

[Research]

## Chemical composition and fatty acid profile of common Kilka , *Clupeonella cultriventris caspia*,

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### ABSTRACT:

The objective of the present study was to determine chemical composition and fatty acid profile of common Kilka, *Clupeonella cultriventris caspia*, in the Caspian Sea. The chemical analysis revealed that the protein and lipid content of common Kilka were 15.05 and 6.5% of the fresh weight, and total MUFA, PUFA and SFA were found to be 37.00, 32.89 and 29.03% respectively. Palmitic acid (16:0) among total SFAs, oleic acid (18:1n-9) among MUFAs, Docosahexanoic acid (DHA) (22:6n-3) among n-3 PUFAs and linoleic acid (LA) (18:2n-6) among n-6 PUFAs were the most abundant fatty acids. The obtained results revealed that common Kilka is a better source of  $\omega$ -3 PUFAs than  $\omega$ -6. DHA and eicosapentaenoic acid (EPA) were the major components of  $\omega$ -3 PUFAs. Levels of DHA and EPA were 20.79% and 6.97% respectively. The PUFA/SFA ratio was 1.13 in common Kilka. This ratio was more than the recommended minimum value (0.45). The EPA/DHA ratio was 0.34 in common Kilka. The present study suggests that common Kilka is rich in essential unsaturated fatty acids of the  $\omega$ -3 family, especially DHA which may be considered nutritionally attractive for human.

**Keywords:** Fatty acid profile, Chemical composition, PUFA, *Clupeonella cultriventris caspia*, Caspian Sea

### INTRODUCTION

Fish has long been recognized as a valuable source of high-quality protein in human diet (Weber et al., 2008). In recent years, fish lipids have also assumed great nutritional significance because of their high polyunsaturated fatty acid levels. Currently there has been a major emphasis on effects of including n-3 fatty acids PUFA in diet (Yashodhara et al., 2009). Eicosapentaenoic acid, 20:5n-3 (EPA) and docosahexanoic acid, 22:6n-3 (DHA) appears to play a key role in ontogenesis, especially neural development, functioning of cardiovascular system and immune system (Gladyshev et al., 2006; Broadhurst et al., 2002). Regular consumption of food with an appropriate content of EPA and DHA provides prevention and treatment of depression,

cardiovascular and some other diseases. Aquatic organisms are known to be the main source of PUFAs; thereby humans obtain principle EPA and DHA by consuming fish, aquatic invertebrates and macro algae (Arts et al., 2001). Fatty acid components of fish oil vary with several factors such as sex, nutrition, catching season, species, maturity, temperature, etc. (Hedayatifard and Moeini, 2003; Üstün et al., 1996). A good source of omega-3 fatty acids is from oily fish. The most abundant fishes of the Caspian Sea are the small clupeids known as Kilka including the common Kilka (*Clupeonella cultriventris caspia*), anchovi (*C. engrauliformes*) and large eyed Kilka (*C. grimmi*) (Svetovidov, 1963). It is a native species of the Caspian Sea and is found

in all parts of the Sea especially along the coastal line. Kilka belongs to pelagic fish and they feed on zooplankton. From 2002 onwards, the frequency of common Kilka increased and reached 24,400 mt in 2009. The common Kilka (*C. c. caspia*) is the predominant species of the southern Caspian Sea comprising 97% of total catches of clupeids in the southern parts of the Sea in 2009. Only the stocks of common Kilka have remained stable, following the severe impacts of *Menemioopsis* (Fazli, 2011). Unfortunately, only a small portion of the Kilka catch is used by human (4%) due to its small size, easy deterioration, impossibility of gutting right away after catching, difficulties in hygienic preservation, packaging, and supply, and the rest is used as fish meal for poultry and in aquaculture (Khoshkhoo et al., 2010; Shabanpour et al., 2007). Noteworthy, clupeids present few positive qualities as well, such as their abundance in the Caspian Sea, low finishing costs, and easy scaling without scaling equipment; therefore, clupeids can be utilized as raw material in the conversion industry. Kilka can be processed into salted, smoked, pickled, sausage, canned, dried and frozen fish. In Iran, Kilka products in market are canned, packed in frozen shape and fresh (Martin, 1994).

A few studies have been conducted on the chemical composition and omega-3 fatty acid content of some commercially important fish species from the south Caspian Sea (Pirestani et al., 2010; Hajirostamloo and Hajirostamloo, 2010; Hedayatifard and Jamali, 2008; Hedayatifard et al., 2002), but there is not enough information in details about the fatty acid content of these fish. Therefore, considering the abundant resources of common Kilka in the Caspian Sea, this study was conducted to investigate the level of fatty acids with special emphasis on omega-3 essential fatty acid and chemical composition of common Kilka.

