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Effect of freshness grade of anchovy (*Engraulis encrasicolus*) on the quality of marinated product stored at 4°C

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Abstract

The aim of this study was to investigate the effects of raw material freshness on the quality of marinated fish. The raw material anchovy (*Engraulis encrasicolus*) was divided into two batches. One batch (A) was kept at ambient temperature (20°C) for 6 h and the other one was kept at 0°C for 72 h. Then, they were marinated by soaking into marination solution containing 3% acetic acid and 8% NaCl. Total volatile basic nitrogen (TVB-N), trimethylamine (TMA), thiobarbituric acid (TBA), para-anisidine (p-Av) values in both marinated samples increased significantly during storage at 4°C. Increases in quality parameters were higher in samples produced with raw anchovy kept at 20°C compared to samples kept at 0°C. Although the sensory scores of both samples decreased during storage, higher scores were obtained for samples kept at 0°C compared to samples kept at ambient temperature. According to the results of the study, it has been determined that the quality of the raw material significantly ($p<0.01$) affects the quality of marinated anchovy.

Keywords: fish; quality; anchovy; marinade; raw material

1. Introduction

Marinated fish are semi-preserved, ready-to-eat with no heat treatment and are high-value delicatessen fish products (Fuentes et al., 2010). Acetic acid and salt are added to fish to retard the action of bacteria and enzymes. This process results in a product with a characteristic flavor and an extended but limited shelf life (Mc Lay, 1972).

The anchovy (*Engraulis encrasicolus*), which is a pelagic fish species belonging to the Engraulidae family, is a common material for fish marinades. Generally, filleted anchovies are soaked to a solution containing acetic acid and salt. After ripening, they are packed with vegetable oil or sauce.

Fish and fish products are perishable materials that deteriorate quickly. The easy deterioration of fish quality is because of the post-mortem biological changes that take place in the body of dead fish (Sikorski et al., 1990). Freshness is a major contribution to the quality of seafood products. For all kinds of seafood products, freshness is essential for the quality of the final product (Alasalvar et al., 2010). Considering the properties of raw material such as freshness, microbial load and physical damage, the initial quality is an important factor affecting the end product quality (Fuselli et al., 1994; Capaccioni et al., 2011). It is known that during marination, microbial activity is inhibited due to the combined effect of salt and acid solutions. It is thought that the marinating process provides food safety with microbial inactivation and therefore, the initial quality of the fish is insignificant.

Based on this wrong thought, this study aimed to investigate the effects of the freshness of the raw material on the quality and storage stability of marinated fish. For this purpose, fish marinade was produced using anchovy in two different freshness grades. Anchovy sometimes exposures to ambient temperature for a few hours before reaching to market or processing plant. In some cases, processors use not only freshly harvested fish but also use refrigerated fish. For this reason, in this study, it was aimed to investigate the effect of freshness grade of anchovy on marinating quality during the storage. For this purpose, the fish were kept at ambient for 6 h and refrigerated temperatures for 72 h before marination.

2. Materials and methods

2.1. Material

The material was anchovy (*Engraulis encrasicolus*) purchased from the main fish market. The fish boxed with crushed ice were transferred to the laboratory immediately. Upon arrival at the laboratory, the fish were divided into the following batches: (A) This batch was kept at ambient temperature (20°C) for 6 h; (B) this batch was kept in boxes with ice at 0°C for 72 h. The fish ware filleted and washed before the marination process.

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2.2. Methods

For the marination process, firstly, marinating brine solutions containing 3% acetic acid and 8% NaCl were prepared. Anchovy fillets were immersed into the brine at 20°C. The ratio of fish to solution was 1:1.5. After 24 h which ripening was completed, the fish were taken from the solutions and put into the boxes with sunflower oil. Packaged fish was stored at 4°C for 4 weeks. During the storage, analyses were done at weekly intervals.

