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This is a refereed journal and all articles are professionally screened and reviewed **ORIGINAL ARTICLE**

Seasonal Evolution of the Biochemical Composition of the Moroccan Mediterranean Cost Anchovy (*Engraulis encrasicolus*).

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Ouafa Hannachi, Mohammed Bouakka, Mohammed Melhaoui, Abdelkader Hakkou; Seasonal evolution of the biochemical composition of the Moroccan Mediterranean cost anchovy (*Engraulis encrasicolus*).

ABSTRACT

The biochemical composition of the edible part of the Moroccan Mediterranean cost anchovy (*Engraulis encrasicolus*) has been determined. A monthly record of a year long cycle displays small seasonal variations of protein contents and mineral matter, respectively from 15% to 17% and from 1% to 1.6% with maximums recorded in winter (17%) for proteins and in summer for (16%) for mineral matter. The amounts of fatty acids show small qualitative and quantitative variations in the year, from 32.5% to 27.1% for saturated fatty acids, 27.3% to 29.7% for unsaturated fatty acids, and 32.5% to 38.1% for polyunsaturated fatty acids. The most abundant fatty acids are palmitic acid (C:16) (21,1 % à 24,2 %), docosahexaenoic acid (C22:6n-3) (17,3 à 21,1 %), followed by oleic acid, (C18:1) (16,19 à 18,38 %), and by eicosapentaneoic acid (C20:5n-3) (6,8 à 10,1 %).

Key words: Anchovy, Protein, Mineral matter, Fat, Seasonal variation, Fatty acid composition.

Introduction

The anchovy (*Engraulis encrasicolus)*, is a Teleostean clupeiform fish, a member of the Engraulidee family, about 6 inches long. This family is spread over the whole Mediterranean basin, including the Black Sea and the Azov Sea. It is a pelagic species, often gathered in large shoals. *Engraulis encrasicolus* lives in shallow waters (33 to 60 feet deep) and sometimes down to 130 feet deep; it can also be found in estuaries [7].

The biochemical composition varies vastly from one species and one individual to another, according to age, sex and the season. These variations are closely linked to food, migration and sexual changes in relation to reproduction periods because the laying requires a large amount of energy. Lipids are the most affected [8], since they represent an important form of storing energy during the biological cycle. This fish uses its body storage during seasonal fasting periods and during gonad formation time. Its fatty acid composition shows its abundance in polyunsaturated fatty acids, particularly in C20: 5 acids (eicosapentaneoic acid) and C22: 6 acids (docosahexaenoic acid) which can amount up to more half of the total fatty acids [4,30]. Those two acids are known to be beneficial in cardiovascular disorders as they reduce infarct risk, re-establish a normal heart beat rhythm and exercise a beneficial effect on artery hypertension [13].

The anchovy is a largely used fish in food industry and its main use is its transformation into salted or marinated semi preserves. Its maturation process seems to be accounted for only by taking into account ecological and biochemical considerations that have to be clarified. Works in this

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respect are not so abundant. The anchovy's biochemical process displays dramatic seasonal variations, in particular in fat content [6,11,26]. The lipid amount of the Azov Sea anchovy can amount to up to 30% of fresh weight and shows seasonal variations.

The study of anchovy's biochemical composition is necessary; it has been carried out in a number of works worldwide, but it is difficult to put forward an accurate composition, since a lot of variations exist between males and females and during the biological cycle.

In this study, we have taken into account the biochemical composition of the Moroccan anchovy in proteins, total fat and mineral matter during its yearly cycle.

Materials and methods

1. Sampling:

1 kg anchovy samples have been collected at least twice a month for a year on fishing boats as they accosted the port of Nador. Male fish have been separated from females and those bigger than 11 cm have been selected and transported to the laboratory at a 3 to 4 °C temperature.

2. Water Quantity Determination:

Water quantity determination is carried out by drying the sample in a drying oven at 105C° until its mass is stable. The loss in weight is calculated as well as the water quantity in the sample. The analysis is carried out on a triple basis and water content is given to the nearest 0.1%.

3. Protein Quantity Determination:

Protein quantity determination is carried out with the Kjeldahl method by determining the total organic nitrogen amount.