## MATERIALS AND METHODS

Fresh Kilka fish were bought from special lantern net equipped Kilka fishing boats in Anzali quay, Guilan province (Iran) and then transferred to the National Research Seafood

Processing Center in Chilled Sea Water tanks with 60% fish, 25% ice and 15% marine water. The fish were immediately deheaded and gutted and then rinsed with cold hygienic water. The moisture content was determined using an oven at 105 °C (AOAC, 2002). The amount of ash was measured by drying the sample in an electrical kiln at 550 °C (AOAC, 2002). The amount of crude protein was determined by Kjeldahl method (AOAC, 2002) and crude fat was measured using the Soxhlet method (AOAC, 2002). Chemical composition was analyzed for fifteen samples. Lipid was extracted by the method of Bligh and Dyer (1959) and was used for lipid quantification and for determination of the fatty acid profile. Fatty acid methyl esters (FAMES) were determined following the methodology described by Metcalf et al. (1996). The fatty acid composition of fish samples was determined using a gas chromatograph (Unicam-4600) equipped with a flame ionization detector (FID) and a 30 mm long×0.25 mm internal diameter capillary column PB×70. The oven temperature was 160 °C for 6 min, followed by an increase to 180 °C at a rate of 20 °C/min and held for 9 min at 180 °C. This was followed by an increase to 200 °C at a rate of 20 °C/min and then was held for 5 min at 200 °C. The injection split ratio was 1:10. The temperatures of the injection port and detector were 250 °C and 280 °C, respectively. Helium was used as the gas carrier. The fatty acids were expressed as percentage of the total fatty acid content. Fatty acids were analyzed in triplicate for fifteen samples and Results were expressed as the percentage of each fatty acid with respect to the total fatty acids. The data obtained were statistically analyzed through analysis of variance using SPSS software.

## RESULTS AND DISCUSSION

The results of proximate analysis of common Kilka are shown in Table 1.

**Table 1.** Proximate composition of fresh common Kilka (gr/100 gr)

Chemical composition	Moisture (%)	Crude fat (%)	Crude protein (%)	Ash (%)
Mean ±SD	75±0.28	6.5±1.14	15.1±0.28	3.0±0.28

Data presented as mean ±SD of common Kilka of fifteen fish, each one in triplicate

Lipid content was  $6.5 \pm 1.14$  % in common Kilka. Pirestanni *et al.*, (2010) and Motalebi and Seifzadeh (2011) found 10.2% and 4% lipid content in common Kilka, respectively. Fish can be grouped into four categories according to their fat content: lean fish ( $\leq 2\%$ ), low fat (2-4 %), medium fat (4-8%) and high fat ( $\geq 8\%$ ) (Ackman, 1989). Common Kilka in this study was categorized in the medium fat group which differed from the findings of Pirestanni *et al.* (2010) who categorized common Kilka as a high fat fish with 10.23% lipid content. This difference may be due to sampling location, sampling season, environmental conditions, size, age of fish and etc.

The protein content is important when considering quality and texture of the fish muscle. Fish muscles that contain small amounts of protein tend to lose much water upon cooking, which ruins the texture of the meat (Afkhami *et al.*, 2011). In the present study, amount of protein in common Kilka was  $15.05 \pm 0.28\%$  which was in agreement with the results of Motalebi and Seifzadeh (2011). Also the amount of ash in this study was 3.00%. Pirestanni *et al.* (2011) and Seifzadeh *et al.* (2009) reported 3.3% and 2.87% ash in common Kilka, respectively. Several studies showed that chemical compounds in fish muscles in different species or even in one species are different and depend on the species, ration and diet composition, size, age, reproductive cycle, salinity, temperature, geographical region, fishing season and genetic factors (Afkhami *et al.*, 2011; Bayir *et al.*, 2006).

In the present study, twenty eight fatty acids in muscle of common Kilka were

identified and evaluated. The fatty acid compositions of common Kilka are shown in Table 2. In addition, the averages of its fatty acids series are shown in Table 3.

MUFA, PUFA and SFA were found to be 37.00, 32.89 and 29.03%, respectively. Distribution of fatty acid contents in common Kilka was MUFA > PUFA > SFA.

The fatty acid content pattern for common Kilka reported by Naseri *et al.* (2010) followed the relative pattern MUFA = PUFA > SFA. They found 32.9, 32.9, and 27.3% of MUFA, PUFA and SFA, respectively, but Pirestanni *et al.* (2010) showed a different pattern of fatty acids profile for common Kilka (MUFA > SFA > PUFA) which is a response to specificity of the local aquatic ecosystem.

