




Zero-trans fats designed by enzyme-catalyzed interesterification of rice bran oil and fully hydrogenated rice bran oil

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Received 2 July 2021 – Accepted 20 September 2021

Abstract – Zero-trans edible fats attractive to be used for shortenings or margarines were designed solely from rice bran oil (RBO). For this purpose, RBO was fully hydrogenated, blended with the original oil at different percentages, and finally, blends were interesterified by an enzyme-catalyzed process. The interesterification process reduced the concentration of trisaturated and triunsaturated triglycerides and increased the concentration of medium saturation degree molecules, thus increasing their compatibility and causing the moderation of the melting point, as compared with blends. Conversely to blends, products showed a high tendency to crystallize under the β' polymorph, which is the preferred one for products destined for many edible applications. Results demonstrated that the proper combination of different technologies (total hydrogenation, blending and interesterification) is a versatile and useful technology for designing zero-trans fats from RBO, attractive for the confection of shortenings or margarines for different applications depending on the proportion of each component in the starting blend. This strategy offers an attractive alternative for the diversification of RBO utilization, a valuable vegetable oil still underexploited, providing attractive fats useful for structuring different type of foods.

Keywords: rice bran oil / enzyme-catalyzed interesterification / total hydrogenation / margarines / shortenings

Résumé – Graisses zéro-trans conçues par inter-estérification enzymatique de mélanges d'huiles de son de riz native et totalement hydrogénée. Des graisses alimentaires zéro-trans pouvant être utilisées comme matière grasse ou dans des margarines ont été élaborées uniquement à partir d'huile de son de riz (RBO). Ainsi, une huile de son de riz a été totalement hydrogénée, puis mélangée dans différentes proportions à l'huile de départ, et finalement, les mélanges ont été inter-estérifiés par voie enzymatique. Le processus d'inter-estérification a réduit la concentration en triglycérides homogènes (saturés et insaturés) et a accru la concentration en molécules de degré de saturation moyen, améliorant ainsi leur compatibilité et modérant le point de fusion, par rapport aux mélanges. À l'inverse des mélanges, les produits ont montré une forte tendance à cristalliser en polymorphe β' , le plus adapté aux produits destinés à de nombreuses applications alimentaires. Les résultats ont démontré que la combinaison adéquate de différentes technologies (hydrogénation totale, mélange et inter-estérification) représente une solution polyvalente et utile pour fabriquer des graisses zéro-trans à partir de RBO, intéressantes pour la confection de matières grasses ou de margarines, et ce pour diverses applications selon la proportion de chaque composant dans le mélange de départ. Cette stratégie offre une alternative intéressante pour la diversification de l'utilisation de l'huile de son de riz, une huile végétale précieuse encore sous-exploitée, en fournissant des graisses intéressantes et utiles pour structurer différents types d'aliments.

Mots clés : huile de son de riz / inter-estérification enzymatique / hydrogénation totale / margarines / matières grasses

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1 Introduction

Several nutritional studies have demonstrated the direct relationship between the intake of *trans* fatty acids (TFA) and the increase in the serum levels of low-density lipoproteins (LDL) and the decrease of high-density lipoproteins (HDL), thus notably increasing the risk of suffering cholesterol risk of cardiovascular diseases (Oteng and Kersten, 2020).

Considering this problem, the World Health Organization has recently launched the REPLACE program, which involves an action package providing a strategic approach to eliminating industrially produced TFA from national food supplies, with the goal of global elimination by 2023 (WHO, 2018).

Although undoubtedly such a program is necessary, it involves a significant challenge for the food industry. Under this scenario, the interesterification of blends of fats from different origins, which has been a convenient and versatile method for designing zero-*trans* edible fats, could offer an alternative technology (Kodali, 2005). Such technology usually involves the blending of liquid oil with fat that provides saturated fatty acids to the blend, responsible for conferring consistency to the interesterified product (Ribeiro *et al.*, 2009; Segura *et al.*, 2011).

Saturated fats from different origin have been successfully used for such purpose, like palm oil (Danthine *et al.*, 2014), palm stearin (Jennings and Akoh, 2010), beef tallow (Segura *et al.*, 2011), or some fully hydrogenated oils (Kloek *et al.*, 2000; Pacheco *et al.*, 2013; Ribeiro *et al.*, 2009). Unlike partially hydrogenated oils, the latter does not contain *trans* fatty acid as long as the process is efficiently continued until completion. Thus, fully hydrogenated oils are attractive raw materials to be used as the hard component of the blends to be interesterified, as mentioned above and reported by other researchers in the several following publications. The chemical interesterification of soybean oil with fully hydrogenated soybean oil was proposed to produce zero-*trans* fats suitable for different types of shortenings, depending on the ratio of each component in the blend (Ribeiro *et al.*, 2009).

More recently, a low *trans* margarine fat analogue to beef tallow was achieved by blending fully hydrogenated palm oil with soybean oil in a ratio 3:4 and processing the blend by lipase-catalyzed interesterification. Product properties were highly improved as compared with the starting blend, showing high potential in formulating a low *trans* fat margarine (Li *et al.*, 2018).

Among much work done exploring different alternatives for applying this technology, previous work regarding the use of rice bran oil (RBO) as the main raw material is very scarce (Segura and Jachmanián, 2020). However, RBO has several advantages due to its fatty acid composition as well as its nutritional properties. It is well known that RBO consumption has beneficial effects on human health, causing a cholesterol-lowering effect and a reduction in aortic fatty streaks (Chandrashekar *et al.*, 2014). Additionally, it was determined that the amount of cholesterol-lowering occurs to a greater extent than expected from the fatty acid composition of the oil, suggesting that other components in the oil were responsible for this effect (Xu *et al.*, 2001). The major components of vitamin E in rice bran are α -tocopherol, α -tocotrienol, γ -tocopherol and γ -tocotrienol. RBO also possesses about

3000 mg/kg of oryzanols, a family of 10 ferulate esters of triterpene alcohol (Xu *et al.*, 2001) reported possessing the capability of lowering cholesterol levels in serum (Chandrashekar *et al.*, 2014; Chung and Kang, 2020). Oryzanols components have too antioxidant functions because their structure includes ferulic acid, a potent antioxidant (Liu *et al.*, 2021). Despite having good nutritional composition and providing health benefits to humans, whereby it has been considered one of the most nutritious and functional oils in nature, RBO is still underutilized (Oi-Ming *et al.*, 2019).

Concerning the fatty acid composition of RBO, it has about 20% palmitic acid (Firestone, 2006), a sixteen-carbon chain length fatty acid that confers some heterogeneity to triacylglycerols (TAG) structure. It has been widely observed that the β' polymorphic form, required in fats destined to shortenings or margarines, is promoted by fats whose TAG contains different kinds of fatty acid moieties (Sato, 2001).

