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COMMERCIAL FISH OIL

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ABSTRACT: The crude commercial fish oil was analysed qualitatively and quantitatively by gas liquid chromatography. The major fatty acids detected in this oil were as follow: 24.8% stearic,23.6% palmitic, 9.84% myristic, 6.56% octadecatetraenoic acids. The physical and chemical properties of crude commercial fish oil were established ,some deodorization methods were applied and the obtained results were discussed.

Key Words: Fish Oil, Composition, Gas-liquid Chromatography,Deodorization.

TİCARİ BALIK YAĞI

ÖZET: Ham ticari balık yağı gaz-likit kromatografisi ile kalitatif ve kantitatif olarak analiz edildi. Saptanan başlıca yağ asidleri %24.8 stearik, %9.84 palmitik, %6.56 miristik, %6.56 oktadekatetraenoik asidlerdir. Ham ticari balık yağının fiziksel ve kimyasal özellikleri incelenmiş, bazı koku giderme metodları uygulanmış ve sonuçlar tartışılmıştır.

Anahtar Sözcükler: Balık Yağı, Kompozisyon, Gaz-Sıvı Kromatografisi, Deodorizasyon.

INTRODUCTION

With the exception of isovaleric acid, all fatty acids found in fish and other similar oils are normal straight-chain compounds.Fish oils are characterized by a relatively high proportion of unsaturated fatty acids, present as triglycerides[1]. .Most of the unsaturated acids are C_{16}(principally palmitoleic), C_{18}(principally oleic with some octadecatrienoic) C_{20} and C_{22} acids.The saturated fatty acids in fish oils make up between about 10-25% of the total acids present,and the principal acid present is palmitic.Myristic acid is the second most important of the saturated acids.

The fatty acid composition of body lipids was detected by gas-liquid chromatography for 14 species of saltwater fish , 3 species of freshwater fish ,and 4 species of shellfish[2]. Fatty acid methyl esters from 4 kinds of marine animal oils were analyzed with both diethyleneglycol succinate polyester and diethylene-glycol adipate polyester columns[3]. Gas choramatographic analysis of oil of 12 kinds of fish showed the predominant fatty acids were linoleic in type and of 20-22C atoms in length[4].

Protein content of fish generally varies between 55-70%.The protein of fish is broken down into intermediate amino acids by the action of enzymes and bacteria[5]. According to Taylor[6] ; fish enzymes must be active in the fish body at or even below the freezing point of water.After death they cause, at ordinary temperatures, rapid enzymic deterioration.

It was assumed that the odor of spoiled fish was caused in part by accumulation of indole, a breakdown product of tryptophan[6]. It has long been known that fish and shell fish , especially if stored for some time , contain appreciable amounts of amines[6].

Highly unsaturated fatty acids in fish oils are oxidatively decomposed during the storage,which unavoidably form low molecular weight acids and/or ketones or aldehydes.Therefore,even though the fish oil freshly pressed from fresh materials has no perceptible odor,the low molecular weight amines contained in the fish oil react,with the lapse of time,with the low molecular weight ketones and/or aldehydes that are formed during the storage and thereby odorous substances are formed,which give forth a nasty smell and cause lowering of the commercial value of fish oil[7].

The deodorization of fats and oils may be made neccessary either because of a naturally developing rancidity in the oil or because of offensive materials formed as a result of processing deodorization methods have included masking[8-12], washing with a selective solvent[13,14], adsorption with fuller's earth or charcoal[15], fermentation[16-18], and steaming at high temperatures and reduced pressures[19,20].

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The fish oil in this study was provided by a factory in the Eastern Black Sea Region of Turkey.The oil was obtained from various fish found at the Black Sea.Since it was a mixture, it was termed as "commercial fish oil" . Before its deodorization, the fatty acid composition of the fish oil was determined.

MATERIALS AND METHODS

Some characteristics of the crude commercial fish oil are given in Table I,which were determined by the conventional methods[21].

