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Production and oxidative stability of a human milk fat substitute produced from lard by enzyme technology in a pilot packed-bed reactor

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Abstract

Residence time and time of production were investigated during the enzymatic production of a specific structured lipid/human milk fat substitute (SL-HMFS), on a kg scale, made from lard and soybean oil fatty acids, using a packed-bed reactor and short path distillation. There were no effects of residence time or time of production on C18:2 and C18:3 incorporation or on acyl migration in the *sn*-2 position. In addition, the SL-HMFS was compared to commercial human milk fat substitute (HMFS) regarding fatty acid composition, content of antioxidants and oxidative stability. Fats were stored at 60 °C for four days and the oxidative stability evaluated by analysis of peroxide value (PV) and volatile secondary oxidation products. SL-HMFS had a lower oxidative stability than did commercial HMFS products or lard, probably due to a lower level of tocopherol in SL-HMFS.

Keywords: Structured lipid; Lard; Human milk fat substitute; Enzyme; Oxidative stability

1. Introduction

Fat in human milk is a major source of energy. Moreover, human milk fat contributes essential structural components for the cell membranes of the newborn (Christie, 1995). The structure of human milk triacylglycerol is unique, as 60–70% of palmitic acid (16:0) is located at the *sn*-2 position and 18:0, 18:1 and 18:2 are preferentially esterified in *sn*-1/3 positions. Since pancreatic lipase selectively cleaves the fatty acids in the *sn*-1 and *sn*-3 positions of the triglyceride, palmitin will mainly be present as 2-monopalmitin, together with, mainly, C18 free fatty acids. 2-monopalmitin is efficiently absorbed while free palmitic acid forms poorly absorbed calcium soaps in the intestine, resulting in reduced absorption of both calcium and fat. Thus, the position of C16:0 in the triglyceride is of considerable significance for the absorption of fat and minerals in infants (Lien, 1994; Lien, Boyle, Tuhas, Tomarelli, & Quinlan, 1997; Lucas et al., 1997). Therefore, the fatty acid composition and distribution in triacylglycerols in infant formulas have recently gained much attention. Human milk fat substitutes have been developed to mimic human milk fat composition and structure (Akimmoto, Yaguchi, & Fujikawa, 1999; Christensen & Hølmer, 1993; Quinlan & Chandler, 1992; Yang, Xu, He, & Li, 2003).

Yang et al. (2003) produced human milk fat substitutes by lipase-catalysed acidolysis of lard with soybean

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fatty acids in a solvent-free stirred reactor on a gramme scale. The HMFS, based on lard and soybean fatty acids, were suitable for infant formula with respect to fatty acid composition, fatty acid distribution of triacylglycerols, and melting properties (Forsyth, 1998; Quinlan et al., 1992) and, most importantly, this HMFS had 71% C16:0 located at the *sn*-2 position.

We have developed continuous packed-bed reactor systems for the production on large scale, of specific structured lipids containing polyunsaturated fatty acids and medium chain fatty acids (Xu, Balchen, Høy, & Adler-Nissen, 1998; Xu, Mu, Høy, & Adler-Nissen, 1999; Xu, Jacobsen, Nielsen, Heinrich, & Zhou, 2002a; Xu, Porsgaard, Zhang, Adler-Nissen, & Høy, 2002b). This system has proved to have advantages of continuity, easy operation, good stability, and lower by-product formation. Therefore, it seems optimal to apply this large-scale system to the production of afore-mentioned human milk fat substitute from lard and soybean fatty acids (Yang et al., 2003). This will provide a better possibility for future production on a industrial scale than a batch reaction system, for the above-mentioned reasons.

When developing new HMFS products, it is important, not only to optimise the nutritional and physical properties, but also to ensure that the oxidative stability of the HMFS is acceptable. During processing and storage, lipid oxidation gives rise to the formation of lipid hydroperoxides, which may be broken down to secondary oxidation products. These products are responsible for unpleasant off-flavours such as rancid and green flavours and odours (Frankel, 1995). In addition, reactive aldehydes, also formed as a result of lipid oxidation, have been suggested to be involved in processes leading to cardiovascular diseases (Kubow, 1993). Thus, in order to maintain an acceptable flavour and a healthy product, it is important that the lipids are oxidatively stable.

