AGRO PRODUCTIVIDAD

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Aromatic amination of refined rice bran oil previously epoxidized with Novozym 435

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ABSTRACT

Objective: To aromatically aminate refined rice bran oil (RBO), a by-product of the rice agro-industry, through a chemical-enzymatic epoxidation based on Novozym 435 and p-xylylenediamine insertion.

Design/Methodology/Approach: Refined RBO was epoxidized with H_2O_2/Novozym 435. The resulting epoxidized rice bran oil (eRBO) was functionalized with the p-xylylenediamine aromatic diamine via epoxy ring-opening (X-eRBO), using $ZnCl_2$ as catalyst. Iodine value (IV), saponification value (SV), and oxirane oxygen content (OOC) were determined to evaluate structural changes in oils. The RBO, the eRBO, and the X-eRBO were identified using FTIR, ^{1}H, and ^{13}C NMR.

Results: The IV, the SV, and the OOC suggest that the synthesis of eRBO and X-eRBO were effective. The increase of molecular weight in eRBO point to the formation of ~6 epoxy rings per original triglyceride. The OOC value of X-eRBO was 22% lower than the OOC value of eRBO, implying that an effective aromatic amination was achieved. The FTIR, ^{1}H and ^{13}C NMR spectroscopy analysis confirmed the epoxidation and amination of the RBO.

Study Limitations/Implications: X-eRBO may be a feasible precursor for value-added products, such as crosslinked polymers or corrosion inhibitors.

Findings/Conclusions: Refined rice bran oil was aromatically aminated after two stages under mild thermal conditions. This result was achieved with an epoxidation sequence with H_2O_2/Novozym 435, followed by functionalization with p-xylylenediamine.

Keywords: Epoxidation, amination, use of agro-industrial residues.

INTRODUCTION

Biorefining is the set of processes that allow chemical products —such as biofuels and high value-added products— to be obtained from biomass. Biorefining raw material includes oilseed crops, domestic or industrial oily residues, and algae, as well as forestry, agricultural, and agro-industrial residues (Ng *et al.*, 2014). Rice (*Oryzae sative* L.) bran is

an agro-industrial by-product, which is extracted from the film that divides the grain and its husk during polishing (Bhosale and Vijayalakshmi, 2015). Its high oleic content (Zúñiga-Diaz *et al.*, 2017) make rice bran a potential source of oil, suitable for human consumption, and a precursor for biodiesel production (Zuñiga-Díaz *et al.*, 2018). To date, the exploitation of rice bran oil (RBO) is limited, particularly in the production of fine chemical products. Raw RBO contains di- and triacylglycerols, phospholipids, waxes, and non-saponifiable lipids, among other components (Garba *et al.*, 2017). Raw RBO that is purified until it reaches a high triglyceride composition is called refined RBO. RBO triglycerides are characterized by C=C unsaturations, which enables the transformation of this oil into value-added chemical products. This transformation can be achieved inserting functional organic groups that gradually provide the physical and chemical properties that are required for products of the polymer, pharmaceutical, and cosmetic industries, among others. To achieve the functionalization of oils, oxirane or epoxy rings must be first formed by inserting an oxygen atom in the unsaturations of the triglycerides —a process known as epoxidation. The subsequent ring-opening enables the functionalization of the oil, forming monomers for subsequent reactions (Pascault and Williams, 2009). Amination is the functionalization that involves the different types of amines; consequently, it is expected to produce precursors which can be tuned to obtain new electrical, rigidity, and adhesiveness properties (Kalita and Karak, 2013). This work reports that rice bran oil can be feasibly functionalized with aromatic amine, using a previous enzymatic epoxidation process based on Novozym 435 lipase. The resulting precursor can be considered for the synthesis of polyurethanes and corrosion inhibitors.

MATERIALS AND METHODS

Reagents. Chemical reagents for analytical grade were obtained from Sigma Aldrich. The refined RBO was obtained from commercial sources.

Physicochemical characterization of the oils

The determinations of IV (Firestone, 1998a), SV (Firestone, 1998b), and OOC (ASTM, 2004) were carried out in triplicate for the various RBO, eRBO, and X-eRBO samples, following international regulations. The IV refers to the amount of iodine that is absorbed per 100 g of the substance under study and it determines the unsaturation level of the C=C bonds of fatty acids. On the other hand, the SV refers to the number of milligrams of KOH required to saponify 1 g of oil and it is a measure of the average molecular weight of a sample. Finally, OOC refers to the amount of oxirane oxygen that is inserted into C=C unsaturations during the epoxidation. Therefore, OOC is a direct measure of the amount of epoxy groups present.

