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Chemical Composition and Nutritional Value of *Engraulis encrasicolus* (Linnaeus, 1758) Caught by Driftnet "Menaide" along Sicilian Coast: a Natural Food for Mediterranean Diet

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Abstract: The anchovy (*Engraulis encrasicolus*, Linnaeus 1758) is a typical Mediterranean fish. In the area of the Gulf of Catania (Sicily, Italy), typically caught with two types of fishing, the driftnet "menaide" (an ancient method of fishing) and also with the purse seine. The aim of this study was to determine the chemical composition of *E. encrasicolus*, to assess the nutritional value and the food safety of this species, comparing the results of the anchovy caught with "menaide" those caught with purse seine. Fatty acids (mainly T3 series) are higher in the anchovies caught by menaide than those fished by purse seine, showing a lower content of saturated fatty acids and an higher content of polyunsaturated fatty acids, especially docosahexaenoic (DHA) and eicosapentaenoic (EPA) acids.. The high content of EPA and DHA produce antithrombotic and anti-inflammatory effects and helps the adjustment of calcium metabolism, lowering LDL cholesterol and increasing HDL cholesterol. So it makes the anchovy particularly suitable in mediterranean diets particularly for children and the elderly.

Key words: Anchovies % Chemical composition % Nutritional value % Atherogenicity and thrombogenicity index % Health benefit % Fatty acids

INTRODUCTION

The fish in general has a chemical composition like to the other meat (chicken, beef etc) with the addition of some specific nutritional properties which are not present in other nourishments. It is widely recognised that fish has a high nutritional value for the human being since it is mainly composed of water ranging from 60 to 80%, of proteins with noble biological value from 15 to 23 %, it is rich in precious mineral salts such as potassium, phosphor and iron and vitamins A and C, but, above all, the quantity and the composition of fats present differs the fish from other food.

These fats are rich in unsaturated molecules, fatty acid and Omega 3, such as the docosahexaenoic acid (EPA) and docosahexaenoic acid (DHA), which can be found only in some fishery product. As far as the Mediterranean fish is concerned, it has a high nutritional value. The proteins provided by these species, are very high, with some exception in the mollusc species. The Mediterranean fish can therefore be included in the category of low fat fishery products. The peculiarity is that these fishes are mainly composed of healthy fats, belonging to the category of the Omega 3. These are precious nutrients that are not available in other foodstuff and are therefore indispensable for the health to prevent cardiovascular and other serious illnesses. Another important property that makes Mediterranean fish a healthy food is the low quantity of cholesterol present in these species (www.inran.it). The T-3 fatty acids are essential dietary nutrients and one of their important roles is providing the fatty acid with 22 carbons and 6 double bonds known as DHA for nervous tissue growth and function. Inadequate intakes of T-3 fatty acids decrease DHA and increase T-6 fatty acids in the brain [1]. DHA is an important structural component of membrane lipids which are widespread in the central nervous system. It has a significant role in retinal tissue, where it represents 80% of PUFA of membrane, allowing them a rapid transmission of light. It is also capable of arresting apoptosis of retinal photoreceptors [2]. A number of positive effects of T3 fatty acids on

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human health have now scientifically been proven. These effects are related to the formation of substances generically called eicosanoids. Among these the most studied are the prostaglandins, there are over thirty types, divided into families: PG1 and PG2 are derived from T6 fatty acids, PG3 comes from T3 fatty acids. Prostaglandins with the major effects on human health are the PG1 and PG3. These inhibit platelet aggregation (antithrombotic effect), inhibit the inflammatory response, regulate calcium metabolism, improve the functioning of the nervous system and immune system, lowering LDL cholesterol, but increasing HDL cholesterol [3].

Several large epidemiological investigations, as well as randomized clinical tests [4, 5], have underscored the cardiovascular benefits of EPA and DHA [6] that are found almost exclusively in seafood. The 2007 recommendations from the American Dietetic Association (ADA) and the Dietitians of Canada (DC) have lately joined in a growing international chorus [7] specifying a healthful intake of EPA and DHA, now set at 500 mg/day. To achieve that intake you must follow the recommendation of the ADA/DC (according to the American Heart Association [AHA]) and aim at consuming "two servings per week, preferably of fatty fish" [4, 8].

