CHEMICAL AND MICROBIOLOGICAL QUALITIES OF DRY-SALTED (LAKERDA) BONITO (*SARDA SARDA*, BLOCH 1793)

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ABSTRACT

*In this study, changes in the chemical and microbiological properties of bonito (*Sarda sarda, *Bloch 1793) salted by dry salting (lakerda) and stored for 6 months at 4 ± 1C were investigated. The fresh gutted and sliced bonitos were used as raw material for salting by using the ratio of fish to salt as 5:1 by weight. Salted fish remained in the liquid pickle formed by salt and the liquid extracted from the fish. This solution was not drained during the storage period. The product is described as "lakerda" in Turkey. In the fresh fish, total volatile basic nitrogen (TVB-N) and trimethylamine nitrogen (TMA-N) values were 11.21 mg/100 g and 1.19 mg/100 g, respectively. During the storage period, TVB-N and TMA values increased slowly to 27.67 mg/100 g and 4.99 mg/100 g, respectively, at the end of the storage period. In the first month after salt processing, the total mesophilic bacteria count was* 4.6×10^2 *cfu/g. In the later months, mesophilic, psychotropic, coliform bacteria and yeast-mold did not reproduce. According to the results, bonito salted by dry salting and stored at 4 ± 1C was in good quality in terms of chemical and microbiological properties during the storage period of 6 months.*

INTRODUCTION

Salting is a fish preservation method based on the penetration of table salt into the tissues and governed by various physical and chemical factors such as diffusion, osmosis and a series of complicated chemical and biochemical processes associated with changes in various constituents of fish (Voskresensky 1965). Depending on the fish composition and size, salting may

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be "dry," where the fish are stacked in salt and the brine formed is allowed to run away, or "wet," where the fish are immersed in a strong brine, or "pickle." The most commonly employed method is a hybrid of the dry and wet methods; the fish are placed in dry salt and eventually become immersed in the liquid pickle formed by solution of the salt in the liquid extracted from the fish (Horner 1992).

Bonito has a limited fishing season, and in Turkey, most of the bonito caught are consumed as fresh or salted. Generally, the traditional salting methods are used in coast areas. Lakerda is one of the salted fish products in Turkey. Generally, lakerda is produced from bonito by dry salting and keeping in brine for several months.

Several studies have been reported on fish salting. Tömek *et al*. (1991) studied the effects of curing period and the use of an antioxidant mixture on the quality of lakerda, which is a kind of salted fish. They found that it is not possible to consume this product longer than 6 months because of the deterioration of the organoleptic properties. Yapar and Tömek (1991) investigated the effects of dry salting and brining methods on change of trimethylamine (TMA) values. Gökoğlu *et al*. (1994) studied the preservation time of trout salted in 15% brine and stored at 4C. They concluded that ripening process of salted fish was completed after 10 days of processing, and the ripened product could be stored in a consumable manner for 7 weeks. Turan and Erkoyuncu (1997) investigated the changes in the chemical and sensory properties of rainbow trout and salmon salted with dry and brine salting methods and at stored for period of 6 months. They found that the total volatile basic nitrogen (TVB-N) value increased 3–3.5 times in dry salting method, and 3–3.8 times in brine salting method. Gudmundsdottir and Stefansson (1997) studied the salt uptake and development of ripening characteristics in spice-salted herring fillets with those of partially gutted (nobbed) and completely gutted herring. They found that the rate of salt uptake was an important regulator of ripening during spice-salting of herring products. Yapar (1999) investigated quality changes in salted anchovy produced using 7.5, 10 and 15% salt concentrations. He found that an increase in salt concentration was effective in the preservation of the quality of the product. Hernandez-Herrero *et al*. (1999) studied TVB-N and other physicochemical and microbiological characteristics as related to the ripening of salted anchovies. Kongpun and Suwansakornkul (2000) investigated histamine formation during salting of Spanish mackerel (*Scomberomorous commerson*). Dabrowski *et al*. (2001) studied microflora of traditionally and vacuum-packed low-salt herring fillets qualitatively and quantitatively. Çelik and Gerek (2002) studied the chemical, physical and sensory changes in the fridge condition of Pike-Perch, pickled in various salt concentrations (15, 20 and 25%). They reported that the effects of both the concentration and duration in brine TVB-N were significant ($P < 0.01$). Karaçam *et al*. (2002) studied the effect of salt concentrations and temperature on the quality and shelf life of brined anchovies, and

