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The kinetics of lipase activity and hydrolytic rancidity of raw, parboiled, and extruded rice bran during storage

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Abstract

The main problem related to rice bran use is that it goes rancid right after its production. The objective of the present study was to apply a mathematical model to evaluate the kinetics of the lipase activity and hydrolytic rancidity of the raw rice bran (RRB), extruded rice bran (ERB), and parboiled rice bran (PRB) stored in low density polyethylene bags at room temperature for 180 days. Extrusion and parboiling were efficient in preventing free fatty acid formation in ERB and PRB. Extrusion reduced the velocity constant of lipase activity as compared to that of RRB while parboiling increased it, and both decreased the lipase activity after equilibrium from 150 days. The extrusion and parboiling treatments increased the velocity constants for the liberation of free fatty acids although the equilibrium was reached with reduced production of free fatty acids in relation to the production of raw rice bran after 150 days of storage. Extrusion proved the best treatment under the storage temperature conditions of rice bran from cultivar BRS Primavera.

Keywords: *Oryza sativa* L.; byproduct; free fatty acids; shelf life.

1 Introduction

Rice is the second most cultivated and consumed cereal in the World, with an estimated production of 730 million tons in 2012 (FOOD..., 2013). Rice bran represents 8% of the total rice weight; it is the layer between the inner white rice grain and the outer husk obtained during the dehulling and polishing performed to obtain the polished rice or polished parboiled rice.

Rice bran contains significant amounts of carbohydrates, proteins, and lipids; lipids are highly susceptible to rancidity, which significantly limits its use. Rancidity is initiated soon after obtaining the bran, resulting in rapid degradation of the lipids and in an increase in acidity (SAUNDERS, 1985-1986) due to enzymatic hydrolytic, especially by the action of lipases (ALENCAR; ALVARENGA, 1991; MALEKIAN et al., 2000; SAUNDERS, 1990a).

One of the ways to conserve rice bran is the inactivation or decrease in the activity of the enzymes responsible for the rancidity by heat treatment (SAUNDERS, 1990b). Thermoplastic extrusion, a traditional method used to stabilize rice bran, causes inactivation of the enzymes promoting a longer shelf life (CARVALHO; BASSINELLO, 2006; LUH; BARBER; BARBER, 1991). The rice bran obtained after the parboiling process requires no additional heat treatment since this process may inactivate the enzymes responsible for lipid degradation (SILVA; SANCHES; AMANTE, 2006; SLAVIN; LAMPE, 1992).

The increase in free fatty acid content of polished rice can be attributed to the activity of rice lipase that degrades the phospholipids releasing free fatty acids at the beginning of the reaction and the triacylglycerides during storage

(AIBRA et al., 1986). Paolucci-Jeanjean et al. (2000) proposed an empirical equation for a two-phase hydrolysis of starch to oligosaccharides. The equation of free fat acid formation in milled rice proposed by Lam and Proctor (2002) is similar to the Paolucci-Jeanjean et al. (2000) model with two expressions, but an additional term represents the initial concentrations of free fat acid on the surface of milled rice. During prolonged storage, the kinetics of lipase activity can show stabilization at a low level of activity tending to equilibrium after 180 days, and consequently the production of fatty acids stabilizes at the maximum level. If this hypothesis is considered valid, the equation of the Azuara, Berinstain and Garcia (1992) model can be used to determine the velocity constants of the reactions, as well as the points of minimal lipase activity and maximum free fatty acid production.

The objective of the present study was to apply the Azuara's model to evaluate the kinetics of the lipase activity and hydrolytic rancidity of raw rice bran (RRB), extruded rice bran (ERB), and parboiled rice bran (PRB) stored in low density polyethylene bags at room temperature for 180 days.

2 Materials and methods

Rice bran obtained from the upland cultivar BRS Primavera was used. The raw rice bran (RRB) was donated by the industry *Arroz Cristal Ltda.*, located in the municipality of Aparecida de Goiânia, GO, Brazil.

Soon after dehulling, a portion of RRB was submitted to extrusion in a single screw extruder (MCI, 1, Itu, Brazil),

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capacity 150 kg h⁻¹, at a stabilized temperature of 110 ± 3 °C, matrix diameter of 133 mm, frequency of 50-60 Hz, and moisture content of 5.6 g (100 g)⁻¹. Extrusion was carried out at *Cicopal - Indústria e Comércio de Gêneros Alimentícios e Higiene Pessoal Ltda*, located in the municipality of Senador Canedo, GO, Brazil. The extruded rice bran (ERB) was then homogenized in a hexagonal-shaped mixer with a capacity for 150 kg per 5 min, at a velocity of 12 rpm, and then dried for 8 h at 60 °C in a incubator (Marconi, MA 035, Piracicaba, Brazil) with air circulation.