Considering those particular characteristics of RBO, in this work, we propose the blending of this oil at different proportions with fully hydrogenated rice bran oil (FHRBO), followed by the enzyme-catalyzed interesterification of the blend, for the obtention of zero-*trans* fats rich in the native bioactive compounds of RBO and with improved crystallization properties, thus attractive to be destined to the confection of zero-*trans* margarine and shortenings.

Enzymes, as naturally occurring biocatalysts, play an increasing role in developing green processes and environmentally benign production technology, particularly for food, as it holds a great promise to enable some processes under mild conditions that chemical catalysts cannot do (Feltes *et al.*, 2012; Pacheco *et al.*, 2013). Thus, considering those advantages, a lipase-catalyzed process was chosen for this work, considering that the milder reaction conditions required would help to preserve the valuable bioactive compounds from RBO (Segura and Jachmanián, 2020; Zhang *et al.*, 2021). Additionally, it is expected that bioprocessing will be applied much more extensively in RBO processing in the future as the development of enzyme techniques and the popularization of RBO progress (Jiang, 2019). Thus, this work proposes an attractive alternative to contribute in this direction.

2 Materials and methods

Refined rice bran oil (ARROZUR SA, Treinta y Tres, Uruguay) was acquired in the local market. Lipase from porcine pancreas was supplied by Sigma-Aldrich (PPL type II, activity equal to 11.6 U/mg, in μ mol of fatty acids hydrolyzed per minute per mg lipase). Lipozyme TL IM (from *Thermomyces lanuginosus*) was kindly provided by Novozymes, Denmark.

Organic solvents, analytical standards and reagents were supplied by Dexin S.R.L, Montevideo, Uruguay (representative of Sigma-Aldrich Company).

2.1 Hydrogenation

Hydrogenation of RBO was performed in a high pressure/high temperature 250 mL reactor (4570 HP/HT Parr Instrument Company, Illinois, USA). The reactor was equipped with a mechanical gas entrainment stirrer to favour

gas/liquid mass transfer phenomena, a 500 mL burette for controlling the amount of H₂ provided to the reactor, a heating jacket, pressure and temperature transducers and a Parr 4848 Reactor Controller. Reactions were performed under H₂ at 15 bar and 120 °C, using 5% Ni/SiO₂ catalyst (Pricat 9910, from Johnson Matthey, London, UK). After a reaction period of 3 hours, FHRBO was achieved, as verified by the analysis of the product by gas chromatography (GC), as described below. Finally, the product was recovered from the reactor and filtered inside an oven at 70 °C by passing through a filter paper (Whatman grade 5) to remove the catalyst, and stored until used.

2.2 Preparation of blends

Nine blends of RBO with 10 to 90% FHRBO (with 10% increments) were prepared by weighing the corresponding amount of each fat inside 15 mL screw cup vials to totalize about 10 g of the blend. Vials were subjected to a stream of nitrogen, hermetically closed and located inside an orbital shaker (Lab Companion, model SI-600) at 70 °C and 200 rpm during 15 min, for complete homogenization.

2.3 Interesterification reactions

Interesterification reaction was performed on pure RBO, or RBO blended with FHRBO. 7 g of the substrate was transferred to a screw cap tube, and 0.7 g of Lipozyme TL IM (previously dried under a vacuum at 60 °C for 30 min) were added. The tube was subjected to a stream of nitrogen, closed and placed inside the orbital shaker at 60 °C, shaker speed was set to 200 rpm and incubation prolonged for 24 h. After the incubation period, the enzyme was separated from products by filtration inside an oven at 70 °C and the lipid fraction kept under nitrogen at -20 °C until analyzed. This procedure was performed in duplicate and the products destined for independent analysis.

2.4 Fatty acid composition

Samples were treated with BF₃/MeOH (boron trifluoride solution in methanol) according to the AOCS method Ce 1b-89 (AOCS, 2017) to convert the triacylglycerols to the corresponding methyl esters. The esters were analyzed by GC, using an equipment Shimadzu GC-2010, equipped with FID and a capillary column Supelco SP2560 (100 m × 0.25 mm × 0.20 μm). The temperature program started at 175 °C, followed by a heating step (5 °C/min) to 220 °C, and remained at 220 °C for 60 min. Nitrogen at 150 kPa at column head was used as the carrier gas, with a split ratio 1:80. Fatty acid composition was determined in duplicate, and average results reported. The identification of the compounds was accomplished by comparing the retention times with that corresponding to the analysis of fatty acids methyl esters standards (provided by Sigma-Aldrich) under identical conditions.

2.5 Triacylglycerol (TAG) composition

The separation and quantitative determination of the triglycerides were performed using high-performance liquid chromatography (HPLC) following the procedure described

below, based on the AOCS Official Method Ce 5b-89 (AOCS, 2017). Samples were dissolved in acetone (5 mg/mL) and directly analyzed using an HPLC Shimadzu Prominence 20A (Shimadzu, Corporation, Kyoto, Japan), equipped with an evaporative light scattering detector Shimadzu ELSD-LTII, two columns Supelcosil TM C18 (25 cm × 4.6 mm × 5 μm). The analysis started delivering a flow rate of 1 mL/min of the mixture acetone/acetonitrile 50:50, followed by an increasing linear gradient of chloroform to achieve the ratio acetone/acetonitrile/chloroform 40:40:20 at 60 min. The solvent composition remained constant for 20 min, and finally returned to the starting composition at 85 min. Peaks were identified using pure TAG standards and considering the order of elution according to the equivalent carbon number (ECN). Two replicate analyses were performed, and the average values were reported.

2.6 Analysis of fatty acid composition at the *sn*-2 position

The nature of fatty acids in the *sn*-2 position of triacylglycerols was determined by hydrolysis with porcine pancreas lipase (PPL), according to the AOCS method Ch 3-91 (AOCS, 1997). The method involves the hydrolysis of oil with the *sn*-1,3-specific PPL, followed by the purification of the 2-monoacylglycerols (2-MAG) produced by thin-layer chromatography. The 2-MAG band is scrapped off, submitted to a derivatization reaction to methyl esters, and analyzed by GC. Two replicate analyses were performed, and average values reported.

2.7 Thermal behavior

Melting thermograms were determined by differential scanning calorimetry (DSC), using a calorimeter TA Q20 (TA Instruments), equipped with a refrigerated cooling system RCS90. The temperature was programmed according to the AOCS method Cj 1-94 (AOCS, 2017), which involves initial heating to 90 °C for the complete melting of the sample, followed by cooling at 10 °C/min to -60 °C, a tempering at -60 °C for 30 min, and a final heating step at 5 °C/min until complete melting. Data processing was performed using TA Universal Analysis 2000 software (version 3.9A). A high purity standard of metallic Indium provided by DSC manufacturer was used for the calibration of the equipment. Two replicate analyses were performed, and the average values reported.