The major fatty acids of common Kilka were oleic acid (C18:1  $\omega$ -9 cis), palmitic (C16:0), DHA (C22:6  $\omega$ -3) and EPA (C20:5  $\omega$ -3). Results of this study showed that the level of Palmitic acid (C16:0) was 19.14%. It was the dominant saturated fatty acid, contributing to approximately 65% of total saturated fatty acids of common Kilka. It occurs naturally in fish, being a source of metabolic energy for their growth (Sargent *et al.*, 2002). Stearic acid (C18:0) was the second major constituent of saturated fatty acids. Naseri *et al.* (2010) showed that palmitic acid was the dominant saturated fatty acid in common Kilka and constitutes about 20.14% of the fatty acid content. Also Gutierrez and Silvi (1993) found that palmitic acid was the predominant saturated fatty acid in both marine and fresh water fish.

**Table 2.**Fatty acid composition of common Kilka (g/100g lipid)

Fatty acid	Name	concentration%	SD
<b>Saturated</b>			
C14:0	Myristic acid	2.6800	0.014
C15:0	Pentadecanoic acid	0.7613	0.001
C16:0	Palmitic acid	19.149	0.014
C17:0	Heptadecanoic acid	0.8109	0.014
C18:0	Stearic acid	4.0267	0.014
C20:0	Arashidic acid	1.3809	0.141
C22:0	Behenic acid	0.2232	0.014
<b>Monoenoic</b>			
C14:1	Myristoleic acid	0.2971	0.001
C15:1	Pentadecanoic acid	0.1676	0.001
C16:1 $\omega$ -7	Palmitoleic acid	3.9520	0.001
C17:1	Heptadecanoic acid	0.6324	0.014
C18:1 $\omega$ -9trans	Elaidic acid	0.1071	0.001
C18:1 $\omega$ -9cis	Oleic acid	26.8892	0.141
C18:1 $\omega$ -7cis	Vaccenic acid	2.1300	0.014
C20:1 $\omega$ -9	Eicosenoic acid	2.0202	0.014
C22:1 $\omega$ -9	Erucic acid	0.1510	0.014
C24:1 $\omega$ -9	Nervonic acid	0.6500	0.014
<b>Polyenoic</b>			
C18:2 $\omega$ -6 trans	Linolelaidic acid	0.1505	0.014
C18:2 $\omega$ -6 cis	Linoleic acid (LA)	2.1243	0.136
C18:3 $\omega$ -3	$\alpha$ - linolenic acid(ALA)	0.2536	0.014
C20:2 $\omega$ -6	Eicosadienoic acid	0.28	0.014
C20:3 $\omega$ -3	Eicosatrienoic acid	0.1511	0.001
C20:4 $\omega$ -6	Arachidonic acid(AA)	0.6300	0.012
C20:5 $\omega$ -3	Eicosapentaenoic acid(EPA)	6.9709	0.141
C21:5 $\omega$ -3	Heneicosapentaenoic acid	0.22	0.015
C22:4 $\omega$ -6	Docosatetraenoic acid	0.63	0.010
C22:5 $\omega$ -3	Docosapentaenoic acid(DPA)	0.6293	0.014
C22:6 $\omega$ -3	Decosahexaenoic acid(DHA)	20.7959	0.142

Data presented as mean  $\pm$ SD of common Kilka of fifteen fish, each one in triplicate

**Table 3.** Average of fatty acids series in common Kilka (g/100gr lipid)

Fatty acids series	Concentration (%)	SD
Saturated fatty acids(SFA)	29.03	0.156
Mono unsaturated fatty acids(MUFA)	37.00	0.189
Poly unsaturated fatty acids(PUFA)	32.89	0.561
PUFA/SFA	1.13	0.161
MUFA+PUFA (UFA)	69.89	0.751
MUFA+PUFA/SFA	2.41	0.014
Omega-3 series ( $\omega$ -3)	29.02	0.311
Omega-6 series ( $\omega$ -6)	3.87	0.136
Omega-9 series ( $\omega$ -9)	29.83	0.120
EPA+DHA	27.76	0.283
DHA/EPA	2.98	0.121
$\omega$ -3/ $\omega$ -6	7.50	0.721

Data presented as mean  $\pm$ SD of common Kilka of fifteen fish, each one in triplicate