4. Fat Quantity Determination:

Total fat extraction is achieved with diethylic ether (d : 0,72; boiling point: 34,5C°). So, a finely grinded sample is put into an thimble-shaped filter and then set into a Soxhlet extractor. After a six hour extraction, the fat contained in the ether is collected in a tared ball. The ether is evaporated and the collected fat is weighed. The fat content is expressed in percentage (in g. of fat in 100g. of dry sample). Measurements are carried out three times.

5. Mineral Matter Quantity Determination:

Cinder or mineral matter contents of the various samples are determined with the gravimetric method after sample calcination. The finely grinded sample is put into a beforehand dried up and weighed melting pot, then calcined in an oven at 600C° for 4 hours until white, or slightly grey cinders (residue), according to the nature of the sample, are obtained. After incineration and cooling down in a dessicator, the sample is weighed and the cinder content is calculated. The mineral matter content is expressed in percentage (in g. of cinders per 100 g. of dry matter) and the analysis has been carried out on a triple basis.

6. Extraction and Analysis of Fatty Acids:

After the beforehand grinding of the fish flesh, the homogenate is directly saponified in a 30% methanolic KOH solution under reflux for 4 hours. The neutral lipids liberated by the saponification are extracted with oil ether and the aqueous phase contains the fatty acids in the form of sodium salts. Fatty acids are then liberated by the acidifying of the phase with 5N hydrochloric acid and then extracted with oil ether. After vacuum-evaporation, the dry extract contains the apolar fatty acids.

The separation and quantification of the fatty acids have been achieved through gas phase chromatography after methylation with BF3/methanol (AOAC, 200e). The chromatographic conditions which have been used were as described by Mugerza *et al.* [21]. 3 µl of the sample and have been injected into a gas phase chromatograph with a FID detector (Perkin-Elmer XL autoSystem) fitted with an SPTM-2560 capillary column (100 m x 0.25 mm). The temperatures of the injector and of the detector are respectively 220C° and 300C° and the temperature of the oven starts with 165C° for 80 min, followed by a 220C° increase with a 4C° gradient/min to remain at that temperature for 50 minutes. The vector gas is hydrogen (20 psi). Identification of the peaks has been achieved by comparing the time length of their retention with those of the pure standard compounds (Sigma) and the quantification of acids has been carried out in relation to heptadecanoic acid methylic ether as internal standard.

Results and dicussion

The anchovy global biochemical composition has been determined on the edible part which represents almost 73% of the total body of the fish by identifying the content of the main constituents of the living matter: water, proteins, mineral matter and fat (Table I).

1. Water Content:

Sketch 1 shows that the average anchovy's water content is 60.2%. This quantity is fairly large and is actually related to the high content of lysine and glutamic acid which hold water, which explains the high elasticity of the flesh of the fish in general [22]. Water content varies according to the fatness of the

fish. In lean fish, it represents 80 to 84% of the muscular mass, whereas in fat fish such as anchovy, it can decrease down to 60%. 70% of the water of the flesh is thought to be located in the myofibrils, 20% in the sarcoplasm and 10% in the connective tissue [17].

2. Protein Content:

The Eastern Mediterranean anchovy contains on average 16% of proteins (sketch 1). These figures thus enable anchovy flesh to be rated among good nutritional quality food. Protein content of fish varies not only in relation to species, but in relation to individuals of a same species [19]. Pelagic fish have a protein content varying from 15 to 20%.

Protein content of the edible part of the anchovy varies very little in relation to season. We recorded a little variation in the protein content from 13 to 14% in the year with a minimum noticed in winter and summer and a maximum in autumn and spring (figure 1). The variation of the protein fraction may be due to the planktonic feed and to climatic changes in the year which influence the general biochemical composition of the fish.

The proteins of the fish can be divided into three classes: myofibril proteins (or structure proteins) (60 to 65%), sarcoplasmic proteins (20 to 30%), extracellular or support proteins (5 to 8%) [5].

The aminic acids which form the muscle proteins of the anchovy are specific to the species. There is a predominance of aspartic and glutamic acids, histidine and taurine in the Pacific anchovy, of aspartic and glutamic acids, leucine and lysine in the European anchovy [4,17,8].

