DETERMINING OMEGA-3 FATTY ACID IN BASA FISH BY FT-IR SPECTROSCOPY AND GAS CHROMATOGRAPHY

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ABSTRACT: *Eating much fish has long been encouraged to prevent heart diseases and strengthen human health; however, there has been still a wonder why fish brings to people the wonderful usefulness like that. It's because of Omega-3 fatty acid found in fish. This famous micro-element helps prevent certain chronic diseases such as heart disease and arthritis, reduce cholesterol, and regulate blood pressure as well. Therefore, it's highly necessary to determine Omega-3 fatty acid in fish. In this paper, we use the method of the FT-IR Spectroscopy and the GC-MS to determine Omega-3 fatty acid in basa fish which are now popularly being fed in the area of Mekong-delta.*

The research results are compared with the different authors' works.

I. INTRODUCTION ABOUT FATTY ACID OMEGA-3

Omega – 3 is the common name of unsaturated fatty acids in which the first double bond fastened with the carbon which is located at third position of alkyl line (n-3).

1. Molecular formula:

- Linolenic acid: $C_{18}H_{30}O_2$
- Eicosapentaenoic acid (EPA): $C_{20}H_{30}O_2$
- Docosahesaenoic acid (DHA): $C_{22}H_{32}O_2$

2. Chemical formula:

- Axít Linolenic:

$CH_3CH_2CH = CHCH_2CH = CHCH_2CH = CH(CH_2)_7COOH$ (18:3n-3)

- Eicosapentaenoic acid:

$CH_3CH_2CH = CHCH_2CH = CHCH_2CH = CHCH_2CH = CHCH_2CH = CH(CH_2)_3COOH$ (20:5n-3)

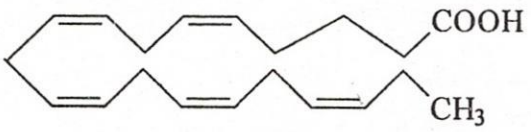
- Docosahesaenoic acid:

$CH_3CH_2CH = CHCH_2CH = CHCH_2CH = CHCH_2CH = CHCH_2CH = CHCH_2CH = CH(CH_2)_2COOH$ (22:6n-3)

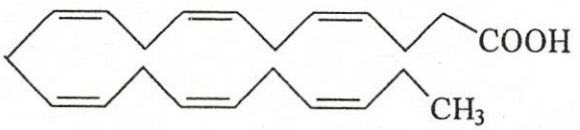
3. Constitutional formula:

- Linolenic acid :

- Eicosapentaenoic acid:



- Docosahesaenoic acid:



α-Linolenic acid (ALA), Eicosapentaenoic Acid (EPA) and Docosahesaenoic Acid (DHA) are fatty acids which are very necessary for human health but our body can't himself create them but have to complement from food. These acids are belonged the fatty acids group namely omega-3 fatty acid. There are much ALA in vegetation oil such as linseed-oil. When it enter my body, our body will change ALA into EPA and DHA. These are two fatty acids which our body can imbibed easily. EPA and DHA have mainly in fish with different contents [5].

The Omega-3 fatty acid can prevent the chronic diseases such as heart disease, arthritis; reduce cholesterol, regulate blood pressure; improve the lung function at asthma persons; reduce the risk of breast cancer at women, ... [5].

The research goal of this work is to define the content of fatty acid omega-3 in basa fish which is currently brought up a lot in Vietnam, the most in The Mekong River Delta which is used for export and national consumption. These results will helped the aquatic farmers, the cooking oil producers, foods and others involved with this kind of acid for more argument and reality foundation to develop their career.

The research method is to use the FT-IR spectroscopy to determine the amount of unsaturated fatty acid in basa fish and Gas Chromatography combined Mas spectrography to name and quantify each fatty acid in basa fat. The results will be compared with the former works and the foreign documents.

II. EXPERIMENTAL AND RESULTS

II.1. Analyzing the total content of unsaturated fatty acid by FT-IR spectroscopy

We use KBr pellet technique for recording FT-IR spectra of the samples. After creating the pellet by hydraulic machine, we coat a very thin and regular layer of fat (which was extracted by Soxhlet) on the surface of the KBr pellet and then record spectrum of it. Fig 1 is FT-IR spectrum which was recorded of basa fish fat.

