

The use of Lipozyme RM IM for modifying the properties of lard

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In the paper, the properties of structured lipids synthesized by the enzymatic interesterification of a blend of lard and rapeseed oil with n-3 fatty acids in the form of their concentrates were reported. A commercially immobilized 1,3-specific lipase was used as a biocatalyst. After interesterification, triacylglycerols were purified by column chromatography. The obtained triacylglycerols were analyzed for the peroxide value determined by the spectroscopic method and for fatty acid composition determined by the chromatographic method.

Enzymatic interesterification of lard resulted in obtaining new fats with the composition of triacylglycerols similar to that of fatty acids in human milk fat. The interesterification has influenced the oxidation resistance.

Key words: lard, structured lipids, interesterification, oxidative stability

Introduction

Enzymatic interesterification has received considerable attention in recent years. The interesterification of lipids catalyzed by lipases is an alternative to the chemical interesterification [1]. Microbial lipases have a great potential for commercial application due to their stability, selectivity and a broad substrate specificity [2]. Catalyzed with lipases, interesterification reactions can be directed in controlled ways, and specific or totally new products can be produced [3]. Interesterification can be used to produce fat products with improved nutritional and dietary properties, e. g., to mimic human milk fat [4].

Human milk is the major source of energy for many infants during the first part of their life. It contains 2–6 % of fat, mainly as triacylglycerols (TAG) which provide about 50 % of energy. The fatty acid composition and the structure of human milk fat are unique. Human milk fat has a high content of saturated palmitic acid which is primarily located at the internal position of TAG. Human milk fat contains also very important long-chain polyunsaturated fatty acids (LCPUFA). The brain, retina and other neural tissues are particularly rich in LCPUFA. Some LCPUFA are precursors for eicosanoid production. These mediators are powerful regulators of numerous cell and tissue functions [5, 6].

Lard is a fat available in large quantities, which has a TAG structure similar to that of human milk fats. As compared with human milk fat, lard has a similar content of palmitic and oleic acids, but it has a lower content of essential fatty acids [7]. One of the options to reach lard in essential fatty acids is enzymatic interesterification.

The aim of this paper was to assess the properties of structured lipids synthesized by enzymatic

interesterification of blend of a lard (L) and rapeseed oil (RSO) with n-3 fatty acids from fish in the form their concentrates (ROPUFA).

Materials and methods

Materials

The mixtures of L, RSO and n-3 fatty acids from fish in the form of their concentrates (ROPUFA), in proportions 7 : 2 : 1, were used in our investigations. Lard and rapeseed oil were purchased commercially, and the ROPUFA 30 n-3 food oil was purchased from DSM Nutritional Products.

Catalyst of enzymatic interesterification

Enzymatic interesterification was catalyzed by the commercial preparation Lipozyme RM IM, which contains immobilized lipase from *Rhizomucor miehei*. The fats were interesterified for 2, 4 and 8 hours at a temperature of 50 °C.

Column chromatography

After interesterification, the fraction of triacylglycerols was purified by column chromatography. The content of polar compounds was analyzed by the standard method ISO 8420:2002.

GC measurements

The obtained TAG were analysed for fatty acid composition. Methyl esters of fatty acids were prepared through saponification of TAG and esterification with methanol, and were analyzed by gas chromatography according to ISO 5508:1990.

Peroxide value (PV) determination

A modified method proposed by Hornero-Mendez et al. [8] was used for the determination of PV. Absorbance at a wave length of 500 nm was measured. Spectrophotometer readings were set to zero using a blank sample. The concentration of lipid hydroperoxides was expressed as PV.

Results and discussion

The main component of fat is triacylglycerols. Fats contain also certain quantities of polar fraction (PF), namely monoacylglycerols (MAG), diacylglycerols (DAG), and free fatty acids (FFA). Non-esterified L displayed 0.6 % of PF. The percentages of PF in non-esterified L and ROPUFA amounted to 0.8 and 0.2, respectively. The values of PF contents for mixtures before and after interesterification are given in Fig. 1. After

interesterification, the concentration of PF has been found to increase. The natural function of lipases is to catalyze the hydrolysis of fats and to give free fatty acids, partial acylglycerols, and glycerol. The reaction is reversible, and the enzymes can be shown to catalyze the formation of acylglycerols from glycerol and free fatty acids under certain conditions.

However, if the water level is reduced, some lipases will continue to catalyze reactions, and at a certain level interesterification begins to dominate over hydrolysis [4, 9].

Blends esterified at the presence of Lipozyme RM IM contained 7.8 to 8.9 % of PF, and the PF content was dependent on the time of the reaction. Macrae [9] reported that most of the FFA and MAG + DAG were formed in the first hour of the reaction. In our study, an equilibrium among triacylglycerols, water, diacylglycerols and free fatty acids was established after 8 hours.

