The Differences of the Structure of Triacylglycerols as a Result of Enzymatic Interesterification of Fat Mixtures With the Omega-3 Family Polyenic Fatty Acids

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Abstract: The studies on the enzymatic restructuring process, with the application of interesterification of the fat mixtures, containing fatty acids of omega-3 group (mono-, di-, tri-, tetra-, penta- and hexaenoic) with a differentiated chain length and degree of saturation, were carried out. The investigations were conducted in a model system, in laboratory scale. The Lipozyme® RM IM of Novozymes® A/S company, Denmark, revealing specificity to the *sn*-1,3 triacylglycerol (TAG), was used as a biocatalyst. The research material consisted of rapeseed oil and fish oil with different levels of EPA and DHA acids. The enzymatic processes of interesterification were conducted in batch system with a stirrer and without solvent. The optimal parameters of the process were determined and the analysis of the obtained product was carried out.

Keywords: restructuring; enzyme; triacylglycerol; EPA; DHA; omega-3

INTRODUCTION

The attempts to obtain the structured lipids (SL) have been undertaken by many researchers in different countries. The reasons for it include wide possibilities which are offered by the enzymatic technologies due to the specificity of the enzymes' action, mild parameters of the process, protecting the raw material from the undesired changes and obtaining a wide range of lipids with a modified structure, creating the conditions for their wide utilization in production of special destination food and drugs [1, 2]. The basic source of obtaining the polyenic long-chained acids from n-3 family (omega-3) is found in fish oils [3]. From the nutritional point of view, the following fatty acids are particularly valuable: all *cis*-eicosa-5,8,11,14,17-pentaenoic (EPA) C20:5 and all *cis*-docosa-4,7,10,13,16,19-hexaenoic (DHA) C22:6. Vegetal oils (e.g. flax, soybean, sunflower and rape seed oil) contain the linolenic acid (all *cis*-octadeca-9,12,15-trienoic) C18:3 which also belongs to n-3 family [4]. Deficiency of omega-3 acids in human body may be the reason for many dangerous disease and therefore, a reasonable consumption of various products, containing the discussed acids, is necessary [5–8]. As its was already mentioned, the application of lipases as biocatalysts offers the possibilities to generate the specific effect on, *inter alias*, the particular positions of triacylglycerol (TAG) molecules – internal *sn*-2 and external ones – *sn*-1,3. The rape seed oil, being a source of linolenic acid, is more resistant to oxidative changes as compared to fish oils.

The aim of the present work was to study the process of enzymatic interesterification of fat composition (fish oil and rapeseed oil), containing polyenic fatty acids with a differentiated chain length. The studies were conducted in a model system, with the laboratory scale.

EXPERIMENTAL

Material and methods. The research material included fish and rape seed oils. The fish oil derived from Norway – it was Denomega ™ 100 (D) and contained 8.8% of eicosapentaenoic (EPA) acid, 13% of docosahexaenoic (DHA) acid. The rape seed oil (R) was produced in Poland and was characterized by 10% content of α-linolenic acid C18:3.

The mentioned oils were used for preparation of the experimental mixture in the weight ratio 2:3, 1:1 and 3:2. The experiment covered – conducting the processes of interesterification with enzyme with a specific action, and analytical tests of the obtained products of the interesterification reaction. The mentioned processes were conducted in periodical reactor with a stirrer, without solvent and with nitrogen. The biocatalyst, called Lipozyme® RM IM (immobilized lipase, obtained from *Rhizomucor miehei*), placed in macroporous anion phenol resin was employed. The discussed lipase catalyses the reaction of interesterification in position *sn*-1,3 of TAG, so it reveals the positional specificity. The parameters of the reaction were as follows: addition of enzyme – 8%, water content in enzyme – 2.5%, temperature of reaction – 60°C, atmosphere of reaction – nitrogen and time of reaction – 4 h. The process was interrupted by filtering off the preparation Lipozyme® RM IM from the reaction mixture.

