



RESEARCH ARTICLE

Monitoring physicochemical attributes and nutritional alterations in lakerda during early-stage and intermediate maturation periods: A preliminary research

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ARTICLE INFO

Article History:

Received: 02.08.2025

Received in revised form: 16.09.2025

Accepted: 20.09.2025

Available online: 30.09.2025

Keywords:

Atlantic bonito

Color

Lakerda

Proximate

Salted fish

TBARs

ABSTRACT

The present study examined several chemical, physical and physicochemical alterations in lakerda made from Atlantic bonito (*Sarda sarda* Bloch, 1793) through traditional production methods, focusing on the 10th and 20th days (L10 & L20). Within this scope, water activity (aw), salt content of the meat, pH value, color analyses and thiobarbituric acid reactive substances (TBARs) analyses, as well as nutritional composition (protein, moisture, ash, lipid, carbohydrate, energy) analyses were performed. According to the analysis results, as the maturation period progressed, there was an increase in the salt content and TBARs amount of lakerda. Additionally, it was revealed that moisture (%) and aw content showed a decreasing trend with the increase in storage time ($p < 0.05$). In addition to all these, it was also determined that color parameters showed significant variability during the storage period; particularly, the L^* parameter showed a decrease during storage, while increases were observed in a^* and b^* values ($p < 0.05$). Furthermore, correlation analyses of the normalized Z-score findings showed that ash and salt changes were positively correlated, whereas ash content was inversely correlated with L^* , pH, and aw. It was also determined that TBARs amount showed an inverse correlation with WI while YI showed an inverse correlation with protein ratio.

Please cite this paper as follows:

Altan, C. O. (2025). Monitoring physicochemical attributes and nutritional alterations in lakerda during early-stage and intermediate maturation periods: A preliminary research. *Marine Science and Technology Bulletin*, 14(3), 139-148. <https://doi.org/10.33714/masteb.1757035>

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Introduction

The usage of traditional methods in the preservation of fish and fish products has a historically deep-rooted past, particularly in communities located in coastal geographies. Among these methods, salting, drying, and fermentation processes hold particularly important places (Özden & Erkan, 2011). Lakerda, one of the traditional products obtained through salting methods, is a food product with high gastronomic value, distinctive aromatic characteristics, prepared primarily from fish with high fat content such as Atlantic bonito and young tuna (Varlık et al., 2004). This gourmet product, enjoyed in communities along the Mediterranean and Black Sea coasts, is important not only for its flavor characteristics but also for food security and sustainability as it enables fish consumption outside the season.

In traditional salted fish production, fish meat is treated with high concentrations of salt to reduce water activity, thereby preventing microbial growth and extending the product's shelf life (Varlık et al., 2011). The osmotic and biochemical reactions occurring during the salting process create lakerda's characteristic texture and aroma profiles; however, the effectiveness of these processes varies according to production conditions, fish species, and salt ratios used (Koral & Köse, 2018). Nevertheless, the quality and safety of traditionally produced salted fish can show great variability depending on the techniques used and hygiene conditions, which may pose risks to consumer health (Erdem et al., 2005). In this context, our study aims to examine lakerda production prepared traditionally by dry salting, particularly in terms of chemical and physical quality characteristics, and to evaluate the changes occurring during the salting process. A review of the research literature on lakerda reveals that most studies—typically excluding fresh samples—begin their analyses at the conclusion of the maturation stage, focusing primarily on the final product characteristics (Kocatepe et al., 2014; Ormanci et al., 2015; Koral & Köse, 2018). The aim of this study is to address this gap by examining more closely the similarities, differences, and relationships among key parameters during both the early maturation and full maturation periods. By analyzing these developmental stages, this research seeks to better understand the progression of changes throughout the maturation process and identify important correlations that may have been overlooked in previous end-point focused studies. In the research, lakerda samples prepared by traditional methods were analyzed on the 10th and 20th days and evaluated in terms of physical, chemical and physicochemical parameters

(water activity, salt content, pH, thiobarbituric acid reactive substances, color analyses) and nutritional composition analyses. In this process, by revealing the changes occurring in lakerda quality at different maturation stages, it was aimed to determine the relationship between the product's storage period and quality and food safety. Additionally, this study will contribute to the literature for future research by revealing the changes between processing steps in terms of quality parameters of traditional lakerda production. However, since no sensory analysis was performed in this research, it has been considered a “preliminary study.” A study incorporating sensory analysis and more parameters will be performed in the forthcoming period.

