PHYSICAL PROPERTIES AND COMPOSITION OF LOW *Trans* CANOLA/PALM BLENDS MODIFIED BY CONTINUOUS ENZYMATIC INTERESTERIFICATION

Keywords: Low *trans* fatty acids; canola oil; palm stearin; margarines; solid fats contents; enzymatic interesterification

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*Three low-*trans *fat blends designed for stick margarines were formulated and modified by enzymatic interesterification in a temperature and moisture controlled packed-column reactor. All the three blends contained 45%, 25% and 30% of hydrogenated canola oil (HCO), palm stearin and canola oil, respectively; the HCO (of iodine values 59.9, 56.2 and 58.7) in each of the blends was made under different hydrogenation conditions. The dropping point of the interesterified blends ranged from 38.2° to 39.8°C. Solid fat contents were 33.4%–38.3%, 18.7%–23.9% and 4.7%–6.5% at 10°, 20° and 35°C, respectively. Crystallization temperatures were 20.6°–24.6°C and all of the interesterified blends crystallized in the β′ polymorphic form in contrast to the control blends, which contained a mixture of β′ and β crystals. Their* trans *fatty acid contents were 12.6%–18.2% and the total* trans *and saturated fat contents were 45.5–49.1 percent.*

INTRODUCTION

Interest in the use of lipases for the interesterification of fats has increased considerably as reflected by the growing number of publications in this area of research. In the literature, both laboratory and pilot scale studies on the performance of microbial lipases for the modification of fats and for the production of triglycerides have been reported (Taylor *et al.*, 1986; Lilly and Dunnill, 1987; Ergan *et al.*, 1990; Malcata *et al.*, 1990).

The modification of fats by enzymatic interesterification to achieve certain physical properties has been attempted by several researchers. A fat containing 1(3)-palmitoyl-3(1)-stearoyl-2-monoolein (POSt) and 1, 3-distearoyl-2-mono-olein

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Original manuscript received: 23 February 1993
Revised manuscript received: 16 May 1994

(StOSt), the main triglyceride components of cocoa butter, can be made by interesterifying palm oil mid-fraction, which contains mainly 1,3-dipalmitoyl-2-mono-olein (POP), with stearic acid or tristearin (Macrae, 1983). The reaction was carried out in an organic medium (petroleum ether) and catalysed by a 1, 3-specific thermostable lipase obtained from *Aspergillus niger*. Fats with appropriate physical properties for margarines, seasonings or frying oils can also be made through enzymatic interesterification of palm oil with other vegetable oils (Muderhwa *et al.*, 1989). The reaction was carried out in a packed-bed reactor using Lipozyme (a thermostable and 1,3 regioselective immobilized lipase preparation) as catalyst. In an attempt to modify the melting properties of butter fat, Kalo *et al.* (1986 and 1990) showed that enzymatic reactions in the absence of an organic solvent were as effective as when the fat was dissolved in hexane. Furthermore, the solid fat content of the product was not affected by the temperature (50° C and 60° C) at which the reaction was carried out.

The polymorphism of a fat is primarily dependent on its chemical composition (Lutton and Fehl, 1970; Hagemann *et al.*, 1975; Small, 1986). Edible fats can be classified into two main groups according to their crystallization habit. Soya bean, safflower, sunflower, corn and canola oils, and lard, tend to crystallize in the β form whereas cottonseed, palm, palm kernel, rapeseed oils, and tallow are β'-tending (Wiedermann, 1978; Vaisey-Genser and Eskin, 1987).

In general, fats containing fairly uniform triglycerides have the tendency to crystallize in the β form and those containing a mixture of various types of triglycerides tend to form β' crystals. Naguib-Mostafa and deMan (1985) reported that hydrogenated canola oil, which contains a large proportion of 18 carbon fatty acids, crystallizes mainly in the β form. However, if a fat also contains a substantial amount of other fatty acids with different chain lengths such as C20 and C22 fatty acids (*e.g.* in rapeseed oil), the homogeneity of the triglycerides is disrupted and β' form crystals are formed instead (Wiedermann, 1978). Cottonseed oil and palm oil, which contain high levels of palmitic acid, also promote the formation of β' crystals (Rivarola *et al.*, 1987).

