Fatty Acid Composition of Oil in Snow Crab (Chionoecetes opilio) by Gas Chromatography/Mass Spectrometry¹

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ABSTRACT

The hepatopancreatic fatty acid extract of the snow crab contains a high percentage (26%) of oddcarbon-numbered fatty acids and a substantial quantity (29%) of methyl-branched fatty acids, as indicated by gas chromatography/mass spectrometry (GC/MS) and gas liquid chromatography (GLC). A wide distribution in chain length of the fatty acids $(C_{10} \text{ to } C_{26})$ and in positional isomers of the linear monoenes are also indicated by GC/MS.

INTRODUCTION

Snow or "Zuwai" crabs (Chionoecetes opilio, O. Faburicius) migrate southward from the Alaskan waters to Japan Sea and to the northern Pacific shores of Japan, and they are highly treasured as winter delicacies in Japan. The male "Matsuba" crab is valuable as a sea-product for the San-in District (Lower Honshu facing the Japan Sea). The young molted soft-shelled Matsuba (pine needle) crab is called "Mizu" (watery) crab, and the small-bodied female snow crab is called "Oya" (parent) crab. In an earlier paper (1), the gas chromatographic analysis on the fatty acid composition of Matsuba crab was reported. We have now determined the structures of the complex fatty acids in the hepatopancreas of the Mizu crab by the gas chromatography/mass spectrometry (GC/MS) technique (2).

The unusual fatty acids were identified as saturated and monoenoic methyl-branched, odd- or even-carbonnumbered molecules, or as monounsaturated acids with several position isomers. Preliminary identification was performed by the gas chromatographic equivalent chain length (ECL) method (3), using both polar and nonpolar stationary liquid phases. Results from this investigation on the hepatopancreatic fatty acid methyl esters have facili-

1Report No. 3 of organo-chemical composition of Cheonoeates opilio.

tated a subsequent thorough study on the fatty acid compositions of extracts from raw and cooked Matsuba, Mizu, and Oya crabs according to their hepatopancreatic, egg, and muscle fractions. These compositions, sixteen in all, will be reported in detail in a separate journal.

EXPERIMENTAL PROCEDURES

Preparation of Sample

Fresh Mizu crabs caught off the Japan Sea shores of San-in District and landed at the Karo seaport in Tottori City on December 5, 1972, were washed with water and weighed at our laboratory in Tottori University. The hepatopancreas was removed with a scalpel from the raw Mizu crab to obtain 922 g (8.4% of total body weight) from 38 crabs. Lipids from the soft tissues were thoroughly extracted by 7 shakings with ethyl ether. The ether extract contained minute flocculants that had to be sedimented by centrifugation at 18,000 rpm (36,000 g) for 10 min (0 C) with a Hitachi 20 PR refrigerated centrifuge. The supernatant was concentrated by removal of the ether with careful use of the rotary evaporator to avoid loss of volatile oils. The ether-insoluble tissues were extracted with ethanol and analyzed for soluble free amino acids (4).

Fatty acids were isolated by initially refluxing the ether extract concentrate (105 g) in 10 vol of acetone over a hot water bath at 60-65 C for 3 hr and allowing the mixture to stand overnight to separate the acetone-solubles from the nonsolubles. Then 360 ml of 2N ethanolic KOH were added to the acetone-soluble material (96 g) and refluxed again for 3 hr to insure saponification of esters. After addition of water, the nonsaponifiables were removed by ether extraction in a separatory funnel. The soap phase was acidified with 1N HC1, and the acids were extracted with ether, washed with water, and dried over anhydrous sodium sulfate before removal of the ether. Yield of the fatty acids was 51.8 g; their index of refraction measured by the Abbe refractometer was 1.4741²⁰/D; iodine value determined by

TABLE I
Mass Number of Diagnostic Ions ^a Generated by Gas Chromatography/Mass Spectrometry in the Identification of Methyl Esters Prepared from Snow Crab (Chionoecetes opilio) Hepatopancreatic Fatty Acid

