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Kinetics and Mechanism of Free Fatty Acid Formation on the Surface of Milled Rice

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The presence of free fatty acid (FFA) is an important factor in determining rice quality for brewing. FFA formation in milled rice during storage was monitored, and a two-parameter semiempirical kinetic model giving product concentration as a function of time is proposed to describe FFA formation on milled rice during storage. The model was tested using sets of data obtained from partially milled rice samples stored at 24, 37, and 50 °C and fully milled rice stored at 37 °C and 70% relative humidity. The predicted values provide very good fits ($R^2 \geq 97\%$) of the experimental data at all storage temperatures. A two-substrate reaction mechanism representing a two-phase process is also presented. Milled rice FFA at a given storage time varied with storage temperatures. The kinetic model and mechanisms proposed could be useful in describing and predicting FFA contents of milled rice during storage and transportation.

KEYWORDS: Free fatty acid; milled rice; kinetics; mechanisms

INTRODUCTION

Free fatty acid (FFA) development on the surface of milled rice adversely affects its flavor quality. FFA levels increase over time as a result of lipid hydrolysis in the residual bran particles on the milled rice surface. Hydrolysis of rice bran phospholipids by phospholipase into FFAs is one of the important reactions responsible for flavor quality changes during short-term storage of milled rice (*1*). Rice lipid FFAs oxidize and subsequently break down to produce off-flavors. These reactions are commercially important in reducing milled rice quality for brewing applications, because brewers are major rice users. Consequently, milled rice with a FFA content of ≥0.1% may not be acceptable for brewing (Steven Malin, 2000; personal communication). Therefore, the ability to describe and subsequently predict potential oxidation problems and understand the kinetics and reaction mechanisms of FFA formation would be useful. Nevertheless, this topic has received little attention, although studies of isolated rice-bran lipases responsible for FFA formation have been conducted (*2*, *3*).

Several kinetic studies of lipase hydrolysis of lipids have been reported in model systems using extracted and purified microbial lipases (*4*−*7*). These include kinetic studies of commercial wheat-germ lipase and synthetic substrates (*8*). These studies applied the classic Michaelis Menten kinetic model to describe the lipase-catalyzed reactions. However, Redondo et al. (*9*) indicated that the Michaelis Menten model is inadequate to explain lipid hydrolysis, because the reaction is too complex to be described by this simple expression. Lipid hydrolysis is a multisubstrate (triglycerides, diglycerides, and monoglycerides) reaction that has been described as reversible, consecutive, and multistep (*10*), making theoretical kinetic analysis and prediction of the reaction difficult. However, Gaouar et al. (*11*) suggested that empirical kinetic models can be very useful when a quantitative description of changes of a complex reaction is desired, rather than a detailed reaction mechanism.

Paolucci-Jeanjean et al. (*12*) proposed an empirical equation for a two-phase hydrolysis of starch to oligosaccharides:

$$P(t) = \frac{at^2}{b^2 + t^2} - \frac{ct^4}{d^4 + t^4} \tag{1}$$

where $P(t)$ is the oligosaccharide concentration at time t (g·dm^{-3}), t is time (h), a and c are concentration constants (g·dm^{-3}), and b and d are time constants (h). The first expression of the equation, $[at^2/(b^2 + t^2)]$, represents an initial increase in oligosaccharide concentration, while the second expression $[ct^4/(d^4 + t^4)]$ indicates a decrease in oligosaccharide concentration because of hydrolysis to smaller oligosaccharides. Equation 1 is similar in form to the fundamental Michaelis Menten equation. However, in this equation, the established relationship is between time and product concentration, whereas the Michaelis Menten equation relates initial substrate concentration to initial reaction velocity. The objective of this study was to develop an empirical mathematical expression of FFA formation on the milled rice surface during early storage that would describe the experimental data and predict FFA development.

MATERIALS AND METHODS

Materials. Commercially milled long-grain rice samples (Riceland Foods, Stuttgart, AR) were obtained at the first-break (partially milled) and after the third-break (fully milled) stages during milling, transported

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under dry ice to the laboratory, and stored at −10 °C. Partially milled rice was included because it retains more surface lipid than fully milled rice and the surface lipid changes would be more easily observed.

Rice Storage and Free Fatty Acid and Total Lipid Analyses. The rice samples were divided into 1.5 kg portions to provide riplicate treatments, placed on perforated trays that were lined with a fine netting material, and stored in a humidity chamber (Precise Humidity Control, PGC, Inc., Black Mountain, NC) at 24, 37, and 50 °C and 70% humidity for 4 days. These conditions were chosen to represent room-temperature conditions, the optimum temperature for rice-bran lipase activity (*13*), and abused storage temperature, respectively. The high humidity of 70% was important to maintain the water−oil interface for lipase activity (*14*).

