

## Production and Quality Determination of Marinade from Different Fish Species

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**Abstract:** In this study the shelf life of bonito (*Sarda sarda*, Bloch 1793) and anchovy (*Engraulis encrasicolus*, Linnaeus 1758) marinades were investigated. Marinades from fresh bonito and anchovy were prepared and stored at +4°C. Shelf life of products was investigated by examining the biochemical composition, chemical and sensory quality during 170 days storage period. According to biochemical composition results, average crude protein values of fresh and marinated bonito and anchovy were 13.25±0.13-11.45±0.64%, 11.89±0.66-10.19±0.04%, average crude fat rates were 17.01±0.29-22.40±0.06%, 11.11±0.24-11.59±0.05%, average moisture rates were 62.45±1.28-59.07±0.07%, 72.04±0.11-70.75±0.19%, average crude ash rates were 3.87±0.04-4.76±0.01%, 2.14±0.07-5.12±0.02%, respectively. The average pH values of bonito and anchovy at the beginning and the end of the storage were in the defined limit values for marinated products. The average values of TMA and POS of fresh and during the storage were under the tolerable limits. The average TVB-N values of fresh bonito and anchovy were 12.13±0.47 mg/100 g, 7.47±0.47 mg/100 g and at the end of the storage they were 17.63±0.77 mg/100 g, 18.67±0.47 mg/100 g, respectively. The average TBA values of fresh bonito and anchovy were 2.16±0.50 mg malonaldehit kg<sup>-1</sup>, 1.45±0.23 mg malonaldehit kg<sup>-1</sup> and at the end of the storage they were 10.93±0.62 mg malonaldehit kg<sup>-1</sup>, 13.47±1.30 mg malonaldehit kg<sup>-1</sup>, respectively. According to result of the sensory analyses the shelf life of marinated bonito was 130 days and marinated anchovy was 155 days.

**Key words:** Marinade, bonito, anchovy, shelf life, proximate composition

### INTRODUCTION

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). 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. Its pro-oxidant activity is reported to accelerate the development of lipid oxidation in marinated and salted fatty fish products (Goulas and Kontominas, 2005; Kiliç and Çakli, 2004).

The marinating process is one of the oldest methods of conservation of fish popular in Europe. Fatty fish like sardines, mackerel, herring and anchovies, as well as several kinds of crustaceans and bivalves are usually used. In general, the shelf-life and safety of non-thermally treated marinated sea-food is due to the used kinds of organic acid, the NaCl concentration and the last pH value. It is well known that a pH 4.5 is enough to

guarantee a high long, despite, in some cases; the products have a strong acidic taste due to the low pH value (Giuffrida *et al.*, 2007).

The term marinades or marinated fish are used to name 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 inspired as ready-to-eat products with no heat treatment (Gram and 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 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. Keeping quality depend largely upon storage temperatures. Marinades are stored at cooler temperatures (4-6°C) keep for a long time (Clucas and Ward, 1991; Gökoglu *et al.*, 2004).

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Sensory methods, microbiological, chemical and biochemical 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 linked to the ability to set quantitative standards (Huss, 1995). The objective of the present research was to assess the chemical quality and sensory attributes, as well as to determine the shelf lives of marinated and brined during storage time at 4°C.

Anchovy and bonito are the most abundant species of fish in the Black Sea. The biomass value of anchovy and bonito are 270,000 tons, 29,690 tons, respectively (Anonymous, 2007).

According to this statistical data there is a possibility of developing new products with these species. One of the possible alternative products is marinade that anchovy marinated is being developed to be added to the supply of fishery products in Turkey.

Marinades are semipreserves; acid, usually acetic acid and salt are added to the fish to retard the action of bacteria and enzymes, resulting in a product with a characteristic flavour and an extended but restricted shelf life of 1-6 months in chilled storage 4°C (McLay, 1972). The preserving principle is the combination of acetic acid and salt. Marination is also used to tenderise or to change taste, textural and structural properties of raw material. Initial quality of raw materials, considering their freshness, microbiological load and physical damage are a significant factor which influences the quality of the end product (Fuselli *et al.*, 1994). Keeping qualities depend largely upon storage temperatures.

Anchovy and bonito are generally consumed as fresh, canned or salted. Generally anchovy are commercially used for marinated fish production and fish meal-oil, but usage of marinated bonito is not common in Turkey. Anchovy is very suitable for marination due to its high fat content. In this study, the usage of anchovy and bonito in marinade production were tried. The aim of the study was to produce anchovy and bonito marinade and to determine their shelf life at 4°C.

