Interesterification of rapeseed oil with anhydrous milk fat and its stearin fraction
I. Modifications of composition

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Introduction

Milk fat in human diet is associated with cardiovascular diseases due to its high content in saturated fatty acids and cholesterol. Most vegetable oils are much healthier as they are rich in unsaturated fatty acids (1,2).

Lipase-catalysed interesterification of oils and fats has arisen as an alternative to hydrogenation or chemical processes to produce margarine and shortenings (3), notably because they do not give rise to undesirable *trans* fatty acids (4). The enzyme exchanges fatty acids from one triglyceride to another, resulting in a redistribution of fatty acids species. For such a reaction, the lipase of *Thermomyceslanuginosa* was shown to be an efficient tool in solvent-free fat blends batches and micro-aqueous conditions (5). Enzymatic interesterification can thus be used to enrich “hard” fats with unsaturated fatty acids (6,7). As an illustration, rapeseed oil (RO) (a choice source of unsaturation-rich residues) was used in the present study to enrich anhydrous milk fat (AMF) with unsaturated C_{18} fatty acids (FA) (oleic, linoleicand linolenic acids). Comparatively, one “harder” fraction of AMF underwent the same reaction. The physico-chemical properties modifications induced by the reaction were followed. The compositional changes are reported in this first part and the consequent physical modifications are presented in a second part.

Materials and methods

Materials. Anhydrous cow’s milk fat was purchased from Corman (Goé, Belgium) and stored at -20 °C. RO was from Vandemoortele (Izegem, Belgium). All reagents and solvents were of analytical grade (Fisher Bioblock Scientific, Tournai, Belgium).

Analytical procedures. FA contents and triglyceride (TAG) profiles were determined by gas chromatography. The technical details were described elsewhere (6).

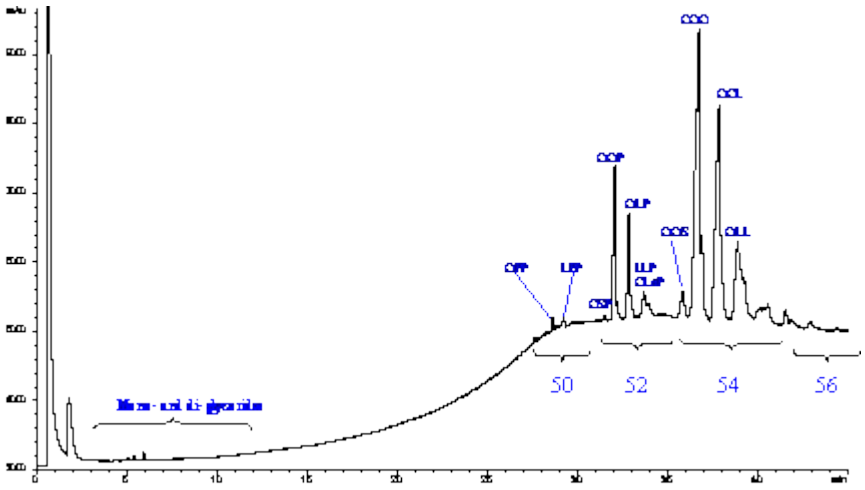
The chromatograms were integrated manually with the aid of HP Chemstation software (Agilent Technologies, Diegem, Belgium) by carbon numbers (CN), that is, the sum of the carbons of the fatty acids from one TAG. The degree of interesterification was defined as:

$ID = (\text{Area CN50/Area CN42})_{IE} - (\text{Area CN50/Area CN42})_{NIE}$

where CN50 and CN42 are the peaks with CN equal to 50 and 42, corresponding, respectively, to a highly variable and a stable group of peaks during the reaction, for interesterified (IE) and non-interesterified (NIE) samples.

Enzymatic interesterification. Lipozyme TL IM (Novozymes A/S, Bagsværd, Denmark) was used for solvent-free interesterification in a 1 L batch reactor as previously described (6).

AMF fractionation. To obtain a stearin fraction (AMFSF) , dry fractionation of AMF was performed using a pilot batch reactor (PEACS, Perwez, Belgium), utilizing the Tirtiaux fractionation procedure and vacuum filtration (stearin yield ≈ 55%).



Results

Fats composition

Figure 1. TAG profile of RO. Some identified species are indicated, with P: palmitate, S: stearate, O: oleate, L: linoleate, Ln: linolenate. The carbon numbers (CN) groups are indicated below the peaks.

