

USING GC-MS TECHNIQUE TO EVALUATE THE OMEGA-3 CONTENT FROM OIL OF OF THREE SPECIES OF IRAQI MARINE FISHES

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ABSTRACT

The current study was conducted from October 2013 until the beginning of September 2014 to estimate the Omega -3 fatty acid for three species of marine fish (*Acanthopagrus arabicus* , *Chelonsubviridis* and *Cynoglossus arels*). The study was conducted during the four seasons winter of 2013, spring of 2014 , summer of 2014 and fall of 2014. The oil was extracted by organic solvent extraction, the fatty acid was estimated using Gas Chromatography connected with mass spectrometry (calculated on the basis of the total amount of the fatty acids), omega-3 percentage of both types DHA, EPA has varied between the fish as it reached 56.5% , 55.9% , 67,13% , 49.22% during winter, spring, summer and autumn respectively. Also it was found ,by the results, that the percentage of omega -3 type EPA was high in the winter as the percentage was 30.99%, followed by the summer as it was 30.42% and the lowest rate was in the autumn as it was 20.33%, while the percentage of the second type of omega -3 DHA was close during the four seasons, as the summer achieved the highest percentage which was 36.71% and the lowest percentage was in the winter as it reached 25.06%.

INTRODUCTION

Fish oils are good sources of fatty acids that are not synthesized in the human body. Fatty acids in fish oil have a very special character compared with other sources, they consist not only of essential fatty acids, but are also an important source of omega-3 fatty acids, especially Eicosapentaenoic acid (EPA, 20:5 n-3) and Docosahexaenoic acid (DHA, 22:6 n-3) and they are the most important fatty acids that are commonly

found in marine fish and arise from phytoplankton and seaweed, which are part of the food chain for fish (1). They have a positive effect on humane health because the liver does not have the ability to manufacture them (2). (3), showed that fatty acid content of omega-3 type in shark was 2500 mg / 100 g and for this it was recommended to be as good and rich sources of omega -3. Omega-3 has an effect in decreasing triglycerides in blood by 20% through balancing between triglycerides storage and its independence (4) and also in decreasing heart clots (5).

The study aimed to extract fish oils and to identify fatty acids type omega-3 found in these fish, and knowing the suitable conditions to form the primarily essential fatty acids that give nutritional value for fish.

MATERIALS AND METHODS

Field study (work):

Three types of marine fish *Acanthopagrus arabicus*, *Chelonsubviridis*, *Cynoglossus arels*. The Samples were collected quarterly from the Iraqi marine waters northwest of the Arabian Gulf, samples were placed in containers filled of ice.

Laboratory work:

Samples were brought to the lab and cleaned and washing by water tap, sensory tests were conducted, and these tests included: (smell and color of the gills and the appearance of the eyes and cohesion of the skin peels and textures and exterior) at a laboratory temperature, these specifications were recorded and stated, according to (6). The smell and colour of the oils extracted were checked depending on the sense of smell and look, then the muscles were extracted and separated from the rest of the parts of each type of the studied fish species .

The guts, heads, skin and bones were removed thereby we have models of the samples of each type of fish, and also meat of each type of fish was mixed separately.

Extraction of oils:

The Oil was extracted from samples of each species of the studied fish using the method of extraction of organic solvent followed by (7) after mincing the samples to ensure that they are small enough. 100 g were taken of the mincing sample of the fish and put in a beaker and then distilled water added and the mixture was mixed by electric mixer, then chloroform and methanol were added to the previous mixture and naturalized by electric mixer on the speed of the 2000 circles for one minute the homogenized mixture was transferred to tubes and transported to the centrifuge , then the mixture separated into three layers, the upper layer of water (methanol layer and

water), semi-solid layer (concentrated with protein) and lower layer (dissolved oil layer in chloroform), and the upper layer was taken out using the dropper and neglected, while the lower layer with semi-solid layer were filtered on filtration paper, and then evaporated and the oil was collected in dry glass bottles and saved by freezing or cooling, The oil produced by this method is known crude oil.

Diagnosing the fatty acids by GasChromatographytechnique connected with mass spectrometry (GC – MS)

A similar esterification was conducted to the fatty acids according to the method described in (8) later the fatty acids were diagnosed in the extracted oil of the types of fish using Gas Chromatography device connected with mass spectrometry type (GC – MS QP210 Ultra, ShimadzuApan) equipped with capillary pole type DB-MS 5 (5% phenyl, 95% methyl polysiloxane) as an inactive (static) phase with dimensions (30 meters in length and a diameter of 0.32 and the thickness of static phase 0.25micrometer) , also High-purity helium gas was used 99.9.

