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Effect of brine and dry salting methods on the physicochemical and microbial quality of chub (*Squalius cephalus* Linnaeus, 1758)

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Abstract

The present study, looks at the physicochemical and microbiological quality changes that occur due to different salting techniques (20% salt concentration) of chub (*Squalius cephalus*) and when stored in 4 ± 0.5 °C. Samples of fish from each group was taken on the 15, 30, 45, 60, 90 and 120th day and was analyzed for nutritional component (crude protein, lipid, moisture, crude ash), pH value, salt content and the microbial flora (total mesophilic aerobic bacteria count, total coliform, total psychrophilic aerobic bacteria count, yeast and mould). It was determined that crude protein, lipid, crude ash and salt amounts in the group where dry salting method was applied were higher than the group where brine salting occurred, in addition protein and lipid values decreased as storage period was longer ($P < 0.05$). It was determined that there is an increase in total aerobic mesophilic, psycrophile bacteria and enumeration of yeast and mould as storage period increased, while coliform bacteria decreased ($P < 0.05$).

Keywords: chub; *squalius cephalus*; quality properties; brine salting; dry salting.

Practical Application: Quality properties of salted chub during storage at 4 ± 0.5 °C

1 Introduction

Salting is one of the oldest food preservation methods. Salting is a process where the common salt (NaCl), sodium chloride, is used as a preservative that penetrates the tissue; hence slows the bacterial growth and deactivates the enzymes. Some of the factors involved in salting of fish which play important role are purity of salt, quantify of salt used, method of salting, and weather conditions, flavor of the product.

In Turkey fish is usually consumed fresh while a small amount is processed in different ways. One of these is salting. Dry salting, brine salting and mixed salting are commonly used methods in salting of fish. Salting of seafood is done with salt. The main purpose of salting is to separate water from the fish and replace it with salt. Thus, the water concertation in fish decreases. Chlorine and sodium ions are carried from brine to fish, and water dipoles are carried from fish to the environment. The rate during this process is high, and it slows during ripening. The determinant factor in this process is the concentration of salt. Moreover, the size and thickness of the fish, whether its skin and scales are removed or not, whether the fish is in the period of rigor mortis or not, the freshness of fish and purity degree of salt are other important factors (Merritt, 1988; Kolsarici & Candoğan, 1997). In salting process, there are 2 stages salting and ripening. The ripening of salted fishes is a biochemical process. During this period, biochemical changes occur in the tissue of fish, and protein and fat enzymes break up which cause these changes (Voskresensky, 1965).

In Turkey, consumption of salted fish is not widespread; however the brined form of *Cyprinids* (*Vimba vimba*, *Capoeta pestai*, and *Carassius carassius*), anchovy (*Engraulis encrasicolus*) and sardine (*Sardina pilchardus*) does occur, and is being consumed with pleasure (Göğüş & Kolsarıcı, 1992).

The aim of this study was to determine the effects of brine and dry salting methods on the nutritional composition of chub and the changes on microbial that arise during storage.

2 Materials and methods

2.1 Material

Fresh chub (*Squalius cephalus* Linnaeus, 1758) was used for salting. The samples that make up the material of this study were actively hunted from Atatürk Dam, Bozova Gülbiten Village coast with a net having mesh size of 18 mm, 50 m depth, 150 m from the shore.10 kg chubs length varying from 15 to 19 cm, weight varying from 51 to 52 g was used in the study. The samples were iced right away, and then transported to laboratory within a field type cooler. Afterwards the heads and fins of the fish were cut and their gills, scales and internal organs were removed and finally washed with water.

2.2 Salting process

The samples were divided into two groups, and dry salting method was applied to one of the groups and brine salting method to the other. The fish, on which both salting technologies were applied, were stored for 120 days at 4 ± 0.5 °C.

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2.3 Brine salting

In the brine salting method, the cleaned fish were placed inside plastic jars of 5 liters, and brine was added until the fish was completely covered and brined in 20% salt solution, fish to salt solution ratio is 1:1. Medium sized dry and clean table salt purchased from the market was used in this process.