To quantitative analyze, we choose the asymmetric stretching vibration ν_{as} (=CH-R) at wavenumber of 3000 cm^{-1} as calculating peak (Fig 1) and CCl_4 as dissolvent [1,2]. Usually, the unsaturated fatty acid in fish fat which has great content is Oleic ($C_{18:1}$) (Table 1), so this acid is chosen as the standard substance for building standard line.

Weigh 49,9 mg of Olenic acid and dilute with suitable amount of CCl_4 solvent to get solutions with corresponding concentrations are 9.52 ppm, 15.87 ppm, 31.74 ppm, and 79.36ppm. Then pour one after another of these solutions into cell (d=0,5cm) and record FT-IR spectrum at the resolution of $4cm^{-1}$ and 10 scans (the number of scans is small because the solvent evaporates very quickly). The corresponding standard line is ploted with these data.

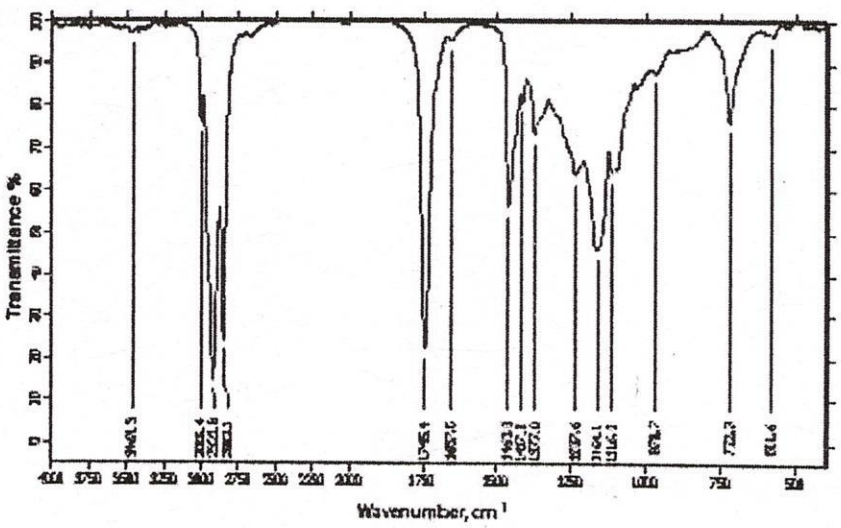


Figure 1. IR spectrum of basa fish fat

Weigh 500,6mg of fat (which was extracted by Soxhlet tool) and dilute by CCl_4 solvent with suitable quantity so that the concentration is in area of standard line. Then, pour this solution into cell as above and record spectrum FT-IR with the same condition like standard solutions. Figure 2 is FT-IR spectra of standard solutions and sample solution. The calculated results show that the total content of unsaturated fatty acids in basa fish fat is 61,01%.

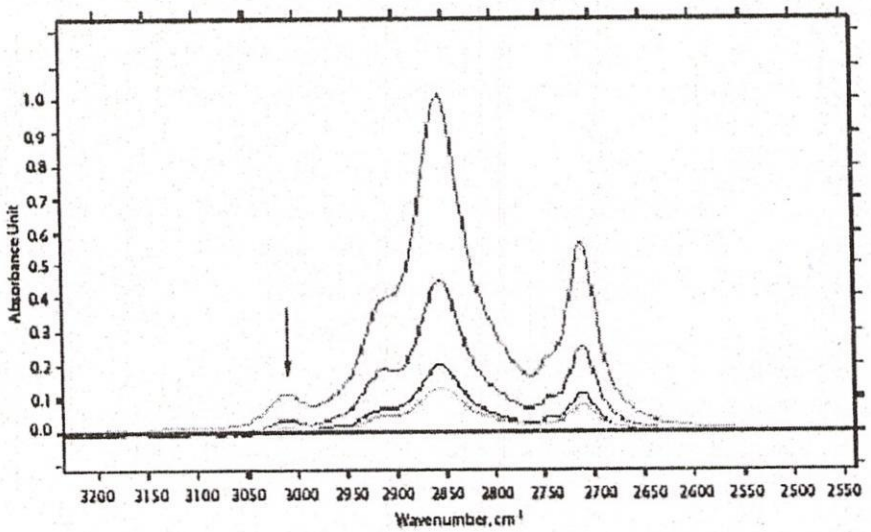


Figure 2. IR spectra of standard and sample

II.2. Analyzing fatty acids by Gas Chromatography (GC) or Gas Chromatography combined with Mass Spectrography(GC-MS)

Because the fat is triglyceride with great moiecular weight and is difficult to evaporate, so the fat must be hydrolyzed in alkali environment, then carry out the methyl ester to change into the ester's components of easier evaporated fatty acid so that it will be analyzed by Gas Chromatography or Gas Chromatography combined with Mass Spectrography [3,6].