This research was initiated as a result of the high levels of *trans* fatty acids found in Canadian and North American stick margarines. In a survey carried out in our laboratory, the *trans* fatty acid contents of Canadian and North American margarines were found to be 22.4% to 44.1% (Postmus *et al.*, 1989). The safety of *trans* fatty acids has been questioned since the publication of a report by an *ad hoc* committee on the composition of margarines and specialty fats (Davignon *et al.*, 1980). Data from more recent publications are also in favour of the same view (Mensick and Katan, 1990; Verschuren and Zevenbergen, 1990).

At present, enzymatic interesterification is not justified economically, and the concept was applied as an alternative to chemical interesterification. The objective of this study was to apply enzymatic interesterification for the modification of the melting and crystallization properties of fats with reduced *trans* fatty acid content.

MATERIALS AND METHODS

Canola oil was purchased from a local supermarket and hydrogenated under various selectivity conditions (Cho *et al.*, 1994). Fractionated palm stearin (PS) was obtained from Lam Soon Bhd., Petaling Jaya, Malaysia.

Enzymatic interesterification

Continuous enzymatic interesterification was carried out in a temperature and moisture controlled bench-top column reactor packed with Lipozyme as described by Cho *et al.* (1994).

Determination of dropping point

Dropping point was determined by the AOCS (1981) tentative method Cc 18-80 using a Mettler FP 83 dropping point cell interfaced with a Mettler FP 80 processor unit (Mettler Instrument, Zurich, Switzerland).

Determination of solid fat content

Solid fat content was determined by the AOCS (1981) recommended procedure Cd 16-81 using a Minispec PC 120 pulsed NMR process analyzer (Bruker Spectrospin Ltd., Milton, Ontario). Prior to analysis, the fat was melted and tempered as follows: 30 min at 60° C, 15 min at 0° C, 30 min at 25° C and 15 min at 0° C. Solid fat contents were then measured at 10°, 20°, 25°, 30° and 35° C following 30 min tempering at each temperature.

Determination of melting and crystallization temperature

The melting and crystallization temperatures were determined by differential scanning calorimetry (DSC) (Kawamura, 1981). Fat samples were first melted and kept in a 60° C oven for at least 15 minutes. About 10 mg of fat was transferred into an open DSC pan with the aid of a micropipette and immediately placed into the cell of a DuPont 900 differential scanning calorimeter maintained at 10° C with crushed ice. Following an equilibration period, the fat was heated from 10° to 60° C at a rate of 5° C/min to record its melting profile. For recording the crystallization temperature, the same fat was equilibrated at 60° C for 5 min and then cooled to 7° C at a rate of 3° C/min using crushed ice.

Determination of polymorphic form of fat crystals

The polymorphic form of fat crystals was determined by powder X-ray diffraction analysis (Naguib-Mostafa and deMan, 1985). The temperature of the fat was maintained at 10° C and they were exposed to the radiation for 2 hours. The diffraction patterns were recorded on a film strip and translated into short spacings for polymorph identification.

Determination of FFA, partial glyceride and triglyceride composition

The amounts of free fatty acids (FFA), monoglycerides (MG) and diglycerides (DG) present in the fats were determined by gas liquid chromatography (GLC) following the method of Goh and Timms (1985). Triglyceride composition was determined by GLC using 3% OV-1 on 80/100 Supelcoport as the column packing material (Litchfield, 1972; d'Alonzo *et al.*, 1981). The details of the operating conditions for the GC have been described by Cho *et al.*(1993).

Determination of fatty acid composition

Fatty acid composition was determined by the method of Shehata *et al.* (1970). The fatty acids were derivatized into their methyl esters and analysed by GLC. They were separated isothermally at 170° C on a glass column (1.8 x 3.0 mm) packed with 10% SP 2330 on 100/200 Chromosorb W AW (Supelcoport Inc., Bellefonte, PA). The details of the analysis have been described previously by Cho *et al.* (1993).