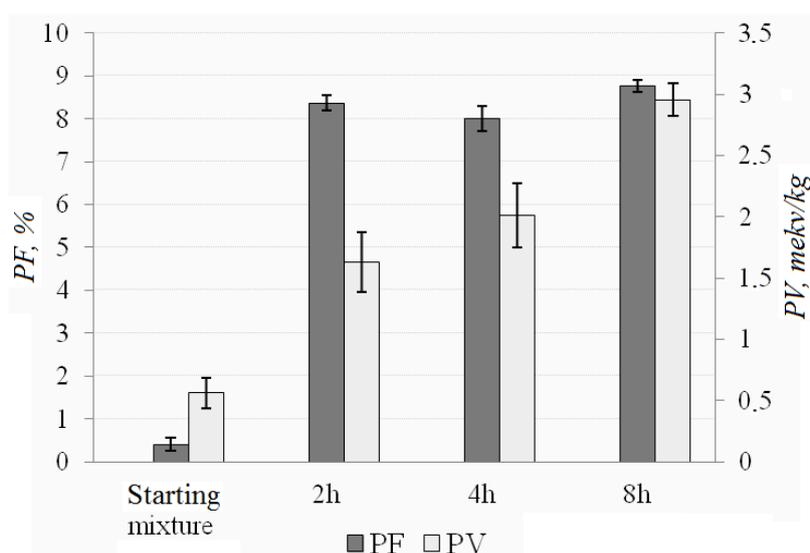


Fig. 1. Polar fraction (PF) and peroxide value (PV) of interesterified fats and starting mixture

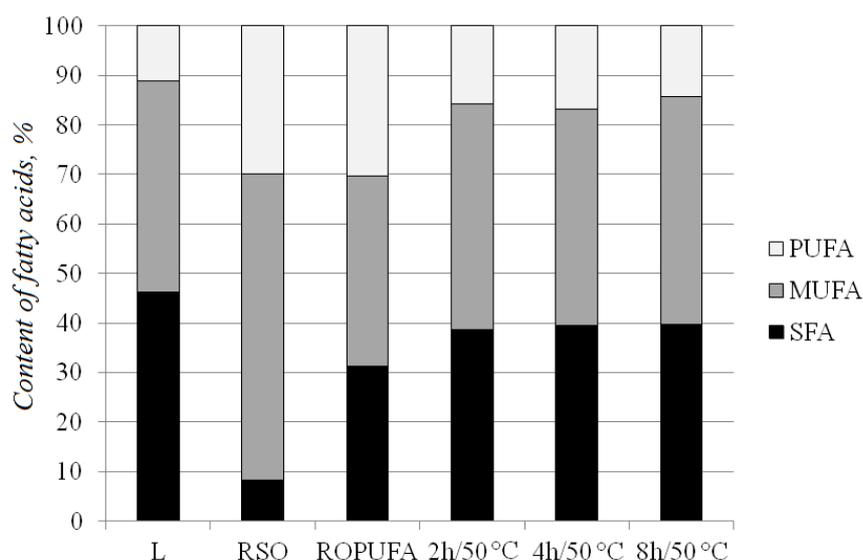


Fig. 2. Content of fatty acids (SFA – saturated fatty acids, MUFA – monounsaturated fatty acids, PUFA – polyunsaturated fatty acids) for raw materials and for blends after interesterification

An increased content of FFA and (DAG + MAG) in the interesterified fatty product can reduce its resistance to oxidation [10]. The TAG fractions isolated from non- and interesterified samples by column chromatography were checked for their hydroperoxide concentration expressed as the peroxide value (PV). The peroxide values for raw materials were as follows: 0.4 mekv/kg for L, 0.8 mekv/kg for RSO, and 1.1 mekv/kg for ROPUFA. Interesterification causes an increase in the peroxide value (Fig. 1). A mixture interesterified for 8 hours is characterized by the highest PV value. In general, most studies have reported a decrease in the oxidative stability of interesterified fats as compared with the initial mixture [11, 12]. Among the different reasons for explaining a worse oxidative stability of interesterified fats, the removal of endogenous antioxidants of interesterified fats has been pointed out as one of the main reasons for this phenomenon [12].

It is worth noting that interesterification causes no changes in the composition of fatty acids, and it is very important from the nutritional point of view. Lard contains mainly palmitic acid (26.8 %) and oleic acid (40.6 %). The addition of rapeseed oil and fish oil to lard increased the content of polyunsaturated (PUFA) fatty acids (Fig. 2). TAG from fats after interesterification showed the PUFA level to range from 14.3 to 15.7 %, while the original lard was characterized by only 11.1 % PUFA content. In human milk PUFA content makes about 18.8 % of total fatty acids [1]. Therefore, the interesterification reaction of a blend of lard, rapeseed oil and n-3 fatty acids from fish oil allowed obtaining new fats with the proportion of PUFA similar to that of human milk fat.

Conclusions

Due to enzymatic interesterification, the unsaturated fatty acids of rapeseed oil and fish oil were incorporated into triacylglycerol structures of lard.

The results of the present research have shown that interesterification influenced the increase of the polar fraction and peroxide value.

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LIPOZIMO RM IM PANAUDOJIMAS TAUKŲ SAVYBĖMS MODIFIKUOTI

S a n t r a u k a

Tirtos struktūruotų lipidų, susintetintų fermentinio peresterinimo būdu iš taukų ir rapsų aliejaus mišinio su n-3 riebalų rūgštimis, savybės. Komercinė 1,3-specifinė imobilizuota lipazė naudota kaip biokatalizatorius. Fermentinio peresterinimo būdu gauti nauji riebalai, kurių riebalų rūgščių sudėtis yra tokia pat, kaip ir žmonių pieno riebalų. Peresterinimas turėjo įtakos riebalų oksidaciniam patvarumui.