Material and Methods

Materials

The Atlantic bonito (*Sarda sarda* Bloch, 1793) fish used in the research were obtained fresh daily from Sinop (Türkiye) fishermen in December 2024. The average length and weight of the fish were determined as 31.40 ± 3.14 cm and 318.54 ± 36.11 g ($n=6$), respectively. Çankırı (Türkiye) rock salt was used as curing salt. The lakerda samples were produced and stored in 5 l glass jars.

Methods

As the first stage, the heads and tails of the fish were cut off, the internal organs were removed, and they were sliced into chunks of approximately 4 cm. After slicing, internal organ remnants were cleaned in detail, and a skewer of approximately 1.50 mm was inserted into their backbones to thoroughly clean the nerves within the spine. The cleaned bonito chunks were soaked in 5% ice-cold salted water solution for half an hour, thus ensuring the removal of all tissue remnants. Subsequently, the chunks were transferred to another ice water container for an additional 15 minutes to ensure complete removal of remnants and then placed in a strainer to drain the water. The dry salting method was preferred in the research, using 300 g of curing salt for every 1 kg of fish slices (30%). To enable analysis replication, lakerda was produced in two separate jars. First, after evenly spreading salt approximately 3 cm high at the bottom of the jars, the fish chunks were tightly arranged in a single row, and a salt layer of approximately 1.50 cm was evenly spread on top. Then, one more layer of fish chunks was tightly placed and covered with salt. After arranging the fish in three rows in the jars, the remaining salt was added to the top layer, ensuring a salt layer approximately 3 cm thick on the upper

surface. As a final step, sterile ceramic plates were placed on top of the salt layer in the upper part of the jars to create weight and apply pressure. The prepared lakerda jars were stored under refrigerator conditions ($4\pm1^{\circ}\text{C}$). The lakerda jars were inverted every 2 days until the analysis days. During the maturation of lakerda (day 10), half of the total amount in the jar was separated to form the day 10 analysis group (L10), and these samples were analyzed on the same day. On the 20th day of maturation, samples were taken from the remaining fish in the jar and analyzed on the same day, designated as the L20 group. Fresh fish meat samples were analyzed on the day the research began. The study was conducted with two replicates, and analyses were performed in triplicate.

Analyses

Nutritional analyses

Protein (P), ash (A), moisture (M), and lipid (L) analyses were performed according to AOAC (2005) (ref: 925.04, 938.08, 954.01 and 991.36), respectively ($n=6$). Total carbohydrate content (%) was calculated by subtracting the sum of four basic nutritional components from 100 (Equation 1) (FAO, 2003). Energy values (kcal) were calculated according to the Atwater conversion factor (Equation 2) (FAO, 2003).

$$\text{Carbohydrate (\%)} = 100 - (P + A + M + L) \quad (1)$$

$$\text{Energy (kcal)} = (L \times 9) + (P \times 4) + (C \times 4) \quad (2)$$

pH, aw (water activity) & salt analyses

pH analysis was performed using a Hanna brand (Model: HI 98100, Hanna Instruments Inc., Smithfield, RI, USA) immersion probe pH meter after homogenizing 5 g of fish meat in 50 mL of pure water (1:10) ($n=6$) (Boland et al., 1981).

Water activity (aw) analyses were measured with a Lab Swift aw device (Novasina AG, Lachen, Switzerland) at an ambient temperature of 22.4°C . Approximately 10 g of homogenized fish meat was placed in the device's measurement cups and measurements were performed ($n=3$).

Salt analysis was performed according to the Mohr method (Yoder, 1919). 5 g of fish meat was homogenized in 100 ml of pure water in an ultra-homogenizer at 3000 rpm for approximately 90 seconds. The obtained homogenate was filtered through Whatman No. 1 filter paper into a flask. 10 ml of the filtrate was transferred to another flask and 2 ml of K_2CrO_4 solution was added to achieve a light orange color. Subsequently, the burette was filled with 0.1 N AgNO_3 and

titration was performed. The consumption at the moment when the color reached brick red was marked and determined as the final consumption after maintaining this color for at least 15 secs. The calculation was performed using the following formula (Equation 3):

$$\text{NaCl (\%)} = \frac{V \times N \times M_{(\text{NaCl})} \times 100}{A \times 1000} \quad (3)$$

