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Study of the Properties of Human Milk Fat Substitutes Using DSC and GC Methods

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Abstract: The development of infant milk fat similar in its structure and composition to that present in human milk receives a considerable amount of attention and has become a challenge for the world's food industry. The aim of this work was an attempt to obtain structured lipids resembling human milk fat (HMF) by modifying mixtures of lard (L) and rapeseed oil (RO) catalyzed by Lipozyme RM IM. The enzymatic reactions were carried out at 70 °C for 4, 8, and 24 h. The second objective was to study the properties of obtained substitutes of human milk fat (HMFS) using gas chromatography, thin layer chromatography, and differential scanning calorimetry (DSC) methods. Chromatographic methods were used to determine the composition of fatty acids (FAs) and the regiospecific structure of triacylglycerols (TAG) of obtained HMFS. DSC methods were used to establish the oxidative stability of HMFS and to calculate their kinetic parameters of oxidation. Structured lipids obtained from a mixture of L and RO after 4 h interesterification in 70 °C were characterized by the closest FA content and their distribution in TAG to HMF. Interesterification influenced the decrease of the induction time of obtained structured lipids.

Keywords: human milk fat substitutes; differential scanning calorimetry; gas chromatography

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1. Introduction

Mother's milk is the major source of nutrients for infants. The ingredient that provides the largest part of the infant's required nutritional energy is human milk fat (HMF) [1]. Therefore, fat in breast milk is the main source of energy for infants, especially in the first period of their life. Additionally, HMF delivers important structural components for neonatal cell membranes [2]. The structure of HMF and its fatty acid (FA) composition are unique. It is characterized by a high content of palmitic acid (20–25%) belonging to saturated fatty acids (SFA) and polyunsaturated fatty acids (PUFA) [3]. Among the PUFA in human milk, there are both essential FAs precursors, linoleic acid (18:2 n-6) and α-linolenic acid (18:3 n-3), and a very long chain of bioactive PUFA of both the n-6 and the n-3 families [4,5]. Triacylglycerols (TAG) are the main component of HMF (98% of HMF). The structure of human milk TAG is special, as 60–70% of palmitic acid (16:0) is found in the internal position of TAG, and unsaturated FAs are positioned externally. The position

of palmitic acid in TAG affects digestion and absorption of fats and minerals from the infant's intestines [1,6].

The World Health Organization and other important health organizations recommend breastfeeding as the best method that provides distinctive benefits for the child and mother. In order to assess the suitability of a given infant formula for feeding, its ability to promote growth and development comparable to that of human milk was determined. The biochemical, functional, and metabolic responses of modified formula-fed infants are compared to those of breast-fed infants [7]. Vegetable oils, which are typically used in infant formulas, contain palmitic acid in the external positions of TAG. The use of vegetable oils as a substitute of HMF in infant formulas may cause calcium deficiency due to the formation of insoluble calcium soaps with saturated FA released by the action of the specific pancreatic lipase [6,8,9]. Recently, many scientific studies have been carried out and attempts have been made to obtain structured lipids (SL) with FA content and TAG structure similar to HMF. SL are often called nutraceuticals or next generation fats. Nutraceuticals are foods or parts of foods that not only provide basic nutrition but also medical and health benefits and prevent and even treat certain diseases. SL are produced by enzymatic or chemical modification of the TAG [10,11]. Due to the numerous advantages of using enzyme catalysts in order to obtain SL with specific functional properties, the number of studies on this type of modification is constantly increasing [6,8]. Enzymatic modification has many advantages compared to chemical methods used during fat modification. Lipases (triacylglycerol acylhydrolases, EC. 3.1.1.3) hydrolyze esters in an aqueous medium. However, if access to water is restricted, interesterification reactions can also be catalyzed with lipases. Lipase catalyzed reactions are performed under milder circumstances and with greater selectivity than chemically catalyzed reactions. Additionally, the use of regioselective lipases allows the FA to be kept in the internal position of the acylglycerols. This is desirable for nutritional reasons and impossible to achieve via chemical catalysis [12].

