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Studies on the Free Fatty Acids of Domestic Butter

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Abstract

Free fatty acids of two brands of domestic butter were isolated directly by a modified silicic acid column chromatography, and were analyzed by gas liquid chromatography. C_{18} FFA congeners ($C_{18:0}$, $C_{18:1}$, $C_{18:2}$ and $C_{18:3}$) were the predominant components (52.83% in brand A and 47.50% in brand B), followed by C_{16}FFA (29.39% in brand A and 30.52% in brand B) and C_{14}FFA (11.85% in brand A and 13.76% in brand B). The other FFA were present as minor components (0.29-3.87%). Concentrations of four FFA (C_4, C_6, C_{10} and C_{12}FFA) which would be expected to contribute strongly to hydrolytic rancidity off-flavors were below individual threshold level, except C_4FFA (56 ppm) in butter B.

Key words: free fatty acids, butter

Introduction

Off-flavors in dairy products caused by release of excessive amount of free fatty acids (FFA, C_4-C_{18}) from milk fat through the action of lipase are known as hydrolytic rancidity or lipase flavors. Occasional occurences of lipolytic rancidity or lipase flavors in both fresh and frozen butter continue to create quality assurance problems and attendant economic losses. Distinctly rancid butter is identified easily by the usual routine indexing methods of flavor scoring and acid degree value (ADV). But detecting excessive free fatty acids by sensory analysis is impossible, and the use of ADV is also unreliable because the approach fails to account for the great differences among the molecular weights and flavor intensities of individual FFA.[1]

Earlier reports on the gas chromatographic quantification of FFA in dairy products have included isolation procedure of fat and FFA esterification procedure in the analysis.[2,3] These procedures frequently lead to distorted FFA profiles that are different from intact samples. But recently a gas liquid chromatographic method for accurately quantifying individual major FFA in butter was developed by Woo and Lindsay.[4] By the development of this method, a statistical approach for routine detection and prediction of hydrolytic rancidity off-flavors in butter using sensory information and quantitative data for individual FFA has been possible.

Consumption pattern for dairy products has been changed along economic growth in our country. However, only a few studies have been done on the lipids of domestic butter[5,6], and it is believed that there is no study on the free fatty acids of domestic butter. The study reported here was undertaken to gain information on the hydrolytic rancidity off-flavors in domestic butter by using the chromatographic method and the GLC method.

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Materials and Methods

Materials

Two brands of butter which were passed 1 day (brand A) and 9 days (brand B) from manufacturing day were purchased from local market during January, 1990. All solvents employed in this study were stored over excess anhydrous sodium sulfate.

Preparation of the column body

The column prepared by the method of Woo & Lindsay.[4]

Four grams of silicic acid and 1g of Celite Analytical Filter Aid were mixed with 30 m*l* of ethyl ether and 10 m*l* of isopropanolic-KOH (dissolving 25g of KOH in 800 m*l* of isopropanol), and the mixture was allowed to stand for 5 min. The slurry then was transferred with ca. 30 m*l* of ethyl ether to a chromatographic column (32 cm by 2.5 cm i.d.) fitted with a small plug of acid-washed glass wool. The column was drained and washed with additional 100 m*l* of ethyl ether. During column preparation, nitrogen pressure was applied to move solvents through the column, and solvent was eluted by the rate of 3-4 m*l*/min.

Preparation of cap material

Table 1. Conditions for GLC analysis of free fatty acids

Gas chromatograph	Pye Unicam model PU 4500
Column	1.8m × 4.0mm glass
Packing material	10% NPGA on 80-100 mesh Chromosorb W AW-DMCS
Column temperature	100-215 °C at 12 °C/min
Detector	FID at 250 °C
Carrier gas	Nitrogen gas, 40 m*l*/min

Ten grams of butter were warmed to 25 °C, stirred, and then were mixed thoroughly with 7g of silicic acid, 15g of sodium sulfate, 3g of Celite, and 1 m*l* of internal standard solution (dissolving 0.12% heptanoic acid in 10% acetonitrile in ethyl ether). The cap material then was acidified by mixing with 0.2 m*l* of 1N sulfuric acid.

Isolation and concentration of FFA fraction

Forty milliliters of 20% petroleum ether in ethyl ether were held above the body of the column, and the cap material was added slowly through the solvent layer. The column then was washed sucessively with 100 m*l* of 20% petroleum ether in ethyl ether and 50 m*l* of 10% acetonitrile in ethyl ether. These eluate were discarded.

Free fatty acids were eluted from the column body with 50 m*l* of 2% formic acid in ethyl ether followed by 100 m*l* of 0.5% formic acid/10% petroleum ether in ethyl ether. Nitrogen pressure also was used to move solvents through the column during isolation.

The eluate containing FFA was vacuum evaporated with a rotary evaporator to ca. 10 m*l* at 30-35 °C. The concentrate then was transferred with 5 m*l* of ethyl ether to a sample concentrate tube. A slow stream of nitrogen was used to distill excess solvent until about 3 m*l* of cloudy solution was obtained. A solution of ethyl ether/acetone (80:20) was added by drop until an absolutely clear solution was obtained. Samples then were capped and warmed to 35 in water bath before sampling for injection into the GLC.

Also TLC method was used to confirm that FFA were separated from other neutral and polar components of an intact butter by this column chromatographic method.

Gas liquid chromatography conditions

The FFA were separated by GLC and the peaks were identified by comparing retention time to those of standard fatty acids. Peak areas were integrated and the percentage of total FFA were determined. Also C_4, C_6, C_{10} and C_{12}FFA were quantified by using response factors. The conditions of GLC are presented in Table 1.

