APPLICATION OF ENZYMATIC INTERESTERIFICATION IN LIPID BLENDS WITH PALM OLEIN

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ABSTRACT

Interesterification is linked to the search for lipid fractions with the absence of trans fatty acids. This process can be either selective or random with respect to the positioning of fatty acids in the triacylglycerol molecule. The aim of this study was to develop enzymatically interesterified fats using Lipozyme® *TL IM*, derived from blends of palm olein (PO) and fully hydrogenated palm oil (FHPO) and to evaluate their chemical composition and physical properties. Blends were prepared in the following proportions: PO:FHPO 100:0, 80:20, 60:40, 40:60, 20:80 and 0:100 (w:w). The blend samples were heated to 70 °C and then 4% (w/w) of the conditioned enzyme was added. Fatty acid composition was determined for the raw materials; while triacylglycerol composition and thermal behavior were analyzed in the blends before and after the interesterification process. As a result, there was a decrease in trisaturated and monosaturated triacylglycerols, accompanied by an increase in disaturated triacylglycerols. Thermal behavior analysis indicated changes in physical properties, including reduction in initial crystallization and final melting temperatures of the interesterificated fats. These analyses confirmed the efficacy of the process in developing fats with different technological profiles.

Keywords: Classes of triacylglycerols. Differential Scanning Calorimetry. Zero trans. Crystallization.

1 INTRODUCTION

The food and oleochemical industries utilize lipases in more sustainable processes. Lipases catalyze interesterification reactions that modify the physical properties of oils or lipid blends by repositioning fatty acids within triacylglycerols molecules. This rearrangement forms new triacylglycerols, there by modifying the triacylglycerol composition, and resulting in a product with different characteristics from the original blend, without the formation of trans fatty acids.[1,2]

In oils and fats, the Lipozyme® *TL IM* is used in enzymatic interesterification, promoting the preferential, though not exclusive, rearrangement of fatty acids in the *sn-1,3* position. This enzyme has been used in the development of technical fats, also known as plastic fats, suitable for a wide range of foods applications including margarines, breads, cookies, chocolates and ice creams. For the development of these fats, a lipid source rich in saturated fatty acids and another rich in unsaturated fatty acids are required as substrates.[3,4]

Two raw materials that can be used in enzymatic interesterification are palm olein and fully hydrogenated palm oil. Palm olein, derived from palm oil fractionation and rich in unsaturated fatty acids, has historically been primarily used in frying applications and is currently losing market share to oils high in oleic acid. Fully hydrogenated palm oil, also known as palm hard fat, is a fully saturated product obtained through total hydrogenation of palm oil. Its use is limited to chemical interesterification and can also be added in small quantities to assist fats crystallization mechanism.[5,6]

The objective of this work was to develop enzymatically interesterified fats, using palm olein (PO) and fully hydrogenated palm oil (FHPO) blends, and to evaluate the effectiveness of the interesterification process.

2 MATERIAL & METHODS

Raw materials and preparation of blends: Palm olein (PO) and fully hydrogenated palm oil (FHPO) were supplied by AGROPALMA® and Lipozyme® *TL IM* (*Thermomyces lanuginosus*) was supplied by Novozymes®. Initially, PO and FHPO were weighed, completely melted and homogenized in the following proportions PO:FHPO =100:0, 80:20, 60:40, 40:60, 20:80 and 0:100 (w/w). The blends were interesterified and were analyzed before and after the reactions.

Enzymatic interesterification (EI) and enzymatic conditioning: For interesterification, the simple blends were heated to 70 °C under stirring at 350 rpm. Then, 4% (w/w) of the conditioned enzyme was added with the residence time is 6 h, the optimized point of the base study. Enzymatic conditioning was carried out by adding the percentage of enzyme to the substrate (commercial soybean oil) at 70 °C, at 350 rpm for 30 min under vacuum.[7] Then, aliquots were removed to monitor acidity, and the substrate was changed until the acidity level of the soybean oil was less than 0.5%, according to the Ca 5 a-40 method.

Fatty acid composition, triacylglycerol composition and thermal behavior: The fatty acid composition was determined by gas chromatography using an Agilent 6850 GC USA chromatograph (Santa Clara, CA, USA) immediately after esterification. Fatty acid methyl esters were separated using the Ce 1f-96 method. The triacylglycerol composition was determined using the Ce 5-86 method. Thermal analysis of the formulations occurred by differential scanning calorimetry (DSC), using TA Instruments

equipment, model Q2000 V4.7A, coupled to an RCS90 refrigeration blend (New Castle, USA), following the Cj 1-94 method.[8,9] Analyses were performed in triplicate.

