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DEVELOPMENT OF ZERO *TRANS* BAKING SHORTENINGS BY ENZYMATIC INTERESTERIFICATION

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Summary. For development of zero *trans* baking shortenings enzymatic interesterification was applied. Both immobilized (Novozym 435, Lipozyme RM IM, Lipase PS "Amano" IM) and native (Lipase A "Amano" 12, Lipomod TM 34P) enzymes were applied as biocatalysts. Palm stearin was explored as a source of palmitic acid. The immobilized enzymes formed new asymmetric triacylglycerols containing palmitic acid. The interesterification process produced the zero *trans* fats with the solid fat content profiles closely matching those of the commercial baking shortening. Not only did the novel structured lipids have comparable physical properties with the commercial baking shortening, but also offered additional health benefits. Thus enzymatic interesterification with immobilized lipases offered great potential in production of fats with expected properties.

Key words: canola oil, palm stearin, enzymatic interesterification, baking shortening, *trans* isomers

INTRODUCTION

Baking goods provide 40–50% of all *trans* fatty acids in human diet. With new labeling regulation it is necessary to eliminate hydrogenated *trans*-containing fats from baking shortenings. Practically *trans* fatty acids are not formed during interesterification, and thus the process has become a viable alternative to hydrogenation to produce specific fats.

The chemistry of interesterification was extensively discussed by Marangoni and Rousseau [1995]. The present and potential applications of interesterification to the strategies of fat modifications have also been reviewed [Gunstone 1998, Xu 2000].

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There are several published papers dealing with formulation of zero *trans* fats by enzymatic interesterification using palm oil and its fractions with other vegetable oils. Ghosh and Bhattacharyya [1997] interesterified palm stearin with sunflower, soybean and rice bran oils utilizing *Mucor miehei* lipase. They reported interesterified products appropriate for potential use as *trans*-free and polyunsaturated fatty acid-rich shortenings and margarine fat bases. Chu et al. [2001] studied the interesterification of palm stearin and palm kernel olein mixture using Lipozyme IM 60. The lipase--interesterified blend was found to be more β'-tending than the commercial samples. However, the authors concluded that the mixture still needed to be optimized to meet its function as a shortening. Lee et al. [2008] studied lipase-catalyzed solid fat with palm stearin and fully hydrogenated soybean and rapeseed oils. The physical properties of the final products showed lower melting points and solid fat content, desirable crystal polymorphism as well as reduction in tocopherols content and a reduced oxidative stability. Reshma et al. [2008] reported interesterification of palm stearin and rice bran oil mixtures using immobilized *Thermomyces lanuginosus* lipase. Their results demonstrated the commercial feasibility of producing new zero *trans* fats with the desired solid fat content profiles to address the environment and health concern of the consumers.

In this paper the use of the palm stearin and canola oil mixture has been described to obtain the high stability zero *trans* structured lipids. Palm stearin in this mixture is the source of palmitic acid, which assures the desired form of lipids crystallization [deMan 1992]. Canola oil in turn has a proper ω-6/ω-3 acids ratio and delivers oleic acid, which is stable towards oxidation and has beneficial effects on human health [Ahmadi and Marangoni 2009].

The main aim of this study was to obtain zero *trans* structured lipids with physical properties similar to the commercial baking shortening. Consequently the suitability of the enzymes used as biocatalysts has been assessed. To the best of Authors' knowledge, the majority of enzymes studied in the present work (Novozym 435, Lipase PS "Amano" IM, Lipase A "Amano" 12 and Lipomod TM 34P) have never been applied as biocatalysts for baking shortenings formulation before. The functionality of the final products has been assessed on the basis of solid fat content, triacylglycerol composition and fatty acid composition of developed structured lipids.

MATERIALS AND METHODS

Materials

Palm stearin (PS), commercially refined, bleached and deodorized regular canola oil (CAN) and the commercial baking shortening were donated by Richardson Oilseed Processing (Lethbridge, Canada). The mixture 25% PS + 75% CAN was prepared by weight.

Lipozyme RM IM and Novozym 435 were donated by Sigma-Aldrich Chemical Co. (St Louis, MO, USA). Lipomod TM 34P was donated by Biocatalysts Ltd (Cardiff, Wales,

UK). Lipase PS "Amano" IM and Lipase A "Amano" 12 were purchased from Amano Enzyme USA Co., Ltd (Elgin, IL, USA).