Several studies were carried out by means of immobilized lipases as catalysts to produce HMFS which mimics HMF. In most of them, SL reminiscent of HMF were obtained via enzymatic acidolysis or interesterification of lard or tripalmitin with free FA from various sources. Lard is the only fat resembling HMF in terms of FA composition and their distribution in glycerol backbone. Compared to HMF, lard is characterized by a similar palmitic and oleic acids content, but it is a worse source of essential FAs. Vegetable oils are rich in polyunsaturated fatty acid. Taking the above-mentioned into account, a mixture of tripalmitin or lard and vegetable oils were selected to obtain a product of similar FAs composition and their distribution in triacylglycerols (TAG) to HMF [13,14].

During the development of new products such as HMFS, it is significant to optimize the physical and nutritional properties, but the oxidative stability of these products should also be guaranteed [2]. Oxidation of lipids is a very important process occurring in food systems. Primary and secondary oxidation products can adversely affect the nutritional value and food safety [15,16]. The production process for SL increases free fatty acids (FFA) concentration, which are responsible for off-flavor and odor characteristic of oxidation. A high concentration of FFA in SL induces an unacceptable rancid and bitter taste. Moreover, structured lipids produced with lipids that contain unsaturated FAs can deteriorate during storage. Unsaturated FAs in SL may be rapidly oxidized to hydroperoxides. They can then be quickly broken down into secondary oxidation products, such as aldehydes, alkenes, and ketones responsible for their unwanted flavor. Numerous products of oxidative degradation of edible oils and fats are detrimental to human healthiness. These products damage vitamins and enzymes and may cause mutations or gastrointestinal problems [17–21].

Many methods are used to monitor the autooxidation of fats and oils. Currently, instrumental methods that are faster, more accurate, more objective, and have a wider detection range are used. Differential scanning calorimetry (DSC) is a nonchemical method that can be used to determine fat quality parameters [22–25].

The purpose of this work was to study the properties of HMFS obtained by enzymatic modification of a mixture of lard and rapeseed oil using DSC as well as chromatography (GC) and thin layer chromatography (TLC).

2. Materials and Methods

2.1. Chemicals and Materials

Lipozyme RM IM, the porcine pancreatic lipase (Type II), and standard compounds were supplied by Sigma-Aldrich (Saint Louis, MO, USA). The rest of the reagents and solvents were purchased from Avantor Performance Materials Poland S.A. (Gliwice, Poland). L and RO were provided by a commercial company. The TLC plates were purchased from Merck (Darmstadt, Germany). The mixtures of L and RO in weight proportions 8:2 were used in this examination. The interesterification process was carried out twice.

2.2. Enzymatic Modification

The method of carrying out the interesterification has been described by Bryś et al. [4]. The temperature of interesterification: 70 °C, the time of modification: 4, 8, or 24 h.

2.3. Peroxide Value and an Acid Value

Peroxide value (PV) of the oils was determined by iodometric technique and acid value (AV) by titration with 0.1 M ethanolic potassium hydroxide in correspondence with ISO standards 3960:2007 [26] and 660:2009 [27], respectively.

2.4. Fatty Acid Composition

The determination of FA composition was carried out by gas chromatographic analysis of fatty acid methyl esters (FAME). FAME were prepared according to the standard ISO method 5509:2001 [28] and injected into a gas chromatograph equipped with an FID detector according to method described by Bryś et al. [4].

2.5. Positional Distribution of Fatty Acids in the sn-2 and sn-1,3 Positions of TAG

Method of determination of positional distribution of FAs in the internal and external positions of TAG has been described by Bryś et al. [4]. The method involved carrying out an enzymatic hydrolysis with the use of regiospecific pancreatic lipase. Subsequently, monoacylglycerols were isolated by using thin layer chromatography. The resulting sn-2 monoacylglycerols were methylated and subjected to chromatographic analysis as described above.

2.6. DSC Measurements

A pressure differential scanning calorimeter (PDSC) was used to determine the oxidative stability of tested oils and fats (DSC Q20 TA). The induction time determination was based on the maximum oxidation rate (maximum rate of heat flow). Method of determination of oxidative stability has been described by Bryś et al. [4]. The differential scanning calorimeter was used for the nonisothermal (dynamic) mode (TA Instruments Q 200). The samples were heated at the rates of 2.5, 5, 7.5, 10, and 12.5 °C per minute. The experiments were conducted in an oxygen atmosphere with an initial pressure of 60–70 kPa (gas flowing—100 mL per minute). For each programmed heating rate (β, °C/min), a minimum three replications were maintained. The onset oxidation temperature was determined (ton, °C) as the intersection of the extrapolated baseline and the tangent line (leading edge) of the recorded exotherm. The kinetic parameters of the oxidation process (activation energy, pre-exponential factor) were calculated [20,29].

