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Nutritional and sensory properties of salted fish product, *lakerda*

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Abstract: The objective of this study was to determine nutritional and sensorial properties of *lakerda*, a traditional salted fish product, produced from Atlantic bonito (*Sarda sarda*). From the point of nutritional characterization, chemical content and amino acid and fatty acid compositions of *lakerda* have been investigated. For determination of sensorial attribute, instrumental texture, instrumental color, and descriptive sensorial analyses were conducted. Chemical composition of *lakerda* was 52.22% water, 14.64% protein, 17.39% lipid, 15.14% ash. Protein of *lakerda* has a well-balanced amino acid composition, with high amounts of Glutamic acid + Glutamine (4.75 g/100 g), Aspartic acid + Asparagine (2.39 g/100 g), Leucine (1.51 g/100 g), and Valine (1.36 g/100 g). Thirty-two fatty acids were identified in lipid of *lakerda*, of which monounsaturated fatty acids were the highest proportion (40.91%). Oleic acid (C18:1) was dominant fatty acid followed by docosahexaenoic acid (DHA; 22:6n−3) and palmitic acid (C16:0) with percentages of 21.68, 18.79, 11.49, respectively. In instrumental properties, hardness, $L*$ (lightness), $a*$ (redness) and $b*$ values (yellowness) of *lakerda* were measured as 2.04 kg, 37.44, 5.23, and 0.47, respectively. The results of this study indicated that *lakerda* has outstanding sensory and nutritional characteristics due to high quality and well-balanced essential amino acid and fatty acid.

Subjects: Food Analysis; Nutrition; Seafood; Sensory Science; Preservation; Processing

Keywords: Atlantic bonito; salted fish; *lakerda*; amino acid; fatty acid; sensorial properties

ABOUT THE AUTHOR

Hasan Basri Ormanci (PhD) is a research assistant at Canakkale Onsekiz Mart University, Faculty of Marine Science and Technology, Fishing and Fish Processing Technology Department, teaching courses on seafood processing technologies, food chemistry, food microbiology, quality control systems in seafood processing factory, and HACCP organizations. He is the author or co-author (many of them along with his PhD advisor, Fatma Arik Colakoglu) of eight international research articles and a lot of national study papers.
His study is on seafood processing, especially fish salting, fish lipids, sensory evaluation and metal contamination in bivalve. This paper is a part of the PhD thesis of him. It is related with the *lakerda* production. *Lakerda*, a salted fish product, has been practiced for over 600 years in Mediterranean region. This study demonstrates the nutritional and sensory characterizations of *lakerda*.

PUBLIC INTEREST STATEMENT

Although *lakerda* has been practiced for over 600 years in Mediterranean region, nowadays, this product has lost popularity and importance for preservation purposes. However, this appreciated product is quite different than the other salted fish products due to specific sensory properties, especially in terms of texture and flavor. Several studies have been conducted concerning *lakerda*, which focus on to determined shelf life and food safety parameters. However, characteristics of this product have not been widely studied. From the point of nutritional characterization, we investigated proximate, amino acid, and fatty acid composition of *lakerda*. For determination of sensorial attribute, instrumental texture, color, and descriptive sensorial analysis were conducted. This study is the first report on the nutritional and sensory characterizations of *lakerda*. Results may benefit the fishing industry, nutritionists and researchers who are striving to improve the nutritive value, processing and marketing of fish.

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1. Introduction

Lakerda, a salted fish product, has been practiced for over 600 years in Mediterranean region (Erkan, Tosun, Alakavuk, & Ulusoy, 2009). Nowadays, this product is primarily produced in coastal areas of Turkey and is locally consumed and it is also exported to several countries. It is appreciated due its specific sensory properties, especially its soft rubbery texture. Generally, *lakerda* is produced from fatty fish. *Lakerda* has basically been produced-from Scombroid fish, particularly large Atlantic bonito (*Sarda sarda*); however, salmon and trout have been used in the production of *lakerda* in the recent years. The production of *lakerda* may be carried out by wet-salting, brining, or a combination of these methods (Ormanci, 2013).

Salting is one of the oldest and commonly used processing techniques for fish preservation all over the world because of simplicity of the process and low production cost (Martínez-Alvarez & Gómez-Guillén, 2013). Salt is effective as a preservative because it reduces the water activity of fish muscle, consequently bacterial growth and enzymatic spoilage are inhibited. On the other hand, the current demand for salted fish is driven more by sensorial alteration purposes, rather than preservation (Mujaffar & Sankat, 2005). Therefore, it could be stated that the aim of producing salted fish "principally *lakerda*" is not only to get a shelf stable product (low moisture and high salt content), but also to promote important sensory changes.

The Atlantic bonito (*Sarda sarda* Bloch, 1793) is the most abundant small tuna species widely distributed in the Atlantic Ocean, including the Mediterranean Sea and the Black Sea. They are a highly migratory fish species that seasonally migrate in Turkish territorial waters (between the Black Sea and North Aegean Sea through the Sea of Marmara) in order to feed and reproduce. In 2012, Turkey's total catch of these commercially important fish was 35,764 tons [Turkish Statistical Institute (TurkStat), 2013] and primarily from the coast of Black Sea. The bonito is appreciated by salted fish industry. Meat color, texture, and nutritional properties, especially high quantity of lipids are the reasons for preference.