2.3. Data analysis

Analyses of the raw samples were made as soon as they arrived at the laboratory. Analyses of samples kept at room temperature and 4°C were carried out 6 h and 72 h, respectively. Analyses of marinated samples were carried out at weekly intervals. Before analyses, marinated fish were removed from the boxes, and drained on filter paper (73 g/m^2 40x40 cm) for 10 min and homogenized using the blender. All analyses were conducted in duplicate samples.

2.3.1. Total volatile basic nitrogen (TVB-N)

10 g sample was washed into the distillation flask and 1 mg magnesium oxide was added with a drop or two of silicone anti-foam solution. Samples were boiled and distilled into 10 ml of 0.1 N HCl solutions in a 500 ml conical flask with added Tashiro-indicator. After distillation, the contents of conical flask were titrated with 0.1 N NaOH (Schormüller, 1968).

2.3.2. Trimethylamine (TMA-N)

A 10 g sample was blended with 90 ml of 5 % trichloroacetic acid (TCA) using an ultra-turrax homogenizer and filtered. A 4 ml aliquot was transferred into test tubes and 1 ml formaldehyde (20 %), 10 ml anhydrous toluene, 3 ml KOH (50 %) solution were added. The tubes were shaken, and a 5 ml toluene layer was pipetted to which 5 ml picric acid (0.02 %) had been added. The supernatant was then transferred to a spectrophotometric cell. Absorbance at 410 nm was measured. At the same time, a series of standards were prepared and measured (Schormüller, 1968).

2.3.3. pH

The pH value was determined by dipping the pH electrode into homogenates of filleted muscle in distilled water (1/1) (Manthey et al., 1988). All measurements were performed at room temperature using pH-meter (WTW Inolab, Weilhem, Germany).

2.3.4. Thiobarbituric acid (TBA)

The thiobarbituric acid (TBA) distillation method was performed as described by Tarladgis et al. (1960). A homogenized 10 g sample was distilled after the addition of 2.5 ml HCl and distilled water solution (1+2). A 5 ml aliquot of the distilled solution was transferred into the stoppered test tube and 5 ml TBA solution (0.288 g TBA/100 ml distilled water) was added. The test tube was shaken and was left in a water bath at 110°C for 35 min. The absorbance was determined by a spectrophotometer (Shimadzu UV 160A, Tokyo, Japan) at 538 nm against a blank containing distilled water and TBA solution. The results were expressed as milligrams of malonaldehyde per kilogram of fish flesh.

2.3.5. Para-anisidine value (p-Av)

Para-anisidine value was determined by the IUPAC method (1987). 0.5 g of fish lipid was dissolved in 25 ml n-hexane and absorbance of the mixture was measured at 350 nm using a UV-Vis spectrophotometer (A_1). Para-anisidine reagent was added to 5 ml of the mixture and held in the dark for 10 minutes before absorbance reading (A_2) at the same wavelength. Value of p-Av was calculated using the following:

$$\text{p-Av} = 25\ (1.2 \times (A_2 - A_1)) / \text{sample weight}$$

2.3.6. Sensory analysis

Sensory analysis was performed by a panel of 5 panellists. The panellists were from the staff of the Fish Processing Department, who had experience evaluating seafood. The panellists evaluated the samples for odour, appearance, taste and texture on a 9-point hedonic scale (Amerina et al., 1965) and overall acceptability was calculated. A score of 9-7 indicated “very good”, a score of 6.9-4.0 “good”, a score of 3.9-1.0 denoted as spoiled.

