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Ayşe Ozyilmaz
Iskenderun Technical University, Faculty of Marine Science and Technology, Department of Marine Technologies, Iskenderun/Hatay, Turkey

Aydin Demirci
Iskenderun Technical University, Faculty of Marine Science and Technology, Department of Marine Technologies, Iskenderun/Hatay, Turkey

Dilsat Bozdogan Konuskan
Mustafa Kemal University, Faculty of Agriculture, Dep of Food Engineering, Hatay, Turkey

Sevil Demirci
Iskenderun Technical University, Faculty of Marine Science and Technology, Department of Marine Technologies, Iskenderun/Hatay, Turkey

Correspondence
Dilsat Bozdogan Konuskan
Mustafa Kemal University, Faculty of Agriculture, Dep of Food Engineering, Hatay, Turkey

Macro minerals, micro minerals, heavy metal, fat, and fatty acid profiles of European hake (*Merluccius merluccius* Linnaeus, 1758) caught by gillnet

Ayşe Ozyilmaz, Aydin Demirci, Dilsat Bozdogan Konuskan and Sevil Demirci

Abstract
The present study was conducted to investigate macro minerals (Mg, Na, P, Ca), micro minerals (Zn, Cu, Fe), heavy metal (Pb) crude lipid level, and fatty acid profiles of European hake (*Merluccius merluccius* Linnaeus, 1758) caught by gillnet in March in 2013 in the northeastern Mediterranean. The levels of the micro minerals were calculated in the shown order; Fe>Zn>Cu for European hake. The average crude lipid level of European hake was calculated to be lower than 1.3%. Additionally, the levels of the saturated fatty acids (SFA) in lipid of the European hake were calculated to be lower than 30%. In addition, the sum of monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) levels were calculated to be 58.95%. Moreover, the average levels of eicosapentaenoic acid (EPA, C20:5n3) and docosahexaenoic acid (DHA, C22:6n3), noble omega-3 fatty acid, in lipid of the European hake were found to be 26.48% and 4.93% respectively.

Keywords: macro-micro minerals, heavy metal, fatty acid, european hake *Merluccius merluccius*

1. Introduction
European hake (Turkish names; bako, berlam or bakalyaro), is one of the important demersal species widely distributed in Mediterranean and is a commercially important fish species constitute a great amount of landings, by volume, along the Mediterranean Sea of Turkey. According the TURKSTAT [1], total catch European hake was reported to be 4100-642 tons in 2005-2014, respectively. Therefore, there is a risk of overexploitation for the Mediterranean hake stocks [2]. This species, has a large bathymetric distribution of 30-80m, is the target for bottom trawl, long line and gillnet fisheries [3, 4]. Especially in artisanal fishing such as gill net, it is exploited as density [5]. Additionally, European hake is also commonly preferred consumed food item because of its delicious taste and its unique aroma. It has a very high price compared to many seafood in fish markets around the region.
A considerable amount of published article support the fact that long chain omega 3 fatty acids (eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have many beneficial effects on some preventing or slowing the some diseases e.g., cardiovascular and anti-inflammatory properties which levels of their consumption are rare or insufficient [6-11]. Nowadays, some considerable attention has been pointed out that consuming fish or sea product have some beneficial actions to promote body functioning.
Elements (e.g., macro minerals, micro minerals, heavy metals) are important parts of fish muscle. Taking them via food has many advantages to human body. Some macro and micro minerals are the essential elements and have benefits to the human while some other elements are toxic and called heavy metals [12]. Even though minerals exist in very small amounts, they have effect on the majorities to function properly [13].
Fish are one of the food items in diets of many countries. Knowing some biologic aspects of the fish (e.g., lipid level, fatty acid profile, and mineral contents) can help consumers, scholars, and processers for many different reasons. There is getting an interest in the beneficial health effects on health issue for body that is related to consumption of fish lipids. Determination of some biochemical properties of European hake might provide valuable information concerning the nutrient value of this species for consumers who wants to know detailed information about

consumed product, the researchers searching on nutrient tables, and for the fish processing industry. The objective of this current study is to investigate macro minerals (Mg, Na, P, Ca), micro minerals (Zn, Cu, Fe), heavy metal (Pb), crude lipid level, and fatty acid profiles of European hake (*Merluccius merluccius* Linnaeus, 1758) caught by gillnet in the northeastern Mediterranean.