Several studies have been conducted concerning *lakerda*. These studies focus on determining shelf life (Caglak, Cakli, & Kilinc, 2012; Erkan et al., 2009) and on food safety (Koral et al., 2013; Turan, Kaya, Erkoyuncu, & Sonmez, 2006) parameters. However, characteristics of this product have not been widely studied. Therefore, the aim of the present study was to investigate the nutritional properties and sensorial attributes of *lakerda*.

2. Materials and methods

2.1. Materials

A total of 120 Atlantic Bonito (*Sarda sarda*) specimens were caught by purse seine on October 2011 in the Dardanelles (Northwestern Turkey) and transported on seawater flake ice to the laboratory within 2 h after the catch. The average body weight and length of the bonito were 868.32 ± 0.24 g and 45.03 ± 0.51 cm, respectively. The fish were headed, gutted, and their dorsal, caudal, and lateral fins were removed then they were cut into (approximately 5 cm thick) pieces. Then their blood clots and bone marrow were completely removed. Granular industrial sea salt (from Aegean Sea; 2–4 mm diameter) was used for the pre-salting and salting stages of this experiment. Typical chemical composition of the salt is shown in Table 1, although the salt was not analyzed in this study.

2.2. Salting protocol and sampling

The salting process was divided into two stages; pre-salting and salting. The pre-salting (brine salting) process were carried out once every 24 h for three days, by immersing fish into a fresh brine solution (10 g of salt in 100 mL of water) at 4 ± 1℃. The ratio of fish to brine was g/L 1:1 (w/v). The fish were removed and strained from the brine after three days. In salting stages, designated as pickle, the fish slices were treated with a granular salt, layered alternately (fish and salt layer) and stored in container.

Table 1. Chemical and ionic composition of the salt from Aegean Sea	
	Content
Chemical Composition	
NaCl (dry) (%)	99.07
NaCl (%)	98.62
$CaSO_4$ (%)	0.17
$CaCl_2$ (%)	0.00
$MgSO_4$ (%)	0.12
$MgCl_2$ (%)	0.22
Water (%)	0.45
Matter insoluble in water (%)	0.43
Ionic composition	
Ca^{2+} (ppm)	501
Mg^{2+} (ppm)	790
SO_4^{2-} (ppm)	2.15

Source: Yaşat Tuz Industry and Commerce Inc.

Thereafter, the fish were stored at 4°C for ripening within 22 days. For sampling, three slices of fresh or salted fish samples were taken randomly and color, hardness, proximate, physicochemical, amino acid, and fatty acid composition were analyzed for each slice separately.

2.3. Proximate and physicochemical analyses
The water was determined on approximately 5 g of minced muscle by oven drying at 105 ± 3°C until a constant weight, following technique 950.46 [Association of Official Analytical Chemists (AOAC), 2000]. Percent protein (Kjeldahl N × 6.25) was determined from a 1 g sample for each treatment by AOAC (2000). Lipid was extracted from samples with a mixture of chloroform, methanol, and water (Bligh & Dyer, 1959). Ash was determined by dry-ashing in a furnace at 550 ± 5°C for 24 h (AOAC, 2000). The pH was measured as described by Ludorf and Meyer (1973), using a digital pH meter (Hanna, Germany). Mohr method was used to determine salt content in fish muscle (AOAC, 2000).

2.4. Determination of hardness and color
Textural properties were measured by compression using a Texture Analyzer TA.XT2 (Stable Micro Systems, UK) equipped with a flat-ended cylindrical plunger (12 mm diameter). The colorimetric values of the sliced fresh and salted fish samples were measured 10 times using a Minolta Chroma Meter CR200b (Minolta Co. Ltd., Osaka, Japan). Colors were expressed in CIELab coordinates. In this system, $L*$ denotes lightness on a 0–100 scale from black to white; $a*$, (+) red or (−) green; $b*$, (+) yellow or (−) blue. The instrument was calibrated to a white standard ($L* = 98.0$; $a* = 0.3$; $b* = 2.4$). The color measurements were performed by leaning the instrument on the surface of the flesh and spot was approximately 8 mm-diameter circle.

