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Research Article

A study of nutritional properties in European pilchard, Golden grey mullet, and Common sole caught in Mersin Bay, North-east Mediterranean Sea

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Keywords

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ABSTRACT

European pilchard (*Sardina pilchardus*, Walbaum, 1792), Golden grey mullet (*Liza aurata*, Risso 1810), and Common sole (*Solea solea*, Quensel, 1806) are the commercially important fish species caught by trawl and purse seine in Mersin Bay, in the Mediterranean. This study was aimed to investigate proximate compositions, carbohydrate levels, energy contents, fatty acid profiles, and the lipid quality indexes of European pilchard, Golden grey mullet, and Common sole caught by trawl. The crude protein, lipid, moisture, and ash content were determined as proximate compositions. The protein levels were determined as the highest levels in sardine (24.08%) followed by the common sole (18.91%) and golden grey mullet (18.01%). An inverse correlation between the moisture contents and the lipid levels among the fish was observed in this study. The ash levels, carbohydrate values, and energy contents were in the following order: European pilchard > Golden grey mullet > Common sole. Palmitic acid (C16:0) and oleic acid (C18:1n9) were the highest level of fatty acids in SFA (saturated fatty acids) and MUFA (monounsaturated fatty acids) for the Golden grey mullet and Common sole, respectively. The levels of eicosapentaenoic acid (EPA) in all fish used in this study were found to be different from each other ($P<0.05$). The amount of docosahexaenoic acid (DHA) were lower than that of EPA in European pilchard and Golden grey mullet. Although the levels of EPA and DHA in fish are found to be in the range of 4.91 - 11.01% and 6.85 - 8.87%, respectively and different from each other, these two fatty acids are good for health.

Introduction

European pilchard, Golden grey mullet, and Common sole caught by trawl and purse seine are three fish species that are economically important for the people around the Mersin Bay, in the north east Mediterranean. The prices for these fish are affordable comparing other fish species. According to Turkish Statistical Institute (Turkstat 2019) report, the share of the total catch for European pilchard, Golden grey mullet, and Common sole were averagely calculated to be 8.6%, 0.2%, and 0.8% from 2017 to 2018 total catch rate of the whole production quantity of the sea fish in Turkey. The reports from 2011 to 2020 relating the total catch rates of the European pilchard, Golden grey mullet, and Common sole show a declining pattern (Turkstat, 2021).

Along with other small pelagic fish, the European pilchard has an important place in the sea. In the last two decades, some fish species have declined in the north eastern Mediterranean Sea, Turkish coastal for that matter, some scientific studies were conducted to gather information to highlight the importance of this issue (Ozyilmaz et al., 2017a, b; Demirci ve Şimşek, 2019; Demirci et al., 2019; Şimşek et al., 2019). Some of the studies were about the changes in biological and ecological traits of these fish and their relationship with environmental variables (Lloret-Lloret et al., 2022), lipid quality (Duricic et al., 2022), seasonal changes in biochemical compositions (Simat et al., 2020), and feeding one year in captivity (Bandarra et al., 2018).

Golden grey mullet is another fish species that belongs to the Mullidae family and has a great value for people around the region. Along with other fish used for this study, golden grey mullet is generally readily available in the fishing season at reasonable prices. Total catch amounts of the Golden grey mullet were reported to be 2182 and 1416 tonnes in 2019 and 2020, respectively (Turkstat, 2021). Along with the other mugilidae,

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Golden grey mullet has a wide range of distribution areas e.g., in tropical, subtropical, and temperate waters as well as coastally in estuaries and freshwater (Nelson 2006). This fish species is economically very important not only for its muscle taste but also as other raw materials e.g., its roe (Hung and Shaw 2006, Turan 2016).

The Common sole has a very special place on tables for the people in the Mediterranean Sea as well as other seas and is one of the economically important and highly demanded fish species. These attributes made common sole very substantial research topic for scientists and producers. In order to catch to demand common sole, there were designs for some projects to provide information about rearing conditions (Parma et al., 2019, Morais et al., 2016).