Interesterification

Vials containing palm stearin and canola oil mixture were flushed with nitrogen and placed in a hybridization oven (Tek Star, Greensboro, NC, USA). The interesterification reactions were performed under the optimal conditions for particular enzymes: 50°C and 8 h for Lipase PS "Amano" IM, 60°C and 8 h for Lipozyme RM IM, 80°C and 4 h for Novozym 435, 50°C and 4 h for both Lipase A "Amano" 12 and Lipomod TM 34P. After thermal equilibration at desired temperature, catalyst was added at 8% (wt/wt). The interesterification was performed with continuous shaking. After predetermined time the interesterification was stopped by removing the enzymes by filtration.

Solid fat content (SFC)

The solid fat content was measured by pulse nuclear magnetic resonance (p-NMR) with a Bruker PC/20 Series NMR Analyzer (Bruker Optics, Milton, ON, Canada) as specified in the AOCS Official Method Cd 16-81 [1993].

Triacylglycerol composition

The composition of triacylglycerols (TAG) was analyzed using reversed phase high performance liquid chromatography (RP-HPLC). Separation was performed on a Finnigan Surveyor liquid chromatograph (Thermo Electron Corporation, Waltham, MA, USA). Two columns connected in series (Gemini 110A, 250 × 3 mm, 5 μm; Phenomenex, Torrance, CA, USA) were used to separate TAG. The gradient elution was applied. 10 μL of a sample was injected and eluting components detected with evaporative light scattering detector (Sedex 75; Sedere, Alfortville, France), operated at 40°C with air pressure of 2.5 bar. TAG were identified by comparison of the retention times of authentic standards (Nu-Chek-Prep Inc., Elysian, MN, USA), calculated as relative retention time to triolein.

Fatty acid composition

Fatty acids were methylated prior to analysis by gas chromatography (GC) based on the AOCS Official Method Ce 1-62 [1993] and then analyzed on Trace GC Ultra gas chromatograph (Thermo Electron Corporation, Rodano, Italy) using a Trace TR-FAME fused silica capillary column (100 m × 0.25 mm, 0.25 μm; Thermo, Waltham, MA, USA). Hydrogen was used as carrier gas with flow rate of 1.5 mL/min. Splitless injection was used utilizing PTV injector. Detector temperature was set at 250°C. 1 μL of a sample was injected with AS 3000 autosampler (Thermo Electron Corporation, Rodano, Italy). Fatty acids were identified by comparison of retention time with authentic standards (standard mixture #617; Nu-Chek-Prep Inc., Elysian, MN, USA).

Statistical analysis

Data were analyzed by single factor analysis of variance (ANOVA) and regression analyses using Minitab 2000 statistical software (Minitab Inc., PA, ver. 13.2). Statistically significant differences between means were determined by Duncan's multiple range tests. Statistically significant differences were determined at $P < 0.05$ level.

RESULTS AND DISCUSSION

Solid fat content (SFC)

Changes in SFC as a function of temperature in the non-interesterified mixture, zero *trans* structured lipids and in the commercial baking shortening are illustrated in Figure 1. SFC profiles of the zero *trans* structured lipids produced by immobilized lipases (Novozym 435, Lipozyme RM IM and Lipase PS "Amano" IM) were the closest match to this of the commercial baking shortening. This pattern of SFC profiles as a function of temperature is characteristic of high stability fats which may be applied for frying, baking or as confectionary fats [Reddy and Jeyarani 2001]. Furthermore, they tended to have lower SFC if compared to non-interesterified mixture and showed a gradual slope with

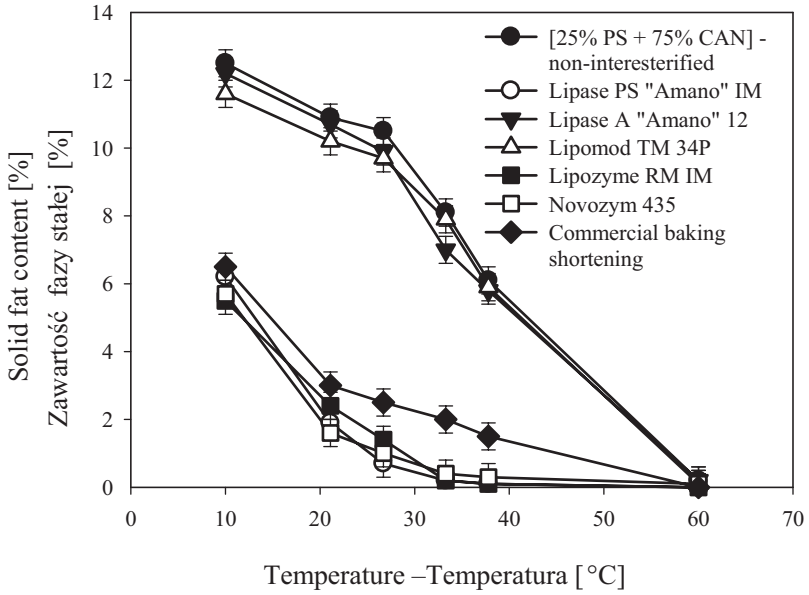

Fig. 1. Solid fat content as a function of temperature for fats studied before and after interesterification (PS – palm stearin, CAN – canola oil)

