생 약 학 회 지
Kor. J. Pharmacog.
5(4):211~216(1974)

Studies of the Components of Purple Laver(Ⅱ)

On Free Fatty Acids

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한국산 「김」의 성분에 관한 연구(Ⅱ)

유리 지방산에 대한 연구

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市販 「김」의 遊離 脂肪酸을 20% DEGS (diethyleneglycol succinate) 칼람을 裝置한 가스 크로마토그라피를 사용하여 separation factor 및 oxymereuration-demercuration法을 利用함으로써 23種의 脂肪酸을 確認하였으며 가스 크로마로그람의 面積으로부터 그들을 각각 定量하였다.

珍奇한 酸은 存在하지 않았으며 $C_{22}F\times1$, C_{26}, oleic acid, palmitic acid가 거의 60%를 차지했다.

Oxymercuration-demercuration法에 依하여 分離된 불포화 脂肪酸중에서 $C_{18}F\times1$, $C_{18}F\times1$, $C_{20}F\times1$는 各各 두個의 幾何異性體가 존재하는 것으로 推定된다.

쇄상 포화 脂肪酸 및 F×1 및 F×2의 쇄상 불포화 脂肪酸은 Ruseva-Atansona 및 Murray등이 言及한 바와 같이 脂肪酸의 炭素數에 대해서 t_R (retention time)을 semi-log plot를 하였을때 直線을 주었다.

The marketed edible Purple Laver is a blend of several species of *Porphyra*(*Rhodophyta*), mainly *Porphyra ternera* K. and *Porphyra yeozoensis* U.[1]

The decreasing activity of cholesterol in blood present in Purple Laver was reported by Kaneta.[2~5]

Katayama reported the constituents of the volatile aicds of Purple Laver.[6]

That the compositions of sterols in Purple Laver were cholesterol, β-sistosterol and stigmasterol and that there is little difference in the compositions of sterols among *Porphyra ternera* K., *Porphyra yezoensis* and Korean edible Purple Laver, were reported by Cook, one of the authors.[1]

Taguchi discovered that 30~60% of fatty acids was $C_{20\sim25}$.[7] In order to study the substance which decreases cholesterol level in blood, we first analyzed the compositions and contents of free fatty acids in Purple Laver.

Experiments

Isolation of Free Fatty Acids of Purple Laver

Purple Laver 196 g was refluxed with 3*l* of benzene for 5 hours. The blackish green-colored oil was obtained by evaporating the benzene successively under reduced pressure. The oily benzene-extract was mixed with small amounts of ether and treated with 5% NaOH. The NaOH layer was acidified with 10% H_2SO_4 and extracted with ether, the resulting ether layer was dehydrated with anhydrous Na_2SO_4

and evaporated under reduced pressure. The resulted semisolid mass was mixed with the ether solution of CH_2N_2 and the mixture was evaporated under nitrogen, therefore, the methyl ester of free fatty acid was obtained(Fig. 1).