The European pilchard, Golden grey mullet, and Common sole are the fish species that are affordable comparing other fish species on the counter and have nutritional values for the consumers. Even though there are few studies conducted on these fish species (Simat et al., 2020; Kucukgulmez et al., 2018; Özogul and Özogul 2007; Gökçe et al. 2004), there should be more studies needed in this field. The aim of this study is to explore the biochemical aspects of the European pilchard, golden grey mullet, and common sole. For this matter, proximate compositions (protein, lipid, moisture, and ash), carbohydrate levels, energy contents, fatty acid profiles, and the lipid quality indexes of these fish were investigated for this study.

Materials and Methods

European pilchard (*Sardina pilchardus*, Walbaum, 1792), Golden grey mullet (*Liza aurata*, Risso 1810), and Common sole (*Solea solea*, Quensel, 1806) are caught by professional fishermen by trawl and purse seine in February, March, and April in Mersin Bay in the Mediterranean (Figure 1). The fish came from both catching methods and three different catching times mixed together and prepared for the biochemical analysis.

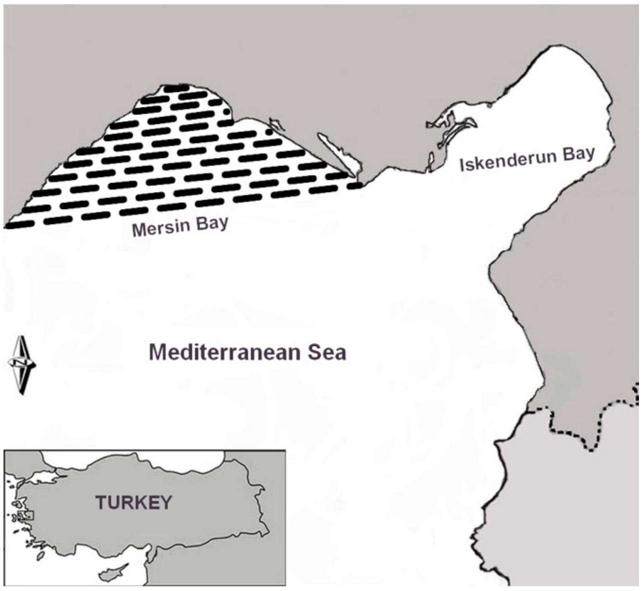

Figure 1. Study area

Proximate compositions, carbohydrate levels, and energy contents

The proximate compositions are composed of the crude protein, lipid, moisture, and ash in this study. The crude protein content was calculated by using nitrogen content obtained by the Kjeldahl method which may be divided into three steps: digestion, neutralization, and titration.

1. Digestion step; The fish samples were practically weighed into a digestion tube with a precision of 0.001 g added 3 Kjeldahl tablets for each tube and then digested by heating it in the presence of sulfuric acid.

2. Neutralization step; After the digestion has been completed, the digestive tube is placed in the Kjeldahl distillation unit. The deionized water and NaOH solution (%40) were added automatically to the distillation unit. The separation of the nitrogen from the digested mixture was performed by steam distillation and trapped the distilled vapors in a dedicated solution of boric acid.

3. Titration step; The nitrogen content is then estimated with titration by using a suitable indicator to determine the endpoint of the reaction. A conversion factor of 6.25 was used for the calculation of protein content (Anonymous, 1992).

Determination of crude lipid was carried out by using a modified Bligh and Dyer method (Hanson and Olley, 1963). For each fish species, approximately 10 g of muscle from the mixed samples was weighed and placed into a homogenization flask. A total of 8 mL of pure water, 20 mL of chloroform, and 40 mL of methanol were added, respectively. After all addition, the mixture was homogenized for 1 minute. An additional 20 ml of chloroform was added and the mixture was homogenized for 0.5 minutes. Finally, 20 ml of water was added and the mixture was homogenized for a further 0.5 minutes. The homogenate was centrifuged for 10 minutes at 3000 rpm. After centrifugation, the aqueous layer was removed by aspiration. A total of 20 ml of the chloroform layer was transferred into a dry pre-weighed round-bottom flask. The chloroform was evaporated by using a rotary vacuum evaporator at 45-55 °C. Final drying was performed in an oven at 105 °C for 30 minutes. The flask was allowed to cool to room temperature in a desiccator for approximately 15 minutes and weighed. The following equation (Percentage of lipid in the fish) was used to calculate the lipid content in the sample.