Rice samples were randomly taken from each treatment every 6 h for 4 days, or until FFA content reached 0.1%, and rice surface FFA and total lipid contents were determined in triplicate, according to the method of Lam and Proctor (*15*), who extracted rice lipids with 2-propanol and subsequently determined FFA levels colorimetrically. The FFA content was calculated as oleic acid and expressed as gram per 100 g of rice.

Kinetic Expression for FFA Concentration. The equation proposed of FFA formation on milled rice is similar to the Paolucci-Jeanjean et al. (*12*) model (eq 1) with two expressions but has an additional term, P_o, where P_o represents the initial concentration of FFA on the surface of milled rice.

$$P(t) = P_o + \frac{at^n}{b^n + t^n} + \frac{ct^m}{d^m + t^m} \tag{2}$$

By analogy to the fundamental Michaelis Menten model, term *a* is the maximum FFA concentration (% FFA) that can be formed in phase one at a given storage temperature, *b* is the time (h) required for attaining half of *a*, *c* is the maximum FFA concentration (% FFA) that can be formed in phase two at a given storage temperature, and *d* is the time (h) required to attain half of *c*. *n* and *m* are nondimensional descriptive function orders, process path indicator, and are constant at a given temperature ($n, m \geq 0$).

The proposed model was used to compute parameters from the experimental data, to produce predicted FFA values at different storage temperatures to generate a thermal plot, and to compare the predicted data to the actual experimental data.

Statistical Analysis. The triplicate milled rice FFA data obtained during 4 days of storage in milled rice at different temperatures and constant relative humidity were analyzed statistically by nonlinear regression (JMP IN 4.0.4, SAS Inc., Cary, NC) using eq 2 in order to optimize values of constants in proposed model, estimate reliability of estimated constants, and predict the fit of the proposed model to experimental data. A three-dimensional plot of FFA formation was generated using the S-Plus 2000 (Professional Release 2) statistical package (MathSoft, Inc., Cambridge, MA).

RESULTS AND DISCUSSIONS

FFA Formation on the Surface of Milled Rice. Figure 1 shows the concentration of FFA formed during storage with time. The data of all three storage temperatures clearly show an increase in FFA content to a maximum of approximately 0.1% during the 4 days of storage. The increase in FFA content may be attributed to the activity of the rice lipase breaking down phospholipids into FFA. Phospholipids and not triacylglycerols have been shown to be the primary substrates for lipases during earlier days of storage of milled rice (*1*). The optimum temperature for lipase from rice bran is 37 °C (*13*), which also was exhibited by the higher rate of FFA formation at this temperature. FFA formation appeared to occur in two phases of successive exponential increases, which makes it possible to adapt the equations of Paolucci-Jeanjean et al. (*12*) to describe and model the reaction.

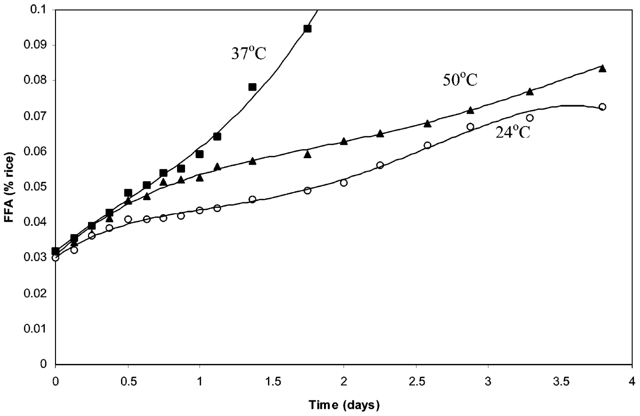

Figure 1. Measured and regression-predicted FFA contents of milled rice as a function of time at 70% relative humidity. Symbols are experimental data; lines are predicted values.

Table 1. Regression-Estimated Values of Parameters in the Proposed Model for FFA Formation[a]

	parameters					
	a (% FFA)	*b* (h)	*n*	*c* (% FFA)	*d* (h)	*m*
partially milled rice						
24 °C	0.03	10.5	4.0	0.03	27.1	6.0
	(0.01)	(3.0)	(1.4)	(0.01)	(5.6)	(2.0)
37 °C	0.05	11.0	4.0	0.04	12.3	6.0
	(0.02)	(3.1)	(1.0)	(0.01)	(3.7)	(1.9)
50 °C	0.04	11.2	4.0	0.03	25.7	6.0
	(0.01)	(3.4)	(1.2)	(0.01)	(4.8)	(1.8)
fully milled rice						
37 °C	0.04	10.8	3.5	0.03	11.7	4.8
	(0.01)	(2.3)	(0.6)	(0.01)	(2.5)	(0.7)

[a] Numbers in parentheses are estimated standard errors of corresponding parameter estimates.