2.8 Solid fat content (SFC)

The solid fat content was determined using a nuclear magnetic resonance (p-NMR) spectrometer (Bruker Minispec PC120) and a TCON 2000 high precision dry bath (0-70 °C) (Duratech, USA), according to the AOCS method Cd 16b-93 (AOCS, 2017). Sample readings were taken in series at 5 °C increments in the temperature range of 10-65 °C.

2.9 Polymorphism analysis

The polymorphic forms were determined by X-ray diffraction spectroscopy according to the AOCS method Cj 2-9593 (AOCS, 2017). Samples were completely melted and

spread over the glass X-ray slide and kept at the temperature to be analyzed (20 °C) for 24 h before the analysis. A Rigaku ULTIMA IV (Rigaku Corporation, Tokyo, Japan) theta-theta powder X-ray diffractometer with a copper X-ray tube was used for the analysis (Cu-K α , $\lambda = 1.5406 \text{ \AA}$). Polymorphic forms were determined according to diffraction patterns in the short spacing region, scanned from 10 to 30° at 0.04° steps with a rate of 10 s by step.

2.10 Statistical analysis

Data were subjected to variance analysis (ANOVA) using the statistical analysis software InfoStat, version 2020 (Di Rienzo *et al.*, 2020). Significant differences between mean values were compared with a Tukey's test with a confidence interval of 95% ($P < 0.05$).

3 Results and discussion

3.1 Fatty acid composition

Table 1 shows the fatty acid composition of both raw materials (RBO and FHRBO). Oleic acid is the major fatty acid in RBO (43.5%), followed by linoleic (32.7%) and palmitic acid (18.6%). This composition is in agreement with that previously reported (Mayamol *et al.*, 2009; Zhang *et al.*, 2021). As expected, major fatty acids in FHRBO were palmitic and stearic acids, at concentrations equal to that of C16:0 and to the total of all C18 fatty acids in the original RBO, respectively. Additionally, minor saturated fatty acids were identified at similar percentages than in the starting RBO.

Neither *cis* nor *trans* unsaturated fatty acids remained in FHRBO, confirming that the full hydrogenation was effectively achieved. The absence of *trans* fatty acids is of significant interest from a nutritional point of view since, as mentioned, this work was focused on the design of zero-*trans* edible fats.

3.2 Triacylglycerol composition

Figure 1a, b shows the HPLC chromatograms of RBO and FHRBO, respectively. As it can be observed, the analytical method permitted a convenient separation of TAG molecules and, due to the absence of trisaturated TAG in RBO, there were no coincidences in the retention time of the peaks in the chromatograms of both raw materials, which simplified the analysis of blends and products.

Table 2 shows the TAG composition of RBO and FHRBO, their blends (B) in different proportions and the products (P) obtained by the interesterification of the blends during 24 h using Lipozyme TL IM as catalyst.

RBO was mostly composed by two types of TAG molecules: 45.5% triunsaturated TAG with 54 carbons (LOO, LLO, OOO and LLL) and 42.9% monosaturated diunsaturated 52 carbons TAG (thus, containing one molecule of palmitic acid: POO, PLO, and PLL), and a minor proportion of disaturated monounsaturated TAG (PPL and PPO). These results are in good agreement with that reported for rice bran oils from different varieties by Zhang *et al.* (2021).

Table 1. Fatty acid composition of rice bran oil (RBO) and fully hydrogenated rice bran oil (FHRBO).

Fatty acid	RBO	FHRBO
C 14:0 (myristic)	0.20 ± 0.01	0.20 ± 0.02
C 16:0 (palmitic)	18.6 ± 0.3	18.8 ± 0.9
C 18:0 (stearic)	1.4 ± 0.2	79.0 ± 0.9
C 18:1 (oleic)	43.5 ± 0.1	nd
C 18:2 (linoleic)	32.7 ± 0.5	nd
C 18:3 (linolenic)	0.8 ± 0.1	nd
C 20:0 (arachidic)	0.40 ± 0.03	1.10 ± 0.01
C 22:0 (behenic)	0.10 ± 0.01	0.30 ± 0.08
C 24:0 (lignoceric)	0.30 ± 0.01	0.40 ± 0.07
SFA	21.0 ± 0.1	99.8 ± 0.2
MUFA	77.0 ± 0.1	nd
PUFA	33.5 ± 0.6	nd

SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; nd: non-detectable.

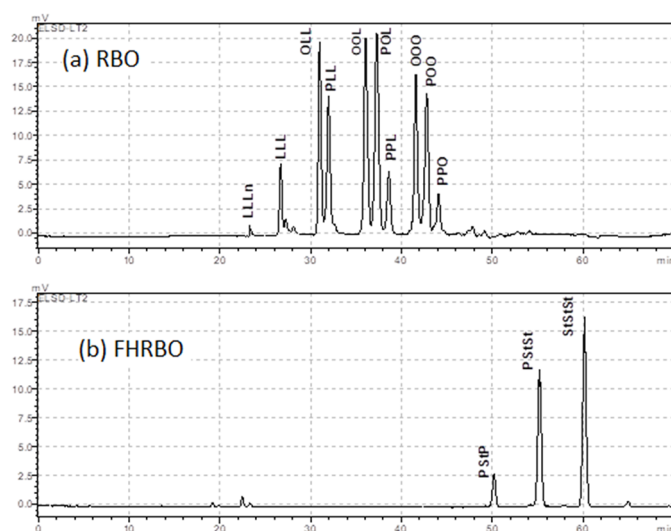


Fig. 1. Chromatograms corresponding to triacylglycerol composition by HPLC of rice bran oil (RBO) (a) and the fully hydrogenated rice bran oil (FHRBO) (b).

Accordingly, StStSt represented near half of the total FHRBO triacylglycerols (50.6%), while PStSt and PStP concentrations were 38.8% and 9.9%, respectively. The relatively high percentage of palmitic acid in the original RBO confers to FHRBO a relative highly heterogeneous TAG composition, which can be determinant on the crystallization behaviour. It is well known that TAG heterogeneity could favour the crystallization of polymorph β' , which is the preferred one for fats destined to shortenings or margarines. TAG heterogeneity of FHRBO is higher than that of other widely used hydrogenated oils, like the known β -tending fully hydrogenated soybean oil, with 63.4% StStSt and 30.8% PStSt (Ribeiro *et al.*, 2009). Conversely, FHRBO heterogeneity is close to the β' -tending fully hydrogenated cottonseed oil, with 40% StStSt and 43% PStSt as the major triacylglycerols (Shi *et al.*, 2005).

