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Seasonal changes in chemical composition and fatty acids of sardines (Sardina pilchardus) from the Dakhla coast (Morocco)

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The sardine (*Sardina pilchardus*) is the most important pelagic fish on the Moroccan coasts. This study was conducted to evaluate the chemical composition and fatty acids profile of sardines caught off the Dakhla coast on a monthly basis over a one-year period (February 2017 to January 2018). The results showed that the total lipid content varied significantly with catching season, being low in winter (1.71 % w/w) and high in summer (16.10 % w/w). These lipids have important nutritional characteristics due to their high level of n-3 fatty acids, especially eicosapentaenoic acid (EPA, 20:5 n-3) and docosahexaenoic acid (DHA, 22:6 n-3). The sum of EPA and DHA varied from 26.4 % to 31.3 % of total fatty acids. Moreover, sardine flesh contained between 36.4 % and 41.6 % of polyunsaturated fatty acids while saturated fatty acids ranged from 32.8 % to 38.9 %. During the one-year period, the protein and ash contents remained constant with average values equal to 18.7 % and 1.46 %, respectively, unlike moisture, which was inversely proportional to fat content. Thus, this species provides a year-long adequate diet element by offering a good source of fat and marine proteins and contributing to n-3 fatty acids intake.

Keywords: Sardine, *Sardina pilchardus*, season, polyunsaturated fatty acids, eicosapentaenoic acid, docosahexaenoic acid

INTRODUCTION

Small pelagics constitute an important part of the fish stocks on the Moroccan maritime coasts. In this area, the total biomass of small pelagics was estimated at 7.59 million metric tons in autumn of 2017. This biomass showed a slight increase of 4.3 % compared to the biomass evaluated in autumn 2015. In 2017, the total catch of small pelagics reached 1,458,155 tons. This production has been dominated by Sardina pilchardus, the most important marine pelagic fisheries resource, which accounted for 73 % (1,060,115 tons) of the total catch. In Morocco, sardines are fished using three primary methods: 1) the purse seine; 2) the pelagic trawlers, including refrigerated sea water trawlers and freezer trawlers, which use the pelagic trawl or semi-pelagic trawl; and 3) the small-scale fishing fleet, which uses the small seine (INRH/DP, 2017).

The beneficial effect of fish consumption on human health is attributed to its high content of n-3 fatty acids, particularly eicosapentaenoic acid (EPA, 20:5 n-3) and docosahexaenoic acid (DHA, 22:6 n-3) (Aidos et al., 2002; Zlatanos and Sagredos, 1993). These n-3 fatty acids have valuable disease prevention benefits and also hold many medicinal properties (Ghaly et al., 2013). Connor et al. (1996) confirmed that the intake of sardines and fish oil during human pregnancy increases the DHA values in newborn infants. Moreover, Patin et al. (2006) found that adding 100 g of sardines two or three times a week to the diets of nursing mothers during lactation improved omega-3 series fatty acids levels in breastmilk. Many articles have described the benefits of n-3 fatty acids in

relation to blood pressure regulation, coronary heart diseases (Sidhu, 2003; Kris-Etherton et al., 2003), atherosclerosis and thrombosis (Schacky, 2000; Schmidt et al., 2005), hypertriglycemia (Banerjee et al., 1992; Goodnight et al., 1982), schizophrenia (Mahadik et al., 2001), stress and depression (Bourre, 2005) and foetal development (Weisinger et al., 2001; Horrocks and Yeo, 1999). However, the most widely discussed benefits are for cardiovascular health (Moyad, 2005; Brouwer et al., 2006; Reiffel and McDonald, 2006; Psota et al., 2006; Jacobson, 2006; Goodnight et al., 1982; Dagnelie et al., 1994; Christensen et al., 1997) and for inflammatory disease prevention and treatment (Karmali et al., 1984; Horrocks and Yeo, 1999; Rogers et al., 1986; Wakimoto et al., 2011; Gebauer et al., 2006).

The seasonal effects on sardine composition have been analyzed as function of catching areas (Algerian Coast, Greece, Adriatic Sea and Portuguese coast) by Bouderoua et al. (2011), Zlatanos and Laskaridis (2007), Leonardis and Macciola (2004) and Bandarra et al. (1997), respectively. However, the seasonal effects on the fatty acid composition of Sardina pilchardus from the Dakhla coast are unknown. The objective of this study is to study the effect of catch season on the chemical and fatty acid composition of sardines from the Dakhla coast to generate accurate data for industry and consumers.