2. Material and Methods

European hake (*Merluccius merluccius* Linnaeus, 1758) caught by gillnet in March in 2013 in the northeastern Mediterranean and immediately iced. Fish were brought to the laboratory for the analysis. Fish muscle mixed to prepare each analysis. All chemicals used in this study were in analytical grade and obtained from Merck (Darmstadt, Germany) and Sigma Aldrich (St. Louis, MO, USA).

2.1 Crude Lipid Analysis: Determination was carried out by using modified Bligh and Dyer method [14]. To perform to analyse, a total of 10 g mixed fish muscle was weighed for each analysis. A total of 40 ml chloroform and methanol were used in the experiment. A detailed information is described ozyilmaz *et al*., [15].

A GC MS (Gas Chromatography Mass Spectrophotometry) was used to determine the fatty acids of European hake. Fatty Acids Methyl Ester's preparation and chromatographic conditions were as given below.

2.2 Fatty Acid Methyl Esters (FAMEs) Preparation: The conversion and separation of FAMEs were carried out as described in Ozyilmaz [16].

2.3 Chromatographic Conditions: Fatty acid determination were performed by using a GC-MS (Gas Chromatography-Mass Spectrometry (GC-MS) [a Hewlett Packard GC (model 6890)] coupled with a Hewlett Packard model 5972A HP 6890 system MS detector. Fatty acids were separated using an HP-INNOWAX Polyethylene Glycol Capillary Column, Model Number: HP 19091N-133.

The oven programme and the identification of individual fatty acids were performed as described in Ozyilmaz [17].

2.4 Extraction and Determination of Macro and Micro Minerals: The wet digestion method was performed to digest the muscle of European hake. The following steps were carried out according to the AOAC Method 975.03 with a few minor modifications. The sample was weighed, and a total of 1.5 mL of 60% perchloric acid (Merck, Darmstadt, Germany) was added to samples. The sample was incubated a few hours. Then, 8.5 mL of 65% HNO_3 (Merck, Darmstadt, Germany) was added to the samples. Next step was heating sample on a hot plate until they were firmly digested. The samples were removed from the hot plate until they were cool. The digests were filtered into a 20 mL volumetric flask, using ash-free filter paper, and filled to 20 mL with ultra-pure water. The determination and quantification of macro and micro minerals were performed by using an ICP-AES (Inductively Coupled Plasma-Atomic Emission Spectrometry, Varian Model-Liberty series II). Calibration curves for each of the individual elements were prepared from ICP Multi element stocks (Merck, Darmstadt, Germany). Wavelengths of Cu, Fe, Pb, Zn, Ca, Mg, and Na, were determined at 324.754, 259.940, 220.353, 213.856, 396.847, 285.213, and 588,995 λ (nanometer), respectively.

2.5 Statistical Analysis: Analysis of descriptive statistics (means and standard deviations) for the results of macro minerals (Mg, Na, P, Ca), micro minerals (Zn, Cu, Fe), heavy metal (Pb), crude lipid level, and fatty acid profiles of European hake were carried out by using SPSS 22.

3. Results and Discussion

The lipid content of fish is generally known to be one of the main changes in the body composition and affected by several reasons e.g., species, stage age, maturity, sex, availability of food season, and diet [15, 16, 17, 18]. According to Perugini diet of Eurepean hake from central Adriatic Sea is mainly small hakes, anchovies, pilchards, herrings, cod fishes, sardines and gadoid species, and squids [8].

The average lipid content of the European hake in this present study was found to be 1.21±0.4%. According to Love [20] fish generally has 0.2-25% fat content. The lipid level finding of this current study is in the range of Love's reporting [20] about fish lipid level. According to Ackman's [21] fish can be classified by its lipid content. He graded the fish in four categories regarding its lipid levels 1) Lean (<2%), 2) Low-fat (2-4%), 3) Medium-fat (4-8), and 4) High-fat (>8%). The European hake used in the present study can be classified as lean fish. Even though it is a lean fish it tastes great and its price is much higher than many fish that can be categorized in different lipid levels.

