AGRO PRODUCTIVIDAD

Colegio de
Postgraduados

Rice bran oil biorefining: functionalization with acrylate

Ponciano-Leyva, Edgar D.[1]; **Zúñiga-Díaz, Jacqueline**[2]; **Porcayo-Calderón, Jesús**[3]; **Hernández-Campos, Francisco J.**[1]; **Salado-Huerta, José E.**[1]; **Acevedo-Quiroz, Macdiel E.**[2]; **Quinto-Hernández, Alfredo**[1*]

[1] Tecnológico Nacional de México-Instituto Tecnológico de Zacatepec. Departamento de Ingeniería Química y Bioquímica. Zacatepec, Morelos, México. C. P. 62780.
[2] Tecnológico Nacional de México - Instituto Tecnológico de Zacatepec. Departamento de Ciencias Básicas. Zacatepec, Morelos, México. C. P. 62780.
[3] Universidad de Sonora. Departamento de Ingeniería Química y Metalurgia. Hermosillo, Sonora, México. C. P. 83000.
* Correspondence: alfredo.qh@zacatepec.tecnm.mx

ABSTRACT

Objective: To obtain acrylated refined rice bran oil (RBO) using a combined functionalization: first, epoxidation with H_2O_2/Novozym 435 lipase, followed by acrylate group insertion.

Design/Methodology/Approach: After being epoxidized with H_2O_2/Novozym 435, the refined rice bran oil was acrylated via epoxy ring-opening, using triethanolamine as catalyst and 4-methoxyphenol as inhibitor. The experimental conditions of temperature (T=100 and 110 °C) and reaction time (t=3 and 4 hours), as well as the ratio of g eRBO (epoxidized oil) to g acrylic acid (1.5 and 2.0) were considered for the functionalization. The functionalizations were monitored using iodine value (IV), saponification value (SV), and oxirane oxygen content (OOC), Fourier transform infrared (FTIR), and nuclear magnetic resonance (^{1}H NMR), which allowed the estimation of the %Acrylation.

Results: The ^{1}H NMR studies indicate that the acrylation of rice bran oil is efficient, which is confirmed with the evolution of IV, SV, and OOC. Using the OOC, the best acrylation condition was identified at T=110 °C, t=3 hours, and ratio of g eRBO to g acrylic acid=1.5, obtaining a %Acrylation of 85.89% via ^{1}H NMR.

Study Limitations/Implications: Partially acrylated rice bran oil may become an intermediate in the biorefining of this oil and be used in the synthesis of crosslinked polymers.

Findings/Conclusions: Refined rice bran oil was efficiently acrylated using two consecutive steps: it was initially epoxidized with H_2O_2/Novozym 435, followed by functionalization with acrylate group.

Keywords: Acrylation, Epoxidation, Biorefining.

Citation: Ponciano-Leyva, E. D., Zúñiga-Díaz, J., Porcayo-Calderón, J., Hernández-Campos, F. J., Salado-Huerta, J. E., Acevedo-Quiroz, M. E., & Quinto-Hernández, A. (2023). Rice bran oil biorefining: functionalization with acrylate. *Agro Productividad*. https://doi.org/10.32854/agrop.v16i12.2509

Academic Editors: Jorge Cadena Iñiguez and Lucero del Mar Ruiz Posadas

Received: February 10, 2023.
Accepted: October 17, 2023.
Published on-line: December 28, 2023.

Agro Productividad, 16(12). December. 2023. pp: 57-63.

This work is licensed under a Creative Commons Attribution-Non-Commercial 4.0 International license.

INTRODUCTION

Rice bran oil (RBO) is a by-product of the milling of rice (*Oryzae sative* L.) seeds. This bran has a 10-25 % oil content (Balat, 2011; Saunders, 1985), depending on the variety of rice, the geographical origin, and the extraction method (Most *et al.*, 2005). Once extracted, the oil is dark green and has a high phosphatide content and a large amount of free fatty acids. When the oil is refined, the phosphatides and free fatty acids are eliminated, increasing the triglyceride concentration. These molecules are esters of three fatty acids attached to a glycerol molecule, and may contain C=C unsaturations.