the best quality was observed with the samples brined at 22 and 26% salt concentrations and stored at 4 ± 1C. Chouliara *et al*. (2004) studied the preservation of salted, vacuum-packaged, refrigerated sea bream (*Sparus aurata*) fillets by irradiation: microbiological, chemical and sensory attributes. Lauritzsen *et al*. (2004) investigated the effects of calcium, magnesium and pH during salt curing of cod (*Gadus morhua* L.). Martinez-Alvarez and Gomez-Guillen (2005) investigated the effect of brine composition and pH on the yield and nature of water-soluble proteins extractable from brined muscle of cod (*G. morhua*). Martinez-Alvarez *et al*. (2005) studied sodium replacement in cod (*G. morhua*) muscle salting process. Basti *et al*. (2006) investigated bacterial pathogens in fresh, smoked and salted Iranian fish. These studies used various salting procedures, salt concentrations and fish. Unfortunately, there is little information on the quality of salted bonito (lakerda), and it is very difficult to compare the results with each other. In the present study, the changes in the chemical and microbiological properties during a storage period of 180 days of the dry-salted (lakerda) bonito stored at 4 ± 1C were investigated.

MATERIALS AND METHODS

The bonito (*Sarda sarda*, Bloch 1793) used in this study were purchased immediately after landing from a commercial vessel. Then, the fish were transported to the laboratory in <30 min. The total weight of fish used in this study was 3500 g. The bonitos were immediately beheaded, gutted and washed in clean running water. The fish were soaked in 5% salted water to remove blood in fish meat. The fish were cut into 7-cm-thick slices. The slices were treated with a mixture of thin and thick granular salt. The salt ratio used in this process was 20% of cleaned fish. First, a little amount of salt was put on the bottom of jars (diameter = 13.2 cm, height = 24.5 cm, approximately 4-L capacity). The fish were rubbed with dry salt and placed in a jar. About 1750 g fish was placed in each of two glass jars. A weight was placed on salted fish in each jar in order to keep the fish below the surface of the brine. The fish remained in the liquid pickle formed by salt and the liquid extracted from the fish (Horner 1992). This solution was not drained during the storage period. The jars were stored at 4 ± 1C for 180 days. The study was carried out in duplicate. Samples were taken for chemical and microbiological analyses at monthly intervals.

Chemical Analyses

The TVB-N content was determined by the method of distillation (Ludorf and Meyer 1973). The method of Boland and Paige (1971) was used for trimethylamine nitrogen (TMA-N) analysis. Each analysis was conducted in duplicate.

Microbiological Counts

Microbiological counts were made on samples of muscle. Ten grams of sample was taken aseptically into a sterile blender containing 90 mL of sterile peptone water (PW) (0.1% w/v) and blended for 2 min at low speed. Further decimal dilutions were prepared in PW. The counts of total aerobic mesophilic and psychrotrophic microorganisms were determined by using plate count agar (PCA) incubated at 35 ± 1C for 48 h for mesophilic microorganisms and at 4 ± 1C for 10–12 days for psychrotrophs. For halophilic bacterial counts, decimal serial dilutions were prepared with the homogenizing solutions including 15% NaCl at the first months and 20% NaCl in the later months. They were plated onto standard PCA containing NaCl in duplicate and incubated at 35 ± 1C for 2 days for mesophilic and at 4 ± 1C for 10–12 days for psychrotrophs. The counts of coliform microorganisms were determined by using violet red bile agar incubated at 35 ± 1C for 24–48 h (Göktan 1990). The counts of yeast-mold were determined by using potato dextrose agar incubated at 35 ± 1C for 72 h.

