Determination of Sucrose Esters of Fatty Acids in Commercial Canned Coffee

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Sucrose esters of fatty acids in commercial canned coffee samples were extracted by the treatment with a silica gel column, identified and purified by gel filtration on a Sephadex LH-20 column, determined by the anthrone reaction, and identified by thin-layer chromatography. The concentrations of the sucrose esters of fatty acids were determined in terms of a commercial emulsifier (P-1570, Ryoto Co., Ltd.*).

Ten commercial canned coffee samples produced in 1981 were used. And a commercial canned coffee sample produced in 1978 was taken from the lot which had suffered from O. A. flat sour spoilage.

Six samples out of 10 produced in 1981 contained the sucrose esters of fatty acids but the others did not. The concentrations of the sucrose esters of fatty acids ranged approximately from 20 ppm to 460 ppm. Among the 6 samples, 4 samples contained more than 250 ppm of the sucrose esters of fatty acids which could prevent O. A. flat sour spoilage. The sample examined microbiologically in previous papers, taken from the lot which had suffered from O. A. flat sour spoilage, contained no sucrose esters of fatty acids.

Introduction

Some sucrose esters of fatty acids prevent obligate-anaerobic (O. A.) flat sour spoilage of canned drinks kept hot in vending machines[1]. Now in Japan, it is said that many companies use sucrose esters of fatty acids in canned coffee as an agent for preventing the spoilage. However, no studies on determination of sucrose esters of fatty acids in commercial canned coffee have been established.

In a previous paper[2], we proposed a method for the determination of sucrose esters of fatty acids in a coffee sample. The present paper deals with the estimation of the concentrations of sucrose esters of fatty acids in commercial canned coffee samples by our method and the comparison with the concentrations necessary for inhibition of the growth of the bacteria causing O. A. flat sour spoilage.

Materials and Methods

Commercial canned coffee samples used for determination of the sucrose esters of fatty acids

As shown in Table 1, all canned coffee samples commercially purchased were products in 1981 by different manufacturers, except for Sample No. 26 of J manufacturer which was produced in 1978. When Sample No. 26 was incubated at 55℃, O. A. flat sour spoilage was observed in some cans from which anaerobic bacteria identified as *Clostridium thermoaceticum* were detected[3,4].

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

Table 1. Commercial canned coffee (with milk and sugar) samples used for determination of the sucrose esters of fatty acids

Manufacturer	Sample No.	Net weight (g)	Date of production
C	15	250	July 29, 1981
K	29	190	Aug. 1, 1981
L	30	250	May 15, 1981
A	31	250	Apr. 29, 1981
M	32	250	July 31, 1981
N	33	250	Jan. 21, 1981
O	34	250	Feb. 20, 1981
P	35	195	Feb. 26, 1981
Q	36	250	Apr. 9, 1981
R	37	250	Sep. 5, 1981
J	26*	250	July 4, 1978

* When cans of the sample were incubated at 55℃, O. A. flat sour spoilage was found in some cans from which some anaerobic bacteria, identified as *Clostridium thermoaceticum*, were isolated[3,4].

Table 2. Compositions of fatty acids and esters of the commercial emulsifier *

Cat. No.	Fatty acid moieties		Esters	
	Palmitic	Stearic	Mono	Di & tri
P-1570	70%	30%	70%	30%

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

Sucrose esters of fatty acids

A commercial emulsifier, P-1570 (Ryoto Co., Ltd.*), with C_{16} and C_{18} fatty acid moieties was used as the sucrose esters of fatty acids. The compositions of the fatty acids and sucrose esters of the emulsifier are shown in Table 2.

Extraction, identification, and determination of the sucrose esters of fatty acids in commercial canned coffee samples

Extraction, identification, and determination of the sucrose esters of fatty acids were carried out by the procedures described previously[2] as shown in Fig. 1.

To examine the recoveries of the sucrose esters of fatty acids (P-1570) in the samples, each sample with a known added amount (200 ppm or 500 ppm) of P-1570 was treated by the same procedures used for the corresponding sample.