Table 2. Triacylglycerol composition of rice bran oil (RBO), fully hydrogenated rice bran oil (FHRBO), their blends, and their interesterification products (minor TAG with concentrations under 1% in all samples were not shown).

TAG	FHRBO	FHRBO (wt.%)																			
		90		80		70		60		50		40		30		20		10		0 (RBO)	
		B	P	B	P	B	P	B	P	B	P	B	P	B	P	B	P	B	P	B	P
StStSt	50.6	45.5	39.6	40.5	28.6	35.4	18.3	30.4	9.3	25.3	7.8	20.2	3.5	15.2	1.4	10.1	0.5	5.1	nd	nd	nd
PStSt	38.8	34.9	32.2	31.0	25.6	27.1	26.0	23.3	13.3	19.4	10.2	15.5	6.1	11.6	2.7	7.8	1.3	3.9	0.7	nd	nd
StOSt	nd	nd	4.3	nd	9.5	nd	10.9	nd	11.4	nd	11.2	nd	8.2	nd	4.8	nd	2.9	nd	0.2	nd	nd
PStP	9.9	8.9	7.8	7.9	7.7	6.9	6.1	5.9	5.9	4.9	4.3	3.9	3.6	3.0	2.9	2.0	1.9	1.0	0.9	nd	nd
StOO	nd	nd	nd	nd	0.7	nd	1.7	nd	3.9	nd	5.6	nd	6.3	nd	5.6	nd	6.0	nd	3.7	nd	0.5
LStSt	nd	nd	3.6	nd	14.0	nd	17.1	nd	21.5	nd	18.0	nd	16.1	nd	11.7	nd	8.8	nd	4.1	nd	nd
PPO	nd	0.3	0.5	0.6	0.4	1.0	0.5	1.3	0.7	1.6	0.6	1.9	0.5	2.3	0.5	2.6	0.5	2.9	0.4	3.2	3.4
POO	nd	1.3	nd	2.5	nd	3.8	nd	5.0	nd	6.3	nd	7.5	nd	8.8	nd	10.1	nd	11.3	nd	12.6	11.1
LOSt	nd	nd	0.4	nd	1.5	nd	3.7	nd	9.2	nd	11.3	nd	14.9	nd	24.2	nd	16.9	nd	15.1	nd	nd
LStP	nd	nd	1.9	nd	4.8	nd	6.8	nd	11.2	nd	11.3	nd	11.9	nd	11.3	nd	9.5	nd	7.1	nd	nd
OOO	nd	1.2	nd	2.4	nd	3.6	nd	4.8	0.3	6.0	0.9	7.1	1.7	8.3	nd	9.5	3.9	10.7	5.9	11.9	7.7
PLO	nd	1.9	nd	3.7	0.8	5.6	2.1	7.5	5.1	9.4	8.4	11.2	11.2	13.1	12.7	15.0	16.9	16.8	19.2	18.7	19.8
PPL	nd	0.5	0.3	1.1	0.6	1.6	0.8	2.1	1.2	2.6	1.8	3.2	3.0	3.7	3.1	4.2	3.8	4.8	3.9	5.3	3.8
LOO	nd	1.7	nd	3.3	nd	5.0	nd	6.6	0.8	8.3	2.1	9.9	3.8	11.6	5.8	13.2	9.4	14.9	14.1	16.6	20.8
PLL	nd	1.1	nd	2.2	nd	3.4	0.2	4.5	0.7	5.6	1.6	6.7	3.1	7.8	4.9	8.9	5.7	10.1	7.9	11.2	8.8
LLO	nd	1.3	nd	2.6	nd	3.9	nd	5.2	0.6	6.5	1.5	7.8	2.8	9.1	5.1	10.4	7.9	11.7	12.2	13.0	17.3
LLL	nd	0.4	nd	0.8	nd	1.2	nd	1.6	nd	2.0	nd	2.4	0.4	2.8	0.7	3.2	1.9	3.6	2.8	nd	5.0

O: oleic; St: stearic; L: linoleic; P: palmitic; *nd*: non-detectable.

Table 2 shows several changes in TAG composition between blends and their corresponding interesterification products, which confirms that the enzyme was very active in the catalysis of the interesterification process. Although results corresponding to the interesterification of pure RBO (0% FHRBO) are shown, pure FHRBO was not possible to be interesterified under identical conditions to the rest of the samples due to its high melting point (over the incubation temperature). In order to simplify the analysis of results, TAGs were grouped in four types (SSS: trisaturated; SSU: disaturated monounsaturated; SUU: monosaturated diunsaturated; UUU: triunsaturated) and their percentages in the blend (B) and product (P) plotted against the rate of FHRBO in starting blend (Fig. 2).

As expected, Figure 2a shows that interesterification caused the diminishing in the level of SSS, provided to samples by FHRBO. Although all products showed a concentration of SSS lower than that in the starting blend, a maximal reduction from 26.5 to 31.0% occurred in mixtures containing from 40 to 60% FHRBO, respectively. Additionally, the concentration of UUU triglycerides from RBO (mostly OOO, LOO, LLO, LLL) diminished too as a consequence of the interesterification process (Fig. 2d), and this group of TAG completely disappeared in those products from blends containing more than 60% FHRBO.

As a result of the diminishing in the concentration of SSS and UUU triglycerides from blends to products, the concentrations of TAG comprised of both saturated and unsaturated fatty acids increased after the interesterification process. Figure 2b shows that the concentration of SSU triglycerides drastically increased in all samples, achieving a maximum of 46.0% in the product from the blend containing 60% FHRBO (with a starting SSU concentration of only 3.4%). This result is mainly a consequence of the appearance of three new TAG

species that were not present in any of the starting materials: StOSt, LStSt and LStP (Tab. 2).

The concentration of SUU triacylglycerides showed a different tendency according to FHRBO percentage (Fig. 2c), while their concentration increased in that samples with less than 60% FHRBO, it decreased in the rest of the samples. Changes in the concentration of this group of TAG can be attributed to the occurrence of two opposite effects: while new TAG like StOO and LOSt were formed, POO from FHRBO completely disappeared after the incubation. Additionally, PLO and PLL diminished in different extent depending on the percentage of FHRBO in the blend.

The reduction of SSS and UUU triglycerides, characteristics from both components of the blends, and the increment in the concentration of TAG with a medium saturation degree, suggests that products should be composed by a blend of TAG species with higher compatibility than the starting blends. The increased concentration of SSU and SUU triacylglycerols promoted by interesterification has been associated with enhanced technological functionality and improved plasticity, thus with higher potential for food applications (Ribeiro *et al.*, 2009). Additionally, it is well known that intermediate melting point triacylglycerides SSU type, which showed the higher increment after interesterification (Fig. 2b), have been associated with a higher oil binding capacity due to their intermediate polarity, allowing them to act as bridges between high-melting TAG and liquid oil, thus playing an essential role in improving the plasticity of fats (Jahaniaval *et al.*, 2002).

