of fish oil adulteration is fairly easy by use of the formula, with the 0.5 correction omitted.

Summary

Typical ultraviolet spectrophotometric absorption curves are presented for unhydrogenated fish oils of commercial importance, before and after alkali isomerization. These curves show the usual maxima for isomerized polyunsaturated fatty acids, including five, and possibly six, double bonds. Tetraene absorption is sufficient to obliterate that of trienes completely. Such a method of analysis affords an easy means of detecting unhydrogenated fish oil contamination in the ordinary vegetable and animal oils and fats as well as a test for the presence of fish oils in admixture with raw linseed oil.

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The Identification of the Fatty Acids of the Fat From a North American Black Bear[1]

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Introduction

RECENTLY Rasmussen, Morgal, and Miller (1) reported the physical and chemical constants of the fat of a North American black bear. Their values were within the range of those found for other species (2-7). They ascribed the differences of the various constants of the fats as due to nature of the fat in the ration consumed during the fattening processes. Their analyses showed the bear fat to contain 10.4% of the saturated fatty acids and 79.4% of unsaturated fatty acids. In view of the high percentage of the latter substance the study of bear fat was continued. The present paper deals with the identification of the various higher fatty acids of bear fat.

Materials

The bear fat used in this study was rendered on December 22, 1945, from adipose tissue taken from the shoulders, neck, and sides of a 4-year old North American male black bear weighing 287 pounds. The bear was killed near Adam's trail in Alger County, Michigan, on November 17, 1945. The fat was removed immediately after the bear was killed and then kept in the frozen state until it was rendered. This was accomplished by placing the fat in a kettle which was set into a container of boiling water and then removing the non-fatty material by straining through a cloth. The fat was a white semisolid at room temperature and had a slight lard odor.

The following constants were observed: melting point 22°C.; refractive index at 20°C. 1.4689, 40°C. 1.4613; specific gravity 100/15 .8776; saponification number 197.6; iodine number (Wijs) 96.0; thiocyanogen number 75.2; per cent free fatty acid (oleic) 6.3; per cent saturated acid 19.6 and per cent unsaturated acid 73.4.

The methods employed for these analyses are those found in the Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists (8) and and Vegetable Fats and Oils by Jamieson (9).

Experimental

Preparation of the Fatty Acids.

The bear fat (532 gm.) was saponified by refluxing for 4 hours with one liter of 98% ethanol containing 130 gm. of potassium hydroxide. After saponification the solution was diluted with 4 liters of distilled water and extracted with Skelly B for 6 hours in a continuous liquid-liquid extraction unit. The ethanolic-aqueous alkali solution was acidified with 188 ml. of concentrated hydrochloric acid and the solution extracted with diethyl ether for 6 hours. The ether was removed in vacuo at 16-mm. pressure and 35°C. The crude fatty acids were dried in vacuo at 4-mm. pressure and 60°C. for 4 hours. A yield of 510 gm. of fatty acid was obtained.

Preparation of the Methyl Esters of the Fatty Acids.

The fatty acids (510 gm.) were converted to the methyl esters by refluxing 4 to 6 hours with 1 liter of absolute methanol containing 5% sulfuric acid (by weight). The methanol was then removed in vacuo and the residual solution was diluted with water and neutralized with 10% sodium carbonate. The fatty acids were extracted with ether and the ether solution was washed with water to remove the last traces of sodium carbonate. The ether was removed in vacuo and the esters dried at 6-mm. pressure and 60°C. for 8 hours. A yield of 491.8 gm. of crude methyl esters was obtained.

Fractionation of the Methyl Esters of the Fatty Acids.

a) Description of Distilling Column. A four-foot Stedman column, having an internal diameter of 25 mm., was used for this study. It was provided with an electrical heating jacket to permit the column to be heated. The one liter distilling flask was heated by a Glas-Col electrical heater. The temperature of both the flask and column was regulated by means of an A. C. variable transformer. A D. M. Smith still head with an enclosed thermometer was used at the top of the column. The reflux ratio was regulated by a

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TABLE I

Fractionation of the Methyl Esters of the Fatty Acids in Bear Fat.

Fraction number	Boiling point in degrees C. at 0.5 mm. pressure	Weight of fraction in grams	Iodine number	Refractive index at 40°C.	Saponification numbers of methyl esters		Melting point of derivative degrees C.		Acid identified
					Found	Calculated	Found[2]	From literature	
Original methyl ester	.....	452.8	85.2	.....	.....	.....	.....	.....	.....
1	106-128	8.4	23.5	1.4332	232.1	231.5	80[3]	81	Myristic
2[1]	128-135(131)	85.5	21.0	1.4348	211.3	207.5 209.0	86[3] 126[4]	86 124.5	Palmitic Palmitoleic
3	135-148	4.9	53.1	1.4401	.....	207.5	86[3]	86	Palmitic
4	148-154(152)	54.5	94.4	1.4462	201.3	189.2	131[4]	132	Oleic
5	154-160(154)	251.4	100.6	1.4465	197.3	188.0 190.5	89[3] 114[5]	90 114	Stearic Linoleic
6	160-170(167)	17.5	87.3	1.4472	195.6	188.0	89-90[3]	90	Stearic
7	170-175(172)	18.2	78.6	1.4483	186.3	189.2 188.0	130[4] 88[3]	132 90	Oleic Stearic
Residue	.....	12.4	.....	.....	.....	.....	.....	.....	.....

[1] Figures in parentheses show temperature in which major portion of the fraction distilled.
[2] Melting points are uncorrected.
[3] p-Bromophenacyl ester (10).
[4] Hydroxy acids (9).
[5] Bromine addition compound (9).

2-mm. stopcock just below the condenser of the still head.

b) Distillation. The crude mixed methyl esters (452.8 gm.) of the fatty acids were fractionated into 7 fractions of different boiling points ranging from 106°C. to 175°C. and 0-mm. pressure, using the column previously described. The data giving the weight of each fraction, heating time, refractive index, iodine number, boiling point, and saponification number are given in Table 1.

c) Identification of Fractions. The saponification numbers agree approximately in some cases with the theoretical values for the methyl esters of C_{14}, C_{16}, and C_{18} fatty acids. The saturated acids were identified by the preparation of their p-bromophenacyl derivatives according to the method outlined by Shriner and Fuson (10). The unsaturated acids were identified by fractionating the distillate by means of the lead salt-ether procedure and preparation of the hydroxy derivative (11) or the bromine addition compound (12). The p-bromophenacyl esters were recrystallized to constant melting point from 95% ethanol and the hydroxy derivatives from absolute ethanol or from 1:1 benzene-ethanol mixture. The melting points of the known (9, 10, 12) and the unknown derivatives are given in Table I. Myristic acid was identified in fraction 1; palmitoleic acid (9:10-hexadecenoic acid; neutral equivalent 286.4, theory 288) in fraction 2; palmitic acid in fractions 2 and 3; oleic acid (9:10-octadecenoic) in fractions 4 and 7; linoleic acid (9:10-, 12:13-octadecadienoic) in fraction 5 and stearic acid in fractions 5, 6, and 7.

Summary

A study has been made of the identification of the fatty acids of the fat from a North American black bear. The methyl esters of the fatty acids from the fat were prepared and fractionated through a Stedman Column. Myristic, palmitic, stearic, oleic, palmitoleic, and linoleic acids were identified by the melting points of the p-bromophenacyl ester of the saturated acids and the hydroxy and bromine addition compounds of the unsaturated acids.

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