2.3.7. Microbiological analyses

For microbiological analysis, a 10 g sample was added to 90 ml of sterile ringer solution and homogenized in a stomacher (Stomacher 80, Seward Medical, and London, UK) for 2 min at low speed at room temperature. Serial decimal dilutions were made and plated onto appropriate culture media (Anonymous, 1992). Aerobic mesophilic counts (APC) were determined by the spread plate method on Plate Count Agar. Plates were incubated at 37°C for 48 h under aerobic conditions. Psychotropic counts were determined by the spread plate method on Plate Count Agar. Plates were incubated at 4°C for 7 days (Anonymous, 1992). *E. coli* was examined by the spread plate method on Violet Red Bile Agar (VRBA). Then overlaid with VRB-MUG and allowed to solidify, then incubated at 37°C for 24 h. These plates were examined under long-wavelength UV lamb for the presence of fluorescent colonies (Anonymous, 1992). Fluorescent colonies were enumerated as *E. coli.* Yeast-molds were enumerated with Potato Dextrose Agar. Petri dishes were incubated at 25°C for 5 days for yeast-mold (Anonymous, 1992). All microbial counts were expressed as base-10 logarithms of colony forming units per gram (log CFU/g).

2.3.8. Statistical analysis

Two replications of the experiment were conducted at separate times and all analyses were performed in duplicates. Data were subjected to analysis of variance followed by Duncan’s Multiple Range Test using the SAS software (Statistical Analysis System, Cary, NC, USA).

3. Result and discussion

TVB-N value of anchovy was found to be 14.68 mg/100g immediately after arrival at the laboratory. TVB-N value of sample (A) kept at ambient temperature was found to be 18.81 mg/100g after 6 h. TVB-N value of sample (B) kept at 0°C was 14.66 mg/100g after 72 h. While there were significant ($p<0.01$) differences in TVB-N values of anchovy samples after keeping at 20°C for 6 h, no differences were observed in samples kept at 0°C for 72 h. Lower TVB-N values were observed after marination compared to raw samples. The ripening process of marinated fish was completed in 24 h.

After ripening, TVB-N values of samples A and B were found to be 14.48 and 10.47 mg/100g, respectively. Similar decreases were reported after marinating process by Maktabi et al. (2015) and Pons-Sanchez-Cascado et al. (2015). TVB-N values of marinated anchovy produced from both raw samples significantly increased during storage at 4°C. Similar increases in storage were reported in previous studies (Aksu et al., 1997; Gokoglu et al., 2004; Gokoglu et al., 2009; Maktabi et al. 2015). TVB-N values of sample B were lower compared to sample A. The TVB-N values of both samples did not reach critical values after 4 weeks of storage (Fig. 1). The level of 30–35 mg TVB-N/100 g has been reported as an acceptable upper limit for seafood (Ludorf & Meyer, 1973; Sikorski et al., 1990). The TVB-N value has been reported to be more useful in assessing the degree of sardine deterioration than evaluating changes in the initial stages of storage (El Marrackhi et al., 1990).

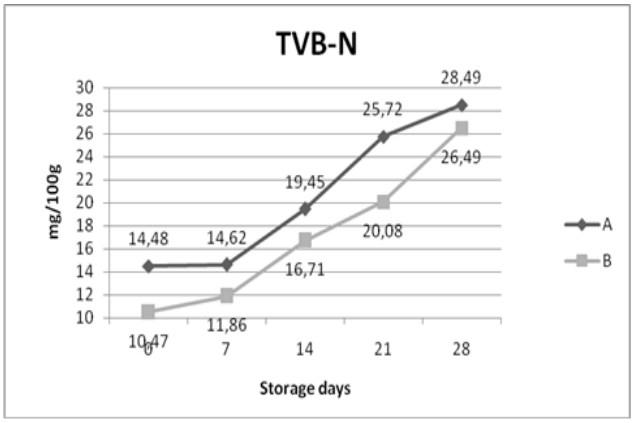

Figure 1. Total volatile basic nitrogen (TVB-N) values of marinated samples A (kept at 20°C) and B (kept at 0°C) during refrigerated storage

The TMA-N value of anchovy was found as 0.022 mg/100g immediately after arrival at the laboratory. Significant ($p<0.01$) differences in TMA-N values of anchovy were found after keeping at 20°C for 6 h and at 0°C for 72 h. TMA-N value of samples kept at 20°C was found to be 0.415 ± 0.099 mg/100g after 6 h. TVB-N value of samples kept on ice was 0.25 ± 0.071 mg/100g after 72 h. TMA-N values of A and B samples were not statistically different from the value of raw fish statistically ($p>0.01$). Significant ($p<0.01$) increases in TMA-N values were found after marinating. TMA-N values of both samples increased significantly during storage (Fig. 2).