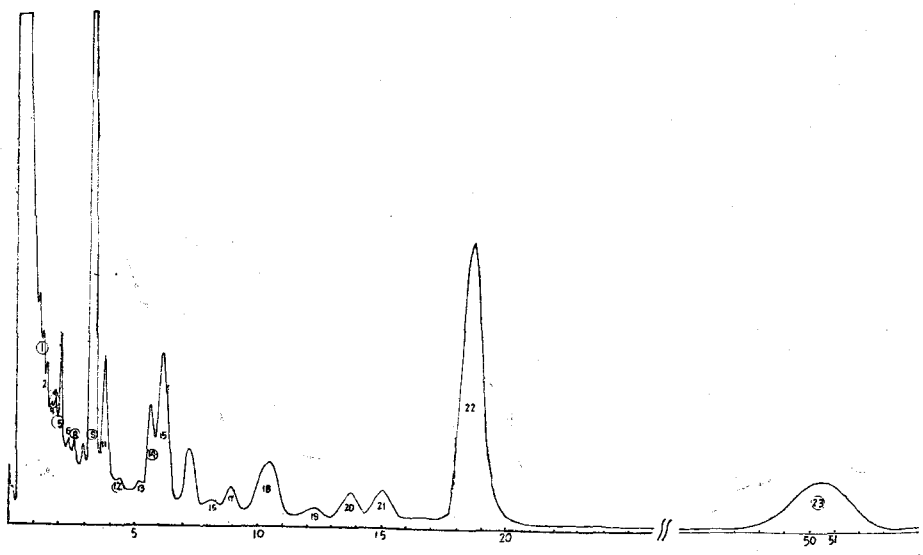

Fig. 1. Gas chromatogram of methyl esters of free fatty acids in Purple Laver
Column: 15% DEGS, 2m×0.4cm Glass, 60ml/min N_2, 185°

Calculation of Separation Factors

The gas chromatographic assay was carried out with the aid of a gas chromatograph, equipped with a flame-ionization detector. A polar column(15% DEGS on Shimalite 60-80 mesh), using a 2m by 0.4cm glass column, was used with nitrogen as the carrier gas.

From the respective peaks resulted from 135°C (150°C) practice of column temperature at the rate of carrier gas N_2 60 ml/min., the calculated separation factor for C_{12}~C_{17} fatty acids methyl esters according to the following LANDOWNE's equation [8] and CLEVAR's equation[9], was shown in Table I.

From the each peaks at 170°C(185°C) practices, the separation factor for C_{17}~C_{26} fatty acids methyl esters was also shown in Table II.

LANDOWNE's equation

$$\text{Separation factor}=\frac{\text{Retention time of saturated component}}{\text{R.T. of preceding saturated component}}$$

CREVA's modified equation

$$\text{Separation factor}=\frac{\text{Retention time of unsaturated acid Me ester}}{\text{R.T. of parent-saturated acid Me ester}}$$

Table I. Retention times and separation factors at 135° and 150°, using 15% DEGS column, of components of free fatty acids of Purple Laver

Peak Number	Retention Time		Separation Factor	
	135°	150°	135°	150°
1 (C_{12})$^{\alpha}$	4.40	3.00	1.38	1.30
2	5.20	3.80	1.18	1.27
3	6.55	4.35	1.06	1.10
4	7.60	4.70	1.23	1.19
5(C_{14})$^{\alpha}$	8.75	5.20	1.41	1.32
6	9.70	5.80	1.11	1.12
7	11.00	6.45	1.26	1.24
8(C_{15})$^{\alpha}$	12.55	7.00	1.43	1.35
9(C_{16})$^{\alpha}$	18.20	10.00	1.45	1.43
10	20.10	10.18	1.10	1.02
11	21.00	12.00	1.20	1.21

α: the fatty acid which could be identified directly by standard sample.

The above separation factor can be formulated: (1) With an increase in the operating temperature of the column, the separation factor for saturated straight

Table II. Retention times and separation factors at 170° and 185°, using 65% DEGS column, of components of free fatty acids of Purple Laver

Peak Number	Retention Time		Separation Factor	
	170°	185°	170°	185°
12(C_{17})$^{\alpha}$	7.05	4.35	1.36	1.32
13	8.45	5.25	1.20	1.21
14(C_{18})$^{\alpha}$	9.55	5.70	1.35	1.31
15(C_{18} : 1)$^{\alpha}$	10.40	6.25	1.09	1.10
11	13.82	8.20	1.08	1.12
17	15.33	8.92	1.20	1.22
18	18.70	10.50	1.07	1.08
19	22.00	12.30	1.26	1.27
20	24.45	13.72	1.03	1.09
21	26.80	15.00	1.13	1.20
22	33.75	17.63	1.05	1.06
23(C_{26})$^{\alpha}$	110.00	50.50	1.34	1.33

α: the fatty acid which could be identified directly by standard sample

chain component decreased. (2) With an increase in the operating temperature of the column, the separation factor for branched-chain saturated isomers, relating to the preceding saturated stright-chain component, also decreased. (3) With an increase in the operating temperature of the column, the separation factor for unsaturated straight-chain componets relative to the parent-saturated staigh-chain component increased.