2.5. Fatty acid composition analysis and GC–MS conditions
To determine the fatty acid composition of the fish samples, fatty acid methyl esters (FAMEs) were prepared using standard International Union of Pure and Applied Chemistry (1979) method. The GC–MS analysis for FAMEs was performed on Thermo Finnigan Trace GC coupled with a Multiplier Quadrupole Mass Selective Detector (GC–MS DSQ) and a Thermo auto sampler AI 3000 injector (Thermo Electron Corporation, Milan, Italy) and operated with Xcalibur Home Page version 1.4 SR1 Software. A capillary column ZB-5MS (5% phenyl methylsiloxane) with a dimension of 30 m × 0.25 mm I.D × 0.25 m film thickness (Phenomenex, Zebron, USA) was used for the separation of fatty acid methyl esters. The initial temperature of 70°C was maintained for 5 min, raised to 200°C at the rate of 5°C/min, and kept at 200°C for 5 min. The temperature was finally raised to 250°C at the rate of 5°C/min and kept at 250°C for 20 min. The split ratio was 1:20, and helium was used as a carrier gas

with the flow rate of 1.0 mL/min. The injector and ion source temperature were 220 and 230°C, respectively. The mass spectrometer was operated in the electron impact (EI) mode at 70 eV in the scan range of 50–650 m/z.

2.5.1. Peak identification and calculations

Peak identification of the fatty acids (FAs) in analyzed fish samples was carried out by comparing the retention times and mass spectra of known standards (Supelco 37 Component FAMEs Mix). GC–MS chromatograms obtained were compared with those of two libraries' (NIST and Wiley) that provide best information about the identification of FAs. The data obtained were analyzed using Qual Browser version 1.4 SR1 (Xcalibur Home Page) software, and calculated FAMEs were presented as percent of total FAMEs of the fish samples. Percentage values were converted to g/100 g wet weight as described by Paul and Southgate (1978).

2.6. Amino acid composition

In order to determine amino acid profiles, samples were hydrolyzed at 110°C for 24 h with 6.0 mol/L hydrochloric acid. The hydrolysates of all samples were filtered through a 0.20 μm PTFE syringe filter, and then all the hydrochloric acid in the hydrolysates was evaporated. After evaporation, all the hydrolysates samples were dissolved in citrate–sodium citrate buffer (0.1 mol/L, pH 2.2) (Chi et al., 2008; Srivastava, Hamre, Stoss, Chakrabarti, & Tonheim, 2006). The levels of amino acids were measured in fish samples using EZ:faast kits (EZ:faast GC/FID Protein Hydrolysate Amino Acid Kit) by gas chromatography according to Badawy, Morgan, and Turner (2008). The procedure of amino acids analysis consisted of a solid-phase extraction step, followed by a derivatization procedure and a liquid/liquid extraction step (Badawy et al., 2008; Kale, Kale, Akdeniz, & Canoruc, 2006). Extracted samples were then analyzed by gas chromatography. Norvaline was used as internal standard. The concentration of the internal standard (IS; Norvaline) in the sample prepared for GC analysis was 200 nmoles/mL. Gas chromatography (Finnigan Trace GC Ultra AI 3000 Thermo Finnigan analyzer, Milan, Italy) was used to determine the amino acids. The column was a Zebron Zebron™ ZB-HAAC 10 m × 0.25 mm capillary GC column. The conditions of the GC device during the injection process: Split 1:15 at 250°C, 2.0 μL; carrier gas: helium 1.0 mL/min; oven program: 35°C/min from 110 to 320°C, hold at 320°C for 1 min; Detector: FID at 320°C. The instrument was calibrated with standard solution of multi amino acids (EZ:faast SD solutions). Tryptophan was not determined.

2.6.1. Estimation of quality of the amino acids

The total amino acid (TAA), total essential amino acids (TEAA), total acid amino acid (TAAA), total sulfur amino acids (TSAA), and total aromatic amino acids (TArAA) were calculated from quantity of amino acids. The predicted protein efficiency ratio (PER) was determined using one of the equations developed by Alsmeyer, Cunningham, and Hapich (1974) as stated below (Adeyeye, 2009):

$$PER = -0.468 + 0.454 \, (Leucine) - 0.105 \, (Tyrosine)$$

The amino acid score (AA_{score}) for the essential amino acids was calculated using the following formula [Food and Agriculture Organization/World Health Organization (FAO/WHO), 1973]:

$$AA_{score} = AAA_{SP} \, / \, AAA_{RF}$$

where AAA_{SP} is the amount of amino acid per sample protein (mg/g); AAA_{RP} is the amount of amino acid per protein in reference protein (mg/g).

2.7. Descriptive sensory analysis

Ten assessors (six female and four male) were selected on the basis of their willingness to participate and previous experience and knowledge on sensory evaluation of salted fish products. Panelists were university staff; ages ranged from 26 to 49 year. The quality of the *lakerda* was assessed using a 5-point descriptive scale and each attribute was quantified from 1 to 5, where 1 = not detected and 5 = extremely strong (Meilgaard, Civille, & Carr, 1999). Panelists received approximately 30 h of

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training. During training, panelists were asked to identify and define color, odor, flavor, and texture attributes of *lakerda*. Twenty-six attributes were determined for color (milky, broken white, yellowish, purplish, and reddish, spotty, uniform), odor (fish smell, flesh odor, soapy, metallic odor, typical odor, sweetness), flavor (salty, metallic flavor, typical flavor, unripe, buttery), and texture (hardness, adhesiveness, cohesiveness, soft, springiness, chewiness, tenderness, and juiciness). Pure water and unsalted bread were provided as palate cleansers.

