Limit of the Solid Fat Content Modification of Butter

Salwa Bornaz, Jacques Fanni and **Michel Parmentier***
Laboratoire de Physicochimie et Génie Alimentaires, Ecole Nationale Supérieure d'Agronomie et des Industries Alimentaires (ENSAIA), 54505 Vandoeuvre les Nancy, France

Three ways have been undertaken to modify solid fat content of butter oil: (i) interesterification, (ii) adjunction of high-melting glycerides and (iii) joint effect of adjunction of high-melting glycerides and interesterification. A solvent-free interesterification, carried out with 1,3-specific lipase from *Mucor miehei*, resulted in an increase of the solid fat content (SFC) by about 114% after 48 h of interesterification. The changes in triglyceride composition induced by this method were followed by quantitative determination of triglycerides of different equivalent carbon number (ECN) and different theoretical carbon number. The major changes in the triglyceride composition occurred mainly in the concentration of three groups of triglycerides with the same ECN (ECN = 38). Adding high-melting glycerides trimyristin (MMM) and tripalmitin (PPP) led to an increase of the SFC measured at 20°C as these proportions increased in the mixture. The joint effect of the addition of MMM or PPP and interesterification was quite significant, mainly for triglycerides that included myristic and palmitic acids. As far as the increase of SFC is concerned, the effect of interesterification decreases when both substrate amounts increase.

KEY WORDS: Butter oil, high-melting glyceride, interesterification, solid fat content.

Using interesterification to modify the physical properties of butter is not a recent idea (1–4). deMan *et al.* (5) and Antila (6) reported that interesterification of milkfat markedly increases its hardness, affects the melting properties and changes the peak ratios in melting curves. However, a better knowledge of triglyceride (TG) compositions (7–9) permits their variation after interesterification to be traced. A tentative explanation of the physical behavior based on chemical composition will be given. This is one of the purposes of the present paper. The second shows the joint effect of the adjunction of high-melting glycerides and interesterification on the physical properties and chemical composition of butter oil.

The purpose of a previous study (unpublished data) was the evaluation and optimization of the environmental factors that govern acyl-exchange reactions between olive oil and trimyristin (MMM) substrates with 1,3-specific lipase (Lipozyme®) from *Mucor miehei* without solvent. Because the specificity of Lipozyme® is not affected by different substrates and media (10), this method has now been applied to more complex systems, such as butter oil, mixtures of butter oil/MMM and mixtures of butter oil/tripalmitin (PPP).

In recent years, a number of reports dealt with interesterification reactions catalyzed by immobilized lipase enzymes. Most of them have been conducted in the presence of solvents (11–15). For instance, the changes induced by a lipase from *Candida cylindracea* are similar to those induced during interesterification catalyzed by sodium methoxide. The most significant changes occur in the concentration of monounsaturated TGs with 36 and 38 acyl carbons, which decrease during interesterification by 45 and 52%, respectively (11). Investigations on the *Pseudomonas fluorescens* lipase-catalyzed interesterification of butterfat in a medium of variable water content in hexane (12) and in a medium of low water content in isooctane at different temperatures (13) show that the TG composition of the treated butterfat was close to the composition calculated according to a random distribution.

The great effect of water activity on enzyme activity should be underlined (16,17). Lipases are known to act in nonaqueous media, although a finite amount of water, associated with protein, must be present to retain conformational integrity, and thereby activity. Hence, for any process, there will be an optimum effect of water activity (a_w) value determined by the balance between the lipase catalyzed syntheses and the catalytic activity of the enzyme (18).

Furthermore, hydrocarbon solvents are often used because they facilitate the reaction by lowering the viscosity of the medium and solubilizing the substrate. The activity of many enzymes is partially dependent on the choice of solvent (19, 20). This constraint can be avoided by using butter oil as a substrate and as a dispersant. Moreover, there are advantages of not using an organic solvent of questionable toxicity, especially in the reduction of cost for the separation of the solvent from the product at the end of the process. However, few papers report on the interesterification of fat in the absence of a solvent (21–24). The effect of interesterification, catalyzed by 1,3-specific *M. miehei* lipase without solvent, on the physical properties and chemical composition of butterfat and mixtures of butterfat with high-melting glycerides, is reported here. Interesterifications were followed by high-performance liquid chromatography. For melting properties, solid fat content (SFC) was determined by differential scanning calorimetry (DSC) (25).

MATERIALS AND METHODS

Materials. 1,3-Specific lipase (Lipozyme®) from *M. miehei*, immobilized on a macroporous anion exchange resin (26), was a generous gift from Novo Industries (Bagsvaerd, Denmark). Tripalmitin (PPP) and MMM with a purity of 90% were purchased from Sigma (St. Louis, MO). Butterfat, provided by Union Beurriere (Vesoul, France), was melted at 60°C and centrifuged to remove the remaining water.