Ozogul *et al* [17] determined the lipid level of European hake and reported 0.76%. This previously reported lipid level European hake is lower than that of fish in this current study. Some of these differences could be the results of different fishing time, the availability of food in the environment, and life stages of the fish.

Unlike other proximate components, lipid levels show a wide range in fish. According to Murua and Motos [22], European hake in Bay of Biscay spawns all year long and the spawning fraction was highest between December and March. The European hake in this present study caught in March in Iskenderun Bay. Therefore the lipid level of the fish could be affected by spawning period.

Additionally, Fatty acid profiles (% of total fatty acids) of European hake used in the present study were given in Table 1. The total amounts of the saturated fatty acids (SFA) in lipid of the European hake were found to be higher than those of in lipid of the monounsaturated fatty acids (MUFA) and lower than that polyunsaturated fatty acids (PUFA). Similar results were previously reported for European hake from Mediterranean Sea, Adriatic Sea, Tyrrhenian Sea, and Ionian Sea [18, 23].

In SFA, palmitic acid (C16:0) was the predominant fatty acid in lipid of European hake followed by Stearic acid (C18:0) with the value of 17.50% and 7.00%, respectively. While the finding of C16:0 in European hake in this current study was found to be lower than that of European hake from Mediterranean Sea, Adriatic Sea, Tyrrhenian Sea, and Ionian [18, 23], the levels of C18:0 in European hake were in accordance with previously reporting.

The average level of oleic acid (C18:1n9) in European hake was found to be the major fatty acid in MUFA with the value of 12.645. Similar findings were reported for European hake from Mediterranean whereas higher values were reported European hake from Adriatic Sea, Tyrrhenian Sea, and Ionian Sea [23].

In PUFA α-Linolenic acid (C18:3n3, ALA), arachidonic acid (C20:4n6, ARA or AA), eicosapentaenoic acid (EPA, C20:5n3) and docosahexaenoic acid (DHA, C22:6n3) were

calculated to be in the shown order; ALA<ARA<EPA<DHA in lipid of European hake. Additionally, a high level of n3, as it was calculated in edible part of European hake in this current study, is very beneficial for body due to its positive health effects which prevent or delay some health problems such as heart related disease risk factors [24].

A total of 8 elements, macro minerals, micro minerals, and heavy metal were determined in flesh of European hake. Determination of minerals and heavy metals in seafood is crucial for labeling requirements and giving knowledge about raw materials in nutritional point of view. This types of information gives consumers a changes to choose healthy products or what they want. Nowadays, it is well known that minerals are important for promoting body function properly. According to Belitz and Grosch [25] minerals had effects on important components of enzymes, hormones, and enzyme activators.

The human body system requires some minerals to maintain the organism; these are called essential minerals. Essential minerals can be categorized to major minerals (called macro minerals) and trace minerals (called micro minerals). These two groups of minerals are important to have a balanced diet for a healthy life, but micro minerals are generally needed in smaller amounts comparing macro minerals [12].

Table 1: Fatty acid profiles (% of total fatty acids) of European hake

Fatty Acids	MM1	MM 2	Mean	SD
C12:0	2.14	1.26	1.70	0.62
C14:0	0.88	0.94	0.91	0.04
C15:0	0.74	0.89	0.82	0.11
C16:0	17.87	17.12	17.50	0.53
C17:0	0.76	0.61	0.69	0.11
C18:0	7.12	6.88	7.00	0.17
C20:0	0.98	0.89	0.94	0.06
SFA	30.49	28.59	29.54	1.64
C16:1	3.70	2.39	3.05	0.93
C17:1	0.90	0.88	0.89	0.01
C18:1n9	12.57	12.71	12.64	0.10
C20:1n9	0.41	0.58	0.50	0.12
MUFA	17.58	16.56	17.07	1.16
C18:2n6	1.99	1.87	1.93	0.08
C18:3n6	0.88	0.98	0.93	0.07
C20:4n6	4.12	3.42	3.77	0.49
n6	6.99	6.27	6.63	0.65
C18:3n3	2.50	2.09	2.30	0.29
C20:5n3	4.92	4.94	4.93	0.01
C22:5n3	1.18	1.92	1.55	0.52
C22:6n3	26.96	25.99	26.48	0.69
n3	35.56	34.94	35.25	1.51
PUFA	42.55	41.21	41.88	2.16
MUFA+PUFA	60.13	57.77	58.95	3.32
n6/n3	0.20	0.18	0.19	0.43
n3/n6	5.09	5.57	5.32	2.33
DHA/EPA	5.48	5.26	5.37	0.50