3.3 Fatty acid regiodistribution

The HPLC analytical method used in TAG analysis is relatively efficient for the separation of the different TAG by

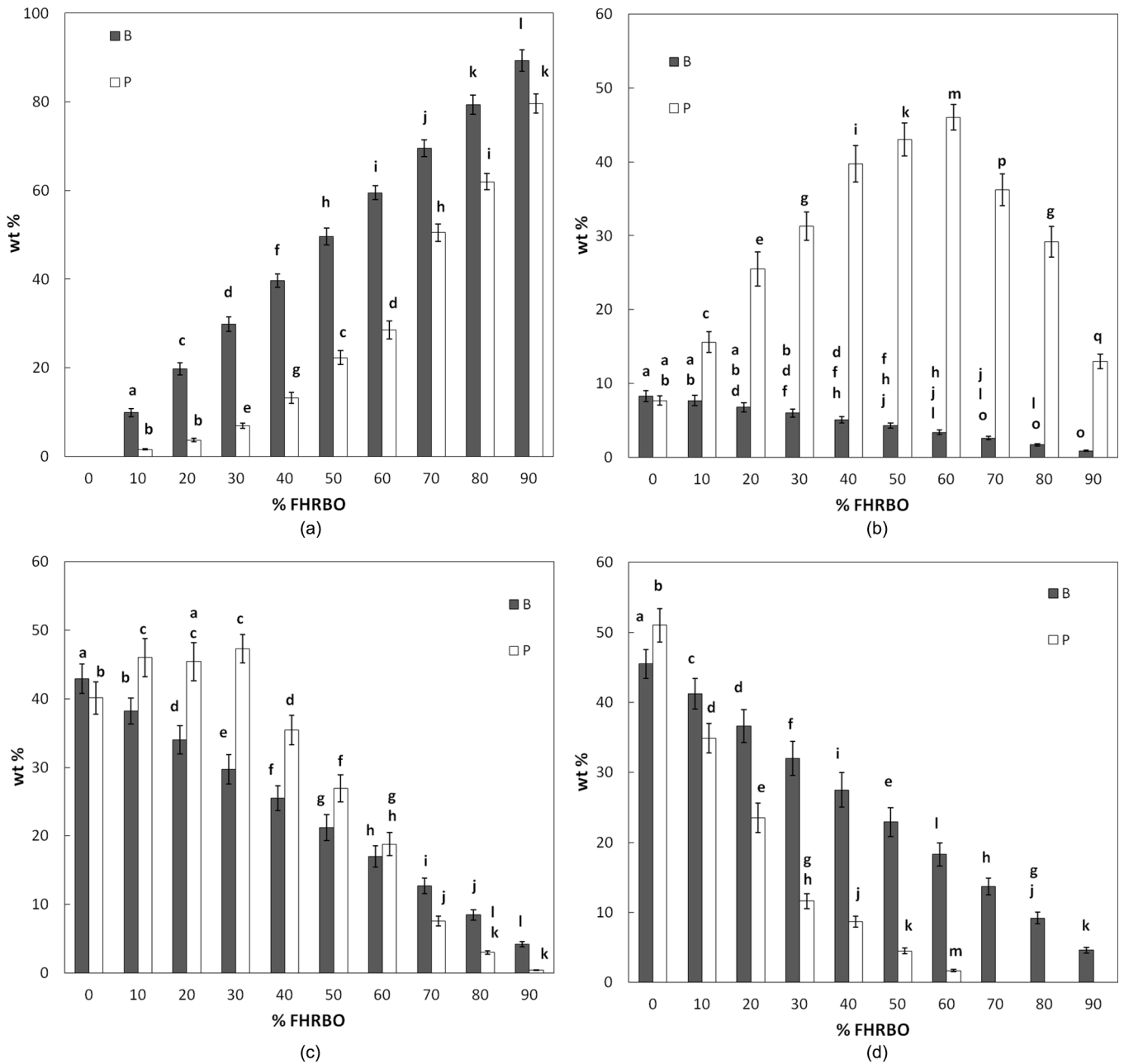


Fig. 2. Composition in type of TAGs of the blends RBO/FHRBO “B” with different percentage of FHRBO and their interesterification products “P”; SSS: triunsaturated TAG (a); SSU: disaturated monounsaturated TAG (b); SUU: monosaturated diunsaturated TAG (c); UUU: triunsaturated TAG (d). The difference between percentages is statistically significant ($P < 0.05$) if bars have different letters.

ECN parameter, as shown in Table 2, but does not discriminate among TAG isomers. Thus, the regiodistribution of the fatty acids cannot be determined by this method.

In order to provide additional information about TAG structure and the distribution of the fatty acids in the glycerol backbone, which is of major interest in determining the properties of these materials, the method of hydrolysis with porcine pancreas lipase (PPL) was performed. Results showed that only 3.1% of *sn*-2 position of RBO triglycerides was occupied by palmitic fatty acid (Tab. 3), suggesting that PStSt and PStP should be the major structures of the heterogeneous TAG from FHRBO. These two heterogeneous TAGs are of major

interest because they are known to have a preference to crystallize in the β' form (Timms, 2003), which is the preferred polymorphic form for fats destined to shortenings and margarines. As shown in Table 2, both PStSt + PStP could represent near half of the total TAG molecules of FHRBO, suggesting that this material could show a high tendency to crystallize in the β' form.

3.4 Thermal analysis

Figure 3a shows the melting thermograms obtained by DSC analysis of RBO and FHRBO and their blends at different

Table 3. Fatty acid composition of *sn*-2 position of rice bran oil (RBO) triacylglycerols.

Fatty acid	<i>sn</i> -2 of RBO (%)
C 14:0	<i>nd</i>
C 16:0	3.1 ± 0.6
C 18:0	0.9 ± 0.2
C 18:1	47.3 ± 0.6
C 18:2	46.0 ± 0.7
C 18:3	0.7 ± 0.2
C 20:0	<i>nd</i>
C 22:0	<i>nd</i>
C 24:0	<i>nd</i>

nd: non-detectable.

proportions. Some parameters of interest determined from thermograms are shown in Table 4. Pure FHRBO shows a single endothermic peak with a maximum at 62.7 °C, corresponding to the melting of the three trisaturated TAG present in this sample. Although each TAG from FHRBO do not have the same melting point, the presence of a single peak suggests that due to the combination of melting and solubilization phenomena, these TAG cannot be differentiated as single peaks by this thermal analysis.

