Lipid Hydrolysis and Oxidation on the Surface of Milled Rice

Henry S. Lam and Andrew Proctor*

Department of Food Science, University of Arkansas, Fayetteville, Arkansas 72704

ABSTRACT: The changes in milled rice FFA content and composition and in conjugated diene (CD) content and bacterial, yeast, and mold counts were determined at 24, 37, and 50°C and 70% RH over 50 d. There was a rapid rate of FFA formation during the first few days of storage, which was optimal at 37°C, but that slowed after 2, 4, and 5 d at 37, 24, and 50°C, respectively. There was a second increase in FFA after about day 12 that increased with increasing temperature, indicating nonlipase hydrolysis. Linoleic and oleic acids were the main components of the total FFA produced on the surface of milled rice. The pattern of CD development followed that of FFA increase. Bacterial growth correlated with increased FFA levels after 12 d of storage, suggesting that bacterial lipase rather than bran lipase may be responsible for rice lipid hydrolysis.

Paper no. J10485 in *JAOCS 80,* 563–567 (June 2003).

KEY WORDS: Conjugated hydroperoxy fatty acids, free fatty acids, microbial growth, milled rice, storage.

Milled rice surface lipids are important to the development of rice off-flavors that reduce rice flavor quality (1) and are commercially important for brewing. Brewers use FFA as an indicator for the potential of future off-flavor development. Milled rice with FFA levels above 0.1% may adversely affect beer flavor quality (Malin, S., personal communication). Residual bran streaks on the milled rice surface contain lipases and lipids with a high proportion of unsaturated FA (2). Rice bran lipids are composed mainly of TG in spherosomes that are disrupted during milling to release the acylglycerols that are degraded by lipases to FFA (3). Unsaturated FFA are oxidized to hydroperoxy FA that subsequently decompose to volatile off-flavors (4).

Milled rice lipid deterioration and development of off-flavor during storage have been reported (5,6). However, none of these studies reported the relationship between rice lipid hydrolysis and oxidation prior to off-flavor development. Lipid hydrolysis is considered to promote lipid oxidation in vegetable oils as FFA are more readily oxidized than esterified FA (7). However, the relationship between residual bran lipid hydrolysis on milled rice lipid oxidation has not been demonstrated. Brown rice FFA and conjugated diene (CD) levels of rice lipid increased simultaneously after 1–6 mon, suggesting a strong correlation between lipid hydrolysis and lipid oxidation (8). We have shown that lipid hydrolysis on milled rice occurs in three stages at 37°C and 70% RH (9). Initially, FFA levels increase rapidly during storage from 0.03 to 0.09% at day 0 to day 2, reach a plateau at day 2, and then increase on day 20 reaching 0.23% on day 50. The first 2 d of storage were critical in determining the commercial quality of milled rice, as during that time, FFA levels rose to almost 0.1%. Lipid oxidation followed a similar trend to that of FFA formation. However, there is no report on the effect of temperature on milled rice surface lipid hydrolysis and oxidation or the role of extraneous factors, such as microorganisms.

Many microorganisms produce lipases (10) and grow at temperature and humidity conditions similar to those of milled rice storage and transport. Simultaneous increase in FFA levels and microbial growth has been reported in rice bran, wheat, corn, soybean, and cottonseed (11). Nevertheless, microorganisms that grow on milled rice during storage and that may affect FFA values have not been determined.

The objectives of this study were to (i) relate the changes in milled rice FFA levels, FA composition, and milled rice surface lipid oxidation with storage time at temperatures of 24, 37, and 50°C; (ii) determine the relationship between milled rice lipid hydrolysis and CD, which are primary oxidation products; and (iii) determine the relationship between mold, yeast, and bacteria growth and lipid hydrolysis and formation of primary oxidation products.

MATERIALS AND METHODS

Rice samples. Commercially milled long-grain rice (Riceland Foods, Stuttgart, AR) was obtained at the first-break stage during milling, transported under dry ice to our laboratory, and stored at −10°C. Undermilled rice was used because it retains more surface lipid than fully milled rice, and the surface lipid changes are more easily observed.