Thanks to these unsaturations, the oil can be transformed into high value-added chemical products, such as those used in the polymer, cosmetic, and food industries. For this purpose, the oils are modified through the addition of functional groups to the unsaturations, a process known as functionalization. Epoxidation is a functionalization in which a C-O-C epoxy group is formed by replacing the C=C double bond and it is usually employed as the initial step for the insertion of subsequent functional groups (Samuelsson *et al.*, 2004). Epoxidation is usually achieved through chemical or chemical-enzymatic processes; in both cases, a catalyst is required in the presence of an atomic oxygen donor (*e.g.*, H_2O_2). Chemical-enzymatic epoxidation has had a better performance than chemical epoxidation; *Candida antarctica* (Novozym 435) is a good option for this process. Meanwhile, acrylation has been studied in different vegetable oils, such as palm (KuShAiri and Choo, 2015; Salih *et al.*, 2015), soybean (Behera and Banthia, 2008; Chu *et al.*, 2014), and linseed or flaxseed (Rana and Evitts, 2015; Wuzella *et al.*, 2012). The typical acrylation strategy consists of the epoxy ring-opening, followed by the insertion of the acrylate group ($CH_2=CHCOO$).

MATERIALS AND METHODS

IV, SV, and OOC of oils

Using international standards, we determined the IV (Firestone, 1998a), the SV, and the molecular weight (MW) (Firestone, 1998b), as well as the OOC (Standard, 2017) of refined (commercially obtained), epoxidized (eRBO), and acrylated (A-eRBO) RBO. These determinations were carried out in triplicate. The IV measures the amount of C=C double bonds present in the oil under study, while the SV enables the inference of its approximate MW, and the OOC determines the percentage of oxirane oxygen that is added to the C=C bonds in a sample.

Epoxidation of rice bran oil (eRBO)

The epoxidation of RBO was carried out using Novozym 435 as a catalyst (Albarrán-Preza *et al.*, 2016), in the presence of H_2O_2. The optimal epoxidation conditions were: 0.1 mol of refined RBO, 1.1 mol of toluene, and 0.018 mol of oleic acid. Once this mixture was completely incorporated, 2.5 g of Novozym 435 were added; immediately, 56.6 mL of 30% H_2O_2 solution at 44 °C were added drop by drop, every 2-3 seconds. The reaction was maintained under these conditions for 24 hours. Once the process was completed, Novozym 435 was recovered, while the organic phase was extracted with a 5 % $NaHCO_3$ solution to remove the catalyst and free fatty acids. The organic phase was also recovered, washed with anhydrous $MgSO_4$ during one hour, and concentrated using a rotary evaporator (Petrović *et al.*, 2002).

eRBO acrylation

The eRBO acrylation was carried out under the following experimental conditions: temperature (T=100 and 110 °C) and reaction time (t=3 and 4 hours), as well as the ratio of g eRBO to g acrylic acid (1.5 and 2.0). For all tests, 1 g eRBO, 0.0025g 4-methoxyphenol, and 0.0025 g triethanolamine were used. Once these components were mixed, the system was subjected to the established temperature and the acrylic acid (5 seconds/drop) was immediately incorporated. The mixture was maintained for the above mentioned time (Salih *et al.*, 2015).

Infrared spectroscopy and nuclear magnetic resonance

The samples obtained were analyzed by infrared spectroscopy (FTIR) and ^{1}H nuclear magnetic resonance (NMR). For this purpose, a Perkin Elmer Spectrum™ One FTIR-ATR spectrometer with a 4,000 to 400 cm^{-1} radiation source and a Bruker Avance™ III HD 400 MHz NMR equipment were used.