## MATERIALS AND METHODS

**Raw material:** Fresh bonito and anchovy were used for marination. Fish were purchased from fisherman in Sinop in December 2005. The mean weight and length of bonito and anchovy were 855±0.020 g, 42.509±0.322 cm, 9.780±0.415 g, 11.521±0.167 cm and 69±0.001 g, 39.741±0.259 cm, respectively. Fish were headed, gutted, filleted, washed and marinated.

**Chemical analysis:** For pH measurement (Orion research Model pH meter) 10 g fish meat samples were homogenised (Ultraturax T25 IKA, 7500 rpm) in 10 mL of distilled water

solution (Curran *et al.*, 1980). Total Volatile Bases (TVB-N, mg N/100g) trimethylamine (TMA, mg/100 g) and thiobarbituric acid (TBA, mg malonaldehyde kg<sup>-1</sup>) were analyzed according to Botta *et al.* (1984), AOAC (1990) and Tarladgis *et al.* (1960), respectively. Dry matter, crude ash and crude protein were determined using the method of AOAC (1984). Lipid was performed according to the method of Bligh and Dyer (1959).

**Sensory analysis:** Sensory analysis of two marinade groups was determined as described by Schormüller (1968). Before sensory assessment, samples were removed from the refrigerator and held for 30 min at room temperature. Bonito and anchovy marinades were served to the panelists to evaluate the sensory attributes (general appearance, colour, odour, texture, flavour) of marinades by using a scoring test of Schormüller (1968). The sensory assessment scale runs from 0-15.1. Sensory evaluations were conducted using six experienced panelists. Score 15 is the best quality, 14,9-13 second quality, 12,9-11 third quality, 10,9-6 fourth quality and a score of 6 or less corresponds to spoiled products.

**Statistical methods:** Data (chemical composition, chemical and sensory analysis) were analyzed by One-way Analysis of Variance (ANOVA) using the SPSS 10.0 for Windows. Significance of differences was defined at  $p = 0.05$ . Statistical comparison was based on 3 samples for each treatment for each specific storage time.

**Process methods:** Before starting this study, preliminary experiments with marination solutions in different concentrations were performed in order to determine the acceptable taste. After the taste tests, it was decided that the best tastes were obtained with one solution. This solution was selected for present study 4% acetic acid and 10% salt. Marination was done by immersing the fish fillets into solutions for 10 days. Fish fillets were packed in glass jars of 4% acetic acid and 10% salt, a fish: liquor ratio of 1:1. In addition to this some spices were added: 2 g red pepper, 2 g cumin, 2 g pepper, 3 garlic seed. Packed fish were stored at 4°C and analysed at every 30 days to determine the shelf life for 170 days.

## RESULTS AND DISCUSSION

In this research, the potential chemical quality indicators assessed to determine the chemical changes in marinated bonito and anchovy fillets during cold storage at 4°C were pH, TBA value, TMA and TVB-N contents. Table 1 shows the results of proximate composition of fresh and marinated anchovy fillets with and fresh and

**Table 1:** Proximate composition of fresh and marinated bonito and anchovy

Components (%)	Bonito		Anchovy	
	Raw	Marinated	Raw	Marinated
Lipids	17.01±0.29	22.40±0.06	11.11±0.24	11.59±0.05
Protein	13.25±0.13	11.45±0.64	11.89±0.66	10.19±0.04
Ash	3.87±0.04	4.76±0.01	2.14±0.07	5.12±0.02
Moisture	62.45±1.28	59.07±0.07	72.04±0.11	70.75±0.19

**Table 2:** Sensory score changes of marinated anchovy and bonito fillets stored at 4°C

Stored time (days)	Anchovy	Bonito
10	12.07±0.43	12.03±0.50
40	14.00±0.00	13.97±0.03
70	14.00±0.00	14.00±0.00
100	11.20±0.34	13.33±0.08
130	5.97±0.42	9.77±0.10
155	5.01±0.02	5.83±0.43

**Table 3:** Changes in pH and TVB-N (mg/100 g) of fresh and marinated samples stored 4°C

Stored time (day)	Anchovy (pH)	Bonito (pH)	Anchovy TVB-N (mg/100 g)	Bonito TVB-N (mg/100 g)
0	6.04±0.01	6.18±0.13	8.14±0.47	7.47±0.47
10	5.39±0.01	5.51±0.01	9.33±0.47	11.67±0.93
40	5.05±0.01	5.09±0.02	4.67±0.47	4.20±0.00
70	5.09±0.00	4.85±0.00	10.73±0.47	12.60±0.00
100	4.96±0.00	4.62±0.02	11.20±0.81	16.80±0.81
130	4.75±0.02	4.61±0.05	16.80±0.81	16.80±0.81
155	4.89±0.07	4.34±0.03	15.40±2.14	15.40±0.81
170	4.59±0.01	4.21±0.01	17.63±0.77	18.67±0.47

marinated anchovy fillets. According to statistical analysis results not significant differences ( $p>0.05$ ) between proximate compositions of fresh and marinated of these fillets were found.