Rice storage and sampling. The rice was divided into four 2-kg portions to provide replicate samples for each temperature, placed on perforated trays, and stored in a humidity chamber (Precise Humidity Control; PGC, Inc., Black Mountain, NC) at 24, 37, and 50°C and 70% humidity for 50 d. Rice samples (40 g) were taken every 6 h from day 0 to day 4 and subsequently every 12 h for the next 46 d.

FFA content of rice surface lipid. Rice kernel surface lipids were extracted and total oil and FFA determined in triplicate according to the method of Lam and Proctor (12). The FFA extracted was measured colorimetrically at 560 nm using a diode array spectrophotometer (Hewlett-Packard, Palo Alto, CA). The FFA content was calculated as oleic acid equivalent from a calibration curve prepared using standard oleic acid. FFA content was expressed as percentage of total extracted lipids and as percentage of milled rice.

*To whom correspondence should be addressed at Department of Food Science, University of Arkansas, 2650 Young Ave., Fayetteville, AR 72704. E-mail: aproctor@uark.edu

Identification and measurement of surface lipid FFA by HPLC. Milled rice (10 g) was mixed with 2 µg of heptadecanoic (margaric) acid as internal standard, and surface lipids were extracted (12). The extract was transferred to a 50-mL round-bottomed flask and flashed with nitrogen gas. The isopropanol solvent was removed under vacuum at 40°C. FFA in the extract were derivatized into their respective *p*-bromophenacyl-esters according to a slightly modified method of Puttmann *et al.* (13). The lipid extract was reconstituted with 2 mL of acetonitrile and added to a vial containing 24 µL each of 50 mmol/L *p*-bromophenacylbromide and 5 mmol/L 18-crown-6 ether in acetonitrile (stored protected from light), and 1 mg of $KHCO_3$. A magnetic bar was inserted, the vial flushed with nitrogen gas, closed tightly, and heated to 75–85°C for 45 min with vigorous stirring. The vial was cooled to near room temperature, and then 40 µL of a 40 g/L solution of formic acid in acetonitrile was added. The resulting solution was heated again for an additional 5 min at the same temperature. The derivatized sample was then cooled to about 5°C in a refrigerator and filtered through a Whatman 0.45 micron syringe filter (Whatman International Ltd., Maidstone, England).

A volume of 10 µL of the filtered sample was injected into the HPLC according to the method of Puttmann *et al.* (13). The HPLC used was a Spectra System (Spectra-Physics Analytical, San Jose, CA) with a P2000 binary gradient pump, an AS1000 fixed-loop autosampler, and a Spectra Focus forward optical scanning detector operated at 254 nm. The absorbance data were collected and analyzed by a Gateway2000 microcomputer (Gateway2000, Inc., Sioux City, SD) using the software package ChromQuest 2.51 (ThermoQuest, San Jose, CA). A reversed-phase Supelcosil (octyl bonded spherical silica) LC-18, 3 µm (25 cm × 4.6 mm) column with a interfaced Supelcosil LC-18, 5 µm (2 cm × 4.6 mm) guard column (Supelco, Bellefonte PA) was used at ambient temperature. Acetonitrile/THF/0.1% H_3PO_4 (50.4:21.6:28, vol/vol/vol) was used isocratically as the mobile phase at a flow rate of 1.0 mL/min. FA standards (linolenic, linoleic, palmitic, oleic, and stearic acids) and formic acid were obtained from Sigma (St. Louis, MO); *p*-bromophenacylbromide and 18-crown-6 ether (chromophore-catalyst reagents) were purchased from Aldrich (Milwaukee, WI). Acetonitrile, THF, and isopropanol were HPLC-grade from Burdick and Jackson (Muskegon, MI). The other reagents were of analytical grade, and Milli-Q water was used throughout.

FA were identified and quantified using known standards. Calibration curves for FA were prepared by analyzing various concentrations of FA standards (palmitic, stearic, oleic, linoleic, and linolenic acids) that were derivatized as bromophenacyl esters with heptadecanoic acid as internal standard.

CD. The CD contents of the total surface lipid extracts were determined in triplicate, as a measure of initial oxidative deterioration of unsaturated lipids on the kernel, according to the method of Nnanna *et al.* (14). The CD content of a 1-mL extract placed in a 1-mm width cuvette was measured colorimetrically at 232 nm using a diode array spectrophotometer (Hewlett-Packard). The CD content was expressed as mg in 100 g of extracted lipids.