The BRS Primavera rice sample was parboiled at the *Cerealista Medeiros* factory under the following conditions: soaking for 5 h at 70 °C, steaming in a continuous autoclave (Pagé, Araranguá, Brazil) with a capacity 10.000 kg h⁻¹, and finally drying in a column dryer (Pagé, Araranguá, Brazil) at 90 °C for 6 h using drying and cooling chambers. After parboiling, the rice was dehusked and milled to obtain the parboiled rice bran (PRB).

The RRB, ERB, and PRB samples were placed in low density polyethylene bags (15 µm thick), heat sealed in a manual sealer (Barbi, M 300T, Itu, Brazil), and stored in cardboard boxes at room temperature, totaling forty-eight individually packed portions for each type of bran, each with a mass of 30 g, for the hydrolytic rancidity (HR) and lipase activity (LA) analyses.

An entirely randomized experimental design was used with three treatments (RRB, ERB, and PRB) and three repetitions during a storage period of one hundred and eighty days. During this period, the LA and HR of the samples of each treatment were evaluated at different times: every three days for the first twelve days, every six days from the twelfth to the thirtieth day, every ten days from the thirtieth to the sixtieth day, every 15 days from the sixtieth to the ninetieth day, and after that, every 30 days up to the end of the experiment. Therefore, at each time, three samples from each treatment were collected at a total of 16 different times, giving a grand total of one hundred and forty-four samples.

The LA and HR analyses were carried out in duplicate at the Grains and Byproducts Laboratory of Embrapa Rice and Beans in Santo Antônio de Goiás, GO, Brazil. The LA analysis was based on the production of free fatty acids as a result of the lipase activity after its extraction and reaction with the added substrate (1 mL of emulsion composed of 30 mg of olive oil with 30 mg of Tween 20 per mL of deionized water); the HR analysis, on the other hand, was based on the free fatty acid content present in the sample, both followed the methodology described by Goffman and Bergman (2003b). The data obtained were evaluated by the analysis of variance (Anova) and non-linear regression, both using the SAS Statistical Software (STATISTICAL..., 2002). The LA and HR results were expressed according to the free fatty acid content in milligrams of caprylic acid (C8:0) by 100 mg of rice bran based on the caprylic acid standard curve built according to the Kwon and Rhee method (1986).

The kinetic behavior of the lipase activity and hydrolytic rancidity were modeled according to Azuara, Berinstain and Garcia (1992), Equation 1.

$$\frac{t}{X} = \frac{1}{s(X_{\infty})} + \frac{t}{X_{\infty}} \quad (1)$$

where: X = values for lipase activity (mg caprylic acid 100 mg⁻¹ of bran) or hydrolytic rancidity (mg of C 8:0 100 mg⁻¹); X_∞ = equilibrium values for the lipase activity or hydrolytic rancidity, respectively; s = velocity constant; t = time (days).

3 Results and discussion

3.1 Lipase activity (LA)

LA is the main factor involved in determining the intensity of the hydrolytic deterioration of rice bran (GOFFMAN; BERGMAN, 2003b). Figure 1a shows the regression equations and the behavior of the lipase in the RRB, ERB, and PRB stored at room temperature for one hundred and eighty days.

Throughout the entire storage period, RRB presented greater lipase activity (P ≤ 0.05) than that of the other bran samples, but before one hundred and fifty days there was practically no difference between the ERB and PRB samples (P > 0.05) although after that time, ERB showed less enzymatic activity (P ≤ 0.05). Both the linear and quadratic effects of storage time were not significant with respect to the lipase activity of RRB (P = 0.6059 and P = 0.8325, respectively). For ERB and PRB (1.13 and 1.11 mg caprylic acid 100 mg⁻¹ of bran, respectively on initial time), only the linear effect of the storage time was significant (P = 0.0458 and P = 0.0019, respectively). The variation in lipase activity for RRB was 0.23-1.64 mg caprylic acid 100 mg⁻¹ of bran, less than that found by Goffman and Bergman (2003b), 0.43-2.28 mg caprylic acid 100 mg⁻¹ of different rice bran.

