Technical

trans Fatty Acids in Milkfat[1]

L. DEMAN and J.M. DEMAN, Department of Food Science, University of Guelph, Guelph, Ontario, N1G 2W1 Canada

ABSTRACT

Milkfat is extremely complex, with ca. 500 different fatty acids reported in the triglycerides. Seasonal feed variation results in higher unsaturated fatty acid levels in summer than in winter. Rumen microbes hydrogenate unsaturated feed lipids to yield a mixture of geometrical and positional isomers which are transmitted to the milk. Total isolated *trans* fatty acids in milkfat reported in the literature range from 2 to 11% with maximal values in summer and minimal values in winter. A study was undertaken about the use of AOCS Method Cd 14-61 for the determination of isolated *trans* levels in milkfat. The triglycerides of milkfat were analyzed using trielaidin as a standard and milkfat methyl esters were analyzed using methyl elaidate as a standard. Corrections were made for the loss of the methyl esters of the short-chain fatty acids. The apparent levels of *trans* found by using the triglycerides were considerably higher than by using the methyl esters. Infrared spectra of pure triglycerides of saturated fatty acids showed measurable absorption at 10.36 μm, whereas methyl esters of saturated and *cis* unsaturated fatty acids did not.

INTRODUCTION

In the cow, ingested feed is subjected to microbial attack in the rumen. The complex plant lipids are hydrolyzed rapidly and the C18 unsaturated fatty acids, consisting mainly of linolenate, undergo extensive hydrogenation and double bond migration to yield a mixture of geometrical and positional isomers. Amount and type of feed can influence the microbial population of the rumen and this can influence hydrogenation and formation of *trans* isomers in milkfat (1).

Trans fatty acids have traditionally been estimated by infrared analysis (2). Isolated *trans* bonds exhibit an absorption band with a maximum at 10.3 μm, arising from the C-H deformation about the *trans* double bond. Quantitation of absorptivity is obtained with the use of primary standards and baseline techniques. The usual procedure has been to analyze the triglycerides. The method is easy and fast.

Unfortunately, the triglyceride moiety also gives rise to absorption at the same wavelength as the *trans* configuration and analyses of the triglycerides result in high apparent *trans* values. The absorption caused by the glyceride linkage at 10.3 μm has been recognized by other workers (3-6). A simple solution to overcome the absorption of the triglyceride molecule is the preparation of methyl esters of the triglycerides. Unfortunately, methyl esters also have their shortcomings. In the determination of *trans* isomers, methyl esters interfere with the *trans* absorption.

Trans fatty acid analysis in butterfat by gas liquid chromatography requires preliminary separation by thin layer chromatography because of the complexity of its fatty acids (7-10). The method is lengthy and is not suitable for routine analysis.

[1] Presented at the 73rd AOCS annual meeting, Toronto, 1982.

Knowledge of *trans* unsaturation content in butterfat is useful in the detection of adulteration. Renewed interest in *trans* fatty acids and a scarcity of information on its content in milkfat have prompted the present study.

MATERIALS AND METHODS

To investigate the extent of the glyceride moiety absorption, several oils were analyzed along with methyl esters. The oils chosen were: coconut oil, because of its long-, medium- and short-chain fatty acids; olive oil, because of its high oleic acid content; sunflower oil, because of its high linoleic acid content; and soy oil, because of its high linolenic acid content. The oils were refined, bleached and deodorized. Several pure triglycerides were also analyzed. They were obtained from Supelco Inc. (Bellefonte, PA), except tributyrin which was obtained from Eastman Kodak. Methyl esters were prepared by the boron trifluoride method (11).