Lipase-catalyzed interesterification. Reactions were carried out in thermostated, stirred batch reactors at 60°C without solvent. The substrate mixture was incubated for 5 min at the proper reaction temperature before adding the enzyme. Because interesterification takes place in a nonpolar medium, the water content in the immobilized lipase was minimized to obtain a favorable esterification equilibrium (16–18). However, a minimum amount of water must be present to activate the lipase (27,28). As observed by Eigtved *et al.* (29), optimal interesterification activity occurred at 0.1% water (w/w) in the immobilized lipase. In the present work, the following parameters were selected: 10 g of butterfat with x% MMM or x% PPP;

*To whom correspondence should be addressed at Laboratoire de Physicochimie et Génie Alimentaires, Ecole Nationale Supérieure d'Agronomie et des Industries Alimentaires (ENSAIA), 2, Avenue de la Fort de Haye, 54505 Vandoeuvre les Nancy, France.

enzyme concentration, 10% (w/w). Assays were done under stirring conditions (400 rpm).

TG determination. The TG composition of butterfat was determined with a high-performance liquid chromatograph equipped with a refractive index detector (LKB 2142; LKB Produkter, Bromma, Sweden) and with a tandem of two 150 mm × 4.6 mm Supelcosil LC-18 columns packed with 5-μm octadecyl-bonded spherical silica (Supelco, Supelcopark, Bellefonte, PA). In the present study, two calculation terms were used for peak identification (9). The first term is "equivalent carbon number" (ECN) and the second term is "theoretical carbon number" (TCN).

SFC. The SFC was determined with a Perkin-Elmer DSC 4 (Norwalk, CT) according to Deroanne *et al.* (25).

RESULTS AND DISCUSSION

Interesterification of butter oil. Thirty-three groups of TGs are separated on Supelcosil columns according to different acyl carbon numbers (CNs) and degree of unsaturation. Corresponding chromatograms are therefore subdivided in classes, each containing four peaks. In one class, the first peak covers saturated TGs, whereas the second, the third and the fourth are mainly represented by, respectively, mono-, di- and triunsaturated TGs. Peak identification is based on the two calculation terms:

$$ECN = CN - 2\,ND \quad [1]$$

$$TCN = CN - f_iND \quad [2]$$

where CN = the acyl carbon number and ND = the double bond number. Furthermore, knowing their fatty acid composition, the amount of TGs can be estimated by a random distribution hypothesis (9).

The changes induced in the TG composition by interesterification, catalyzed 1,3-specific lipase, are shown in Figure 1. The great complexity of butter oil TGs prevented the study of interesterification kinetics. Interpretation was based only on the concentration of precise TG components before (t = 0) and after the interesterification reaction (t = 48 h). The concentration changes of the 33 first peaks are displayed in Figure 2.

Most of the saturated TGs are in a higher proportion in the interesterified fat than in the untreated fat (peaks corresponding to TCN = ECN = CN = 48, 46, 44, 42, 40, 36). Only two groups of saturated TGs show a slight decrease after interesterification. The first one is mainly formed by SSM and PPS (TCN = 50), and the second by LaLaM, BuPS and MMP (TCN = 38) where S = stearic, M = myristic, P = palmitic, La = lauric and Bu = butyric acids.

The most significant changes after interesterification occur mainly in the concentration of three groups of TGs with the same ECN = 38 corresponding to monounsaturated, diunsaturated and triunsaturated TGs, respectively. The first and second groups decrease after interesterification by 45 and 30%, respectively, whereas the last peak, formed by triunsaturated TGs, increases approximately

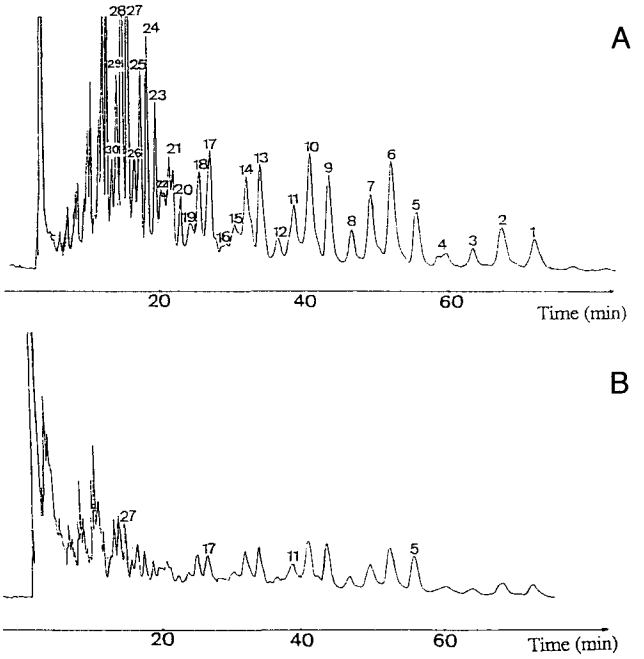

FIG. 1. High-performance liquid chromatographic chromatogram of butter oil before interesterification (A: t = 0 h) and after lipase-catalyzed interesterification (B: t = 48 h).

by 50%. In the same way, Table 1 shows that most triunsaturated TGs are quantitatively more important after interesterification.

The main changes induced by sodium methoxide or by nonspecific lipase (from *Candida cylindracea*) catalyzed interesterifications remain in the concentrations of 36 and 38 acyl-carbon monounsaturated TGs, which decrease during interesterification by 45 and 52%, whereas the triunsaturated TGs in the CN range from 44 to 50 increase by 26% (11,13). Solvent-free interesterification products, obtained at 50 and 60°C, are similar to those obtained at the same temperature in isooctane (13,21). However, fat interesterified at 40°C displays higher proportions of saturated TGs in the acyl CN range 46–54 than the corresponding reaction product in isooctane.