3 RESULTS & DISCUSSION

They major fatty acids for PO the oleic acid C18:1 (43.3%), palmitic acid C16:0 (37.5%), linoleic acid C18:2 (10.8%) and stearic acid C18:0 (4.4%), with a total of 44.3% saturated fatty acids and 55.7% unsaturated fatty acids. As for FHPO, C18:0 (55.0%) and C16:0 (42.0%) were identified. The results agree with Normative Instruction (NI) nº 87, of 2021, and with what is reported in the literature.[10,11]

Table 1 Triacylglycerol composition of PO:FHPO blends before and after interesterification.

NC	TAG	Before interesterification (PO:FHPO%)						After interesterification (PO:FHPO EI %)					
		100:0	80:20	60:40	40:60	20:80	0:100	100:0	80:20	60:40	40:60	20:80	0:100
C48	PPP	0.54	2.23	3.80	5.34	6.71	8.00	6.65	7.56	8.22	9.01	9.47	10.59
	MOP	1.70	1.32	0.90	0.69	0.27	-	1.31	1.23	1.06	0.78	0.33	-
C50	PPS	3.56	8.59	17.00	25.24	32.78	39.29	2.00	7.30	12.56	18.40	24.35	30.46
	POP	26.95	24.57	18.33	12.05	6.14	-	21.39	18.20	14.44	10.16	5.75	-
	PLP	8.57	7.27	5.41	3.53	1.39	-	6.93	5.92	4.99	3.84	2.69	-
C52	PSS	1.09	8.72	17.16	25.78	33.76	40.81	0.33	3.04	6.95	13.84	22.45	33.62
	POS	8.95	4.55	3.36	2.23	1.05	-	4.25	11.20	14.72	14.44	9.76	-
	POO	23.48	22.26	16.68	10.98	5.17	-	21.44	14.31	8.31	3.89	1.27	-
	PLS	-	-	-	-	-	-	1.37	3.47	4.88	5.27	4.56	-
	POL	10.08	7.81	6.03	-	1.82	-	12.54	8.51	5.40	2.74	1.01	-
	PLL	1.97	1.44	0.74	-	-	-	2.10	1.37	0.85	0.46	-	-
C54	SSS	-	2.34	4.75	6.95	9.31	11.26	-	1.95	1.37	3.43	7.34	12.85
	SOS	-	-	-	-	-	-	0.48	4.19	4.00	5.23	4.63	-
	SOO	3.02	2.22	1.64	1.22	0.50	-	2.15	3.92	4.16	2.67	1.20	-
	OOO	5.90	3.37	2.62	1.39	0.57	-	8.32	2.32	2.83	2.30	2.92	-
	SOL	-	-	0.84	-	-	-	6.10	3.17	2.60	1.88	0.95	-
	OOL	2.14	0.80	-	-	-	-	1.77	-	1.49	0.26	-	-

Abbreviations: NC: Number of carbons, EI: enzymatic interesterification, M: myristic acid, P: palmitic acid, La: lauric acid S: stearic acid, O: oleic acid, L: linoleic acid (-) unidentified

The major triacylglycerols (TAGs) of PO were: POP (26.95%), followed by POO (23.48%), POL (10.08%), POS (8.95%) and PLP (8.57%). For FHPO, the main TAGs found were PSS (40.81%), PPS (39.29%), SSS (11.26%) and PPP (8.00%). Like those described in the literature for the raw materials used[12,13]. For blends, as expected, the TAGs POP and POO predominated for the lowest concentrations of FHPO. As the concentration of PO decreases in the blends, the contents of PSS and PPS increase and the contents of in POP and POO decrease.

For interesterified blends, the complexity of molecular composition resulting from interesterification promoted a more uniform and diverse distribution among TAG species. This includes the emergence of new TAGs and alteration in the levels of existing ones. Interesterification increased the content of POS and PPP, while reducing POO and POP levels. New TAGs such as PLS, SOS and SOL were formed during the process.