Levels of monoenoic fatty acids, such as oleic acid (C18:1  $\omega$ -9) and palmitoleic acid (C16:1 $\omega$ -7) in common Kilka were 26.88% and 3.95% respectively. Oleic acid was the most abundant of mono unsaturated fatty acids in common Kilka and palmitoleic acid was the second major MUFA. Gutierrez and Silva (1993) reported that oleic acid is the prominent MUFA and it was found in higher levels in fresh water fish than in marine fish. The obtained results revealed that common Kilka is more of  $\omega$ -3 series of PUFA than  $\omega$ -6 series and their amounts were 29.02 % and 3.87% for  $\omega$ -3 PUFA and  $\omega$ -6 PUFA, respectively. Therefore, common Kilka is a better source of  $\omega$ -3 PUFA than  $\omega$ -6 PUFA. The most abundant  $\omega$ -6 PUFA was linoleic acid (LA; C18:2  $\omega$ -6) about 2.12%, followed by arachidonic acid (AA; C20:4  $\omega$ -6) and docosatetraenic acid (C22:4  $\omega$ -6). DHA and EPA were the major components of  $\omega$ -3 PUFA. Levels of DHA and EPA were 20.79% and 6.97% respectively. DHA was the prominent PUFA  $\omega$ -3 in common Kilka

and the ratio of DHA/HPA was 2.98. Gutierrez and Silva (1993) analyzed fatty acid composition of nine marine fish and found that in all marine fish except sardines, level of DHA was higher than EPH. DHA is one of the most useful polyunsaturated fatty acids (PUFA) with pharmaceutical potential and importance for the prevention and control of various human diseases and disorders such as cardiovascular disease, inflammation, allergy, cancer, immune response, diabetes, hypertension and renal disorders. DHA is also known as "brain food" as it is highly concentrated in the membranes of brain cells and retinal cells of eye (Youdimet *al.*, 2000; Uauy and Valenzuela, 2000). While EPA is the most important EFA of the  $\omega$ -3 series in human diet, it is the precursor to the 3-series ecosanoids and has been recognized as beneficial for human health by reducing the risk of cardiovascular disease (Hall *et al.*, 2008). The obtained results on

evaluation of fatty acid contents of common Kilka revealed that common Kilka is a principle dietary source of  $\omega$ -3 PUFA especially DHA. EPA and DHA are usually synthesized de novo by algae, while higher trophic organisms such as zooplankton and fish obtain these important molecules through bioaccumulation or by converting PUFA  $\omega$ -3 precursors to DHA and EPA via elongation and desaturation (Sargent and Whittle 1981). There are two specific essential fatty acids: linoleic acid (LA; C18:2  $\omega$ -6) and alpha-linolenic (ALA; C18:3  $\omega$ -3). It is from these two "parent" essential fatty acids that  $\omega$ -3 and  $\omega$ -6 fatty acid "families" are derived through a series of enzyme catalyzed desaturation and elongation reactions, that generally take place in the cell cytosol and mitochondria. ALA is metabolized to DHA via EPA and docosapentaenoic (DPA), whereas LA is metabolized to arachidonic acid via gamma ( $\gamma$ )-linoleic acid (GLA) or eicosadienoic acid (C20:2 $\omega$ -6) as two pathways are active. But this bioconversion is slow and usually does not meet the physiological demands of the organisms (Sargent and Whittle, 1981). According to Sargent *et al.* (1989) marine fish have limited abilities to convert shorter fatty acid chains to elongated and desaturated chain. This suggests that marine fish should meet a major part of their essential fatty acid ( $\omega$ -3 PUFA) demand through their diet. This means that common Kilka probably obtained high levels of  $\omega$ -3 PUFA through their diet by eating zooplankton that has fed on marine algae. Marine algae are primary synthesizers of omega3 fatty acids. The importance of micro algae as a resource of PUFA has been recently reviewed (Patil *et al.*, 2005). Therefore, levels of EPA and DHA increase in common Kilka through bioconversion of PUFA n-3 precursors and specially bioaccumulation of them. EPA and DHA play a vital role in the development and functioning of nervous system, photoreception and the reproductive system (Pirestani *et al.*, 2010). The level of EPA+DHA is important in human diet and health and its levels is 27.76% in common Kilka. Also the total