Melting properties. The melting curves of interesterified and untreated butterfat differ noticeably (Fig. 3). In both cases, two endothermic peaks appear. The first peak is located at low-temperature and results from low melting glycerides (LMG). The second one, which appears at high temperature, is associated to high-melting glycerides (HMG). After interesterification, the LMG area decreases by 56%, whereas the HMG area increases by 38% (Table 2).

The SFC, as determined by DSC at 20°C, increases after interesterification by about 114% (Fig. 4). These results differ greatly from chemical interesterification, where the increase of SFC at the same temperature is about 40% and reaches only 2% with *C. cylindracea* (11), but is about 60% after *P. fluorescens* lipase interesterification (21). By using a lipase from *C. cylindracea*, Kemppinen *et al.* (15) show that, below 20°C, the SFC of the interesterified fat is lower than the SFC of the untreated fat. The phenomenon is generally inverted above 25°C. According to Kalo

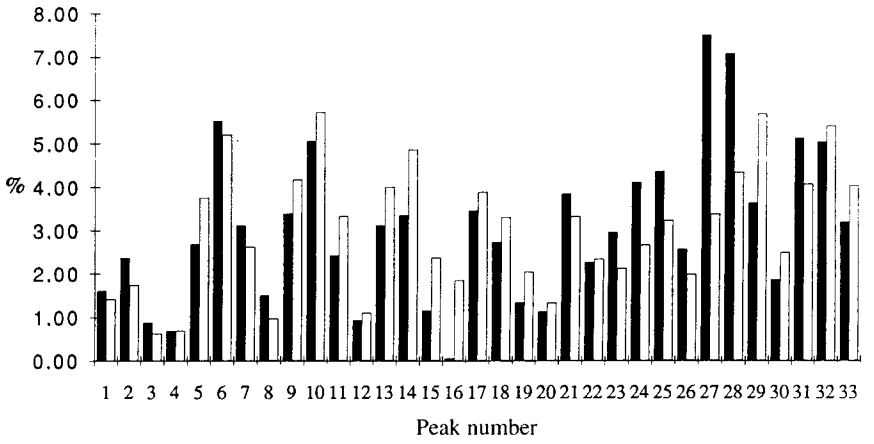

FIG. 2. Composition (%) of triglycerides in the products before and after interesterification of butter oil (t = 0 h: ■, t = 48 h: □).

TABLE 1

Triunsaturated Triglycerides Most Affected by Interesterification[a]

Number of TG groups (cf. Fig. 1)	ECN	TCN	Predominant TGs
12	46	44.56	OOL
16	44	42.53	OLL
		42.70	OOLn
27	38	36.76	LLnLn
		37.00	CLL
		37.64	CPLn
		37.03	CyOL
		37.06	CoCO
31	36	34.92	LnLnLn
		35.00	CyLL
		35.03	CoOL
		35.06	BuOO

[a]TG(s) = triglyceride(s), ECN = equivalent carbon number, TCN = theoretical carbon number, O = oleic acid, L = linoleic acid, Ln = linolenic acid, C = capric acid, P = palmitic acid, Cy = caprylic acid, Co = caproic acid, Bu = butyric acid.

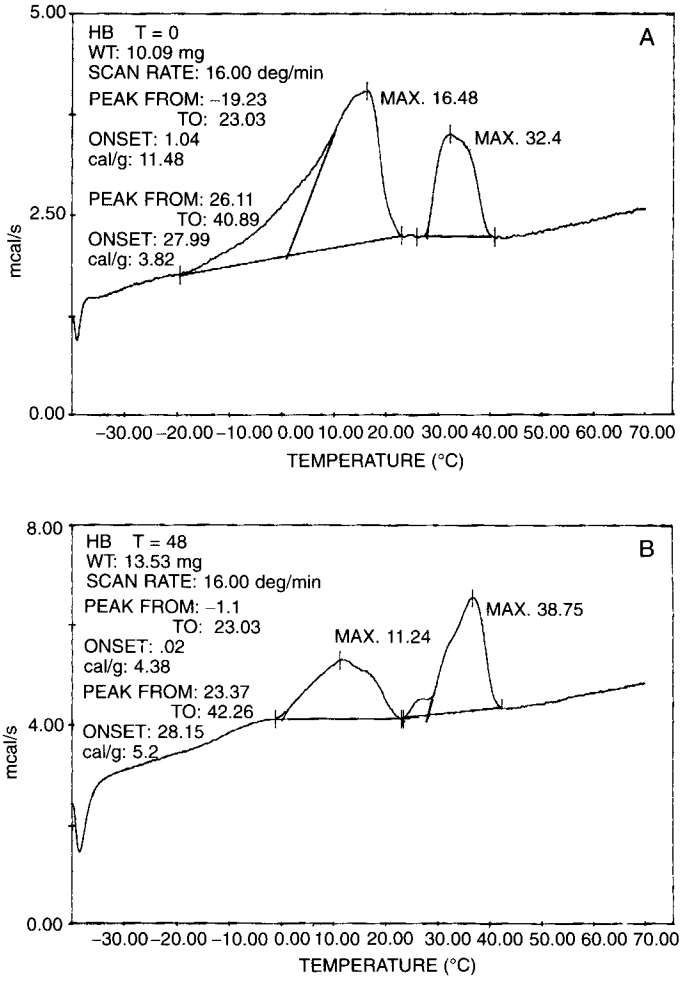

FIG. 3. The melting curves of interesterified (t = 48 h) and untreated butterfat.

et al. (30), hydrolysis occurring during interesterification can be regarded as the most important factor that affects changes in the low temperature range, whereas the increase of high-melting TGs at higher temperature may explain the high SFC values (11,31).