TAG profile of RO is presented on Fig. 1. TAG species spread from CN of 50 to 56; some identified TAGs are indicated. The area percents of these different CN

groups are reported on Fig. 2 together with those of AMF and of AMFSF. Both these fats have much more complex profiles with TAGs spreading from CN of 20 to 54. AMFSF had less CN of 34 to 40 than AMF and more CN of 44 to 50.

The FA compositions are given in the table of Fig. 1. RO has a total of 91% of unsaturated C18, whereas AMF and AMFSF have respectively 30.2 and 31.2% palmitate, 11 and 13.3% stearate, and 11.2 and 8.8% FA with 10 or less carbons.

Fatty acids	AMF (area%)	AMFSF (area %)	Rapeseed oil (area %)
4:0	4.52 ± 0.09	2.91 ± 0.08	-
6:0	2.24 ± 0.13	1.85 ± 0.05	-
8:0	1.31 ± 0.09	1.26 ± 0.02	-
10:0	3.08 ± 0.08	2.80 ± 0.04	-
10:1	0.34 ± 0.02	0.30 ± 0.01	-
12:0	3.88 ± 0.04	4.65 ± 0.15	-
13:0	0.08 ± 0.01	0.10 ± 0,00	-
14:0	12.05 ± 0.04	12.31 ± 0.10	-
14:1	1.15 ± 0.02	1.01 ± 0.01	-
15:0	1.18 ± 0.13	1.25 ± 0.01	-
16:0	30.17 ± 0.13	31.15 ± 0.17	4.87 ± 0.02
16:1	1.80 ± 0.02	1.52 ± 0.02	0.21 ± 0.01
17:0	0.53 ±0.02	0.60 ± 0.03	-
18:0	10.97 ± 0.05	13.27 ± 0.67	1.60 ± 0.05
18:1	22.65 ± 0.06	22.49 ± 0.37	61.84 ± 0.33
18:2	3.44 ± 0.06	1.93 ± 0.03	20.38 ± 0.23
18:3	0.61 ± 0.03	0.59 ± 0.12	8.94 ± 0.22
20:0	-	-	0.47 ± 0.03
20:1	-	-	1.09 ± 0.03
22:0	-	-	0.23 ± 0.02
22:1	-	-	0.19 ± 0.00
24:0	-	-	0.07 ± 0.00
24:1	-	-	0.10 ± 0.03