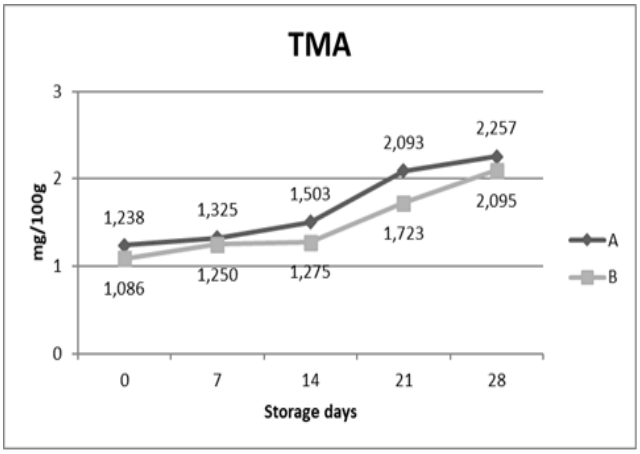

Figure 2. Trimethylamine (TMA) values of marinated samples A (kept at 20°C) and B (kept at 0°C) during refrigerated storage

This increase was very slow in the early stages of storage. The increase in TMA-N values was faster after 14 days. On the 28th day of storage, TMA-N values reached 2.25 and 2.09 mg/100g in samples A and B, respectively. In previous studies, similar increases have been reported during refrigerated storage (Ozden & Baygar, 2003; Gokoglu et al., 2004; Sallam et al., 2007). TMA-N values did not exceed acceptable limit values during 4 weeks of storage. TMA value of 5-10 mg/100 g sample was reported as the acceptability limit of the fish (Sikorski et al., 1990). TMA-N results from the reduction of TMA-N oxide by bacterial activity and partly by intrinsic enzymes and is often used as an index of the freshness of marine fish (Villareal & Pozo, 1990). In this study, raw material quality affected TMA-N values during storage. Lower TMA-N values were found in marinated fish produced using more fresh raw materials.

The mean pH value of the samples just after arrival at the laboratory was measured as 6.90 ± 0.02. The values did not change significantly after keeping at 20°C and 0°C. However, after marination, significant decreases were found due to acetic acid penetration into the fish muscle. Similar decreases were reported in previous studies (Sen & Temelli, 2003; Gokoglu et al., 2004; Maktabi et al., 2015). The pH values of both sample (A and B) significantly ($p<0.01$) increased during the storage (Fig. 3). At the first 7 days of storage, sharply increase was observed. Increases in pH values of the samples occurred slowly on other storage days. At the end of storage, pH values of sample A and B reached 4.41 and 4.34, respectively. During the storage of marinades, heterofermentative lactic acid bacteria can grow and cause the breakdown of amino acids. Thus, the formation of carbon dioxide and other decarboxylation products is observed. These products bind acetic acid and the pH of the marinade rises (Shenderyuk & Bykowski, 1989).

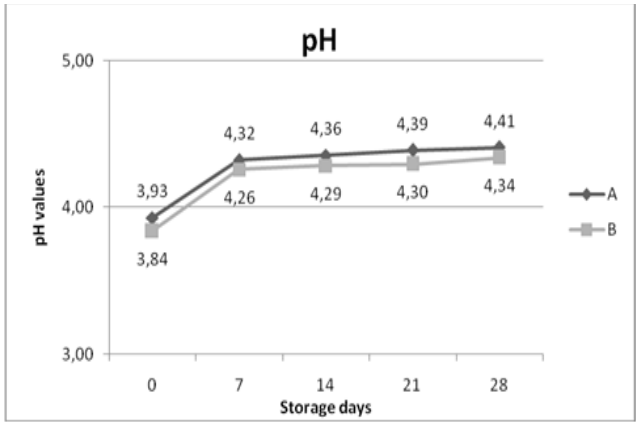

Figure 3. pH values of marinated samples A (kept at 20°C) and B (kept at 0°C) during refrigerated storage