The separation process was conducted in accordance with the thermal program GC on 50° for a minute then raised to 150°C for two minutes at a rate of 5° per minute and then raised 280° at a rate 5° per minute , the temperature was stabilized on 280°C for a minute. The injection process was carried out using automatic injector type AOC - 20is, SHIMADZU.

RESULTS AND DISCUSSION

Omega -3 amount of fatty acids was determined, it was in high percentages in some species, and of significant change; as a result of the different types of fish and the difference of fat composition in the areas of the fish's body that varied relatively with each other.The differences that happened in the fatty acid percentagesare due to the condition of food and heat.

The results showed the percentage and the amount of omega–3 in both types which got to, during the winter, 56.05% . The highest percentage of EPA was in *A.arabicus*, reaching 11.6% and the lowest value was in *C.subviridi* 8.74%, while the percentage of the DHA was close in all types. *C.arels* achieved the highest percentage of 8.77% and the lowest percentage in *A.arabicus* 8.04%. This indicates to increase the proportion of the EPA during the winter compared with DHA as shown in Figure (1). It is less than what has (9) in a study on fish oils in Ireland and found that the amount of fatty acid EPA in fish oils 18.2% to the type of fatty acid DHA 12.2%.

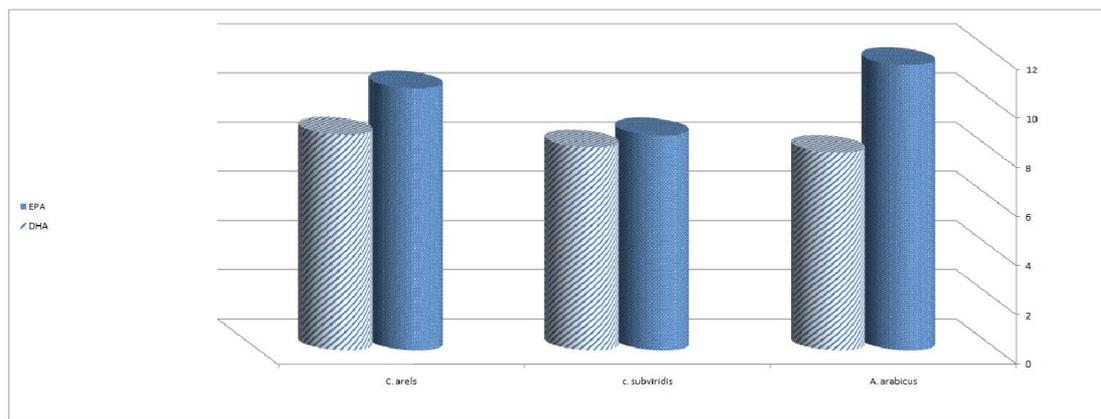


Fig.(1) the percentage of omega- 3 in *A. arabicus* , *C. subviridi* and *C.arels*during winter

But in the spring the proportion of omega - 3 was about 55.29%, the highest percentage of EPA was in *A.arabicus*, reaching 12.64% and the lowest value was in *C.arels* 4.55%, while the percentage of the DHA was the highest in *C.arels* which was 12.94% and the lowest value in *A.arabicus* 5.45%, as in Figure (2). The difference between the percentage of the two types of omega-3 acid due to the nature of the food and the season. These results agreed with (10).

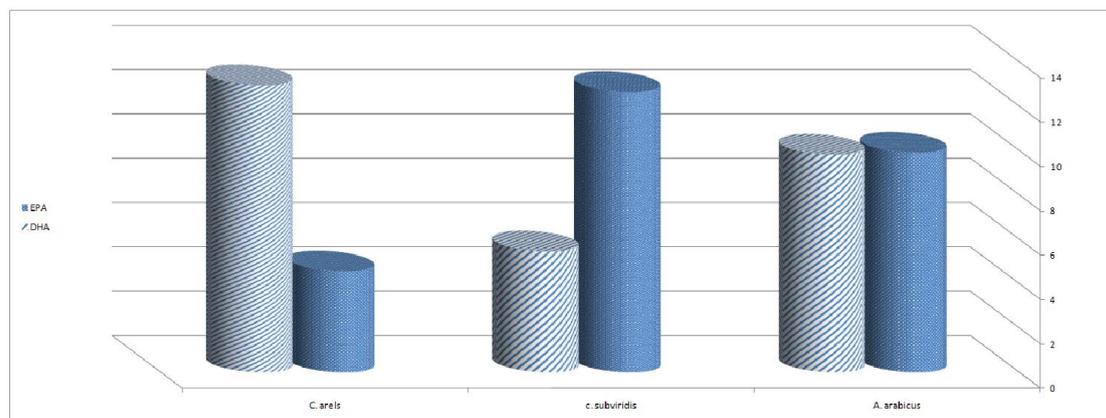


Fig (2) Omega-3 percentage of both types *A.arabicus* , *C. subviridi* and *C.arels* during spring season