For RRB, ERB, and PRB, the highest lipase activities were observed at the beginning of the storage period, a fact also reported by Goffman and Bergman (2003a) when investigating raw rice bran samples from two rice cultivars stored in open plastic bags at room temperature (20 °C to 25 °C) for five months. According to these authors, the maximum lipase activity at the beginning of storage was due to the high triacylglyceride content (bran from rice cultivars 'Cypress' and 'Earl' differing in oil concentration 23.5 and 18.3 mg of triacylglycerol per 100 mg of bran, respectively), which decreased with the decrease in the concentration of this lipase substrate.

Rice bran has different types of lipase, such as the phospholipases, glycolipases, and esterases. Depending on the type of heat treatment applied to the bran, the complete inactivation of these enzymes may or may not occur (LUH; BARBER; BARBER, 1991). Bhardwaj, Raju and Rajasekharan (2001) found significant heat stability for the rice bran lipase. Inactivation of the lipases by the heat treatments applied to the bran samples in this study resulted in a gradual fall in the activity of the enzyme during storage. The enzyme was active up to 40 °C, and the activity decreased sharply to 65% at 60 °C, and then it decreased gradually (BHARDWAJ; RAJU; RAJASEKHARAN, 2001). It is likely that the parboiling at 70 °C for 5 hours used in this study was not sufficient to inactivate 100% of lipolytic activity. The increased lipase activity after 120 days is probably