In the preparation of butterfat methyl esters, some loss of the short-chain fatty acid methyl esters is encountered. For this reason the butterfat triglycerides were analyzed for their fatty acid composition by the method of Shehata et al. (12). The methyl esters obtained for the infrared analyses were also analyzed for their fatty acid composition. Corrections were made in the final *trans* results for the loss of short-chain methyl esters. Butterfat contains long-, medium- and short-chain fatty acids. For this reason, methyl elaidate and coconut methyl esters were chosen for the preparation of the calibration curve instead of methyl stearate as recommended in the IUPAC method (6). Trielaidin was used for the calibration curve of the triglyceride analyses as outlined in the AOCS method (2). Methyl elaidate and trielaidin were obtained from Serdary Research Laboratories Inc., London, Ontario.

Samples of Ontario butter were collected throughout a whole year. The butter was melted in the oven at 60 C, and the fat was decanted through fast filter paper and stored in the freezer until analyzed.

A Beckman model 4230 infrared spectrophotometer and fixed thickness absorption cells with NaCl windows and path length of 0.5 mm were used for the infra red analyses. Samples were dissolved in carbon disulfide.

RESULTS AND DISCUSSION

At present, there are five methods in the literature that describe the *trans* isomer determination in methyl esters by infrared analysis.

(1) AOCS Method Cd 14-61 (2). Baseline is drawn from 10.02-10.59 μm. Calibration curve is methyl elaidate.
(2) AOAC Method 26.061 (13). Baseline is drawn from 10.02-10.59 μm. Correction factors for methyl esters are: long-chain fatty acid: % *trans* corrected = (% *trans* calculated + 1.5)/1.015, lower and medium-chain fatty

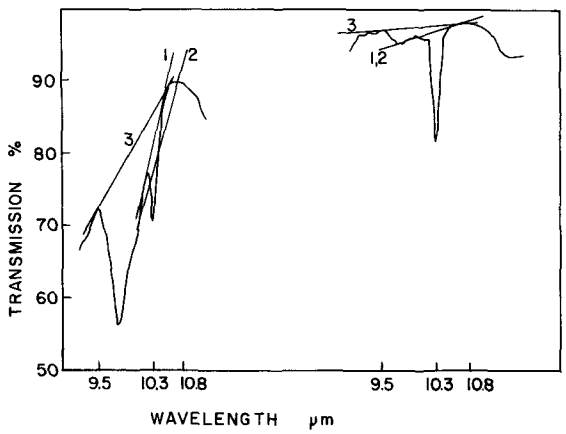

FIG. 1. Methods of drawing baseline for quantitative infrared *trans* determination. Left: 1.6 g/L methyl elaidate and 20 g/L coconut oil methyl esters in carbon disulfide; right: 1.6 g/L methyl elaidate in carbon disulfide. (1) AOCS method; (2) IUPAC method; (3) method of Hirayama et al. (1978).

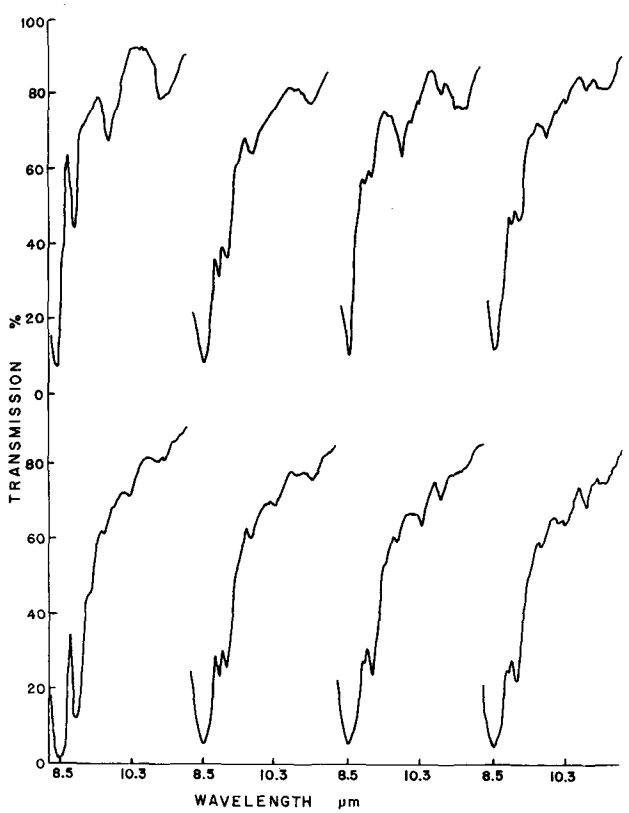

FIG. 2. Absorption at 10.3 μm in the infrared for methyl esters (top) and triglycerides (bottom) of coconut, olive, sunflower and soy oil (from left to right, respectively). Concentration 20 g/L in carbon disulfide.