Model To Describe the Experimental Data of FFA Formation. Table 1 shows the estimate of the parameters in the model for each storage temperature and rice degree of milling. From the standard error of the estimates, it is clear that the parameters were precisely estimated by the proposed model (eq 2). The values of the parameters *a*, *b*, *c*, and *d* indicate that the rate of formation of FFA in phase one was higher than that during phase two at all storage temperatures. This effect may be explained by a reaction mechanism in which different phospholipid substrates are hydrolyzed at each of the two phases, as implied by the model. The analogy with consecutive reactions in which the two ester bonds in a phospholipid molecule are degraded in succession is apparent. This type of mechanism may suggest a changing accessibility of the lipase enzymes to the phospholipid substrates at each of the two phases of the model. The estimated values of the exponential show that the constant *m* was higher than *n*, which reflects the longer period over which FFA was formed during phase two as compared to that during phase one. The total time ($b + d$) estimated for phospholipid hydrolysis at 37 °C was lower than that at both 24 and 50 °C. This observation supports the findings that 37 °C is the optimum temperature for rice lipase activities (*13*).

Model To Predict FFA Formation on Milled Rice. Figure 1 also shows that FFA concentrations calculated according to eq 2 satisfactorily fit the experimental data for FFA formation on milled rice during the 4 days of storage. This supports the theory of consecutive reactions occurring as suggested by the proposed model. The percentage variance accounted for by the

Table 2. Correlation of Measured and Regression-Predicted FFA

sample	temp (°C)	R^2	RMSE[a]
partially milled rice	24	0.98	0.021
	37	0.99	0.018
	50	0.97	0.026
fully milled rice	37	0.97	0.028

[a] Root-mean-square error.

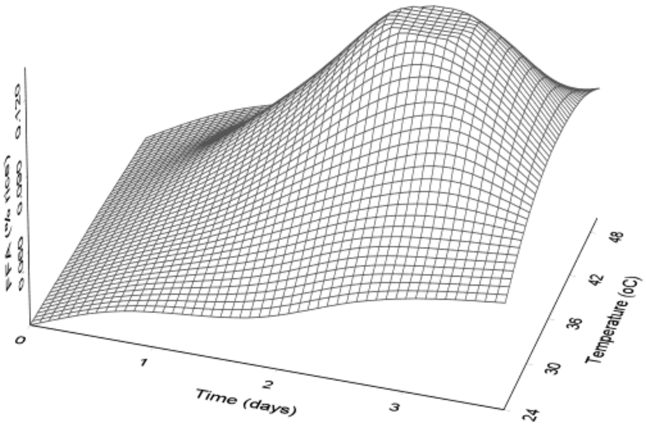

Figure 2. Predicted FFA content as a 3-D function of storage time and temperature.

proposed model (R^2) at each storage temperature is quite high, and the RMSE is satisfactory (**Table 2**).

Reaction Mechanisms of FFA Formation on Milled Rice. We were interested in finding a model for FFA formation on milled rice during early storage. The kinetic model and the estimated values of the model constants suggest that the mechanism may consist of two consecutive reactions. Therefore, to explain eq 2, we propose two successive equilibria:

$$E + PL \rightleftharpoons E{-}PL \rightarrow MPL + FFA + E \qquad (3)$$

$$E + MPL \rightleftharpoons E{-}MPL \rightarrow GP + FFA + E \qquad (4)$$

The first (eq 3) describes the reversible binding of the phospholipase (E) to phospholipid (PL) and the subsequent hydrolysis of the latter to monoacyl phospholipids (MPL). The monoacyl phospholipids are then hydrolyzed in the second (eq 4) to release glycerophosphates (GP), FFA, and the free enzyme.

Three-Dimensional Plot of FFA Formation on Milled Rice. Figure 2 shows the calculated FFA data (from eq 2) presented as a function of time at the three storage temperatures. Observance along the time axis shows the double and consecutive exponential curves for FFA formation, as seen in the experimental data. This observation is indicative of the simple consecutive reactions used in the model. The temperature axis shows the maximal concentration of FFA attained and the difference in apparent FFA concentration above and below 37 °C of storage. At a similar level of FFA concentration, below 37 °C the FFA level is still increasing with time, whereas the FFA level is already declining with time above 37 °C. This observation is a clear indication of the temperature dependence of the lipase-catalyzed formation of FFA on milled rice.

FFA formation on milled rice can be represented by semiempirical equations at constant storage temperatures. Formation of FFA followed two consecutive reactions patterns. The kinetic model proposed could provide a basis for predicting FFA concentration on milled rice. It should also stimulate detailed kinetic studies on FFA formation on milled rice.

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