The initial TBA value of the raw material was 2.12 mg/kg. It was observed that the TBA values of raw materials did not change significantly after 6 h at 20°C and 72 h at 0°C. After marination, significant decreases were found in the TBA values of both samples. TBA analysis is an important quality index indicating fat oxidation. Oxidative rancidity is complex spoilage and especially occurs in fatty fishes (Connell, 1980). During lipid oxidation, malonaldehyde (MA), a minor component of fatty acids containing three or more double bonds, is formed as a result of the breakdown of polyunsaturated fatty acids. It is usually used as an indicator of the lipid oxidation process, both for the early appearance as oxidation occurs and for the sensitivity of the analytical method (Shahidi & Zhong, 2005).

TBA values of marinated samples increased significantly during storage ($p < 0.01$) (Fig. 4). Lower ($p<0.01$) TBA values were found for sample B compared to sample A throughout storage. At the end of the retention period, TBA values of sample B reached limit values, while sample A exceeded the acceptable level. TBA value should be less than 3 mg malonaldehyde/kg in good quality material and not be more than 5 mg/kg. The limit level is reported as 7-8 mg/kg by Schormüller (1968 & 1969). Increases in TBA values have been reported for marinated anchovy (Gunsen et al., 2011), pacific saury (Sallam et al., 2007) and rainbow trout (Maktabi et al., 2015).

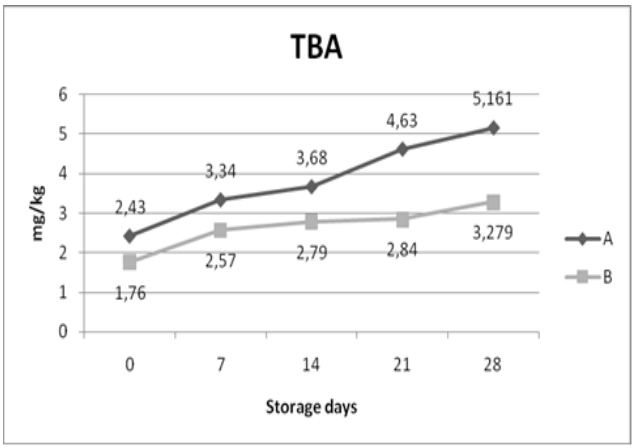

Figure 4. Thiobarbituric acid (TBA) values of marinated samples A (kept at 20°C) and B (kept at 0°C) during refrigerated storage

The initial para anisidine (p-Av) value of the raw sample was found to be 6.81. This value increased significantly ($p<0.01$) after keeping at 20°C for 6 h. However, the samples were kept unchanged at 0°C for 72 h. After marinating, p-Av increased significantly for both samples. Significant increases were observed during storage (Fig. 5).

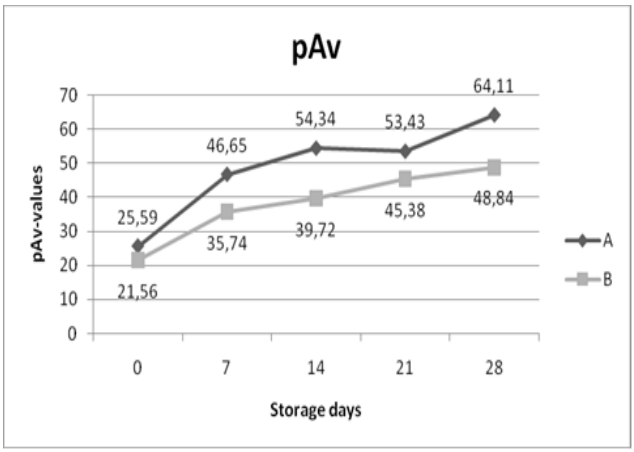

Figure 5. Para-anisidine (P-AV) values of marinated samples A (kept at 20°C) and B (kept at 0°C) during refrigerated storage