The fish species that we studied (*Engraulis encrasicolus*) is a pelagic species that belongs to the Engraulidae family. It has a very slim body with the typical coloration of pelagic fish: a dark back to avoid detection by birds and a silver belly, which is confused with the appearance of the surface of the water when observed from below.

In Italy, this fish is commonly consumed fresh during spring and summer, but it can be preserved in salt which is another traditional method of consumption. The anchovies are usually caught by the purse seine with light source, but only in few places (i.e. the Gulf of Catania, Sicily) they are also caught by a driftnet called "menaide". In this study we've evaluated the chemical composition, the microbial values, the crude composition and the eventual presence of pathogens in Mediterranean anchovies *E. encrasicolus* in order to define the nutritional value, the impact of processing on the ideal characteristics of the product and the potential healthy properties if regularly present in human diet. Particurarly the anchovies by "menaide". The salted anchovies are also consumed, so we have also taken samples already processed anchovies.

MATERIALS AND METHODS

Twenty samples of *E. encrasicolus* were taken directly on landing at the Marin fisheries of Catania (Sicily, Italy), Fig. 1.

Landing data for some fishing vessels and for the two different fishing métiers were collected during the survey. The fish samples were collected directly from fishermen of Catania and Ognina after their landing and then immediately frozen until analysis to ensure the integrity of the tissue samples. We also collected 10 samples of salted anchovies (caught by menaide) from typical local seafood distributors.

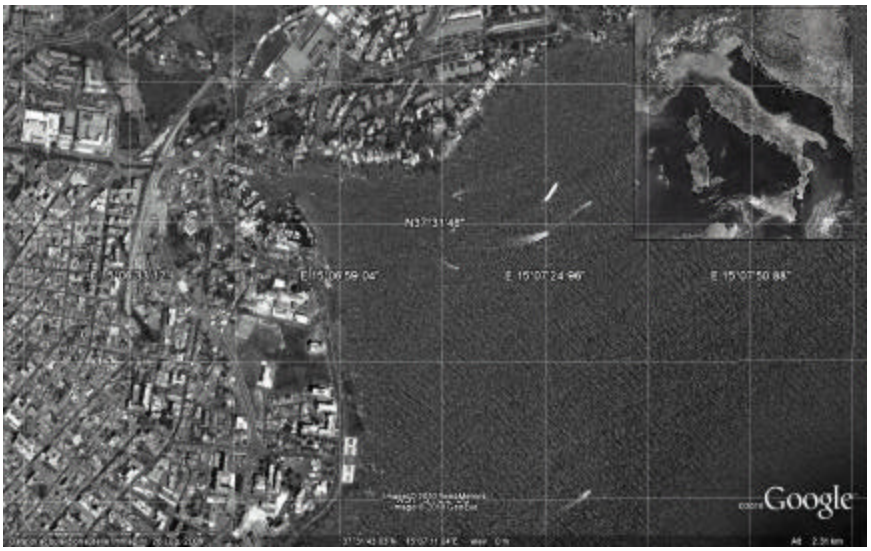

Fig. 1: Study area (the Gulf of Catania, Sicily, Italy)

Types of Fishing: Purse seine: this method is used to encircle fish schools in mid-water, close to the surface, by a netting wall with small meshes. The lower part of the net is then closed to prevent escapement by diving. The purse seine is always operated from a vessel, varying in size from small coastal purse seiners of 15m in length to large ocean going purse seiners as large as 100m length. When a fish school has been located, the catching operation starts by dropping a surface buoy with a line connected to the end of the seine. As the vessel moves forward, the drag from the buoy line will pull the purse seine overboard and the seine is paid out in a circle around the fish school. Light is used to attract fish. In darkness the light will either attract the target species directly or indirectly by attracting and illuminating prey organisms. In modern purse seine fisheries, hydro-acoustic equipment (sonar) is widely used for locating fish schools and also for monitoring the position of the school relative to the gear during the setting of the seine. Menaide driftnet: these systems are employed at night without the aid of light or echo-sounder to attract and detect fishing schools. Fishing boats remain tied to the nets, once they are set at sea. During the fishing season, every night several sets are made according to the catches. There is no net hauler or other gear handling equipment on the boat.