Microbial growth on milled rice. Bacteria, yeasts, and mold-colony-forming units (cfu) on milled rice were determined in duplicate on samples taken after 12 and 36 h, and 8, 14, 22, and 38 d of storage, according to the method of Jonsson *et al.* (15).

Statistical analysis. The data obtained from total FFA, FFA composition, CD analysis, and microbial cfu in milled rice were subjected to a one-way ANOVA. The calculations were performed with JMP 4.0 software (Cary, NC). The difference of means between pairs was resolved by means of confidence intervals using the LSD range test. Level of significance was set at $P < 0.05$.

RESULTS AND DISCUSSION

FFA content of rice surface lipid. Figure 1A shows the change in milled rice FFA levels, expressed as a percentage of the extracted surface lipids. FFA levels changed in three distinct stages at all temperatures, as reported by Lam *et al.* (9) at 37°C. The most rapid increase in FFA occurred during the first few days (days 1–5) that constituted the first stage. During this time, most FFA was produced at 37°C with lower FFA levels produced at the lower and higher temperatures. This implies that bran lipase is most likely responsible for the increasing FFA values, since 37°C is the optimal temperature for rice bran lipase activity (16). The differences in FFA content during the first 2 d are shown in Table 1. The results show that increasing FFA levels were significantly different at all storage temperatures, with the highest levels at 37°C and the lowest levels at 24°C. The rapid formation of FFA during the early days of storage could be related to the activity of rice endogenous lipases. The rate of increase then dramatically stabilized, and there was no net increase in FFA until around day 12. Stabilization of FFA values could be due to lipase product inhibition (17) or equilibrium between FFA formation and oxidative breakdown. The length of the time of static FFA levels was shorter as temperature increased. The largest increase in FFA occurred in the third and final stage, after day 12, when FFA levels increased. This was probably not due to bran lipase activity, as FFA production related

TABLE 1
Changes in Total Surface FFA (% surface lipid) on Milled Rice During First 2 d of Storage[a]

	Temperature (°C)		
Time (h)	24	37	50
0.0 (Control)	3.44[a]	3.42[a]	3.40[a]
6.0	3.85[a]	4.16[c]	3.94[b]
12.0	4.36[a]	5.15[c]	4.91[b]
18.0	4.40[a]	5.74[c]	5.49[b]
24.0	4.62[a]	6.31[c]	5.61[b]
30.0	4.70[a]	6.85[c]	5.96[b]
36.0	4.97[a]	8.34[c]	6.12[b]
42.0	5.46[a]	10.53[c]	6.59[b]
48.0	5.69[a]	10.52[c]	7.01[b]

[a]Values are means of triplicate determinations. Means on the same row superscripted by different roman letters are significantly different ($P < 0.05$).