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 and Chamul, 2000). In fact, the variation in the chemical composition of fish is closely connected 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 rightly applied (Murray and Burt, 1969).

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

Bonito and anchovy marinades were served to the panelists to evaluate the sensory attributes (appearance, colour, odour, texture, flavour) of marinades by using a

scoring test of Schormüller (1968). Sensory results of marinated bonito and anchovy fillets are presented in Table 2. No significant differences in the overall acceptability scores for 70 days were detected between marinated bonito and anchovy at 4°C ( $p>0.05$ ). However, significant differences ( $p<0.05$ ) were determined between marinated bonito and anchovy at 100 days.

The changes in pH, TVB-N (mg/100 g) of samples are shown in Table 3. The pH in fresh fish flesh is often between 6.0 and 6.5. In the post-mortem period, decomposition of nitrogenous compounds leads to an increase in pH in the fish flesh. The increase in pH indicates the loss of quality (Shenderyuk and Bykowski, 1990). Marinades have a low pH due to acetic acid content.

During the storage period pH value increased according to storage time, but pH value is not a criterion of spoilage. It has to be supported by other chemical and sensory analyses (Ludorff and Meyer, 1973; Varlik *et al.*, 1993). In this study, pH values in raw anchovy and bonito flesh were found 6.04 and 4.59 and 6.18 and 4.21, respectively at the end of the storage period. pH levels of marinated anchovy and bonito decrease from 5.39-4.59 and 5.51-4.21, respectively at the end of the storage period. There was a significant difference ( $p<0.05$ ) in pH between samples marinated during storage time.

The pH value in sardine found by El Marrakchi *et al.* (1990), Varlik (1994) and Gökoglu *et al.* (1998) were 5.83, 6.35, 6.2 and between 3.84-4.19, respectively. Poligne and Collignan (2000) determined that the pH levels of anchovies pickled with acetic acid increased from 3.90-4.21 after 20 days of storage and then remained constant until the end of the storage. Aksu *et al.* (1997) fixed that pH levels of anchovy marinated with 2 and 4% and stored at 4°C from 4.25 and 4.18-4.53 and 4.31, respectively. It was also reported that pH value in anchovy marinated 4% acetic acid changed from 3.89-3.95 during the storage of 8 months. These results were very similar to our findings on the increase of pH value during storage.

The TVB-N is used for the determination of the spoilage level of fish during storage (Kietzman *et al.*, 1969; Cobb and Vanderzant, 1975; Oehlenschläger, 1981; Yapar, 1999). During the storage of the marinades TVB-N values increased considerably in anchovy and bonito fillets. The initial TVB-N in raw bonito and anchovy fillets was found 8.14 and 7.47, respectively. Kiliç and Çakli (2004) determined a considerable decrease in TVB-N, from 10.3-6.5, after the marinating process of sardine fillets in a solution containing 7% acetic acid and 14% salt. During storage, increase in the TVB-N value was noticed by the days, 70, 100, 130, 155 and 170 for bonito and anchovy

fillets marinated. By the end of the storage period, a significant increase ( $p < 0.05$ ) in such values to relatively high levels of 17.63 and 18.67 mg/100 g were detected for fillets marinated bonito and anchovy, respectively but these marinated samples 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 indicating the significant effect of acetic acid in the reduction of chemical changes in marinated fish. Ludorff and Meyer (1973) reported an acceptability level of 35 mg TVB-N/100 g in sea fish. A rejection limit of 25 mg/100 g has been proposed for fresh water fish (Giménez *et al.*, 2002). Total volatile basic nitrogen content of fish is in general an indicator of the freshness. Yet, based on the results obtained in this study, TVB-N is a poor indicator of marinated fish freshness, as also proposed by the TVB-N value of 8.31 mg/100 g in anchovy marinated using 2% acetic acid increased to 15.18 mg/100 g, the TVB-N value of 7.79 mg/100 g in anchovy marinated using 4% acetic acid increased to 13.48 mg/100 g and the TVB-N value of 7.41 mg/100 g in anchovy marinated using 6% acetic acid increased to 12.34 mg/100 g after storage of 150 days (Aksu *et al.*, 1997).

A similar pattern of the increase in TVB-N has been reported in marinated sardine (Gökoglu *et al.*, 2004; Kiliç and Çakli, 2005) brined chub mackerel (Goulas and Kontominas, 2005) and brined anchovies (Karaçam *et al.*, 2002) during refrigerated storage. On the other hand, Pons-Sanchez-Cascado *et al.* (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. These findings are very similar to our findings.