2.8. Statistical analysis

The data were subjected to one-way analysis of variance (ANOVA) with a Tukey's multiple comparison test. The suitability of data for ANOVA was tested using Anderson–Darling Test for normality and Levene's Test for equal variances (homogeneity). The software used was PASW® Statistics 18 for Windows (IBM SPSS Inc., Chicago, IL). Significance of differences was defined at $p < 0.05$.

3. Result and discussion

3.1. Proximate and physicochemical composition

The chemical composition of food materials, primarily protein, fat, and minerals, has an important role on human system. Sufficient provision of these nutrients is essential for maintaining prosperous health. Processing methods have considerable effect on the nutritional value of fish, and in variations of protein, lipid, and water contents. In the present study, water, protein, lipid, and ash contents were rated at 63.25, 17.52, 14.65, and 2.40%, respectively, in Atlantic bonito (*Sarda sarda*), whereas 52.22% water, 14.64% protein, 17.39% lipid, and 15.14% ash ratios were determined in *lakerda* (Table 2). During salting, the mass transfer occurs basically between salt and water: the fish muscle takes up salt and loses water (Chaijan, 2011; Oliveira, Pedro, Nunes, Costa, & Vaz-Pires, 2012). Nutritional components, such as protein, lipid, and ash, were increased due to the loss of water in fish muscle in the salting process (Brás & Costa, 2010; Chaijan, 2011). However, protein content was decreased in some cases, such as transference of water soluble proteins to the salt solution (Abbas Bakhiet & Khogalie, 2012; Clucas & Ward, 1996). From the result, protein content was determined significantly lower ($p < 0.05$) than the fresh fish (Table 2). The major changes in the protein fraction of the salted fish are caused by the increased NaCl concentration, which increases protein degradation. Consequently, the pH value decrease is explained by the

Table 2. Proximate, physicochemical, and instrumental sensorial composition of Atlantic bonito and *lakerda*

Parameters	Fresh	Lakerda
Proximate composition		
Protein (%)	17.52 ± 0.49[a]	14.64 ± 0.67[b]
Water (%)	63.23 ± 1.33[a]	52.22 ± 0.80[b]
Lipid (%)	14.65 ± 0.34[b]	17.39 ± 0.83[a]
Ash (%)	2.40 ± 0.19[b]	15.14 ± 0.19[a]
Physicochemical composition		
Salt content (%)	2.34 ± 0.12[b]	19.86 ± 0.25[a]
pH value	6.44 ± 0.01[a]	5.90 ± 0.02[b]
Hardness and color properties		
Hardness (kg/cm^2)	0.48 ± 0.15[b]	2.04 ± 0.08[a]
L^* value	49.03 ± 0.43[a]	37.44 ± 0.52[b]
a^* value	6.64 ± 0.27[a]	5.23 ± 0.17[b]
b^* value	−0.81 ± 0.16[b]	0.47 ± 0.30[a]

Notes: Values are expressed as mean ± SE (standard error) of three determinations ($n = 3$) for proximate and physicochemical composition; 10 determinations for hardness and color properties ($n = 10$).
Means with different superscript lowercase letters in the same row indicate significant differences ($p < 0.05$).

increase of the ionic strength of the solution inside of the cells (Goulas & Kontominas, 2005; Leroi & Joffraud, 2000). This is confirmed by our data where pH decreased from 6.44 to 5.90 ($p < 0.05$; Table 2).

3.2. Hardness and color of Atlantic bonito and lakerda

The hardness value of Atlantic bonito and *lakerda* are shown in Table 2. In raw fish, the hardness value was found 0.48 ± 0.15 kg/cm², whereas in *lakerda* it was 2.04 ± 0.08 kg/cm². Texture of fish muscle has been related to water content and lipid content (Jittinandana, Kenney, Slider, & Kiser, 2002). During the salting, the texture of fish muscle is affected due to change of the muscle proteins (Barat, Rodriguez-Barona, Andres, & Fito, 2002). Especially, hardness properties increase because of increasing protein denaturation and a reduction of the hydration of proteins (Brás & Costa, 2010). To our knowledge, no information exists on the hardness value of salted Atlantic bonito in the literature; however, the data can be compared with other salted fish products. For example, Martínez-Alvarez, Borderías, and Gómez-Guillén (2005) reported that the hardness of salted cod (*Gadus morhua*) muscle (18% (w/v) brine) ranged between 0.94 and 1.61 kg. In addition, dry salted Atlantic salmon were stated to be about 2.35 kg by Gallart-Jornet et al. (2007b). These results show that the current study is consistent with those of above mentioned study; however, it should be emphasized that several factors have been reported to affect the hardness of salted fish including salt concentration, composition, processing, ripening time, fish species, size, composition and freshness (Gallart-Jornet et al., 2007a, 2007b; Lauritzen et al., 2004; Martínez-Alvarez et al., 2005). In addition, texture of *lakerda* is described as soft rubbery by gourmets. Therefore, they are generally compared with Turkish delight. Instrumental hardness values of Turkish delight were reported as 1.03–3.43 kg (Cam, 2010; Ipek, 2009; Uslu, Erbas, Turhan, & Tetik, 2010). In this context, *lakerda* and Turkish delight have similar texture in terms of hardness.