V: Volume of AgNO_3 consumed (ml)

N: Normality of AgNO_3 (0.1N)

$M_{(\text{NaCl})}$: Molecular weight of NaCl (58.44 g/mol)

A: Amount of samples used (g)

Thiobarbituric acid reactive substances (TBARs) analyses

TBARs analysis was performed according to Weilmeier & Regenstien (2004) and Khan et al. (2006). After adding 50 μl of butylated hydroxytoluene (BHT) and 45 ml of trichloroacetic acid (TCA) to 5 g of fish meat, all fish samples were homogenized in an ultra-homogenizer for 3 minutes each. Then, Whatman No. 1 filter paper was placed in funnels and the homogenate was filtered into Erlenmeyer flasks. 5 ml aliquots from the filtrates were placed into test tubes, then 5 ml of thiobarbituric acid reagent prepared with 10% glacial acetic acid was added to each, and they were kept on a hot plate set to $75\text{--}79^{\circ}\text{C}$ for 30 minutes. At the end of the period, the tightly closed test tubes were rapidly cooled by immersing in cold water and read at 532 nm in a spectrophotometer against blank.

Color analyses

Color parameters (L^* , a^* & b^*) were determined using a Konica Minolta CR-400 colorimeter (light projection tube model: A33a, Tokyo, Japan) with an 8.0 mm closed aperture. Measurements were performed at 6 different locations, with each measurement point at least 3 cm apart. In all analyses, the CIE Lab^* color space recommended by the International Commission on Illumination (CIE) was used (Hunter & Harold, 1987). In this system, the L^* parameter represents lightness (0: black, 100: white), the a^* parameter represents the red-green axis, and the b^* parameter represents the yellow-blue axis (Hunter & Harold, 1987; Bekhit et al., 2019).

The following color indices were calculated from these primary color coordinates; hue angle (Equation 4) (h ; rad), chroma (Equation 5) (C), total color difference (Equation 6) (ΔE), whiteness index (Equation 7) (WI), and yellowness index (Equation 8) (YI) (Pathare et al., 2013). While C indicates color saturation, h parameter is qualitatively defining the dominant

color. *WI* and *YI* are used to interpret changes in whiteness and yellowness more quantitatively and comprehensively, respectively; these parameters hold a particularly important position in the context of drying or product deterioration (Škrlep & Čandek-Potokar, 2007). ΔE quantitatively reveals the total color change between experimental samples (both smoke-threatened groups) and reference samples (fresh fillets). All calculations were performed based on L^* , a^* , and b^* parameters using the following formulas (Rhim et al., 1999; Pathare et al., 2013):

$$h = \tan^{-1} (b^*/a^*) \quad (4)$$

$$C^* = \sqrt{a^{*2} + b^{*2}} \quad (5)$$

$$\Delta E = \sqrt{(a^*_{lakerda} - a^*_{fresh\ fillet})^2 + (b^*_{lakerda} - b^*_{fresh\ fillet})^2 + (L^*_{lakerda} - L^*_{fresh\ fillet})^2} \quad (6)$$

$$WI = \sqrt{(100 - L^*)^2 + a^{*2} + b^{*2}} \quad (7)$$

$$YI = 142.86 \times b^*/L^* \quad (8)$$

Statistical analyses

Data evaluation was performed using one-way analysis of variance (ANOVA) through Minitab Software (Version 21.4 for Windows, Minitab Inc., State College, PA, USA). Data were presented as mean $\pm$ standard error (mean $\pm$ se) and evaluated for homogeneity of variances at a significance level of $p < 0.05$. Graphs, visuals, and Pearson correlation analyses with Euclidean distance were created using OriginPro Software (Version 2022, Windows version, Northampton, MA, USA).

