# Analysis of Sucrose Esters of Fatty Acids in Coffee Drinks [*1]

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A method for the determination of sucrose esters of fatty acids was investigated and adjusted for coffee drinks as follows:

A commercial emulsifier, P-1570 (Ryoto Co., Ltd.[*2]), was used as the sample of sucrose esters of fatty acids. Thirty-two g of silica gel and 10 ml of a coffee sample were thoroughly mixed and mixed with 100 ml chloroform to make the slurry for a silica gel column (2.5 x 20 cm). The sucrose esters were eluted from the silica gel column with 300 ml of 17.5 % methanol in chloroform.

After the effluent was evaporated to dryness, the residure was dissolved in 10 ml of N, N-Dimethylformamide (DMF). One ml of the solution was applied on top of a Sephadex LH-20 column (2.5 x 200 cm) which was prepared with DMF. The sucrose esters were eluted with DMF. Ninety fractions, each containing 5 g of effluent, were obtained.

The amounts of the sucrose esters in each fraction and the combined fractions for the sucrose esters were determined colorimetrically with the anthrone reagent as the commercial emulsifier.

Qualitative analysis of the sucrose esters was performed by the chromatogram on the Sephadex LH-20 column and the silica gel thin-layer chromatogram of the combined fractions for the sucrose esters.

The recovery of the sucrose esters in the coffee sample by this method was approximately 95 %.

## Introduction

In a previous paper[1], we showed that some kinds of sucrose esters of fatty acids could prevent a new type of flat sour spoilage, obligate-anaerobic (O. A.) flat sour spoilage, at 300 ppm in canned coffee and at 5000 ppm in canned *shiruko* (a sweet bean drink). From these findings, we suggested that the addition of the sucrose esters of fatty acids should effectively prevent the spoilage of canned drinks kept hot in vending machines.

From the beginning of the hot vending systems in Japan, it has been said that the sucrose esters of fatty acids sometimes had been used in canned coffee for hot vending as the emulsifier. And now it is generally said that many companies, following our suggestion, use the sucrose esters of fatty acids as an agent preventing O. A. flat sour spoilage in canned coffee.

Many studies on separation, purification, identification, and determination of sucrose es-

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*1 A New Type of Flat Sour Spoilage —Ⅷ.

*2 Present name of the company: Mitsubishi-Kasei Food Co.

ters of fatty acids have been reported[2-34]. However, almost all the analytical studies have been carried out to examine the conditions necessary for production and quality control, the compositions of esters, and the chemical structures by thin-layer chromatography[2-16], column chromatography[7, 8, 15-22, 28], gas chromatography[11, 16, 23-26], paper chromatography[20, 27, 28], optical rotations[27, 29], etc.[8, 19, 30, 31, 34] And a few reports on the determination of sucrose esters of fatty acids in foods have been appeared[14, 27]. There have been no papers published on the determination of sucrose esters of fatty acids in canned coffee.

The present paper deals with a method for the extraction, identification, and determination of sucrose esters of fatty acids in coffee samples.

## Materials and Methods

*Sucrose esters of fatty acids*

The sucrose esters of fatty acids used were five kinds of commercial emulsifiers, S-570, S-1570, S-1670, P-1570, and P-1670 (Ryoto Co., Ltd.*), with $C_{16}$ and $C_{18}$ fatty acid moieties. The compositions of the fatty acids and sucrose esters of the emulsifiers are shown in Table 1.

*Preparation of the coffee sample*

The coffee sample was prepared by the method for the coffee medium described previously[1], except that the procedure to remove bacteria from sugar by filtration was omitted.

The concentrations of P-1570 in the coffee sample were 0 ppm and 1000 ppm.

*Quantitative determination of the sucrose esters of fatty acids*

As Kinoshita and Oyama reported[5], the quantitative determination of sucrose esters of fatty acids could be performed by the method of Wise et al.[33] using the alcoholic anthrone reagent. However, N, N-Dimethylformamide (DMF) ("Special grade") was used in this study instead of water as a solvent of sucrose esters of fatty acids.