The objective of this work was to optimise production on a kg scale, of a specific structured HMFS product (SL-HMFS) based on lard and soybean free fatty acids, with respect to the incorporation of polyunsaturated fatty acids, as well as to minimizing acyl migration, particularly of C16:0 in the *sn*-2 position. A second objective was to determine the oxidative stability of the HMFS product compared to commercial HMFS products.

2. Materials and methods

2.1. Materials

Lard (FFA% 0.05, PV 1.7 meq/kg)(Dragsbæk, Thisted, Denmark) was purchased in the local market. Soybean fatty acids were purchased from Brøste A/S (Lyngby, Denmark). Fatty acid composition of soybean fatty acids was (mol%): 14:0 (0.1), 16:0 (12.5), 18:0 (3.6), 18:1*n*-9 (24.4), 18:1*n*-7 (1.7), 18:2*n*-6 (52.0) and 18:3*n*-3 (5.0). The fatty acid composition of lard was as indicated in Table 1. lipozyme RM IM, an immobilised lipase from Rhizomucor miehei (s*n*-1,3 specific), was supplied by Novozymes A/S, Bagsvaerd, Denmark. The water content of lipozyme RM IM was 3.0%. Fatty acid methyl ester standards were purchased from Sigma (St. Louis, MO). All other reagents and solvents were of analytical grade. Laitao 06 (L06) and Laitao 07 (L07), blends of vegetable oils, were kindly donated by Arla Foods amba ARINCO (Videbæk, Denmark), while Betapol 45 (B-pol), a commercial specific structured lipid, was donated by Loders Croklaan (Wormerveer, Holland).

2.2. Production of human milk fat substitute structured lipid, HMFS-SL.: enzymatic process

Liquid lard, fatty acids of soybean oil (1:1, w/w) and 0.1 wt% water were mixed. The mixture was pumped through a packed-bed reactor (a glass column: $L = 95$

Table 1
Fatty acid composition in TAG and at *sn*-2 position for SL-HMFS and reference fats

FA	Hum[a]	FA profile of TAG					FA profile of *sn*-2				
		HMFS-SL	Lard	B-pol	L06	L07	HMFS-SL	Lard	B-pol	L06	L07
C8:0	nd	0	0	1.1	0.8	0	0	0	0.3	0.4	0
C12:0	5.4	0.2	0.1	12.7	5.6	0.3	0.4	0.4	14.0	9.5	0.3
C14:0	8.1	1.65	1.8	4.6	2.8	0.9	4.5	4.4	4.7	1.6	0.4
C16:0	31.1	31.2	29.5	22.3	29.6	30.1	71.9	73.9	31.7	9.7	6.4
C18:0	8.0	8.6	16.2	2.9	3.9	3.3	3.4	3.1	1.4	0.7	0.5
C18:1*n*-9	36.5	27.1	35.3	38.3	35.9	47.8	10.1	9.5	28.3	50.2	64.8
C18:1*n*-7	nd	1.8	2.6	0.9	1.3	1.3	0.7	0.6	0.4	0.8	0.9
C18:2*n*-6	10.1	23.8	9.2	13.6	15.7	13.6	4.8	3.2	16.4	23.2	24.0
C18:3*n*-3	0.7	2.3	0.8	1.3	2.3	1.4	0.6	0.5	2.0	2.9	2.0

Values are given as mol%; nd, not detected; FA, fatty acid. Only fatty acids which were present at levels >1.0% for at least one of the lipids are listed. Hum, Human milk fat; HMFS-SL, structured lipid based on lard and soybean oil; Lard, lard; B-pol, commercially produced structured HMFS; L06 and L07, blends of vegetable oils used as HMFS.

[a] Jensen et al. (1999).