MATERIALS AND METHODS

Sampling

Samples of sardines (Sardina pilchardus) were collected monthly from commercial landings of pelagic trawlers in the fishing harbor of Dakhla (Morocco), from February 2017 to January 2018. The fish samples were iced after landing and during transportation. Upon arrival at the laboratory, the edible flesh was removed and minced. The chemicals and solvents used were of analytical grade.

Chemical Analysis

Crude protein content (percentage of total nitrogen x 6.25) in homogenized samples was carried out by colorimetric method using the Kjeldahl digestion method. Moisture content was determined by heating 2 g of each sample to a constant weight in a crucible set in an oven fixed at 105°C. Ash value was measured by incinerating the sample in a muffle furnace set at 550 °C for 12 h. These analyses followed the AOAC official methods (AOAC, 2000). The resulting data are expressed as percentage of wet samples.

Total lipid content in edible flesh samples was determined gravimetrically after extraction using the Bligh and Dyer method (1959). The results are expressed as grams of lipid per 100 g of wet weight.

Fatty acids analysis

Fatty acid methyl esters of total lipids were methylated following the method used by Hammond (1986). More specifically, fifty milligrams of the sample were refluxed in 5 ml of reagent, which consisted of concentrated sulphuric acid-toluene-methanol (1:10:20 v/v/v), for one hour at 90°C. After cooling, water (3 ml), hexane (2 ml) and the internal standard (1 ml) were added. The internal standard used was methyl esters of C19 (nonadecanoic acid methyl ester). The hexane layer was recovered and dried over anhydrous sodium sulphate. At that point, it was considered ready for injection.

Gas chromatography was conducted on an Agilent 7890B Series gas chromatograph, coupled with a fused silica capillary column DB 23.30 m x 0.53 mm with a 0.50 µm film thickness (Supelco, Inc.) and an FID detector. The temperature of the injection device was 250°C, while that of the detector was 280°C. The oven temperature was increased from 100°C to 240°C at 5°C/min after an initial

period of 2 min. The final temperature was maintained for 10 min. The Identification of FAMEs was based on the retention time of each fatty acid methyl ester and on the comparison between anonymous peaks and those of reference standards (Sigma-Aldrich. Co). The fatty acid methyl esters were quantified using internal standards method.

Statistical analysis was carried out using R software version 3.6.1 (R Core Team, 2019) with RStudio version 1.1.463 interface (RStudio Team, 2019). An analysis of variance (ANOVA) was used. Significance was compared to $\alpha = 0.05$ ($n = 3$). Results are expressed with mean ± standard deviation.

RESULTS AND DISCUSSION

The chemical composition of Sardina pilchardus was determined over one year and the results are illustrated in Figures 1, 2, 3 and 4.

The fat content of Sardina pilchardus from the Dakhla coast demonstrated a significant seasonal effect. The total lipids content ranged from 1.71 % to 16.10 % (w/w). The highest fat content was recorded in August (summer) and the lowest in February (end of winter). The fat content increases when the moisture decreases while it decreases when the moisture increases. The moisture content varied from 63.7 % to 77.6 %. Nevertheless, the protein and ash content remained constant during the year.

In February, however, low values of fat content were obtained, which can be attributed to the lack of feed resources and the fish reproductive cycle. Amenzoui et al. (2004) have reported lack of feed for Sardina pilchardus at Laâyoune region of Morocco during winter because of the minimal production of zooplankton. In addition, several studies indicated that sardine reproduction largely occurs during winter and thus, the sardine uses its fat resources in this period to reproduce (Furnestin and Furnestin, 1959; Amenzoui et al., 2004; Ettahiri et al., 2003; Zlatanos and Laskaridis, 2007). The fat reserves accumulate during spring and summer, seasons favorable to climate and trophic plans. In these seasons, spawning is minimal and zooplankton production is maximal. Moreover, during this period, sardines grow also their energy reserves, which are used for metabolism and gonad maturation during the winter (Amenzoui et al., 2004; Zlatanos and Laskaridis, 2007; Furnestin and Furnestin, 1959; Ettahiri et al., 2003; Macciola, 2004; Okada and Morrissey, 2007). Similar observations were reported by Tomasini et al. (1989) for Sardina pilchardus W. from Oran (Algeria).