$$\% \text{ Lipid (B \& D)} = \frac{W_L}{W_S} \times \frac{V_1}{V_2} \times 100$$

Where:

WL= Weight of the lipid extracted (g)

WS= Weight of the sample (g)

V1 = Total volume of chloroform that used for lipid extraction (mL)

V2= Volume of chloroform used for evaporation (mL)

Fatty Acid Compositions

A Gas Chromatography-Mass Spectrometry (GC-MS) was used to determine fatty acids of the lipids belonging to European pilchard, golden grey mullet, and common sole. After obtaining lipid from each of these three fish, fatty acid methyl esters were prepared. The GC-MS (a Hewlett Packard GC, model 6890) and coupled with Hewlett Packard (model 5972A, HP 6890 system) MS detector was used for the analysis. Separations of fatty acids were performed with HP-INNOWAX Polyethylene Glycol Capillary Column (Model number HP 19091N-133, 0.25 mm * 30m * 0.25 μm) and HP 6890 automatic injection system was used.

First of all, the injection and detector temperatures were set at 250°C and 270°C, respectively. The split ratio was arranged at 1:20 with a total injection volume of 1 μl. secondly, the injector was washed three times with iso-octane in order to avoid any contagion. Finally, the post-injection, injector program was also set to triple wash of injector for the next injection.

The oven temperature was programmed initially at 120°C and held for 3 minutes. Then, the temperature was increased to 250°C with a 10°C per minute ramp rate and hold at this temperature for nine minutes. Total separation was achieved in 25 minutes. Identification of individual fatty acids was made by comparing the retention time of FAME standard (Supelco 47085U PUFA No: 3) and Supelco 37 component Fame mix (Supelco 47885-U). Confirmation of fatty acid methyl esters was also performed by using the MS database library (FAMEDBWAX). Further information was provided in Özyılmaz and Palalı (2014).

Lipid Quality Indexes

The identified fatty acid composition of the European pilchard, Golden grey mullet, and Common sole were practically used to calculate atherogenicity Index (AI) and thrombogenicity Index (TI). The equations given below are used to calculate Lipid Quality Indexes, AI, and TI which were reported by Ulbricht and Southgate (1991).

AI=[(4xC14:0)+C12:0+C16:0]/[(ΣPUFA-n6+ΣPUFA-n3)+ΣMUFA]

IT=[(C14:0+C16:0+C18:0)/(0.5xMUFA+0.5xPUFA-n6+3xPUFA-n3+PUFA-n3/PUFA-n6)]

Also, the polyene index (PI) was calculated by using the following equation which gives an idea about the PUFA damage that was previously reported by Lubis and Buckle (1990).

(PI) = (C20:5 + C22:6)/C16:0

Statistical analysis

Statistical analysis was performed with SPSS (22.0). Significance was established at $P<0.05$. The data of this study regarding different fish species were subjected to one-way analysis of variance (ANOVA), and a mean comparison was carried out by using Duncan's Multiple Range Test. Before the ANOVA analysis was performed, the homogeneity of variances was tested.

Results

Proximate compositions, carbohydrate levels, and energy contents of the European pilchard, Golden grey mullet, and Common sole were given in Table 1. The average protein levels of the fish were found to be in the range of 18% to 24% which is good for the consumers. The golden grey mullet and common sole are lean fish with values of 0.67% and 0.32%, respectively, while the European pilchard is low-fat fish with an average level of 2.44% in their lipid amounts.

There is an inverse correlation between the moisture content and the lipid level in fish in this current study. The ash content of the fish diverged ($P<0.05$) from 1.17 to 1.61 (Table 1). Even though carbohydrate and energy amounts in all fish used in this study differed from each other, these differences were found to be statistically significant only in European pilchard to the other two fish species. Although, the data of the carbohydrate and energy amounts in Golden grey mullet and common sole showed certain differences. These differences were found to be statistically insignificant ($P>0.05$).