Figure (3) shows omega- 3 percentage in both types of marine fish during the summer where it reached 67.14%, which the highest percentage of omega–3 acid during the four seasons, as *C.arels* achieved the highest percentage of both types EPA and DHA,

reached to 11.14%, 14.65% respectively, and the lowest value for the EPA was in *C.subviridi* 9.53%, and the lowest proportion of DHA was found in *A.arabicus* 10.4% and the difference in omega-3 content due to the feeding techniques and the type of food consumed by the fish (11).

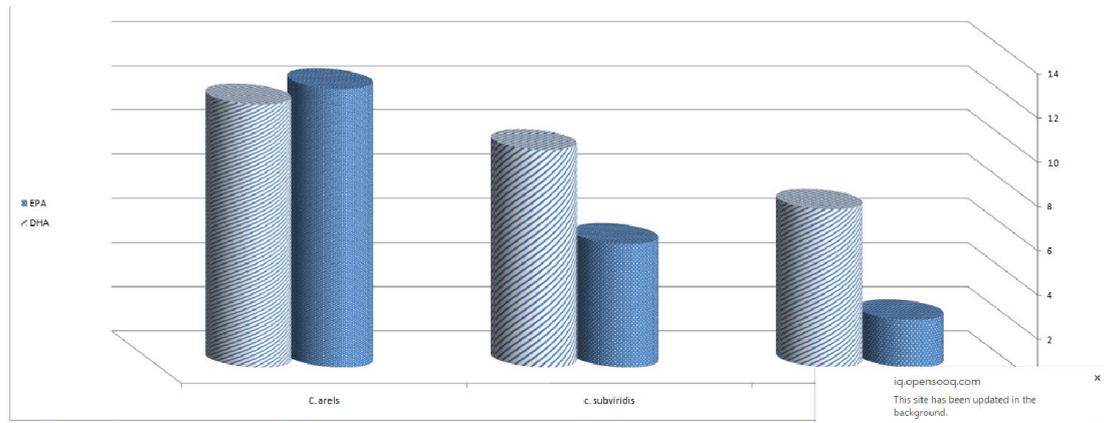


Fig (3) Omega-3 percentage of both types in *A. arabicus* , *C. subviridi* and *C. arels* during summer season.

showed for the autumn that there was about 49.12% of omega – 3 of both types EPA, DHA in the studied fish. The *C. arels* achieved the highest percentage of both types EPA and DHA, reached to 11.14%, 14.65% respectively , and the lowest value for both types was found in *A.arabicus*2.14% , 7.15% respectively as shown in fig (4). This difference in omega - 3 percentage depends on the nature of fish food (12).

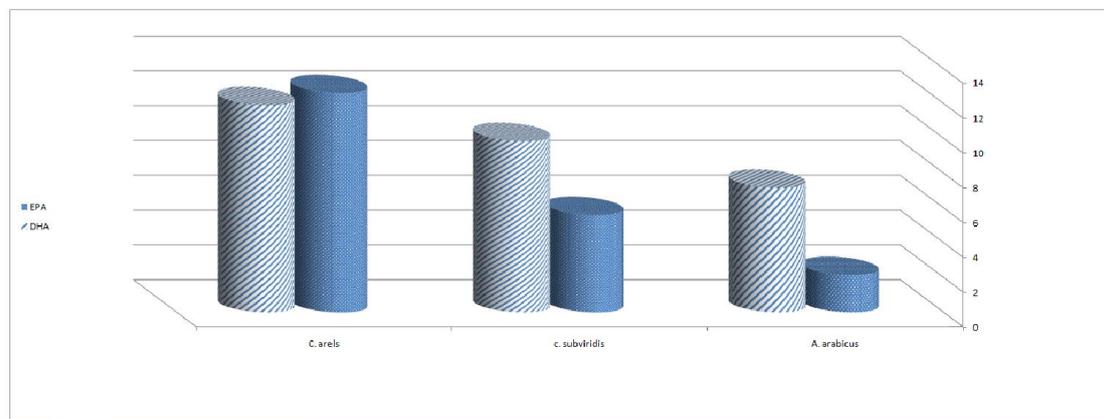


Fig (4) Omega-3 percentage of both types *A. arabicus* , *C. subviridi* and *C.arels* during summer season

When Comparing marine fish content of omega-3 between seasons of the year we find that the fish in the summer, with the highest content of omega-3 compared to other seasons, as shown in Figure (5). *C.arels* has contained the highest percentage of omega -3 type DHA during all seasons and the highest percentage of EPA during the summer and autumn.