Results and Discussion

Nutritional Analyses

The protein, lipid, moisture, ash, carbohydrate and energy values of fresh Atlantic bonito meat were determined as $23.16 \pm 0.12\%$, $6.62 \pm 0.35\%$, $68.40 \pm 0.16\%$, $1.63 \pm 0.04\%$, $0.24 \pm 0.02\%$ and 153.33 ± 1.56 kcal/100 g, respectively. Altan & Turan (2016) and Altan et al. (2022) reported the protein, lipid, moisture, ash, carbohydrate and energy values of fresh Atlantic bonito as 24.43% and 22.18%, 14.62% and 17.65%, 59.40% and 58.05%, 1.33% and 0.67%, 0.22% and 1.44%, 230.06 and 253.77 kcal/100 g, respectively. As seen from the researchers' findings, the biggest difference among Atlantic bonito's nutritional components is observed in lipid ratios, as the lipid variability exhibited by Atlantic bonito according to its sexual maturity

status during the fishing period and many other environmental conditions emerges more distinctly than in many other fish species (Zaboukas et al., 2006; Balçık Mısıır et al., 2014). Atlantic bonito experienced a small amount of protein loss after being produced to lakerda, but this loss showed a slight increase on the 20th day of storage (L20) (Figure 1) ($p < 0.05$). The observed protein loss during lakerda production (Figure 1) is primarily attributed to proteolytic enzyme activity and salt-induced protein denaturation mechanisms (Thorarinsdottir et al., 2002; Marchetti et al., 2022). During the salting process, endogenous proteases (principally cathepsins B, D, and L) remain active at refrigeration temperatures, catalyzing the hydrolysis of myofibrillar and sarcoplasmic proteins into smaller peptides and free amino acids (Chéret et al., 2007; Zhao et al., 2022). This proteolytic breakdown, combined with the osmotic extraction of water-soluble proteins during salt penetration, expresses the progressive protein decrease from fresh meat to L20. Similarly, Ormanci et al. (2015) and Erkan et al. (2020) also recorded a slight decrease in protein content after producing and maturing lakerda from Atlantic bonito ($p < 0.05$). The reason for such decreases is thought to be due to peptides and free amino acids released during protein denaturation.

Compared to the lipid content found in fresh fish meat (6.62%), it increased after transformed to lakerda, with the highest value determined in the L20 group (Figure 1) ($p < 0.05$). Saygun & Tokur (2021) observed an increase in lipid values after salted marination of Atlantic bonito. However, Erkan et al. (2020) reported a lipid loss after the salting process ($p < 0.05$). The reasons for such differences in lipid changes may be influenced by many parameters such as salting techniques, meat structure and lipid amount in fish used in production, penetration rate of salt used, temperature variations in application conditions.

Following the salting of fresh Atlantic bonito, moisture loss was observed in lakerda (Figure 1) and moisture loss increased according to storage time ($p < 0.05$). Due to osmoregulation, water exits while salt enters the fish meat, and therefore water and salt inflows and outflows reach equilibrium and stabilize at the end of osmoregulation. Various researchers have also reported this moisture decrease in lakerda production (Ormanci et al., 2015; Erkan et al., 2020).

The ash content, initially determined as 1.63%, increased significantly with water exit from the meat and salt entry (Figure 1) ($p < 0.05$). Particularly as storage time increased (L20), more salt entry and moisture decrease were observed until osmoregulation was completed, and the highest crude ash content was determined in the L20 group ($p < 0.05$).

