spectively. In the case of heptachlor epoxide, the difference between the enantiomers was not so remarkable (about 2.3 times) as compared with the above two compounds. Recently, cyclodiene insecticides have been limited by their use as agricultural chemicals, because of their carcinogenic and chronic toxicity. Our present findings may suggest that optically active forms of these insecticides should be further investigated on their mode of action, biochemical metabolism in animals, and possible use as agricultural chemicals.

LITERATURE CITED

Bluestone, H., Lidov, R. E., Knaus, J. H., Howerton, P. W., U.S. Patent 2576666 (1951).
Brooks, G. T., Harrison, A., Lewis, S. E., *Biochem. Pharmacol.*, **19**, 255 (1970).
Carlson, A. W., U.S. Patent 3118913 (1964).
Crabbe, P., "ORD and CD in Chemistry and Biochemistry", Academic Press, New York, N.Y. 1972, p39.
Kleiman, M., U.S., Patent 2598561 (1952).
Kleiman, M., Goldman, A., U.S. Patent 2672486 (1954).
Imai, K., Marumo, S., Ohtaki, T., *Tetrahedron Lett.*, 1211 (1976).
Iwaki, S., Marumo, S., Saito, T., Yamada, M., Katagiri, K., *J. Am. Chem. Soc.* **96**, 7842 (1974).
Loew, P., Johnson, W. S., *J. Am. Chem. Soc.* **93**, 3765 (1971).
Mori, K., *Tetrahedron* **30**, 4223 (1974).
Mori, K., *Tetrahedron* **31**, 1381 (1975). Mori, K., *Agric. Biol. Chem.* **40**, 415 (1976).
Mori, K., *Agric. Biol. Chem.* **40**, 415 (1976).
Mori, K., Mizumachi, N., Matsui, M., *Agric. Biol. Chem.* **40**, 1611 (1976a).
Mori, K., Takigawa, T., Matsui, M., *Tetrahedron Lett.*, 3953 (1976b).
Riley, R. G., Silverstein, R. M., Moser, J. C., *Science* **183**, 760 (1974).
Woodward, R. B., Katz, T. J., *Tetrahedron* **5**, 70 (1959).

Akio Miyazaki*[1]
Tokuji Hotta[1]
Shingo Marumo[2]
Michihiko Sakai[3]

[1]Osaka Agricultural Research Center
Shakudo, Habikino-shi, Osaka 583, Japan
[2]Department of Agricultural Chemistry
Nagoya University
Nagoya 464, Japan
[3]Agricultural Chemicals Division
Takeda Chemical Industries, Ltd.
Kyoto 606, Japan

Received for review October 25, 1977. Accepted January 30, 1978.

Modification of the Mojonnier Fat-Testing Method for Soy-Protein-Lipid Concentrate

Application of acid hydrolysis to the Mojonnier (modified Roese-Gottlieb) procedure has been studied for the determination of the lipid content of soy-protein-lipid concentrate. The time-saving new method was found to give significantly greater test values than the conventional ether-extraction method.

The standard ether-extraction (EE) procedure employing the Soxhlet apparatus for the determination of the fat content of soybean meal, soybean flour, and other similar products is known to give low values and, therefore it has been sometimes used to estimate free-fat content (Hand et al., 1964). Also, it is time-consuming. Further, acid treatment of the sample has been reported to give more accurate results (Genotti, 1968; Zhukov and Vereschagin, 1976). So, it was proposed to suitably modify the Mojonnier method (Milk Industry Foundation, 1959), widely used for dairy products, with incorporation of acid hydrolysis for soy-protein-lipid concentrate (precipitate of soymilk prepared by the method of Nelson et al., 1975).

EXPERIMENTAL SECTION

Materials. The Mojonnier equipment used was the Model D (Mojonnier Bros. Co., Chicago). The chemicals were as follows: hydrochloric acid (BDH), analytical grade, specific gravity, 1.18; 95% ethyl alcohol, specific gravity, 0.817 at 15.6 °C; petroleum ether (BDH), bp 40–60 °C and ethyl ether (Sarabhai M. Chemicals), laboratory grade.

Methods. The following variables were studied: (a) the amount of sample, (b) addition of warm water, (c) acid hydrolysis with different time–temperature combinations, (d) addition of alcohol to avoid gelling, and (e) addition of ethyl and petroleum ethers.

The alcohol levels for different extractions with different treatments were decided through preliminary trials which also included varying levels of samples as well as water. Those combinations giving quick and satisfactory separation of the two phases in the extraction flask were adopted for complete test. The experimental tests were compared with the classical EE procedure (AOAC, 1975).

RESULTS AND DISCUSSION

The results of the Mojonnier procedure with various acid treatments and those of the EE procedure have been presented in Table I. The percent fat test after first extraction with some treatments was very low, and, so, they were discarded. Among the remaining treatments, some gave a fat test fairly close to that given by EE method whereas others showed appreciably higher test values. The results with seven treatments showing higher test values were subjected to statistical analysis.