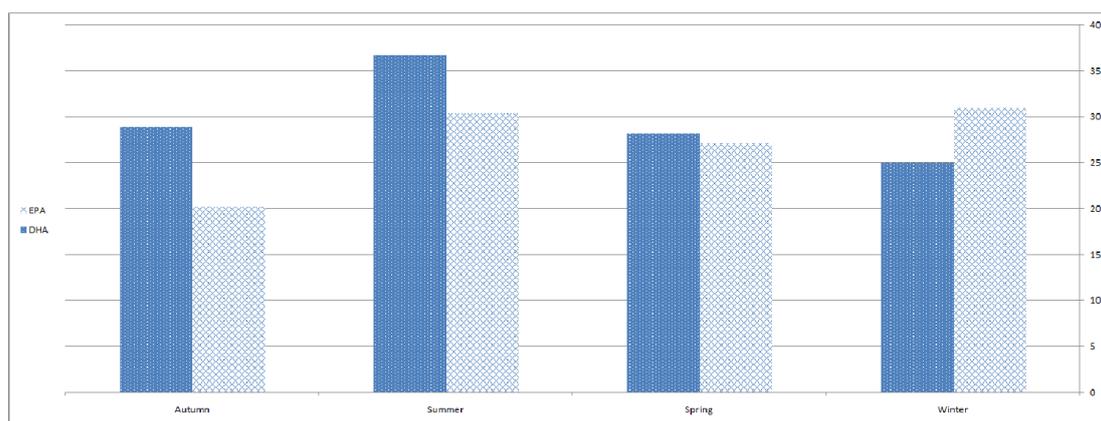


Fig (5) Omega-3 percentage of both types in *A. arabicus* , *C. subviridi* and *C.arels* during the four seasons

These differences due to the season of the year (Jangaard, *et al.*, 1967) and the structure of food (13) or the type of fish (11) and the depth of fishing water (14). These results were agreed with (15) data in terms of that edible marine fish in the Arabian Gulf contain high amounts of unsaturated fatty acids, particularly fatty acids of the type omega-3, and with the results of (16) concerning marine fish in Norway. (17) showed in their study about the quality of fatty acids in cooked and frozen sardines that there are significant differences even between the same class of sardines. These results agreed with (15) in his study of the importance of the nutrition of the edible marine fish in Qatar Coast region, and those results were confirmed by (18), who studied the fat content, fatty acids, cholesterol and the amount of energy in some kind of fresh and cooked marine fish in the United Arab Emirate, and with (19) of omega-3 content in some marine fish in Turkey, and also with (20) in determining the type and structure of fatty acids in some marine fish in Turkey, also with (21) of the content and structure of fatty acids in marine fish on the south west coast of Brazil.

استخدام تقنية GC- MS لتقييم محتوى الاوميكا-3 فيزيوت ثلاث انواع من الاسماك البحرية العراقية

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الخلاصة

اجريت الدراسة الحالية من بداية تشرين الاول 2013 ولغاية ايلول 2014 لتقدير الاحماض الدهنية نوع اوميكا-3 لثلاثة انواع من الاسماك البحرية وهي الشانك *Acanthopagrus arabicus* والبياح الاخضر *Chelonsubviridis* ولسان الثور الاملس *Cynoglossus arels* ، اجريت الدراسة اثناء الفصول الاربعة شتاء 2013 و ربيع 2014 وصيف 2014 وخريف 2014 . تم استخلاص الزيت بطريقة الإستخلاص بالمذيب العضوي ، وقدرت الأحماض الدهنية باستخدام كرماتوكرافي الغاز المتصل بمطياف الكتلة (محسوبة على أساس المجموع الكلي للأحماض الدهنية الكلية) ، وقد تباينت نسبة الاوميكا-3 بين الانواع السمكية بنوعها EPA ، DHA اذ بلغت 56.05% ، 55.29% ، 67.13% ، 49.22% خلال فصل الشتاء ، الربيع ، الصيف والخريف على التوالي. كذلك وجد من خلال النتائج ان نسبة الاوميكا-3 نوع EPA كانت مرتفعة في فصل الشتاء اذ بلغت نسبتها 30.99% ويليها فصل الصيف اذ كانت 30.42% واقل نسبة كانت في فصل الخريف اذ بلغت 20.33% ، اما النوع الثاني للاوميكا-3 DHA فكانت نسبتها متقاربة خلال الفصول الاربعة اذ حقق فصل الصيف اعلى نسبة لها وهي 36.71% وادنى نسبة كانت في فصل الشتاء اذ بلغت 25.06% .

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