The following analytical methods were applied: determination of fatty acid composition, by gas chromatography (Hewlett-Packard device 6890, column 60 m, BPX 70), according to PN-ISO 5508; determination of fatty acid composition in position sn-2 of TAG, using pancreatic lipase according to PN-EN ISO 6800 and using Grignard reagent [9, 10]; determination of the composition of the interesterification product by a column chromatography according to AOCS Cd 11c-93: determination of acid value according to PN-ISO 660.

RESULTS AND DISCUSSION

The results obtained in the work are illustrated in the successive Tables 1 and 2 and Figures 1 and 2. The obtained products of enzymatic interesterification consisted of the mixtures of acylglycerols (triacylglycerols – TAG, diacylglycerols – DAG, monoacylglycerols – MAG and free fatty acids – FFA). In consequence of interesterification of the reaction mixture of TAG in the presence of biocatalyst – Lipozyme® RM IM, the interchange of fatty acids occurred in external positions of TAG whereas the internal position *sn*-2 of TAG was not subjected to essential changes.

The interesterification process was aimed at maximization of triacylglycerols' fraction – TAG and the optimal conditions of the reaction were determined in this respect. Chromatographic analysis allowed the determination of the presence of fatty acids in the model mixtures with different levels of EPA and DHA acids. The model mixture R/D (rapeseed oil – fish oil, 2:3) showed 13.3% of saturated fatty acids (including palmitic acid – 7.7%) and 86.7 % of

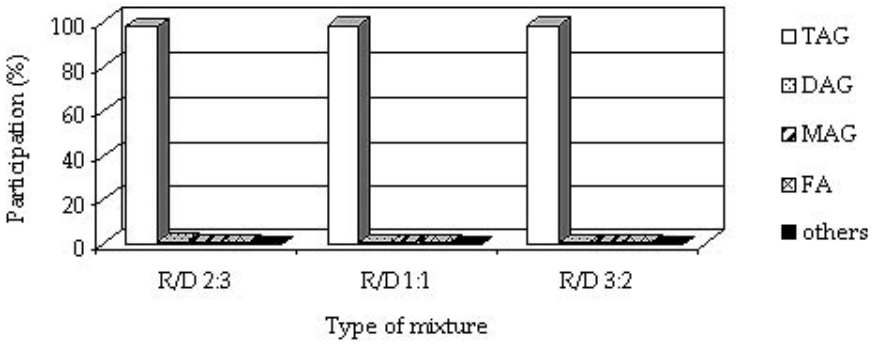

Figure 1. Participation of the polar and non-polar fractions in the model mixtures for the enzymatic interesterification

Table 1. Participation of the polar fraction (MAG, DAG, FFA) and nonpolar (TAG) in the mixtures and products of the enzymatic interesterification model mixtures R/D 2:3, 1:1, 3:2

Type of mixtures (m)	Fraction participation in the product (%)									
	TAG		DAG		MAG		FA		others	
	m	p	m	p	m	p	m	p	m	p
R/D 2:3	98.0	85.1	0.9	5.9	0.8	1.5	0.3	7.2	0.0	0.3
R/D 1:1	98.2	86.5	0.8	5.6	0.7	1.3	0.3	6.3	0.0	0.3
R/D 3:2	98.3	88.3	0.8	4.8	0.7	1.2	0.3	5.5	0.0	0.2

m–mixure; p –product

Table 2. The selected fatty acids composition in *sn*-2 and *sn*-1,3 TAG before and after enzymatic interesterification. The percentage participation of fatty acids in *sn*-2 position. Biocatalysts Lipozyme® RM IM. The model mixture R/D 2:3 w/w