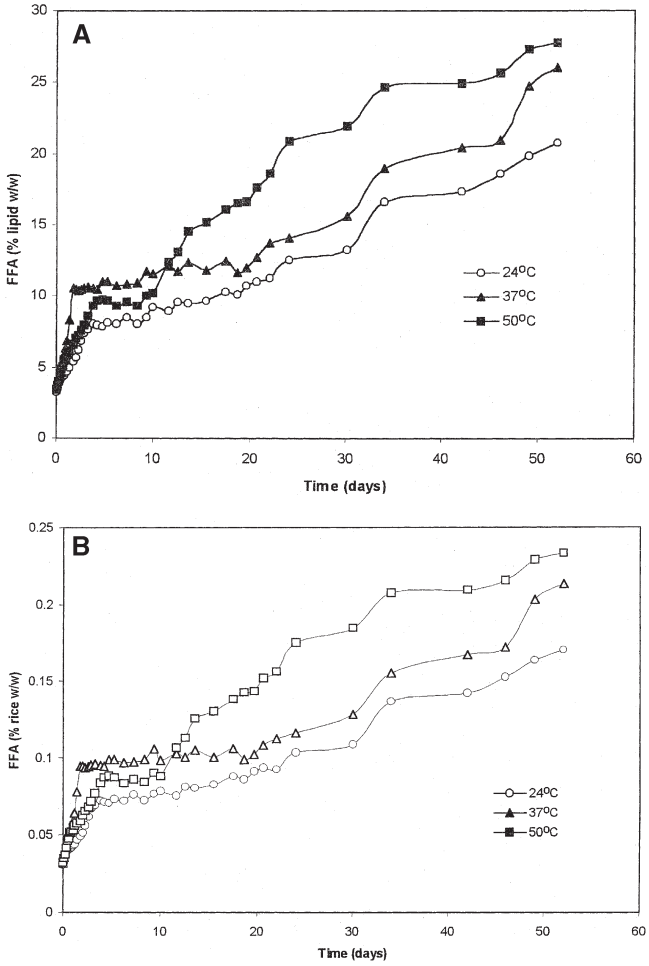

FIG. 1. Changes in the content of total FFA on the surface of milled rice stored at 70% RH for 50 d: (A) (% lipid w/w) LSD (0.05) = 0.03; (B) (% rice w/w) LSD (0.05) = 2.26.

directly to temperature, but the rate of increase was less than that observed in the first stage.

Figure 1B shows the change in milled rice FFA levels, expressed as a percentage of milled rice, to illustrate the commercial significance of the rice FFA levels. Since milled rice FFA levels of >0.1% exceed those acceptable for brewing applications, we found that 37°C produces unacceptable FFA levels faster than other temperatures. Furthermore, this occurs rapidly on or around the period of static FFA levels. The subsequent FFA increase is probably not related to bran lipase activity but results in FFA levels rising above 0.1% at all the storage temperatures.

Identification and measurement of surface lipid FFA by HPLC. Changes in levels of specific FA comprising the total FFA in the milled rice surface lipid extract, of rice stored at 37°C is shown in Figure 2. Data are expressed as a percentage of total extracted oil. In order of predominance, the FA are: linoleic, oleic, palmitic, linolenic, and stearic acids. The trend in linoleic and oleic acids levels was similar to those of total FFA (Fig. 1A) and shows that they were probably the main contributors to the total FFA content of milled rice and its subsequent deterioration. However, linolenic acid increased during the initial 48 h, leveled off, and did not change significantly for the rest of the storage duration. Since linolenic acid is more prone to oxidation than all of the other FA measured, its levels probably reflect an equilibrium between formation and oxidative degradation, and it may be formed and oxidized rapidly. The proportions of the various FFA in the extracts after 21 d of storage (data not reported) were similar to those obtained at the beginning of the study and reflect the actual FA composition in rice bran oil (18).

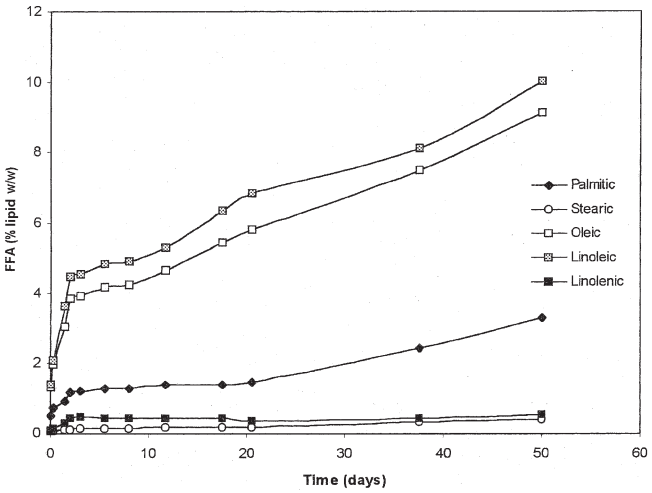

FIG. 2. Changes in individual FFA content of milled rice surface during storage at 37°C and 70% RH. LSD (0.05) = 0.21.