3. Mineral Matter Content:

Mineral matter content, also named cinder content, is determined after incineration of the fish flesh at 600C° . Our results show that the studied flesh of the anchovy contains on average 1.4% of mineral matter (sketch 1). Let's point out that our results are close to those of the literature.

Mineral matter content of the edible part of the anchovy slightly changes with the seasons of the year. As a matter of facts, in the males, we record a variation from 1.26 to 1.38% in the course of the year with a minimum recorded in January and a maximum in April. For the females, this content varies between 1 and 1.6 with a maximum in winter (figure 2).

4. Fat Content:

The anchovy contains on average 7.25% of fat (sketch 1); this amount vastly varies with sex, season, food and the sexual cycle. In the fat fish such as the anchovy which is very rich in lipids, fat is mainly made up of triglycerides which are made of polyunsaturated fatty acids such as eicosapentaneoic acid or C20:5n-3 and docosahexaenoic acid or C22:6n-3. These acids are thought to be very wholesome, particularly in the protection of the cardiovascular system [13,20,29,18,9].

The lipid fraction represents the constituent which undergoes the most dramatic changes in the year in the anchovy. The variation in a particular species often displays a typical seasonal curb with a minimum usually recorded around the period of biological rest [14].

The fat content in the edible part of the studied anchovy is related to season. So, in the females, we record a variation of the lipid content from 3.7 to 8.2% in the year with a minimum recorded in November and a maximum in April. Furthermore, in the males, this content reaches its climax in the end of winter and during spring, up to 6.92% (figure 1). Spiros *et al*., [27]; Karaçam [14] have also found that the fat content of the anchovy is maximum at the end of winter, beginning of spring and minimal at the end of summer.

The decrease of the lipid content in spring is mainly due to expenditure of triglycerides stored in the fish tissues during the maturation of the reproductive tissues (gonads). Furthermore, the mobilization of the muscles and the liver lipids brings about a dramatic fluctuation of the lipid content in these tissues during the gonad development. About 40% the body fat are transferred to the ovaries and eggs in the mature reproducing capelin [12]. These lipids are used during the fasting, reproduction and maturation periods [23].

Though, the fat matter content and the fatty acid composition are not constant. They are linked to the species' cycle of life and to external factors, such as temperature, salinity, and the fat content of their food [1,10].

The qualitative analysis of the fatty acids extracted from the anchovy flesh was carried out by gas phase chromatography (GPC) (sketch 2). The fat content of the edible part of the Mediterranean anchovy varies very little with the seasons. So, the whole of the saturated fatty acids of the studied samples varies from 31 to 34%, the minimum of which being registered during February (31.64%). Palmitic acid (C:16) represents the largest part of the saturated fatty acids with 24.17% recorded during December. These results confirm those obtained by several authors on the Mediterranean anchovy [28,15,24,30].

According to a seasonal interpretation, the whole of the saturated fatty acids reaches its peak in winter and tends to decrease in spring time. This amount is larger than that recorded by Lya *et al*., (19.96% in December and 20.66% in January) but closer to the one found by Barlow and Pike [2].

The mono-unsaturated fatty acid fraction amounts to 28 to32% with a maximum recorded in March (32.21%) and a minimum in July (28.64%). These results make it possible for us to show that the

Table I: Global biochemical composition of the Moroccan Mediterranean anchovy.
The figure of each season represents a three month average and each month represents a three experiment average.

Water (%)	Proteins (g/100g of dry matter)	Fat matter (g/100g of dry matter)	Cinders (g/100g of dry matter)
60,2±2,5	16±1.3	7,25±2,8	1,6±0,8

Table II: Seasonal evolution of the fatty acid content of the Moroccan Mediterranean anchovy C (in g/100g. of gry matter).
The figure of each season represents a three month average and each month represents a three experiment average.

