TERNARY SYSTEMS FOR OBTAINING ENZYMATICALLY INTERESTERIFIED LIPID BASES

Estefânia P. Rodrigues[1]*, Mayara R. Lopes[1], Juliana C. Hashimoto[1], Odalys G. Cabrera[1], Ana P. B. Ribeiro[1]

[1] Faculty of Food Engineering – State University of Campinas.
** Corresponding author's email address: estefaniapratesrodrigues@gmail.com*

ABSTRACT

The enzymatic interesterification process is an important industrial alternative to produce zero trans fats with different plasticity profiles. The objective of the work was to produce fats for specific applications with different plasticity profiles using fats normally used in industry: fully hydrogenated vegetable oils (hardfat) from soybeans and palm kernels and high oleic sunflower oil. The ternary mixtures were formulated in the following proportions (mass/mass): high oleic sunflower oil 90%: palm kernel hardfat 05%: soy hardfat 05% to high oleic sunflower oil 05%: palm kernel hardfat 45%: soy hardfat 45 %. The enzymatic interesterification was carried out with Lipozyme® *TL IM* and 4% mass of the conditioned enzyme was added to the ternary mixtures, carrying out the enzymatic interesterification for 6 h. Interesterification promoted modification of the triacylglycerol composition, with the formation of new groups of triacylglycerols. For thermal behavior, there was an increase in the initial crystallization temperature and enthalpy of the ternary mixtures, delaying the crystallization process. Given this, we conclude that interesterified ternary mixtures have different plasticity profiles and can be used in a wide variety of products in the food industry.

Keywords: Zero trans fats. Enzymatic Interesterification. Ternary Mixtures.

1 INTRODUCTION

Fats are used for a wide range of products in the food, cosmetics and pharmaceutical industries, providing texture, sensory properties, and physical properties that consumer acceptance.[1,2]

The mixing method is widely used to develop specific fatty products sensory properties. The consistency and crystallization behavior of the products is determined by the phase behavior in the mixtures, which is affected by factors such as the molecular weight of the is triacylglycerols (TAG) and polymorphism, for example. However, the mixing process is limited in terms of obtaining new lipid bases, as the triacylglycerol composition is kept unchanged.[3, 4, 5]

The interesterification process represents an important alternative for lipid modification, especially enzymatic interesterification. In this process, two reactions occur: the partial hydrolysis of TAGs and the resynthesis of esters. Consequently, there will be the reorganization of fatty acids within a TAG or exchange of fatty acids between TAGs.[7] The lipases used can present selectivity (selective quality or characteristic) and regiospecificity, and due to this selectivity, it is possible to control the redistribution of fatty acids, obtaining lipids matrices with pre-determined TAG composition. From the use of sn - 1,3 lipases, fatty acid exchanges will only occur in positions 1 and 3 of the TAGs.[8,9]

The products obtained from the total hydrogenation of oils and fats, hardfats, are fully saturated products and can be used as raw materials to obtain interesterified fats. They are characterized by high melting point, low cost and different compositions. Fully hydrogenated soybean oil and fully hydrogenated palm kernel oil have been used in the production of interesterified fats aiming different functionalities required for food applications. The objective of this work was to obtain and characterize enzymatically interesterified fats, formulated with fully hydrogenated soybean and palm kernel oils and high oleic sunflower oil in different proportions, targeting lipid bases with different plasticity profiles.

2 MATERIAL & METHODS

The raw materials used were fully hydrogenated palm kernel oil – FHPkO – supplied by Agropalma; fully hydrogenated soybean oil – FHSO – supplied by Cargill; high oleic sunflower oil – HOSO – supplied by Cargill and Lipozyme® *TL IM* supplied by Novozymes. The ternary mixture for enzymatic interesterification was constituted by the HOSO:FHSO:OPETH fractions, in the following percentages: **IE HARDFAT** : 50% FHPkO + 50 FHSO; **IE OIL** : 100% HOSO; **IE90** : 90% HOSO + 5% FHPkO + 5% FHSO; **IE80** : 80% HOSO + 10% FHPkO + 10% FHSO; **IE70** : 70% HOSO + 15% FHPkO + 15% FHSO; **IE60** : 60% HOSO + 20% FHPkO + 20% FHSO; **IE50** : 50% HOSO + 25% FHPkO + 25% FHSO; **IE40** : 40% HOSO + 30% FHPkO + 30% FHSO; **IE30** : 30% HOSO + 35% FHPkO + 35% FHSO; **IE20** : 20% HOSO + 40% FHPkO + 40% FHSO and **IE10** : 10% HOSO + 45% FHPkO + 45% FHSO.