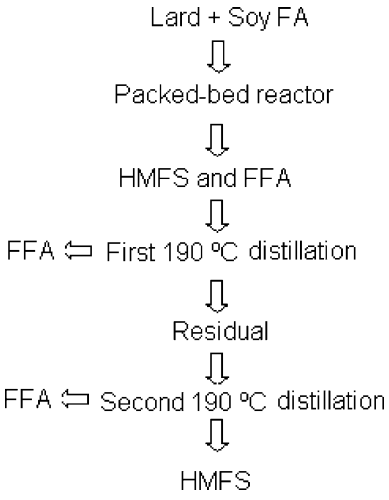

Fig. 1. Process steps of HMFS production. HMFS-human milk fat substitutes.

cm, o.d. = 5.5 cm, i.d. = 5 cm, packed with lipozyme RM IM, and maintained at 65 °C). The reaction system was placed in a 55 °C operation-room to avoid solidification of reactants or products in the flowing pipes (see also Fig. 1). When experiments were conducted to study the effect of residence time, samples were taken after three residence times in each residence time set-up (1.5, 2.0, 2.5 and 3 h). One column was packed and used for the whole set of experiments in the residence time study within three days. For operation stability studies, a new column was packed. The substrates were pumped through the columns under the following conditions without any change: temperature 65 °C, water content 0.1 wt% (based on total substrates), substrate molar ratio (soybean fatty acids:lard) 3:1, and residence time 1.5 h. Samples were withdrawn from the outlets at defined times.

2.3. Short path distillation to purify the product

The short path distillation (KD6, UIC GmbH, Alzenau-Hörstein, Germany) was used for the purification of products to remove free fatty acids under the following conditions: evaporator temperature 190 °C, condenser temperature 60 °C, heat exchanger temperature 100 °C, feeding speed 2.4 l/h and vacuum below 0.001 mbar. The major part of the equipment was constructed from stainless steel. The vacuum system included a diffusion pump and two vamp pumps. The heating of the evaporator was provided by the jacket circulated with heated oil from an oil bath (Xu et al., 2002a).

2.4. Determination of total fatty acid composition and fatty acids in the sn-2 position

Fatty acids were dissolved in heptane and fatty acid methyl esters (FAME) were prepared from total lipids through transesterification catalysed by KOH in methanol (Christopherson & Glass, 1969). The FAME were analysed by gas-liquid chromatography, using a Hewlett–Packard 6890 Chromatograph with flame-ionisation detection (Hewlett–Packard GmbH, Waldbronn, Germany), and a fused silica capillary column (SP-2380, 60 m, ID 0.25 mm, Supelco, Bellefonte, PA). Helium was the carrier gas. A split ratio of 1:20 was applied. The column flow was constant at 1.2 ml/min. Initial oven temperature was 70 °C for 0.5 min and temperature programming was as follows: 15 °C/min to 160 °C, 1.5 °C/min to 200 °C, which was maintained for 15 min, followed by a rate of 30 °C/min to 225 °C, which was then maintained for 5 min. Fatty acids from 8:0 to 22:6*n*-3 were analysed and identified, if present from external standards (Nu-Chek-Prep,Inc. Minnisota, USA). Regiospecific analyses of the fats were performed by degradation of the triacylglycerol molecules with allylmagnesium bromide as Grignard reagent (Becker, Rosenquist, & Hølmer, 1993). The *sn* 2-monoacylglycerol fraction was isolated by thin-layer chromatography on boric acid-impregnated thin-layer chromatography-plates developed twice (2×60 min) in chloroform:acetone (90:10 v/v), methylated, and analysed by gas-liquid chromatography as described above.

Measurements were done in triplicate. Absolute standard deviations were less than 1.1%.

2.5. Oxidative stability experiment

Five fat batches were evaluated (SL-HMFS, lard, B-pol, L06 and L07). Fat samples were stored in brown glass jars with plastic lids in the dark at 60 °C for up to three days. At each sampling time one jar was used for determination of PV, volatile oxidation products and antioxidants, as well as, FFA, at the start and end of the experiment. Samples were taken at the start of the storage experiment and after 3, 6, 24, 48 and 72 h. Jars were stored at −30 °C until analysis and thawed before analysis. Replicates were withdrawn from the same bottle.

