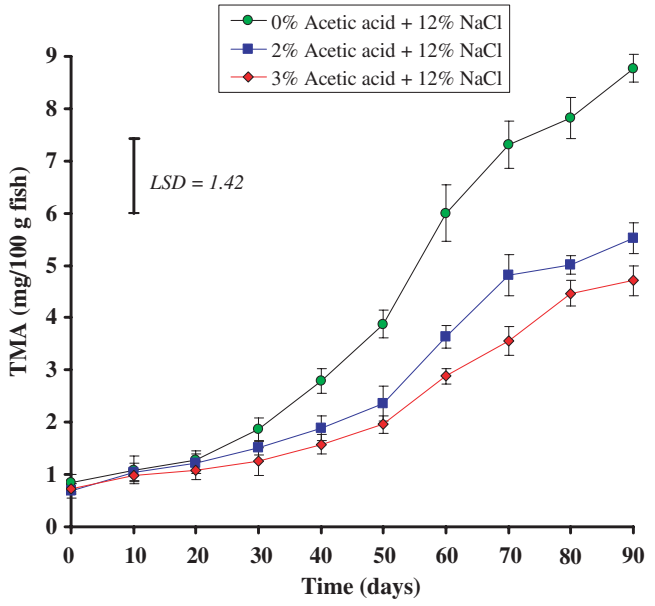

Fig. 3. Changes in the trimethylamine (TMA) concentration in brined and marinated Pacific saury fillets during vacuum-packaged storage at 4 °C. Values represent means ± SE of three samples; LSD is defined at $P < 0.05$.

A TMA value of 5–10 mg/100 g sample was reported as the limit for the acceptability of fish (Sikorski, Kolakowska, & Burt, 1990). In this study, a TMA value of 6 mg/100 g coincided with the onset of spoilage (day 60) for the brined saury fillets, while fillets marinated in 2% and 3% acetic acid did not reach such a value, even by day 90 of storage.

3.2.5. Lipid oxidation

The highly unsaturated lipids in fat-rich fish are easily susceptible to oxidation that results in a rancid smell and taste as well as alterations in texture, colour and nutritional value (Ólafsdóttir et al., 1997). TBA (thiobarbituric acid) values is a widely used indicator for the assessment of degree of lipid oxidation. It has been suggested that a maximum TBA value, indicating the good quality of the fish, is 5 mg malonaldehyde/kg, while fish may be consumed up to a TBA value of 8 mg malonaldehyde (MA)/kg (Schormüller, 1969).

In the present study, the TBA value of fresh raw Pacific saury was 0.37 mg MA/kg. After the treatment processes, sharp increases ($P < 0.01$) in the initial TBA values to high levels of 0.87, 0.72 and 0.63 were measured for brined, 2% acetic acid- and 3% acetic acid-marinated fillets, respectively. During storage, there was a tendency towards an increase in TBA values up to a maximal point (at 50–70 days of storage), followed by a gradual decrease to lower values (Fig. 4). Even so, significantly ($P < 0.01$) higher values of 2.82, 1.88 and 1.61 mg MA/kg, in comparison with the initial values, had been attained by the end of the storage for brined, 2% acetic acid- and 3% acetic acid-marinated fillets, respectively. The decrease in TBA content after the peak point has been attributed to the interaction between MA and decomposition products of protein to give tertiary degradation products (Fernández, Pérez-Alvarez, & Fernández-López, 1997; Reddy & Setty, 1996). The present result indicated that oxidative rancidity in marinated fillet samples remained relatively low throughout the entire period of vacuum-packaged storage at 4 °C and its level was within the acceptability limits for fish consumption.