3 First, the enzyme was conditioned using high oleic sunflower oil, following the specifications indicated by the manufacturer. This step reduces the initial moisture content of the enzyme, to reduce the production of FFA, MAG and DAG by hydrolysis of TAGs during the reaction, Martinez (2022).[10] The determination of the acidity of the oil and the conditioning process were carried out until a maximum of 0.5% oleic acid was obtained in the sample. The process was carried out as follows: **1)** deaeration: oil at

70 °C, with addition of the enzyme at 450 rpm/vacuum; **2)** drying: with high oleic sunflower oil at 70 °C at 450 rpm/vacuum; **3)** determination of acidity: final acidity of the oil <0.5% free fatty acids; **4) c**entrifugation and oil removal.

Enzymatic interesterification was done according to Martinez (2022).[10] After mixing the fractions, the samples were heated to 70 °C with stirring at 300 rpm in the Hei-Tec P/N 505-30000-00 system, coupled with a Pt 1000 temperature sensor (Heidolph, Germany), followed by the addition of 4% (m/m) of the conditioned enzyme (Lipozyme® *TL IM*) under N_2. The reaction time was 6 h at 70 °C.

The analytical methodologies followed the protocols and conditions recommended by the official methods of the American Oil Chemists' Society – AOCS.[11] The enzymatically interesterified mixtures were characterized in terms of triacylglycerol composition, according to AOCS Ce 5-86[11] and identification of TAG groups were carried out by comparing retention times,[12] thermal behavior was determined according to AOCS Cj 1-94[11], using the following parameters for evaluating results: initial crystallization and melting temperatures (Ti_c and Tf_f), peak crystallization and melting temperature (Tp_c and Tp_f), enthalpy of crystallization and melting (ΔH_i and ΔH_f) and final crystallization and melting temperatures (Tf_c and Tf_f).[13]

4 RESULTS & DISCUSSION

The TAGs are esters of a glycerol molecule and three fatty acid molecules, each fatty acid can have a different number of carbon atoms. In this way, TAGs are classified by the parameter number of carbons (NC), which refers to the sum of the number of carbon atoms in the acyl chains of fatty acids.[14] The TAG compositions for raw materials and enzymatically interesterified ternary mixtures were determined according to the number of carbons in the chain and expressed as a percentage (%). For IE Oil, new groups were formed, such as C34 to C48, even in small quantities. The groups with the highest percentage were C52 and C54, with 23.27% and 73.67%, respectively. For IE Hardfats, groups C32 to C56 were formed. The predominant TAG groups were C42 with 19.46% and C48 with 20.84 %.

For the interesterified mixtures, new TAGs were formed as expected, but the groups that are the majority in the original raw materials prevail. The groups with the highest quantity were C42, C44, C46 and C48, with a significant presence of groups C52 and C54.

Figure 1 A and B show the crystallization profile of the ternary mixtures after interesterification with Lipozyme® *TL IM* after 6 h of reaction with the parameters of interest for evaluation: initial crystallization temperature (Ti_c), crystallization peak temperature (Tp_c), final crystallization temperature (Tf_c), peak height (Ap_c) and enthalpy of crystallization (ΔH_c).

For IE Hardfats, Ti_c and Tfc were 37.21 °C and - 4.71 °C, respectively. The difference between the crystallization curves highlights the change in the crystallization phenomena caused by the reduction in the TAGs with three saturated fatty acids (represented as SSS) and the increase in the TAGs with two saturated and one unsaturated fatty acids (represented as SSU) and TAGs with one saturated and two unsaturated fatty acids (represented as SUU).