We use this method to investigate the dependence of fatty acid components on the temperature of fat extraction: extracted at cold temperature (4^0C), extracted by the method of Soxhlet (70^0C), and fry. The Fig 3 is chromatogram of fat at cold temperature. The table 1 shows the results of fat components follow the extraction temperature.

Table 1: The change of fat components follow the extraction temperature of fat

No	Fatty acids		Contents with different extraction methods		
			Cold (4^0C)	Fry	Soxhlet (70^0C/6h)
1	Lauric	$C_{12:0}$	0,24	0,19	0,07
2	Myristic	$C_{14:0}$	4,39	5,91	4,44
3	Pentadecanoate	$C_{15:0}$	0,25	0,24	0,13
4	Paimitic	$C_{16:0}$	22,97	30,19	32,04
5	Hexadecenoic	$C_{16:1}$	3,50	2,27	1,33
6	Hexadecanoic	$C_{17:0}$	0,33	0,25	0,14
7	Stearic	$C_{18:0}$	9,63	8,46	7,76
8	Oleic	$C_{18:1}$	39,94	40,01	41,17
9	9,11-Octadecadienoic	$C_{18:2}$	9,76	5,85	8,03
10	Linolenic	$C_{18:3}$	0,41	0,40	0.21
11	Eicosanoic	$C_{20:0}$	0,47	0,29	0,35
12	11-Eicosenoic	$C_{20:1}$	1,66	1,41	1,80

13	11,14-Eicosadienoic	$C_{20:2}$	0,53	0,37	0,81
14	Arachidonic	$C_{20:4}$	0,72	0,40	-
15	Eicosapentaenoic	$C_{20:6}$	0,31	0,72	0,40
16	Docosanoic	$C_{22:0}$	0,21	-	-
17	Docosahexaenoic	$C_{22:6}$	0,32	0,13	-
18	Total saturated fat		38,69	45,53	44,93
19	Total unsaturated fat		61,31	54,47	55,07
20	Total one-bond unsaturated fat		45,22	43,69	44,30
21	Total multi-bond unsaturated fat		16,09	10,78	10,77