Separation of Methyl Esters of Saturated and Unsaturated Fatty Acids

In order to separate methyl esters of saturated and unsaturated fatty acids, we utilized CREVAR's method[9], involving preparation of mercuric acetate adducts of unsaturated acids and separation of these adducts from the methyl esters of saturated acids by column chromatography, which had been used in analysis of *Ludwigia alternofolia* L. for fatty acids.

The free fatty acid methyl esters 20mg and 100mg of mercuric acetate were placed in a test tube and 8 m*l* of a solution containing 5% distilled water and 0.3% glacial acetic acid in ethanol was added. The tightly sealed tube was heated in a water bath at 50°C for a few minutes. The solvents and excess acetic acid were evaporated under nitrogen at room temperature. The resulted solid was mixed with 20m*l* of benzene, and the benzene solution dehydrated and evaporated, yielding 5.1mg, methyl esters of unsaturated fatty acids. Their gas chromatograms were shown in Fig. 2.

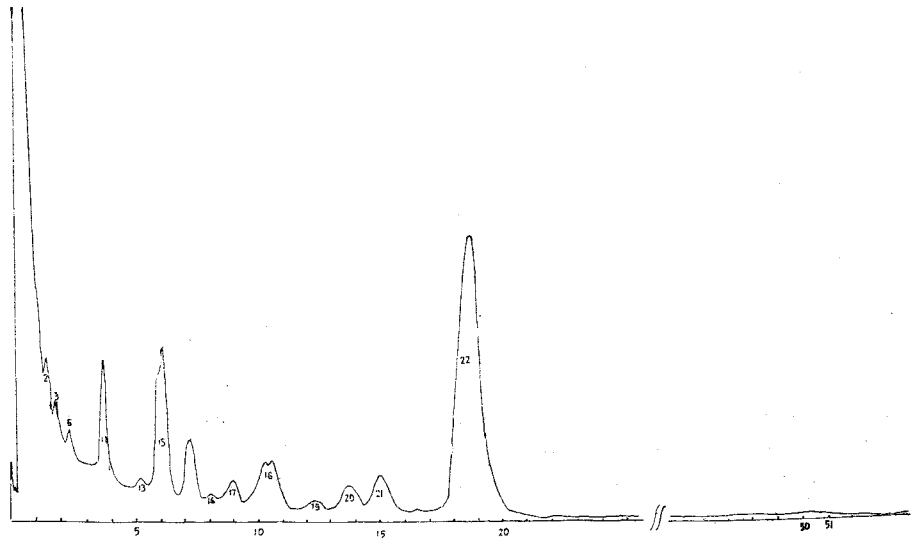

Fig. 2. Gas chromatogram of methyl esters of free unsaturated fatty acids in Purple Laver. Column: 15% DEGS, 2m×0.4cm Glass, 60ml/min N_2, 185°

Quantitative Analysis of Methyl Esters of Free Fatty Acids

Quantitation as percent of total recovered amounts was accomplished by measuring the area of each peak as triangle under following conditions. 15% DEGS on Shimalite 50~60 mesh, 60 m*l*/min N_2, Col. Temp. 170°C

Results and Discussion

With the aid of separation factor and mercuric method, 23 kinds of free fatty acids were identified and analyzed quantitatively by measuring peak area. The result was shown in Table III.

Table III. Identification of respective methyl esters of free fatty acids and percent composition.