MM: each mixed muscle was composed of six individual fish muscle, SD: Standard Deviation

Macro minerals, Ca (Calcium), Mg (Magnesium) Na, (Sodium), and P (Phosphorus), in muscle of European hake were shown in Table 2. Seafood contain considerable amounts of minerals such as Ca, Mg, Na, P, and K (Potassium) because of the importance of seafood mineral content, edible part of European hake's mineral content were searched and results were given in Table 2. The average macro minerals were found to be in the range of 876.84 to 195.47 ppm. Similar results were found in some other fish species regarding their macro mineral contents [26, 27, 28].

Table 2: Macro minerals of European hake (mg/kg)

Macro Minerals	MM 1	MM 2	MM 3	Mean	SD
Ca (Calcium)	197.20	166.74	222.46	195.47	27.90
Mg (Magnesium)	196.62	202.35	215.74	204.91	9.81
Na (Sodium)	485.24	490.64	480.83	485.57	4.91
P (Phosphorus)	889.33	839.73	901.47	876.84	32.71

MM: Each mixed muscle was composed of six individual fish muscle, SD: Standard Deviation

Micro minerals [Fe (Iron), Zn (Zinc), Cu (Copper)] and heavy metal [Pb (Lead)] in muscle of European hake were depict in Table 3. A very high intake of Pb may cause many health problems and affect some organs such as liver, kidney [29].

As it mentioned before, body generally needs micro minerals in very small amounts. Even though Fe is considered to be a trace mineral, the amount needed for Fe is more than comparing all other micro minerals. A balanced Fe in diet is considered to be very crucial for avoiding some health problems [25, 30].

In a similar study regarding Cu and Zn levels, Perugini [19] reported than mean levels of Cu and Zn in muscle of European hake a place near Jabuka Pit, a depression in the center of the Adriatic/Italy, as 5.67 mg/kg and 22.93 mg/kg. These values were higher than those obtained in this study.

Table 3: Micro minerals and heavy metal of European hake (mg/kg)

Micro Minerals and Heavy Metal	MM 1	MM 2	MM 3	Mean
Fe (Iron)	0.457	0.112	0.352	0.307
Zn (Zinc)	1.254	1.275	1.280	1.270
Cu (Copper)	0.069	0.152	0.009	0.077
Pb (Lead)	0.001	0.001	0.002	0.001

MM: Each mixed muscle was composed of six individual fish muscle

Like all heavy metals, Pb is a toxic heavy metal and potentially harmful to most organisms at some level [31]. The average level of Pb in muscle is at acceptable levels for human consumption designated by various health organizations (FAO and WHO) According to FAO and WHO, tolerable daily intake of Pb by for a 70 kg person is 0.24 mg/day. Findings regarding the mean value of Pb in European hake in this present study in Mediterranean region. In this current study the same data was calculated to be lower than that of provided information. Some scientific studies showed that bottom-dwelling fish species may accumulate higher levels of heavy metals than pelagic ones [32, 33].

4. Conclusion

Consequently, it can be concluded that macro and micro minerals were found to be adequate for a well-balanced diet. Even though the lipid levels of European hake is in lower amount, it has rich fatty acid compositions. Moreover, the average level of Pb which is a toxic heavy metal was present at levels below its hazard level. Even though European hake has got lower lipid level, it is tastes perfect and contains excellent fatty acid beneficial for health.

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