Determination of *trans* fatty acid content

Total isolated *trans* fatty acid isomers was determined by the AOCS (1981) method Cd 14-60 using methyl elaidate (Nu-Chek Prep Inc., Elysian, MN) as the reference material. The fats were derivatized into their methyl esters using 14% boron trifluoride in methanol (Pierce Chemicals, Rockford, IL) before analysis.

RESULTS AND DISCUSSION

Enzymatic interesterification produced softer fats, thus was reflected by their dropping points as compared with those of the control blends (*Table 1*). Since the interesterified blends contained high levels of DG (*Table 2*) the change in dropping point was probably not solely due to the change in the triglyceride composition brought about by interesterification. The dropping points of the interesterified blends were on average 5.2° C higher than those of commercial stick margarines. The values reported in the literature range between 32.3° and 35.0° C (deMan *et al.*, 1983; Postmus *et al.*, 1989). This suggests that the composition of the blends made in the present study would need to be adjusted if they were to be used in stick margarines.

The solid fat content of the blends was lowered by enzymatic interesterification (*Figure 1*). The parallel shift in the solid content profiles of all the three blends indicates that the high and medium as well as the low melting triglycerides were all modified during the process. As with their dropping points, part of this change could have been brought about by the presence of high levels of DG as discussed above. A typical stick margarine contains about 25%, 13% and 4% solid at 10°, 20° and 35° C, respectively (Vaisey-Genser and Eskin, 1987). The interesterified blends reported in *Figure 1* contained 33% – 38%, 18% – 24% and 5% – 7% solid fat at the same temperatures. These interesterified blends contained less than 40% solids at 10° C and therefore may still be spreadable depending on the chilling and working conditions used during packaging (Weiss, 1983).

A shift in both the major and minor endothermic peaks towards lower temperature ranges indicates that both the high and medium melting triglycerides in the blends had been modified (*Figure 2*). Exothermic peaks were not observed in any

TABLE 1. EFFECT OF ENZYMATIC INTERESTERIFICATION ON THE DROPPING POINTS OF FAT BLENDS

Fat Blend	Dropping point (°C)	
	Control	Interesterified
A	43.7	38.4
B	42.8	39.8
C	42.2	38.2

Standard error of difference between treatment means = 0.36
The control blends were not interesterified.
All the fat blends contained 45%, 25% and 30% of hydrogenated canola oil, palm stearin and canola oil, respectively.
Blends A, B and C contained hydrogenated canola oil (I.V. = 59.9, 56.2 and 58.7, respectively) made under non-selective conditions (140°C, 310.1 KPa), AOCS conditions (175°C, 103.4 KPa) and selective conditions (200°C, 51.7 KPa), respectively.

TABLE 2. FREE FATTY ACID AND PARTIAL GLYCERIDE CONTENT OF CONTROL AND INTERESTERIFIED FAT BLENDS

Fat blend	Composition (%)			
	Free fatty acid	Mono-glyceride	Di-glyceride	Total
A (CN[a])	0.7	1.1	2.4	4.3
A (I[b])	2.0	1.3	6.8	10.2
B (CN)	0.7	1.1	0.8	2.7
B (I)	4.9	1.4	9.4	15.7
C (CN)	0.7	1.1	1.0	2.8
C (I)	2.1	1.3	6.6	9.9

[a] CN – Control
[b] I – Interesterified
The control blends were not interesterified.
All the fat blends contained 45%, 25% and 30% of hydrogenated canola oil, palm stearin and canola oil, respectively.
Blends A, B and C contained hydrogenated canola oil (I.V. = 59.9, 56.2 and 58.7, respectively) made under non-selective conditions (140°C, 310.1 KPa), AOCS conditions (175°C, 103.4 KPa) and selective conditions (200°C, 51.7 KPa), respectively.

sample, but distinct double endothermic peaks were present in all the interesterified blends. These may indicate either a polymorphic transition (Hagemann, 1988) or the melting of the various components present in the blends. More elaborate analysis needs to be performed in order to confirm any polymorphic transition.