Pure RBO (B-0) showed two melting peaks, the highest one at -19.8 °C and the smaller one at -10.3 °C. As expected from the fatty acid composition of RBO, it did not show any melting peak at temperatures over 0 °C (Fig. 3a). Thus, RBO will remain liquid over 0 °C, playing a role of “solvent” when blended with the high melting point of FHRBO.

Concerning the thermal behaviour of the blends, Figure 3a shows that they combine properties from both components. As the percentage of FHRBO decreased, the height and area of the high temperature melting peak showed by pure FHRBO decreased too, due to the progressive dilution of its trisaturated TAG. Additionally, the completion melting temperature of blends ($T_{C,B}$) shifted from 66.9 °C to 55.2 °C when the percentage of FHRBO diminished from 90 to 10%, respectively (Tab. 4). The relatively high melting point of the blend containing as little as 10% of FHRBO suggests that the trisaturated TAGs from FHRBO should have a very low solubility in the liquid RBO, thus remaining crystallized in the blend until the temperature overpasses their melting point. Similar behaviour has been reported for blends of fatty materials with low compatibility, such as soybean oil blended with fully hydrogenated soybean oil (Ribeiro *et al.*, 2009).

It can also be observed that as the percentage of RBO increased, the peak located close to -20 °C gradually increased too. Thus, DSC analysis indicates that the thermal behaviour of the blends is mostly the result of the combination of the thermal behaviour of both components, expressed according to their proportion in the blend.

Figure 3b shows the melting thermograms of the products obtained by interesterification of the different blends RBO/FHRBO. Concerning pure RBO, the interesterification caused a shifting of the major peak from -19.8 to -7.7 °C. Considering that the TAG composition of RBO was not

significantly different from that of the interesterified RBO (Tab. 2), changes in the fatty acids regiodistribution could have contributed to this change of the melting thermogram.

The thermogram of the product derived from the blend containing 90% FHRBO (P-90) shows three endothermic peaks, P_1 , P_2 and P_3 , with increasing peak temperature and area, respectively. Additionally, the thermogram has an exothermic peak (P_e) between P_2 and P_3 , which evidences the occurrence of a polymorphic transition from a less to a more stable polymorph, probably from α to β' form. Considering that peak P_3 is located at a temperature range close to that of the single peak shown by the blends with a high percentage of FHRBO, it should correspond to the melting of the same group of TAG (trisaturated TAGs from FHRBO: StStSt + PStSt + PStP). This result is in agreement with the TAG composition of P-90, which has a total trisaturated TAG of 79.6% provided by FHRBO to the blend and that remained partially unreacted after the interesterification (Tab. 2). According to the diminishing of trisaturated TAG concentration in the products obtained from the blends with a lower percentage of FHRBO, P_3 area became progressively smaller. Additionally, the completion melting temperature of the products ($T_{C,P}$) shifted from 61.6 to 33.5 °C when the percentage of FHRBO diminished from 90 to 10%, respectively, a much more drastic decrease than that shown by the non-interesterified blends. This result is consistent with increased compatibility between the new TAG species in the products, as a consequence of the reduction of trisaturated TAG caused by interesterification, particularly StStSt, which has the highest melting point and very low solubility in the liquid oil.

3.5 Solid fat content (SFC)

The ratio between the amount of solid fat crystals and that of the liquid oil is determinant of fat plasticity and, additionally, influences the physical and sensory properties of an edible fat (Johnson, 2008). It is also well known that the texture and plasticity of a fatty material are strongly linked to SFC and with how this parameter varies with temperature (Lai *et al.*, 1999).

Figure 4 shows the percentage of solid fat content (SFC) determined by RMN analysis as a function of temperature for the different blends RBO/FHRBO and their interesterification products.

While B-90 reached 0% SFC at 65 °C, P-90 did at 60 °C, only 5 °C below the corresponding blend. The situation was different in samples with low percentages of FHRBO, while 0% SFC was achieved by B-10 at 55 °C, the corresponding product (P-10) did at a temperature 20 °C lower (35 °C). This result is coherent with the reduction of the concentration of the high melting point SSS molecular species as a consequence of the interesterification process, effect enhanced in products form blends with lower levels of FHRBO.

Results are in agreement too with the corresponding low variation in T_{CB} values determined by DSC analysis of the different blends. In contrast, T_{CP} values decreased drastically as the percentage of FHRBO diminished (Tab. 4).

A big difference can be observed in the variation of SFC of blends and products in the range from 10 to 25 °C, while blends

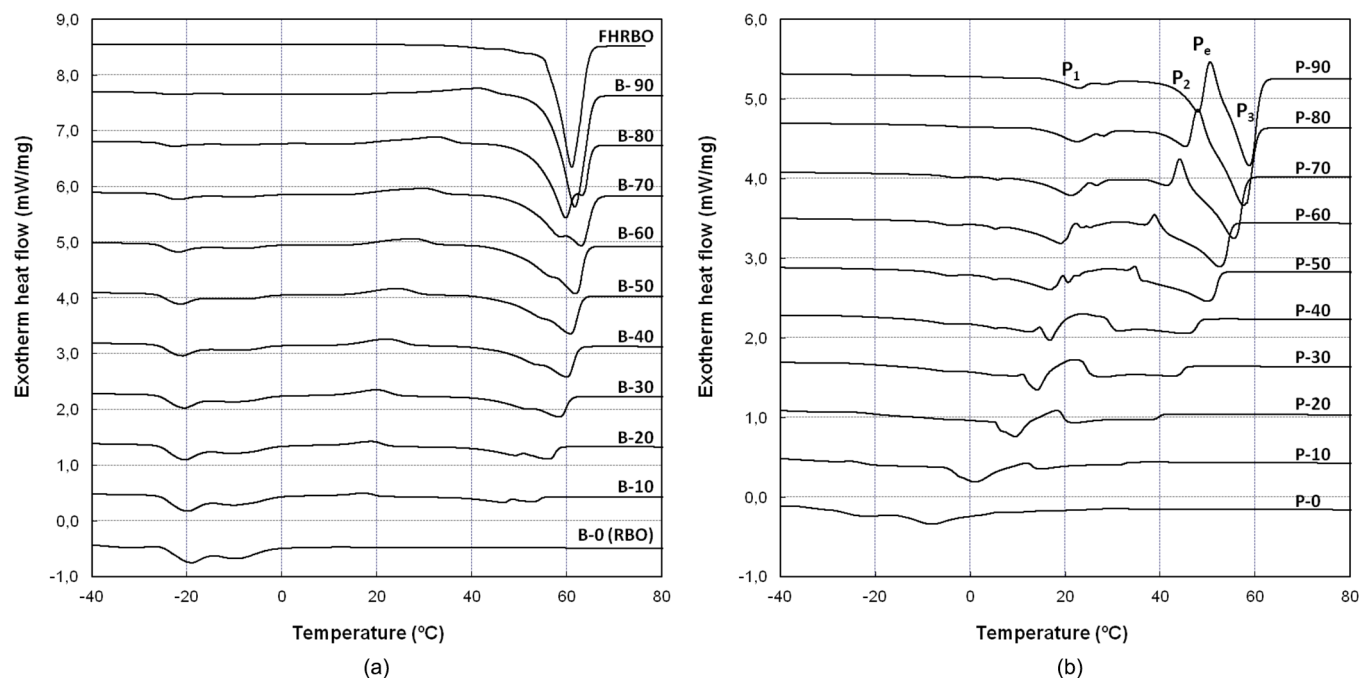


Fig. 3. Melting thermograms of the blends RBO/FHRBO at different proportions (a) and their interesterification products (b). Notes: “B-x”: “Blend–% FHRBO”; “P-y”: “Product–% FHRBO”.