Net dimensions range between 150 and 350 m in length, between 10 and 20 m in height, with a mesh of 2.5-2.6 cm.

Microbiological Analysis: We investigated: Total aerobic bacterial count at 32°C (by official method ISO 4833:2003), Total coliforms (by official method ISO 6887-3:2003), *Escherichia coli* (by official method ISO 7251:1993), *Staphylococcus aureus* (by official method ISO 6888-1:1999), *Listeria monocytogenes* (by official method ISO 11290-2:1996), *Listeria sp.* (by official method ISO 11290-2:1996). Two answers for the analysis were given for each parameter.

Chemical Analysis: Samples received in the laboratory, were weighed, gutted, threaded and homogenized. On each sample were analyzed: Moisture, according to the AOAC official method 950.46 B (a); Percentage of ash, according to the official method AOAC 28.023 (b); Protein content, obtained by multiplying by 6.25 the percentage of nitrogen determined by the method Kijeldhal, using a Büchi apparatus mod. K 314; Total lipids according to the method of Folk, Lees and Stanley [9] modified by Ways and Hanahan [10]; Total Volatile Basic Nitrogen (TVB-N) according to the reg. 95/149/EC. The method is applicable for TVB-N concentrations from 5 mg/100 g to at least 100 mg/100 g.

Atherogenicity Index (AI) and Thrombogenicity Index (TI) were determined according to Ulbricht e Southgate [11]. Two answers for the analysis were given for each parameter.

The analysis of crude composition and AI and TI indices were also carried out on salted anchovies.

Parasitologic Analysis: A research on the parasite Anisakis sp., responsible for zoonosis anisakiasis was also carried out. 30 specimens of *Engraulis encrasicolus* were eviscerated and the viscera and the celomatic cavities were carefully checked for anisakidae larvae presence.

RESULTS AND DISCUSSIONS

The composition of fish products (water content, high content of unsaturated fatty acids) makes them so valuable from a nutritional point of view, but it is at the same time the cause of their particular perish ability. The fish meat, considered sterile at the time of capture, may be also contaminated by the numerous micro-organisms present in water and, consequently, on the fish itself, at the gills, skin and gastrointestinal tract. The microbial load present on fish depends, sequentially, on several factors such as season, water temperature, the possible pollution from municipal waste (mesophilic microorganisms), the method of harvesting and further processing. The results of microbiological tests carried out are presented in Table 1. By analyzing the results we can see that the total bacterial count, total coliforms and *Escherichia coli* values are within the limits suggested in the literature [12]. Regarding the presence although minimal of *Staphylococcus aureus*, this is surely due to the handling suffered by samples after fishing. *Listeria* sp. and *Listeria monocytogenes* are absent. This parameter is undoubtedly of great importance because its presence is due to improper hygiene practices during the handling and marketing of the product.

The analysis of the crude composition (Table 2) of all samples shows similar values regarding of water content and ash, despite being caught with different tools. Anchovies caught in the *menaide* have a greater fat content and a lower total protein content than those caught with purse seine.

Table 1: Microbiological tests on samples (TABC=Total Aerobic Bacterial count; TC= Total Coliform)

	TABC 32°C	TC	*Escherichia coli*	*Staphylococcus aureus*	*Listeria sp.*	*L. monocytogenes*
	CFU/g	MPN/g	MPN/g	CFU /g	P/A in 25g	P/A in 25g
Purse seine	76.000	148	0	11	Absent	Absent
Menaide	115.000	174	0	16	Absent	Absent

n= 20

Table 2: Crude composition of samples

	Water content	Ash	Protein	Fat	
Samples	% mean±SD	% mean±SD	% mean±SD	% mean±SD	*n*
Purse seine	24.63±0.50	2.41±0.02	23.20±1.22	0.96±0.01	20
Menaide	25.48±0.50	2.56±0.16	22.12±1.01	1.57±0.71	20
Salted	53.46±2.97	29.95±3.01	25.58±1.00	4.21±0.92	10

Table 3: Percentage distribution of fatty acids (mean values)