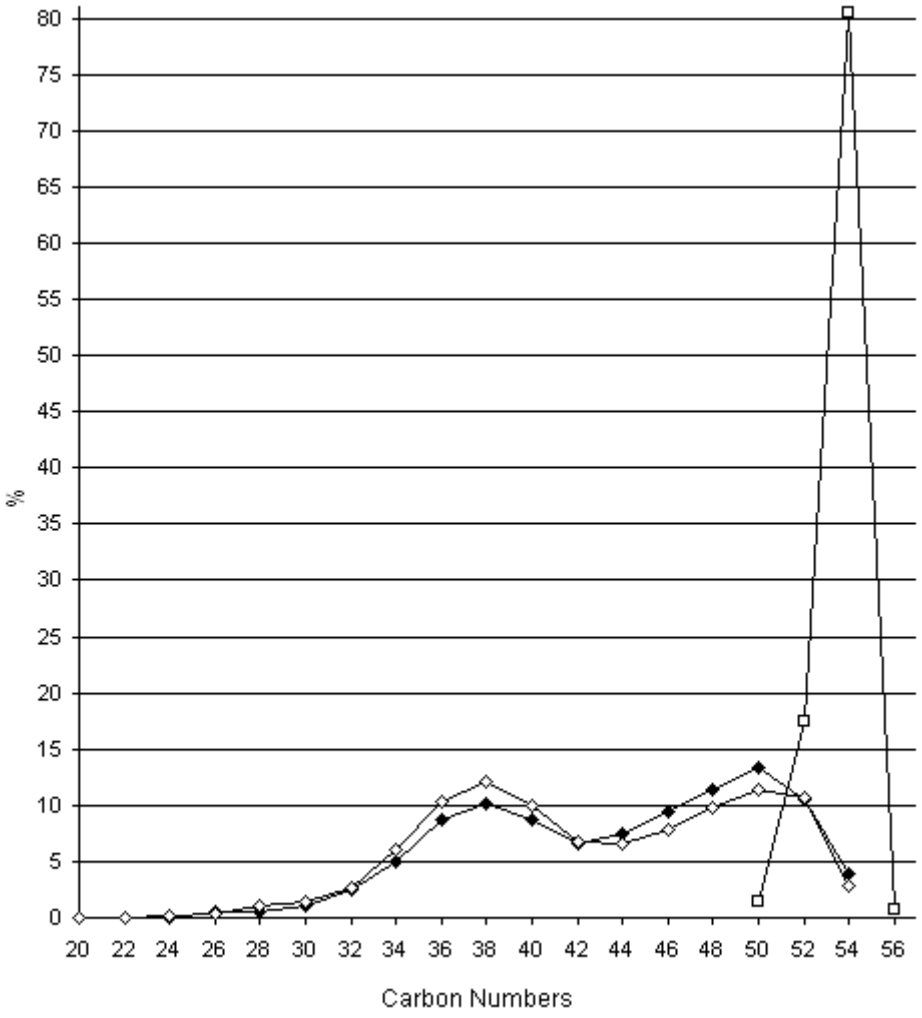


Figure 2. Composition in TAG species by CN (graph) and in main fatty acids (table) of the fats used in this study (area %): AMF (◇), AMFSF (◆) and RO (○).

Interesterification

Lipozyme TL IM is a commercial immobilised lipase able to carry out interesterification reactions, exchanging FAs from one TAG to another and resulting in a redistribution of FAs species amongst the *sn*-1 and *sn*-3 positions of TAGs. Fig. 3 illustrates the change of TAG species occurring with the reaction, i.e. a decrease of TAGs with CN of 34 to 40 and CN of 54, and an increase of TAGs with CN of 46 to 52. Using the ratio CN50/CN42, an interesterification degree (ID) was defined to monitor the reaction as shown by Fig. 4. A quasi-equilibrium was reached after 4 to 6 hours of reaction.

The content in free FAs remained lower than 1 % (Fig. 5). Free FAs are a by-product of the reaction as the lipase hydrolyses TAGs in the extent of the available water content (8). In the present medium water content was around 0.04% as determined by volumetric Karl Fischer titration (not shown) (6).

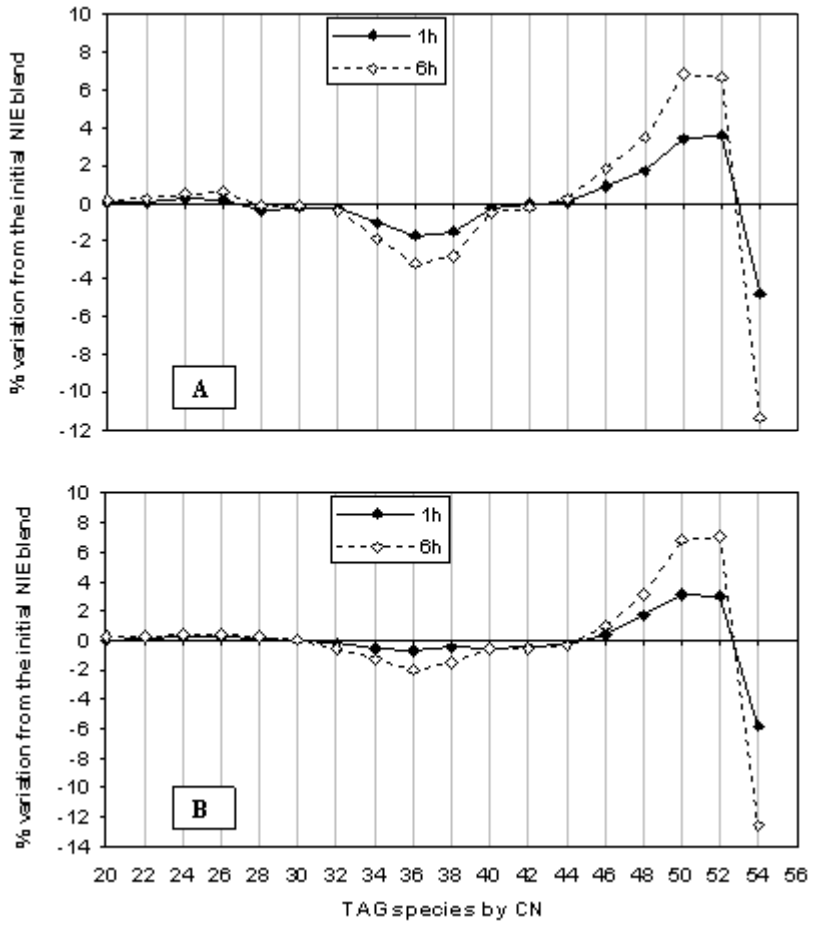


Figure 3. % variation of the content of the different TAG species (by CN) after 1h (◆) and 6h (◇) interesterification when reported to the initial non-interesterified (A) AMF/RO (70/30) and (B) AMFSF/RO (70/30) blends.