*Silica gel column for the separation of the sucrose esters of fatty acids and sucrose*

Chromatograms of sucrose esters of fatty acids have been obtained by silical gel column[7, 15-18, 20, 28]. We used silica gel column

Table 1. Compositions of fatty acids and esters of the commercial emulsifiers*

| Cat. No. | Fatty acid moieties | | Esters | |
|---|---|---|---|---|
| | Palmitic | Stearic | Mono | Di & tri |
| S-570 | 30 % | 70 % | 30 % | 70 % |
| S-1570 | 30 | 70 | 70 | 30 |
| S-1670 | 30 | 70 | 75 | 25 |
| P-1570 | 70 | 30 | 70 | 30 |
| P-1670 | 70 | 30 | 75 | 25 |

* Approximate concentrations according to Ryoto Co., Ltd., Tokyo, Japan.

* Present name of the company: Mitsubishi-Kasei Food Co.

for separating the sucrose esters of fatty acids and sucrose, and methanol-chloroform as eluting solutions as described by Otake[18].

A glass column (2.5 x 30 cm) with a porous plate at the bottom and equipped with a stopcock was used. Ten g of silica gel (Kieselgel 60, 70-230 mesh ASTM, Merck) was weighed in a beaker and mixed with 40 ml of chloroform (〝Special grade〞). The slurry was transferred to the glass column. Then, 32 g of silica gel and 10 ml of a coffee sample were thoroughly mixed. The fine slurry prepared by stirring in 100 ml of chloroform into the mixture was applied on top of the silica gel column. The silica gel column was washed with 100 ml of chloroform. The silica gel column thus prepared is shown in Fig. 1 schematically. The flow rate of the eluting solutions was fixed at 100 ml/hr.

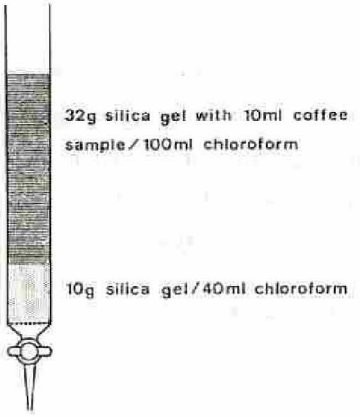


Fig.1. Silica gel column for seperation of sucrose esters of fatty acids and sucrose

*Gel filtration of the sucrose esters of fatty acids and sucrose on Sephadex LH-20 column*

Konishi et al. have described a possibility of separation of the compositions of sucrose esters of fatty acids by a Sephadex LH-20 column[21]. We employed Sephadex LH-20 for gel filtration of the sucrose esters of fatty acids and sucrose with a modification as follows:

A glass column (2.5 x 220 cm) with a porous plate at the bottom and equipped with a stopcock was used. About 200 g of Sephadex LH-20 (Pharmacia Fine Chemicals) was allowed to stand to swell for 24 hr in contact with DMF and then was carefully poured into the glass column. The height of gel bed was adjusted to 200 cm. Thereafter, the gel bed was allowed to settle for 24 hr with DMF flowing through the column. The column temperature was room temperature and the flow rate was controlled at 60 g/hr. One ml of the samples (DMF solutions of the sucrose esters of fatty acids and/or sucrose) was applied on top of the gel bed. The eluent was DMF. Ninety fractions, each containing 5 g of effluent, were taken, using an automatic fraction collector. The amount of the sucrose esters of fatty acids in each fraction was determined colorimetrically after the treatment with the alcoholic anothrone reagent.