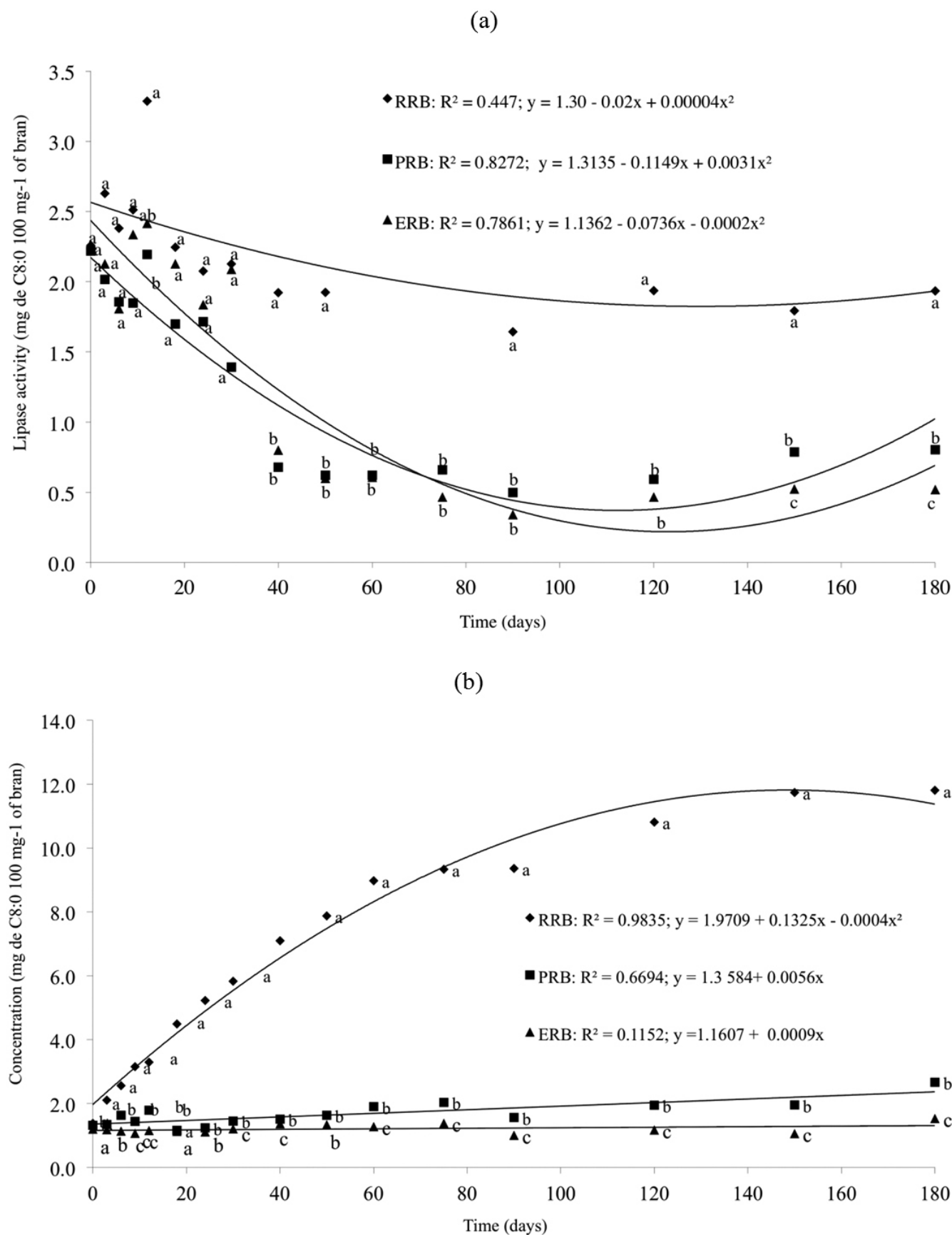


Figure 1. Lipase activity (mg caprylic acid 100 mg⁻¹) (a) and hydrolytic rancidity (mg caprylic acid 100 mg⁻¹) (b) in the raw rice bran (RRB), extruded rice bran (ERB), and parboiled rice bran (PRB) stored at room temperature.

due to microbial lipase activity, which may have occurred after this period.

Throughout one hundred and thirty five days of storage of rice bran, Saunders (1990a) observed less lipase activity in ERB than in RRB, a fact also observed in the present study. According to the same author, the extrusion temperature inversely affects enzyme activity. The extrusion temperature used in the present study ($110 \pm 3^\circ\text{C}$) was sufficient to slow down the lipase activity, but not to inactivate it.

Hammond (1994) concluded that the bran obtained from parboiled rice required no additional heat treatment for stabilization, that is, the parboiling process was sufficient to inactivate some of the enzymes, including the lipases, increasing the product shelf life. This was also observed in the present study since the enzymatic activity of the rice bran obtained from the parboiled rice showed a similar behavior to that of the rice bran stabilized by extrusion.

The graphical representation of the regression of the lipase activity is shown in Figure 2a, while the values calculated for the model parameters and the coefficients of determination (R^2) are presented in Table 1. The regression equations obtained for the enzymatic activities of RRB, ERB, and PRB as a function of storage time, using the Azuara, Berinstein and Garcia (1992) model, were significant ($P < 0.0000$) and explained 97%, 90%, and 90% of the responses, respectively. The treatments showed considerable variation with respect to the velocity constants (s_1), that is, after extrusion, the lipase reaction velocity in the product decreased by more than three times when compared to that of RRB (Table 1). However, for the parboiled bran, the lipase activity velocity practically doubled when compared to that of the non-treated sample (Table 1). A negative sign in the velocity constant (s_1) indicates that the lipase activity is decreasing with storage time. The value of the equilibrium constant (LA_∞) for RRB was 1.8295, almost two fold greater than that of PRB and three fold greater than that of ERB, indicating that the heat treatments decreased the lipase activity for longer storage times at room temperature. Lipase activity is a parameter that should not be analyzed alone since the lipases are part of a group of enzymes with different specificities and probably the lipase activity does not determine the degree of rice bran rancidity alone.

3.2 Hydrolytic rancidity (HR)

The free fatty acid contents of ERB and PRB increased during the storage period, but the variation was small, from

1.19-1.53 mg for C 8:0 100 mg^{-1} and from 1.32-2.67 mg for C 8:0 100 mg^{-1} , respectively. However, the variation was high for RRB, from 1.38-11.81 mg for C 8:0 100 mg^{-1} , indicating that ERB and PRB were practically stabilized by the heat treatments with respect to free fatty acid formation (Figure 1b).

The regression equations for HR were not significant for ERB ($P = 0.8770$) and PRB ($P = 0.3092$), and the coefficients of determination were low, showing that only 12% and 67% of the variation in the responses was explained by the models, respectively (Figure 1b). Thus, for ERB and PRB, the variation in the production of free fatty acids during storage was not significant, indicating that the storage time did not interfere with the formation of these fatty acids.