The instrumental color determinations on Atlantic bonito and *lakerda* are given in the Table 2. Instrumental color values are based on the reflectance of light at specific wavelengths from the fish muscle surface (Lauritzen et al., 2004). The L^*, a^*, and b^* values of *lakerda* were found to be different in comparison to the fresh sample ($p < 0.05$). Compared to the fresh sample, *lakerda* has less lightness and redness due to the removal of blood from the muscle, and more yellowness due to oxidation as an undesired result of the salting process. The main reaction responsible for the color changes in fish muscle during salting is lipid oxidation. NaCl promotes lipid oxidation due to reaction between free radicals and oxygen in the presence of initiators (metal, light, and heat), consequently resulting in metmyoglobin formation and discoloration in fish meat. In general, our results are in agreement with Caglak et al.'s (2012) on the instrumental color determination of bonito *lakerda*. However, with respect to the b^* value, our result were found to be lower than the reported value. These differences may be concerned with species of raw material, time of ripening or may be due to variations in salting methods that may have a profound effect on the meat color in fish (Åsli & Mørkøre, 2012). For example, Jónsdóttir et al. (2011) stated that there were significant differences in b^* values between the injected + brined (−6.4) and the kench salted (−3.5) cod fillets.

3.3. Amino acid content

The composition of amino acids is the factor determining the quality of protein in foods. In general, high protein foods were also high in the contents of amino acids including the essential amino acids. The amino acid profiles of the fish samples—Atlantic bonito and *lakerda* are presented in Table 3. The predominant amino acids were determined non-essential amino acids, Glu + Gln (4.75 ± 0.06 g/100 g), Asp + Asn (2.39 ± 0.07 g/100 g), followed by predominant essential amino acids, Leu (1.51 ± 0.03 g/100 g) and Val (1.36 ± 0.02 g/100 g), in *lakerda* sample. The quantities of Glu + Gln, Val, and Met + Cys were significantly higher ($p < 0.05$) in *lakerda* compared to raw material. Contrary to this, quantities of Asp + Asn, His, and Ser were significantly lower ($p < 0.05$). One of the major effects imparted by salt is loss of nutrients due to the salting process, which happens mainly to some amino acids due to transferring from the tissue to the salt solution (Ferraro et al., 2011). In this study, the total amino acid (TAA) contents increased after salting, but variations were not significantly different

Table 3. Amino acid profile of Atlantic bonito and *lakerda* (g/100 g)				
Amino acid	**Recommended daily intake***		**Fresh**	***Lakerda***
	mg/kg body weight	**g/70 kg body weight**		
Valine (Val)*	26	1.82	1.11 ± 0.07[b]	1.36 ± 0.02[a]
Leucine (Leu)*	39	2.73	1.46 ± 0.09	1.51 ± 0.03
Isoleucine (Ile)*	20	1.40	0.98 ± 0.02	1.09 ± 0.06
Threonine (Thr)*	6.5	0.46	0.91 ± 0.05	0.83 ± 0.02
Methionine + Cystine (Met + Cys)*	15	1.05	0.52 ± 0.04[b]	0.78 ± 0.01[a]
Phenylalanine + Tyrosine (Phe + Tyr)*	25	1.75	1.32 ± 0.05	1.29 ± 0.02
Lysine (Lys)*	30	2.10	1.16 ± 0.02	1.12 ± 0.04
Histidine (His)*	10	0.70	1.33 ± 0.03[a]	0.68 ± 0.02[b]
Tryptophan (Trp)*	4	0.28	ND	ND
Alanine (Ala)			1.03 ± 0.07	1.05 ± 0.06
Glycine (Gly)			0.90 ± 0.03	0.80 ± 0.03
Serine (Ser)			0.74 ± 0.03[a]	0.61 ± 0.01[b]
Proline (Pro)			0.84 ± 0.01	0.77 ± 0.03
Aspartic acid + Asparagine (Asp + Asn)			3.34 ± 0.17[a]	2.39 ± 0.07[b]
Hydroxyproline (Hyp)			0.09 ± 0.00	0.08 ± 0.00
Glutamic acid + Glutamine (Glu + Gln)			2.78 ± 0.08[b]	4.75 ± 0.06[a]
Total amino acid (TAA)			18.52	19.13
Total essential amino acids (TEAA)	175.5	12.29	8.79 (47.48)	8.67 (45.34)
Total acid amino acids (TAAA)			6.12 (33.04)[b]	7.14 (37.31)[a]
Total sulfur amino acids (TSAA)			0.52 (2.83)[b]	0.78 (4.10)[a]
Total aromatic amino acids (TArAA)			2.65 (14.32)[a]	1.98 (10.34)[b]
PER**			2.96	3.80

Notes: Values are g/100 g of total amino acid expressed as mean ± SE (standard error) of three replicates (*n* = 3; where otherwise noted), ND, not determined.