Rys. 1. Zawartość fazy stałej w funkcji temperatury w badanych tłuszczach przed przeestryfikowaniem i po przeestryfikowaniu (PS – stearyna palmowa, CAN – olej canola)

a wide plastic range from 10 to 60°C. These changes are caused by fatty acids rearrangement within and among the TAG molecules during the interesterification and creation of new lower melting TAG species [Kowalska and Gruczynska 2016, Kowalska et al. 2016]. The relatively low effect of interesterification by the native enzymes (Lipase A "Amano" 12, Lipomod TM 34P) on the SFC values was observed.

Triacylglycerol composition

The TAG composition as determined by RP-HPLC is shown in Table 1. The commercial baking shortening contained mainly LOO, LOP, OOO and POO which amounted to 73.8% of the total TAGs. The palm stearin and canola oil mixture before interesterification consisted of following TAG species: LnOO, LOO, LOP, OOO, POO, POP, PPP, and POS of which LOO, OOO and POP were in the highest amounts and constituted 64.8% in total. In the interesterification products obtained by the immobilized enzymes (Novozym 435, Lipozyme RM IM and Lipase PS "Amano" IM) new asymmetrical triacylglycerols occured containing palmitic acid in the molecules (PLLn, POLn). According to deMan [1992] the presence of such asymmetrical triacylglycerols (in which sn-1 and sn-2 or sn-2 and sn-3 positions are occupied by two saturated or two unsaturated fatty acids) favours desired polymorphic form β′. Comparing to the commercial baking shortening the interesterification structured lipids obtained with Novozym 435 had significantly lower content of LnOO, LOO, LOP, OOO and POO, and significantly higher content of POP and PPP. For the interesterification products by Lipozyme RM IM and Lipase PS "Amano" IM, significantly lower content of PPP and POS were noticed. PPP is one of the TAG species exhibiting a natural tendency to crystallize in an undesired polymorphic form β [deMan 1992]. Interesterification performed by Lipase A "Amano" 12 and Lipomod TM 34P resulted in less pronounced changes in triacylglycerol composition with the exception of LOO, LOP and POO which content was significantly lower and PPP that exhibited the opposite trend.

Fatty acid composition

The amounts of beneficial for human health unsaturated fatty acids in the interesterification products were higher than in the commercial baking shortening by about 10.0–33.0%. However, linoleic and linolenic acids are particularly susceptible to thermal and oxidative degradation. This fact indicates the importance of temperature effect on their deterioration during high temperature processes. At elevated temperatures non-volatile secondary oxidation products such as short-chain glycerol-bound aldehydes, acids, ketones and alcohols with potential detrimental health effects may be formed. As they remain in the fats, they are absorbed and subsequently ingested [Velasco et al. 2002]. As anticipated, *trans* fatty acids were not formed as a result of interesterification and their content in the structured lipids was as low as in the non-interesterified mixture and amounted to 0.5%. That is of a great importance from a nutritional point of view since the amount of *trans* isomers in fat affects the *trans* level in food products subjected to high temperature. The presence of *trans* isomers in the commercial shortening (8.2%) indicated that it contained partially hydrogenated fat (Table 2).

Table 1. Triacylglycerol composition of the commercial baking shortening and the palm stearin (PS) and canola oil (CAN) mixture before and after interesterification

Tabela 1. Skład triacylogliceroli handlowego szorteningu piekarskiego i mieszaniny stearyny palmowej (PS) z olejem canola (CAN) przed przeestryfikowaniem i po przeestryfikowaniu