2.6. Determination of tocopherols

The tocopherol content of the oils was measured according to the AOCS method (1992), using an HPLC with fluorescence detector, and a Spherisorb column (S5W, 25 cm, 4.6 mm i.d., Spherisorb, Norwalk, CT, USA). After dilution of the oil sample with *n*-heptane, the tocopherol isomers (α-, β-, γ- and δ-tocopherol) were determined simultaneously, using an external standard. Analyses were done in duplicate. Amounts were calculated from standard curves. Tocopherol equivalents were calculated as: $\alpha\text{-TE} = 1 * [\alpha\text{-toc}] + 0.1 * [\gamma\text{-toc}] + 0.03 * [\delta\text{-toc}]$.

2.7. Determination of citric acid

The content of citric acid in the fats was determined using a UV-based assay kit (R-biopharm GmbH, Darmstadt, Germany). Measurements were performed on a Shimadzu UV-160 spectrophotometer (Shimadzu Scientific Instruments Inc., Columbia, MD). Analyses were made in triplicate.

2.8. Determination of FFA

FFA was determined according to the AOCS method using titration with NaOH and phenolphthalein as indicator (1997a). The amount of FFA was calculated as % oleic acid. Analyses were done in duplicate.

2.9. Determination of primary oxidation products by PV

PV was determined according to the IDF method described by Shanta and Decker (Shantha & Decker, 1994). The absorption was measured on a Shimadzu UV-160 spectrophotometer (Shimadzu Scientific Instruments Inc., Columbia, MD). Analyses were done in triplicate.

2.10. Determination of volatile secondary oxidation products

About 4 g of oil were weighed into a pear shaped glass flask, together with 4-methyl-1-pentanol as an internal standard. Headspace volatiles from the oil were released by heating to 75 °C and purging with nitrogen for 30 min while collecting on Tenax tubes. Trapped volatiles were separated and quantified on a GC (HP5890, Hewlett–Packard, Avondale, PA, USA) with capillary column (J&W Scientific DB 1701, 30 m, i.d. 0.32 mm, film thickness 1.0 μm; Folsom, California, USA) and a flame ionization detector. The oven temperature programme was: 3 min isothermal at 35 °C, increase 3 °C/min to 140 °C, increase 5 °C/min to 170 °C, increase 10 °C/min to 240 °C and 8 min isothermal at 240 °C. Identification of compounds was done by retention time and confirmed by GC-MS (mass spectrometry) and by spiking with external standards. Concentrations were calculated from standard curves and the internal standard. Results from the analyses are given as ng/g oil. Analyses were done in triplicate.

3. Results and discussion

3.1. Production of HMFS in packed-bed reactor

Packed-bed reactors are the most frequently used reactors in immobilised lipase-catalysed operations on a large scale. They are best used continuously on a commercial scale in order to minimise labour and overhead costs and to facilitate process control. Packed-bed reactors need relatively low power input and have the lowest reactor volume, due to the high enzyme/substrate ratio maintained. The highest enzyme-to-substrate ratio will result in the highest reaction rate and the shortest reaction time needed to reach a certain extent of conversion (Xu, 2002). Based on our previous results in batch reactors for the production of human milk fat substitutes (Yang et al., 2003), the acidolysis of lard with soybean fatty acids was conducted on a large-scale packed-enzyme reactor in the present study.

Residence time should be the primary consideration for the continuous production of HMFS from lard, in terms of acyl incorporation (Yang et al., 2003). However, residence time (1.5–3.0 h) had no significant effect on C18:2*n*-6 and C18:3*n*-3 incorporation in the present system (Fig. 2). The results indicated that reaction equilibrium was reached in less than 1.5 h residence time.