The results for fat content obtained in this study are similar to those reported by Shirai et al. (2002), who observed a low fat content, 1.8% (w/w), in the winter (February) for the Japanese sardine (Sardinops melanostictus). However, our results from July to September are higher than those recorded by the same authors for the summer (7.2 %). In addition, our results for February were higher than the lipid content found in samples of Sardina pilchardus caught off Tunisia (1.16 g/100 g) (Selmi and Sadok, 2007). Our results are in accordance, nonetheless, with those of Beltran and Moral (1991), who reported a lipid fraction of 10.9% for Sardina pilchardus W. fished in June from the Mediterranean Sea off Spain’s Castellón coast. Garcia-Moreno et al. (2014) similarly found that in Spanish Sardina pilchardus, the highest lipid content (17.7 %) was observed in samples in July. These results are consistent with those of Bandarra et al. (1997), who found that the total lipids in samples of this species ranged between 1.2 % and 18.4 % and with the results reported by Bouderoua et al. (2011), the total lipid content of Sardina pilchardus on the Algerian coast varied from 2.9% in winter to 11.3% in summer.

The results of our study show that the sardine fat content is approximately 10 times higher in summer than in winter which are greater than the results reported by Bouderoua et al. (2011) for Algerian Sardina pilchardus. However, Leonardis and Macciola (2004) registered a high variability of sardine lipid content, with the total average value for lean sardine (January–March) at 2.7 %,

ranging from 1.8 % to 3.5 %, and the total lipid content of sardine filets caught during August–October at an average 13.8 %, varying from 10.6 % to 16.9 %.

All studies conducted on the lipid content of Sardina pilchardus have revealed a significant seasonal dependency (Leonardis and Macciola, 2004; Zlatanos and Laskaridis, 2007; Selmi and Sadok, 2007; Tomasini et al., 1989; Bandarra et al., 1997), which is common for fish from the same geographic area and is affected by the genetic cycles of fish species (Tomasini et al., 1989; Luzia et al., 2003). The responsible factors are exogenous and endogenous, such as highly seasonal feeding (availability of, competition for, and composition of food), reproductive cycles, species, size, geographic area, season, migratory behavior, environmental water temperature, water salinity, age, and sexual maturity stage (Hardy and Keay, 1972; Rajasilta, 1992; Shirai et al., 2002; Amenzoui et al., 2004).

The results of our study showed that the sum of water content and fat content constitute almost 80 % of the wet weight. Similar observations were reported by Aidos et al. (2002) and Okada and Morrissey (2007). This sum of fat and moisture content can vary depending on species and catching area, as these values were between 75 % and 84 % for Sardina pilchardus from different geographical areas (Garcia-Arias et al., 2003; Gökodlu et al., 1998; Garcia-Moreno et al., 2014). This rule can be used as a general tool for estimating fat content from a net water content analysis. The moisture content in our samples are low compared to the results (79.1 %), reported for Sardina pilchardus in Greece by Vareltzis et al. (2012).

Sardine is also a nutritious source of protein, which is the most interesting aspect of the fish as food (Vareltzis et al., 2012). The results of our study showed that protein content remained constant during the one-year period. The average value of protein was 18.7 % of wet weight. These results are in contrast with a study of Bouderoua et al. (2011), in which the protein content of Sardina pilchardus from the Algerian coast was found higher in the summer than the winter. However, Bandarra et al. (2001) noted that protein content remained fairly constant during a one-year period for horse mackerel (Trachurus trachurus L.) caught on the Portuguese coast, with values ranging from 18.3 % to 19.9 %. Our results showed that in addition to protein, ash content was also independent of the catching season.