*Thin-layer chromatography (TLC) for qualitative analysis of the effluents of the gel filtration on Sephadex LH-20 column*

Qualitative analysis of the sucrose esters of fatty acids in the effluents of Sephadex LH-20 column was carried out by the method of Kinoshita[4]. However, pre-coated TLC plates [DC-Fertigplatten Kieselgel 60 (ohne Fluoreszenzindikator), 10 x 20 cm, Schichtdicke 0.25 mm, Merck] were purchased, and DMF was used as the solvent of samples.

## Results and Discussion

*Anthrone reactions of the emulsifiers and sucrose*

Solutions containing known amounts of 5 kinds of the emulsifiers and sucrose were subjected to the above procedure by using the alcoholic anthrone reagent. As shown in

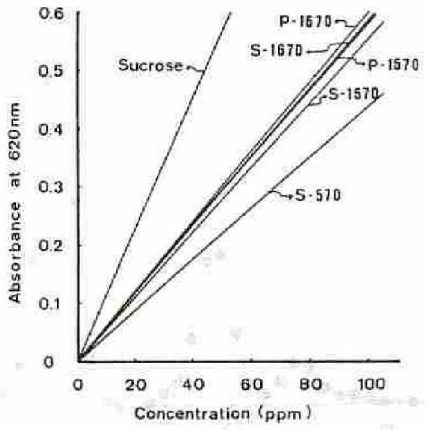


Fig.2. Anthrone reactions of the emulsifiers and sucrcse

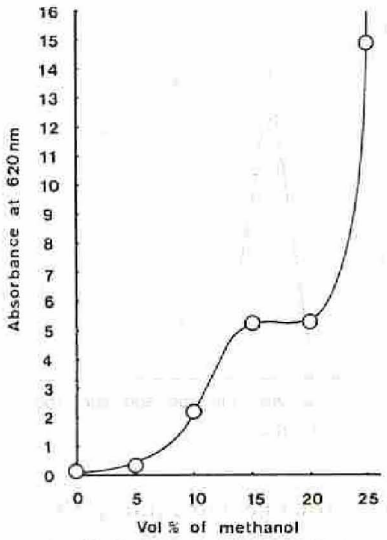


Fig.3. Effect of the eluting solutions on the elution of the emulsifier (P-1570) and sucrose in the coffee sample from the silica gel column

Fig. 2, the intensities of color per ppm increase nearly in proportion to the ratio of sucrose in the emulsifiers. The result is expected from the paper of Mima and Kitamori[7] which reported that the intensities of color per mol were all equal for sucrose, sucrose monoester, and diester.

As the emulsifiers used for a food additive are the mixtures of at least 6 kinds of sucrose esters of fatty acids as shown in Table 1, the line of p-1750 in Fig. 2 was used for a calibration curve. Therefore, by using the calibration curve, the concentration of sucrose esters of fatty acids was obtained as P-1570.

*Elution of P-**1570** in the coffee sample from the silica gel column*

The coffee sample containing 1000 ppm of P-1570 was subjected to 6 silica gel columns as described above. From each column, P-1570 was eluted with 300 ml of 0%, 5%, 10%, 15%, 20%, and 25% methanol ("Special grade") in chloroform, respectively. After each effluent was evaporated to dryness in a water bath, the residue was dissolved in 10 ml of DMF. The DMF solution was subjected to the above procedure by using the alcoholic anthrone reagent.

Absorbances at 620 nm of 300 ml of eluting solutions of various methanol contents are shown in Fig. 3. As shown in Fig. 3, the increase of the ratio of methanol in the eluting solutions increased the amounts of eluted materials which colored by treating with the alcoholic anthrone reagent, with a shoulder of the curve at 15-20% methanol in the eluting solutions. If the colorations were only by P-1570, more than 90% of P-1570 were eluted with 15-20% methanol in chloroform. Although, Otake[18] described that sugar could be eluted from a silica gel column when methanol-chloroform was replaced with DMF, 25% methanol in chloroform showed an extreme increase of the absorbance which could be explained by the elution of sucrose.