	Menaide	*Purse seine*	Salted
Satured	%	%	%
C14:0	3,97	4,48	4,60
C15:0	0,69	0,84	0,86
C16:0 iso	0,07	0,07	0,11
C16:0	20,67	21,16	20,13
C17:0 iso	0,07	0,12	0,09
C17:0	1,04	1,38	1,02
C18:0	4,56	4,64	3,88
C20:0	0,19	0,25	0,20
C22:0	0,20	0,25	0,17
C23:0	0,05	0,08	0,04
C24:0	0,21	0,18	0,19
Monounsatured			
C14:1	0,15	0,19	0,20
C16:1T7	4,10	3,32	3,56
C17:1	0,35	0,49	0,29
C18:1T 9	6,44	7,81	4,84
C18:1T 7	3,20	3,03	2,52
C18:1T 5	0,06	0,06	0,08
C20:1T11	0,14	0,12	0,67
C20:1T 9	0,42	0,55	
C20:1T 7	0,24	0,15	0,24
C22:1T 11	0,27	0,29	0,56
C22:1T 9	0,17	0,17	0,35
C24:1T 9	0,25	0,15	0,20
T 3 Polyunsaturated			
C18:3T 3	0,39	0,45	0,68
C18:4T 3	0,48	0,59	1,11
C20:3T 3	0,07	0,10	0,10
C20:4T 3	0,28	0,25	0,36
C20:5T 3	7,51	6,90	9,95
C21:5T 3	0,24	0,21	0,28
C22:5T 3	1,40	1,18	2,11
C22:6T 3	29,13	27,62	30,29

Continued Table 3: Percentage distribution of fatty acids (mean values)

	Menaide	*Purse seine*	Salted
T 6 Polyunsaturated			
C18:2T 6	1,06	1,20	1,08
C18:3T 6	0,07	0,07	0,07
C20:2T 6	0,24	0,28	0,24
C20:3T 6	0,09	0,07	0,02
C20:4T 6	1,65	1,82	0,91
C22:4T 6	0,16	0,21	0,22
C22:5T 6	1,06	1,13	0,90
C16:4T 1	0,07	0,06	0,07
C16:2T 4	0,40	0,39	0,47
C16:3T 4	0,13	0,07	0,11
TOTALS			
Saturated	31,72	33,45	31,28
Monounsatured	15,78	16,32	13,51
Polyunsaturated	44,42	42,60	48,94
Poli T 3	39,51	37,30	44,87
Poli T 6	4,32	4,78	3,43
T3/T6	9,14	7,80	13,08

Table 4: (S/P) Saturated/unsaturated ratio, (AI) atherogenic and (TI) thrombogenic indexes (SD: standard deviation)

	S/P	AI	TI	
	% ± SD	% ± SD	% ± SD	*n*
Purse seine	0,51 ±0.50	0,67 ±0.02	0,23 ±1.22	20
Menaide	0,48 ±0.50	0,61 ±0.16	0,21 ±1.01	20
Salted	0,46 ±2.97	0,62 ±3.01	0,18 ±1.00	10

The data, comparable with other reported in the literature [13-15], are justified by a difference in size of the samples analyzed. As a matter of fact those from the purse seine fishing are smaller. The results obtained confirm that an increase in size corresponds to a slight decrease in the total protein content and an increase in total fat, mainly due to an increase of accumulated lipid under the skin. It is different for the processed product, where we have higher percentage of water detected - due to the presence of salt that retains more water - as well as of ash content. The protein have values slightly increasing compared to the fresh product, while fats are the component that has a noticeable increase. The fat content in the analyzed species can be considered low, being all the obtained values less than 3% of the product fresh.

Table 3 shows the fatty acid composition of both the species caught with the two different gears and the transformed ones (salted).

From the nutritional point of view, the anchovies caught with the menaide have a better distribution of fatty acids and a lower and better saturated content in polyunsaturated fatty acids, especially EPA and DHA. The high fatty acid content of the T3 series recorded in both the samples caught with the two different gears, enhances their nutritional value.

The antithrombotic and anti-atherogenic effect, linked to fatty acid composition, can be assessed through indices AI (atherogenic index) and TI (thrombogenic index) calculated according to Ulbricht and Southgate [11] and reported in Table 4.

From capture to death of the fishery products till the time of consumption, chemical modifications are involved. They depend on many factors, such as the intrinsic properties of the fish (species, stage of development, diet, living environment) and the treatment suffered after being caught.