In relation to IE Oil, the results for the crystallization parameters had no difference compared to the simple raw material. There were three crystallization peaks for mixtures IE80 to IE30; mixtures IE90 and IE20 gave 2 crystallization peaks and IE10 one crystallization peak. There is an increase in Tic with the increase in hardfats in the mixtures. While IE90 crystallized at a temperature of 4.25 °C, the IE10 mixture began crystallizing at 35.57 °C.

In relation to Tp_c, according to the incorporation of hardfats in the mixtures, IE90 showed a Tpc of 0.65 °C and IE10 equal to 33°C. Tif and Tic values were higher as the concentration of hardfats in the mixtures increased. ΔHc represents the energy required for crystallization, and this parameter was shown to be proportional to the hardfat content in the original and interesterified mixtures. The IE90 mixture, with 10% of the hardfats, required 2.52 (J/g) while IE10, with 90% of hardfat, required 83.04 (J/g).

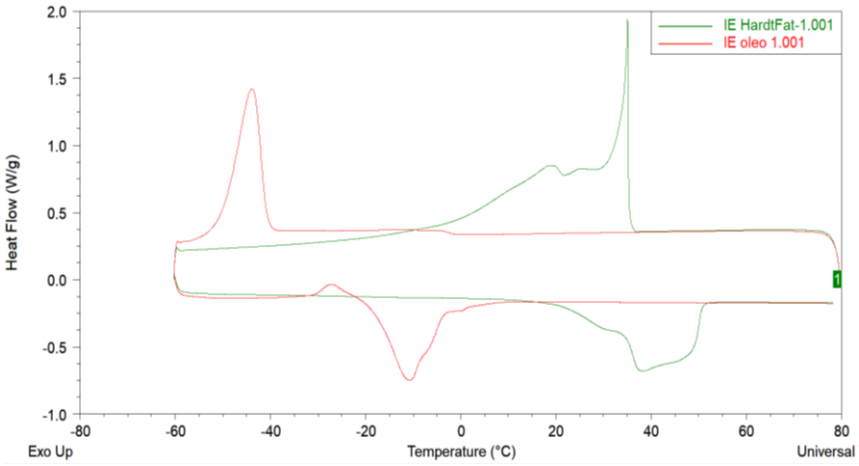
Figure 1 A and B show the melting profile of the ternary mixtures after the interesterification catalyzed by Lipozyme® *TL IM* after 6 h of reaction with the parameters of interest for evaluation: initial melting temperature (Ti_f), melting peak temperature (Tp_f), final melting temperature (Tf_f), peak height (Ap_f) and enthalpy (ΔH_f).

IE Hardfats presented only one melting peak, with Ti_f equal to 20 ºC and Tf_f equal to 52 ºC, similar to the corresponding simple mixtures. The melting events for HOSO and for IE Oil, were characteristic of oils, due to the high content of unsaturated TAGs, whose melting points are low.

There two melting peaks for the mixtures IE50 to IE20; the first peak corresponds to the fraction with a low melting point and the second peak corresponds to the TAGs with a higher melting point. The melt profiles were relatively simple and correspond ed closely to the composition TAGs. Tif, increased, as there was an increase in the proportions of FHSO and FHPkO in the mixtures. As occurred in crystallization, the behaviors found in melting are related to the TAG composition of the mixtures.

There is an increase in Tp_f as hardfats are incorporated into the mixtures. Only small differences in Tp_f were observed in relation to enzymatically intersterified mixtures. The lowest ΔH_f values were associated with the mixture with a greater amount of HOSO, which is associated with less heat release during fat solidification.