Fig. 1. TLC chromatogram of each eluting fractions

Adsorbent: Silica gel G (0.25mm)
Solvent system: hexane-diethyl ether-formic acid (80:20:2, V/V)
A: Fraction 1, eluates of 20% petroleum ether in ethyl ether
B: Fraction 2, eluates of 10% acetonitrile in ethyl ether
C: Fraction 3, eluates of 2% formic acid in ethyl ether and 0.5% formic acid/10% petroleum ether in ethyl ether
D: Standard free fatty acid (Oleic acid)

Results and Discussion

Separation of free fatty acids

Each eluted fraction through the column was identified by thin layer chromatography (TLC) on silicagel G(0.25mm layer) with hexane-diethyl ether -formic acid (80:20:2, V/V) as developing solvents, and Fig. 1 shows the results.

FFA were contained in fraction 3, and the results indicated that FFA were clearly separated from other components of an intact butter.

Free fatty acid composition of butter

A gas chromatogram of the FFA from butter is

Table 2. Free fatty acid compositions of butter

Sample	Free fatty acids (Weight %)							
	C_4	C_6	C_8	C_{10}	C_{12}	C_{14}	C_{16}	C_{18}congeners
Butter A	1.15	0.37	0.46	1.78	2.17	11.85	29.39	52.83
Butter B	1.54	0.34	0.29	2.18	3.87	13.76	30.52	47.50

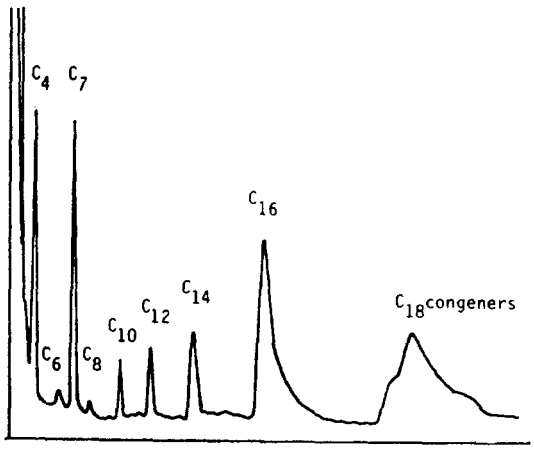

Fig. 2. Gas liquid chromatogram of free fatty acids isolated from butter

shown in Fig. 2, and analytical data for butter samples are given in Table 2.

There were no pronounced differences between FFA compositions of butter A and B. In butter A and B, C_{18}FFA congeners were the predominant components (52.83% in A and 47.50% in B), followed by C_{16}FFA (29.39% in A and 30.52% in B) and C_{14}FFA(11.85% in A and 13.76% in B). The other FFA were present as minor components (0.29%-3.87%).

Flavor quality of butter

Recently, Woo *et al.* has developed a statistical method for routine detection and prediction of hydrolytic off-flavors in butter using sensory information and quantitative data for individual FFA. He concluded that C_4 and C_6 FFA were primarily responsible for the rancid and goaty flavors, whereas C_{10} and C_{12} FFA were principally responsible for the soapy and bitter flavors, but C_8 and C_{14}-C_{18} FFA played minor roles.[7] Thus C_4, C_6, C_{10} and C_{12}FFA were individually quantified in order to estimate flavors of domestic butter samples employed in this study, and their concentrations are shown in

Table 3. FFA(C_4, C_6, C_{10} and C_{12}) concentrations of butter

Sample	Free fatty acids (ppm)			
	C_4	C_6	C_{10}	C_{12}
Butter A	35	10	48	60
Butter B	56	11	70	126
Threshold level[a]	40	15	250	200

[a] Woo *et al.*[7]

Table 3.

C_4, C_6, C_{10} and C_{12}FFA concentrations in butter A were 35, 10, 48 and 60 ppm, and in butter B 56, 11, 70 and 126 ppm, respectively. According to the study of Woo *et al.*[7], threshold concentrations of these 4 FFA in butter were 40, 15, 250 and 200 ppm, respectively. Therefore we could find that these 4 FFA concentrations in butter A were below threshold level, but in butter B C_4FFA concentration was 1.4 folds of threshold level. Accordingly butter B will give slightly rancid flavors. But we cannot conclude that all the samples of butter B will give rancid flavors, because there are differences in distribution systems and in quality control of the product after it is delivered to markets. Thus further studies are necessary to clarify how the distribution systems for butter affect flavors.

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국산 버터 중의 유리지방산에 관한 연구

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국내에서 생산되고 있는 버터의 유리지방산 조성을 분석하기 위하여 silicic acid column chromatography 방법을 이용하여 버터로부터 직접 유리지방산을 분리 용출시켜 농축한 후 GLC로 분석하였다. 국산 버터 2종류 제품을 분석한 결과, 버터 A의 유리지방산 조성에 있어서는 C_{18} 유리지방산($C_{18:0}$, $C_{18:1}$, $C_{18:2}$ 및 $C_{18:3}$) 함량이 52.83%로 가장 높았고, 그 다음으로 palmitic acid가 29.39%, myristic acid가 11.85% 함유되어 있었으며, 버터 B의 경우에는 C_{18} 유리지방산이 47.50%, palmitic acid가 30.52%, myristic acid가 13.76% 함유되어 있었고, 그외의 유리지방산은 소량씩 함유되어 있었다. 한편 함량면에서는 적지만 버터의 풍미에 중요한 영향을 미치는 것으로 알려진 butyric, caproic, capric 및 lauric acid의 농도를 분석한 결과, 버터 A에서는 각각 35, 10, 48, 60 ppm, 버터 B에서는 각각 56, 11, 70, 126 ppm이었다.