content of EPA and DHA in 100g of muscle tissue of common Kilka was 1.81 g. The recommended daily dose of EPA and DHA for a healthy person is 0.5 g (Polak Juszczak and Komar-Szymczak, 2009) that is contained in 27.6 g of common Kilka. For cardiovascular patients, this dose should be two fold higher. Our results showed that the content of  $\omega$ -9 was 29.83% in common Kilka. The  $\omega$ -9 fatty acids are effective in decreasing LDL cholesterol blood and also for increasing HDL cholesterol blood. They also have better capability for increasing HDL than  $\omega$ -3 and  $\omega$ -6 and also the capability to impede the production of eicosanoid structure (stimulants causing cancer in animal tests) (Suseno *et al.*, 2010). The  $\omega$ -3/ $\omega$ -6 ratio is a good index for comparing relative nutritional value of fish oils (Pigott and Tucker, 1992). For the well-being of human health, the consumption of fish and fish products rich in  $\omega$ -3 PUFAs and poor in  $\omega$ -6 PUFAs is essential. An increase in the human dietary  $\omega$ -3/ $\omega$ -6 fatty acid ratio is essential in the diet to help prevent coronary heart disease by reducing plasma lipids and to reduce cancer risk (Kinsella *et al.*, 1990). The  $\omega$ -3/ $\omega$ -6 ratio was found to be 7.5 in common Kilka. This is similar to the results of Naseri *et al.* (2010) who found this ratio to be more than 7.6 for common Kilka. Also, in the present study, the ratio of  $\omega$ -3/ $\omega$ -6 PUFAs was within the range found for marine fish. The ratio of  $\omega$ -3/ $\omega$ -6 PUFAs in total lipids of fresh water fish varies mostly between 0.55 and 5.6 whereas it ranges between 4.7 and 14.4 in marine fish (Henderson and Tocher, 1987; Hearn *et al.*, 1987; Cengiz *et al.*, 2010)

In most industrial western societies, ratio of  $\omega$ -3/ $\omega$ -6 is currently about 1:15-20 (Simopoulos, 2002) whereas nutrition experts recommend a  $\omega$ -3/ $\omega$ -6 ratio of more than 1:4 (Valencia *et al.*, 2006). Another useful key factor for evaluation of fish nutrition quality is PUFA/SFA ratio. Values of PUFA:SFA ratios greater than 0.45 are recommended (HMSO, 1994). The PUFA:SFA ratio was 1.13 in common Kilka which was more than the recommended minimum value. Naseri *et al.* (2010) showed that ratio of PUFA: SFA was 1.21

in *C. c. caspia* which is similar to our findings. However Hajirostamloo and Hajirostamloo (2010) found it to be 0.53 in *C. c. caspia*. The present study suggests that common Kilka may be a preferable and valuable food owing to its high DHA and EPA for human consumption.

Therefore, considering the abundant resources of common Kilka in the Caspian Sea and high levels of omega-3 fatty acids in this invaluable species, it seems very necessary to increase human consumption of this species rather than use it for fish meal production.

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## بررسی ترکیبات شیمیایی و پروفایل اسید چرب ماهی کیلکای معمولی (*Clupeonella cultriventris caspia*)

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### چکیده:

هدف از انجام مطالعه حاضر تعیین ترکیب شیمیایی و پروفایل اسیدهای چرب بافت عضلانی ماهی کیلکای معمولی (*Clupeonella cultriventris caspia*) بود. آنالیز تقریبی عضله ماهی کیلکا معمولی نشان داد که مقدار پروتئین و چربی به ترتیب 15/05 و 6/5 درصد می‌باشد. میزان اسیدهای چرب تک غیراشباع (MUFA)، اسیدهای چرب چندغیراشباع (PUFA) و نیز اسیدهای چرب اشباع (SFA) در ماهی کیلکا به ترتیب 37/00، 32/89 و 29/03 درصد بود. پالمیتیک اسید (16:0)، اولئیک اسید (18:1n-9)، دکوزاهگزانوئیک اسید (22:6n-3) (DHA) در بین اسیدهای چرب اشباع، اسیدهای چرب تک غیراشباع و نیز اسیدهای چرب چند غیراشباع فراوان ترین اسیدهای چرب بودند. نتایج حاصله نشان داد که کیلکای معمولی منبع سرشاری از اسیدهای چرب  $\omega$ -3 است. دکوزاهگزانوئیک اسید و ایکوزاهگزانوئیک اسید مهم ترین اسیدهای چرب از گروه  $\omega$ -3 بود. مقدار DHA و EPA به ترتیب 20/79 و 6/97 درصد بود. نسبت  $\omega$ -3/ $\omega$ -6 در ماهی کیلکای معمولی 7/5 بود. همچنین نسبت PUFA/SFA در این ماهی برابر 1/13 بود. این نسبت بیشتر از نسبت توصیه شده (0/45) می‌باشد. نتایج حاصل از این تحقیق نشان داد که ماهی کیلکای معمولی از نظر اسیدهای چرب چندغیراشباعی امگا-3 و به ویژه DHA منابع غذایی و شیلاتی بسیار ارزشمند بوده و در کاهش ابتلا به بیماری‌های قلبی و عروقی موثر می‌باشد.

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