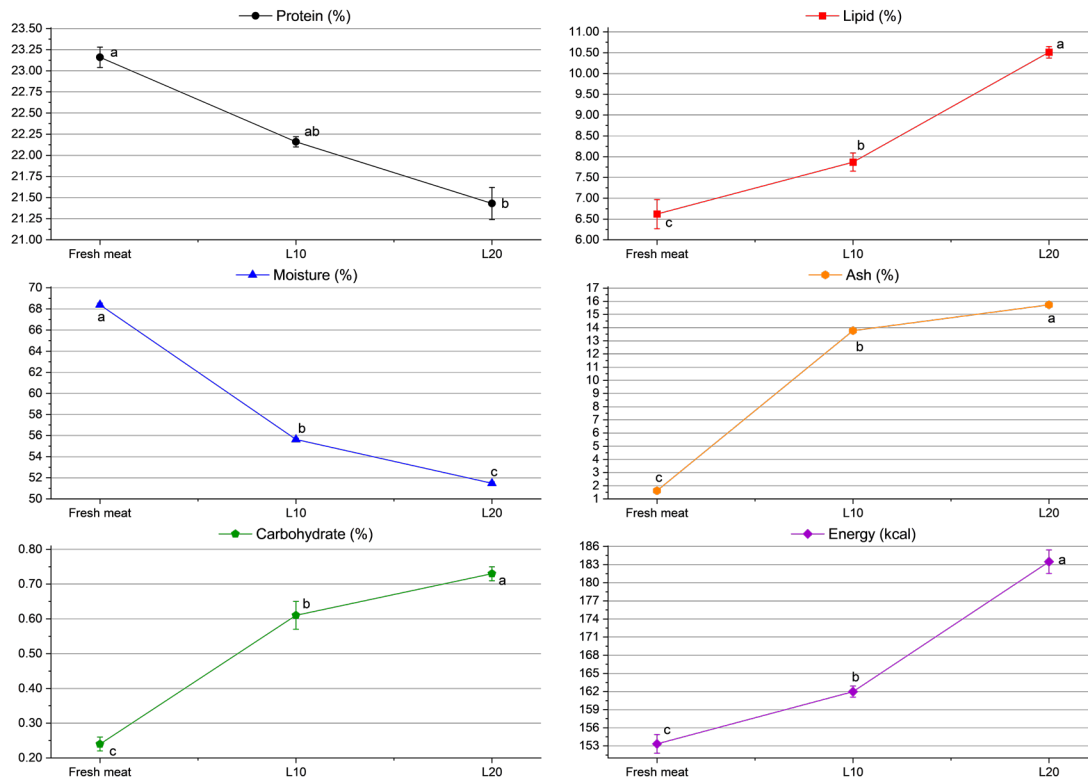


Figure 1. Nutritional composition findings of fresh bonito meat and lakerda groups (Each point in the same-colored line, labeled with different letters, is significantly different from each other [$p < 0.05$])

Carbohydrate values were determined in the range of 0.24% to 0.73%. Our carbohydrate findings are similar to the literature (Ormancı et al., 2015; Erkan et al., 2020).

An increase in energy content was observed due to proportional weight loss after water exit from the fish meat (Figure 1) ($p < 0.05$). This increase was observed to be highest in the L20 group, which is expected to have the highest energy level due to having the highest lipid and lowest moisture content.

pH, aw & Salt

The pH, aw and salt contents detected in fresh fish fillets were determined as 6.91 ± 0.06 , 0.986 ± 0.001 and $0.17 \pm 0.02\%$, respectively. Altan & Turan (2016), Koral & Köse (2018), Balçık Mısıır & Koral (2021) and Altan et al. (2022) determined pH and aw values in fresh Atlantic bonito as 5.96, 6.16, 6.21, 5.59 and 0.985, 0.994, 0.993 and 0.978, respectively. When the researchers' findings are evaluated, it is seen that pH varies between 5.59 and 6.21, while aw ranges between 0.978 and 0.993 (Figure 2). Although the pH level detected in our research is slightly higher than those of other researchers, our aw findings align with the aw findings detected by the researchers. It is known that pH depends on many variables such as fishing conditions, sexual maturity, habitat conditions, etc. (Tülsner, 1994).

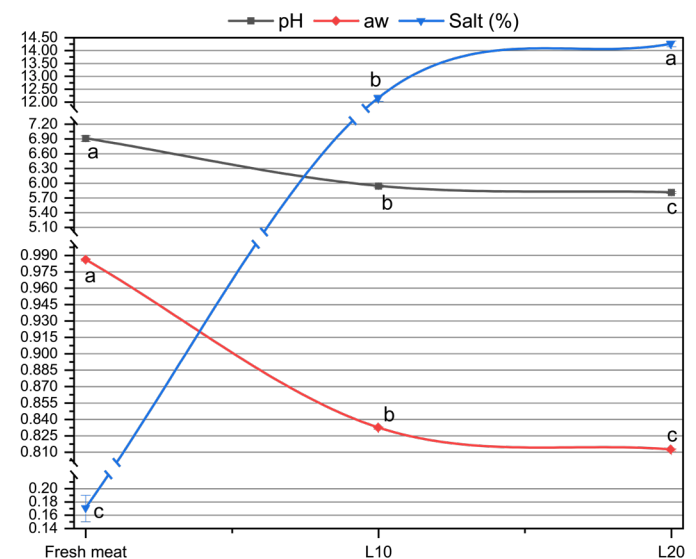


Figure 2. pH, aw and salt amount (%) findings of fresh bonito meat and lakerda groups (Each point in the same-colored line, labeled with different letters, is significantly different from each other [$p < 0.05$])

A decrease in pH values was observed after the salting process (Figure 2) and this decrease was determined to increase as storage time progressed ($p < 0.05$). Erkan et al. (2020) determined decreases in pH as storage time increased in their research where they stored salted Atlantic bonito. The researchers' findings are similar to our pH findings.