Results

Rough estimations of the concentrations of the sucrose esters of fatty acids in the commercial canned coffee samples after the treatment with the silica gel column

To estimate preliminarily the concentrations of the sucrose esters of fatty acids in each commercial canned coffee sample, the N, N-Dimeth-

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ylformamide (DMF) solution (10 ml) obtained after the treatment of each sample (10 ml) with the silica gel column was diluted with DMF 10 times and subjected to the anthrone reaction. The absorbances at 620 nm and the concentrations, roughly estimated on the assumption that

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 N, N-Dimethylformamide (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)
3) Thin-layer chromatography of the concentrated combined fractions (approximately 1 ml)

Fig. 1. Procedures for extraction, identification and determination of the sucrose esters of fatty acids in coffee samples[2].

Table 3. Rough estimations of the concentrations of the sucrose esters of fatty acids in the commercial canned coffee samples after the treatment with the silica gel column

Sample No.	Absorbance at 620 nm *1	Concentration *2
15	0.391	670 ppm
29	0.107	185
30	0.201	340
31	0.310	530
32	0.327	565
33	0.197	335
34	0.168	290
35	0.091	155
36	0.112	195
37	0.208	355
26	0.029	50

*1 After the treatment of a coffee sample (10 ml) with the silica gel column and evaporation of the solvent, the DMF solution obtained (10 ml) was diluted 10 times with DMF and subjected to the anthrone reaction.

*2 Estimated roughly on the assumption that the color development was only due to the sucrose esters of fatty acids (P-1570) with no interference.

the color development was only due to the sucrose esters of fatty acids with no interference, are shown in Table 3. It appeared that the commercial canned coffee samples contained 50 ppm-670 ppm of the sucrose esters of fatty acids.

For the recovery tests in Sample Nos. 15 and 32, 20 ml aliquots of the samples added with 500 ppm of the sucrose esters of fatty acids(P-1570) were used, i.e., the effluents from 2 silica gel columns were evaporated to dryness in a evaporating frask, and the residure was dissolved in 10 ml of DMF. And a 1.0 ml aliquot of the DMF solution was subjected to gel filtration on the Sephadex LH-20 column. Each sample without the addition of P-1570 was treated by the same means.

For the recovery tests in the other samples, 50 ml aliquots of the samples added with 200 ppm of P-1570 were used, i.e., the effluents from 5 silica gel columns were evaporated to dryness in a evaporating flask, and the residure was dissolved in 10 ml of DMF. And a 1.0 ml aliquot of the DMF solution was subjected to gel filtration on the Sephadex LH-20 column. Each sample without the addition of P-1570 was treated by the same means.

Gel filtration on the Sephadex LH-20 column

Gel chromatograms of all the samples on the Sephadex LH-20 column are shown in Fig. 2-Fig. 12.

The gel chromatograms of Sample Nos. 15, 32, 31, 37, and 35 (Figs. 2, 3, 4, 5, and 6) indicate that in both the experiments with the samples with (-●-) and without (-o-) P-1570 added, materials which colors with the alcoholic anthrone reagent were eluted in the fractions for P-1570 (Fraction Nos. 61-75) giving the pattern with 2 peaks similar to that of P-

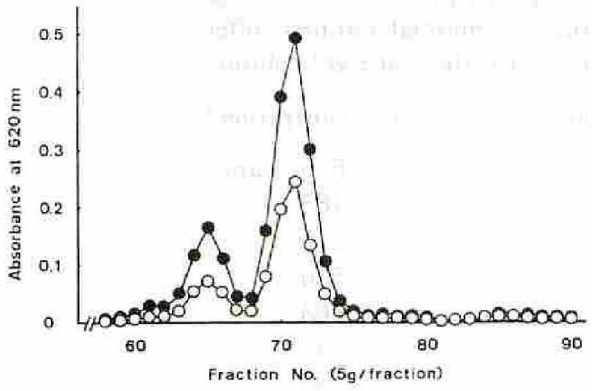

Fig.2. Gel filtrations of Sample No.15 and Sample No. 15 with the sucrose esters of fatty acids (P-1570) added on the Sephadex LH-20 column

-○- : Sample No.15, -●- : Sample No.15 with 500 ppm of the sucrose esters of fatty acids (P-1570) added.