2.4 Dry salting

An amount of salt was put on the bottom of plastic jars of 5 liters. The fish were rubbed with dry salt and placed in the jars. The concentration of salt was adjusted to be 20% of the fish's weight (fish weight: salt weight [5:1]). In this method salt having the same properties in brine salting was used.

2.5 Analysis

For the first two months, analyses of these samples were performed once every 15 days, and then they were performed on a monthly basis after that 60th, 90th and 120th days. During each analysis period, 5 fish were chosen randomly from each group among the samples stored in the refrigerator, and then their fillets were drained and homogenized prior to analyses excluding the microbial analyses.

Proximate analysis

To determine the crude protein amount in the fish samples the EN ISO16634-1 method was used (Müller, 2014), lipid amount was determined using ISO 1443:1973 method (International Organization for Standardization, 1973) moisture was determined using Ludorff & Meyer (1973) method and crude ash content was determined using the burning method (Association of Official Analytical Chemists, 2000).

Physicochemical analysis

Samples of fish flesh (10 g) were homogenized in sterile blenders with 10 mL of distilled water. pH value was measured using (INOLAB pH 7310 brand) pH meter at 16 ± 1 °C (Association of Official Analytical Chemists, 2000). Salt amount was determined using the Mohr method and resisting the titration with 0.1 N $AgNO_3$ accompanied by K_2CrO_4 indicator (Karl, 1994).

Microbiological analysis

Plate Count Agar (PCA, Merck, 1.05463) feedlot was used to get the total mesophilic aerobic bacteria count (TMBC) and total psychrophilic aerobic bacteria count (TPBC), 25 g sample was weighted using 225 ml sterile peptone water which was mixed in the stomacher (Mayo, HG 400V, Italy) and made homogenous. A diluent of 10^{-1} was prepared in this manner, and other sub-dilutions were prepared from that dilution. 1 ml was taken from the prepared dilutions, and 3 parallel inoculations were performed using the pour plate method. TMBC was left in incubation for 72 hours at 30 ± 1 °C (International Organization for Standardization, 2003). And **T**PBC was incubated for 10 days at 7 ± 1 °C (International Organization for Standardization, 2003). Tempo (Biomerieux TEMPO® TC, 80 006) (Biomerieux, 2011) device is used to determine total coliform count (TC) group bacteria in the sample groups.

The computer program Tempo preparation unit was used to record sample information and recent dilution rate using the device and through the window by pressing F8 key. The cards used for the analysis were read by the barcode reader. Bacteria number was given by the Tempo device as log cfu/g. Tempo yeasts-moulds is an automatic test done by the Tempo device and it counts yeast and mould in 72-76 hours at 25 °C. The method used for analysis is the same that was used for coliform bacteria analysis, just the incubation was for 5 days at 20 °C temperature (Biomerieux TEMPO® YM, 80 001) (Biomerieux, 2013).

2.6 Statistical analysis

Statistical analysis was performed using "SPSS 18.0 for Windows software". Difference in the means between the groups was analyzed using T test. Duncan's multiple range tests were applied in order to do multiple means comparison. Statistical significance was set at $p < 0.05$.

3 Results and discussion

The changes in the nutrient composition of chub used in the study was examined in the raw material and salted products in storage, and the results obtained from the research are given in Table 1. When the crude protein values of dry and brine salting groups were compared, the crude protein rate of dry salting (DS) was higher than for brine salting (BS) method, and

Table 1. Proximate and physicochemical analysis of chub samples.