From the silica gel column prepared with the coffee sample containing 1000 ppm of P-1570, P-1570 was eluted with 15%, 17.5%, and 20% methanol in chloroform. The volume of the fractions collected was 100 ml. The frac-

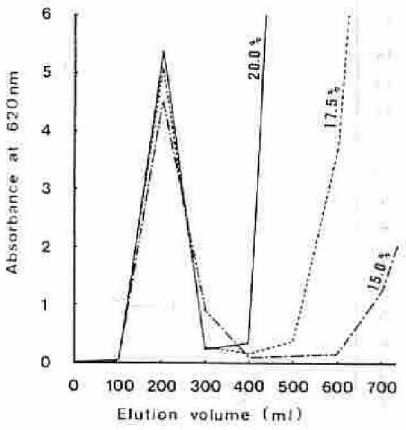


Fig.4. Silica gel chromatograms of the coffee sample with the three eluting solutions

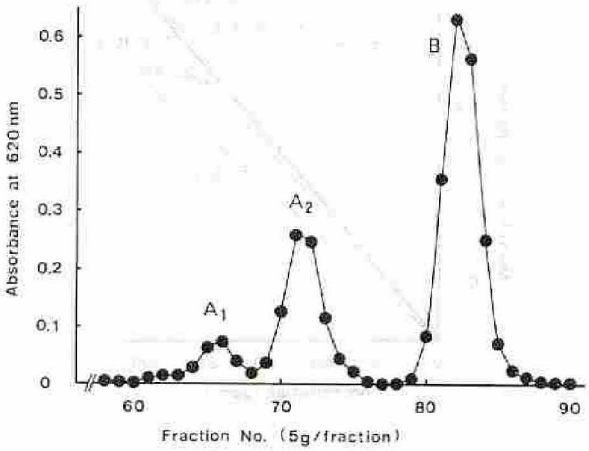


Fig.5. Gel filtration of the emulsifier (P-1570) and sucrose on the Sephadex LH-20 column

$A_1$ and $A_2$ : the emulsifier (P-1570), B: sucrose

tions were evaporated to dryness in a water bath. The residue was dissolved in 10 ml of DMF. The DMF solution was subjected to the anthrone reaction.

As shown in Fig. 4, the elution of P-1570 was sufficient with each 300 ml of all the three eluting solutions. Higher ratio of methanol in the eluting solution caused faster elution of sucrose.

From these results, it is concluded that the emulsifier is eluted with 300 ml of 17.5% methanol in chloroform from the silica gel column at the flow rate of 100 ml/hr.

*Gel filtration of the sucrose esters of fatty acids and sucrose on the Sephadex LH-20 column*

A gel chromatogram of the DMF solution containing both 1000 ppm of P-1570 and 1000 ppm of sucrose on the Sephadex LH-20 column is shown in Fig. 5. It appears that the former two peaks ($A_1$ and $A_2$) are the ones of the emulsifier and the latter (B) is of sucrose, because when the emulsifier and sucrose were subjected on the Sephadex LH-20 column separately, the former was eluted in the same fractions (Fraction Nos. 61-75) as the two peaks ($A_1$ and $A_2$) and the latter was eluted in the same fractions (Fraction Nos. 79-89) as the peak (B). When commercial granulated sugar was used instead of sucrose, the same result was obtained.

It appears that the separation between the emulsifier and sucrose is satisfactory. Combined fractions for the emulsifier (Fraction Nos. 61-75) were subjected to the anthrone reaction quantitatively. The recovery of the emulsifier was 94.6%.

*Extraction, identification, and determination of the sucrose esters of fatty acids in the coffee sample*

From the results described above, extraction, identification, and determination of the sucrose esters of fatty acids in coffee samples were performed as shown in Fig. 6. The amount of the sample used depended on the concentration of the emlsifier in the sample, i.e., the number of silica gel column used were varied with the concentration of the emulsifier in the sample.