The crystallization temperatures of the interesterified blends indicate that they may be too hard at 15° C (*Table 3*). During the manufacture of margarines, fats are crystallized at this temperature for further processing and handling (Haighton, 1976). The crystallization temperatures of commercial stick margarines made from canola oil range from 18.5° to 21.5° C (Postmus *et al.*, 1989). The values are lower than those obtained in the present study because the interesterified blends made were harder, as indicated previously by their dropping points and solid fat content. Also in the study carried out by Postmus *et al.*, (1989), the start of the exothermic peaks were taken as the crystallization temperature whereas in the present study the peak temperature was recorded.

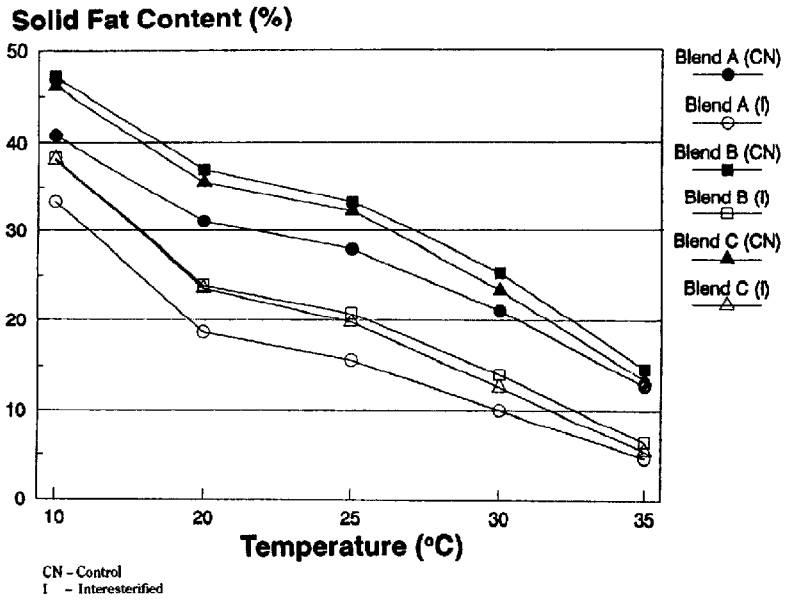
The interesterified blends contained only β' crystals whereas all the control blends contained a mixture of β and β' crystals (*Table 4*). Again, the presence of high levels of DG in the interesterified blends, as discussed earlier, could have influenced the polymorphism of the fats. Diglycerides are known to retard the β to β' transition when added to β-tending fats (Hernqvist *et al.*, 1981; Reddy and Prabhakar, 1986). Therefore, the presence of only β' crystals in the processed blends could have been due to the combined effect of interesterification and the presence of diglycerides. In margarines and cake shortenings, the β' crystal form is desirable to provide a smooth texture and good cake volume. Normally margarines are cooled under controlled temperature conditions in order to stabilize fat crystals in the β' form (Ward, 1988). However, upon storage under fluctuating temperature conditions they tend to revert to β crystals, which then exhibit a sandy texture in the fat. The polymorphism of the blends made in the present study was improved by enzymatic interesterification. However, the stability of the β' crystals is not known and needs to be investigated in a future study.

Although slight differences in the *trans* fatty acid content were observed between the control and interesterified blends (*Table 5*), these changes were not expected. The higher *trans* fatty acid content present in blends B and C was due to the incorporation of hydrogenated canola oil made under more selective conditions than for blend A. As expected, the amount of 18:1 was also higher in the hydrogenated canola oil made under more selective conditions (*Table 6*). This therefore, explains the higher *trans* fatty acid content present in blends B