RESULTS AND DISCUSSION

In this study, changes in the chemical and microbiological properties (during the storage period of 6 months) of bonito (*S. sarda*, Bloch 1793) salted by dry salting (lakerda) and stored at 4 ± 1C were investigated.

Table 1 shows the changes in TVB-N and TMA values during the storage period of dry-salted bonito, while Table 2 shows the TVB-N and TMA levels in other studies.

According to the TVB-N results shown in Table 1, a strong relation between the TVB-N value and the storage period ($y = 3.9706\text{Ln}(x) + 20.121$,

TABLE 1.
CHANGES IN TOTAL VOLATILE BASIC NITROGEN (TVB-N) AND TRIMETHYLAMINE NITROGEN (TMA-N) VALUES OF DRY-SALTED BONITO

Storage period (months)	TVB-N (mg/100 g)	TMA-N (mg/100 g)
0	11.21 ± 0.00	1.19 ± 0.48
1	19.23 ± 0.35	3.59 ± 0.50
2	23.69 ± 0.56	3.86 ± 0.08
3	24.43 ± 0.37	4.43 ± 0.06
4	25.12 ± 0.56	4.04 ± 0.05
5	26.47 ± 0.56	4.96 ± 0.24
6	27.67 ± 1.20	4.99 ± 0.17

$n = 4$; (±) represents standard error.

TABLE 2.
TOTAL VOLATILE BASIC NITROGEN (TVB-N) AND TRIMETHYLAMINE NITROGEN (TMA-N) LEVELS FOUND IN OTHER STUDIES

Product	Storage time and temperature	TVB-N (mg/100 g)	TMA-N (mg/100 g)	Reference
Dry-salted (rainbow trout)	165 days, 4C	12.61	–	Turan and Erkoyuncu 1997
Dry-salted (salmon)	165 days, 4C	14.71	–	Turan and Erkoyuncu 1997
Dry-salted (3 days), then was soaked in 15% brined (trout)	49 days, 4C	38.00	–	Gökoğlu *et al*. 1994
Dry-salted (anchovy)	9 weeks, 20C	35.64	–	Hernandez-Herrero *et al*. 1999
7.5, 10, 15% dry-salted (anchovy)	10 weeks, 4C	–	19.23, 4.23, 3.85	Yapar 1999
12, 15, 20% dry-salted (trout)	150 days, 5C	–	27.53, 14.71, 12.77	Yapar and Tömek 1991

$r = 0.99$) was determined. The initial TVB-N value in fresh fish was 11.21 mg/100 g fish. TVB-N values increased to 27.67 mg/100 g fish during the period of storage as a result of decomposition of nitrogenous substances. The rate of increase was higher at first months than the later times in which a gradual increase was observed throughout storage. TVB-N value is an important parameter for determining the freshness of fish products. TVB-N value is affected by species, catching region and season, age and sex of fish (Gökoğlu *et al*. 1998). Ludorff and Meyer (1973) regarded a TVB-N content of 25 mg N/100 g as very good, 30 mg N/100 g as good, 35 mg N/100 g as marketable and TVB-N values over 35 mg N/100 g as spoiled in seafood. In our study, the highest TVB-N value determined was below this spoilage level. Khuntia *et al*. (1994) reported that salted pink perch was found to have 1.6 times longer shelf life than the fatty mackerel at both ambient (26.8C) as well as cooler (2.5C) temperatures. In the rainbow trout salted with dry salting (25%) method and stored at 4 ± 1C for 165 days, the TVB-N content was 12.61 at the end of the storage period. In the same study, in the salmon salted with dry salting (25%) method and stored at 4 ± 1C for 165 days, the TVB-N content was 14.71 mg/100 g (Turan and Erkoyuncu 1997). The difference in TVB-N results may be attributed to the different fish used. Gökoğlu *et al*. (1994) reported that in trout salted with dry salt for 3 days, soaked in 15% brine and stored at 4C, the TVB-N value increased gradually from 2.2 to 38.0 mg/100 g during a research period of 49 days. The authors reported that ripening process of salted fish was