RESULTS AND DISCUSSION

IV, SV, MW, and OOC monitoring

Table 1 shows the determinations of the IV, SV, MW, and OOC for the RBO and eRBO. The average IV of the refined RBO samples was estimated at 90.63±1.36, matching the findings of previous reports: 90 (Gupta *et al.*, 2016), 91.38 (Zúñiga-Diaz *et al.*, 2017), and 95.40 (Hanmoungjai *et al.*, 2011). Similarly, a SV of 192.10±2.06 was obtained for RBO, based on a MW of 877.19 g/gmol and a OOC of 0.06±0.02; the latter suggests the absence in practice of epoxy groups. The average SV of refined RBO was slightly higher than the previously reported results —187.60 (Hanmoungjai *et al.*, 2001) and 182.35 (Zúñiga-Diaz *et al.*, 2017)— which implies a lower MW of the refined RBO. The IV, SV, MW, and OOC were also determined for the eRBO. The IV was reduced to 0.52±0.16, suggesting an almost complete disappearance of the C=C double bonds after epoxidation. Considering that the RBO is composed of 81.97% unsaturated fatty acids (oleic acid, linoleic acid, and linolenic acid (0.87%) (Most *et al.*, 2005), the RBO could be efficiently functionalized. The eRBO SV was estimated at 170.82±3.48, obtaining an eRBO MW of 985.61 g/gmol. The difference between the MW of the RBO and the eRBO proves an epoxidation with the insertion of 6-7 atomic oxygen atoms into the RBO.

The increase of OOC to 5.12±0.60 in eRBO confirmed that the insertion of the epoxy group was even more efficient than in previous reports. Therefore, the average OOC of the eRBO increased ~85 times with respect to the OOC of the refined RBO. In comparison, the traditional method based on H_2O_2/formic acid (Gupta *et al.*, 2016) only increases OOC by ~4.7 times after epoxidation. The OOC was also determined after the functionalizations with an acrylate group, for all the experimental conditions (temperature, reaction time, and ratio of g eRBO to g acrylic acid). The maximum OOC reduction (to 3.63±0.18) was found under the following conditions: T=110 °C, t=3 hours, and a ratio

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

Oil	IV	MW	SV	OOC
	(g I_2/100g)	(g/gmol)	(mg KOH/g)	
RBO	90.63±1.36	877.19	192.10±2.06	0.06±0.02
eRBO	0.52±0.16	985.61	170.82±3.5	5.12±0.60
A-eRBO	72.33±1.25	1162.79	145.86±3.00	3.63±0.18

of g eRBO to g acrylic acid of 1:1.5. These results imply that the greatest destruction of epoxy rings took place in these conditions due to the insertion of the acrylate group. The resulting sample is now called A-eRBO and its IV, SV, and OOC were determined (Table 1). The dramatic increase in the IV (to 72.33±1.25) in the A-eRBO sample suggests the insertion of the acrylate group (CH_2=CHCOO), given the new increase in the presence of C=C bonds. The SV was estimated at 145.86±2.93 and its MW at 1,162.79 g/gmol, indicating a partial acrylation of the eRBO. A simple analysis suggests that the ~16% increase in the MW of A-eRBO (with respect to the MW of eRBO) implies an insertion of 2-3 acrylate groups of the 6-7 epoxy groups available in eRBO.

Infrared spectroscopy analysis

RBO and eRBO samples were analyzed using infrared spectroscopy. Figure 1 shows the FTIR spectra for these oils. The RBO FTIR spectrum (Figure 1a) matches the findings of Rohman *et al.* (2011) and Zúñiga-Diaz *et al.* (2017); however, there is an absence of the weak signal at ~1711 cm^{-1}, which indicates the presence of free fatty acids. Such absence is attributed to the refinement of the RBO. Finally, the eRBO FTIR spectrum (Figure 1b) shows a strong signal (at 824 cm^{-1}), which is consistent with the presence of the epoxy group (Vlček and Petrović, 2006).

^{1}H NMR analysis of the A-eRBO functionalized oil

Figure 2 shows the ^{1}H NMR spectrum of partially acrylated rice bran oil (A-eRBO). A generic structure of the A-eRBO obtained in this work can be seen in this figure. Resonant protons associated with unreacted epoxy groups are observed in the 2.93 ppm (–CH– of epoxy group), 1.57 ppm (adjacent –CH– protons to epoxy group), and 3.14 ppm (adjacent –CH– protons of two epoxy groups) signals (Lopez Tellez *et al.*, 2008). Patterns of proton signals typical of triglycerides are also observed. Some include the triplet centered at 0.86 ppm (protons of the $–CH_3$ methyl group at the ends of the carbon chains of the triglyceride), the signal at 1.27 ppm ($–CH_2–$ methylene protons of the saturated chains of the $–CH_2–CH_2–CH_2–$ fatty acid), and the signal at 1.65 ppm, resulting from