Residence time is also the primary factor for acyl migration at the *sn*-2 position. We have previously demonstrated that acyl migration is less in a packed-bed reactor than in the batch stirred tank reactor (Xu et al., 1998). Low acyl migration is important to keep C16:0 at the *sn*-2 position of the produced TAG, to ensure an optimal absorption of TAGs and calcium in infants (Christie, 1995). In this study, residence time (1.5–3.0 h) had little effect on acyl migration. The contents of C16:0 were 29.8, 28.4, 28.6 and 30.2 mol% after 1.5, 2, 2.5 and 3 h, respectively. At the *sn*-2 position the contents of palmitic acid after 1.5, 2, 2.5 and 3 h were 72.1, 71.1, 70.8, and 71.5 mol%, respectively. Previously, we found that in the range 40–70 °C, temperature was not a crucial factor for the reaction performance (Xu,

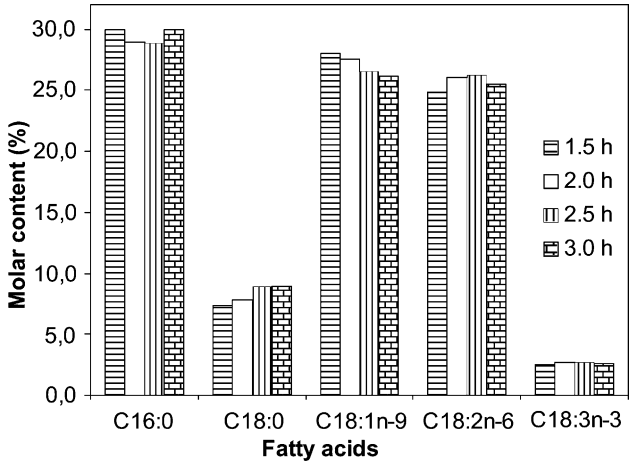

Fig. 2. Effect of residence time on acyl incorporation in the continuous acidolysis of lard with soybean fatty acids shown as content of fatty acids in TAG. Conditions: Packed-bed column (L = 950 mm, o.d. = 55 mm, i.d. = 50), packed with lipozyme RM IM, temperature 65 °C, water content 0.1 wt% (based on total substrates), substrate molar ratio (soybean fatty acids:lard) 3:1.

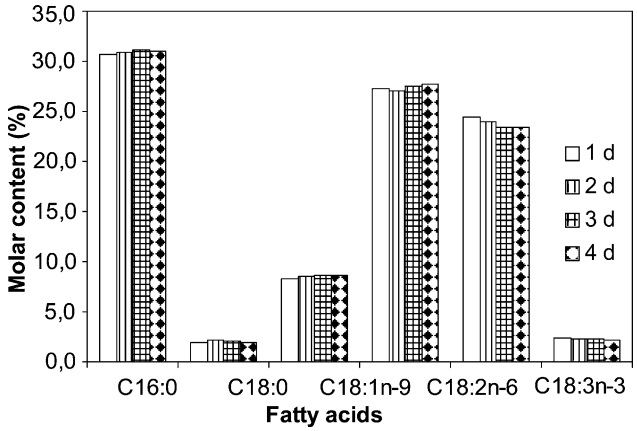

Fig. 3. Operation stability of the continuous acidolysis of lard with soybean fatty acids over four days (d), shown as content of fatty acids in TAG. Conditions: Packed-bed column (L = 950 mm, o.d. = 55 mm, i.d. = 50), packed with lipozyme RM IM, temperature 65 °C, water content 0.1 wt% (based on total substrates), substrate molar ratio (FA/lard) 3:1, and residence time 1.5 h.

2000). Therefore, the effect of temperature was not investigated in the present study. The efficacy of incorporation and the stability of the positional distribution of fatty acids during operation were also investigated. During the 4 days of production, no significant reduction was found for acyl incorporation (Fig. 3). The composition of fatty acids at the *sn*-2 position did not change either. Thus, the contents of C16:0 at the *sn*-2 position were 71, 72, 73 and 73 mol% after 1, 2, 3 and 4 days, respectively. This finding showed that there was no reduction in C16:0 at the *sn*-2 position, indicating that there was no increase of acyl migration during four days of operation. These results imply that it is possible to obtain a satisfactory stability of the enzymatic process in such a system. The production was stopped after 4 days because of the large amount of raw materials used.