completed at the 10th day of processing, and the ripened product could be stored in a consumable manner for 7 weeks. A similar result was also found by Hernandez-Herrero *et al*. (1999) in which the TVB-N increased linearly in fish muscle and brine during ripening. Karaçam *et al*. (2002) found that the storage period and temperatures increased the level of TVB-N of anchovies brined significantly ($P < 0.001$). They also found the best quality with the samples that were brined at 22 and 26% salt concentrations, and stored at 4 ± 1C.

TMA-N, which is one of the spoilage products of spoiling fish, has a typical fishy odor (Huss 1995). TMA results from the reduction of trimethylamine oxide by bacterial activity and, possibly, partly from intrinsic enzymes and is often used as an index of freshness of marine fish (Gökoğlu *et al*. 1998). The level of TMA-N found in fresh fish rejected by sensory panels varies between fish species, but is typically around 10–15 mg TMA-N/100 g in aerobically stored fish (Huss 1995). A level of 8 mg TMA-N/100 g fish is generally regarded as the limit of acceptability for human consumption (Varlık *et al*. 1993).

According to the TMA-N results shown in Table 1, a strong relation was determined between the TMA-N value and storage period ($y = 0.8924\text{Ln}(x) + 3.3195$, $r = 0.98$). The amount of TMA-N in fresh fish was 1.19 mg/100 g. The TMA-N contents increased gradually to 4.99 mg/100 g fish during the period of storage as a result of decomposition of nitrogenous substances. However, the TMA-N value found after 6 months of storage was below the level of spoilage (>8 mg/100 g). In a study with anchovy salted at 7.5, 10 and 15% salt concentrations and stored for 10 weeks at 4 ± 1C, TMA-N values were 19.23, 4.23 and 2.32 mg/100 g, respectively (Yapar 1999). In another study, Yapar and Tömek (1991) found that TMA-N increases slower in dry-salted trout than brined. They also showed that the TMA-N values of trout salted at 12, 15 and 20% salt concentrations and stored for 150 days, were 27.53, 14.71 and 12.77 mg/100 g, respectively.

Mesophilic and psychrotrophic bacterial counts were $<10^1$, although the coliform, yeast and mould were not found at the start of the experiment. After salting, the mesophilic bacteria count was 4.6×10^2 cfu/g for first month. This may have occurred because of salt or any contamination during salting process. At other months, the mesophilic bacteria count was $<10^1$. During the storage period, psychrotrophic, coliform, yeast and mold did not appear. In a study reported by Karaçam *et al*. (2002) on anchovy brined at 14, 18, 22 and 26% salt concentration and stored at refrigerator temperature for 150 days, the psychrotrophic bacteria count was 3.47 log cfu/g at the 30th day for 26%; the mesophilic bacteria count was 3.92 log cfu/g at the 60th day for 26% salt concentration. They found that the salt concentrations had a significant ($P < 0.05$) effect on bacterial change, excluding the concentrations between 14 and 18%, and 22 and 26%. They also found that halophile bacteria did not

appear on anchovy brined at 22 and 26% salt concentrations. In a study reported by Hernandez-Herrero *et al*. (1999) on anchovies stored at 20C for 9 weeks, psychrotrophic bacteria and slight and moderate halophilic/halotolerant bacteria counts decreased appreciably ($P < 0.05$) during the first 2 weeks, and thereafter the counts decreased gradually. They found that the extremely halophilic counts showed an increasing trend during ripening from 0.22 to 4.9 log cfu/g. Basti *et al*. (2006) also found that *Coliforms* in heavy-salted fish were negative.

CONCLUSION

According to the TVB-N, TMA and microbiological results, bonito (lakerda) salted by dry salting and stored in brine at refrigerator temperature (4 ± 1C) for 6 months can be safely consumed.

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