The chemical composition of Sardina pilchardus is dominated by moisture, followed by protein, fat and ash. According to Gökodlu et al. (1998), mean moisture, protein, fat and ash contents of Sardina pilchardus from the Marmara Sea on Turkey’s coast in June were 69.9%, 20.7%, 14.1% and 1.95% respectively. Similar results were also reported by Garcia-Arias et al. (2003) for Spanish Sardina pilchardus fillets, where moisture, protein, fat and ash levels were equal to 60.7 %, 20.7 %, 15.4 % and 3.26 %, respectively. Moreover, for the same species from Spain in July, Garcia-Moreno et al. (2014) observed mean water, protein, fat and ash values at 57.5%, 16.7%, 17.7% and 2.7%, respectively.

The levels of individual fatty acids in Sardina pilchardus during the year are presented in Table 1.

A wide variety of fatty acids were detected in the total lipids of Sardina pilchardus. The PUFAs constituted the majority of them, followed by the SFAs and MUFAs. A similar distribution of fatty acids groups was obtained by Selmi and Sadok (2007) for Sardina pilchardus caught in Tunisia. In their case, levels of PUFAs, SFAs and MUFAs equaled 44.0 %, 37.0 % and 11.3 %, respectively. Zlatanos and Laskaridis (2007) also reported a similar distribution of fatty acids groups for Sardina pilchardus from the Mediterranean, with PUFAs, SFAs and MUFAs values at 38.1 %, 34.6 % and 18.0 %, respectively. The same configuration was registered by Garcia-Arias et al. (2003) for Sardina pilchardus from Spain and by Pacetti et al. (2013) for the same species from the Adriatic Sea. Beltran and Moral (1991), by contrast, found that the most important fatty acids group in the total lipids of Sardina pilchardus from Spain was SFAs (31.8 %), followed by PUFAs (27.7 %) and MUFAs (26.6 %). Leonardis and Macciola (2004) also registered a different distribution, in which the total lipids of Sardina pilchardus from the Adriatic Sea were equally divided among SFAs (38.3

%), MUFAs (31.2 %) and PUFAs (30.4 %).

In our study, the SFAs fraction varied from 32.8 % to 38.9 %. In this group, the main fatty acid was palmitic acid (16:0), which was followed by myristic acid (C14:0). Similar results were obtained by other authors for Sardina pilchardus from different areas (Beltran and Moral, 1991; Garcia-Arias et al., 2003; Zlatanos and Sagredos, 1993; Zlatanos and Laskaridis, 2007). Bouderoua et al. (2011) reported the same distribution for three of four analyzed samples of Sardina pilchardus from the Algerian coast. In contrast, some studies reported that the dominant SFA was C16:0, followed by C18:0 and C14:0 (Selmi and Sadok, 2007; Pacetti et al., 2013).

In this study, it was observed that the catching season significantly influences ($p < 0.05$) the percentage of palmitic acid which varied between 19.9% and 23.5 % of total fatty acids in sardine flesh. Pacetti et al. (2013) reported that spawning season influences C16:0 content, which they found was higher in sardine fillets from the spawning period (24.5 %) than the nonspawning period (21.6 %). These findings, however, do not corroborate the results of Bandarra et al. (1997), who reported that C16:0 percentage is not influenced by season.

The total monoene content ranged from 24.0 % to 28.1 %, with C18:1 n-9 being the most important MUFA (comprising between 10.6 % and 14.1 %), followed by C16:1 n-7, with values ranging between 7.62 % and 10.6%. Similar distribution was found by other authors for Sardina pilchardus (Zlatanos and Sagredos, 1993; Selmi and Sadok, 2007; Pacetti et al., 2013; Bouderoua et al., 2011; Saglik and Imre, 2001). Of the 12 samples, analyzed over one year by Bandarra et al. (1997), C16:1 was abundant only in sardines fished in January. Similarly, Zlatanos and Laskaridis (2007) found that the most important MUFA was C16:1 in samples from February, April and December, while in the other samples C18:1 was the dominant MUFA (June, August and October) in Sardina pilchardus fat from Spain. However, Garcia-Arias et al. (2003) determined that the dominant MUFA was C18:1 (1.66 %), followed by C22:1 (1.12 %) and C16:1 (1.05 %), and Beltran and Moral (1991) reported that the dominant monoene was C16:1 (12.1 %). Both of those studies analyzed Sardina pilchardus lipids from Spain.