acids: % *trans* corrected = (% *trans* calculated + 3.0)/1.030.

(3) Hirayama et al. (1978). Baseline is drawn from 9.5-10.6 μm. Calibration curve is methyl elaidate.

(4) IUPAC method 2.207 (6). Baseline is drawn from 10.0-10.8 μm. Calibration curve is a mixture of methyl elaidate and methyl stearate.

(5) Allen (14). No baseline or calibration curve. The ratio of the absorbances of the ester bond and the *trans* bond is used to calculate the *trans* content: % *trans* = 121.86 ($A_{10.3}/A_{8.55}$) − 9.18.

TABLE I

Isolated *trans* Content in Oils and Their Derived Methyl Esters (AOCS Method)

Oil	Triglycerides % *trans*	Methyl esters % *trans*
Soy	6.9	3.0
Olive	2.5	0
Sunflower	4.5	0.9
Coconut	3.7	0

TABLE II

Apparent Isolated *trans* Content of Some Triglycerides (AOCS Method)

Triglycerides	Apparent *trans* %
Tributyrin	25.3
Tricaprylin	4.6
Trilaurin	3.3
Trimyristin	4.2
Tripalmitin	2.6
Tristearin	3.4

Huang and Firestone (15) have critically evaluated Allen's method against the AOCS method and suggested a methyl ester matrix with the same composition as the sample matrix for the determination of the two constants in the formula given above. Because of the complexity of butterfat fatty acids, this method was not investigated. Figure 1 shows the different baselines that result from using the different methods. The IUPAC baseline drastically cuts the absorption peak when coconut methyl esters are present and leaves little to be measured when the amount of *trans* content is small. For this reason, the IUPAC baseline techniques were also not investigated. A good feature of the IUPAC method is that it recommends a standard curve that is prepared from methyl elaidate and methyl stearate in varying proportions to the same total amount of 200 mg. Addition of methyl stearate compensates for the interference with *trans* absorption due to methyl ester content.

Figure 2 shows the absorption curves for coconut, olive, sunflower and soy oil triglycerides and methyl esters. There is no absorption at 10.3 μm in the spectra of methyl esters of olive and coconut oil. There is only a slight absorption in the case of soy and sunflower methyl esters. This may be caused by extraction and processing procedures (2). Table I shows the calculated apparent *trans* content in both triglycerides and methyl esters as calculated by the AOCS method, using trielaidin and methyl elaidate for the preparation of the calibration curves. Several pure triglycerides were also analyzed. Their apparent *trans* content is shown in Table II. The calibration curves for methyl esters are shown in Figure 3. Curve number 1 is the calibration curve of methyl elaidate only, calculated according to the AOCS method. Curve number 2 contains the same amount of methyl elaidate as curve number 1 plus 200 mg of coconut methyl esters per 10 mL of carbon disulfide. AOCS baseline techniques were used for this curve. Curve number 3 is the calibration curve of methyl elaidate using the baseline technique of Hirayama et al. (5). Addition of coconut methyl esters did not change this calibration curve. The difference between calibration curves 1 and 2 is equivalent to 4 mg methyl elaidate. In other words, methyl esters containing no *trans* will interfere with the absorption of isolated *trans*