Means with different superscript lowercase letters in the same row indicate significant differences (*p* < 0.05).

Values in parenthesis are percentage of TAA.

*Essential amino acids and recommended daily intake values according to World Health Organization/Food and Agriculture Organization/United Nations University (WHO/FAO/UNU, 2007).

**Calculated g/100 g protein of amino acid.

(*p* > 0.05; Table 3). When compared to raw samples, the amounts and proportions (%) of total aromatic amino acids (TArAA; Phe, Tyr, Trp, and His) decreased (*p* < 0.05), whereas total sulfur amino acids (TSAA; Met and Cys) increased (*p* < 0.05) in *lakerda*.

Total essential amino acid (TEAA) ratios were determined lower in *lakerda* (45% of TAA) (Table 3), when compared to fresh fish. However, it has conclusively been shown that the total essential amino acids (TEAA) ratio of *lakerda* has respectively very high values (45%), given Food and Agriculture Organization/World Health Organization/United Nations University (FAO/WHO/UNU, 1985) standards state that 39, 26, and 11% are considered adequate protein rates for infants, children, and adults. In addition, requirement of the essential amino acids for adults (70 kg) is about 12.29 g per day [World Health Organization/Food and Agriculture Organisation/United Nations University (WHO/FAO/UNU), 2007] and 142 g of *lakerda* meets the daily requirement value. The protein efficiency ratio (PER) of *lakerda* was determined significantly higher compared to the standard reference PER of fish, that has PER of 2.7 (FAO/WHO/UNU, 1985). In general, PER below 1.5 approximately is described as poor quality; PER between 1.5 and 2.0 is described as an intermediate quality; and PER above 2.0 is described as high quality (Friedman, 1996; Oluwaniyi, Dosumu, & Awolola, 2010). On the other hand, the

use of amino acid scores has been proposed (Sarwar et al., 1984) as a more accurate alternative when compared to the use of PER (Friedman, 1996). This proposal being used evaluating protein quality worldwide (Iqbal, Khalil, Ateeq, & Sayyar Khan, 2006; Zhao, Zhuang, Song, Shi, & Zhang, 2010) is also supported by the Food and Agriculture Organization/World Health Organization (FAO/WHO, 1991). Table 4 compares the amino acid scores of present study to reference amino acid pattern of young people and adults (FAO/WHO/UNU, 1985), all the essential amino acid scores were higher than the reported value which are considered to be 100% (Harper & Yoshimura, 1993).

3.4. Fatty acid content

The composition of FAs is shown in Table 5. A total of 32 FAs were identified in this study. Unsaturated fatty acids (UFAs) contents were found to be 77.91% for fresh sample and 78.83% for *lakerda*, and these ratios were higher when compared to those of saturated fatty acids' (SFA). Palmitic acid (16:0) was the primary saturated fatty acid, followed by stearic acid (18:0) (Table 5).

Fish oils are now regarded as an excellent source of UFA, especially polyunsaturated fatty acid (PUFA) (Navarro-Garćıa, Pacheco-Aguilar, Bringas-Alvarado, & Ortega-Garćıa, 2004). However, PUFA is more oxidative than SFA. In addition, processing techniques, particularly salting, promote oxidation in fish. In the present study, PUFAs were higher in raw material, whereas salted sample showed a higher content ($p < 0.05$) of monounsaturated fatty acids (MUFAs). Oleic acid (18:1) was identified as the major MUFA in both fresh fish and *lakerda*.

It is known that PUFAs (separated $n−3$ and $n−6$) are the most important FAs for human health and nutrition (Weiss, Barrett-Connor, & von Muhlen, 2005; Wong, 2005). In our study, PUFA of not only Atlantic bonito, but also *lakerda* can be considered as a good source of the $n−3$ series fatty acids (Table 5), particularly of eicosapentaenoic acid (EPA) and DHA.

The $n−3$: $n−6$ ratio has been suggested to be a useful indicator for comparing relative nutritional values of fish oils (Zhao et al., 2010). According to current World Health Organization (WHO) recommendations, a daily ratio of $n−3$: $n−6$ in a balanced human diet should be lower than 1:5 (Vujković, Karlović, Vujković, Vörösbaranyi, & Jovanović, 1999). The $n−3$: $n−6$ ratio of *lakerda* was within the recommended value (Table 5) and it contains a balanced lipid composition for nutritional purposes. In addition, for a balanced and healthy diet, Simopoulos, Leaf, and Salem (1999) reported that recommended daily intake of two essential PUFAs (EPA + DHA) were 0.65 g/100 g in wet weight. Our