2.7. Statistical Analysis

Statistical analysis has been described by Bryś et al. [4]. Multiple Range Test (Tukey's method) was used not only to compare analyzed samples but also to identify which

interesterified fat presented was the closest to HMF in terms of its FAs composition and distribution in TAG.

3. Results

3.1. Comparison of the Composition of FAs and the Structure of TAG of Obtained SL with HMF

3.1.1. FAs Composition in Studied Fats

We have come to the following conclusions: lard contains (Table 1, Figure 1) large amounts of SFA, among others palmitic acid (27.65%) and stearic acid (18.00%). This fat also contains significant quantities of oleic acid (38.0%), which belongs to monounsaturated fatty acids (MUFA), and linoleic acid (9.50%), which belongs to PUFA. The predominant FA in rapeseed oil was oleic acid (61.07%). Rapeseed oil also contains α-linolenic acid (9.26%) and linoleic acid (19.21%) in considerable amounts. The content of the SFA in this oil is very low and does not exceed 8%. Taking the above mentioned into account, rapeseed oil was used in our research to enrich lard in essential FAs.

Table 1. FAs composition of raw materials and mixtures after interesterification (wt%).

Fatty Acid	L [1]	RO [1]	LR70_4 [1]	LR70_8 [1]	LR70_24 [1]
C10:0	$0.14 \pm 0.01_{b}$	-	$0.10 \pm 0.01_{a}$	$0.10 \pm 0.01_{a}$	$0.10 \pm 0.01_{a}$
C12:0	$0.13 \pm 0.01_{b}$	-	$0.10 \pm 0.01_{a}$	$0.11 \pm 0.01_{a}$	$0.10 \pm 0.01_{a}$
C14:0	$1.70 \pm 0.04_{c}$	-	$1.41 \pm 0.01_{ab}$	$1.49 \pm 0.01_{b}$	$1.33 \pm 0.04_{a}$
C16:0	$27.65 \pm 0.08_{d}$	$4.44 \pm 0.02_{a}$	$24.2 \pm 0.1_{c}$	$24.8 \pm 0.1_{c}$	$23.2 \pm 0.7_{b}$
C16:1 cis	$2.17 \pm 0.06_{d}$	$0.22 \pm 0.01_{a}$	$1.73 \pm 0.04_{b,c}$	$1.81 \pm 0.01_{c}$	$1.70 \pm 0.01_{b}$
C17:0	$0.38 \pm 0.02_{c}$	$0.12 \pm 0.01_{a}$	$0.32 \pm 0.02_{b}$	$0.33 \pm 0.04_{b,c}$	$0.32 \pm 0.02_{b}$
C17:1 cis	$0.22 \pm 0.01_{b}$	$0.06 \pm 0.01_{a}$	$0.21 \pm 0.01_{b}$	$0.21 \pm 0.01_{b}$	$0.21 \pm 0.01_{b}$
C18:0	$18.00 \pm 0.07_{c}$	$1.98 \pm 0.01_{a}$	$14.4 \pm 0.1_{b}$	$14.5 \pm 0.2_{b}$	$14.5 \pm 0.1_{b}$
C18:1 cis	$38.0 \pm 0.1_{a}$	$61.07 \pm 0.07_{e}$	$42.4 \pm 0.4_{c}$	$41.8 \pm 0.3_{b}$	$43.4 \pm 0.4_{d}$
C18:2 n-6	$9.50 \pm 0.06_{a}$	$19.21 \pm 0.03_{c}$	$11.21 \pm 0.01_{ab}$	$11.12 \pm 0.02_{a}$	$11.25 \pm 0.07_{b}$
C18:3 n-3	$0.78 \pm 0.01_{a}$	$9.26 \pm 0.02_{d}$	$2.38 \pm 0.04_{c}$	$2.32 \pm 0.03_{b,c}$	$2.31 \pm 0.01_{b}$
C20:0	$0.24 \pm 0.01_{a}$	$0.70 \pm 0.01_{c}$	$0.31 \pm 0.01_{b}$	$0.30 \pm 0.01_{b}$	$0.30 \pm 0.01_{b}$
C20:1 cis	$0.75 \pm 0.04_{a}$	$1.59 \pm 0.01_{d}$	$0.93 \pm 0.04_{b,c}$	$0.89 \pm 0.01_{b}$	$0.98 \pm 0.03_{c}$
C20:2 n-6	$0.36 \pm 0.03_{b}$	$0.40 \pm 0.01_{ab}$	$0.31 \pm 0.01_{a}$	$0.31 \pm 0.01_{a}$	$0.33 \pm 0.04_{a}$