RRB was considered inadequate for consumption on the twenty-fourth day of storage since the maximum tolerated limit for free fatty acids is $5\text{ mg } 100\text{ mg}^{-1}$ according to Malekian et al. (2000). The free fatty acids can increase the sensitivity of fat-rich foods to oxidative rancidity since the products formed include the unsaturated fatty acids: oleic, linoleic, and linolenic acids; all highly susceptible to oxidation (ARAÚJO, 2004). This could justify the probable tendency for a decrease in the hydrolytic rancidity curve of the raw rice bran since the free fatty acids could be originated from other products, such as peroxides and secondary oxidation products. Therefore, based on the data obtained in the present study, one can imagine that the heat treatment applied to ERB and PRB was efficient in inactivating the enzymes and consequently reduced the production of free fatty acids.

Goffman and Bergman (2003b) evaluated the hydrolytic rancidity and lipase activity of twenty-four rice varieties for a period of 24 hours and observed a significant positive correlation between hydrolytic rancidity and lipase activity ($r = 0.89$), indicating that the lipolytic process, that is the formation of free fatty acids, is highly influenced by the lipase activity. This can be seen in Figures 1a and 1b, which show similar behavior for each bran with respect to the two parameters determined. The highest lipase activity and the greatest free fatty acid formation were found for the RRB. ERB and PRB showed less lipase activity and less hydrolytic rancidity throughout the entire storage period.

The experimental data were fitted to the Azuara, Berinstein and Garcia (1992) model (Equation 1) by linear regression. Figure 2b shows the graphical representations of the regressions for the hydrolytic rancidity, while the values calculated for the parameters of the model and the coefficients of determination (R^2) are presented in Table 2. The regression equations obtained

Table 1. Parameters of the Azuara, Berinstein and Garcia (1992) model obtained for lipase activity in the raw rice bran (RRB), extruded rice bran (ERB), and parboiled rice bran (PRB) stored in low density ($15\text{ }\mu\text{m}$) polyethylene bags at room temperature for 180 days.

Treatment	Lipase activity			
	P	R^2	$s_1(\text{day}^{-1})$	LA_∞
RRB	<0.0000	0.9658	-0.3418	1.8295
PRB	<0.0000	0.9003	-0.6872	0.6879
ERB	<0.0000	0.8955	-0.1165	0.4470

R^2 : Coefficient of determination. P: Significance level of the model. s_1 : Velocity constant (day^{-1}). LA_∞ : Lipase activity at equilibrium (mg caprylic acid per 100 mg).

Table 2. Parameters of the Azuara, Berinstein and Garcia (1992) model obtained for hydrolytic rancidity in the raw rice bran (RRB), extruded rice bran (ERB), and parboiled rice bran (PRB) stored in low density ($15\text{ }\mu\text{m}$) polyethylene bags at room temperature for 180 days.

Treatment	Hydrolytic rancidity			
	P	R^2	$s_2(\text{day})^{-1}$	HR_∞^b
RRB	<0.0000	0.9645	0.0347	13.333
PRB	<0.0000	0.9145	0.0792	2.2878
ERB	<0.0000	0.9445	0.4748	1.2685

R^2 : Coefficient of determination. P: Significance level of the model. s_2 : Velocity constant (day^{-1}). HR_∞ : Hydrolytic rancidity at equilibrium (mg caprylic acid per 100 mg).