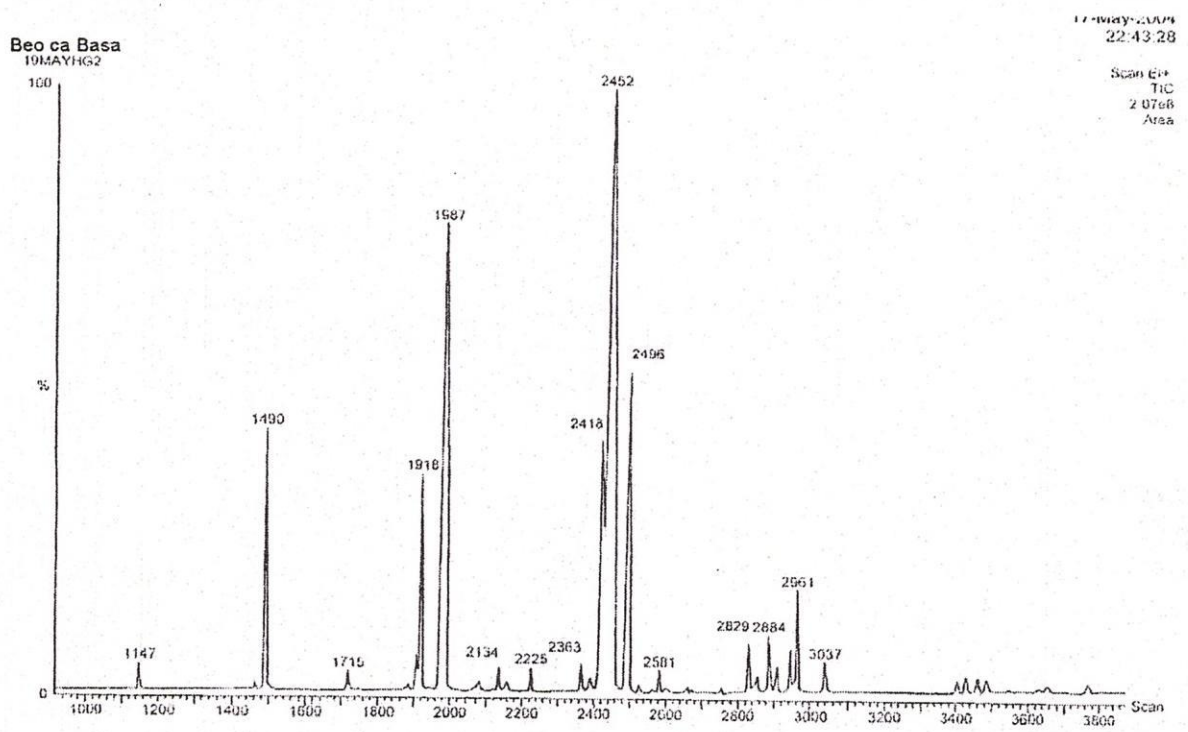


Figure 3. Chromatogram of fat extracted at cold temperature

III. DISCUSSION

Analysing the components of basa fish fat, shows that: main components of fatty acid are $C_{16:0}$ and $C_{18:0}$, of one-double bond unsaturated fatty acid is $C_{18:1}$, of multi-double bond unsaturated fatty acid is $C_{18:2}$. The total contents of saturated acid, of one double bond acid and of multi-double bond acid obey the order: one-double bond > saturated > multi-double bond. This is suitable to the results of former authors [4,7]. Furthermore, we can see the appearance of 3 kinds of fatty acid omega-3 are ALA, EPA and DHA in basa fish fat. However, the quantity of saturated fatty acid in basa fish meat is more than codfish.

The result of analyzing total unsaturated fatty acid by the FT-IR method is 61,01%. This is very suitable with the analyzed result by the extraction method at cold temperature which is 61,31%. Meanwhile, extracting by the method of Soxhlet at 70^0C is 55,07%. This shows that there are changes of total unsaturated fatty acid follow the extraction temperature.

Investigating the change of fatty acid components follow the extraction temperature of fat also shows that the high temperature make higher the total content of full fatty acid from 38,7% to 45,1% and make lower the quantity of unsaturated fatty acid from 61,3% to 54,5%. Especially, at high temperature with long time, fatty acid omega-3, most is DHA – the very necessary substance for human's brain, will disappear totally.

IV. CONCLUSIONS

With this work, firstly we defined that there are three kinds of fatty acid omega-3 in basa fish fat with the content more than 1% per total of fat quantity, specially there's the appearance of DHA which is necessary for nutrition demand of human and have the property to prevent cancer which our body can't create. However, DHA is very sensitive with oxygen, therefore if processing fish at high temperature will make change completely this acid.

At high temperature, the analyzing results show that the rate change of fatty acids: total saturated fatty acids increase, meanwhile total of unsaturated acids decrease. This will make less quality of basa fish fat.

Can depend on the analyzing the quantity of unsaturated fatty acid by FT-IR method in CCl_4 solvent to define the quality of fat, oil from animal and plants.

ỨNG DỤNG QUANG PHỔ HỒNG NGOẠI BIẾN ĐỔI FOURIER (FT-IR) VÀ SẮC KÝ KHÍ ĐỂ XÁC ĐỊNH AXÍT BÉO OMEGA-3 TRONG MỠ CÁ BASA

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TÓM TẮT: *Con người đã biết ăn nhiều cá để chống lại bệnh tim mạch, tăng cường sức khoẻ, nhưng họ chưa biết tại sao cá lại có tác dụng như thế. Đó chính là nhờ trong cá có acid béo omega-3. Nguyên tố vi lượng nổi tiếng này giúp ngăn ngừa những bệnh mãn tính như bệnh tim, viêm khớp; làm giảm lượng cholesterol, điều hoà huyết áp,... Nhu cầu xác định omega-3 trong các loại cá là rất cần thiết. Trong bài báo này, chúng tôi dùng phương pháp phổ hồng ngoại biến đổi Fourier (FT-IR) và sắc ký khí ghép khối phổ (GC-MS) để xác định omega-3 trong mở cá basa, loại cá mà hiện nay được nuôi rất nhiều ở nước ta nhất là ở đồng bằng sông Cửu Long.*

Các kết quả này được so sánh với các công trình trước đây và của nước ngoài.

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