[1] Data expressed as means ± S.D. The different lower-case letters (a–e) in the same row indicate significantly different values ($p < 0.05$).

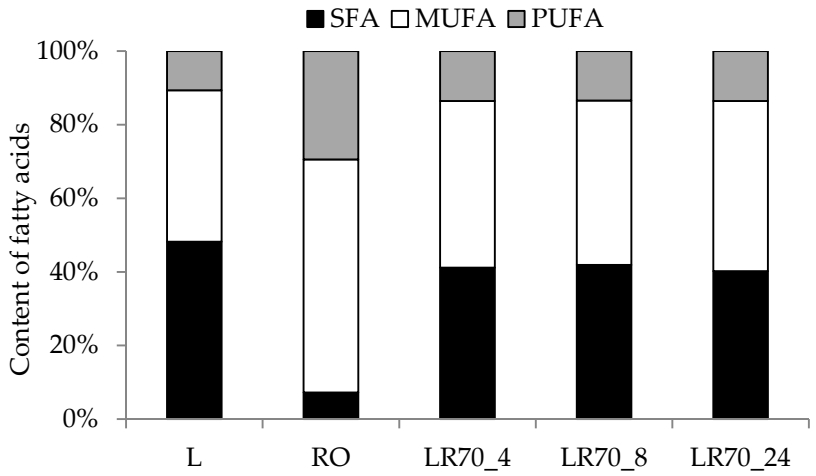

Figure 1. Content (wt%) of the fatty acids (FAs) for raw materials and mixtures after interesterification.

The results of the determination of the FA composition of the obtained SL are presented in Table 1 and Figure 1. Mixtures after interesterification showed 13.9% of PUFA, while the original lard only amounted to 10.2% of those acids. TAG of interesterified fats contained 23.2–24.8% of palmitic acid and from 41.8% to 43.4% of oleic acid. The essential FAs from

vegetable oil were incorporated into TAG structures of lard. The fats after enzymatic modification contained about 2.4% of α-linolenic acid belonging to n-3 essential FAs.

3.1.2. FAs Distribution in TAG in Studied Fats

The FA composition in external and internal TAG positions of most abundant acids present in SL is shown in Table 2. Palmitic acid was the most common FAs located in internal position (41.6%) of SL after 4-h interesterification. Extending the reaction time reduced the palmitic acid content in the sn-2 position of TAG to 26.8% and increased the oleic, linoleic, and stearic content in that position. Meanwhile, to confirm the distribution of FAs between regiospecific positions of TAG, it is necessary to recalculate the FA composition in internal position in the relative concentration of FA in the internal position. As reported by Lopez-Lopez et al. [30], the composition in FA in external and internal positions in TAG is not an optimal way to present data of sn-2 FA composition without considering the total percentage of FAs in total TAG molecules. Thus, Figure 2 shows the percentage of the most abundant FAs in the sn-2 position of the TAG, expressed as the relative FA [(sn-2 FA × 100%)/(3 × total FA in TAG)] in SL. The percentage of palmitic acids in sn-2 of TAG in SL ranged from 38.4% to 57.3%. The percentage of linoleic acid at the sn-2 of TAG in SL ranged from 26.6% to 30.3% and the percentage of oleic acid ranged from 30.9% to 38.4%. It was also observed that after 24 h of interesterification, the FA distribution in TAG appeared to be close to statistical.

Table 2. FA composition in the internal and external positions of triacylglycerols (TAG) of raw materials and mixtures after interesterification.