Saturated fatty acids	January	February	March	April	July	August	September	October	November	December
C12:0	0,11 ± 0,02	0,10 ± 0,03	0,09 ± 0,02	0,08 ± 0,04	0,09 ± 0,07	0,14 ± 0,03	0,15± 0,05	0,22 ± 0,08	0,15 ± 0,03	0,12 ± 0,05
C14:0	5,11 ± 0,52	5,17 ± 0,33	4,49 ± 0,72	4,58 ± 0,84	5,69 ± 0,27	5,14 ± 0,73	4,84 ± 0,47	5,42 ± 0,38	4,55 ± 0,53	4,50 ± 0,44
C15:0	0,91 ± 0,12	1,10 ± 0,13	0,99 ± 0,32	1,18 ± 0,14	0,69 ± 0,17	0,94 ± 0,23	1,20 ± 0,27	0,92 ± 0,18	0,95 ± 0,13	0,87 ± 0,14
C16:0	22,31 ± 2,02	21,10 ± 2,30	21,89 ± 1,92	22,28 ± 2,04	21,19 ± 2,07	22,74 ± 1,93	22,00 ± 0,97	22,22 ± 1,98	23,15 ± 2,03	24,17 ± 2,07
C18:0	4,11 ± 0,62	4,17 ± 0,43	4,49 ± 0,72	4,58 ± 0,84	4,69 ± 0,37	4,14 ± 0,53	4,02 ± 0,35	4,42 ± 0,38	4,25 ± 0,73	4,42 ± 0,29
Total	32,55	31,64	31,95	32,7	32,35	33,1	32,26	33,2	33,05	34,08
Monoinsaturated fatty acids	January	February	March	April	July	August	September	October	November	December
C14:1	0,71 ± 0,12	0,76 ± 0,23	1,09 ± 0,32	0,88 ± 0,14	1,09 ± 0,17	0,84 ± 0,23	0,57 ± 0,05	0,82 ± 0,38	0,75 ± 0,33	0,60 ± 0,49
C16:1	8,11 ± 0,92	7,10 ± 0,73	8,09 ± 0,82	6,88 ± 0,44	6,79 ± 0,97	6,14 ± 0,73	5,95 ± 0,47	8,22 ± 1,18	8,15 ± 1,03	7,07 ± 0,89
C18:1	16,31 ± 2,02	17,10 ± 1,30	17,89 ± 1,92	18,38 ± 1,04	16,19 ± 1,07	15,94 ± 0,93	16,21 ± 1,07	17,22 ± 1,08	17,15 ± 1,03	17,12 ± 1,25
C18:1n-7	2,11 ± 0,52	2,17 ± 0,33	2,49 ± 0,72	2,10 ± 0,84	1,99 ± 0,27	2,14 ± 0,73	1,98 ± 0,47	2,42 ± 0,38	3,05 ± 0,13	2,40 ± 0,24
C20:1	2,21 ± 0,12	2,27 ± 0,23	2,39 ± 0,62	1,98 ± 0,34	2,39 ± 0,17	2,14 ± 0,73	1,99 ± 0,49	2,42 ± 0,28	2,15 ± 0,23	2,30 ± 0,26
C22:1	0,23 ± 0,02	0,24 ± 0,03	0,26 ± 0,02	0,23 ± 0,04	0,19 ± 0,09	0,15 ± 0,05	0,32 ± 0,08	0,35 ± 0,07	0,32 ± 0,05	0,15 ± 0,05
Total	29,68	29,64	32,21	30,45	28,64	27,35	27,02	31,45	31,57	29,64
Polyinsaturated fatty acids	January	February	March	April	July	August	September	October	November	December
C18:2n-6	1,11 ± 0,52	1,17 ± 0,33	1,49 ± 0,72	0,88 ± 0,24	1,39 ± 0,27	1,14 ± 0,23	0,99 ± 0,35	1,42 ± 0,18	1,15 ± 0,23	1,75 ± 0,19
C18:4n-3	1,53 ± 0,17	1,37 ± 0,21	2,01 ± 0,12	1,78 ± 0,27	1,48 ± 0,17	1,24 ± 0,43	1,68 ± 0,26	1,92 ± 0,08	1,85 ± 0,21	1,90 ± 0,59
C20:4n-6	0,81 ± 0,22	0,70 ± 0,13	0,99 ± 0,12	1,11 ± 0,14	0,69 ± 0,17	1,64 ± 0,19	1,88 ± 0,27	1,83 ± 0,19	0,95 ± 0,33	0,95 ± 0,18
C18 :3	1,41 ± 0,22	1,22 ± 0,13	2,49 ± 0,64	2,18 ± 0,17	1,33 ± 0,15	0,74 ± 0,33	0,75 ± 0,15	0,92 ± 0,29	0,95 ± 0,33	0,94 ± 0,29
C20:5n-3	9,11 ± 1,12	9,17 ± 0,93	9,09 ± 1,82	8,88 ± 1,44	6,79 ± 0,97	7,14 ± 0,93	9,21 ± 1,18	8,22 ± 1,18	9,15 ± 1,03	10,02 ± 1,49
C22:5n-3	2,76 ± 0,16	2,78 ± 0,23	2,29 ± 1,12	1,88 ± 0,24	2,09 ± 0,17	1,87 ± 0,23	1,95 ± 0,17	1,82 ± 0,28	2,05 ± 0,23	1,95 ± 0,34
C22:6n-3	18,31± 2,32	21,12± 2,37	19,77± 1,72	20,18 ± 2,54	18,89 ± 2,47	20,14 ± 1,53	18,85 ± 1,70	17,82 ± 1,80	18,55 ± 2,83	17,29 ± 1,25
Total	35,04	37,53	38,13	36,89	32,66	33,91	35,31	33,95	34,65	34,8