Storage time (day)	Crude Protein (%)		Lipid (%)		Moisture (%)		Crude Ash (%)		pH		Salt (%)	
	DS	BS	DS	BS	DS	BS	DS	BS	DS	BS	DS	BS
Raw material	20 ± 1^{abc}	20 ± 1^{b}	7 ± 0^{a}	7 ± 0^{c}	75 ± 1^{d}	75 ± 1^{bc}	2 ± 0^{a}	2 ± 0^{a}	7 ± 0^{a}	7 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}
15	21 ± 0^{bc}	15 ± 6^{a}	6 ± 0^{a}	7 ± 1^{bc}	62 ± 2^{c}	$77 \pm 2^{c*}$	11 ± 1^{b}	$2 \pm 1^{b*}$	6 ± 1^{a}	7 ± 1^{a}	21 ± 1^{d}	$10 \pm 1^{c*}$
30	21 ± 1^{bc}	$13 \pm 1^{a*}$	6 ± 1^{a}	4 ± 1^{a}	60 ± 2^{bc}	$74 \pm 2^{ab*}$	13 ± 1^{cd}	$9 \pm 1^{b*}$	6 ± 1^{a}	6 ± 1^{a}	17 ± 2^{c}	$9 \pm 1^{bc*}$
45	22 ± 2^{c}	$15 \pm 1^{a*}$	6 ± 1^{a}	4 ± 1^{a}	59 ± 2^{bc}	$73 \pm 2^{ab*}$	13 ± 1^{c}	$8 \pm 1^{b*}$	6 ± 1^{a}	7 ± 1^{a}	13 ± 2^{b}	$7 \pm 1^{b*}$
60	19 ± 1^{ab}	$13 \pm 2^{a*}$	7 ± 2^{a}	5 ± 1^{ab}	61 ± 1^{c}	$78 \pm 2^{c*}$	13 ± 1^{cd}	$4 \pm 1^{b*}$	6 ± 1^{a}	7 ± 1^{a}	15 ± 1^{bc}	$8 \pm 2^{b*}$
90	18 ± 1^{a}	$12 \pm 1^{a*}$	6 ± 2^{a}	5 ± 1^{ab}	56 ± 1^{a}	$71 \pm 2^{a*}$	20 ± 2^{d}	$12 \pm 1^{b*}$	6 ± 1^{a}	7 ± 1^{a}	16 ± 1^{bc}	$8 \pm 1^{bc*}$
120	18 ± 1^{a}	$11 \pm 1^{a*}$	5 ± 1^{a}	4 ± 1^{a}	57 ± 2^{ab}	$75 \pm 2^{bc*}$	19 ± 2^{cd}	$10 \pm 2^{b*}$	6 ± 0^{a}	8 ± 1^{a}	15 ± 2^{bc}	$9 \pm 1^{bc*}$

n = 3; (mean value ± standard deviation), DS: Dry salted, BS: Brine salted, $P < 0.05$. (*) shows statistical differences of "Two-tailed Independent T-test" between two different applications (DS or BS) of same parameters (crude protein, lipid, moisture, crude ash, pH, and NaCl) in same line. Different letters (a-d) in same column show statistical differences of "Duncan Multiply Range Test" among different storage times (0-120 days) for same application (DS or BS) and parameter (crude protein, lipid, moisture, crude ash, pH, and NaCl).

the difference between the groups was statistically significant ($P < 0.05$). The reason for the protein rate being higher in dry salted samples compared to brine salted samples is water loss, and it may be due to increase of dry matter and protein. When the two groups are assessed internally, it was determined that there is a statistically significant relation between the protein value in BS group and the days, and in DS and the days ($P < 0.05$). In a study performed by Bilgin et al. (2007) on salted products, the protein rate in dry salted *Salmo trutta magrostigma* species was lower than its brine samples. This may be due to difference in specie being used. In a study performed on salted pearl mullet *Chalcalburnus tarichi*, it was determined that the high rate of protein was in salted products when compared to fresh fish (Kılınççeker & Küçüköner, 2003). Al-Asous & Al-Harbı (2016) discovered that protein value of the salted bluespot mullet increased throughout the storing process. In a study done by Yapar (1999), he noted that the protein rate decreases the longer the product was in storage both in the brine and dry salting methods applied to *O. mykiss*, but the decrease was higher in the brine group. In another research similar to this study, a decrease in protein amount was determined by using different salting techniques that were applied to *V. vimba tenella* (Işıklı, 2000). Pourashouri et al. (2015) and Latifa et al. (2014) found that the protein was lower in salted fish when compared to when the fish was fresh. While it was determined that high salt concentration causes protein loss, however in this study high salt concentration doesn't cause protein loss in dry salting method only in in brine (Kolsarici & Candoğan, 1997). The reason for this may be due to the fish species being used, the salt amount, difference of salting methods and storage period.