	Type of Sample	FAs (wt%)			
		C16:0	C18:0	C18:1 cis	C18:2 n-6
sn-2 MAG	L	$74.6 \pm 0.5_{e}$ [1]	$4.7 \pm 0.3_{b}$	$11.6 \pm 0.8_{a}$	$3.1 \pm 0.2_{a}$
	RO	$2.9 \pm 0.3_{a}$	$0.15 \pm 0.07_{a}$	$45.2 \pm 1.1_{c}$	$29.7 \pm 1.1_{c}$
	LR70_4	$41.6 \pm 0.7_{d}$	$10.2 \pm 0.4_{c}$	$39.3 \pm 1.9_{b}$	$8.9 \pm 0.8_{b}$
	LR70_8	$34.9 \pm 0.6_{c}$	$11.9 \pm 0.4_{d}$	$43.6 \pm 0.3_{c}$	$9.6 \pm 0.1_{b}$
	LR70_24	$26.8 \pm 1.3_{b}$	$13.1 \pm 0.4_{d}$	$49.9 \pm 0.3_{d}$	$10.2 \pm 0.6_{b}$
sn-1,3 DAG	L	$4.2 \pm 0.2_{a}$	$24.6 \pm 0.2_{d}$	$51.2 \pm 0.4_{c}$	$12.7 \pm 0.1_{b}$
	RO	$5.20 \pm 0.07_{a}$	$2.89 \pm 0.02_{a}$	$69.0 \pm 0.3_{d}$	$14.0 \pm 0.3_{c}$
	LR70_4	$15.5 \pm 0.3_{b}$	$16.5 \pm 0.2_{c}$	$44.0 \pm 0.9_{b}$	$12.4 \pm 0.4_{a,b}$
	LR70_8	$19.7 \pm 1.1_{c}$	$15.7 \pm 0.8_{b}$	$40.9 \pm 0.6_{a}$	$11.9 \pm 0.3_{a,b}$
	LR70_24	$21.5 \pm 0.6_{d}$	$15.2 \pm 0.2_{b}$	$40.1 \pm 0.1_{a}$	$11.8 \pm 0.3_{a}$

[1] Data expressed as means ± S.D. The different lower-case letters (a–e) in the same row indicate significantly different values ($p < 0.05$).

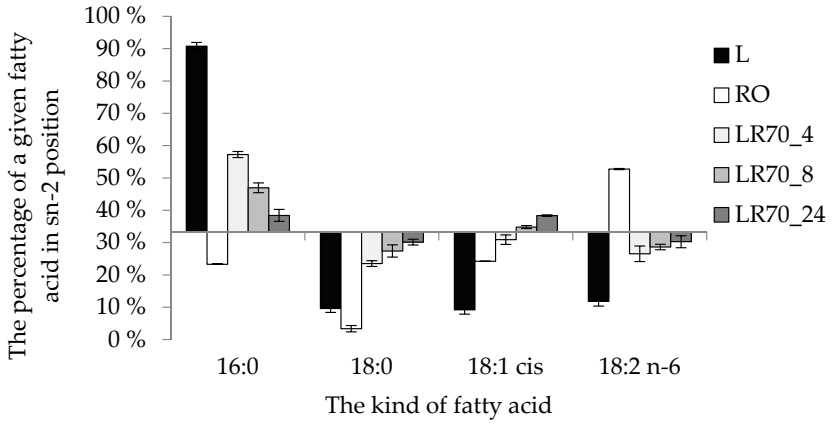

Figure 2. The percentage of a given FA in internal position of TAG of raw materials and modified fats, expressed as the relative FA [(sn-2 FA × 100%)/(3 × total FA in TAG)].

3.2. The Quality Assessment of Studied Fats

During the development of the SL, it is not only important to optimize the FA composition and distribution in TAG but also to provide acceptable oxidative stability of SL. There are many methods used for monitoring the autoxidation of lipids. One of the most popular techniques used to evaluate the oxidative stability of fats and oils is DSC [31,32]. Basic PDSC parameter used to evaluate the resistance of the fat being analyzed to its oxidative decomposition is induction time. Fats with a shorter induction time are less stable than those for which the induction time obtained at identical temperature is longer [33].