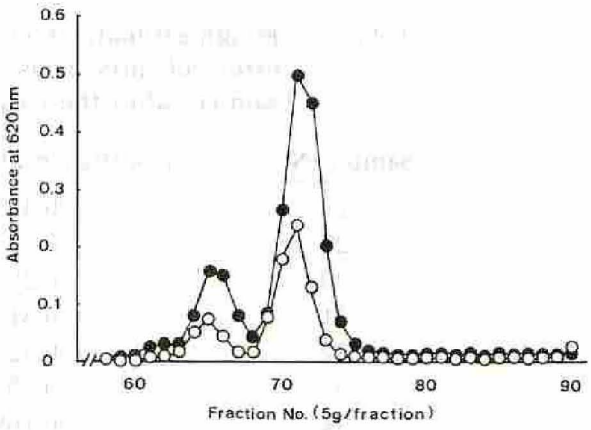

Fig.3. Gel filtrations of Sample No. 32 and Sample No. 32 with the sucrose esters of fatty acids (P-1570) added on the Sephadex LH-20 column

-○- : Sample No.32, -●- : Sample No.32 with 500 ppm of the sucrose esters of fatty acids (P-1570) added.

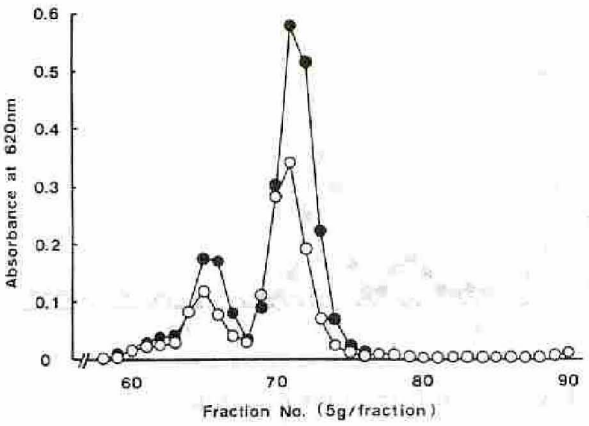

Fig.4. Gel filtrations of Sample No. 31 and Sample No. 31 with the sucrose esters of fatty acids (P-1570) added on the Sephadex LH-20 column

-○- : Sample No.31, -●- : Sample No.31 with 200 ppm of the sucrose esters of fatty acids (P-1570) added.

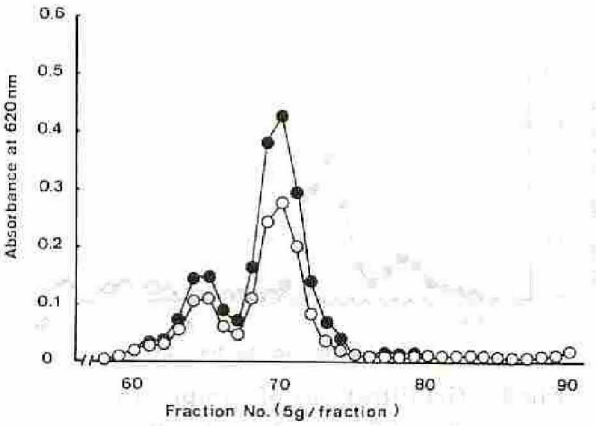

Fig.5. Gel filtrations of Sample No. 37 and Sample No. 37 with the sucrose esters of fatty acids (P-1570) added on the Sephadex LH-20 column

-○- : Sample No.37, -●- : Sample No.37 with 200 ppm of the sucrose esters of fatty acids (P-1570) added.

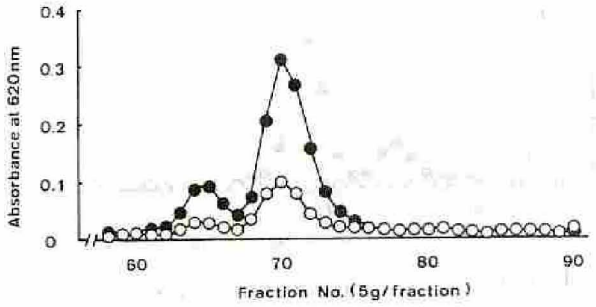

Fig.6. Gel filtrations of Sample No. 35 and Sample No. 35 with the sucrose esters of fatty acids (P-1570) added on the Sephadex LH-20 column

-○- : Sample No.35, -●- : Sample No.35 with 200 ppm of the sucrose esters of fatty acids (P-1570) added.