Coffee sample (10 ml)
↓ Adsorbed on silica gel (Merck's Kieselgel 60)
Silica gel column chromatography
↓ Eluted with 17.5% methanol-chloroform
↓ Evaporated to dryness
↓ Dissolved in 10 ml of DMF
Gel filtration on Sephadex LH-20
↓ Eluted with DMF
Qualitative and quantitative analyses
1) Color development of each fraction (5 g/fraction) by the anthrone reaction
2) Color development of combined fractions (Fraction Nos. 61-75) by the anthrone reaction
3) Thin-layer chromatography of the concentrated combined fraction (approximately 1 ml)

Fig. 6. Procedures for extraction, identification and determination on the emulsifier in coffee samples

The coffee sample containing 1000 ppm of the emulsifier (P-1570) was performed by the procedure. Twenty ml of the coffee sample was used, i.e., the effluents from 2 silica gel columns were evaporated to dryness in a flask, and the residure was dissolved in 10 ml of DMF. And also the coffee sample without the emulsifier was treated in the same way.

Gel chromatograms of the coffee samples with and without the emulsifier on the Sephadex LH-20 column are shown in Fig. 7. The gel chromatogram of the coffee sample containing 1000 ppm of the emulsifier shows that the emulsifier was eluted in the same fractions (Fraction Nos. 61-75) as the two peaks in Fig. 5. The gel chromatogram of the coffee sample without the emulsifier shows that no colored materials with the alcoholic anthrone reagent were eluted in the same fractions. In both gel chromatograms, no color developments were found in the fractions for sucrose (Fraction Nos. 79-89). This means that no sucrose was eluted from the silica gel column in both experiments.

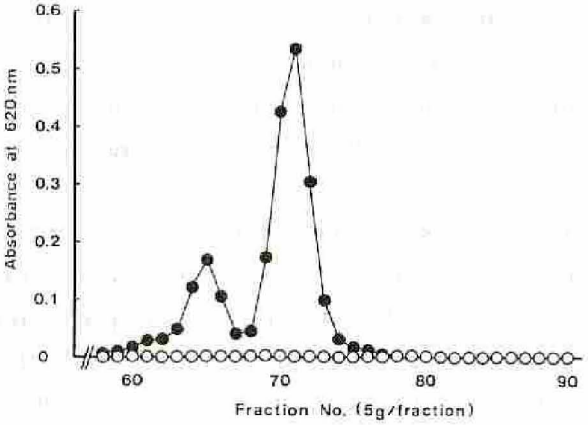


Fig.7. Gel filtration of the coffee samples with and without the emulsifier (P-1570) on the Sephadex LH-20 column
● : with the emulsifier,
○ : without the emulsifier.

Table 2. Determination of the emulsifier (P-1570) in the coffee samples

| Sample | Conc. of P-1570 | Absorbance at 620 nm | | Recovery after the treatment with | |
|---|---|---|---|---|---|
| | | Silica gel*1 | Sephadex*2 | Silica gel*1 | Sephadex*2 |
| A | 1000 ppm | 0.576 | 0.141 | 90.5% | 94.6% |
| B | 0 ppm | 0.046 | 0.001 | - | - |

*1 After the treatment of the coffee sample (20 ml) with the silica gel column, the DMF solution obtained (10 ml) was diluted with DMF 20 times and used in the color development with the anthrone reagent.

*2 After the treatment of the DMF solution (1.0 ml) with the Sephadex column, the combined fractions (Fraction Nos. 61-75) were used in the color development with the anthrone reagent.

Table 2 shows the absorbances at 620 nm and recoveries of each sample after the treatments with the silica gel column and the Sephadex LH-20 column. The recovery of the coffee sample with the emulsifier after the treatment of the Sephadex LH-20 column was the same as that of the DMF solution containing 1000 ppm of the emulsifier. Therefore, it is concluded that the loss of the emulsifier during the treatment with the silica gel column is negligibly little, and that the 94.5% recovery is satisfactory.