A decrease in aw levels was observed after salt entry into the fish meat (Figure 2) and the amount of this decrease observed depending on storage time increased ($p < 0.05$). A decrease in moisture and aw due to osmoregulation is expected.

The salt amounts detected on the 10th (L10) and 20th days (L20) of storage were determined as 12.45% and 14.26%, respectively (Figure 2) ($p < 0.05$). The correlation coefficients (r) detected between the amount of salt transfer to meat by days and changes in crude ash, moisture and aw values were determined as 0.94, -0.91 and -0.83, respectively. The salt ratios detected by Koral & Köse (2018) in lakerda at the end of weeks 1 and 2 were reported as 13.17% and 13.77%, respectively. It is possible to say that the salt ratios determined in our research follow a similar pattern.

TBARs

The TBARs content of fresh Atlantic bonito used in our research was determined as 0.62 ± 0.03 mg MDA/kg. Koral & Köse (2018) and Erkan et al. (2020) reported TBA values in fresh Atlantic bonito and lakerda as 0.49 and 3.74; 0.77 and 5.17 mg MDA/kg, respectively. After salt entry into the fish meat, the oxidative effect began to emerge rapidly and the TBARs value reached 6 mg MDA/kg on the 10th day (Figure 3). In the subsequent 10-day period, the TBARs value exceeded 7.50 mg MDA/kg, beginning to approach consumable limits ($p < 0.05$). The TBARs values detected in our research were found to be slightly higher compared to the literature but still remained below the limit value (8 mg MDA/kg) (Varlık et al., 2011). It is reported that these changes in TBARs content may occur due to the lipid structure of the fish or differences in processing or storage stages (Koral & Köse, 2018). However, the rapid TBARs increase from 0.62 to over 6 mg MDA/kg (Figure 3) reflects the pro-oxidant effect of salt on lipid oxidation through multiple mechanisms (Mariutti & Bragagnolo, 2017). Salt acts as a pro-oxidant by; disrupting cell membranes and releasing pro-oxidant enzymes and metal ions; reducing the activity of antioxidant enzymes such as catalase and glutathione peroxidase; and promoting the dissociation of iron from heme proteins, catalyzing lipid peroxidation (Lee et al., 1997; Hernández et al., 2002; Grunwald & Richards, 2006; Mariutti & Bragagnolo, 2017; Tunieva & Kotenkova, 2017; Domínguez et al., 2019; Wu et al., 2022). The particularly high oxidation rate in our research may be attributed to Atlantic bonito's high content of polyunsaturated fatty acids, which are highly susceptible to oxidation (Balçık Misir et al., 2014).

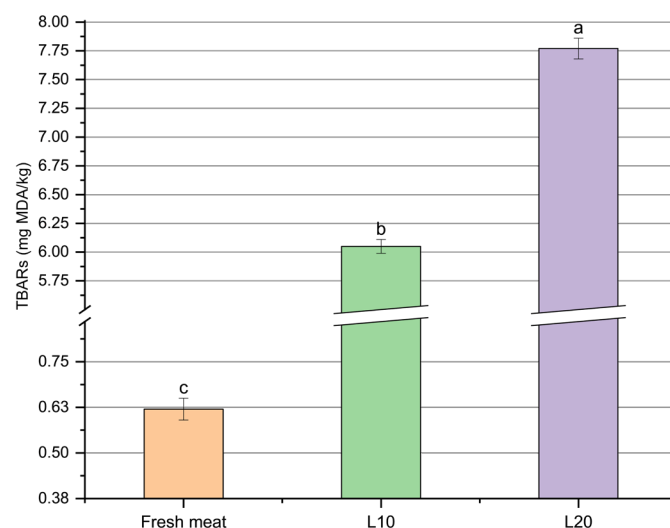


Figure 3. TBARs amounts determined in fresh bonito meat and lakerda groups (The labels on the bars with different letters are significantly different from each other [$p < 0.05$])