Based on the triacylglycerols composition, acyl migration may have ocurred during interesterification, which can lead to an increase in saturated fatty acids at the *sn-2* position. Acyl migration has been reported in other studies.[14]

TAGs can be categorized into classes such as: trisaturated (SSS), disaturated (SUS or SSU), monosaturated (SUU) and triunsaturated (UUU). Figure 1 (a) shows the percentage distribution of these classes before and after enzymatic interesterification. In this work, the percentages of SSS (PPS and PSS) and SUU (POO) decreased while SUS (POS) increased suggesting that Lipozyme® *TL IM* may not have acted t in a completelly specific manner throughout the reaction. Based on the composition of the raw materials and reaction parameters, the formation of SSU/SUS was favored over the formation of SUU, the target triacylglycerol class in interesterification blends.

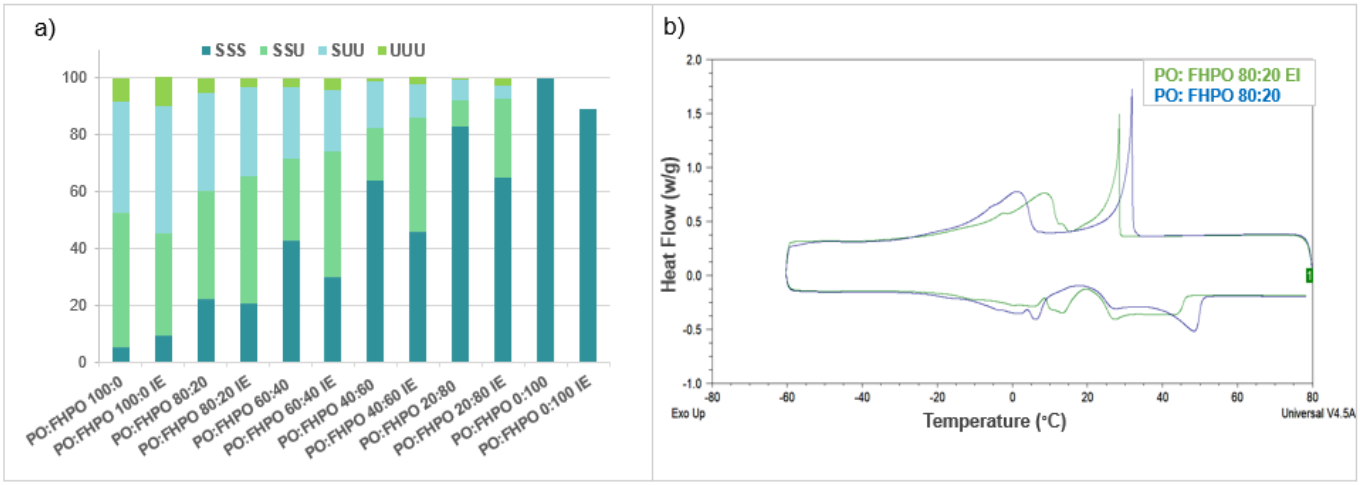

Figure 1 Triacylglycerol classes (a); trisaturated (SSS), disaturated (SUS or SSU), monosaturated (SUU) and triunsaturated (UUU) before and after enzymatic interesterification (EI) and (b) thermal behavior of the PO:FHPO 80:20 blend before and after EI.

The thermal behavior was determined, Figure 1 (b), for the blends before and after the reaction, examining the impact of molecular rearrangement on the resulting fats. Parameters such as initial temperature (Ti), final temperature (T f), maximum peak temperature (TMaxP), peak height (Ap) and enthalpy (ΔH) were evaluated for both crystallization (c) and melting (f) processes.

Generally, the first crystallization peak corresponds to trisaturated SSS and desaturated SUS while the second peak relates to monosaturated SUU and triunsaturated UUU. The modifications resulting from enzymatic interesterification were influenced by the decrease in SSS TAGs and primarily by the increase in SSU/SUS TAGs For crystallization (upper curve), interesterification caused a reduction in Ti and TMaxP (Figure 1 (b)) accompanied by the decrease in ΔH and Ap. In melting (lower curve), there was a decrease in Tf for most blends. Changes observed in the thermogram of thermal behavior and TAGs composition underscore the efficacy of enzymatic interesterification in these blends.

4 CONCLUSION

Zero trans fats were developed with different profiles through enzymatic interesterification using Lipozyme® *TL IM* lipase. The thermal behavior confirmed lipid modification in enzymatically interesterified blends, evidenced by the emergence of new peaks in thermal events with a decrease in initial crystallization and final melting temperatures. Interesterified fats derived from palm olein and palm hard fat offer a favorable cost-benefit ratio and are supported by the industrial availability of lipid sources, there by enhancing the technological applicability and valorization of lipid raw materials.

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