Table 4. Melting peak temperature (T_p) and completion melting temperature (T_c) of the different blends rice bran oil (RBO)/fully hydrogenated rice bran oil (FHRBO) and their interesterification products, determined from the melting thermograms obtained by DSC analysis (Fig. 3).

% FHRBO	Melting peak temperature (°C)		Completion melting temperature (°C)	
	Blend ($T_{P,B}$)	Product ($T_{P,P}$)	Blend ($T_{C,B}$)	Product ($T_{C,P}$)
100	62.7	–	67.3	–
90	61.7	58.8	66.9	61.6
80	59.9	57.7	66.5	60.8
70	63.1	55.6	66.1	58.4
60	61.9	52.6	64.7	55.4
50	60.8	50.1	63.5	53.0
40	60.0	45.6	62.8	48.6
30	58.5	28.3	61.0	45.5
20	56.3	21.5	58.4	40.6
10	47.0	15.1	55.2	33.5
0	–19.8	–7.7	4.4	10.4

did not show high variations in SFC within these temperatures, products showed much lower SFC at 25 than at 10 °C. Such behaviour is important because it will involve a noticeable reduction in product hardness from refrigerator to room temperature.

It is interesting to analyze the solid fat content at 37 °C ($SFC_{37^\circ C}$) because of its influence on “mouth feel”. According to Figure 4, products show a drastic reduction in $SFC_{37^\circ C}$ as compared with the corresponding blends, which obviously is a direct consequence of the decrease in the concentration of high melting trisaturated TAG due to the interesterification (Fig. 2a). While B20, B30 and B40 had 16, 24 and 34%

solids at 37 °C, their corresponding products had 12, 5 and 2% solids at the same temperature, respectively. Thus, these products offer raw materials with low $SFC_{37^\circ C}$, which suggests that undesired mouthfeel like waxiness, graininess or sandiness, should not be noted during their intake as food components.

SFC also provides information about the “plastic range” of a fat, defined as the temperature range over which it can be molded and spread, thus being neither too hard nor too soft. The sharpness of the melting range and other factors like crystal morphology determines the temperature range within which a fat could be considered plastic. As a reference, it has

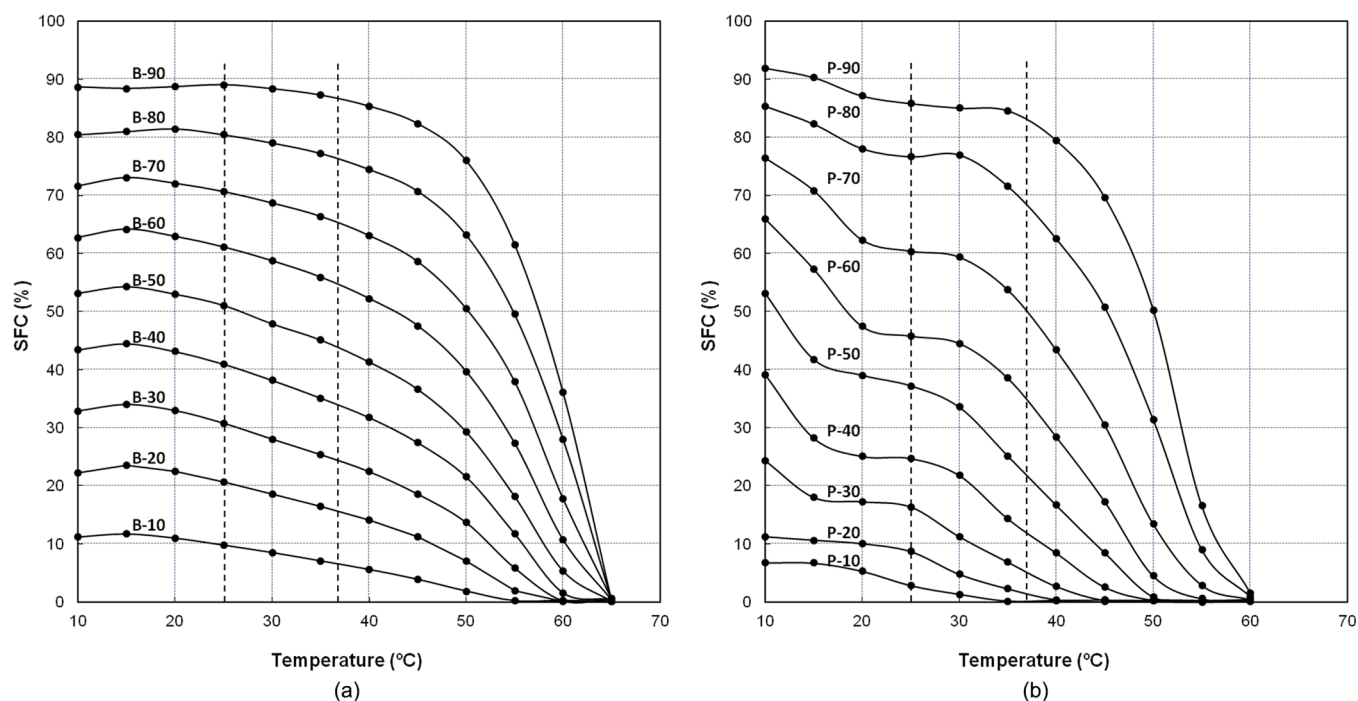


Fig. 4. Variation of solid fat content (SFC) with temperature for the blends RBO/FHRBO (a) and their interesterification products (b). Notes: “B-x”: “Blend–% FHRBO”; “P-y”: “Product–% FHRBO”.

been considered that a convenient spreadability is achieved if SFC is within a range of roughly 15–35%, and temperatures corresponding to both SFC limits defined the plastic range of a fat (DeMan, 1992).