Nevertheless, it should be pointed out that all calculation methods of SFC can be different (nuclear magnetic resonance for example). Comparisons should therefore be undertaken carefully (32).

In previous studies (9,33), it has been shown that butter firmness is influenced by four TGs groups: TG1, TG2, TG3 and TG4, mainly represented by POO, MOO, ClaO + CyMO + CoPO + BuSO and BuPO + CoMO + CoPL, respectively, where O = oleic, M = myristic, Cla = capric, Cy = caprylic, Co = caproic, La = lauric and L = linoleic acids. TG1 and TG2 are correlated negatively with firmness, whereas TG3 and TG4 are positively correlated.

TABLE 2

Low-Melting Glyceride (LMG) Area and High-Melting Glyceride (HMG) Area During Interesterification

Time (h)	LMG (cal/g)	HMG (cal/g)
0	11.5	3.8
6	7.0	5.7
24	5.7	5.0
48	5.1	5.3

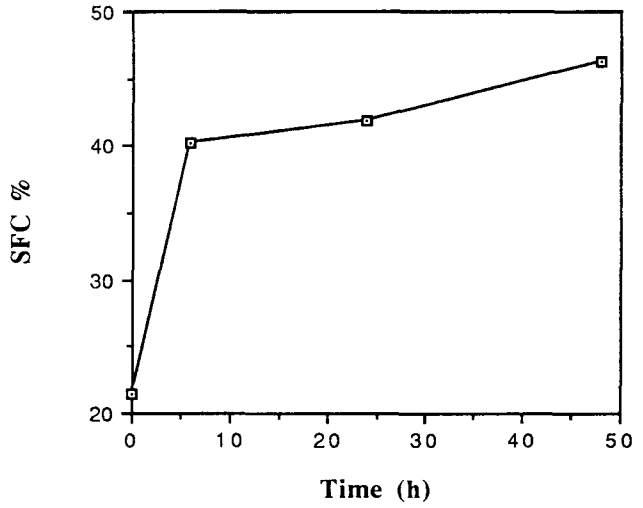

FIG. 4. Solid fat content (SFC) of butter fat determined by differential scanning calorimetry at 20°C during interesterification.

Based on the Haighton equation (34) [Firmness α $(SFC)^2$], confirmed in the present experimental conditions (unpublished data), the relation between these four groups of TGs and firmness can easily be translated to a similar relation between TGs and SFC.

After interesterification, the SFC of butterfat at 20°C and the proportion of TG2 both increase, whereas the amounts of TG1, TG3 and TG4 decrease. This observation tends to invalidate the model. However, this model only fits French "natural" butter. Significant TG variations induced by interesterification, ruled by a random distribution, prevent any comparison with seasonal and regional variations. The model is therefore not valid in the present case.

On the basis of the abovementioned observations, can a relation between the prevalent TGs and firmness be used as a new detection method of adulterated butterfat? More studies will be required to answer this question.

On the other hand, despite the knowledge of the composition of butter TGs, the data obtained in the present work remain insufficient for explaining the fundamentals of interesterification mechanisms, as two arrangements (saturated and unsaturated TGs) are mainly favored during the process.

Interesterification of mixtures of butter oil with HMG. The effects of high-melting TG/butter oil ratio on the interesterification reaction have been studied with 4.7, 9, 16.7 and 23.1% MMM or PPP as reaction media under the conditions previously described. The joint effect of the addition of MMM or PPP and interesterification on the TG composition is considerable (Figs. 5 and 6). The major change in TG composition of the mixture MMM/butter oil occurs in the concentration of peaks 9 (TCN = 46) 10 (TCN = 45.53), 11 (TCN = 45.06), 13 (TCN = 44), 14 (TCN = 43.53) and 17 (TCN = 42).

Concerning the mixture PPP/butter oil, the major change in TG composition occurs in the percentage of peaks 1 (TCN = 50), 5 (TCN = 48), 6 (TCN = 47.53), 9 (TCN = 46) and 13 (TCN = 44).

It should be emphasized that the proportion of these peaks, after 48 h of interesterification, increases with increasing MMM or PPP because all these peaks are mainly formed by TGs including myristic acid or palmitic acid (Table 3). The proportion of other peaks shows no distinct pattern (Figs. 5 and 6).

Prior to interesterification (t = 0), increasing amounts of MMM or PPP result in a higher SFC at 20°C. Moreover, at low concentration (<17%), the addition of PPP has more influence on SFC than the addition of MMM. This can be solely explained by the difference in the melting points (m.p.) of PPP [(PPP) = 65.5°C] and MMM [(MMM) = 57°C]. At high concentration (23.1%), the phenomenon is inverted (Table 4).