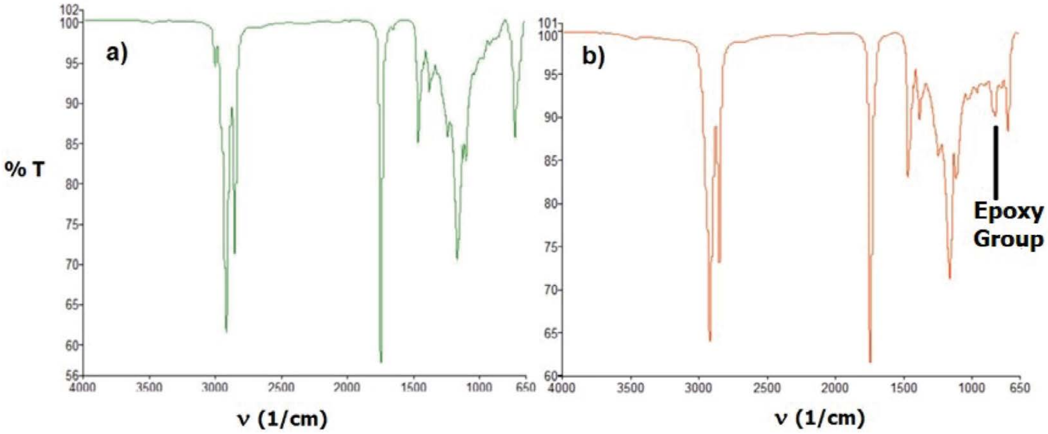

Figure 1. FTIR spectra of refined RBO (green line) and epoxidized eRBO (red line).

the resonances of the β methylene $-CH_2$ protons of the glycerol portion of the triglyceride in $-C{=}OCH_2\text{-}CH_2$ (Salih *et al.*, 2015). The insertion of the acrylate group was verified by four signals in the ^1H NMR spectrum (Salih *et al.*, 2015). The signals in the 3.58-3.75 ppm (Hk) region are attributed to the protons of the –CH–OH methine group that previously were part of the epoxy group. Likewise, the proton of a neighboring carbon to this methine group is identified in the region at 4.07-4.15 ppm (Hl): the αproton with respect to atomic oxygen of the $-CH-CH-O-C{=}O<$ acrylate groups, identified as a quartet at 4.07-4.16 ppm. Finally, the signals of the $CH_2{=}CH-$ (vinyl) group in the acrylate group were also identified. In conclusion, the two vinyl protons in $CH_2{=}CH$ (Hm, Hn) and its methine proton (Ho) are observed at 5.80-5.86, 6.40-6.48, and 6.18 ppm, respectively. Nevertheless, the three protons in $CH_2{=}CH$ are not equivalent and therefore have different δ chemical shifts, as a consequence of their orientation (Fu *et al.*, 2010). No signals corresponding to free acrylic acid —which are usually observed at the 10-13 ppm range— were found.

The %Acrylation was determined as an internal reference pattern, based on the areas of the CH_3 methyl group signals. Equation 1 establishes the %Acrylation, following previous reports (Zhang *et al.*, 2011).

$$\%Acrylation = \frac{a_{Acrylate}}{a_{Epoxy} + a_{Acrylate}} \times 100 \qquad (1)$$