The results of PDSC measurements are shown in Table 3. The PDSC tests for SL performed at constant temperature of 120 °C showed that their induction times (27.2–53.0 min) were reduced compared with the starting fats (70.9 min for L and 72.0 for RO). The determinant of primary oxidation products in fat is a peroxide value (PV). PV of L, RO, and SL are presented in Table 3. PV of blends after enzymatic modification amounted from 4.1 to 5.7 mmol O_2/kg of fat. Therefore, this modification causes an increase in PV compared to raw materials (2.7 mmol O_2/kg for L and 3.4 for RO). Results obtained also show that PV for mixture interesterified for long time (24 h) was lower than for those interesterified for short time (4 h). The determinant of free FAs contents in fat is an acid value (AV). The results of AV for mixtures after interesterification, for L and RO, are given also in Table 3. It has been found that after interesterification the AV increased. AV of blends esterified in the presence of Lipozyme RM IM amounted from 5.4 to 11.0 mg KOH/g of fat. The FFA content significantly depends on the reaction time. The fats obtained after 8 h of interesterification are characterized by the highest content of free fatty acids, while after 24 h of modification, the content of fatty acids was the lowest.

Table 3. Acid value, peroxide value, and oxidation induction time of raw materials and mixtures after interesterification.

Type of Sample	AV [1] (mg KOH/g)	PV [1] (mmol O_2/kg)	IT [1] (min)
L	$0.86 \pm 0.06_{b}$	$2.7 \pm 0.2_{a}$	$70.9 \pm 0.8_{a}$
RO	$0.58 \pm 0.05_{a}$	$3.4 \pm 0.1_{b}$	$72.0 \pm 0.6_{a}$
LR70_4	$8.7 \pm 0.1_{d}$	$5.7 \pm 0.2_{e}$	$27.2 \pm 1.4_{d}$
LR70_8	$11.0 \pm 0.6_{e}$	$4.8 \pm 0.3_{d}$	$34.7 \pm 0.2_{c}$
LR70_24	$5.4 \pm 0.2_{c}$	$4.1 \pm 0.3_{c}$	$53.0 \pm 1.8_{b}$

[1] Data expressed as means ± S.D. The different lower-case letters (a–e) in the same column indicate significantly different values ($p < 0.05$).

3.3. Kinetic Analysis of Oxidation

If there is a need to determine the oxidative stability of fats stored and processed under various conditions, it is necessary to provide their kinetic parameters [17]. In Table 4, the experimental initial (onset) oxidation temperatures (T_{on}) obtained at five heating rates are shown. The T_{on} experimental values as a function of heating rates (β) were recalculated on ruthless onset temperatures (T_{on}, K). Substituting values of T_{on} and β into the Equation (1):

$$Log\ \beta = a\left(\frac{1}{T_{on}}\right) + b \quad (1)$$

where a and b are adjustable coefficients, we found a linear dependence ($R^2 > 0.96$ in each case).

Table 4. Parameters T_{on} obtained for five rates of heating sample during the thermo-oxidative processes of interesterified fats.

Heating Rates (°C/min)	T_{on} (°C)		
	LR70_4	LR70_8	LR70_24
2.5	140.2 ± 1.6	150.9 ± 0.1	149.7 ± 1.3
5.0	147.8 ± 0.6	156.4 ± 1.9	161 ± 1
7.5	153.0 ± 0.4	166.6 ± 1.7	168.2 ± 0.6
10.0	162.0 ± 0.2	171.3 ± 0.9	172.7 ± 0.4
12.5	164.4 ± 1.3	174.9 ± 1.0	176.4 ± 0.2

Under an assumption that the degree of reaction is a constant value independent of the heating rate when a DSC curve reaches its peak, activation energy (E_a) was calculated for each sample (Equation (2)):

$$E_a = -2.19\, R\, \frac{d \log \beta}{d\left(\frac{1}{T}\right)} \quad (2)$$

where β—the heating rate (°C/min), R is the gas constant and T—temperature (K).

According to the Equation (3), other kinetic parameters, such as the pre-exponential factor (Z) of fat oxidation, was calculated:

$$Z = \frac{\beta E_a e^{\frac{E_a}{RT}}}{RT^2} \quad (3)$$

The regression analyses of the data and kinetic parameters for the SL are listed in Table 5.

Table 5. Statistical and kinetic parameters characterizing thermal-oxidative decomposition of interesterified fats. Ea—activation energy (kJ/mol), Z—pre-exponential factor.