Fig. 8 shows a TLC plate of the fractions for the emulsifier. A is a control only with the emulsifier (P-1570), B is a sample from the fractions of the coffee sample with the emulsifier, and C is a sample from the fractions of the coffee sample without the emulsifier. The emulsifier was applied as DMF solution to give 300 $\mu$g per application. About 150 $\mu$l of the DMF solution of the coffee sample with the emulsifier was also applied to give about 300 $\mu$g and the DMF solution from the coffee sample without the emulsifier was treated in the same way. No spot was formed in the thin-layer chromatogram of the coffee sample without the emulsifier. The same spots were found in both thin-layer chromatograms of the emulsifier and the coffee sample with the emulsifier. The results indicates that the color developed with the combined fractions for the emulsifier (Fraction Nos. 61-75) was actually due to the emulsifier.

From the results described above, it is concluded that the procedure shown in Fig. 6 is satisfactory to know the concentration of the sucrose esters of fatty acids in canned coffee samples as the emulsifier (P-1570).

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* Present name of the company: Mitsubishi-Kasei Food Co.

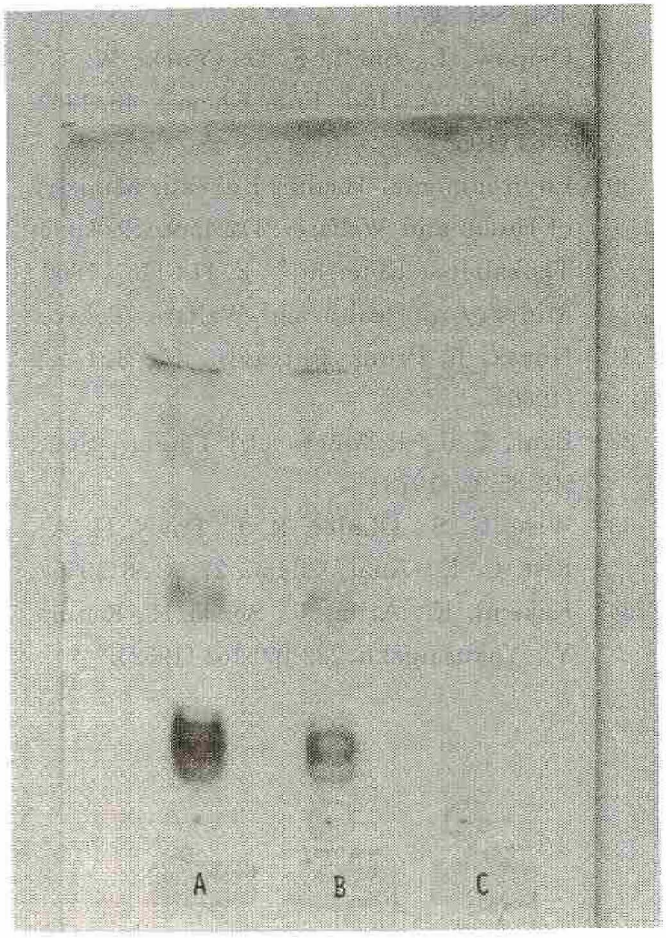


Fig. 8. Thin-layer chromatograms of the emulsifier (P-1570) and the samples from the combined fractions of the coffee solutions with and without the emulsifier (P-1570)

A: the emulsifier (P-1570), B: the sample from the combined fraction of the coffee solution with the emulsifier (P-1750), C: the sample from the combined fraction of the coffee solution without the emulsifier (P-1570). Developing solvent: chloroform-methanol-acetic acid-water (80:10:8:2), chromgenic reagent: 50% $H_2SO_4$ [4].

providing us with the samples of sucrose esters of fatty acids.