TABLE 3. EFFECT OF ENZYMATIC INTERESTERIFICATION ON CRYSTALLIZATION TEMPERATURE OF FAT BLENDS

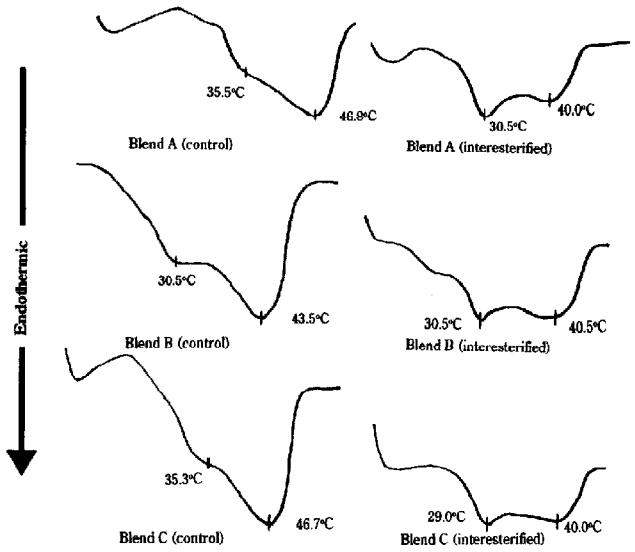
	Crystallization temperature (° C)	
Fat blend	**Control**	**Interesterified**
A	28.4	22.2
B	27.9	24.6
C	27.4	20.6

Standard error of difference between treatment means = 0.66
The control blends were not interesterified.
All the fat blends contained 45%, 25% and 30% of hydrogenated canola oil, palm stearin and canola oil, respectively.
Blends A, B and C contained hydrogenated canola oil (I.V = 59.9, 56.2 and 58.7, respectively) made under non-selective conditions (140° C, 310.1 KPa), AOCS conditions (175° C, 103.4 KPa) and selective conditions (200° C, 51.7 KPa), respectively.



The standard errors of difference between treatment means at 10°, 20°, 25°, 30° and 35° C were 0.84, 1.36, 1.63, 1.11 and 0.67, respectively.
The control blends were not interesterified.
All the blends contained 45%, 25% and 30% of hydrogenated canola oil, palm stearin and canola oil, respectively.
Blends A, B and C contained hydrogenated canola oil (I.V. - 59.9, 56.2 and 58.7, respectively) made under non-selective conditions (140° C, 310.1 KPa), AOCS conditions (175° C, 103.4 KPa) and selective conditions (200° C, 51.7 KPa), respectively.

Figure 1. Effect of enzymatic interesterification on solid content of fats.



The control blends were not interesterified.
All the blends contained 45%, 25% and 30% of hydrogenated canola oil, palm stearin and canola oil, respectively.
Blends A, B and C contained hydrogenated canola oil (I.V. - 59.9, 56.2 and 58.7, respectively) made under non-selective conditions (140° C, 310.1 KPa), AOCS conditions (175° C, 103.4 KPa) and selective conditions (200° C, 51.7 KPa) respectively.

Figure 2. Effect of enzymatic interesterification on the DSC melting profile of fat blends.

TABLE 4. EFFECT OF ENZYMATIC INTERESTERIFICATION ON THE CRYSTALLIZATION HABIT OF FAT BLENDS

	Polymorphic form	
Fat blend	**Control**	**Interesterified**
A	β >>> β'	β'
B	β >>> β'	β'
C	β >>> β'	β'

The control blends were not interesterified.
All the fat blends contained 45%, 25% and 30% of hydrogenated canola oil, palm stearin and canola oil, respectively.
Blends A, B and C contained hydrogenated canola oil (I.V. = 59.9, 56.2 and 58.7, respectively) made under non-selective conditions (140°C, 310.1 KPa), AOCS conditions (175°C, 103.4 KPa) and selective conditions (200°C, 51.7 KPa), respectively.