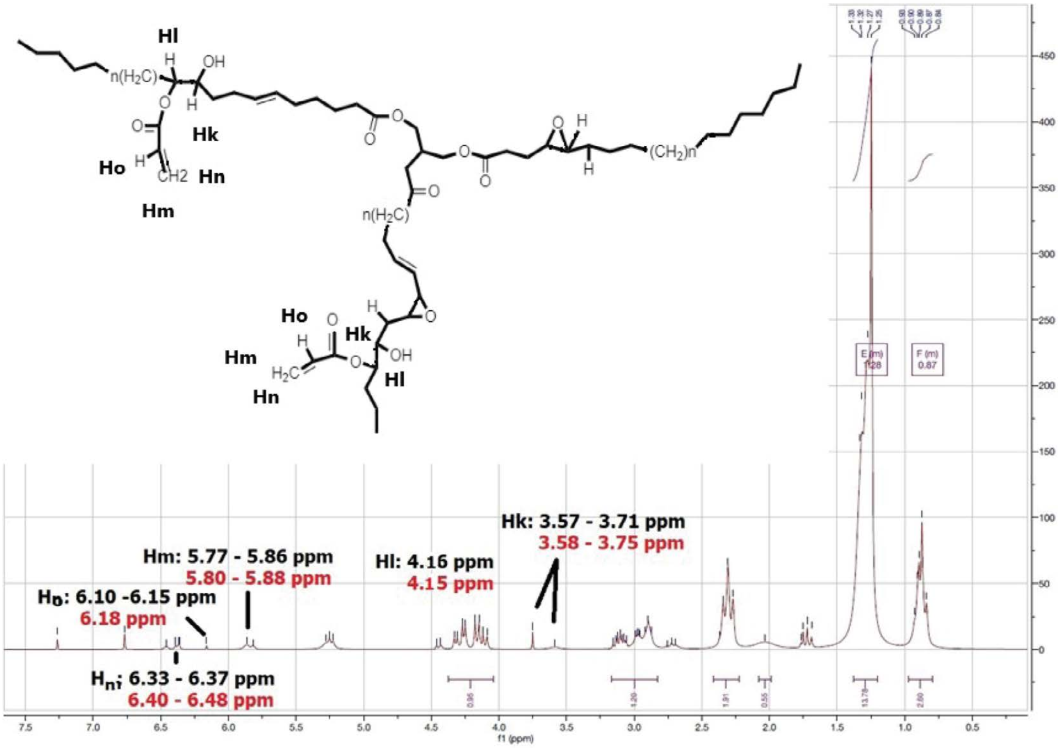

Figure 2. ^1H NMR analysis of rice bran oil partially acrylated with acrylic acid (A-eRBO). Values in bold typography are reference values (Fu *et al.*, 2010; Salih *et al.*, 2015).

where a_{Epoxy} is the integrated area of the associated protons of the methine epoxy at 2.76 ppm and $a_{Acrylate}$ is the integrated area of the acrylate protons in the 5.80-6.48 ppm range. The ^{1}H NMR spectrum (Figure 2) indicates that an 85.89% Acrylation was obtained with a_{Epoxy}=296.72 ua and $a_{Acrylate}$=1,806.8 ua. These results are comparable to previous reports of acrylated oils (Scala and Wool, 2002; Sharma *et al.*, 2006).

CONCLUSIONS

Refined rice bran oil can be feasibly functionalized with an acrylate group, through an intermediate epoxidation using H_2O_2, in the presence of Novozym 435 lipase. According to the ^{1}H NMR analysis, the best %Acrylation (85.89%) was estimated for the following conditions: T=110 °C, t=3 hours, and ratio of g eRBO to g acrylic acid of 1:1.5. The evolution of acrylation was confirmed by the of IV, SV, OOC, and MW values recorded.

ACKNOWLEDGEMENTS

The authors would like to thank the Tecnológico Nacional de México for financing this research (Proyecto No.15266.22-P de Investigación Científica, Desarrollo Tecnológico e Innovación).

REFERENCES

Albarrán-Preza, E., Corona-Becerril, D., Vigueras-Santiago, E., & Hernández-López, S. (2016). Sweet polymers: Synthesis and characterization of xylitol-based epoxidized linseed oil resins. *European Polymer Journal*, 75, 539-551.

Balat, M. (2011). Potential alternatives to edible oils for biodiesel production–A review of current work. *Energy Conversion and Management, 52*(2), 1479-1492.

Behera, D., & Banthia, A. K. (2008). Synthesis, characterization, and kinetics study of thermal decomposition of epoxidized soybean oil acrylate. *Journal of applied polymer science, 109*(4), 2583-2590.

Chu, X. M., Liu, S. J., & Zhao, F. Q. (2014). Preparation of acrylated epoxidized soybean oil with excellent properties.

Firestone, D. E. (Ed.). (1998a). AOCS, Method 920.158 (15th ed.). American Oil Chemists' Society.

Firestone, D. E. (Ed.). (1998b). AOCS, Method 920.160 (15th ed.). American Oil Chemists' Society.

Fu, L., Yang, L., Dai, C., Zhao, C., & Ma, L. (2010). Thermal and mechanical properties of acrylated expoxidized-soybean oil-based thermosets. *Journal of applied polymer science, 117*(4), 2220-2225.