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Total C atoms in backbone	Saturated fatty acids						Unsaturated fatty acids	
	Linear	Branched				Linear	Branched	
		Monomethy1	Dimethyl	Trimethyl	Tetramethyl		Monomethyl	
12	214							
13	228	242					209	
14	242	256				209	223	
15	256	270	284		312	223		
16	270	284	298	312	326	237	251	
17	284	298	312	326		251		
18	298	312	326			265	279	
19	312	326				279		
20	326	340				293	307	
21	340					307		
22	354	368				321	335	
23	368	382				335		
24	382					349		

^aSaturated esters identified by M⁺, monoenoic esters by (M-31)⁺.

the Wijs method was 87; and the neutralization value by 0.5N KOH titration was 188.1.

For gas liquid chromatography (GLC) and GC/MS analyses, the free fatty acids were converted to methyl esters by refluxing in anhydrous methanol with *p*-toluenesulfonic acid as catalyst. The use of diazomethane was avoided because of potential hazards, thereby incurring some losses of volatile methyl esters during their preparation.

GLC

Fatty acid methyl esters from the Mizu crab hepatopancreas was analyzed by GLC on polar diethylene glycolpentaerythritol adipate (LAC-2-R 446 or Resoflex 446) and nonpolar Apiezon L liquid phases. ECL determination was based on a standard mixture of methyl esters of C_8 to C_{24} *n*-alkanoates. Conditions for Resoflex 446 were 5% liquid phase on Gas Chrom Q, acid-washed, silylated, 60/80 mesh; glass coiled column, 1/4-inch o.d., 10 ft long; helium carrier gas, 50 ml/min, 30 psig; column oven temperature ' programmed at 140 C plus 0.5 C/min to 200 C (hold); injection port 200 C, flame ionization detector oven 250 C; Packard 7401 gas chromatograph. Conditions for Apiezon L were identical except for a 4 ft column length.

GC/MS

The fatty acid methyl esters were separated by preparative thin layer chromatography (TLC) into (a) nonpolar or saturated, (b) monoenoic, (c) intermediate mono/dienoic, (d) "dienoic," and (e) polar constituents, and together with the original methyl ester mixture were analyzed by GC/MS. The conditions for preparative TLC and the identification of branched structures by GC/MS were described earlier by Spencer and Tallent (2). The stationary phase for the 6 ft GC/MS column was the polar Silar 5CP, 3% on Gas Chrom Q, instead of the nonpolar 5% Apiezon L. Position isomers of monoenes were determined by GC/MS as described by Plattner et al. (5).

RESULTS AND DISCUSSION

GC/MS Structure Determination

Components identified by GC/MS according to their saturation, branching, and monounsaturation are listed in Table I. No diunsaturated components were detected by GC/MS or by GLC. Values in the table indicate the diagnostic mass number of the ions by which the components were identified (2). Double bond positions were determined for all linear monoenes from C_{16} to C_{24} , and their relative abundance is reflected in the composition by GLC and GC/MS given in Table II. Positions of methyl substituents and unsaturation in the branched fatty acids were not determined.

Branched trimethyl pentadecanoic $(15:0Me^3)$ acid was not detected, although the tetramethyl pentadecanoic $(15:0Me^4)$ homolog was clearly indicated by GC/MS. The high molecular weight components (C₂₀ or greater) were predominantly monomethyl-branched monoenoic fatty acids.

GLC Composition

Fatty acid composition was determined by the combination of GC/MS and ECL methods. Table II lists the ECL values and the percentages of all components, based on analyses using both polar Resoflex 446 and nonpolar Apiezon L columns, ECL values of the GC/MS diagnostic peaks are also listed to corroborate the identification of peaks in the Resoflex 446 chromatograms. The iodine value calculated from the composition given in Table II is 54, the experimental value by the Wijs method being 55 for the methyl ester sample.