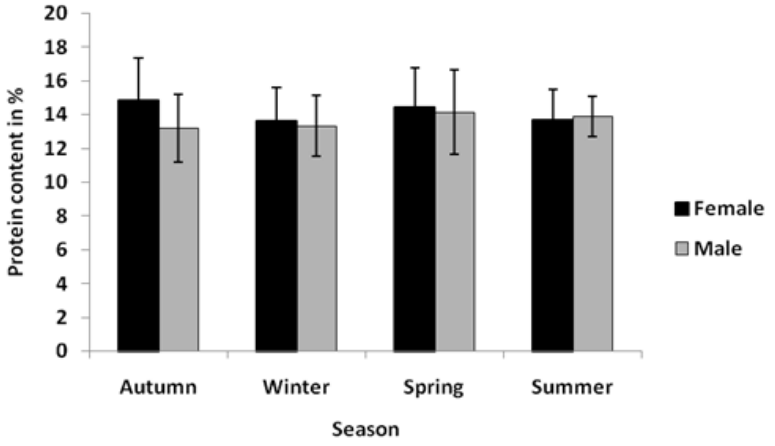

Fig. 1: Seasonal evolution of the protein content of the Moroccan Mediterranean anchovy *Engraulis encrasicolus* (in g/100 g. of dry matter).
The figures represent a three experiment average.

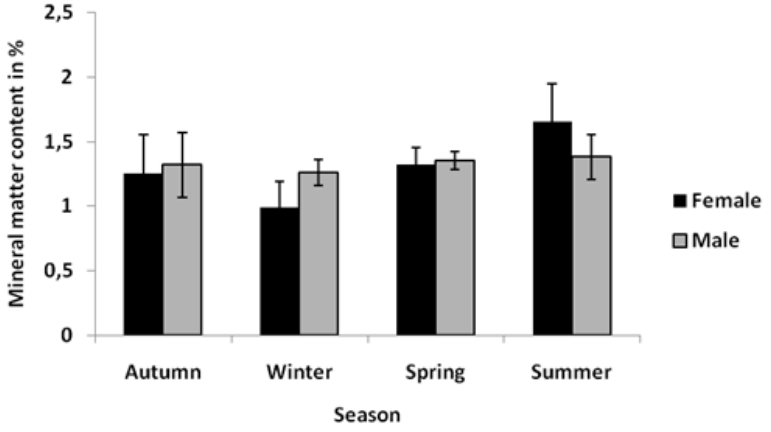

Fig. 2: Seasonal evolution of the mineral matter content of the Moroccan Mediterranean anchovy *Engraulis encrasicolus (in g/100g. of dry matter).*
The figure of each season represents a three month average and each month represents a three experiment average.