Sample (fat) Próbka (tłuszcz)	Triacylglycerol composition [relative %] – Skład triacylogliceroli [%]*, **									
	PLLn	LnOO	POLn	LOO	LOP	OOO	POO	POP	PPP	POS
Commercial baking shortening Handlowy szortening piekarski	–	5.99 ±0.04^{a}	–	18.29 ±0.09^{a}	11.22 ±0.08^{a}	22.33 ±0.10^{a}	22.00 ±0.10^{a}	7.72 ±0.06^{c}	7.29 ±0.02^{c}	5.02 ±0.02^{a}
TAG of non-interesterified mixture – Triacyloglicerole mieszaniny fizycznej przed przeestryfikowaniem										
[25% PS + +75% CAN]	–	8.73 ±0.04^{a}	–	19.88 ±0.09^{a}	6.24 ± 0.02^{b}	29.02 ±0.11^{a}	5.51 ±0.04^{c}	15.88 ±0.04^{b}	8.98 ±0.05^{c}	1.26 ±0.02^{b}
TAG of interesterification products – Triacyloglicerole produktów przeestryfikowania										
Novozym 435	1.11 ±0.01^{a}	0.30 ±0.01^{b}	3.33 ±0.02^{a}	1.92 ±0.01^{c}	3.71 ±0.02^{c}	14.20 ±0.08^{b}	11.36 ±0.08^{b}	21.15 ±0.09^{a}	25.35 ±0.10^{a}	3.55 ±0.02^{a}
Lipozyme RM IM	0.50 ±0.01^{b}	5.94 ±0.04^{a}	2.96 ±0.02^{a}	17.29 ±0.08^{a}	11.46 ±0.08^{a}	20.76 ±0.09^{a}	22.76 ±0.10^{a}	6.72 ±0.04^{c}	2.82 ±0.01^{d}	0.67 ±0.01^{b}
Lipase PS “Amano” IM	0.47 ±0.01^{b}	5.77 ±0.04^{a}	2.92 ±0.02^{a}	16.14 ±0.08^{a}	10.71 ±0.08^{a}	20.72 ±0.09^{a}	23.70 ±0.09^{a}	6.50 ±0.04^{c}	2.68 ±0.02^{d}	0.64 ±0.01^{b}
Lipase A “Amano” 12	–	5.88 ±0.04^{a}	–	14.98 ±0.06^{b}	6.53 ±0.05^{b}	24.83 ±0.10^{a}	6.38 ±0.05^{c}	7.96 ±0.06^{c}	18.98 ±0.08^{b}	4.50 ±0.03^{a}
Lipomod TM 34P	–	7.09 ±0.04^{a}	–	15.79 ±0.08^{b}	6.67 ±0.04^{b}	22.29 ±0.10^{a}	4.74 ±0.03^{c}	5.96 ±0.04^{c}	15.93 ±0.07^{b}	6.90 ±0.04^{a}

*All values are averages of triplicate analyses from three repetitions – Podane wartości stanowią średnie z trzech analiz wykonanych w trzech powtórzeniach.

**Ln – linolenic, L – linoleic, O – oleic, P – palmitic, S – stearic acids – Kwasy: Ln – linolenowy, L – linolowy, O – oleinowy, P – palmitynowy, S – stearynowy.

Values with different superscript letters $^{(a-d)}$ within each column are significantly different at $P < 0.05$ level – Wartości różniące się indeksami górnymi $^{(a-d)}$ w kolumnach są statystycznie różne na poziomie istotności $P < 0{,}05$.

Table 2. Fatty acid composition of the commercial baking shortening and triacylglycerols of the palm stearin (PS) and canola oil (CAN) mixture before and after interesterification

Tabela 2. Skład kwasów tłuszczowych handlowego szorteningu piekarskiego i triacylogliceroli mieszaniny stearyny palmowej (PS) z olejem canola (CAN) przed przeestryfikowaniem i po przeestryfikowaniu