Table 1. Proximate compositions, carbohydrate levels, and energy contents of the European pilchard, Golden grey mullet, and Common sole

	European pilchard	Golden grey mullet	Common sole
Protein (%)	24.08 ± 0.94^{a}	18.01 ± 0.52^{b}	18.91 ± 0.49^{b}
Lipid (%)	2.44 ± 0.26^{a}	0.67 ± 0.14^{b}	0.32 ± 0.05^{c}
Moisture (%)	70.37 ± 1.42^{a}	79.07 ± 0.80^{b}	79.43 ± 0.53^{b}
Ash (%)	1.61 ± 0.05^{a}	1.37 ± 0.04^{b}	1.17 ± 0.07^{c}
Carbohydrate (%)	1.51 ± 0.95^{a}	0.89 ± 0.38^{b}	0.18 ± 0.05^{b}
Energy	523.64 ± 20.87^{a}	345.21 ± 11.92^{b}	335.93 ± 10.31^{b}

[a,b,c] Values within same row with different superscripts diverge significantly at $p<0.05$

On the other hand, the fatty acid profiles and lipid quality indexes of the European pilchard, golden grey mullet, and Common sole were tabulated in Table 2. While a total of 27 fatty acids was determined for European pilchard and golden grey mullet, a total of 25 fatty acids were determined for Common sole in the present study. The highest saturated fatty acids and monounsaturated fatty acids were observed in European pilchard followed by Golden grey mullet and Common sole, respectively. The average levels of PUFA (polyunsaturated fatty acids) in golden grey mullet and common sole were found to be 37.77% and 37.15 % which are close to each other.

The highest saturated fatty acid components were in the following order: palmitic acid (C16:0)> myristic acid (C14:0)>stearic acid (C18:0) for European pilchard and C16:0>C18:0>C14:0 for Common sole in this study. Additionally, lauric acid (C12:0), pentadecanoic acid (C15:0), heptadecanoic acid (C17:0), and arachidic acid (C20:0) were found in lower amounts in all fish used in this study. The individual monounsaturated fatty acid components differed from each other regarding their existence levels. The levels of palmitoleic acid (C16:1n9) were the major fatty acid in monounsaturated fatty acid components for European pilchard, while those of oleic acid (C18:1n9) were the

Table 2. The fatty acid profiles and lipid quality indexes of the European pilchard, Golden grey mullet, and Common sole

Fatty Acids	European pilchard	Golden grey mullet	Common sole
C12:0	0.12±0.01[a]	1.13±0.07[b]	0.28±0.01[c]
C14:0	11.20±0.20[a]	3.83±0.14[b]	2.13±0.10[c]
C15:0	0.62±0.01[a]	0.55±0.07[a]	1.31±0.06[b]
C16:0	23.56±0.48[a]	20.75±0.67[b]	14.91±0.71[c]
C17:0	0.41±0.01[a]	0.82±0.03[b]	1.59±0.03[c]
C18:0	5.34±0.11[a]	6.77±0.28[b]	4.83±0.07[a]
C20:0	0.34±0.01[a]	1.55±0.06[b]	2.48±0.01[c]
∑SFA	**41.57[a]**	**35.40[b]**	**27.52[c]**
C16:1 n9	13.90±0.22[a]	5.72±0.28[b]	3.16±0.04[c]
C16:1 n7	0.42±0.01[a]	0.28±0.01[a]	1.39±1.48[a]
C17:1	1.40±0.04[a]	0.21±0.01[b]	0.44±0.01[c]
C18:1 n9	5.20±0.22[a]	11.48±0.01[b]	7.89±0.00[c]
C18:1 n7	4.16±0.04[a]	2.96±0.05[b]	2.93±0.04[b]
C20:1n9	0.17±0.01a	1.24±0.04[b]	1.86±0.01[c]
∑MUFA	**25.24[a]**	**21.87[b]**	**17.66[c]**
C22:2	0.58±0.01[a]	0.96±0.05[b]	ND
C18:2 n6	1.08±0.04[a]	5.77±0.09[b]	4.08±0.07[c]
C18:3 n6	0.45±0.01[a]	0.52±0.01[b]	0.91±0.02[c]
C20:2n6	0.12±0.01[a]	1.24±0.05[b]	1.36±0.15[b]
C20:3n6	0.38±0.00[a]	1.52±0.01[b]	1.78±0.48[b]
C20:4n6	2.65±0.04[a]	3.81±0.01[b]	4.08±0.13[c]
C22:4n6	0.19±0.01[a]	0.83±0.01[b]	1.81±0.01[c]
C22:5n6	0.55±0.00[a]	0.49±0.41[a]	2.10±0.31[b]
C18:3 n3	0.36±0.00[a]	1.96±0.02[b]	0.81±0.01[c]
C18:4 n3	0.95±0.01[a]	1.02±0.01[a]	1.81±0.04[b]
C20:4n3	0.47±0.03[a]	0.84±0.01[b]	ND
C20:5n3	11.01±0.55[a]	9.03±0.04[b]	4.91±0.02[c]
C22:5n3	1.37±0.01[a]	2.48±0.07[b]	3.95±0.06[c]
C22:6n3	8.87±0.28[a]	6.85±0.01[b]	7.41±0.15[b]
∑PUFA	**29.02[a]**	**37.77[b]**	**37.15[b]**
n6	**5.41[a]**	**14.17[b]**	**16.11[c]**
n3	**23.03[a]**	**22.65[a]**	**21.05[a]**
n6/n3	**0.24[a]**	**0.63[b]**	**0.77[c]**
PUFA/SFA	**0.70[a]**	**1.07[b]**	**1.36[c]**
AI	**1.27[a]**	**0.63[b]**	**0.44[c]**
PI	**0.85[a]**	**0.77[a]**	**0.83[a]**
TI	**0.46[a]**	**0.36[b]**	**0.27[c]**