3.2. Short-path distillation

Modified TAG and FFA were the main components in the acidolysis products of lard with soy FFA. FFA should be removed from the products before using them for food applications. Because FFA and TAG are heat sensitive and have high-boiling points, thermal decomposition at very high temperatures under atmospheric pressure easily takes place. Short-path distillation is operated at lower temperatures than batch deodorisation. The lower temperatures are supposed to give a gentler but also less efficient purification than batch deodorisation. When optimised, short-path distillation may be a feasible way to purify TAG from FFA. For better separation and efficiency, it is necessary to conduct distillation several times at a constant temperature (Szelag & Zwierzykowski, 1983). The residue from the foregoing distillation is the starting material for the next distillation. After the second distillation at 190 °C, the final HMFS product contained 0.26% FFA, which is acceptable for fat products.

3.3. FA composition and structure

A sufficient intake of the essential fatty acids, linoleic acid and α-linolenic acid is very important for newborn children because their stores at birth are small and they may show signs of deficiency within a week if essential fatty acids are not supplied in the diet (ESPGAN Committee on Nutrition, 1977). The optimal parameters found in the previous investigations on stability during production were applied for the production of 15 kg of human milk fat substitutes (SL-HMFS) from lard and soybean fatty acids. There was a significant difference between fatty acid compositions of SL-HMFS and the other formula fats (Table 1). SL-HMFS had the highest content of linoleic acid, a high content of α-linolenic acid and a low content of oleic acid compared with lard, B-pol and L07. The content of linoleic acid in infant milk formula must be between 3.7 and 34.5% of the total fat, while for, α-linolenic acid, there is only a lower limit of 0.6% (http, 070704). In addition, the ratio of linoleic acid to α-linolenic acid must be between 5 and 15 (http, 070704). In the fats of this study, the ratio of the two fatty acids were 10.4, 11.5, 10.5, 6.8 and 9.7 for SL-HMFS, lard, B-pol, L06 and L06, respectively, compared with 14.4 in human milk. Thus, all the fats, including SL-HMFS, had the right amount of linoleic and α-linolenic acid. Lard, on the other hand, had a high content of C18:0 (16.2%). C16:0 in the *sn*-2 position is efficiently absorbed while free palmitic acid from the *sn*-1 and *sn*-3 positions released by pancreatic lipase results in reduced absorption of both calcium and fat. Thus, it is important that the main part of C16:0 is positioned at *sn*-2 in HMFS as it is in human milk fat. C18:0, together with C16:0, when located at the *sn*-1 and -3 position, is also thought to cause formation of precipitates of calcium (ESPGAN Committee on Nutrition, 1977; López-López et al., 2001; Quinlan, Lockton, Irwin, & Lucas, 1995). In the SL-HMFS, produced in this study, and in lard, C16:0 was mainly at the *sn*-2 position (72–74%), whereas less than 35% of C16:0 was located at the *sn*-2 position in the other fats. Even though lard had a high content of C18:0, only a minor part of it was located at the *sn*-2 position.

Thus, due to the fatty acid composition and the position of the fatty acids in the TAG SL-HMFS seem optimal for use as a HMFS.

3.4. Antioxidant content

The content of tocopherol was constant for all fats over the entire storage period (Table 2). However, in

Table 2
Tocopherol and citric acid contents of fats used in storage experiment

	SL-HMFS	Lard	B-pol	L06	L07
α	nd	20–28	62–70	168–179	246–254
γ	nd	1.1–6.2	79–97	291–314	41–58
δ	nd	nd	25–29	90–96	2.3–3.3
Citric acid	nd	7.2–8.7	nd	37–41	8.6–10.6

Values are μg/g and given as means ± SD. $n = 3$. For interpretation of code names please refer to Table 1. nd: not detectable.
The range indicates the min. and max. values measured during storage at 60 °C. $n = 2$.