PUFA levels varied between 36.4 % and 41.6 %. The major PUFAs were EPA and DHA, and although the percentages of these fatty acids varied throughout the year. EPA (C20:5) remained the most important single fatty acid within this fraction, with levels varying from 17.3 % to 23.7 %. Next, came DHA (C22:6), with values ranging between 5.82 % and 13.5 %.The high level of polyunsaturated fatty acid is characteristic for Sardina pilchardus. These results are in agreement with findings from Bandarra et al. (1997), who pointed out that PUFAs were the major group in the lipids of Sardina pilchardus harvested from the Portuguese coast, with values ranging between 39.8 % and 50.2 %. These findings are also in accordance with those reported by Gaméz-Meza et al. (1999) for whole sardines (Sardinops sagax caeruleus) from the Gulf of California in Mexico, where the EPA/DHA ratio varied between 1.35 and 2.48. In fact, EPA higher than DHA could be considered a characteristic for Sardina pilchardus from Dakhla due to the type of feed available to the species as EPA is a mean fatty acid of plankton (Shirai et al., 2002). Our findings are also in accordance with those of Bandarra et al. (1997), who concluded that EPA levels were higher than DHA levels from May to November (1994) and in April (1995) in Sardina pilchardus fat, where EPA ranged from 10.7% to 26.0 % and DHA varied from 9.61 % to 22.2 %. Young (1986) also reported a higher content of EPA than DHA in Sardina pilchardus lipids from different coasts (South Africa, Peru and Japan). These findings are not in agreement, however, with those obtained by researchers who noted DHA content higher than EPA levels (Beltran and Moral, 1991; Garcia-Arias et al., 2003; Selmi and Sadok, 2007; Bouderoua et al., 2011; Zlatanos and Sagredos, 1993; Zlatanos and Laskaridis, 2007; Pacetti et al., 2013; Leonardis and Macciola, 2004; Saglik and Imre, 2001). Some EPA/DHA ratios have ranged from 0.19 (Selmi and Sadok, 2007) to 0.73 (Garcia-Arias et al., 2003). Moreover, Zlatanos and Sagredos (1993) obtained a value for this ratio of 0.38, while Zlatanos and Laskaridis (2007) obtained a ratio between 0.37 and 0.61 during their 6-month analysis. Shirai et al. (2002) found that DHA levels were higher than EPA yearlong for Japanese Sardinops melanostictus, except in July, when EPA content (18.9 %) was higher than DHA (10.7%). This

suggests that values can differ depending on feeding activity, seasonal variation of plankton (Shirai et al., 2002) and sexual state. The EPA/DHA ratio was found varying from 0.19 in spawning season to 0.39 in non-spawning season in Sardina pilchardus from the Adriatic Sea (Pacetti et al., 2013).

The configuration of fatty acids in fish oil was similar throughout the year. However, changes in the percentage of mean essential fatty acids occurred throughout the year. The DHA values were lower between April and November, while EPA percentages were higher during the same period in comparison to the rest of the year. This observation was also reported by Bouderoua et al. (2011). This increase in EPA concentration can be attributed to diet (Shirai et al., 2002; Chakraborty et al., 2015). First, in this period, food is abundant and sardines are well fed since they are consuming zooplankton, which is rich in EPA. Second, DHA is a prominent constituent of lipid composing membrane, whose value can decrease, especially in the post-spawning period (Bandarra et al., 1997).

The sum of EPA and DHA varied between 26.4 % (October) and 31.3 % (May). The lowest combined levels were obtained in the summer period, which matches the high levels of fat in sardines. The negative correlation of the fat with the n-3 fatty acid percentage is probably a characteristic of sardines, since Zlatanos and Laskaridis (2007) reported a similar correlation with combined EPA-DHA levels, which varied between 28.0 % and 37.2%. Consistent with this, Bandarra et al. (1997) obtained a high sum of EPA and DHA (35.8 %) in May in the fat of Sardina pilchardus from the Portuguese coast.

In the present study, it was observed that SFAs increased in the fatty season mainly between June and October, at the expense of PUFAs, which reached the lowest values in that same period. The ratio of PUFAs to SFAs varied from 0.95 to 1.28. Many studies on Sardina pilchardus have reported values within that interval (Garcia-Arias et al., 2003; Selmi and Sadok, 2007; Zlatanos and Laskaridis, 2007). Bouderoua et al. (2011) noted that the ratio of PUFAs to SFAs ranged from 1.24 to 1.29, indicating significant differences between seasons, an effect that was also observed in the present research. A lower ratio (0.74) was reported by Saglik and Imre (2001) for Mediterranean Sardina pilchardus.