TABLE II

Fatty Acid Composition of Snow Crab Hepatopancreatic Extract

Resoftex Silar Apiezon Identification shorthand ⁴ GLC and GC/MS 10.0 10.0 10.0 10:0 1race 12.0 12.0 12:0 0.2 0.2 13.0 13.0 13:0 0.05 0.2 13.5 13.5 13:0 0.1 0.1 14.0 14.0 14.0 14:0 2 14.3 14.3 14.3 14.7 14:1 0.1 14.5 14.6 14:0Me ¹ 0.2 0.2 15.0 15.0 15:0 0.7 15:3 15:5 15.9 15.9 16.2 15:0Me ¹ 0.1 16.3 16.3 15.7 16:1(9) 7 16.3 16.3 15.7 16:1(9) 7 16.3 16.3 15.7 16:1(9) 7 16.3 16.4 16:1Me ¹ 0.6 16.9 17.2 16:0Me ² 0.1 16.7 16:0Me ⁴	Equivalent chain length Percent by									
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$\begin{array}{cccccccccccccccccccccccccccccccccccc$	21.5	21.3	21.8	21:0Me1	1					
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$\begin{array}{cccccccccccccccccccccccccccccccccccc$	21.9		22.2	21:0Me ²	0.1					
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	22.0	22.0	22.0	22:0	0.2					
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	22.3	22.3	21.7	22:1(9)	0.5					
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	22.3	22.3	21.7	22:1(11)	2					
22.5 22.5 22.8 $22.0Me^{1}$ 0.1 22.7 22.6 $22.1Me^{1}$ 2 23.0 23.0 23.0 23.0 0.1 23.3 23.3 22.7 23.1615 1 23.5 23.8 $23.0Me^{1}$ 1 23.8 $$ 23.6 $23.1Me^{1}$ 2 24.0 24.0 24.0 0.1 0.1 24.3 24.3 23.7 $24.1(13)$ 0.2 24.3 24.3 23.7 $24.1(15)$ 1 24.7 $$ 24.6 $24.1Me^{1}$ 2 25.3 $$ 24.6 $24.1Me^{1}$ 2 25.7 $$ 25.6 $25.1Me^{1}$ 0.2 25.7 $$ 25.6 $25.1Me^{1}$ 2 26.7 $$ 26.6 $26.1Me^{1}$ 1	22.3	22.3	21.7	22.1(13) 22:1(15)	0.5					
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	22.5	22.5	22.8	22:0Me ¹	0.1					
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	22.7	22.7	22.6	22:1Me ¹	2					
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	23.0	23.0	23.0	23:0	0.1					
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	23.3	23.3	22.7	23:1(15) 23:0Mel	1					
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	23.8	23.3	23.6	23:1Me ¹	2					
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	24.0	24.0	24.0	24.0	0.1					
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	24.3	24.0	23.7	24:1(13)	0.2					
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	24.3	24.3	23.7	24:1(15)	1					
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	24.7		24.6	24:1Me ¹	2					
$26.7 \qquad \qquad 26.6 \qquad 26.1 \text{Me}^{} \qquad 2$	25.3		24.7	25:1 25:1Mal	0.2					
	26.7		26.6	26:1Me ¹	1					

^aLinear backbone carbon number:number of unsaturations in backbone (position of unsaturation) methyl branching with total branches as superscript; e.g., 20:1(11) is eicos-11-enoic acid and $20:1Me^1$ is monomethyl-branched eicosenoic acid.

Special Characteristics of Crab Oil Fatty Acids

Three outstanding features can be stressed for the hepatopancreatic fatty acid extract of the snow crab. It contains a high percentage of odd-carbon-numbered fatty acids, as indicated by both GLC and GC/MS. It also contains a substantial quantity of methyl-branched fatty acids, as clearly indicated by GC/MS; although this feature was first suspected from their GLC ECL values, which suggested the presence of *iso* and *anteiso* branching similar to those found in marine mammals (2). The third feature is the unusually wide dispersion of positional isomers in the monoenes indicated by GC/MS. Altogether, these characteristics demonstrate the peculiar manner in which the snow crab attempts to maintain a lowered melting point and an increased fluidity for its lipid constituents.

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