SL-HMFS, none of the tocopherols were detectable. The α-tocopherol content was highest in L07 (approx. 250 μg/g), followed by L06 (approx. 174 μg/g), with significantly less in B-pol and lard (approx. 66 and 24 μg/g, respectively). γ- and δ-tocopherol were highest in L06 followed by B-pol and L07 and less in lard. In lard, δ-tocopherol was not detectable and the γ-tocopherol levels were very low. The contents of α-tocopherol equivalents in the fats were L07 > L06 >> B-pol > lard > SL-HMFS (approx. 256, 207, 76 and 25 μg/g, respectively). Citric acid was mainly present in L06 (39 mg/kg) and in lower amounts in lard and L07 (8 and 10 mg/kg) (Table 2). No citric acid was found in SL-HMFS or B-pol, and as for the tocopherols, there were no significant differences in the amounts before storage and after 72 h of storage at 60 °C. The results in Table 2 demonstrate that, already at the start of the experiment, the contents of antioxidants were very different in the five fats. The high content of tocopherol in L06 and L07 probably reflects the natural content of the vegetable oils from which they were blended. Lard has a low natural content of antioxidants. The low contents of antioxidants in SL-HMFS and B-pol are probably a result of the purification procedure after interesterification. We have previously observed a lower content of antioxidants in structured lipids than in the lipids from which they were produced (Nielsen, Xu, Timm-Heinrich, & Jacobsen, 2004; Timm-Heinrich, Xu, Nielsen, & Jacobsen, 2003).

The FFA content was low in all samples and during 72 h of storage at 60 °C there was no change in the amount of FFA. The FFA content was higher in SL-HMFS and in lard (both 0.3%) than in L06 (0.15%), L07 (<0.1%) and B-pol (<0.1%).

3.5. Primary oxidation products

At the start of the storage experiment, the PV of SL-HMFS was highest (2.2 meq/kg), followed by lard (1.7 meq/kg) (Fig. 4). PV of the other fats were below 0.35 meq/kg. During the first 24 h of storage at 60 °C, the PV of SL-HMFS increased to 7.7 meq/kg. Thereafter, PV remained stable. On the other hand, the PV of lard, which was similar to the PV of SL-HMFS at the start of the storage, did not increase during storage. Likewise,

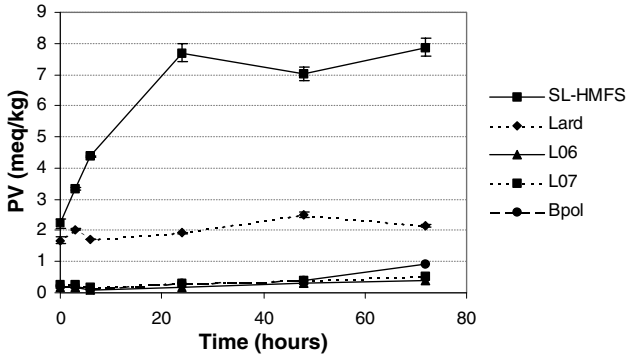

Fig. 4. Development of peroxides during storage of structured HMFS produced from lard and soybean fatty acids (SL-HMFS) and reference fats at 60 °C for up to 72 h. Values are calculated as meq O_2/kg and given as means ± SD. $n = 3$. SL-HMFS: structured lipid based on lard and soybean oil, lard, B-pol, L06 and L07.

the PV of B-pol, L06 and L07 did not increase markedly during 72 h of storage. In B-pol, PV reached 0.9 meq/kg and in L06 and L07 PV reached 0.4 and 0.5 meq/kg, respectively. Thus, due to the low content of antioxidants in SL-HMFS, this fat is not protected from oxidation to the same degree as the other fats, resulting in an increase in PV. In addition, the higher content of FFA in SL-HMFS and lard may have resulted in increased oxidation. Very little information on the stability of HMFS is available in the literature.

3.6. Volatile secondary oxidation products

Twenty-three volatiles were identified. These compounds could be divided into two major groups according to the difference in development of the volatiles in the fats during storage. The first group was comprised of 19 compounds, which were found at higher levels in SL-HMFS during storage than the levels of the other fats. The second group was comprised of 4 compounds that developed differently. In the first group, 12 compounds, namely butanal, butenal, pentanal, pentenal, 1-penten-3-ol, octane, hexanal, 2-hexenal, 2-heptenal, octenal, 2,4-decadienal and 2,4-nonadienal, increased significantly during storage in SL-HMFS whereas the increase was very modest in the other fats (as an example see Fig. 5). The other seven compounds of the first group, 1-octen-3-ol, octanal, nonanal, 2-nonenal, 2-decenal, 3,5-octadien-2-one and 2,4 heptadienal, were also present at higher levels in SL-HMFS than in the other fats. However, the difference between SL-HMFS and the other fats were much lower for these compounds.