The effect of interesterification on these mixtures mainly occurs at the beginning of the reaction. When the amount of added MMM or PPP remains within 0 and 17%, the SFC at 20°C increases throughout the interesterification reaction, particularly during the first six hours. Afterwards, the SFC remains nearly constant (Figs. 7 and 8).

The ratio SFC_{48}/SFC_0 (SFC after 48 h of interesterification/SFC prior to interesterification) decreases as MMM or PPP increases (Figs. 9 and 10). With a maximum amount of PPP (23.1%), the SFC_{48}/SFC_0 ratio approaches 1 as if no interesterification occurs. With a maximum amount of MMM (23.1%), SFC_{48}/SFC_0 remains below 1. In the latter case, the effect of the interesterification counteracts the effect of the adjunction of MMM. This can be explained by overlaying two opposite effects: (i) The first effect concerns the adjunction of high-melting TGs (MMM or PPP). These high substrate concentrations lead to an increase of SFC in the product. However, during the interesterification reaction, randomization occurs, together with a decrease of the concentration of MMM or PPP and the SFC. (ii) The second effect concerns the interesterification reaction. In fact, as mentioned above, this reaction leads to an increase of SFC. There is a balance between these two effects. Obviously, the first effect dominates the second. The interesterification of butterfat/tallow mixtures in the presence of a sodium methoxide catalyst shows the same tendency. In fact, the changes in the TG composition induced by interesterification tend to approach zero when the amount of tallow increases. The interesterification effect is more pronounced in the mixtures as butterfat content increases (35).

These results have to be compared with the study of Graille *et al.* (36) on the 1-3 regioselective interesterified

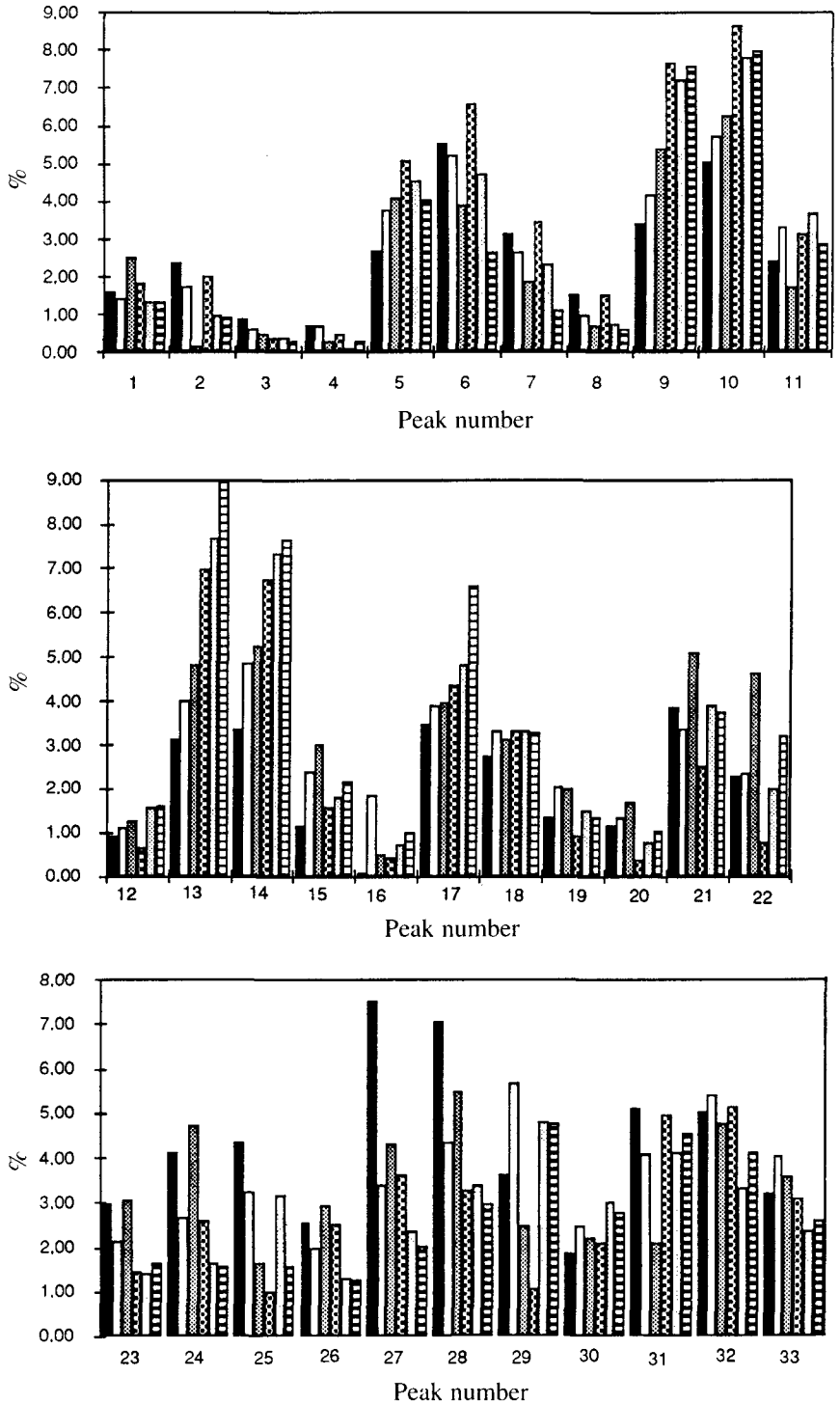

FIG. 5. Effect of adjunction of trimyristin (MMM) and interesterification on the composition (%) of triglycerides of butterfat. Solid black box: before interesterification, white box: after interesterification, gray box: after interesterification with 4.8% MMM, dark gray box: after interesterification with 9.1% MMM, dotted box: after interesterification with 16.7% MMM, striped box: after interesterification with 23.1% MMM.