Table 4. Essential amino acid score (%) of Atlantic bonito and *lakerda*				
Amino acid	Standard WHO/FAO/UNU (2007) (g/100 g protein)	Essential amino acid composition (g/100 g protein)		Essential amino acid score (%)	
		Fresh	*Lakerda*	Fresh	*Lakerda*
Val	2.6	6.36 ± 0.31	9.26 ± 0.12	245	356
Leu	3.9	8.32 ± 0.54	10.32 ± 0.20	213	265
Ile	2.0	5.58 ± 0.12	7.46 ± 0.38	279	373
Thr	1.5	5.21 ± 0.27	5.68 ± 0.12	347	379
Met + Cys	1.5	4.96 ± 0.34	9.40 ± 0.14	331	627
Phe + Tyr	2.5	7.51 ± 0.29	8.85 ± 0.13	300	354
Lys	3.0	6.59 ± 0.12	7.66 ± 0.28	220	255
His	1.0	7.62 ± 0.15	4.66 ± 0.15	762	466
Trp	0.44	ND	ND	ND	ND
∑Essential	18.44	52.15 ± 1.42	63.29 ± 0.42		

Notes: ND—Not determined.

Table 5. Fatty acid compositions (%) of Atlantic bonito and *lakerda*		
Fatty acid	**Fresh**	*Lakerda*
C12	0.03 ± 0.01	0.03 ± 0.01
C13	0.02 ± 0.00	0.03 ± 0.00
C14	2.56 ± 0.24	2.68 ± 0.28
C15	0.55 ± 0.02	0.60 ± 0.06
C16	13.83 ± 0.29	11.49 ± 2.87
C17	0.58 ± 0.06	0.71 ± 0.00
C18	3.84 ± 0.40	4.73 ± 0.08
C20	0.33 ± 0.05	0.44 ± 0.05
C21	0.05 ± 0.01	0.06 ± 0.01
C22	0.16 ± 0.03	0.21 ± 0.03
C23	0.04 ± 0.01	0.05 ± 0.01
C24	0.12 ± 0.03	0.12 ± 0.05
$\sum$SFA	22.10 ± 0.11	21.17 ± 2.98
C14:1	0.31 ± 0.09	0.22 ± 0.03
C15:1	0.03 ± 0.02	0.05 ± 0.01
C16:1	8.36 ± 0.41	8.70 ± 1.04
C17:1	0.65 ± 0.01	0.72 ± 0.04
C18:1n−9c	19.05 ± 0.98	21.68 ± 1.35
C18:1n−9t	3.27 ± 0.04	2.83 ± 0.22
C20:1n−9	2.68 ± 0.34	3.62 ± 0.31
C22:1n−9	2.05 ± 0.30	3.02 ± 0.56
C24:1	0.05 ± 0.01	0.07 ± 0.01
$\sum$MUFA	36.45 ± 0.87[b]	40.91 ± 1.30[a]
C18:2n−6c	4.13 ± 0.51	4.98 ± 0.11
C18:2n−6t	1.04 ± 0.39	0.85 ± 0.08
C18:3n−3	0.56 ± 0.07	0.33 ± 0.05
C18:3n−6	0.29 ± 0.07	0.31 ± 0.03
C20:2	0.70 ± 0.04	0.78 ± 0.05
C20:3n−3	0.87 ± 0.09	1.09 ± 0.11
C20:3n−6	1.22 ± 0.09	1.04 ± 0.09
C20:4n−6	2.46 ± 0.11	2.30 ± 0.25
C20:5n−3 (EPA)	6.86 ± 0.28	6.69 ± 0.81
C22:6n−3 (DHA)	22.52 ± 0.99	18.79 ± 2.27
C22:2	0.81 ± 0.03	0.76 ± 0.10
$\sum$PUFA	41.46 ± 0.94	37.92 ± 3.41
$\sum n$−3	30.81 ± 1.07	26.89 ± 3.02
$\sum n$−6	9.14 ± 0.22	9.49 ± 0.37
n−3:n−6	3.38	2.83

Notes: Values are percentage of total fatty acid expressed as mean ± SE (standard error) of three replicates ($n = 3$; where otherwise noted).

Means with different superscript lowercase letters in the same row indicate significant differences ($p < 0.05$).

results indicated that, EPA + DHA value in *lakerda* was 3.99 g/100 g in wet weight (Table 6) and sufficient daily intake value was 16.65 g. In this regard, *lakerda* appears to be the most valuable food in terms of EPA + DHA intake.

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Table 6. Fatty acid compositions (g/100 g wet weight) of Atlantic bonito and *lakerda*										
	Lipid	**∑SFA[a]**	**∑MUFA[a]**	**∑PUFA[a]**	**∑n−3[a]**	**∑n−6[a]**	**EPA[a]**	**DHA[a]**	**EPA + DHA[a]**	**PQ[b]**
Fresh	13.19	2.91	4.81	5.47	4.06	1.21	0.90	2.97	3.87	16.78
Lakerda	15.65	3.31	6.40	5.93	4.21	1.48	1.05	2.94	3.99	16.30

[a]Values (g/100 g wet weight) were obtained with conversion of the percentile values to units the formulae recommended.

[b]Quantity of product (g/100 g wet weight) which can provide a human with the recommended daily quantity of EPA + DHA fatty acids of 0.65 g.