In this study, C16:0 values were higher than C22:6 levels in all analyzed samples. The C22:6/C16:0 ratio was between 0.25 and 0.64. In the literature, Beltran and Moral (1991) reported a C22:6/C16:0 ratio of 0.62, while Garcia-Arias et al. (2003) noted a ratio of 0.86. Both studies were conducted on Sardina pilchardus from Spain. The C22:6/C16:0 ratio was higher (1.32) for the same species from Tunisia (Selmi and Sadok, 2007). The results of Zlatanos and Laskaridis (2007), indicated that the C22:6/C16:0 ratio was variable throughout the year, ranging from 0.8 (June) to 1.0 (December). These results align with those in the literature, where C16:0 was reported as the most abundant fatty acid in four studies on the Mediterranean sardine (Karakoltsidis et al., 1995; Leonardis and Macciola, 2004; Saglik and Imre, 2001; Beltran and Moral, 1991).

Our results indicated that the n-3 PUFA/n-6 PUFA ratio varied between 7.42 (May) and 12.14 (April). Results obtained by Bouderoua et al. (2011) for Sardina pilchardus from the Algerian coast also fell within this range. Our results were lower than those obtained by Garcia-Arias et al. (2003), who noted n-3 PUFA content as 27 times higher than that of n-6 PUFA for Sardina pilchardus W. from Spain. In addition, the n-3 PUFA/n-6 PUFA ratio has been observed to decrease when moving from spawning to non-spawning Sardina pilchardus from the Adriatic Sea (Pacetti et al., 2013). From a nutritional point of view, these results illustrate that sardines are the best source of n-3 fatty acids at all times throughout the year.

Research studies that have investigated the n-3 fatty acid percentage of Sardina pilchardus (Karakoltsidis et al., 1995; Saglik and Imre, 2001; Zlatanos and Sagredos, 1993) have not always produced similar results. The fat content and fatty acid profiles of the sardines are not constant. As discussed above, the profiles are related to the life cycle of the fish as well as external factors, including temperature, salinity, geographical area and fatty acid composition of zooplankton

(Bandarra et al., 2001; Rajasilta, 1992, Gaméz-Meza et al., 1999). Nevertheless, several studies have concluded that sardine is the best source of essential fatty acids and have recommended its consumption (Zlatanos and Sagredos, 1993; Horrocks and Yeo, 1999; Leaf et al., 1999; Luzia et al., 2003; Kris-Etherton et al., 2003; Karmali et al., 1984; Saglik and Imre, 2001; Okada and Morrissey, 2007; Zlatanos and Laskaridis, 2007; Gaméz-Meza et al., 1999).

Conclusion

Evaluation of the chemical composition of Sardina pilchardus fished in the Dakhla coast (Morocco) at different seasons confirms that the fat content exhibits important seasonal dependency, which is typical of pelagic species. The minimum fat value obtained in February (1.71 % w/w) and the highest fat percentage (16.1 % w/w) was found in August. The percentage change in fat is reflected in the percentage of moisture. Water and fat constitute the same proportion of the net weight (about 80%). Nevertheless, the protein and ash contents remained constant during the year with average values equal to 18.74 % and 1.46 %, respectively.

Sardine flesh contained between 24.0 % and 28.1 % of monounsaturated fatty acids, with C18:1 n-9 being the most important MUFA while saturated fatty acids ranged from 32.8 % to 38.9 %. In this group, the main fatty acid was palmitic acid (16:0). The polyunsaturated fatty acid content ranged from 36.4 % to 41.6 %. SFAs increased in the fatty season mainly between June and October, at the expense of PUFAs, which reached the lowest values in that same period. The ratio of PUFAs to SFAs varied from 0.95 to 1.28. EPA constituted the most important essential polyunsaturated fatty acid with levels varying from 17.3 % to 23.7 %, followed by DHA (C22:6), with values ranging between 5.82 % and 13.5 %. In spite of change in percentages of these fatty acids that was significantly influenced by catching season, sardines constitute a yearlong valuable source of essential fatty acids.

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