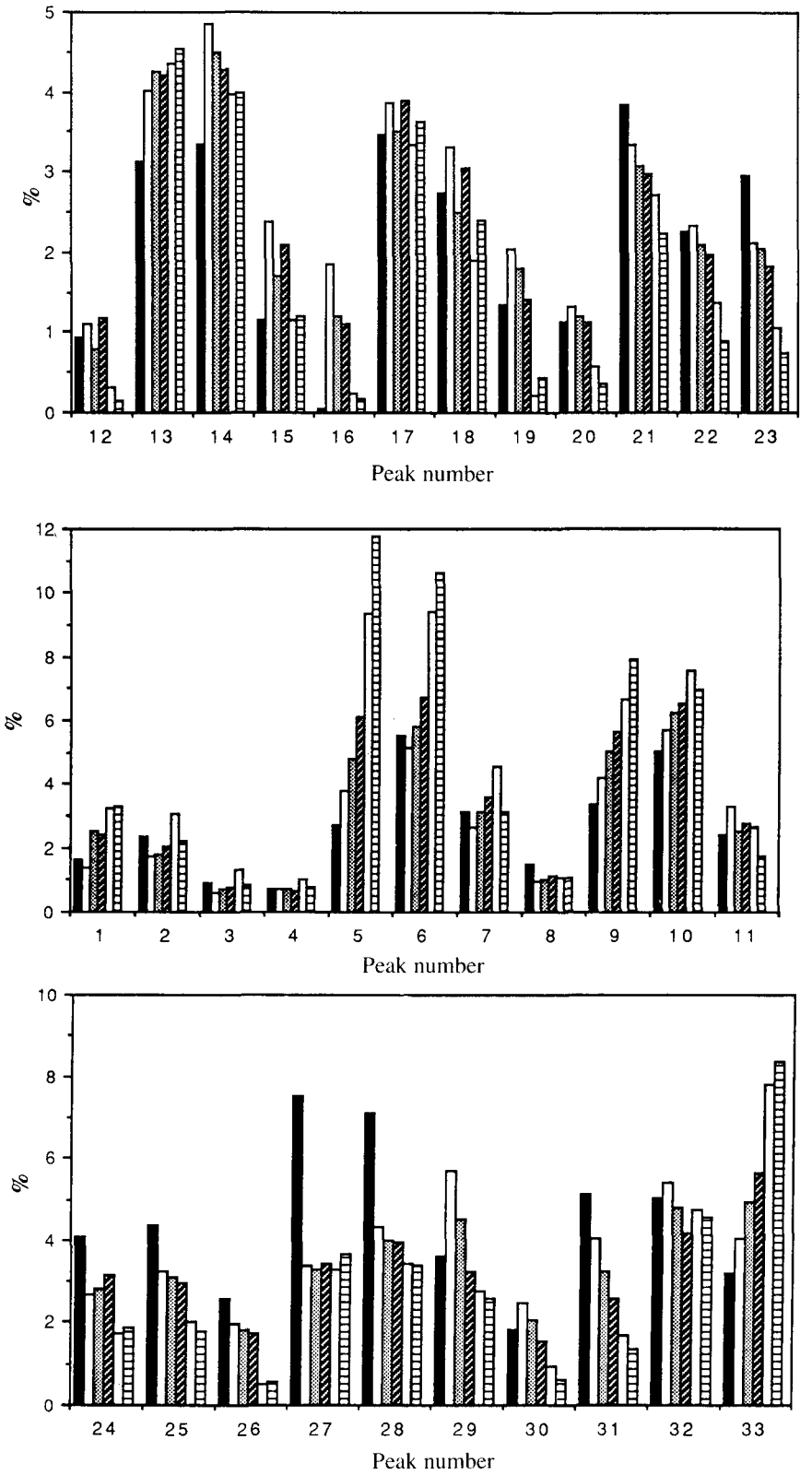

FIG. 6. Effect of adjunction of tripalmitin (PPP) and interesterification on the composition (%) of triglycerides of butterfat. Solid black box: Before interesterification, white box: after interesterification, gray box: after interesterification with 4.8% PPP, dark gray box: after interesterification with 9.1% PPP, dotted box: after interesterification with 16.7% PPP, striped box: after interesterification with 23.1% PPP.

TABLE 3

Identification of Some Peaks from Butterfat Chromatogram[a]

Number of peaks	Predominant triglycerides
A	
9	MPP
10	MPO
11	MOO
13	MMP
14	MMO
17	MMM
B	
1	PPS
5	PPP
6	PPO
9	PPM
13	PMM

[a]A: Peaks affected by adjunction of trimyristin (MMM); B: peaks affected by adjunction of tripalmitin (PPP). O = oleic acid; S = stearic acid.