At the end of storage, para-anisidine values of samples A and B were measured as 64.11 and 48.84, respectively. The p-anisidine test provides useful information on non-volatile carbonyl compounds formed in oils during processing and is often used to detect secondary oxidation products. The para-anisidine value of good quality fish oil is reported to be less than 20 (Hamilton et al., 1998). In this study, para-anisidine values of marinated anchovy exceeded limit value. The increase in p-Av of all samples indicates that primary lipid oxidation products (hydroperoxides), which may indicate an advanced stage of lipid oxidation, are significantly decomposed into secondary oxidation products (carbonyls). Higher p-Av values were obtained in sample A compared to sample B. Raw material quality affected the oxidation level of marinated fish during storage.

Aerobic mesophilic count (APC) of raw fish was found to be 3.23 log CFU/g. Aerobic mesophilic counts were found as 4.30 and 4.04 log CFU/g for the samples kept at 20°C and 0°C respectively. Keeping at ambient temperature caused a significant ($p<0.01$) increase in APC. While the psychotropic count of raw fish was found as 3.78 log CFU/g, for samples kept at 20°C and 0°C, it was determined as 3.59 and 3.82 log CFU/g, respectively. After marinating, microorganisms were not detected in marinated samples A and B. Marinating the fish inhibited microorganisms due to their salt and acid content. The antimicrobial effect of acetic acid, especially bacteria and yeast, has been reported (Sen & Temelli, 2003). In a previous study, microorganisms could not be isolated during the storage of marinated fish and it was reported that microorganisms were inhibited after marination (Erkan et al., 2000: Fuselli et al., 1994).

Sensory scores significantly ($p<0.01$) decreased after keeping at 20°C and 0°C. In sample A, a lower score was observed after 6 h at 20°C compared to sample B. Sensory scores of marinated fish decreased significantly ($p<0.01$) during storage (Fig. 6). The decrease in sensory scores was faster in sample A than in sample B. After storage, the sensory score of sample A reached the limit value of the deterioration. However, the score of example B was still within the limits of good quality.

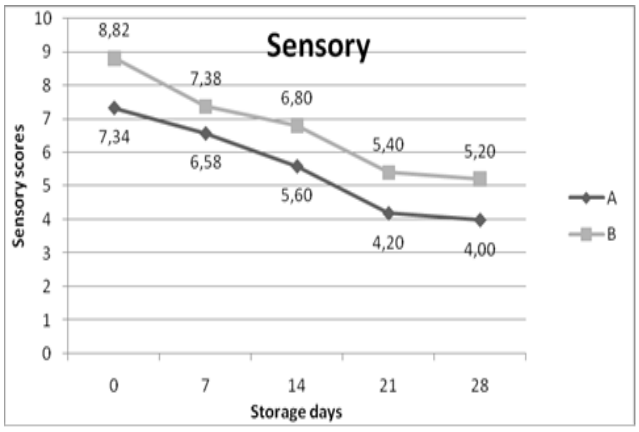

Figure 6. Sensory acceptability scores of marinated samples A (kept at 20°C) and B (kept at 0°C) during refrigerated storage.

4. Conclusions

The quality of fish products depends on the quality of raw material. Processing does not improve the quality of fish, protects only existing quality. It is accepted that organic acids used for marination extend the shelf-life of the product and provide safety. It is known that during marination, microbial activity is inhibited due to the combined activity of salt and acid solutions. Thus, it is believed that marination almost makes sterilization effect and therefore, the initial quality of fish is insignificant. The results of the present study clearly showed that the quality of raw material significantly affected the quality of the end product. Lower quality was observed in samples kept at 20°C during the storage. Especially lower sensory scores were found for this sample. For this reason, use of quality material should be a rule in fish processing operations.

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