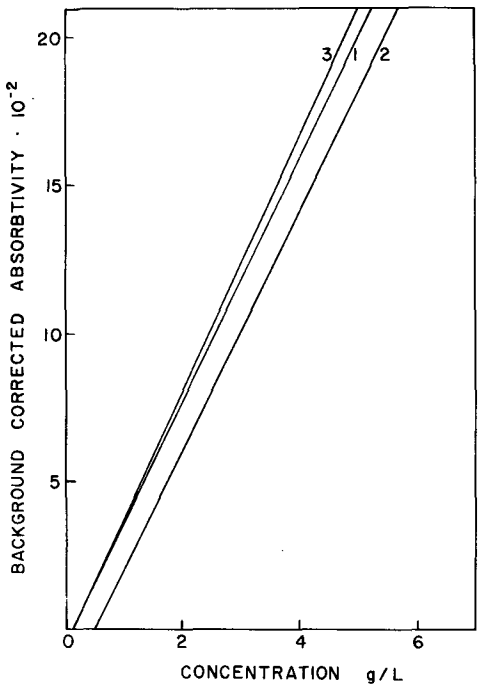

FIG. 3. Calibration curve for *trans* determination. (1) methyl elaidate, using AOCS method; (2) methyl elaidate plus 20 g/L coconut oil methyl esters, AOCS method; (3) methyl elaidate, using the method of Hirayama et al. (1978). Solvent: carbon disulfide.

double bonds to the extent of 2 mg methyl elaidate per 100 mg methyl esters in 10 mL of carbon disulfide. This level of interference increased in a constant ratio when sample weight was increased up to 800 mg in 10 mL carbon disulfide.

The *trans* content of Ontario butter is presented in Figure 4. The open circles represent the *trans* content in the triglycerides calculated according to the AOCS method. The closed circles show the *trans* content in the methyl esters calculated according to the AOCS method. The data represented by the crosses were obtained by correcting the values of the closed circles for methyl ester interference (2 mg methyl elaidate/100 mg sample). The triglyceride values range from 9.9% in the summer to 7.4% in the winter. The uncorrected methyl ester values range from 3.7% in the summer to 2.0% in the winter. Corrected methyl ester values of *trans* content range from 5.7% in the summer to 4.0% in the winter. Corrected values of *trans* content of the methyl esters calculated according to the AOAC method range from 6.5% in the summer to 4.9% in the winter. The *trans* content of butterfat varied only slightly during the winter season, but increased when cows went on pasture in the spring.

There have been several reports in the literature on *trans* content of butterfat. Smith et al. (7) examined four samples and found an average value of 4% *trans* determined by the AOCS method with methyl elaidate as standard. Gas chromatography combined with thin layer chromatography resulted in 1.9% *trans*. Parodi (16) examined butterfat methyl esters using the AOAC method and obtained results ranging from 4.3 to 7.6%. The following researchers used triglycerides according to the AOCS method: deMan (17), 6.6-4.9% *trans*; Woodrow and deMan (18), 8.6% *trans*, Bartlet and Chapman (19), 5.8-12% *trans*; Galoppini and Lotti (20), 11.37% *trans* and (21), 9.6-8.1% *trans*. Cornwell et al. (22) found 5% *trans* in winter and 9.7% *trans* in summer by means of fractional distillation of the methyl esters and infrared analyses.

The differences in reported *trans* levels in butterfat may be the result of seasonal and other variations. The diversity of methods used is likely to play a role in these differences. Using the infrared method with methyl esters and preparation of the standard curve with methyl elaidate and coconut methyl esters should yield more uniform results.

ACKNOWLEDGMENT

The Natural Sciences and Engineering Research Council of Canada and the Ontario Ministry of Agriculture and Food provided financial support.