In the second group, the level of 3-pentanol was highest in lard throughout the storage experiment. The levels of heptanal were similar in L07 and in SL-HMFS. 2-Pentylfuran increased most in lard during the first 24 h of storage whereafter its concentration was stable. Consequently, the level of 2-pentylfuran was initially highest

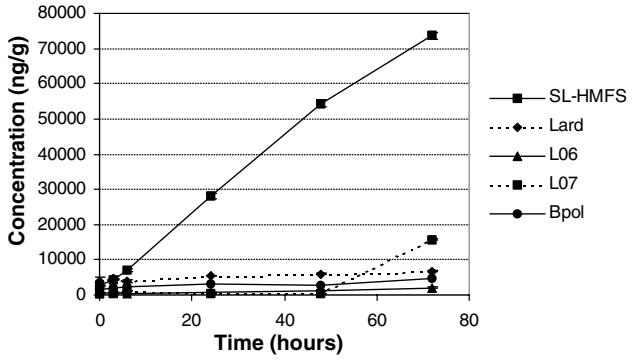

Fig. 5. Development of hexanal during storage of structured HMFS produced from lard and soybean fatty acids (SL-HMFS) and reference fats at 60 °C for up to 72 h.

Table 3
Concentration (ng/g) of selected volatiles after 72 h of storage of fats at 60 °C ($n = 3$)

	SL-HFMS	Lard	Bpol	L06	L07
Pentanal	12483	473	512	421	343
Hexanal	73620	6983	4591	2096	1939
Heptanal	3568	0	497	585	1299
Octanal	2670	1081	1099	397	203
Decanal	1039	1351	689	0	13099
tt-2,4-Nonadienal	10542	0	0	0	0
tt-2,4-Decadienal	4660	0	0	0	0
2-Pentylfuran	571	14208	160	153	205
t-2-Heptenal	24931	1359	543	0	0
t-2-Octenal	10040	780	449	0	0
t-2-Nonenal	1728	672	796	0	0
c-2-Decenal	3398	1315	519	0	0

The relative standard deviation was <15%.

in lard and, from 48–72 h of storage, the pattern was similar to the 19 compounds in the first group. Decanal developed very differently from the other volatiles. In SL-HMFS, the level increased up to 24 h and then slowly fell, while there was a similar increase in lard, which continued after 24 h. After 72 h of storage, the level was highest in lard, followed by SL-HMFS and L07.

Table 3 shows the concentration of some major aldehydes and pentyl furan after 72 h of storage at 60 °C. The concentration was significantly higher in SL-HMFS for 11 of the 12 aldehydes, followed by lard, L07, B-pol and L06.

The significant increase in PV and secondary oxidation products in SL-HMFS during storage is probably related to its higher content of linoleic acid compared to the other fats, as well as to its low content of antioxidants. The relatively high PV observed in lard may be a result of the production method. For example, lard is not refined and therefore peroxides formed during slaughtering and butchering are not removed. Neither PV nor volatile concentrations increased during storage, which indicated that lard had a relatively high oxidative stability, despite its low level of antioxidants. This finding can most likely be ascribed to the low content of PUFA in lard compared to the other fats in this study. In summary, the PV and the volatiles data showed that the order of oxidative stability was: L06 = B-pol = L07 > lard >> SL-HMFS.

Volatile aldehydes constitute the major part of the secondary oxidation products responsible for off-flavour. Thus, the higher content of these compounds in SL-HMFS than in the other fats is expected to result in a less palatable product. Especially, the dienals have very low threshold values, and are thus important for off-flavour (Frankel, 1998).

In summary, we have developed a simple production method (on a kg scale) for the specific structured HMFS product based on lard and soybean free fatty acids with a fatty acid composition in TAG and *sn*-2 resembling that of human milk fat. The oxidative stability, however, was not as high as the stability of commercial vegetable blends or a commercial structured lipid. The lower oxidative stability was probably a result of the production method, where antioxidants are removed. Future studies may demonstrate whether increased stability can be obtained by addition of antioxidants during production.

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