Peak Number	Free Fatty Acid Identification	Percent of Composition
1	C_{12}	0.24
2	C_{12} : 1	1.10
3	C_{13} : 1	0.34
4	C_{14} iso	—
5	C_{14}	—
6	C_{14} : 1	—
7	C_{15} iso	0.07
8	C_{15}	0.24
9	C_{16}	0.75
10	C_{17} iso	—
11	C_{16} : 1	3.56
12	C_{17}	0.50
13	C_{17} : 2	0.36
14	C_{18}	7.44
15	C_{18} : 1	9.03
16	C_{19} : 1	1.90
17	C_{19} : 2	1.03
18	C_{20} : 1	2.70
19	C_{20} : 2	0.63
20	C_{21} : 1	1.43
21	C_{21} : 2	4.37
22	C_{22} : 1	28.12
23	C_{26}	17.42

For the normal saturated fatty acids and normal unsaturated fatty acids containing up to two double bonds, the number of carbon was accurately linear with retention time, being semilog-plotted, as indicated Ruseva-Atansona *et al.*[10~12] The result was shown in Figs. 3 and 4.

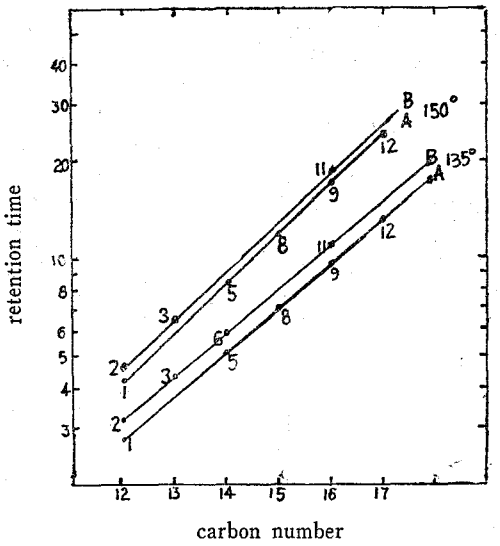

Fig. 3. Retention times of methyl esters of free fatty acids(C_{12}—C_{17}) in Purple Laver, plotted on a log scale against their carbon numbers. Results obtained at 135°~150°C on a 15% DEGS column
A: saturated fatty acids
B: monoalkenic fatty acids

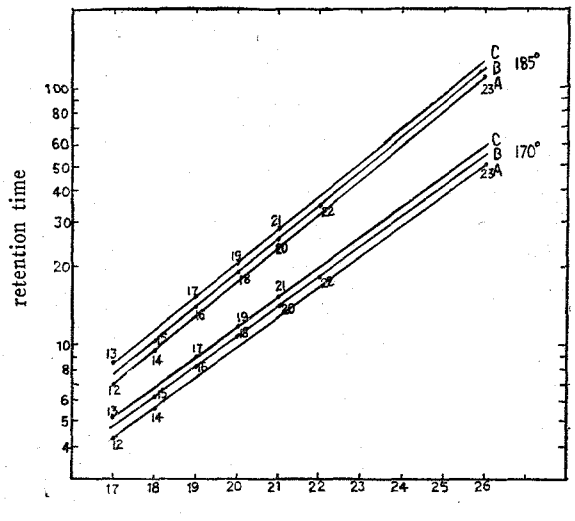

Fig. 4. Retention times of methyl esters of free fatty acids (C_{17}~C_{26}) in Purple Laver, plotted on a log scale against their carbon numbers. Results obtained at 170~185°C on a 15% DEGS column. A: saturated fatty acids B: monoalkenic fatty acids C: dialkenic fatty acids

Among the separated esters of the unsaturated fatty acids with the aid of mercuric acetate method, C_{13} : 1, and C_{18} : 1, and C_{20} : 1 were splitted to two peaks which suggested that they are the geometric isomers.

The contents of the free fatty acids with C_{24} : 1, C_{26} : 1, C_{16} and C_{18} were nearly 60% and unusual fatty acids did not exist.

〈Received 15 November 1974〉

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