In our study, the lipid values were 7 ± 0% and the raw material gradually decreased in both salting methods, and on the 120th day, they had decreased to 5 ± 1% in dry salted group and to 4±1% in brine salted group. According to the statistical assessment performed, it was determined that the difference between two groups when it comes to lipid values was significant ($P < 0.05$). In dry salting method, the loss in lipid amount was less. There was no statistically significant found between the lipid values in BS group and the storage days, and same can be said of DS group and the days ($P > 0.05$). The results obtained in this study shown similarity with the results in Tömek & Yapar (1990), Turan & Erkoyuncu (1997), and Ürküt & Yurdagel (1985). In a study performed by Bilgin et al. (2007) on salted products, the total lipid content was found to be 2.551 ± 0.157% in fresh fish, and on the 180th day of storage, this value decreased to 1.332 ± 0.119% in dry salted samples and to 1.039 ± 0.030% in brine salted samples. Our results are in agreement with the results from this research. That the reason for the decrease in the lipid content in salted products may depend on the draining and removal of fat from the fish's tissue and as result of compaction of tissue due to osmotic pressure and denaturation of protein caused by salt (Yapar, 1989). While the moisture rate was determined to be 75 ± 1% in raw material, it increased to 77 ± 2% in samples treated with brine salting in the first 15 days, however it decreased to 62 ± 2% in samples treated with dry salting method. At the end of 120th day, decrease in moisture rates was observed and recorded as 75 ± 2% in brine salting group and 57 ± 2% in the dry salting group. It was determined that the moisture loss in dry salting was higher compared to brine salting. According to the statistical assessment performed, it was determined that the difference between two groups with respect to moisture values was significant ($P < 0.05$). While statistically significant relation was observed between the moisture value and the days in BS group ($P < 0.05$), there was no such statistically significant between the % moisture value and the days in the group where dry salting was applied ($P > 0.05$). Beyond this during the salting process, outrun of water occurs due to the penetration of the salt in the muscular tissues of the fish, and therefore dry matter rate increases while humidity rate decreases along with water outrun. An increase of 15.57% of dry matter in the sardine fish (*S. pilchardus*) stored for 10 months after being salted with 20% concentration of salt was observed. This means there was a decrease of moisture content (Ürküt & Yurdagel, 1985). Some researchers (Turan & Erkoyuncu 1997) have determined that the dry matter amount in *O. mykiss* and *S. salar* which were salted using different salting methods increased during storage period at 4 °C when compared to the initial values. The obtained results support our results.

According to statistical assessment performed for the two groups with respect to ash values, it was determined that the difference was significant ($P < 0.05$). Ash amount was 2 ± 0% in raw material, however there were increases and decreases during the storage period for both groups, and by the end of 200th day, it reached 10 ± 2% in samples treated with brine while it was 19 ± 2% in samples treated with dry salting. Despite not being able to determine a statistically significant relation between the ash value and the days in BS group ($P > 0.05$), it was determined that there was a statistically significant relation between the ash value and the days in DS group ($P < 0.05$).

It is known that inorganic matter increases in salted fish and it depends on preservation period and salt rate. Inorganic matter and salt rates increase along the storage period in anchovy stored that had been salt cured and were in storage for 29 weeks, and that this condition affects other components (Kolsarici & Candoğan, 1997). The obtained findings support our study.