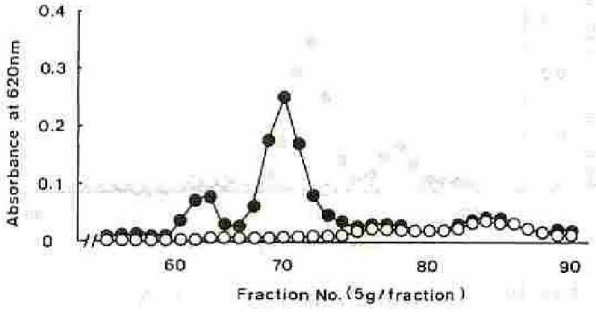

Fig.7. Gel filtrations of Sample No. 34 and Sample No. 34 with the sucrose esters of fatty acids (P-1570) added on the Sephadex LH-20 column

-○- : Sample No.34, -●- : Sample No.34 with 200 ppm of the sucrose esters of fatty acids (P-1570) added.

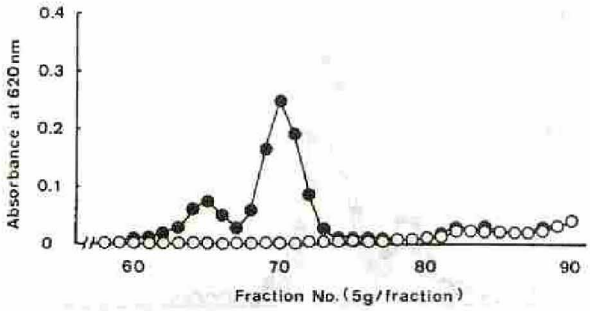

Fig.8. Gel filtrations of Sample No. 36 and Sample No. 36 with the sucrose esters of fatty acids (P-1570) added on the Sephadex LH-20 column

-○- : Sample No.36, -●- : Sample No.36 with 200 ppm of the sucrose esters of fatty acids (P-1570) added.

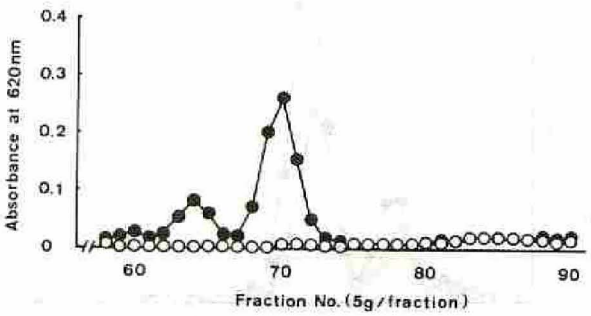

Fig.9. Gel filtrations of Sample No. 33 and Sample No. 33 with the sucrose esters of fatty acids (P-1570) added on the Sephadex LH-20 column

-○- : Sample No.33, -●- : Sample No.33 with 200 ppm of the sucrose esters of fatty acids (P-1570) added.

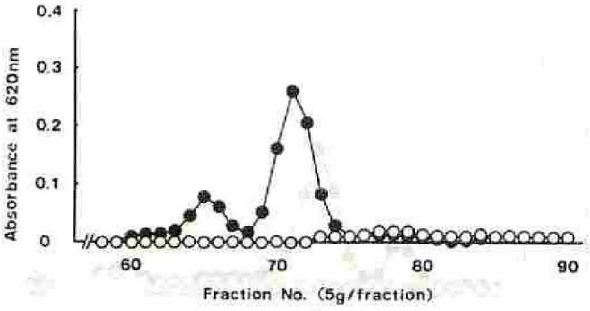

Fig.10. Gel filtrations of Sample No. 29 and Sample No. 29 with the sucrose esters of fatty acids (P-1570) added on the Sephadex LH-20 column

-○- : Sample No.29, -●- : Sample No.29 with 200 ppm of the sucrose esters of fatty acids (P-1570) added.