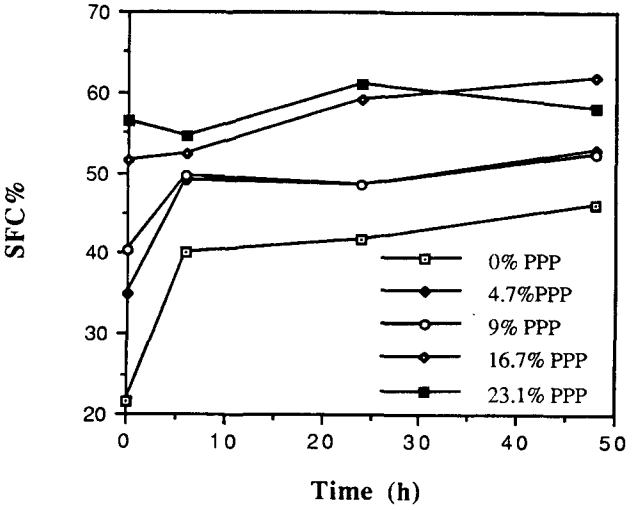

FIG. 8. Effect of interesterification on solid fat content (SFC) of the mixture tripalmitin (PPP)/butter oil (%) from differential scanning calorimetry at 20°C.

TABLE 4

Effect of Adjunction of MMM and PPP on Solid Fat Content (SFC) of Butterfat Determined by Differential Scanning Calorimetry at 20°C[a]

Concentration (%)	SFC (MMM) (%)	SFC (PPP) (%)
0	21.5	—
4.7	31.7	34.8
9.0	35.5	40.4
16.7	47.2	51.6
23.1	60.3	56.4

[a]See Table 3 for other abbreviations.

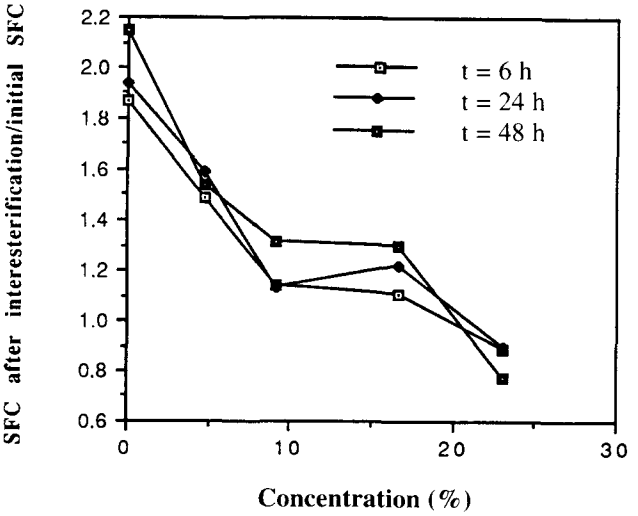

FIG. 9. Ratio of solid fat content (SFC) after 48 h interesterification time (SFC_{48}) over SFC before interesterification (SFC_0) vs. concentration of trimyristin.

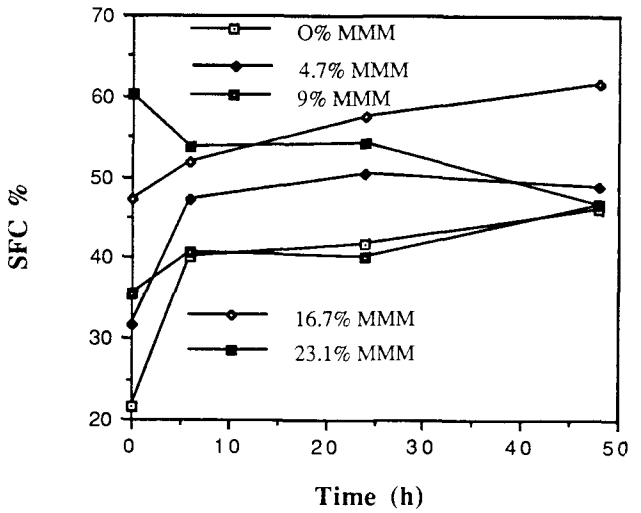

FIG. 7. Effect of interesterification on solid fat content (SFC) of the mixture trimyristin (MMM)/butter oil (%) from differential scanning calorimetry at 20°C.

mixtures of 30:70 palm stearin/palm kernel oil and 70:30 palm oil/coconut oil. These authors show that the mixtures obtained are characterized by a distinctly lower solid content than that of the respective unprocessed ones. Furthermore, the solid content of the interesterified mixtures varies with the time spent in the reactor, falling gradually during the interesterification reaction. This behavior is, to a certain extent, in keeping with the evolution shown in Figures 7 and 8, particularly in the case of a high adjunction of PPP and MMM.

In conclusion, interesterification is an effective way to increase the SFC of fat. However, if the fat already contains a high level of SFC—for example, by adjunction of high-melting fractions—this procedure loses most of its advantages.