The pH value is used as an indicator of degree of freshness or spoilage of a fish. In fresh fish pH is close to neutral, first it decreases due to lactic acid arising from death, however then it increases because of deterioration. The reason for this increase is the disruption to the oxidation-reduction balance along with the effect of enzymes and bacteria, and the changes in the concentration of free hydrogen and hydroxyl ions (Işıklı, 2000; Varlık et al., 1993). The increase in pH indicates the loss of quality (Latifa et al., 2014). In our study, the pH value started with 7 ± 0 in fresh fish and it was 6 ± 0 in the 120th day in the fish treated with DS and 7 ± 1 for the fish treated with BS. Therefore it was observed that there is no regular increase or decrease in the pH measurements, and it was determined that there was a statistically significant relation between the pH values in DS and BS groups ($P < 0.05$). It was also observed that there was no statistically significant relation between the pH value in BS group and days in storage, and same can be said for the DS group ($P > 0.05$). In a study performed on a vimba vimba fish (*V. vimba tenella*), it was determined that the pH value decreased to 6.85 from 6.99 on the 7th day, and then increased

to 7.37 for samples which used brine method, and that these values changed to 6.61 for (DS) and 6.71 for (BS) after being in storage for 118 days (Işıklı, 2000). Therefore it exhibited an irregular change during the preservation period. Another study performed using salted trout, showed that the pH value of the samples which were brine salted was higher when compared to dry salted samples (Bilgin et al., 2007), and this supports the results of our study.

In the research, according to statistical assessment made in respect to % salt amounts recorded along the storage period, it was determined that there was a statistically significant relation between the salt values used in dry salted and brine salted groups ($P < 0.05$). On other hand it was determined that there was no statistically significant relation between the salt values used in the group where the BS was applied and storage days ($P > 0.05$), and that there was statistically significant relation in between the salt values of the group to which DS was applied and the days ($P < 0.05$). The salt amount measured in raw material was (0 ± 0%) and to be 15 ± 2% in the DS group and 9 ± 1% in BS group by the 120th day in storage. The results are similar to the results found in Yapar (1989) study in which the salt rate was determined to be 15.74% in dry salted trout and 11.74% in brine salted trout. Turan & Erkoyuncu (1997) have discovered that salt penetration in the fish depends on fat content of fish, and that the process is slower in fatty fish and that it is higher in dry salting compared to brine. Tömek & Yapar (1990) revealed in their study on trout that there is an increase in salt penetration along with the increase in preservation period. The obtained results are in agreement with the results other researches have been able to obtain so far.

During the study, two types of salting methods were applied, and microbial changes were measured in chubs and a storage period was given all this date can be seen in Table 2.

The total mesophilic aerobic bacteria count (TMBC), was determined to be 5 ± 0 log cfu/g in raw material and 7 ± 0 log cfu/g in the DS group and 7 ± 0 log cfu/g in the BS group by the end of 120 days of storage. When it came to changes in viable bacteria number, a statistically significant relation was determined to exist between the DS and BS groups ($P < 0.05$).

While a decrease in bacterial development was expected due to the effect of salt, what actually occurred is a significant increase was observed in the number of total viable bacteria and it depended on the ripening period. The reason for this bacterial increase could be that the bacteria adapted to the environment and the skin that was not removed became a microbial source during the process. Todorov (1975) research shows that the total number of bacteria increases along the ripening period. Küçüköner & Akyüz (1992) found $7.5x10^5$-$7.5x10^6$ cfu/g, and Nino de Onshuus (1974) between $6.5x10^6$-$1.67x10^8$ cfu/g. Patır et al. (2006) reported that the total mesophilic aerobic bacterial count in salted grey mullet (*Chalcalburnus tarichii*) stored at 4 ± 1 °C ranged from 2.0 to 5.0 log cfu/g, with average 3.94 log cfu/g. Al-Asous & Al-Harbı (2016) reported that the total mesophilic viable count in salted wild mullet (*Valamugil seheli*) ranged from 3.62 to 4.15 cfu/g. The data determined in our study and the data determined in these studies shows agreement.

While total psycrophile bacteria count was determined to be 6 ± 0 log cfu/g in raw material, by the 120th day in storage it was determined to be 6 ± 0 log cfu/g in the group treated with DS and as 7 ± 0 log cfu/g in the group treated with BS. The change of psycrophile bacteria that occurred had a statistically significant relation between the two groups ($P < 0.05$) Al-Asous & Al-Harbı (2016) reported that the total psychotropic viable counts in salted fish stored in a refrigerator ranged from 3.43 ± 0.34 to 4.49 ± 0.52 log cfu/g.