Color

The L^* , a^* , b^* , h , C , WI and YI values detected in fresh Atlantic bonito fillets were determined as 55.09 ± 0.46 , 1.71 ± 0.09 , 9.78 ± 0.19 , 1.40 ± 0.01 , 9.93 ± 0.21 , 54.02 ± 0.48 and 28.36 ± 0.65 , respectively. Ormancı et al. (2015) detected L^* , a^* and b^* values in fresh Atlantic bonito as 49.03, 6.64 and -0.81, respectively, while Balçık Mısır & Koral (2021) determined these parameters as 45.08, 11.09 and 21.10, respectively. As seen from the researchers' findings, quite different results can emerge in color parameters. The primary reasons for these differences are the sexual differences and maturity of the fish. Additionally, the diversity of species that fish feed on during that period directly affects the pigments in the meat tissue.

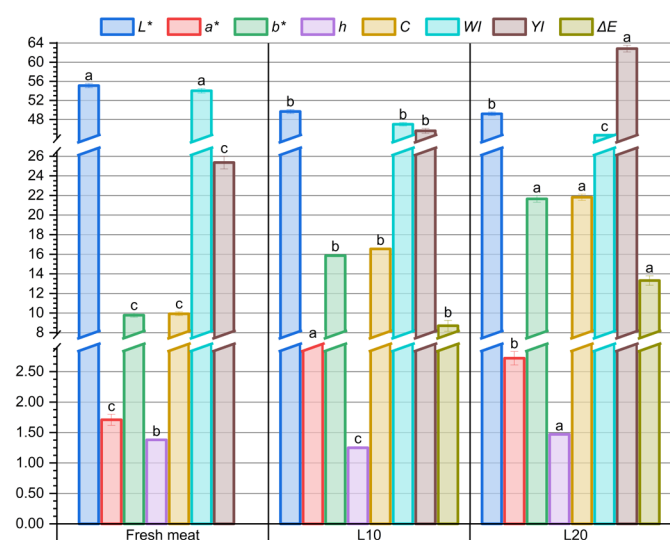


Figure 4. Color parameter findings determined in fresh bonito meat and lakerda groups (In each same-colored bars, labels on the top with different letters are significantly different from each other [$p < 0.05$])

After Atlantic bonito was salted and transformed to lakerda form, a decrease was observed in L^* parameters, while an increase was detected in a^* and b^* parameters (Figure 4). An increase in a^* and b^* parameters in salted products due to oxidation is expected. Additionally, a positive correlation between L^* parameter and water loss has been reported (Altan, 2025, Huang et al., 2025). Similar to the changes in our research, allied decreases and increases in L^* , a^* and b^* parameters after lakerda production from Atlantic bonito have also been reported by other researchers (Ormanci et al., 2015; Balçık Mısıır & Koral, 2021; Selçuk & Ayvaz, 2022).

The visually perceivable equivalent of the h (rad) value detected in fresh Atlantic bonito is a vivid and bright yellowish-green tone. Slight changes were observed in h values in lakerda groups ($p < 0.05$). While the yellowish tone was slightly higher in the h value of the L10 group, a slight increase in greenish tones occurred in the L20 group (Figure 4). However, in terms of h values, it is possible to say that it would be quite difficult for the human eye to perceive the tonal difference, especially when there is no side-by-side comparison of both lakerda groups.

The C parameter determined in unprocessed Atlantic bonito meat increased after lakerda production, and the increasing trend in the C parameter continued as storage time increased ($p < 0.05$). It is possible to say that the C parameter showed more than a two-times increase throughout the 20-day lakerda storage group from fresh fish meat (Figure 4). It can be said that the most important component of this increase is the b^* parameter.

The WI value decreased after lakerda production and this decreasing trend continued according to storage time ($p < 0.05$). As a result of the relative decrease in L^* parameter after lakerda production and the increase in a^* and b^* parameters, WI values decreased (Figure 4).

An increase was observed in YI values of fish converted to lakerda, and this increase was determined to become more pronounced with the progression of storage time (Figure 4) ($p < 0.05$). This increase in YI values is expected with the acceleration of the oxidation process and the yellowish color specific to lakerda becoming more prominent.