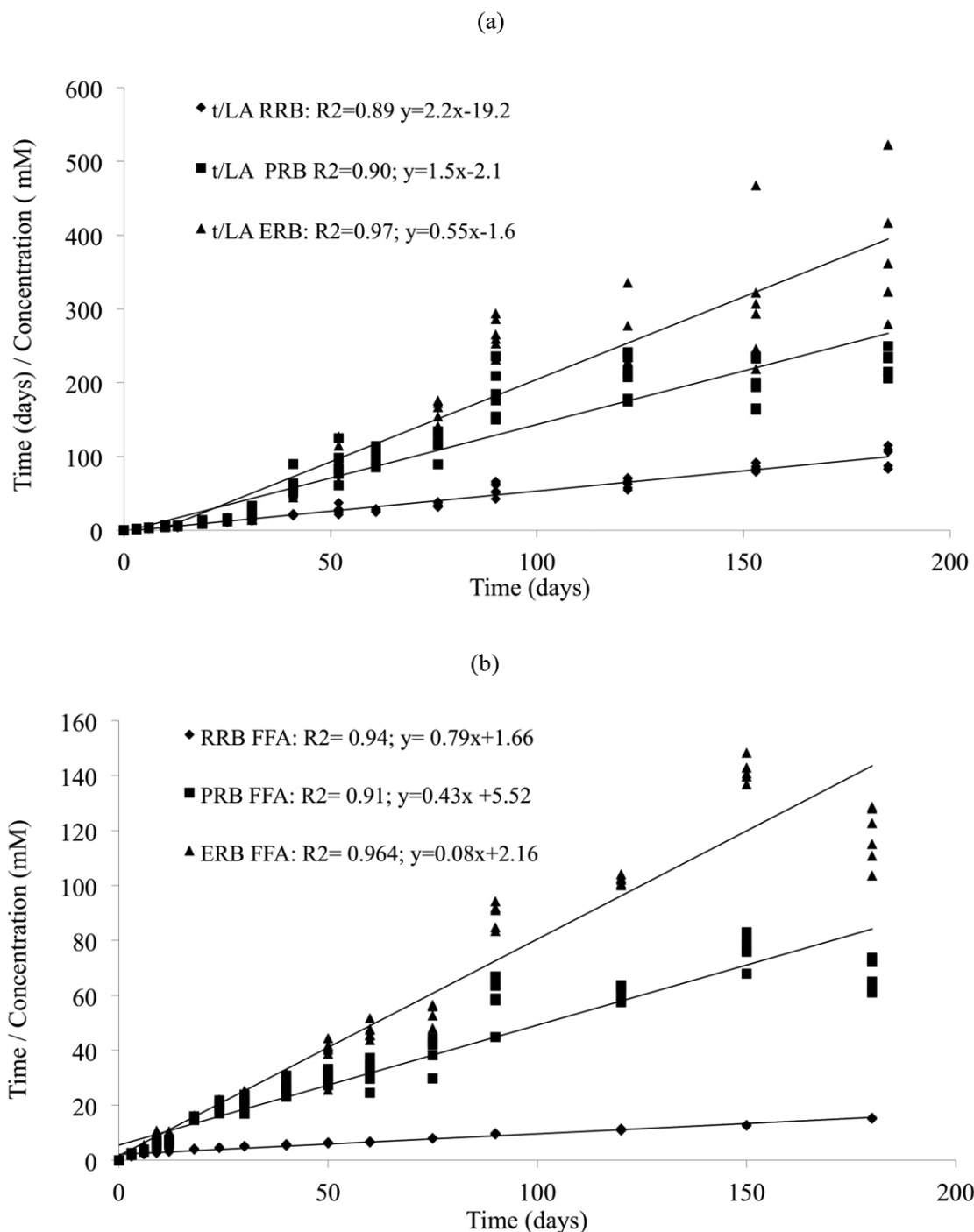


Figure 2. Linearization of the lipase activity (mg caprylic acid per 100 mg) (a) and of the accumulation of free fatty acids (mg caprylic acid per 100 mg) (b) in the raw rice bran (RRB), extruded rice bran (ERB), and parboiled rice bran (PRB) stored at room temperature according to the Azuara, Berinstein and Garcia (1992) model.

for hydrolytic rancidity in the treatments of RRB, ERB, and PRB as a function of storage time applying the Azuara, Berinstein and Garcia (1992) model were significant ($P < 0.0000$) and explained 96%, 91%, and 94% of the responses, respectively.

With respect to the velocity constants for hydrolytic rancidity (s_2), the parboiling treatment caused an increase of

over two fold as compared to that of RRB, while the extrusion treatment showed an increase of about thirteen fold as compared to that of RRB and five fold as compared to that of PRB, showing that the extrusion and parboiling treatments, under the same storage conditions, increased the reaction velocity constant. For RRB, the equilibrium constant (RH_{∞}) showed higher values of up to 10 times than those of ERB, indicating that the extrusion

treatment reached equilibrium with hydrolytic rancidity values much lower than those for the RRB and PRB samples.

Further research is required to make the use of rice bran in human feeding feasible, such as an investigation of the aspects related to the better conservation of the rice bran (packaging, production conditions, and storage) and a biological evaluation of its nutrients and functional components.

4 Conclusions

The extrusion of rice bran was effective in decreasing the velocity constant for the activity of lipase as compared to that of the raw rice bran by decreasing this enzyme activity after reaching equilibrium during longer storage periods. Parboiling increased the velocity constant for the activity of lipase in the rice bran as compared to that of RRB and decreased the lipase activity at the equilibrium during longer storage periods at room temperature.

For hydrolytic rancidity, the extrusion and parboiling treatments increased the velocity constant although equilibrium was reached with lower values for the fatty acid contents compared to that of the raw rice bran after long storage periods.

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