Rice bran oil epoxidation

The epoxidation of RBO was carried out using Novozym 435 lipase as catalyst (Ortiz *et al.*, 2019). In order to synthesize eRBO, 12.0 g RBO, 0.98 g oleic acid, 2.5 g catalyst, and 52.84 g toluene (as a solvent) were mixed for 2 hours at 49 °C. Next, 62.77 g H_2O_2 (30%) were added drop by drop. Subsequently, the system was allowed to react for 24 hours with a gentle mechanical stirring. The catalyst was separated from the resulting mixture using vacuum filtration. During the next stage of the process, the catalyst was repeatedly rinsed with toluene and left to dry at room temperature to be used again. The filtered mixture was then washed with distilled water. An organic layer was extracted and mixed for ~1 hour with 10:1 anhydrous Na_2SO_4 (mixture: Na_2SO_4) to eliminate moisture and unreacted H_2O_2. Finally, the solids were removed by vacuum filtration and the toluene or water was extracted with a rotary evaporator (Petrović *et al.*, 2002).

eRBO aromatic amination

The reaction conditions to achieve amination were temperature-molar ratio of the eRBO to the amount of catalyst ($ZnCl_2$), and a fixed time of 3 hours. The reaction temperatures were 70, 77, and 85 °C, while the eRBO:$ZnCl_2$ molar ratios were 0.84, 0.92, and 1.00, resulting in 9 tests. In each one, 1.02 mmol eRBO was mixed with 2.00 mmol p-xylylenediamine and an amount of $ZnCl_2$ depending on the molar ratio. Once the reaction was completed, the product was cooled at room temperature, dissolved in ethyl acetate, and transferred to a separatory funnel. To remove $ZnCl_2$ and unreacted amine, nine successive washes were immediately carried out with 50 mL of solutions (two washes with a pH=4 acid solution, two washes with a saturated solution of $NaHCO_3$, two washes with a pH=13 basic solution, and three washes with distilled water). Finally, the organic layer was recovered and kept in a vacuum desiccator for 24 hours at 30 °C (Lopez-Tellez *et al.*, 2008).

Infrared spectroscopy and nuclear magnetic resonance

The oil samples were subjected to an infrared spectroscopy (FTIR) analysis using a PerkinElmer® Spectrum™ One FTIR-ATR spectrometer with a 4,000 to 400 cm^{-1} radiation source. Nuclear magnetic resonance (NMR) analysis was achieved with a Bruker Avance™ III HD 400 MHz NMR instrument that uses $CDCl_3$ as solvent. ^1H and ^{13}C NMR spectra were obtained at 500 and 125 MHz, respectively.

RESULTS AND DISCUSSION

Physicochemical characterization: IV, SV, MW, and OOC

Table 1 shows the determinations of IV, SV, and OOC for the RBO and eRBO. The corresponding molecular weights (MW) obtained from the SV are also included.

Table 1. IV, SV, MW, and OOC of the refined RBO and eRBO.

Sample	IV	SV	MW	OOC
	(g I_2/100g)	(mg KOH/g)	(g/gmol)	
RBO	90.40±0.67	191.58±3.92	878.5	0.07±0.02
eRBO	11.61±0.12	171.90±4.62	979.14	5.60±0.65

The average IV for the refined RBO samples was estimated at 90.40±0.67, fully matching previously reported values for RBOs of different varieties and regions: 90 (Gupta *et al.*, 2016), 91.38 (Zúñiga-Diaz *et al.*, 2017), and 95.40 (Hanmoungjai *et al.*, 2000). After epoxidation, the IV was reduced to ~12% (11.61±0.12). This decrease suggests a significant loss of C=C unsaturations, as a consequence of enzymatic epoxidation based on H_2O_2/Novozym 435. Previously, Gupta *et al.* (2016), using entirely chemical and not enzymatic strategies, reported an IV of raw eRBO of ~32.2, showing that the process based on Novozym 435 is more efficient. The average SV for the refined RBO of this study was 191.58±3.92, which was also consistent with previous reports: 182.35 (Zúñiga-Diaz *et al.*, 2017) and 178.18 (Hanmoungjai *et al.*, 2000). Based on this SV, the molecular weight of the refined RBO was estimated at 878.50 g/gmol, which is lower than other estimates of raw RBO (923.36 g/gmol) (Zúñiga-Diaz *et al.*, 2017). This effect is possibly associated with the absence of the components removed during the refining of the RBO. The SV of eRBO (171.90±4.62) allowed the calculation of a molecular weight of 979.14 g/gmol. The difference in molecular weights between eRBO and RBO suggests that each triglyceride was epoxidized by the insertion of an average of 6 oxygen atoms and consequently formed epoxy rings; this proof matches the significant decrease of IV in eRBO. Finally, the evolution of the OOC enabled a direct monitoring of the appearance or disappearance of epoxy groups. The OOC in refined RBO was 0.07±0.02, confirming an absence of epoxy or oxirane rings in the initial sample. Gupta *et al.* (2016) have reported a 0.68 OOC of raw RBO, with which the determination of this study can be compared. Once the epoxidation was completed, the OOC in eRBO increased 8.2 times (5.60±0.65) compared to the OOC of the refined RBO. A similar increase (4.7 times) has been reported in raw RBO using chemical epoxidation (Gupta *et al.*, 2016); once more, this result implies that Novozym 435 lipase improves the performance of an epoxidation. The greatest OOC reduction (to 1.28) was recorded after functionalization, with p-xylylenediamine at 70 °C and an eRBO:$ZnCl_2$ molar ratio of 0.92. These were the best conditions for partial aromatic amination, at the fixed reaction time. The OOC reduction is the result of the destruction of epoxy rings by the insertion of the aromatic amino group.