Parameter	T_{on} Based Value		
	LR70_4	LR70_8	LR70_24
a	−4912.3	−5090.0	−4949.3
b	12.33	12.46	12.10
R^2	0.96	0.96	0.99
E_a	89.40	92.63	90.07
Z	4.08 × 1010	4.41 × 1010	1.93 × 1010

The activation energies calculated from the experimental data ranged from 89.40 kJ/mol to 92.63 kJ/mol and the pre-exponential factors ranged from 1.93 × 1010/min to 4.41 × 1010/min. There were no statistically significant differences among the activation energies in the mixture interesterified for a different period of time.

4. Discussion

HMF provides approximately 50% of infants' total energy intake and consists mainly C18:1, C16:0, and C18:2 FAs [34]. The long-chain polyunsaturated FAs (LCPUFA) are also present in HMF at levels tied to the mother's eating habits. The main LCPUFAs in HMF are arachidonic acid (ARA), docosahexaenoic acid (DHA), and eicosapentaenoic acid (EPA). DHA and ARA are membrane FAs necessary for development and growing of the infants' central nervous system. FAs such as DHA and ARA also influence bone mineralization, bone mass growth, and the synthesis of eicosanoids, i.e., tissue hormones. Therefore, the human milk fat substitutes should also contain these LCPUFAs in appropriate amounts [35]. In comparison with HMF, lard is a source of palmitic and oleic acids at a similar level, but it is characterized by less content of linoleic and higher amounts of

stearic acid [12]. Rapeseed oil (RO) contains twice as many of MUFA and PUFA compared to HMF. The addition of the rapeseed oil to lard increased the unsaturated FAs contents.

Brest milk fat contains about 40.6% of the SFA 39.1% of the MUFA and 19.5% of the PUFA [30], while in the case of obtained SL, SFA amount was determined at the level of from 40.2 to –41.9%, MUFA-from 44.7–46.2%, and PUFA-13.4–13.6%, including significant essential FAs from n-3 group and from n-6 group, like α-linolenic acid and linoleic acid. Content of oleic and palmitic acid for obtained SL is similar to HMF. Obtained SL does not contain valuable DHA, EPA, ARA, but it is a source of precursors of these FAs. FAs such as DHA, EPA, and ARA can be formed in the human body by metabolism of FAs, i.e., α-linolenic acid and linoleic acid. Therefore, the enzymatic modification of a mixture of lard and rapeseed oil allows for an accumulation of new fats with oleic and palmitic acids at quantities comparable to HMF, as well as containing crucial essential FA from the omega-3 group.

The types of FAs and its distribution in the molecule of TAG affect the physical properties of fats as well as the behavior of fats during digestion and absorption [36,37]. Breast milk contains approximately 70% palmitic acid in the middle position of TAG, while in most vegetable oils this FA is located in the outer positions of the TAG. Taking into account the obtained results, it can be concluded that lard, like HMF, contains about 85.3% palmitic acid in the internal position of TAG, while the percentage of this FA in the internal TAG position in RO was only 23.3%.

The selective pancreatic lipase plays an significant role in the digestion of fat; it hydrolyzes TAG molecules in the external positions to free FAs and 2-monoglyceride. Therefore, the products of human fat digestion will be 2-monopalmitin that is effectively absorbed and unsaturated free FAs. However, if free palmitic acid is present after hydrolysis, insoluble calcium soaps may form in the intestine. This reduces the absorption of both calcium and fat. Therefore, the position of palmitic acid in TAG is important for the absorption of minerals and fat in infants [2,5]. Given the results on the distribution of FAs in the TAG molecules of investigated SL, it can be stated that this distribution is very similar to that of HMF. The percentage of palmitic acids in internal position of TAG in all SL exceeded 33%, which means that it is mostly in the sn-2 TAG position. Considering the percentage of unsaturated FAs in the internal TAG position in SL, it can be deduced that they are mainly in the external TAG positions. These are especially linoleic acid and oleic acid. Similar results were obtained in the work related to enzymatic modification of lard with milk thistle oil [4]. Taking into account the obtained results, it can be concluded that the time of interesterification affects the distribution of FAs in the TAG both in the middle and in the outer position. The modification of fats with the use of Lipozyme RM IM occurred predominantly in the outer positions of the TAG. Because interesterification in the presence of regiospecific enzymes only occurs at the external positions, the sn-2 position of the TAG remains unchanged [33]. Changes in internal position of TAG may be caused by possible acyl migrations within and between TAG molecules during a longer interesterification period, as evidenced by Xu et al. [38].