In terms of the ΔE parameter, which expresses the total color change compared to fresh fish meat, the L20 group was detected to be higher than the L10 group (Figure 4) ($p < 0.05$). The increase particularly in b^* and YI values resulted in the L20 group having a higher ΔE value.

Correlation Analysis

Upon the analysis performed by normalizing all data along with their parallels and converting them to Z scores, the relationships of all findings with each other are clearly visible (Figure 5). As a result of the increase in crude ash content, decreases were observed in L^* , pH and aw values, while increases were seen in salt values. That is, the increase in crude ash is significantly related to the increase in salt content and the decrease in pH, aw and L^* values. However, although the increase in crude ash and moisture loss has an inverse and relatively strong relationship, it was still not sufficient to statistically demonstrate this connection ($p > 0.05$). As seen in the graph, interestingly, no direct statistical connection was detected between lipid data and any other data, only a relatively strong relationship was determined with b^* and YI . Protein was found to be inversely related to b^* , C and YI ($p < 0.05$), and a linear relationship was found between YI and b^* and C parameters ($p < 0.05$).

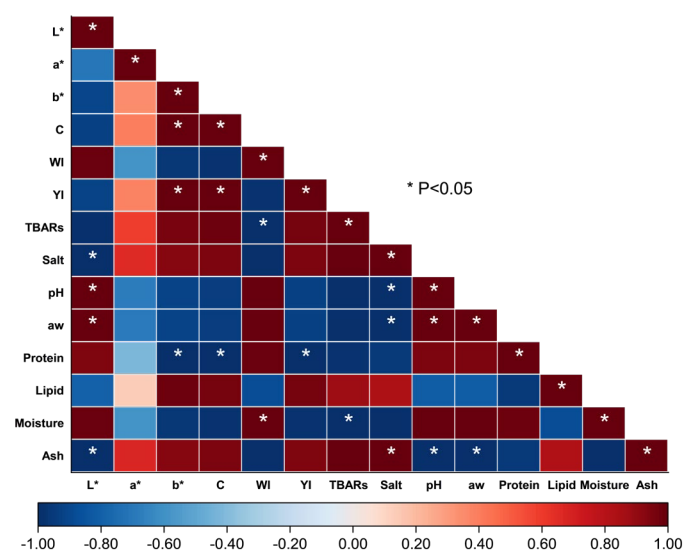


Figure 5. Euclidean distance correlation results of the analysis findings

Conclusion

In the research, the 10th and 20th days within the maturation phase of lakerda were examined through various physical and chemical analyses. The research findings showed that as storage time increased in terms of nutritional composition, decreases were observed in protein and moisture values, while increases occurred in lipid, ash, carbohydrate and energy values. As a result of salt penetration into the meat, the salt content in the meat increased during storage, whereas pH and aw values decreased. While the lowest TBARs content was observed in fresh fish meat, the highest was detected in the L20

group. The L^* value determined in fresh fish meat decreased as storage increased but increases occurred in a^* ($L10>L20$) and b^* values. With the increase in b^* value due to the effect of salt, significant changes also occurred in h , C , WI , YI and ΔE values according to storage periods. It can be stated that, for industrial or large-scale production, the use of natural or nature-identical antioxidants would be highly beneficial under the conditions of this study, especially from the 20th day onwards, as the TBARS values were found to approach the acceptable limit. The addition of such antioxidants would significantly reduce lipid oxidation. Furthermore, antioxidants would help to prevent increases in both the b^* value and the YI , thus supporting better color stability and contributing to the production of visually higher-quality products. Moreover, in addition to the findings obtained in the research, it is aimed to increase the depth of the research by adding analyses such as texture profile analysis, total volatile basic nitrogen analysis, and total free amino acids and fatty acids in future studies.

Acknowledgements

No financial or moral support was received from any institution or individual for this research. The author would like to thank SSYUB (Samsun and Sinop Provinces Aquaculture Producers' Union) for providing chemicals and consumables used in the analyses, and SEANOP Aquatic Products Processing, Quickfreezing, and Storage Facility for providing support with laboratory use.

Compliance With Ethical Standards

Conflict of Interest

The author declares that there is no conflict of interest.

Ethical Approval

For this type of study, formal consent is not required.

Funding

Not applicable.

Data Availability

All data generated or analysed during this study are included in this published article.

AI Disclosure

The authors confirm that no generative AI was used in writing this manuscript or creating images, tables, or graphics.

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