Sample (fat) Próbka (tłuszcz)	Fatty acid composition – Skład kwasów tłuszczowych [g/100 g]*								
	14 : 0	16 : 0	18 : 0	18 : 1 (9-cis)	18 : 2 (all-cis)	18 : 3 (all-cis)	20 : 0	20 : 1 (9-cis)	Σ *trans*
Commercial baking shortening Handlowy szortening piekarski	0.34 ±0.01^{a}	15.92 ±0.02^{a}	9.11 ±0.02^{a}	45.29 ±0.05^{b}	12.02 ±0.02^{b}	5.38 ±0.02^{b}	0.44 ±0.01^{a}	0.52 ±0.01^{b}	8.22 ±0.02^{a}
TAG of non-interesterified mixture – Triacyloglicerole mieszaniny fizycznej przed przeestryfikowaniem									
[25% PS + +75% CAN]	0.39 ±0.01^{a}	15.22 ±0.02^{a}	2.21 ±0.01^{b}	54.43 ±0.07^{a}	16.94 ±0.02^{a}	7.24 ±0.02^{a}	0.56 ±0.01^{a}	1.15 ±0.01^{a}	0.50 ±0.01^{b}
TAG of interesterification products – Triacyloglicerole produktów przeestryfikowania									
Novozym 435	0.38 ±0.01^{a}	15.44 ±0.02^{a}	2.82 ±0.01^{b}	54.77 ±0.05^{a}	15.98 ±0.02^{a}	7.07 ±0.02^{a}	0.54 ±0.01^{a}	1.27 ± 0.01^{a}	0.50 ±0.01^{b}
Lipozyme RM IM	0.39 ±0.01^{a}	15.29 ±0.02^{a}	2.60 ±0.01^{b}	54.99 ±0.05^{a}	16.02 ±0.02^{a}	7.09 ±0.02^{a}	0.55 ±0.01^{a}	0.96 ± 0.01^{a}	0.52 ±0.01^{b}
Lipase PS “Amano” IM	0.28 ±0.01^{a}	15.21 ±0.02^{a}	2.92 ±0.01^{b}	54.94 ±0.05^{a}	16.38 ±0.02^{a}	7.01 ±0.02^{a}	0.56 ±0.01^{a}	1.11 ±0.01^{a}	0.46 ±0.01^{b}
Lipase A “Amano” 12	0.38 ±0.01^{a}	15.99 ±0.02^{a}	2.65 ±0.01^{b}	55.55 ±0.05^{a}	15.72 ±0.02^{a}	7.08 ±0.02^{a}	0.56 ±0.01^{a}	0.98 ±0.01^{a}	0.45 ±0.01^{b}
Lipomod TM 34P	0.29 ±0.01^{a}	15.29 ±0.02^{a}	2.72 ±0.01^{b}	54.92 ±0.05^{a}	15.99 ±0.02^{a}	7.44 ±0.02^{a}	0.56 ±0.01^{a}	1.10 ±0.01^{a}	0.47 ±0.01^{b}

* All values are averages of triplicate analyses from three repetitions – Podane wartości stanowią średnie z trzech analiz wykonanych w trzech powtórzeniach.

Values with different superscript letters $^{(a-b)}$ within each column are significantly different at $P < 0.05$ level – Wartości różniące się indeksami górnymi $^{(a–b)}$ w kolumnach są statystycznie różne na poziomie istotności $P < 0{,}05$.

CONCLUSIONS

Enzymatic interesterification by Novozym 435, Lipozyme RM IM and Lipase PS "Amano" IM offered efficiency in production of novel zero *trans* structured lipids that might be applied as baking shortenings. Not only did the interesterification products have comparable solid fat content profiles with the commercial baking shortening, but also offered additional human health benefits as absence of *trans* isomers and higher content of oleic, linoleic and linolenic acids. However further studies should be aimed at development of economically viable method of introducing immobilized enzymes in production of structured lipids as shortenings for high temperature applications.

OTRZYMYWANIE SZORTENINGÓW PIEKARSKICH ZERO *TRANS* METODĄ PRZEESTRYFIKOWANIA ENZYMATYCZNEGO

Streszczenie. Szorteningi piekarskie zero *trans* otrzymywano metodą przeestryfikowania enzymatycznego. Katalizatorami reakcji były zarówno enzymy immobilizowane (Novozym 435, Lipozyme RM IM, Lipase PS "Amano" IM), jak i natywne (Lipase A "Amano" 12, Lipomod TM 34P). Źródłem kwasu palmitynowego była stearyna palmowa. W lipidach strukturyzowanych powstających w obecności lipaz immobilizowanych występowały nowe niesymetryczne triacyloglicerole zawierające w cząsteczkach kwas palmitynowy. W wyniku przeestryfikowania otrzymano nowe tłuszcze zero *trans* o profilach zawartości fazy stałej zbliżonych do handlowego szorteningu piekarskiego przygotowanego z dodatkiem tłuszczów częściowo uwodornionych. Otrzymane tłuszcze miały nie tylko właściwości fizyczne i chemiczne zbliżone do handlowego szorteningu piekarskiego, ale również oferowały dodatkowe korzyści zdrowotne. Przeestryfikowanie enzymatyczne z wykorzystaniem enzymów immobilizowanych może zatem stanowić potencjalną metodę otrzymywania tłuszczów o programowanych właściwościach.

Słowa kluczowe: olej canola, stearyna palmowa, przeestryfikowanie enzymatyczne, szortening piekarski, izomery *trans*

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