The level of TBA and the pattern of its increase in brined saury of this study was similar to that reported for various fish species brined in different concentrations of NaCl solution during vacuum-packaged refrigerated storage, including threadfin bream (Jeevanandam, Kakatkar, Doke, Bongiwar, & Venugopal, 2001), sea bream (Chouliara et al., 2004) and chub mackerel (Goulas & Kontominas, 2005), in which the TBA value increased to the maximal level at a certain period during storage, and thereafter it decreased gradually. However, much higher TBA

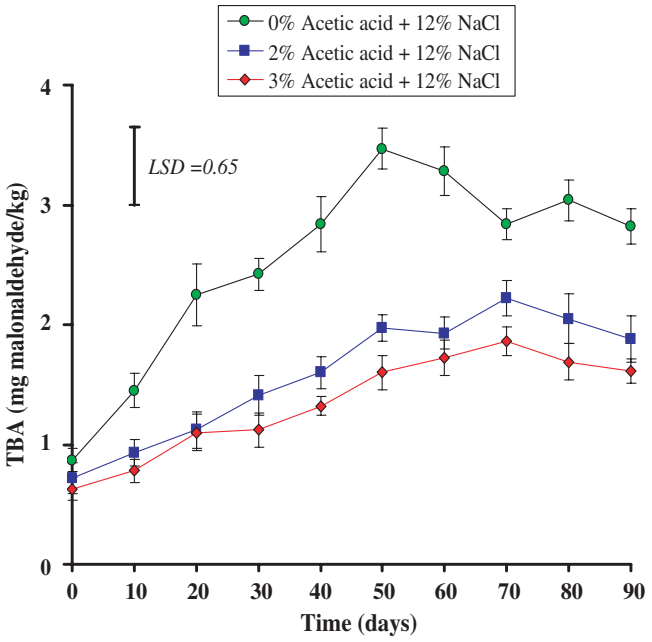

Fig. 4. Changes in the lipid oxidation (TBA value) in brined and marinated Pacific saury fillets during vacuum-packaged storage at 4 °C. Values represent means ± SE of three samples; LSD is defined at $P < 0.01$.

levels were reported in salted anchovies during 9 weeks of ripening in cans at 20 °C (Hernandez-Herrero et al., 2002), and also in non-pasteurized sardine marinade in tomato sauce during 6 months of storage at 4 °C (Kilinc & Cakli, 2005). On the other hand, fluctuations in the TBA levels during refrigerated storage of anchovies that were brined in different concentrations of salt (14–26%) have been reported by Karaçam et al. (2002), who also concluded that TBA may not be a reliable criterion for anchovies salted under the condition used.

NaCl has a pro-oxidative effect that enhances the activity of iron toward lipid peroxidation (Kanner, Harel, & Jaffe, 1991). By the day 20 of storage and afterwards, significantly lower differences ($P < 0.05$) were noticed in the TBA values of marinated fillets when compared with the brined fillets (Fig. 4). This could be indicative of the possible role of acetic acid, in marinated fillets, in reducing the pro-oxidative effect of NaCl.

3.3. Sensory evaluation

Sensory evaluation is the most popular way of assessing the freshness of fish. It is fast, simple, and provides immediate quality information. The sensory characteristics of fish are clearly visible to the consumer and are essential for consumer satisfaction (Reineccius, 1990).

Sensory attributes of brined and marinated Pacific saury fillets (pooled data over the storage period) are presented in Table 3. No significant differences in the overall acceptability scores were detected between brined and marinated samples before storage (data not shown), although the marinated samples received higher scores. Sensory scores of both brined and marinated fillets were in the typical categories of appearance, texture and juiciness. Significant differences ($P < 0.05$), however, were determined for colour, odour, rancidity, tenderness, as well as flavour and aftertaste between the brined and marinated samples. No differences were found, in the sensory attributes analyzed, between the 2% and 3% acetic acid-marinated fillets. At day 60 of storage, panellists considered brined samples to be unfit for human consumption as the samples were described as soft, dark in colour with slight bitter taste and lack of the typical product odour and presence of slight ammonia off-odour. However, no off-odour or off-flavour could be detected by any of the 15 panellists in the marinated samples which were organoleptically evaluated until the end of the storage period (day 90).