REFERENCES

1. Posati, L., J.D. Kinsella and B.K. Watt, J. Am. Diet. Assoc. 66: 482 (1975).
2. Official and Tentative Methods of the American Oil Chemists' Society, AOCS, Champaign, IL, 1973, Method CD-14-61.
3. Firestone, D., and M. De La Luz Villadelmar, J. Assoc. Off. Anal. Chem. 44: 459 (1961).
4. Firestone, D., and P. LaBouliere, Ibid. 48: 437 (1965).
5. Hirayama, H., Z. Shimoda, S. Nakasato, Y. Murase, T. Shige, T. Takahoshi, H. Watanabe, S. Yoneyama, M. Yamashita, K. Yamanaka and H. Yoshida, Yukagaku 27: 606 (1978).
6. Standard Methods for the Analysis of Oils, Fats and Derivatives, 6th edn., IUPAC, Pergamon Press, 1979.
7. Smith, L.M., W.L. Dunkley, A. Franke and T. Dairiti, JAOCS 55: 257 (1978).
8. Conacher, H.B.S., J. Chromatogr. Sci. 14: 405 (1976).

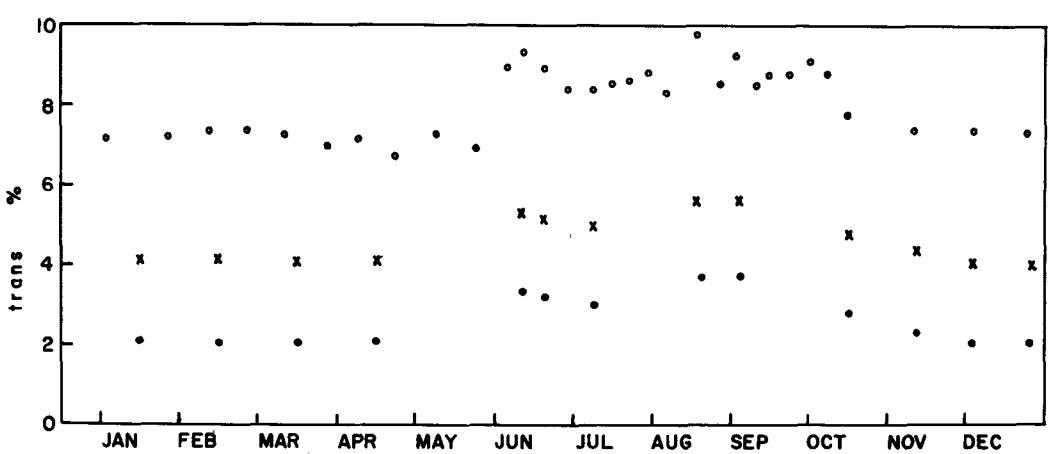

FIG. 4. *trans* content of Ontario butter. ○: *trans* content using AOCS triglyceride method. ●: *trans* content using AOCS methyl ester method. X: AOCS methyl ester method corrected for methyl ester interference.

9. Conacher, H.B.S., and J.R. Iyengar, J. Assoc. Off. Anal. Chem. 61: 702 (1978).
10. Conacher, H.B.S., J.R. Iyengar and J.L. Beare-Rogers, Ibid. 60: 899 (1977).
11. Solomon, H.L., W.D. Hubbard, A.R. Prosser and A.J. Sheppard, JAOCS 51: 424 (1974).
12. Shehata, A.Y., J.M. deMan and J.C. Alexander, Can. Inst. Food Technol. J. 3: 85 (1970).
13. Official Methods of Analysis of the Association of Official Analytical Chemists, 12th edn., AOAC, Washington, DC, 1975.
14. Allen, R.R., JAOCS 46: 552 (1968).
15. Huang, A., and D. Firestone, J. Assoc. Off. Anal. Chem. 54: 1288 (1971).
16. Parodi, P.W., Austr. J. Dairy Technol. 26: 60 (1971).
17. DeMan, J.M., J. Dairy Res. 28: 81 (1961).
18. Woodrow, W., and J.M. deMan, Biochim. Biophys. Acta 152: 472 (1968).
19. Bartlet, J.C., and D. Chapman, Agric. Food Chem. 9: 50 (1961).
20. Galoppini, G., and G. Lotti, Chim. Ind. 46: 795 (1964).
21. Galoppini, G., and G. Lotti, Ric. Sci. 8: 1049 (1965).
22. Cornwell, D.G., R. Blackderf, G.L. Wilson and J.B. Brown, Arch. Biochem. Biophys. 46: 364 (1953).