Literature data indicate that fats after interesterification are characterized by a reduced oxidative stability compared to the starting blends [19,20,23]. The obtained results confirm that the enzymatic modification of fats reduces their oxidative stability. The induction time for samples interesterified for a long time (24 h) is higher than for those interesterified for short time (4 h). The results obtained by DSC are consistent with tests of peroxide value (PV) determination. The high PV is a measure of the content of primary oxidation products from which secondary oxidation products can be formed very quickly. Bryś et al. [23] report that there is an inverse relationship between PV and induction time. An increased index of PV in the blends after interesterification may reduce its oxidative stability. This parameter is a very important factor that affects the quality of oils, fats, and foods. Usually, foods containing high amounts of unsaturated FAs are characterized by a low oxidative stability. Within the FA family, PUFA are highly unstable molecules and are susceptible to oxidation processes, which results in the formation of free radicals,

polymers and hydroperoxides which may lead to quality loss, both in terms of technology and health [20]. The presence of free FAs in the oils can induce oxidation due to the catalytic effect of FAs carboxyl groups on the formation of free radicals. Frega et al. [39] investigated the effect of free FA content on the oxidative stability of vegetable oils. The scientists observed the pro-oxidative effect of free FAs with all filtered oils. A higher reduction in oxidative stability was also observed in the case of modified oils and fats, when the product after interesterification contained a higher level of free FAs, monoacylglycerols and diacylglycerols [20]. Triacylglycerols are the core components of fats. Fats also include certain quantities of incomplete acylglycerols and free FAs. As a result of the hydrolysis process in the presence of lipases free FAs, partial acylglycerols and glycerol are obtained. The hydrolysis reaction is reversible and, if the water level is lowered, the formation of new acylglycerols will dominate the hydrolysis [40]. Oxidative stability does not only depend on the FA composition and free FA content. Stability may also be affected by the content of antioxidants. Vegetable oils like rapeseed oil contain natural antioxidants such as tocopherols, tocotrienols, phenolic compounds, or phytosterols. The inferior stability of mixtures of lard and rapeseed oil after interesterification, as compared to the starting materials, may be related to the loss of these natural antioxidants during the process [20,41]. The oxidative stability of fats and oils is also related to the distribution of FAs in the TAG molecules. During interesterification the distribution of FAs changes and it may affect the oxidative stability [42].

The activation energies for the thermal oxidative decomposition of the fats obtained in this work correlate with the data mentioned in the literature [24]. The results indicate that E_a should not be the only parameter of comparison for analyzed fats and oils. Interesterified lard with rapeseed oil is a very complex blend, containing mainly triacylglycerols. These compounds are highly diverse in terms of chain length and degree of unsaturation of FAs and their position. Such variety influences the oxidative stability. During the measurement at the same time there are some reactions that are characterized by a different constant rates and DSC detects only the reactions with the highest exothermic effects. This can be an explanation of why changes in the effective activation energy, according to the compensation theory, have no physical significance [24].

5. Conclusions

The blends of lard and rapeseed oil after enzymatic modification using a biocatalyst-immobilized lipase were suitable for the production of HMFS because they are characterized by a FAs composition similar to that of HMF. The distribution of FAs in the TAG molecules of investigated SL is also very similar to that of HMF. The percentage of palmitic acids in internal position of TAG in all SL exceeded 33%, which means that it is mostly in the sn-2 TAG position. The time of interesterification affects the distribution of FAs in the TAG both in internal and external positions. The FA distribution in TAG after 24 h of interesterification appeared to be close to statistical. Interesterification influenced the reduction of the induction time of obtained structured lipids. The presence of free FAs formed during interesterification can reduce the oxidative stability.

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Abbreviations for Figures and Tables

L	lard
RO	rapeseed oil
LR70_4	blend of L and RO (8:2), modification for 4 h at 70 °C
LR70_8	blend of L and RO (8:2), modification for 8 h at 70 °C
LR70_24	blend of L and RO (8:2), modification for 24 h at 70 °C
AV	acid value
PV	peroxide value
IT	oxidation induction time

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