[a,b,c] Values within same row with different superscripts diverge significantly at $p<0.05$; AI (atherogenic index); TI (thrombogenic index) ; PI (polyene index)]; ND: Not Detec

highest for golden grey mullet and Common sole. The vaccenic acid (C18:1n7) and eicosenoic acid (C20:1n9) of all fish used in this study differed from each other and these differences were found to be statistically insignificant ($P<0.05$). The levels of arachidonic acid (ARA, C20:4n6), EPA, and DHA levels were calculated to be in the range of 2.65-4.08%, 4.91-11.01%, and 6.85-8.87%.

According to Turkstat (2021) reports from the year of 2011 to the year 2020, the quantities of caught European pilchard, Golden grey mullet, and Common sole were in a degreasing pattern. These fishes are important for people who care for healthy food with low payment. Information about some biochemical aspects of the European pilchard, Golden grey mullet, and Common sole in the study may provide useful information.

Discussion

The average lipid amount of the European pilchard in February, March, and April in this study was found to be 2.44 %. The lipid amount of the European pilchard caught in the central Adriatic fishing region southwest of the long island was reported to be an average of 2.24 % (1.18%, 1.91%, and 3.64%) in February, March, and April (Simat et al., 2020). This average value stated for the European pilchard in the study of Simat et al. (2020) is very close to the finding of the European pilchard used in this current study. Additionally, Lloret et al. (2022) also reported the lipid amount of two different sardine species (*Engraulis encrasicolus* and *Sardina pilchardus*) in three different locations (L'Escala, Barcelona, and Tarragona) in the Northwestern Mediterranean Sea diverged and lower than 2.50% in February, March, and April. Moreover, Bandarra et al. (1997) measured the lipid levels of the European pilchard caught on the Portuguese coast (Peniche) in February, March, and April at 1.9%, 1.2%, and 1.3%, respectively. These previously reported values of lipid levels in European pilchard (1.46%) were lower than those of lipid values in European pilchard in this current study.

On the other hand, the protein and lipid levels of the golden grey mullet in this study were measured to be 18.01% and 0.67%, respectively. Kucukgulmez et al. (2018) reported that the protein and lipid levels of the Golden grey mullet were in the range of 16.09-18.40% and 0.13-4.00% throughout a year. The protein and lipid levels of the Golden grey mullet were in the range of the study reported by Kucukgulmez et al. (2018). Özogul et al. (2009) reported that the lipid levels of the Golden grey mullet were 2.29 % which is higher than that of the golden grey mullet used in this study. This could be the result of the region and seasonal differences. According to Lloret et al. (2022), along with many attributes, the lipid content of fish depends on various kinds of environmental factors and seasonal changes which do not have to be in long-term changes.