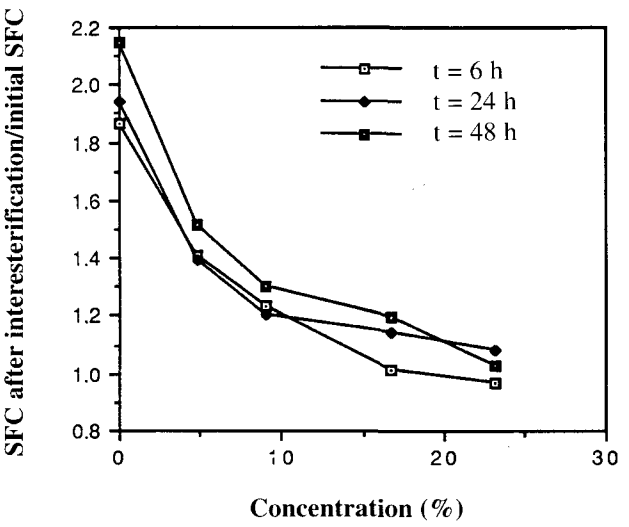

FIG. 10. Ratio of solid fat content (SFC) after 48 h interesterification time (SFC_{48}) over solid fat content before interesterification (SFC_0) vs. concentration of PPP.

ACKNOWLEDGMENTS

The authors thank J. Hardy for valuable advice and Société Coopérative Union Beurriere for providing cream.

REFERENCES

1. deMan, J.M., *J. Dairy Res. 28*:85 (1961).
2. Kherkhoven, E., and J.M. deMan, *Ibid. 49*:1086 (1966).
3. Parodi, P.W., *Ibid. 46*:633 (1979).
4. Parodi, P.W., *Ibid. 48*:131 (1981).
5. deMan, J.M., J.E. Dobbs and P. Sherman, *Int. Dairy Congress C*:43 (1966).
6. Antila, V., *Meijeritiet. Aikak. 27, 1*:72 (1966).
7. El-Hamdy, A.H., and E.G. Perkins, *J. Am. Oil Chem. Soc. 58*:867 (1981).
8. Frede, E., and H. Thiele, *Ibid. 64*:521 (1987).
9. Bornaz, S., G. Novak and M. Parmentier, *Ibid. 69*:1131 (1992).
10. Ergan, F., and M. Trani, *Ibid. 68*:19 (1991).
11. Kalo, P., *Milchwissenschaft 41*:82 (1986).
12. Kalo, P., H. Huotari and M. Antila, *Fat Sci. Technol. 91*:276 (1989).
13. Kalo, P., *Meijeritiet. Aikak. 47, 1*:29 (1989).
14. Kaimal, T.N.B., and M. Saroja, *Biotechnol. Lett. 10*:337 (1988).
15. Kemppinen, A., P. Kalo and M. Antila, *Meijeritiet. Aikak. 46, 1*:48 (1988).
16. Muderhwa, J., M. Pina and J. Graille, *Oléagineux 43*:427 (1988).
17. Ergan, F., M. Trani and G. André, *Biotechnol. and Bioengin. 35*:195 (1990).
18. Halling, P.J., *Fat Sci. Technol. 2*:74 (1990).
19. Zaks, A., and A.J. Russell, *Proc. Natl. Acad. Sci. USA 82*:3192 (1988).
20. Zaks, A., and A.M. Klibanov, *Science 224*:1249 (1985).
21. Kalo, P., *Milchwissenschaft 45*:281 (1990).
22. Muderhwa, J.M., M. Pina, D. Montet, P. Feuillard and J. Graille, *Oléagineux 44*:35 (1989).
23. Muderhwa, J.M., M. Pina and J. Graille, *Rev. Fr. Corps Gras 36*:11 (1989).
24. Elliot, J.M., and K.L. Parkin, *J. Am. Oil Chem. Soc. 3*:171 (1991).
25. Deroanne, C., J.M. Marcoen, J.P. Watelet and M. Severin, *J. of Therm. Analysis 11*:109 (1977).
26. Eigtved, P., Danish Patent Application DK 4025/83 (1983).
27. Macrae, A.R., *J. Am. Oil Chem. Soc. 60*:243 (1983).
28. Yokozeki, K., S. Yamanaka, K. Takinami, Y. Hirose, K. Sonomoto and S. Fukui, *J. of Appl. Microbiol. Biotech. 14*:1 (1982).
29. Eigtved, P., T.T. Hansen and H. Sakaguchi, *J. Am. Oil Chem. Soc. 63*:463 (1986).
30. Kalo, P., P. Elo, and M. Antila, *Milchwissenschaft 43*:416 (1988).
31. Kalo, P., A. Kemppinen and M. Antila, *The Proceedings of the World Conference on Biotechnology for the Fats and Oils Industry*, 1988, p. 323.
32. Lambelet, P., *Leben.-Wiss.u.-Technol. 16*:90 (1983).
33. Bornaz, S., J. Fanni and M. Parmentier, *J. Am. Oil Chem. Soc. 70*:1075 (1993).
34. Haighton, A.J., *Ibid. 53*:397 (1976).
35. Kalo, P., *Meijeritiet. Aikak. 44, 2*:37 (1986).
36. Graille, J., M. Pina, D. Montet and J.M. Muderhwa, *Elaeis 4*:1 (1992).

[Received August 23, 1994; accepted August 31, 1994]