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## References

1) Nakayama, A., Sonobe. J., Shinya, R.: J. Food Hyg. Soc. Japan, 23, 25-32 (1982).
2) Gee, M.: J. Chromatog., 9, 278-282 (1962).
3) Mima, H., Kitamori, N., Kanzawa, T.: Kogyo Kagaku Zasshi, 65, 833 (1962).
4) Kinoshita, S.: ibid., 66, 450-455 (1963).
5) Kinoshita, S., Oyama, M.: ibid., 66, 455-458 (1963).
6) Linow, F., Ruttloff, H., Täufel, K.: Naturwissenschaften, 50, 589-690 (1963).
7) Mima, H., Kitamori, N.: J. Amer. Oil Chem. Soc., 41, 198-200 (1964).
8) Otake, T., Tamate, E.: Kogyo Kagaku Zasshi, 67, 1586-1589 (1964).
9) Otake, T., Tamate, E.: ibid., 68, 1896-1900 (1965).
10) Kinoshita, S., Oyama, M.: ibid., 69, 1040-1044 (1966).
11) Wachs, W., Gerhardt, K.: Tenside, 2, 6-10 (1965).
12) Ranny, M.: Abh. Deut. Akad. Wiss. Berlin, Kl. Chem., Geol. Biol., 216-224 (1966).
13) Weiss, T. J., Brown, M., Zeringue, H. J. Jr., Feuge, R. O.: J. Amer. Oil Chem. Soc., 48, 145-148 (1971).
14) Kaimenkasseizai Bunseki Kenkyukai (ed.): "Kaimenkasseizai Bunsekiho" p. 377-378 (1975) Saiwai Shobo, Tokyo.
15) Zeringue, H. J., Feuge, R.O.: J. Amer. Oil Chem. Soc., 53, 567-571 (1976).
16) Gupta, R. K., James, K., Smith, F. J.: J. Amer. Oil Chem. Soc., 60, 1908-1913 (1983).
17) Lemieux, R. U., McInnes, A. G.: Can. J. Chem., 40, 2394-2401 (1962).
18) Otake, T.: Seito Gijutsu Kenkyu Kaishi, No. 12, 25-34 (1963).
19) Otake, T., Tamate, E.: Kogyo Kagaku Zasshi, 67, 809-812 (1964).

20) Bares, M.: Prum. Potravin, 17, 375-379 (1966).
21) Konishi, K., Inoue, H., Taniguchi, N.: J. Chromatog., 54, 367-372 (1971).
22) Kaufman, V. R., Garti, N.: J. Liq. Chromatogr., 4, 1195-1205 (1981).
23) Gee, M., Walker, H. G. Jr.: Chem. & Ind., 829-830 (1961).
24) Lemieux, R. U., McInnes, A. G.: Can. J. Chem., 40, 2376-2393 (1962).
25) Kimura, R., Takashita, Y., Takashima, A., Sakamoto, T., Murata, T., Ito, K.: Eisei Kagaku, 24, 123-127 (1978).
26) Tsuda, T., Nakanishi, H.: J. Assoc. Off. Anal. Chem., 66, 1050-1052 (1983).
27) Kröller, E.: Fette, Seifen, Anstrichmittel, 65, 482-488 (1963).
28) Zajic, J., Bares, M.: J. Chromatog., 28, 484-488 (1967).
29) Osipow, L., Snell, F. D., York, W. C., Finchler, A.: Ind. Eng. Chem., 48, 1459-1462 (1956).
30) Environmental Health Bureau, Ministry of Health and Welfare: "Dai Ippan Shokuhin Tenkabutsu Koteisho" p. 113-115 (1960) Ministry of Health and Walfare, Tokyo.
31) Novak, J.: Prum. Potravin, 17, 421-422 (1966).
32) Baur, F. J.: J. Amer. Oil Chem. Soc., 50, 85-92 (1973).
33) Wise, C. S., Dimler, R. J., Davis, H. A., Rist, C. E.: Anal. Chem., 27, 33-36 (1955).
34) Kakemi, K., Arita, T., Sezaki, H., Katsura, Y.: Yakuzaigaku, 22, 160-163 (1962).