Storage time has no effect ($P > 0.05$) on the appearance, juiciness, or tenderness of saury fillets; whereas colour, odour, texture, rancidity, flavour and aftertaste and the overall acceptability scores significantly decreased ($P < 0.05$) as the storage time increased (data not shown). Likewise, it has been reported that the sensory scores of marinated and brined fish significantly decreased with the

Table 3
*Mean sensory attribute scores of brined and marinated Pacific saury fillets during vacuum-packaged storage at 4 °C

Sensory attributes	Brined fillets (0% acetic acid + 12% NaCl)	Marinated fillets (2% acetic acid + 12% NaCl)	Marinated fillets (3% acetic acid + 12% NaCl)
Appearance	6.54 ± 0.21^{x}	6.42 ± 0.18^{x}	6.35 ± 0.14^{x}
Colour	5.87 ± 0.17^{y}	5.23 ± 0.13^{x}	5.12 ± 0.20^{x}
Odour	6.48 ± 0.19^{y}	5.79 ± 0.13^{x}	5.64 ± 0.15^{x}
Texture	5.49 ± 0.16^{x}	5.58 ± 0.11^{x}	5.55 ± 0.14^{x}
Rancidity	6.44 ± 0.22^{x}	7.20 ± 0.18^{y}	7.32 ± 0.13^{y}
Juiciness	5.52 ± 0.09^{x}	5.63 ± 0.14^{x}	5.57 ± 0.17^{x}
Tenderness	6.32 ± 0.11^{y}	6.58 ± 0.18^{x}	6.65 ± 0.10^{x}
Sour (vinegar) taste	7.05 ± 0.17^{y}	5.18 ± 0.18^{x}	5.45 ± 0.10^{x}
Salty taste	5.54 ± 0.17^{x}	6.12 ± 0.21^{y}	6.33 ± 0.19^{y}
Flavour and aftertaste	5.48 ± 0.15^{x}	6.32 ± 0.11^{y}	6.13 ± 0.13^{y}
Overall acceptability	6.29 ± 0.22^{x}	7.13 ± 0.18^{y}	6.88 ± 0.12^{y}

*Pooled data over the storage time.
$^{x–z}$ Scores in the same row followed by different superscripts are significantly different ($P < 0.05$).

increase of storage time (Gökoğlu et al., 2004; Karaçam et al., 2002).

It is known that acetic acid induces a specific vinegar taste. No difference ($P > 0.05$) in the intensity of the vinegar taste was detected between the 2% acetic acid- and 3% acetic acid-marinated fillets. Despite both brined and marinated fillets receiving the same concentration of salt (12%) during the treatment process, the salty taste was significantly ($P < 0.05$) lower in marinated than in brined samples. It is more likely that acetic acid tended to suppress the salt perception in the marinated fillets. Similar findings have been also recognized in anchovies marinated in acetic acid and salt solutions (Poligne & Collignan, 2000). In this regard, Breslin (1996) pointed out that, when two compounds that elicit different taste qualities are mixed in solution at moderate or strong concentrations, the mixture will often yield a taste sensation that is less intense than the simple sum of the component tastes, and he concluded that subthreshold salt concentrations tended to suppress sourness and vice versa.

A significant difference ($P < 0.05$) was detected, for the overall acceptability, between the brined and marinated samples, while no differences were detected between the two marination processes. Saury fillets, marinated in 2% acetic acid, received the highest overall acceptability score (7.13), followed by 3% acetic acid-marinated samples (6.88), while the brined fillets exhibited the least acceptable scores (6.29). Nonetheless, all of the analyzed samples were considered as acceptable (achieved overall scores of more than 5) during all the occasions of the sensory analysis.

The increase in psychrotrophic populations of bacteria, as well as in the chemical indicators (pH, TVB-N, TMA and TBA), determined in this study, generally coincided with the sensory scores detected by the sensory panel. A good correlation between sensory scores and chemical indices of quality has been determined for many ready-to-eat seafood products during their storage (Dondero, Cisternas, Carvajal, & Simpson, 2004; Goulas & Kontominas, 2005).

4. Conclusion

The present study concluded that marination of Pacific saury in solutions containing 12% NaCl and 2% or 3% acetic acid can retard the microbial growth, delay the chemical changes, improve or maintain the sensory attributes and extend the shelf life of the product during refrigerated storage; therefore, marinating with combinations of acetic acid and salt solutions can be used as a safe method for preservation of fatty fish.

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