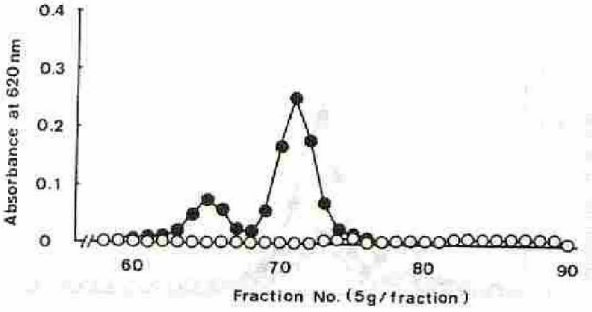

Fig.11. Gel filtrations of Sample No. 30 and Sample No. 30 with the sucrose esters of fatty acids (P-1570) added on the Sephadex LH-20 column

-○- :Sample No.30, -●- :Sample No.30 with 200 ppm of the sucrose esters of fatty acids (P-1570) added.

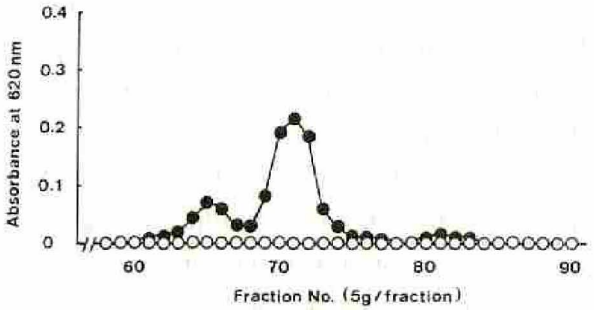

Fig.12. Gel filtrations of Sample No. 26 and Sample No. 26 with the sucrose esters of fatty acids (P-1570) added on the Sephadex LH-20 column

-○- : Sample No.26, -●- : Sample No.26 with 200 ppm of the sucrose esters of fatty acids (P-1570) added.

1570[2]. No materials which colors with the alcoholic anthrone reagent were eluted in the fractions for sucrose (Fraction Nos. 79-89)[2] in all the experiments.

The gel chromatograms of Sample Nos. 34, 36, 33, 29, 30, and 26 (Figs. 7, 8, 9, 10, 11, and 12) indicate that in the experiments with the samples (-o-) no materials which colors with the alcoholic anthrone reagent were eluted in the fractions for P-1570, and that in the experiments with the samples added with P-1570 (-●-) materials which colors with the alcoholic anthrone reagent were undoubtedly eluted in the fractions for P-1570, giving the pattern with 2 peaks of P-1570. No materials which colors with the alcoholic anthrone reagent were eluted

Table 4. Determination of the sucrose esters of fatty acids in the commercial canned coffee samples

Sample No.	P-1570 added	P-1570 found	Recoveries (%)
15	0 ppm	463 ppm	98.6
	500	956	
29	0	4	93.5
	200	191	
30	0	2	96.0
	200	194	
31	0	252	87.0
	200	426	
32	0	459	102.6
	500	972	
33	0	5	95.0
	200	195	
34	0	22	87.0
	200	196	
35	0	82	101.5
	200	285	
36	0	6	84.5
	200	175	
37	0	238	83.5
	200	405	
26	0	2	92.5
	200	187	

in the fractions for sucrose in all the experiments.

Determination of the sucrose esters of fatty acids in the commercial canned coffee samples

Combined fractions for the sucrose esters of fatty acids (Fraction Nos. 61-75) were subjected to the anthrone reaction and the concentrations in terms of P-1570 were determined as described previously[2]. The concentrations and recoveries in all the samples are shown in Table 4. Duplicate determinations were performed and the mean values of both experiments were recorded.

Table 4 shows that Sample Nos. 15 and 32, Sample Nos. 31 and 37, Sample No. 35, and Sample No. 34 contained approximately 460 ppm, 250 ppm, 80 ppm, and 20 ppm of the sucrose esters of fatty acids similar to P-1570, respectively, and that the other samples (Sample Nos. 29, 30, 33, 36, and 26) contained negligible amounts of the sucrose esters of fatty acids. The recoveries ranged from 83.5% to 102.6% and the average was 92.9%.