[Received June 8, 1982]

Degumming of Soybean Oil: Quantitative Analysis of Phospholipids in Crude and Degummed Oil[1,2]

L.D. RACICOT and **A.P. HANDEL**, Department of Food Science and Technology, University of Nebraska, Lincoln, NE 68583-0919

ABSTRACT

Phospholipids of crude and degummed soybean oils were isolated and separated by column chromatography and thin layer chromatography. Standards and specific spray reagents were used to identify the phospholipids. Phospholipids identified in the crude and degummed oils were phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, phosphatidic acid, lysophosphatidylcholine and lysophosphatidylethanolamine. Several unknown phosphorus-containing compounds were present. Samples of crude and degummed oil were collected from four soybean oil processors over four consecutive days. Total and individual phospholipids were quantitated by determining the phosphorus content. The total phosphorus content of crude oil varied among companies, with the average ranging from 453 to 676 ppm. The average total phosphorus content of the degummed oil of the four processors ranged from 12 to 84 ppm. Processors removed an average of 86-98% of the phosphorus present in the crude oil during the degumming process. There was also a daily variation in phosphorus removal within the individual companies. During the degumming process, the proportion of phosphatidylcholine decreased and the proportion of unknown, nonpolar phosphorus compounds increased in samples from all companies. Significantly higher proportions of phosphatidic acid and lyso compounds were found in the degummed oil of some but not all companies.

INTRODUCTION

Crude soybean oil must undergo several refining steps to produce a final product with bland flavor and odor. The first step is degumming with water (with or without a degumming agent such as phosphoric acid) to remove easily hydrated materials which are primarily phospholipids. Degumming removes 76-98% of the phosphorus-containing compounds (1) and residual phospholipids are removed during subsequent refining with caustic soda. High levels of residual phospholipids require greater amounts of caustic for refining, which increases the loss of neutral oil. The presence of excess phospholipids in the final oil causes darkening of the oil and poor flavor stability (2).

Chapman (3) found crude soybean oil to contain 39.0% phosphatidylcholine, 23.3% phosphatidylethanolamine, 20.3% phosphatidylinositol, 4.8% phosphatidic acid, and 12.5% unknown compounds. Hvolby (4) classifies phosphatidylcholine, phosphatidylethanolamine and phosphatidylinositol as hydratable phospholipids and phosphatidic acid and lysophosphatidic acid as nonhydratable. Nielsen (5) determined that the nonhydratable phosphorus compounds also include inorganic phosphate, inositolmonophosphate, and glycerophosphate. He attributes these phosphorus compounds to decomposed materials which are present in soybeans, with only small amounts formed during processing of the oil. Mounts et al. (6) attributes an increase in nonhydratables in exported beans to splitting and breakage of the seeds during transport which could activate enzymes that cause decomposition of phospholipids. Similar decomposition occurs during storage (7) and in beans that have been freeze-damaged (8).

The purpose of this investigation was to identify and quantitate the phospholipids not removed during the degumming of soybean oil. Crude and degummed soybean oil samples from four oil processors were analyzed for total phosphorus and individual phospholipids were quantitated.

MATERIALS AND METHODS

Samples

Crude and degummed soybean oils were collected on four consecutive days from four different soybean oil processing plants in Lincoln, NE, Decatur, IL, Fort Wayne, IN, and Stuttgart, AR. Samples were collected from one of the processors at two times during the year (November and February).

Separation Techniques

Crude and degummed oils were separated into neutral and polar lipids by a chromatographic system based on that of Hirsch and Ahrens (9). Chromatographic columns (10 X 100 mm) with a 200 mL solvent reservoir were packed with 5 g of 100-200 mesh Bio-Sil A (Bio-Rad

[1]Presented at the 72nd AOCS annual meeting, New Orleans, 1981.
[2]Paper No. 7056, Journal Series, Nebraska Agricultural Experiment Station.