A



B

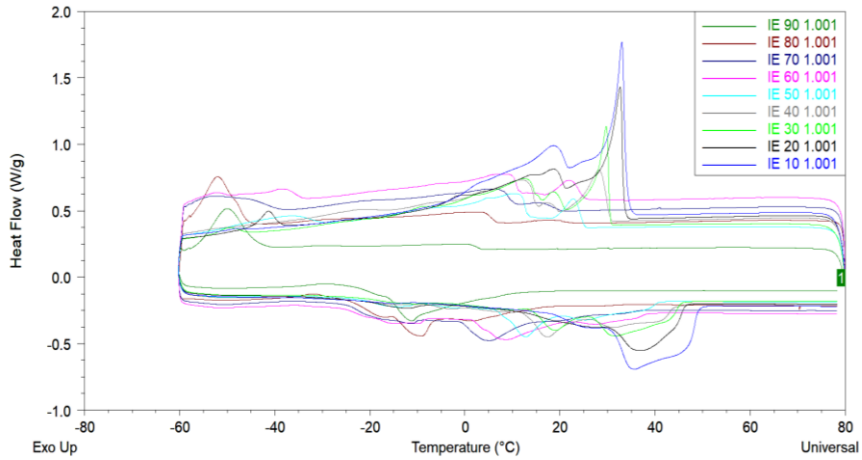


Figure 1 Crystallization and melting curves obtained by differential scanning calorimetry (DSC) for interesterified raw materials enzymatically (A) and for enzymatically interesterified ternary mixtures (B).

5 CONCLUSION

In conclusion, the enzymatic interesterification of ternary mixtures of fats and oils is a viable alternative to produce new lipid bases with different plasticity profiles to be used in the food industry.

REFERENCES

[1] BAYES-GARCIA, L., CALVET, T., ANGEL CUEVAS-DIARTE, M., UENO, S., SATO K. 2015. J. Phys. Chem. B, 119 (12), 4417- 4427.
[2] MARANGONI, A. G., ACEVEDO, N., MALEKY, F., CO, E., PEYRONEL, F., MAZZANTI, G., PINK D. 2012. Soft Matter, 8 (5), 1275 -1300.
[3] LIU, C., MENG, Z., CHAI, X., LIANG, X., PIATKO, M., CAMPBELL, S., LIU, Y. 2019. Food Chem, 286, 636 - 643.
[4] WATANABE, S., YOSHIKAWA, S., ARISHIMA, T., SATO, K. 2018. JAOCS, 95 (4). 447- 460.
[5] ZHANG, L., UENO, S., SATO, K. 2018. J. Oleo Sci, 67. 679-687.
[6] WIRKOWSKA-WOJDYLA, M., BRYS, J., GÓRSKA, A., OSTROWSKA-LIGEZA, E. 2016. LWT. 74, 99 –105.
[7] RONNE, T. H., YANG, T., UM, H., JACOBSEN, C., XU, X. 2005. J. Agric. Food Chem., v. 53, 5617- 5624.
[8] XU, X., GUO, Z., ZHANG, H., VIKBJERG, A. F., DAMSTRUP, M. L. 2006. Chemical and enzymatic interesterification of lipids for use in food. *In*. GUNSTONE, F. D. Modifying Lipids for Use in Food. Woodhead Publishing. 234 – 272
[9] GHOTRA, B. S., DYAL, S. D., NARINE, S. S. 2002. Int. Food Res. Ontario, v.35, 1015 -1048.
[10] MARTINEZ, A. P. P. B. 2022. Optimization of the enzymatic interesterification reaction in a system batch for high melting point model lipid matrice. Master's. University of Campinas, São Paulo. 248.
[11] AMERICAN OIL CHEMISTS' SOCIETY - AOCS. 2009. Official methods and recommended practices of the American Oil Chemists Society. AOCS (ed) 5th ed. AOCS Press, Urbana.
[12] ANTONIOSI-FILHO, N., MENDES, O. L, LANÇAS, F. M. 1995. Chromatogr. v. 40, p. 557– 562.
[13] CAMPOS, R. 2005. Fat Crystal Networks. *In:* Fat Crystal Networks. New York: Marcell Dekker Inc., 267– 349.
[14] DOMODARAN, S. 2019. Lipídios. *In:* Química de alimentos de Fennema. 5. Ed, Artmed (ed), Porto Alegre1104.

ACKNOWLEDGEMENTS

The authors acknowledge the financial support received from the National Council for Scientific and Technological Development (CNPq), process numbers: 140425/2022-5 and 310936/2021-7, at the State University of Campinas (UNICAMP).