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

The combined fractions for the sucrose esters of fatty acids (Fraction Nos. 61-75) of gel filtration on the Sephadex LH-20 column, used

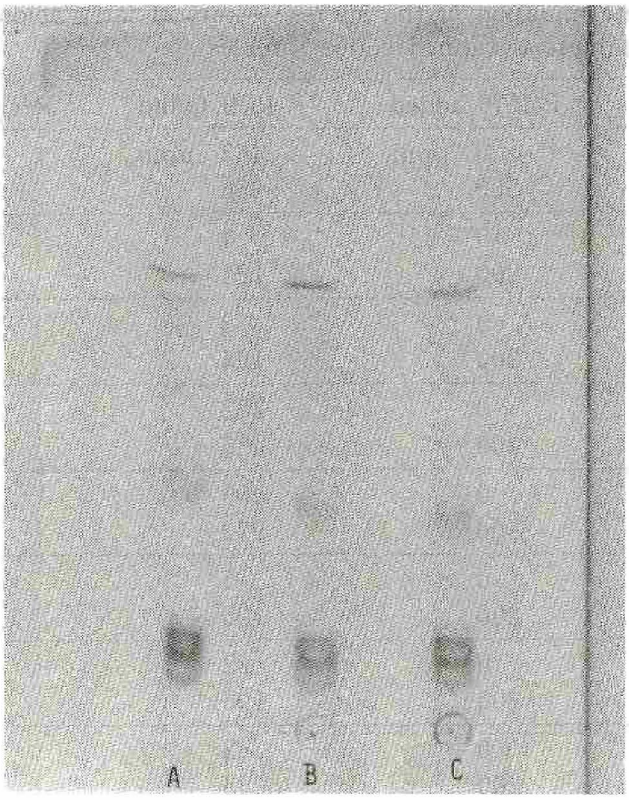

Fig.13. Thin-layer chromatograms of Sample No. 15
A: the emulsifier (P-1570), B: Sample No. 15, C: Sample No. 15 with 500 ppm of P-1570 added.
Developing solvent: chloroform-methanol-acetic acid-water (80:10:8:2), chromgenic reagent: 50% H_2SO_4[2].

for the determination of the sucrose esters of fatty acids, were subjected to the qualitative analysis by the TLC method described previously[2].

The typical TLC plates are shown in Figs. 13 and 14. Fig. 13 shows a TLC plate of Sample No. 15, typical of the samples containing the sucrose esters of fatty acids (Sample Nos. 15, 32, 31, 37, 35, and 34). Fig. 14 shows a TLC plate of Sample No. 36, typical of the samples containing no sucrose esters of fatty acids (Sample Nos. 29, 30, 33, 36, and 26).

As shown in Fig. 13, Sample No. 15 (B) and Sample No. 15 added with P-1570 (C) gave similar spots to those of P-1570 (A). On the TLC plates of the other samples containing the sucrose esters of fatty acids (Sample Nos. 32, 31, 37, 35, and 34), the same results were obtained.

As shown in Fig. 14, Sample No. 36 (B) gave no spots, but Sample No. 36 added with P-1570 (C) gave similar spots to those of P-1570 (A). On the TLC plates of the other samples containing no sucrose esters of fatty acids (Sample Nos. 29, 30, 33, and 26), the same results were obtained.

Discussion

The gel chromatograms on the Sephadex LH-20 column (Fig. 2 - Fig. 12) indicate that Sample Nos. 15, 32, 31, 37, and 35 contained some