FA	FA in TAG (%)	FA in TAG positions (%)				Participation in *sn*-2 (%)	
		sn-2		*sn*-1,3			
		before	after	before	after	before	after
16:0	7.7	5.6	5.8	8.75	8.65	24.2	25.1
16:1	5.6	1.4	1.4	7.7	7.7	8.3	8.3
18:0	2.0	1.6	1.5	2.2	2.25	26.7	25.0
18:1	36.2	26.7	26.5	40.9	41.0	24.6	24.4
18:2	9.3	14.0	14.6	6.95	6.65	50.2	52.3
18:3 (n-3)	4.4	6.9	7.0	3.15	3.1	52.3	53.0
20:5 (n-3) EPA	5.2	8.7	8.7	3.45	3.45	55.8	55.8
22:6 (n-3) DHA	8.1	10.8	10.0	7.05	7.15	42.0	41.2

unsaturated fatty acids (oleic acid – 34.8%; linolenic acid 9.3%; linolenic acid – 4.4% , eicosapentaenoic (EPA) – 5.2% and docosahexaenoic acid (DHA) – 8.1%. In the remaining model mixtures (R/D – 1:1 and 3:2) the content of fatty acids EPA and DHA was found on the lower level while that one C18 acids (oleic, linolenic and linolenic acids) was on a higher level.The optimum time of reaction was established empirically at 4 hours. The prolongation of the time of reaction did not have any effect on further progress of reaction and did not increase TAG fraction. Analysis of the model product (R/D (2:3), as obtained in reaction with Lipozyme® RM IM showed its following composition: TAG 85.1%, DAG 5.9%, MAG 2.9%, FFA 5.8% and the remaining ones 0.3% (e.g. Figures 1 and 2, Table 1). Although the above mentioned results have specified the summary content of FA in positions *sn*-1,3, the allowed calculation of the percentage participation of the particular fatty acids in position *sn*-2 and by this, in external positions *sn*-1,3 (e.g. Table 2). According to the assumption, FA composition in external positions of TAG before and after interesterification should be differentiated whereas that one in the internal TAG position should become unchanged. Based on the obtained results, it may be stated that the changes in the internal position *sn*-2 did not practically occur. When evaluating the results in positions *sn*-1,3 of TAG which were assumed as being of the same value, we cannot – on these grounds – determine the quantity of the particular fatty acids in position *sn*-1 or *sn*-3 because it is the summary result. We may, however, suppose that the translocation of acyls in these positions had place but there is no direct evidence of this fact. The analysis of the product, as specifying the polar (MAG, DAG and FA) and non-polar (TAG) fractions is a indirect proof of rearrangement of acyls in external positions of TAG. Direct determination of TAG structure in position *sn*-1 and separately, in *sn*-3 requires the stereo-specific analysis of triacylglycerols to be applied [11].

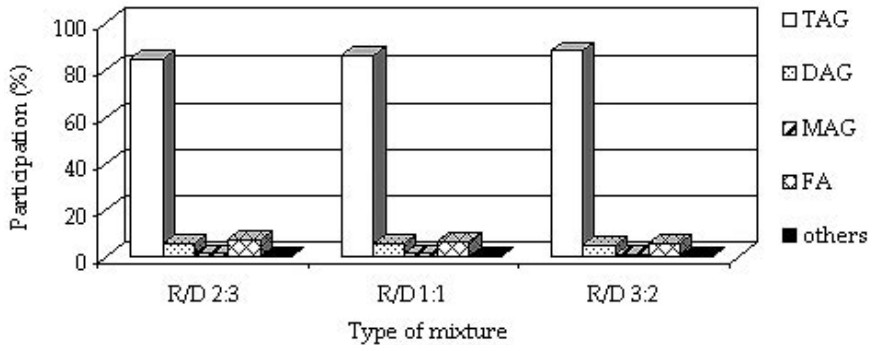

Figure 2. Participation of the polar and non-polar fractions in the products of the enzymatic interesterification

CONCLUSIONS

In the case of enzymatic interesterification reaction with the participation of specific biocatalyst, i.e. Lipozyme® RM IM, the TAG structure was subjected to change as a result of translocation of acyl groups in TAG molecules, mainly in *sn*-1,3 positions. The slight changes in the *sn*-2 TAG position were observed although this position should be unchangeable. In the product, as result of the enzymatic interesterification process of the rapeseed and fish oil mixtures, polar (DAG, MAG) and non-polar (TAG) fractions as well as FA were found. The main components in the original mixtures were constituted by TAGs.

Acknowledgement: The paper was performed with the frames of PBZ/KBN/021/PO6/99, financed by the National Committee of Scientific Studies (KBN) during the years 2001–2004.

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