Infrared spectroscopy analysis

Figure 1 shows the FTIR spectra of refined RBO (black line), epoxidized RBO (blue line), and RBO functionalized with p-xylylenediamine (yellow line). The FTIR spectrum of the refined RBO is consistent with previous works (Silverstein and Bassler, 1962; Zúñiga-Diaz *et al.*, 2017). In contrast, the FTIR spectrum of eRBO shows a band in the 842-824 cm^{-1} region, associated with the oxirane group (Petrović *et al.*, 2002). This band can still be observed after functionalization with the aromatic amine. The FTIR spectrum of X-eRBO shows the band of the epoxy group, as well as the appearance of strong bands at 1639 and 1550 cm^{-1}, assigned to secondary and primary amine groups, respectively (Lopez Tellez *et al.*, 2008).

Another proof of the epoxy ring-opening and functionalization is the 3,613-3,149 cm^{-1} band which is related to the vibration of the OH group, which occurs simultaneously when

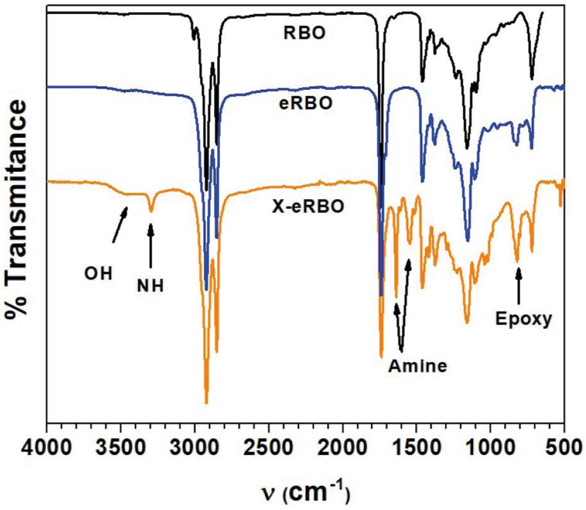

Figure 1. FTIR spectra of refined RBO (black line), eRBO (blue line), and X-eRBO (yellow line).

p-xylylenediamine is inserted into the triglyceride structure. The band centered at 3,294 cm^{-1} proves the primary N-H vibration of unreacted p-xylylenediamine.

^{1}H and ^{13}C NMR analyses of X-eRBO functionalized oil

Figure 2a shows the ^{1}H NMR spectrum for X-eRBO. The observed signals enable the identification and proposal of a generic structure (see box in the figure). The signals found in this work (indicated in parentheses) are consistent with previous reports (Lopez Tellez et al., 2008). The conserved oxirane rings are identified by three signals: protons –CH– of the epoxy group (Ha) at 2.9 ppm, –CH– adjacent to epoxy groups (Hb) at 1.45 ppm, and –CH– adjacent to two epoxy groups (Hc) at 3.1 ppm. The signals that confirm the functionalization of X-eRBO correspond to displacements in $–CH_2–NH–$ and $–CH_2–NH_2Ph$ protons (3.6 ppm) associated with methylene protons (Hd, Hg, at 3.9 ppm), and aromatic protons (He, Hf, at 7.2 ppm). After the oxirane ring-opening, an OH group (Hh, at 2.75 ppm) and a secondary amine (Hi, at 3.65 ppm) were formed.

Figure 2b shows the ^{13}C NMR spectrum of X-eRBO. Considering that the X-eRBO sample remains epoxidized and comparing it with a ^{13}C NMR spectrum of epoxidized canola oil (Madankar *et al.*, 2013), it is easy to see that both are practically identical, which confirms that X-eRBO remains partially epoxidized. The 53-60 ppm signals are associated with the carbon of the epoxy group, while those between 68 and 62 ppm come from the carbon resonance of glycerol in the α and β carbon atoms, respectively. The 128-ppm signal confirms the amination of RBO, since it matches the carbons of p-xylylenediamine (SpectraBase, 2021).