Additionally, previously published data relating to the Common sole proximate composition (the ash, moisture, protein, and lipid levels) provided by Ozogul et al. (2011) and Gökçe et al. (2004) were very close to the Common sole in this study. Özogul and Özogul (2007) reported the lipid level of the Common sole was lower than 1% which is in parallel with the level of the common sole in this study.

The carbohydrate amounts in European pilchard, Golden grey mullet, and Common sole were in the range of 1.21±1.03-1.50±0.54 % which are higher than those reported for three freshwater fish species (in the range of 0.15±0.07% - 0.55±0.03% (Ozyilmaz et al., 2016). Additionally, the energy value of European pilchard, Golden grey mullet, and the Common sole was in the range of 369.30±14.84%-445.12±11.46%. Ozyilmaz et al. (2016) reported carbohydrate levels of three freshwater fish species in the range of 335.93±10.31%-523.64±20.87% which are in parallel with this study.

The average saturated fatty acid amounts of the common sole in this study were found to be %27.52. Özogul and Özogul 2007 reported the saturated fatty acid levels of the Common sole from the northeastern Mediterranean coast of Turkey as %29.60. The results regarding the saturated fatty acid amounts of the common sole were different from each other. The reason for that could be the catching seasons. Additionally, the highest saturated fatty acid components were in the following order: C16:0>C14:0>C18:0 for European pilchard in this study. Bandarra et al. (2018) reported similar results for the European pilchard. The highest saturated fatty acid components were in the following order: C16:0>C18:0>C14:0 for golden grey mullet in this study. Kucukgulmez et al. (2018) reported similar results for golden grey mullet caught from the northeastern Mediterranean coast of Turkey in summer, winter, and spring. Özogul et al. (2009) also reported the same pattern for Golden grey mullet. This current study and the aforementioned studies are in good agreement.

The average levels of the EPA were higher than that of DHA in golden grey mullet in this study. Kucukgulmez et al. (2018) stated that the levels of the Golden grey mullet have the same stations in four seasons. Özogul et al. (2009) also reported the mean amount of the EPA was higher than that of DHA in golden grey mullet caught by trawlers in the Mediterranean Sea. Having higher amounts of EPA in polyunsaturated fatty acids could be one of the characteristic attributes of the golden grey mullets.

The ratio of n6/n3 and PUFA/SFA are some of the most important attributes from point of nutritional view. The ratio of n6/n3 of European pilchard, Golden grey mullet, and Common sole used in this study was in the range of 0.24-0.77 which is lower than the maximum recommended level (recommended 4 at the highest). Additionally, the ratio of PUFA/SFA for all fish in this study was in the range of 0.24-0.77 and 0.70-1.36 which is higher than 0.45 (recommended as the lowest level). The ratio of n6/n3 higher than 4 and the ratio of PUFA/SFA lower than 0.45 are not recommended by HMSO UK, (1994).

This study clearly shows that the ratio of n6/n3 and PUFA/SFA is in the safe range which knowledge was provided by health departments. Based on the data of this present study, the European pilchard, Golden grey mullet, and Common sole used in this current study are highly valuable sources of the fatty acids like EPA and DHA which should be added to the diet of the human nutrition to get benefits avoiding some diseases (Tsoupras et al, 2022; Nesheim et al, 2015; Perk et al, 2012; Kinsella et al, 1990). The taking of a sufficient amount of fish oil is recommended to get the health benefits the fish lipid can provide. Therefore, European pilchard, Golden grey mullet, and Common sole used in this study relating to results of their biochemical components can be recommended to get that benefits to maintain healthier life.

Conclusion

As a result, this study contains very important information that might be useful to consumers, producers, and scholars. Because these three economic fish species are affordable to many people around the region. Knowing biochemical components give an idea to people who are interested in this issue. The sea products have the ability to help out maintaining a healthy diet and readily available ones should be consumed to get nutritional benefits. The presented study gives this knowledge to people who want to learn biochemical aspects of the European pilchard, Golden grey mullet, and Common sole.

COMPLIANCE WITH ETHICAL STANDARDS

Authors' Contributions

Authors contributed equally to this paper.

Conflict of Interest

The author declare that there is no conflict of interest.

Ethical Approval

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

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