Fresh fish naturally contain Trimethylamine Oxide (TMAO). TMAO is a flavourless 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 and Nychas, 1999; Özogul *et al.*, 2006). TMA-N results from the decrease of TMAO by bacterial activity and partly by intrinsic enzymes and is often used as an index of

freshness of marine fish. The quantity of TMA found in fish is used as an index of spoilage. In this study, TMA-N values of both samples marination not significantly increased ( $p > 0.05$ ) during the storage at 4°C. TMA-N values in marinated bonito fillets were higher than marinated anchovy fillets. A TMA value of 5-10 mg/100 g sample was reported as the limit for acceptability of fish (Sikorski *et al.*, 1989). In fresh fish, the TMA-N value is about 1 mg/100 g; in spoiled samples it is above 8 mg/100 g (FAO, 1986). The TMA-N value of raw bonito and anchovy materials were found to be 1.17 and 0.83 mg/100 g TMA-N, respectively. TMA-N levels of the samples were lower the limit level. According to variance analysis, there were no significant differences ( $p > 0.05$ ) in TMA-N values between the beginning and the end of the marination process. As it can be seen from Table 4, there were fluctuations at the TMA-N levels with samples stored at 4°C. Therefore, although the TMA-N test is commonly used in the determination of spoilage in fish products, it can be concluded that it may not be a reliable quality criterion for anchovies and bonito salted under the conditions used in this study. Kiliç and Çakli (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. Similar results were reported with marinated fish in the previous researches (Aksu *et al.*, 1997; Dokuzlu, 2000; Gökoglu *et al.*, 2004; Sallam *et al.*, 2007).

The changes in TMA-N (mg/100g) and TBA (mg malonaldehit kg<sup>-1</sup>) of samples are shown in Table 4.

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 (Olafsdottir *et al.*, 1997). Thiobarbituric acid values are a widely used indicator of the quality of the fish, whether it was frozen, chilled or stored with ice (Tarladgis *et al.*, 1960; Vareltzis *et al.*, 1988). It has been proposed that a maximum TBA value is 5 mg malonaldehyde kg<sup>-1</sup>, while the fish may be consumed up to a level of 8 mg malonaldehyde kg<sup>-1</sup> in TBA value (Schormüller, 1969). During storage, there was a tendency towards an increase in TBA values up to a maximal point. TBA value of fresh bonito and anchovy fillets was found

Table 4: Changes in TMA-N (mg/100 g) and TBA (mg malonaldehit kg<sup>-1</sup>) of fresh and marimated samples stored at 4°C

Time (days)	Ancovy (mg/100 g) TMA-N	Bonito TMA-N (mg/100 g)	Ancovy TBA (mg malonaldehit kg <sup>-1</sup> )	Bonito TBA (mg malonaldehit kg <sup>-1</sup> )
0	0.83±0.12	1.17±0.08	2.16±0.50	1.45±0.23
10	0.86±0.22	1.02±0.35	2.18±0.04	4.27±0.13
40	1.23±0.14	1.27±0.36	5.75±0.26	5.88±0.34
70	1.26±0.12	0.86±0.46	9.65±0.94	6.68±0.86
100	0.95±0.08	1.47±0.15	10.93±0.62	4.11±0.26
130	1.25±0.16	1.98±0.15	*	5.15±0.45
155	1.08±0.10	1.38±0.06	*	6.97±0.52
170	0.50±0.13	0.80±0.05	*	13.47±1.30

\*Not made analisess because of unconsumption

to be 1.45 and 2.16 mg malonaldehyde kg<sup>-1</sup>, respectively. After the treatment processes, TBA value of marinated anchovy fillets increased from 2.18 mg malonaldehyde kg<sup>-1</sup> to 10.93 mg malonaldehyde kg<sup>-1</sup> during at 100 days of storage. TBA value of marinated bonito fillets increased from 4.27 mg malonaldehyde kg<sup>-1</sup> to 13.47 mg malonaldehyde kg<sup>-1</sup> during 170 days at 4°C. According to statistical variance analysis, this increase was significant (p<0.05). The data obtained in the present study suggest that TBA values of marinated fish are within the good quality limits after 70 days of processing in barrels at 4 °C. The levels of TBA of this study were similar to that reported for various fish species that the TBA value increased to the maximal level at a certain period during storage (Yapar, 1998; Kilinç and Çakli, 2005; Sallam, 2007).

### CONCLUSION

This study concluded that marination of bonito and anchovy in solutions containing 10% NaCl + 4% acetic acid can extend the shelf life of the product during refrigerated storage 4°C; therefore, marination process can be used as a safe method for preservation of fatty fish. After storage of 130 days bonito marinated was found unconsumable according to the results of sensory analysis and 155 days anchovy marinated was found unconsumable according to the results of TBA values sensory analysis.

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