Gas Liquid Chromatography

Methyl esters of the fish oil fatty acids were analysed using a Varian Model 1400 gas chromatography provided with a flame ionization detector and a disc integrator. For the analysis, methyl esters of the fish oil fatty acids which were prepared by methanolysis with methanol sulphuric acid[22] , 2 feet and 1/8 inch stainless steel packed column with 15% OV-275 on 80/100 CWAW was used.The column was run at programmed temperature from 100°C to 200°C at a rate of 2°C/ min.The other operating conditions of the chromatography were: injection port temperature 160°C, detector block temperature 230°C and nitrogen flow rate 20 mL/minute .In all cases, areas under the peaks were measured with disc integrator and the composition were expressed as wt percent.

Table I: Some characteristics of the crude commercial fish oil

Refractive indice (n_D^{30})	1.483
Iodine value	64.93
Sponification value	187.4
Acid value	10.15
Reichert-Meissl value	1.76
Polenske value	0.6
Unsaponifiable matter (%)	0.46

For qualitative and quantitative analysis, as a first step, methyl esters of the fish oil fatty acids were prepared by methanolysis with methanol sulphuric acid [22] . The total fatty esters of the oil were hydrogenated in a methanolic medium using platinum oxide (Adams' catalyst) for about 5-6 hours [23]. Methyl esters of pure fatty acids were used as reference standards for the C_8-C_{24} saturated acids and $C_{16:1}$, $C_{18:1}$, $C_{18:2}$, $C_{18:3}$ unsaturated acids.The chain lengths of the peaks which had no standards were identified according to the retention time data as presented in Table II. The chain length of unsaturated acids were further verified by comparison with the chromatogram of the methyl ester samples before and after hydrogenation.

Disc integrator was used for the calculation of the peak area.The linearity of the detector response was checked by injecting an increasing amount of model mixtures of known methyl esters and by comparing the peak height ratios of any two components of different concentrations.

Deodorization Methods

For the deodorization of the fish oils,several methods were investigated.

The Salting Out and pH Effect

This method of "salting out" proteins,probably depends upon several physical phenomena, the two most important ones are the suppression of the charge on the surface of the protein by the salt ions of opposite charge and the removal of water shell formed around the protein molecule by the competition of the ions of the salt for that water[24].

At pH values between 4 and 8, at which most fractination processes are carried out ,the net charge on a protein molecule is much smaller than the one in the extremely acid and alkaline ranges. But, the total number of positive and negative groups reach a maximum somewhere in this range and the solubility reaches a minimum[25].

The pH tests have demonstrated that, the best precipitation of proteins in fish oil occured at pH=4.5 and 5.5 buffer systems.

PH=4.5	pH=5.5
0.374g $CH_3COONa.3H_2O$	0.2506g $C_6H_8O_7.H_2O$
0.13mL 100% CH_3COOH	3.2mL 1 N NaOH
19.77mL dist.H_2O	16.8mL dist.H_2O

3 grams of $(NH_4)_2SO_4$ is appropriate for 1 gram of oil. 1 part of oil ,4 parts of buffer system and solid $(NH_4)_2SO_4$ were stirred at 40°C for 3 hours.The amount of salt , temperature and reaction period were determined experimentally.After centrifugation of the mixture,the upper oil phase was separated by decantation.The deodorizated fish oil was thus obtained.

Solvent Effect

The addition of solvent to an aqueous system lowers its dielectric constant, which means, in effect, that the electrical forces between charged particles in the solution are increased ,thus reducing the solubilities of substances such as proteins[24].

1 part of oil, 3 part of 95% ethanol (or acetone) were kept in deep freeze at -17°C , for 5 days.The amounts and storage periods used were experimentally determined.The mixture was filtrated, and after distillating the solvent, the deodorizated fish oil was obtained.

Kieselgel Effect

Kieselgel is an adsorbent and precipitates undesired materials. 1 part of oil, 3 parts of hexane and 1/40 part of Kieselgel 60G were stirred at 30°C, for 2 hours.The reaction period and temperature used were as mentioned in the literature[15]. The amount of Kieselgel was determined experimentally.The mixture was filtrated and after the distillation of hexane, an oil was obtained, which almost contained no proteins.

Formaldehyde Effect

Formaldehyde reacts with amide groups of the protein. In acidic medium N-methylene bisamides and in basic medium N-Hydroxymethylamides are obtained[26]. 1 part of oil and 1/10 part of formaldehyde were stirred for 15 minutes by heating. The mixture was neutralized by 5% NaOH. After the addition of water, the mixture was transferred into the separating funnel. The oil phase was separated, and oil without protein was obtained. The amounts and reaction periods were determined experimentally.