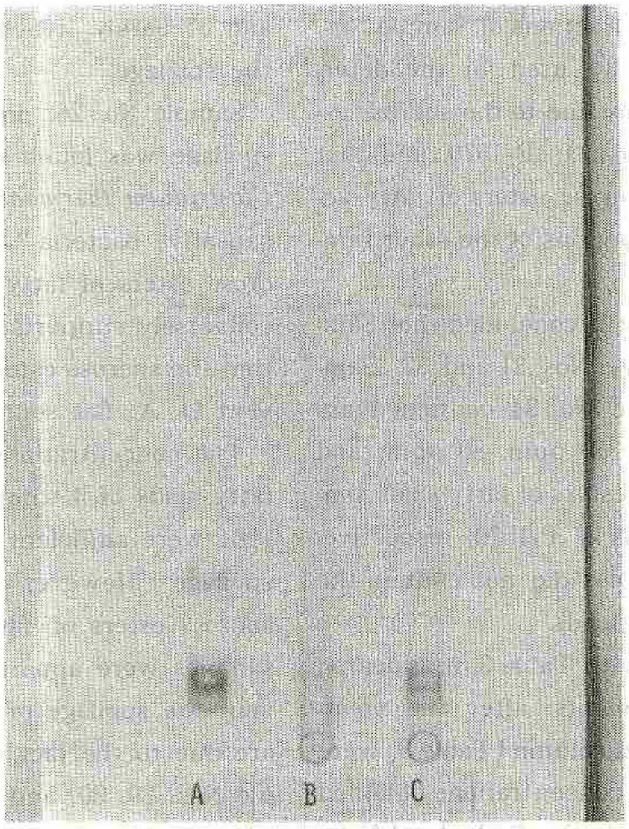

Fig.14. Thin-layer chromatograms of Sample No. 36
A: the emulsifier (P-1570), B: Sample No. 36, C: Sample No. 36 with 200 ppm of P-1570 added.
Developing solvent: chloroform-methanol-acetic acid-water (80:10:8:2), chromgenic reagent: 50% H_2SO_4[2]

sucrose esters of fatty acids which had similar compositions of fatty acids and sucrose esters to P-1570 but the others (Sample Nos. 34, 36, 33, 29, 30, and 26) did not.

The determination of the sucrose esters of fatty acids in the combined fractions for the sucrose esters of fatty acids (Table 4) indicate that Sample Nos. 15, 32, 31, 37, and 35 contained approximately 80-460 ppm of the sucrose esters of fatty acids, and moreover indicate that Sample No. 34 which showed no typical two peaks in the gel chromatogram contained approximately 20 ppm of the sucrose esters of fatty acids but the others contained negligible ones.

The thin-layer chromatograms of the combined fractions indicate that the color developments of the combined fractions of Sample Nos. 15, 32, 31, 37, 35, and 34, used for the determinations, were entirely due to the sucrose esters of fatty acids similar to P-1570, and that there were not the sucrose esters of fatty acids in the combined fractions of the other samples.

From these results, it is concluded that Sample No. 15 and 32, Sample Nos. 31 and 37, Sample No. 35, and Sample No. 34 contained approximately 460 ppm, 250 ppm, 80 ppm, and 20 ppm of the sucrose esters of fatty acids similar to P-1570, in terms of P-1570, respectively, and the other samples did not contain the sucrose esters of fatty acids.

Comparing Table 4 with Table 3, it appeared that the color developments after the treatment with the silica gel column (Table 3) were not proportional to the concentrations obtained after the treatment with the Sephadex LH-20 column (Table 4). The reason why the concentrations estimated preliminarily after the treatment with the silica gel column were higher than the concentrations obtained after the treatment with the Sephadex LH-20 column should be that the absorbances at 620 nm of the color development after the treatment with the silica gel column included impurities like coffee pigments.

We reported previously that the sucrose esters of fatty acids prevent O. A. flat sour spoilage at 300 ppm in canned coffee[1]. Therefore, though it must be taken into consideration that the components of coffee with milk and sugar affect the inhibition of the sucrose esters of fatty acids on the growth, germination and/or outgrowth of the bacteria causing O. A. flat sour spoilage, it is safe to say that the concentrations of the sucrose esters of fatty acids in Sample Nos. 15 and 32 were sufficient to prevent O. A. flat sour spoilage, and the concentrations in Sample Nos. 31 and 37 barely reached the level to prevent the spoilage.

Sample No. 26, in which O. A. flat sour spoilage was found in some cans from which *Clostridium thermoaceticum* was isolated as the causative bacteria[3,4], did not contain the sucrose esters of fatty acids. This result further conversely supports our previous conclusion that the sucrose esters of fatty acids could prevent O. A. flat sour spoilage[1].

The concentrations of the sucrose esters of fatty acids of 4 samples out of 10 produced in 1981 were sufficient to prevent O. A. flat sour spoilage. However, the concentrations of the sucrose esters of fatty acids of the other 6 samples were apparently either too low to prevent the spoilage or almost nil. These results are due to the fact that the samples like Sample Nos. 29, 30, and 36 are products in August, May, and April, respectively, and might not have been intended for hot vending. But the samples like Sample Nos. 33, 34, and 35 which were produced in January, February, and February, respectively, and most likely to have been intended to be sold by hot ven-

dors, contained either too little or nil to prevent the spoilage. For these samples, some other measures than the addition of sucrose esters of fatty acids might have been adopted to prevent the spoilage.

Acknowledgments

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