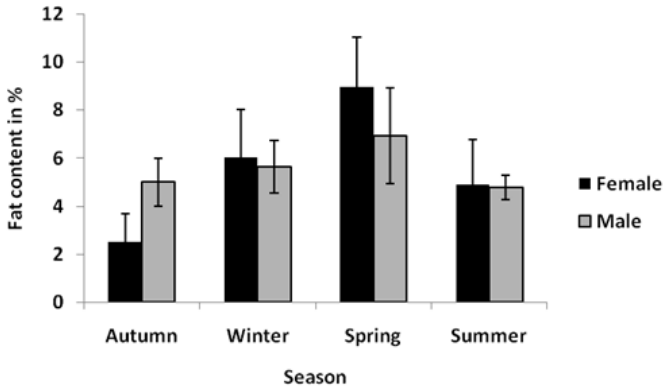

Fig. 3: Seasonal evolution of the fat content of the Moroccan Mediterranean anchovy *Engraulis encrasicolus (in g/100g. of gry matter).*
The figure of each season represents a three month average and each month represents a three experiment average.

mono-unsaturated fatty acids of the Mediterranean anchovy reach their maximum amount in spring. Nevertheless, the amounts we found are larger than the ones reported by Lya *et al*.

Oleic acid (C:18.1) is the more abundant among the mono-unsaturated fatty acids (18.38%) and this proportion slightly varies in the year (16.19% recorded in July and 18.38% in April). This amount is larger than the one found by Lya et al. and by Barlow and Pike [2].

The polyunsaturated fatty acids represent the largest fraction (31 to 36%) of all the fatty acids of the studied anchovy, with a minimum in summer (31.18%) and a maximum in February (36.16%).

Eicosapentaneoic acid (C20:5n-3) and docosahexaenoic acid (C22:6n-3) are the more abundant of the polyunsaturated fatty acids in the anchovy. These results are closer to the ones obtained by Lya *et al*. (17.44% and 19..16% of docosahexaenoic acid) and by Barlow and Pike [2] (16% and 14%). These results confirm Bimbo's [3]. The more abundant fatty acids after C16 are C22:6n-3 with 17.27 to 21.12% and C20:5n-3 with 6.79 to 10.02%, oleic acid (C18:1n-9) and myristic acid (C14:0). These results are in accordance with the literature [15,18,24,30].

The comparison of the seasonal variations between total fat and fatty acid content has shown a negative correlation between the fatty acid content and the n-3 fatty acids of the anchovy, which means that n-3 fatty acid percentages have been low during the months of high fatty acid content. The opposite relation has been found for the saturated fatty acids which contents have increased during the months of high fatty acid content. That suggests that different biological functions are enabled by the various fatty acids in the same species during the biological cycle. The saturated fatty acids are probably used to store energy. Consequently, their content increases during the feeding periods [10,25]. The negative correlation between the total fatty acid content and n-3 fatty acids is probably a typical feature of the clupeidae order.

The anchovy fatty acid content appears to fluctuate dramatically [8]. This fat is made of neutral lipids (77.6%), phospholipids (22.12%) and glycolipids (0.3%). Their fatty acid composition brings out their richness in polyunsaturated fatty acids, particularly in C20:5 acids (eicosa-pentanoic acid) and C22-6 acids (docosahexaenoic acid) which can amount to more than half of all the fatty acids [4,30]. Those two acids are known to be beneficial in the prevention of cardiovascular disorders, in decreasing the infarct risk, in restoring a normal heart beat and in having a positive effect on artery hypertension [13].

Conclusion:

The global biological composition of the edible part of the Moroccan Mediterranean anchovy (*Engraulis encrasicolus*), such as its content in proteins, mineral matter and total lipids, and its fatty acid composition point to the nutritional quality of anchovy's muscles. The muscles contain about 15 to 17% of the total proteins which vary very little in the year. On the other hand, the fat matter contents show dramatic changes in the year and in relation to sex (4 to 8%). The anchovies fished in Oriental Moroccan Mediterranean are very rich in unsaturated fatty acids which amount to 60 to 68% of all the fatty acids with a predominance of docosahexaenoic acid (C22:6n-3) (17.3 to 21.1%), oleic acid (C18.1) (16.19 to 18.38%) and eicosapentaneoic acid (C20:5n-3) (6.8 to 10.1%).

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