When it came to changes in yeast-mould count, it was determined that there was a statistically significant relation between raw material and DS and BS groups ($P < 0.05$). The yeast-mould numbers were 3 ± 0 log cfu/g in the fresh fish however it varied along the storage period, and by the end of 120th day, the number was 3 ± 0 log cfu/g in DS group and as 3 ± 0 log cfu/g in BS group.

Depending on raw material and storage period, it was determined that there was no statistically significant relation between groups regarding coliform bacteria numbers in products to which salting was applied to ($P > 0.05$). Coliform group bacteria was 2 ± 0 log cfu/g in raw material and it reached 5 ± 0 log cfu/g on the 15th day in the group treated with dry salt, and below detectable level (<10 cfu/g) in both groups in the remaining

Table 2. The microbial flora of salted chub during storage at 4 ± 0.5 °C (log cfu/g).

Storage time (day)	According to the groups							
	TMBC		TPBC		Yeasts-Moulds		TC	
	DS	BS	DS	BS	DS	BS	DS	BS
Raw material	5 ± 0^{a}	5 ± 0^{a}	6 ± 0^{a}	6 ± 0^{a}	3 ± 0^{e}	3 ± 0^{a}	2 ± 0^{b}	2 ± 0^{b}
15	6 ± 0^{c}	$8 \pm 0^{d\star}$	6 ± 0^{b}	$8 \pm 0^{d\star}$	1 ± 0^{b}	$4 \pm 0^{ab\star}$	5 ± 0^{c}	$<10^{a\star}$
30	6 ± 0^{d}	$8 \pm 0^{d\star}$	7 ± 0^{d}	$8 \pm 0^{e\star}$	$<10^{a}$	$3 \pm 0^{a\star}$	$<10^{a}$	$<10^{a}$
45	6 ± 0^{b}	$8 \pm 0^{e\star}$	6 ± 0^{c}	$8 \pm 0^{f\star}$	2 ± 0^{c}	$5 \pm 0^{c\star}$	$<10^{a}$	$<10^{a}$
60	7 ± 0^{d}	$8 \pm 0^{f\star}$	6 ± 0^{d}	$9 \pm 0^{g\star}$	3 ± 0^{e}	$4 \pm 0^{b\star}$	$<10^{a}$	$<10^{a}$
90	7 ± 0^{e}	$7 \pm 0^{c\star}$	7 ± 0^{e}	$8 \pm 0^{c\star}$	7 ± 0^{f}	$5 \pm 0^{c\star}$	$<10^{a}$	$<10^{a}$
120	7 ± 0^{e}	7 ± 0^{b}	6 ± 0^{a}	$7 \pm 0^{b\star}$	3 ± 0^{d}	$3 \pm 0^{a\star}$	$<10^{a}$	$<10^{a}$

n = 3; (mean value ± standard deviation) DS: Dry salted BS: Brine salted, $P < 0.05$. *shows statistical differences of "Two-tailed Independent T-test" between two different applications (DS and BS) of same parameters (TMBC, TPBC, YM and TC) in same line. Different letters (a-g) in same column show statistical differences of "Duncan Multiply Range Test" among different storage times (0-120 days) for same application (DS or BS) and parameter (TMBC, TPBC, YM and TC).

days in storage. Todorov (1975) research shows that coliform was found on the surface of Atlantic mackerel however it could not be found in the fish. The reason for the difference between our research and ours is the fish species, salting techniques, and storage period and storage temperature.

4 Conclusions

According to the obtained results, the quality was lower in fish treated with brine salting method, and pH value and microbial values (TMBC, TPBC, Yeasts-Moulds) were higher when compared to dry salting. A way to fix this may be to use a salt solution with higher concentration in order to increase lasting period of brine products. In the light of the findings obtained in this study, it was concluded that *Squalius cephalus* is a species more suitable for dry salting method. But chub is a fish with high fat rate, and using a fish with a lower fat concentration would give better results in salting process.

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