TABLE 5. FATTY ACID COMPOSITION AND *Trans* CONTENT OF CONTROL AND ENZYMATICALLY INTERESTERIFIED FAT BLENDS

Fatty Acid	Compositions (%)					
	Blend A (CN)[a]	**Blend A (I)[b]**	**Blend B (CN)**	**Blend B (I)**	**Blend C (CN)**	**Blend C (I)**
12:0	0.1	0.0	–	0.0	0.1	0.1
14:0	0.4	0.4	0.3	0.4	0.4	0.4
16:0	17.3	17.2	16.9	17.1	17.3	17.0
16:1	0.1	0.1	0.0	0.2	0.1	0.2
18:0	14.9	15.0	13.8	14.3	12.8	13.2
18:1	53.7	53.5	55.2	54.3	55.6	55.4
18:2	8.4	8.5	8.3	8.3	8.3	8.3
18:3	3.5	3.5	3.6	3.6	3.5	3.5
20:1	1.0	1.0	1.0	1.1	1.0	1.0
22:0	0.3	0.3	0.3	0.3	0.4	0.3
22:1	0.5	0.5	0.5	0.5	0.5	0.5
Other	–	–	0.1	–	0.1	0.0
Trans	13.3	12.6	17.3	15.1	17.9	18.2
S + T[c]	46.2	45.5	48.7	47.2	48.8	49.1

[a]CN - Control
[b]I - Interesterified
[c] Includes all saturated and *trans* fatty acids.
All the fat blends contained 45%, 25% and 30% of hydrogenated canola oil, palm stearin and canola oil, respectively.
Blends A, B and C contained canola oil hydrogenated under non-selective conditions (140°C, 310.1 KPa), AOCS conditions (175°C, 103.4 KPa) and selective conditions (200°C, 51.7 KPa), respectively.

TABLE 6. FATTY ACID COMPOSITION OF COMPONENT FATS AND OIL USED IN THE FORMULATION OF FAT BLENDS

Fatty Acid	Compositions (%)				
	Canola oil	HCO[a] (S)[b]	HCO (AOCS)[c]	HCO (NS)[d]	Palm Stearin
12:0	–	–	–	–	0.2
14:0	0.1	0.1	0.1	0.1	1.3
16:0	3.7	3.9	3.9	3.9	56.5
16:1	0.3	0.3	0.3	0.3	–
18:0	2.0	24.1	26.3	28.9	4.7
18:1	61.8	68.0	65.8	63.5	30.2
18:2	20.3	0.4	0.3	0.1	6.7
18:3	8.9	0.9	1.0	1.0	0.5
20:1	1.5	1.4	1.3	1.3	–
22:0	0.3	0.4	0.4	0.4	–
22:1	0.4	0.6	0.7	0.6	–
Other	0.8	–	–	–	–

[a] Hydrogenated canola oil
[b] Selective conditions (200° C, 51.7 KPa)
[c] AOCS conditions (175° C, 103.4 KPa)
[d] Non-selective conditions (140° C, 310.0 KPa)

and C. Fats hydrogenated under selective conditions normally contain higher levels of *trans* fatty acid than those hydrogenated under non-selective conditions (Patterson, 1983) and this trend was also observed in the present study.

The *trans* fatty acid content of the interesterified blends was between 12.6% and 18.2% (*Table 5*). These values were much lower than in commercial canola or soya bean based stick margarines, which contain 22.4% to 44.1% *trans* fatty acid (Postmus *et al.*, 1989). The total *trans* and saturated fatty acid content in the interesterified blends was 45.5% – 49.1%, while that of the commercial stick margarines as reported by Postmus *et al.* (1989), ranged from 41.6% to 59.4 per cent. The *trans* and saturated fat content in the blends made in the present study can still be further reduced because their dropping points and solids contents at various temperatures were too high as discussed earlier. Therefore more liquid oil can still be incorporated into the blends and the nutritional quality of the blends can be further improved.

Enzymatic interesterification was effective in modifying the dropping point and solid fat content of hydrogenated canola oil/palm stearin/canola oil blends designed for stick margarine formulation. Presently, commercial stick margarines contain 22.4% – 44.1% of *trans* fatty acids. The blends made in this study contained 12.6%–18.2% of the same fatty acids. Therefore, a marked reduction in the *trans* content of the blends for stick margarines were observed.

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