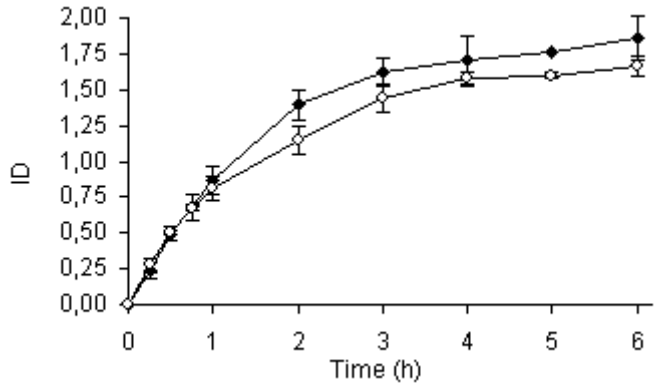


Figure 4. ID for the blends AMF/RO (70/30, wt/wt) (○) and AMFSF/RO (70/30, wt/wt) (◆).

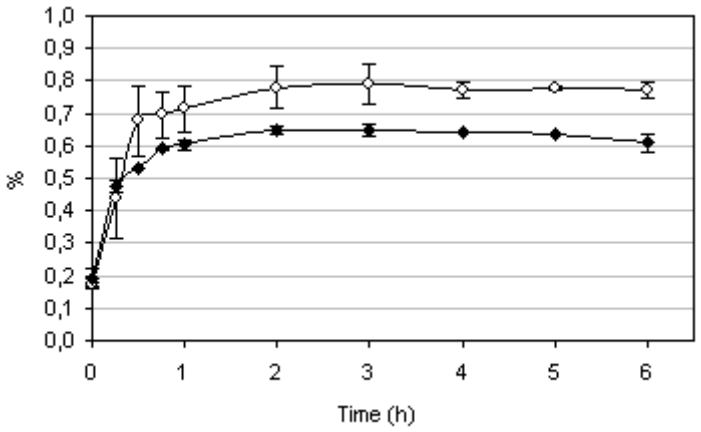


Figure 5. Free fatty acids in the medium throughout interesterification for the blends AMF/RO (70/30, wt/wt) (○) and AMFSF/RO (70/30, wt/wt) (◆).

Conclusions

New fats enriched in unsaturated C_{18} were obtained by enzymatic interesterification of AMF/RO and AMFSF/RO blends. In this first part were shown the compositional changes occurring throughout the reaction. The corresponding changes in physical properties of the fats are presented in the second part of this work.

References

1. Wijendran V., Hayes K.C. Dietary n-6 and n-3 fatty acid balance and cardiovascular health. *Annu. Rev. Nutr.* 2004. 24:597–615.

2. Seppänen-Laakso T., Vanhanen H., Laakso I., Kohtamäki H., Viikari J. Replacement of butter on bread by rapeseed oil and rapeseed oil-containing margarine: effects on plasma fatty acid composition and serum cholesterol. *Br. J. Nutr.*1992. 68:639-654.

3. Osborn H.T., Akoh C.C. Structured lipids-novel fats with medical, nutraceutical, and food applications. *Comprehensive Rev. Food Sci. Food Technol.* 2002. 3:110-120.

4. Lichtenstein A. *Trans* fatty acids, blood lipids and cardiovascular risk: where do we stand. *Nutr. Rev.* 1993. 51:340–343.

5. Zhang H., Xu X., Nilsson J., Mu H., Adler-Nissen J., Høy C.-E. Production of margarine fats by enzymatic interesterification with silica-granulated *Thermomyces lanuginosa* lipase in a large scale study. *JAOCS* 2001. 78:57–64.

6. Aguedo M., Hanon E., Danthine S., Paquot M., Lognay G., Thomas A., Vandenbol M., Thonart P., Wathelet J.-P., Blecker C. Enrichment of anhydrous milk fat in polyunsaturated fatty acid residues from linseed and rapeseed oil through enzymatic interesterification. *J. Agric. Food Chem.* 2008. 56:1757-1765.

7. Kowalski B., Tarnowska K., Gruczynska E., Bekas W. Chemical and enzymatic interesterification of a beef tallow and rapeseed oil equal-weight blend. *Eur. J. Lipid Sci. Technol.* 2004. 106:655-664.

8. Osório N.M., da Fonseca M.M.R., Ferreira-Dias S. Operational stability of *Thermomyces lanuginosa* lipase during interesterification of fat in continuous packed-bed reactors. *Eur. J. Lipid Sci. Technol.* 2006. 108:545–553.