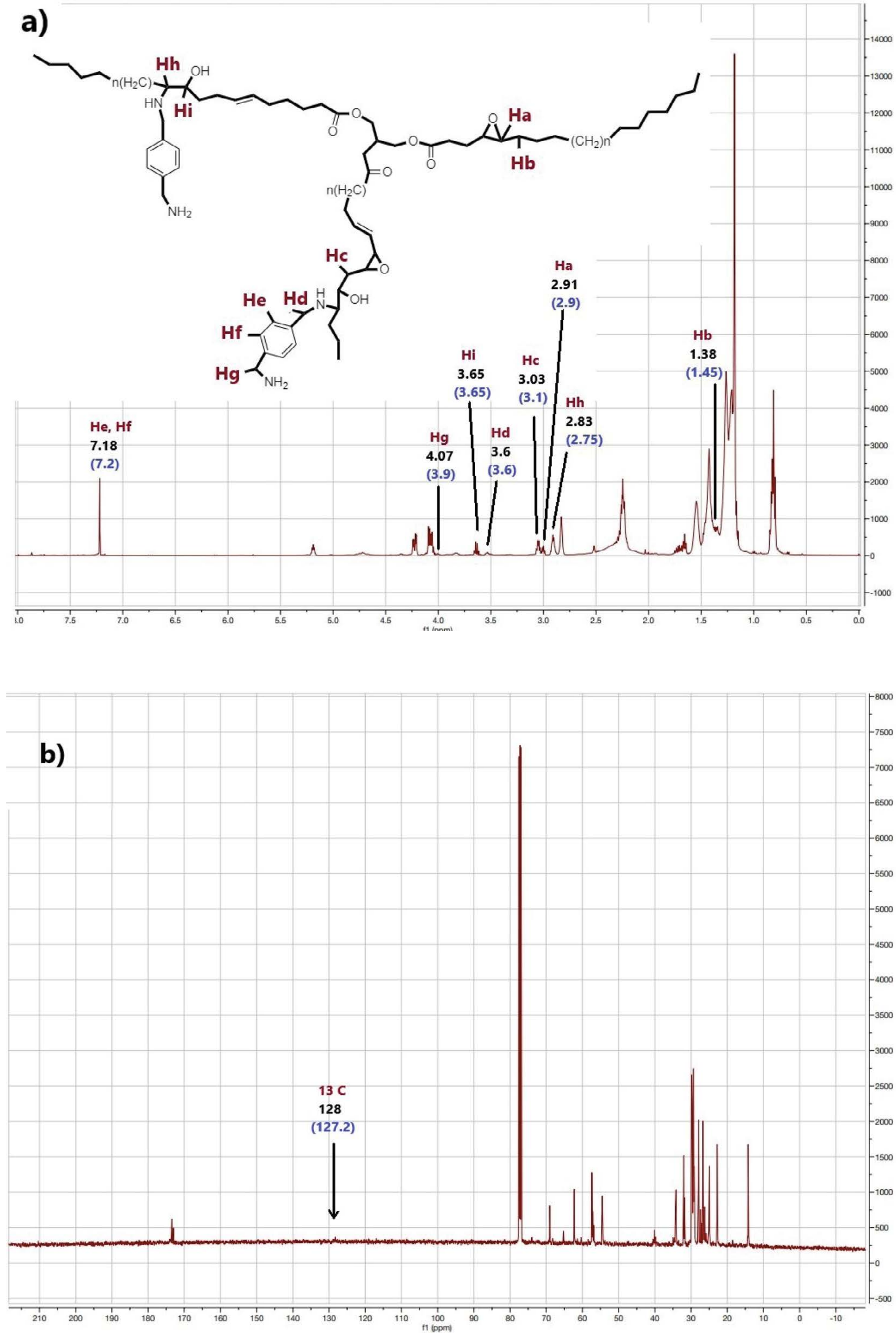

Figure 2. a: ^{1}H and b) ^{13}C NMR analyses of rice bran oil partially aminated with p-xylylenediamine (X-eRBO). b: ^{13}C NMR spectrum of X-eRBO.

CONCLUSIONS

Rice bran oil, a by-product of the rice industry, can be functionalized with the p-xylylenediamine aromatic diamine. The prior epoxidation of this oil using Novozym 435 lipase results in a more efficient strategy for the insertion of oxirane groups than with previously reported procedures. The most efficient aromatic amination occurred under conditions of 70 °C and an eRBO:$XnCl_2$ molar ratio of 0.92. The amination with the p-xylylenediamine resulting from the RBO was partial and confirmed by FTIR, ^{1}H, and ^{13}C NMR analyses.

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