Steam Distillation Effect

Since the volatile amines and proteins cause fishy odor,they were removed from the oil by steam distillation[19,20].

The deodorizated fish oils were compared by colour reaction.First,the deodorizated fish oils were hydrolized by 6N HCl at 30°C,for a day.(15 mL. 6N HCl was used for 5 g. oil) and all the proteins in the oil were changed to amino acids.Separated acid phase was concentrated to 80% and 5 mL. Of concentrated acid phase was boiled with 0.5 mL. Ninhydrin reagent which reacts with amino acids to give blue colour. The obtained colour intensity is due to concentration of amino acid and was measured by spectrophotometer at 520 nm. Oils obtained by the two best deodorization methods, were stored for 5 months,and measured again by spectrophotometer.The results are given in Table III.

RESULTS AND DISCUSSION

Firstly, the crude commercial fish oil was analysed qualitatively and quantitatively by gas-liquid chromatography.Our investigation was undertaken to determine the fatty acid composition of the used fish oil and also to find out the importance of the oil as a fatty acid source.

The retention times and weight percentages of the fish oil's fatty acids methyl esters were determined on 15% OV-275 column before and after hydrogenation. The results are presented in Table II.

The data shows that the amount of constituents of the fatty acids varied widely among themselves. The prominent saturated fatty acids, according to weight percentages, were found to be 24.8% stearic,

23.46% palmitic, 9.84%myristic, 2.47% pentadecanoic acids, unsaturated fatty acids were 6.56% octadecatetraenoic, 6.56% octadecatrienoic and 5.2% palmitoleic acids.

This commercial fish oil can be used in industry as source of stearic and palmitic acids' productions.

The crude fish oil was attempted to be deodorized by steam distillation and by treatment with ethanol, acetone, kieselgel, formaldehyde, ammonium sulphate at different pH values. In this work, commercial fish oil was deodorizated by removing proteins and volatile amines from the oil. The methods in used $(NH_4)_2SO_4$, ethanol, formaldehyde and acetone were developed due to denaturating of proteins in the fish oil. Kieselgel is an adsorbent and precipitates great molecules such as protein. Steam distillation was removed volatile amines from the oil. The change of fish oil odour was investigated by colour reaction. The results obtained are given in Table III. Formaldehyde method was the best deodorization one according to the results and during storage of fish oil thr odour increased due to the concentration of the remained protein.

Table II:Retention values and weight percentages of the methyl ester before and after hydrogenation.

Before Hydrogenation			After Hydrogenation	
RT(minute)	WT %	Carbon Number	WT %	RT(minute)
6.5	1.18	8:0	0.83	6.5
10	1.62	10:0	0.91	9.5
14.25	1.13	12:0	1.08	14.5
21.5	9.84	14:0	12.5	20
22	2.0	?	1.83	22
24.5	2.47	15:0	3.54	23.5
31.75	23.46	16:0	31.5	30
33	5.2	16:1	-----	-----
40.5	24.8	18:0	36.78	38
43.5	2.67	?	8.92	43.5
44.5	1.49	18:1	-----	-----
45.5	4.37	18:2	-----	-----
51	6.56	18:3	-----	-----
54.25	0.79	19:0	1.20	56
57	6.56	18:4	-----	-----
65.5	1.13	20:0	0.83	65.5
75	4.63	20:2	-----	-----

Table III:The colour intensity with ninhydrin at 520 nm.

Sample	Measurements at once	Measurements 5 months later
The crude oil	0.450	-----
The oil with acetone	0.445	-----
The oil with ethanol	0.324	-----
The oil with $(NH_4)_2SO_4$ at pH=4.5	0.323	-----
The oil with steam distillation	0.320	-----
The oil with $(NH_4)_2SO_4$ at pH=5.5	0.300	-----
The oil with kieselgel	0.233	0.329
The oil with formaldehyde	0.180	0.196

Fish oils are usually in paint, varnish, cemicals, soap, and leather industries and in medicine and cosmetics. Since the bad odour of the fish oil creates problems in industry, its deodorization has become an important issue.

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