As indicated in Table II, the percent fat obtained with the EE procedure was significantly higher than that obtained after the first extraction with the Mojonnier method with or without acid treatment. However, all the seven treatments yielded appreciably higher fat values when the extraction was extended to second and third stages. Two treatments (nos. 9 and 12) giving the highest test values did not vary appreciably from each other. In the case of treatment no. 12 involving sample hydrolysis with 5 mL of acid, while the second extraction resulted in significantly higher test values, compared to the first extraction, the third extraction showed no further rise in the fat test

0021-8561/78/1426-0977$01.00/0

Table I. Effect of Various Treatments

Sr. no.	Sample,[b] g (less than)	Treatment	Amount of alcohol, 1st 2nd, 3rd extraction	No. of replicates	Mean fat test, % Extraction no. 1st	2nd	3rd
1	0.5	Nil, usual Mojonnier method (9 mL of water, and 3 mL Amm. Hydroxide)	10, 5, 0	4	22.9	28.1	28.0
2	0.5	2 mL HCl (2 min shaking, no heating)	8, 2, 2	4	24.0	27.5	28.0
3	0.5	4 mL Hcl (same)	6	4	15.4		
4	0.5	6 mL HCl (same)	4	2	12.3		
5	0.8	2 mL HCl (60 °C, 20 min)	7, 3, 0	2	21.1	27.1	27.6
6	0.8	4 mL HCl (same)	3	2	16.2		
7	0.8	2 mL HCl (80 °C, 10 min)	7, 3, 0	2	23.0	27.7	27.8
8	0.8	4 mL HCl (same)	6, 2, 0	4	25.8	30.0	30.0[a]
9	0.8	6 mL HCl (same)	6, 3, 0	4	27.0	30.1	30.1[a]
10	0.8	8 mL HCl (same)	2	2	4.5		
11	0.8	10 mL HCl (same)	2	2	4.4		
12	0.8	5 mL HCl (same)	6, 2, 0	4	26.7	30.1	30.1[a]
13	0.8	2 mL HCl (5 min in boiling water bath)	7, 3, 0	4	26.5	28.5	28.5
14	0.8	4 mL HCl (same)	5, 2, 0	4	27.3	29.6	29.7
15	0.8	6 mL HCl (same)	8, 2, 0	4	22.8	29.9	30.0[a]
16	0.8	5 mL HCl (same)	7, 3, 0	4	24.9	29.7	29.7[a]
17	0.8	2 mL HCl (10 min in boiling water bath)	6, 3, 0	4	28.3	29.4	29.4
18	0.8	4 mL HCl (same)	8, 2, 0	4	25.6	30.0	29.9[a]
19	0.8	3 mL HCl (same)	8, 4, 0	4	27.7	29.9	29.9[a]
20		Standard ether extraction with Soxhlet method			28.9	28.9	28.9

[a] Selected for statistical analysis. [b] Sample amount greater than indicated gave gelation and resulted in incomplete extract separation.

Table II. Comparison of Selected Treatments[a]

Extraction no.	Mean test, % by weight Treatment number 8	9	12	15	16	18	19	20	F value[d]	CD[b]
I	25.8	27.0	26.7	22.8	24.9	25.6	27.7	28.9	28.45**	1.98
II	30.0	30.1	30.1	29.9	29.7	30.0	29.9	28.9[c]	65.03**	0.41
III	30.0	30.1	30.1	30.0	29.7	29.9	29.9	28.9[c]	95.57**	0.36

[a] Figures as per Table I. [b] Critical difference. [c] Same figure as that for first extraction. [d] ** = significant at the 1% level.

Table III. Optimum Number of Extractions

Treatment no.	Mean test, % by weight Extraction number 1st	2nd	3rd	F value	CD	SD[a]
12	26.7	30.1	30.1	57.62	0.79	0.50

[a] Standard deviation.

(Table III).

The following method is recommended for the determination of the lipid content of soybean protein–lipid concentrate: (1) weigh accurately 0.7 to 0.8 g of the sample into the Mojonnier flask; (2) add 7 to 8 mL of warm water and mix well; (3) digest with 5 mL of concentrated hydrochloric acid, at 80 °C for 10 min (with intermittent shaking), and cool under tap water; (4) add 5 mL of alcohol, 25 mL each of ethyl and petroleum ethers, shaking for 90 s after each addition; (5) centrifuge for 30 s and transfer the ether extract into a weighed fat dish; (6) carry out second extraction with 2 mL of alcohol and 25 mL each of the ethers, shaking for 60 s; (7) evaporate the ether on the hot plate for 10 min and remove the last traces of the ether in the vacuum oven for 7 to 8 min before cooling and weighing the dish.

ACKNOWLEDGMENT

The authors wish to thank G. R. Patil of the Dairy Technology Division for his valuable suggestions during the course of this investigation and to Bhupal Singh of the Dairy Economics, Statistics and Management Division, for his guidance in the statistical analysis of the data.

LITERATURE CITED

Association of Official Analytical Chemists, "Official Methods of Analysis", 12th ed, Washington, D.C., 14.080, 14.081, 1975, p 234.

Genotti, G., *Tech. Molitoria* 19(22), 625–29 (1968).

Hand, D. B., Steinkraus, K. H., Van Buren, J. P., Hackler, L. R., El-Rawi, I., Pallesen, H. R., *Food Technol.* 18(12), 139 (1964).

Milk Industry Foundation, "Laboratory Manual", 3rd ed, Washington, D.C., 1969, p 264.

Nelson, A. I., Steinberg, M. P., Wei, L. S., U.S. Patent 3901978 (1975).

Zhukov, A. V., Vereshchagin, A. G., *J. Am. Oil Chem. Soc.* **53**(1), 1 (1976).

A. A. Patel
S. K. Gupta*

Division of Dairy Technology
National Dairy Research Institute
Karnal (Haryana) 132001, India

Received for review August 31, 1977. Accepted February 16, 1978.