The law strictly controls the quality of the products, sets binding limits on the marketing and on the consumption. In case the organoleptic examination of the product does not respect the freshness or shows improper maintenance, laboratory tests for chemical and microbiological assessment of the fish shall be carried out. Among the most important it is the determination of total volatile basic nitrogen (TVB-N), which mainly comprises trimethylamine, ammonia and dimethylamine.

Table 5: TVB-N levels at time T0 and time T3 (SD= standard deviation)

	T_0	T_3	
	mg/100 g ± SD	mg/100 g ± SD	*n*
Purse seine (whole)	20,81 ± 3.16	30,46 ± 0.75	20
Purse seine (eviscerated)	22,65 ± 1.79	27,76 ± 2.06	20
Menaide (whole)	17,87 ± 1.76	23,61 ± 0.65	20
Menaide (eviscerated)	20,06 ± 1.72	23,87 ± 0.70	20

The European Commission on 8 March 1995, adopted Decision 95/149/EC, which establishes the method to determine the concentration of TVB-N in fish and fishery products and the acceptable limits for some species of fish. The values of the analysis (Table 5) performed immediately after the Fisheries (T0) are in all cases well below the lower limit values mentioned in the reference table (Commission Decision 95/149/EC) relative to other fish species. From the data obtained, we noticed that the ABTV during storage, after three days (T3) is lower for anchovies menaide compared to those of the purse seine, as well as at (T0). Indeed, fishing with the menaide, which allows not only a selection of size but also reduces stress for the animal, increases the time-life of the product. The evisceration, however, does not seem to affect the freshness of the product.

To assess the quality of the anchovies considering the presence of parasites we examined the presence of Anisakis. Larval stages of Anisakis represent a potential risk to human health following consumption of fish or marine mollusks cephalopods, parasitized, raw or undercooked. The larvae are located in elective abdominal cavity and organs and once the dead fish, larvae leave the abdominal cavity to invade the muscles, this migration takes place after a certain period of time increasing the risk of human infection if the fish is not immediately gutted [16].

Some transformation processes, such as storage at low temperature, the heat and curing, cause the death of the parasite, especially when it is localized in cavitary organs.

The outcome of the search for Anisakis is negative for all the samples examined that were obtained by fishing by purse seine and by menaide. This result is in line with the percentages of frequency of infestation in fish caught in Italian waters [12] in which, for the species studied were detected at a low frequency percentage of infestation with Anisakis compared to other fish species. When stored properly the fish caught by menaide deteriorates less quickly.

In conclusion, fish consumption (and particularly of the blue fish) has shown to be the only realistic way to increase dietary quantities of PUFAs long-chain T3 such as eicosapentaenoic acid and docosahexaenoic acid and re-establish a more balanced T6:T3 ratio in the diets of human beings. It has been ascertained that fish plays a preventive role in the occurrence of cardiovascular diseases. In any case the suggestion that ‘a person is well advised to eat more fish’ remains good advice [17].

The results of this research show that the distribution of fatty acids, particularly those in the T3 series, is better for the anchovies fished by menaide. They present a lower content of saturation and a higher content in polyunsaturated fatty acids, especially EPA and DHA. The species under study, however, show a high content of fatty acids. This makes the anchovy particularly suitable for diets and especially for the nourishment of children and the elderly, since the high content of fatty acids produces antithrombotic and anti-inflammatory effects, the adjustment of calcium metabolism, thus improving the functions of the nervous system and the immune system while lowering LDL cholesterol and increasing HDL cholesterol.

Summing up, eat blue fish, particularly anchovies is very good for health. The anchovies caught by menaide have higher nutritional value and play their potential healthy properties if regularly present in human diet.

The commercial value of anchovy caught by “menaide” is very much higher than the one usually found on the national market. The anchovies caught by this method, are particularly well adapted for salting because of their size which is usually bigger than those fished by purse seine. After capture the heads of the fish are removed while still in the net, in order to remove blood and entrails. The bleeding of fishes is done to preserve the quality of the fish meat and improve the muscle appearance and flavour and allows a rapid chilling. The removed blood eliminates also the possibility of lipid oxidation while fish is stored frozen [18].

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