Gupta, N. K., Yadav, P. K. S., Eadara, R., & Singh, R. P. (2016). Synthesis of epoxy resin from waste ricebran oil. *Polymers from Renewable Resources, 7*(1), 21-32.

Hanmoungjai, P., Pyle, D. L., & Niranjan, K. (2001). Enzymatic process for extracting oil and protein from rice bran. *Journal of the American Oil Chemists' Society, 78*(8), 817-821.

KuShAiri, A., & Choo, Y. M. (2015). Optimisation on synthesis of acrylated epoxidised palm olein using response surface methodology. *Journal of Oil Palm Research, 27*(4), 366-376.

Lopez Tellez, G., Vigueras-Santiago, E., Hernandez-Lopez, S., & Bilyeu, B. (2008). Synthesis and thermal cross-linking study of partially-aminated epoxidized linseed oil. *Designed Monomers and Polymers, 11*(5), 435-445.

Most, M. M., Tulley, R., Morales, S., & Lefevre, M. (2005). Rice bran oil, not fiber, lowers cholesterol in humans. *The American journal of clinical nutrition, 81*(1), 64-68.

Petrović, Z. S., Zlatanić, A., Lava, C. C., & Sinadinović-Fišer, S. (2002). Epoxidation of soybean oil in toluene with peroxoacetic and peroxoformic acids—kinetics and side reactions. *European journal of lipid science and technology, 104*(5), 293-299.

Rana, A., & Evitts, R. W. (2015). Synthesis and characterization of acrylated epoxidized flaxseed oil for biopolymeric applications. *International Polymer Processing, 30*(3), 331-336.

Rohman, A., Che Man, Y. B., & Riyanto, S. (2011). Authentication analysis of red fruit (*Pandanus conoideus* Lam) oil using FTIR spectroscopy in combination with chemometrics. *Phytochemical Analysis, 22*(5), 462-467.

Salih, A. M., Ahmad, M. B., Ibrahim, N. A., Dahlan, K. Z. H. M., Tajau, R., Mahmood, M. H., & Yunus, W. M. Z. W. (2015). Synthesis of radiation curable palm oil–based epoxy acrylate: NMR and FTIR spectroscopic investigations. *Molecules, 20*(8), 14191-14211.

Samuelsson, J., Sundell, P.-E., & Johansson, M. (2004). Synthesis and polymerization of a radiation curable hyperbranched resin based on epoxy functional fatty acids. *Progress in organic coatings, 50*(3), 193-198.

Saunders, R. M. (1985). Rice bran: Composition and potential food uses. *Food Reviews International, 1*(3), 465-495.

Scala, J. L., & Wool, R. P. (2002). The effect of fatty acid composition on the acrylation kinetics of epoxidized triacylglycerols. *Journal of the American Oil Chemists' Society, 79*(1), 59-63.

Sharma, B. K., Adhvaryu, A., & Erhan, S. Z. (2006). Synthesis of hydroxy thio-ether derivatives of vegetable oil. *Journal of agricultural and food chemistry, 54*(26), 9866-9872.

Standard, A. (2017). D1652-97, Standard Test Methods for Epoxy Content of Epoxy Resins. In Annual book of ASTM standards.

Vlček, T., & Petrović, Z. S. (2006). Optimization of the chemoenzymatic epoxidation of soybean oil. *Journal of the American Oil Chemists' Society, 83*(3), 247-252.

Wuzella, G., Mahendran, A. R., Müller, U., Kandelbauer, A., & Teischinger, A. (2012). Photocrosslinking of an acrylated epoxidized linseed oil: kinetics and its application for optimized wood coatings. *Journal of Polymers and the Environment, 20*(4), 1063-1074.

Zhang, X., Chen, L., Zhang, Q., Huang, Y., & Li, J. (2011). Synthesis and characterization of epoxidized acrylated natural rubber cross-linked by star-shaped polystyrene.

Zúñiga-Diaz, J., Reyes-Dorantes, E., Quinto-Hernandez, A., Porcayo-Calderon, J., Gonzalez-Rodriguez, J. G., & Martinez-Gomez, L. (2017). Oil extraction from "Morelos Rice" bran: Kinetics and raw oil stability. *Journal of Chemistry*, 2017.