3.5. Sensory profile

Sensory tests, of course, have been conducted by human beings to evaluate the goodness and badness of food (Meilgaard et al., 1999) via their senses (i.e. tasting, smelling, touching, etc.). The descriptive analysis involves the detection (discrimination) and the description of both the qualitative and quantitative sensory aspects of a product by trained panels. The Figure 1 has presented the average values obtained in the evaluation of different attributes related with: color, odor, flavor, and texture of *lakerda* by the sensory panel. The predominant characteristics of *lakerda* were perceived as broken white (4.7) and uniform (3.9) for color evaluation. Salted fish odor (4.5) and sweet odor (4.2) were observed as typical attributes of *lakerda*. The flavor of *lakerda* was dominantly described by panelist as "salted fish" (4.2) and "salty" (4.0). In addition, predominant texture was perceived as chewy (3.6) and cohesive (3.5) followed by soft (3.1) and juicy (3.0).

Although *lakerda* is produced by salting techniques, there are differences from other salted fish products in terms of sensorial properties. Previous studies on salted fish products reported that the texture was hard and juiceless (Lauritzsen et al., 2004; Martinez, Salmerón, Guillén, Pin, & Casas, 2012),

Figure 1. Mean values and standard errors of descriptive sensorial attributes of *lakerda*.
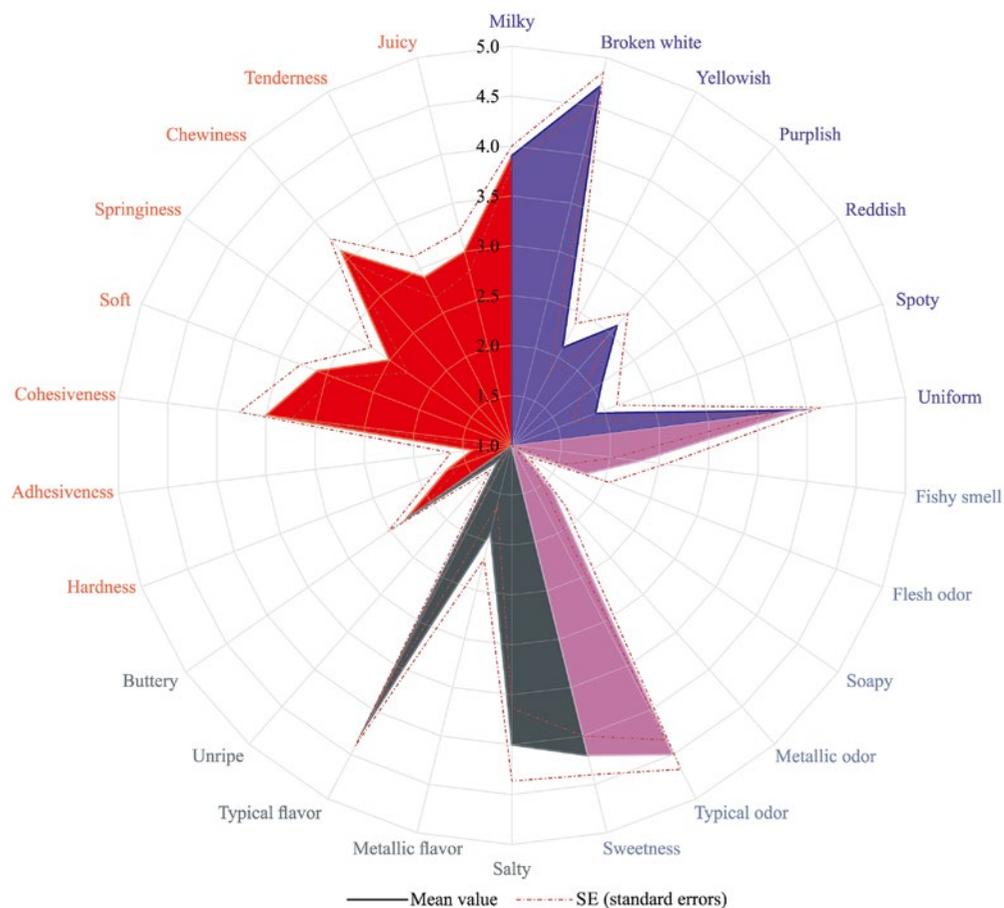
while color was yellowish brown (Chaijan, 2011; Czerner, Tomás, & Yeannes, 2011). However, the results of this study showed that *lakerda* has soft rubbery texture, similar to the texture of the Turkish delight and bright broken white color.

4. Conclusion

In summary, this paper demonstrates the nutritional and sensory characterizations of *lakerda*, a traditional and highly appreciated salted fish product. *Lakerda* has a broken white, milky, and uni-form color; salted fish and sweat-smelling odor; salty flavor and salted fish-like flavor and cohesiveness and chewiness in texture. It was determined in this study that *lakerda* has outstanding sensory and nutritional characteristics with its high protein quality and is a lipid source with a well-balanced composition of essential amino acids and fatty acids. In this respect, these results may benefit the fishing industry, nutritionists and researchers who aim to improve the nutritive value, processing and marketing of fish.

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