Figure 4b shows that P-50 matches such requirement from about 30 to 40 °C, thus surely being too hard at temperatures over room temperature. Conversely, P-30 had a SFC high enough to be considered plastic at temperatures below 25 °C, while over this temperature, SFC falls below the minimum of 15% required. Thus, P-40 appears as the more suitable material in terms of plasticity, with a plastic range from 12 to 35 °C, which covers the widest temperature range containing room temperature.

3.6 Polymorphism

The diffractogram of pure FHRBO in the short spacing region shows two strong diffraction peaks at 3.8 and 4.2 Å (Fig. 5a), which indicates that the crystallization of the sample generated the β' polymorph. This result is highly promising considering that this is the more convenient polymorph for the products of interest and the absence of any other diffraction peaks corresponding to polymorphic forms different from β' . It is well known that the existence or non-existence of a polymorph depends heavily on the composition and the position of the fatty acids on the glycerol unit (Foubert *et al.*, 2007).

X-ray diffraction analysis confirmed that the percentage of palmitic acid in FHRBO confers to this fat a heterogeneity high enough to generate a stable β' polymorph. Thus, although FHRBO contains 50.6% of the β -tending StStSt, the presence of two known β' -tending TAGs, PStSt and PStP (Timms, 2003), totaling 49.7%, was enough to promote β'

crystallization. This result is in agreement too with the fact that β' -polymorph is usually the most stable one if the triglyceride is asymmetrical (Foubert *et al.*, 2007), like PStSt.

The situation changed as the percentage of RBO was progressively increased in the blends. As shown in Figure 5a, the diffraction peak at 4.6 Å corresponding to β polymorph appeared when B-80 was analyzed, accompanied by the relative diminishing in the intensity of peaks corresponding to β' polymorph. As RBO percentage increased from B-80 to B-20, the relative intensity of peaks changed, suggesting an increasing ratio from β to β' polymorph. Results can be explained considering that the heterogeneous β' -tending TAGs from FHRBO are more soluble than StStSt in the liquid RBO, thus, as the proportion of RBO increased, the proportion of StStSt in the solid fraction increased too, conferring to the blend a higher preference to crystallize in the β form.

Concerning the interesterification products (Fig. 5b), the diffractogram of P-80 shows, like that of B-80, peaks indicating the presence of both β and β' polymorphs in their solid fraction. But, unlike B-60, the P-60 diffractogram shows only the peaks corresponding to β' polymorph. Such behaviour can be associated with the reduction in the concentration of StStSt from 30.4% in M-60 to only 9.3% in P-60. Additionally, the overall TAG composition of products is much more heterogeneous than that of the original blend, which can also contribute to enhancing the β' tendency of the products.

4 Conclusions

Results showed that the relatively high content of palmitic acid in rice bran oil was enough to confer the required heterogeneity to triacylglycerols to promote the crystallization

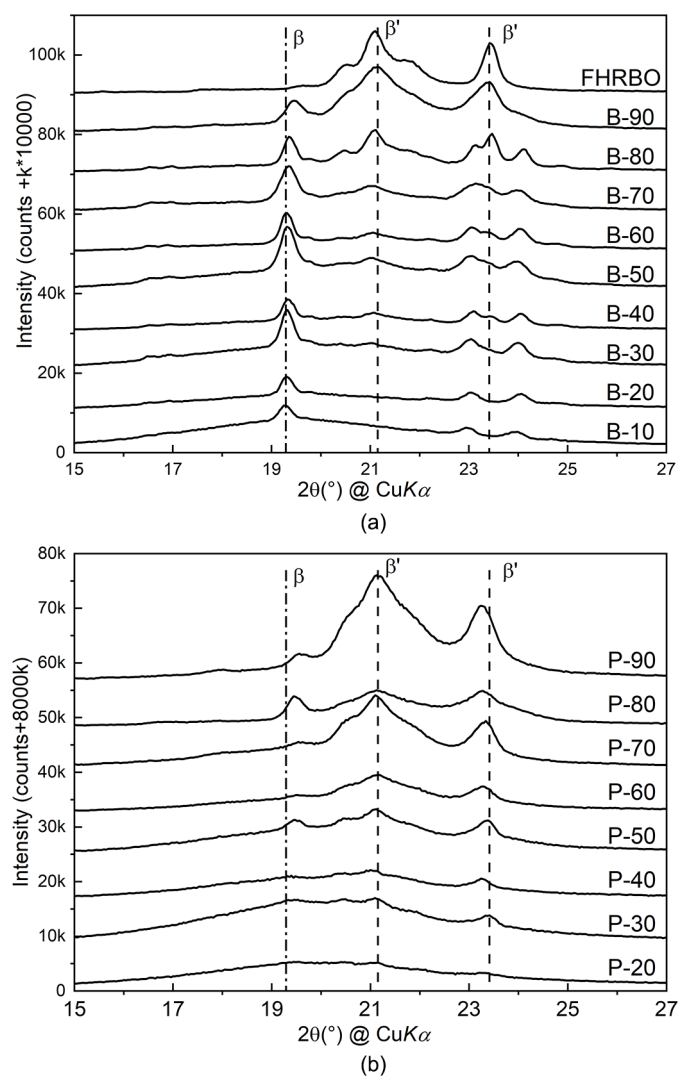


Fig. 5. Powder X-ray diffraction patterns collected at 20 °C for the blends RBO/FHRBO (a) and their interesterification products (b).

of the fully hydrogenated oil in the β' form. Additionally, this polymorphic form was also predominant in the interesterification products, a valuable property in fats destined for edible applications. Conversely to the low compatibility showed by the trisaturated and triunsaturated TAGs provided by both components to the blends, the interesterification process produced new TAGs species with an intermediate unsaturation degree and improved compatibility. These changes impacted on the thermal behaviour of the products, reducing their melting point and solid fat content. Thus, by conveniently choosing the proportion of each component in the starting blend, it is feasible to design products with different properties according to that required by the edible application to be destined.

Results demonstrated that the proposed methodology involving fully hydrogenation, blending, and interesterification offers a valuable tool for producing zero-*trans* products using solely RBO as the starting raw material, which could diversify the utilization of a valuable vegetable oil still underexploited.

Acknowledgments. The authors thank to CSIC (Comisión Sectorial de Investigación Científica, Universidad de la República), PEDECIBA (Programa de Desarrollo de las Ciencias Básicas), CAP (Comisión Académica de Posgrado, Universidad de la República), ANII (Agencia Nacional de Investigación e Innovación) for the financial support and scholarships.

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Cite this article as: Callejas Campioni N, Suescun Pereyra L, Badan Ribeiro AP, Jachmanián Alpuy I. 2021. Zero-trans fats designed by enzyme-catalyzed interesterification of rice bran oil and fully hydrogenated rice bran oil. *OCL* 28: 46.