CD hydroperoxy FA. Figure 3 shows the oxidation of milled rice surface lipid as measured by CD and expressed as a percentage of the total surface lipid extract. The oxidation of milled rice lipid to form CD is a measure of linoleic and linolenic acids oxidation. Table 2 shows that CD was formed at significantly different levels during the first 10 d of storage at all temperatures, indicating that temperature did influence the rate of linoleic and linolenic acid oxidation. Increasing CD development occurred with increasing temperature between day 12 and around day 25, but little change occurred

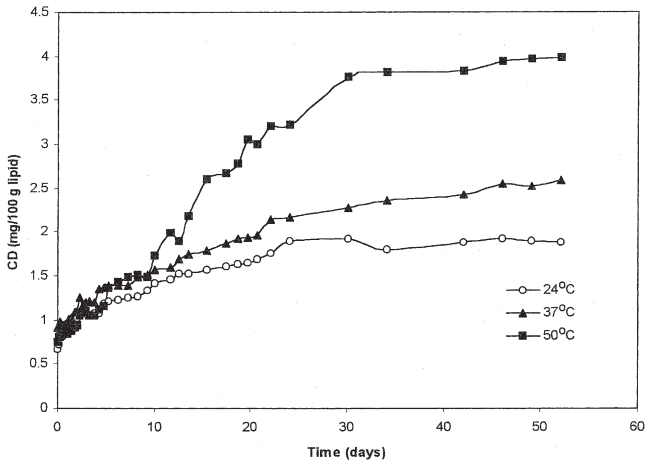

FIG. 3. Changes in the content of conjugated diene (CD) on milled rice surface stored at 70% RH for 50 d. LSD (0.05) = 0.04.

TABLE 2
Changes in Conjugated Dienes (% surface lipid) on Milled Rice During 10 d of Storage[a]

	Temperature (°C)		
Time (d)	24	37	50
0.00 (Control)	0.67[a]	0.69[a]	0.65[a]
0.25	0.79[a]	0.91[b]	0.82[a]
0.50	0.82[a]	0.93[b]	0.84[a]
0.75	0.85[a]	0.96[b]	0.84[a]
1.00	0.93[b]	0.97[b]	0.84[a]
1.25	0.95[b]	1.01[c]	0.89[a]
1.50	0.95[b]	1.03[c]	0.89[a]
1.75	0.97[b]	1.07[c]	0.91[a]
2.00	1.03[b]	1.08[c]	0.94[a]
2.50	1.07[a]	1.17[b]	1.05[a]
3.00	1.08[a]	1.21[b]	1.07[a]
4.00	1.09[a]	1.34[b]	1.12[a]
5.00	1.19[a]	1.38[b]	1.36[b]
6.00	1.22[a]	1.39[b]	1.42[b]
7.00	1.25[a]	1.40[b]	1.48[c]
8.00	1.27[a]	1.48[b]	1.52[b]
9.00	1.33[a]	1.51[b]	1.54[b]
10.00	1.42[a]	1.56[b]	1.58[b]

[a]Values are means of triplicate determinations. Means on the same row superscripted by different roman letters are significantly different ($P < 0.05$).

after day 30. The observed increase in CD content after 12 d could have been due to the depletion of antioxidants in stored rice. The changes in CD content followed a similar trend of formation as FFA (Fig. 1A) and may be due to the fact that CD content measures the conjugated hydroperoxy FA (19). Although CD other than FFA will be included in the analysis, the change in CD content was probably related more to the FFA content than oil acyglycerides, since FFA are oxidized more rapidly than acylglycerides. Chan and Coxon (20) also reported that the formation of peroxide during autoxidation of PUFA paralleled increase in the amount of CD.

Microbial growth on milled rice. Figure 4 shows the effects of microbial growth on the development of FFA on milled rice stored at 37°C. There was no significant ($P < 0.05$) change in bacterial growth between day 1 and day 13 of storage. However, bacterial counts increased by more than one log unit after day 22. There was no significant increase in yeast growth throughout the storage duration. Mold growth was observed only after 12 d of storage but did not significantly increase during subsequent storage. The increase in lipid hydrolysis was independent of the microbial growth during the first 12 d of storage but corresponded to microbial growth from day 21 onward. Changes in bacterial growth appeared to have had a stronger influence on lipid hydrolysis than on either yeast or mold growth.

The large increase in FFA shown in Figure 1 after 21 d of storage could be attributed to the breakdown of TAG by bacterial lipases. Microbial growth and activity increase at higher temperature (21), hence the higher level of FFA formation at 50°C after 21 d of storage. Approximately 10% of total bacterial species growing on rough rice were shown to be lipolytic and were mainly *Pseudomonas alcaligenes* (22). Mold growth also could have contributed to the increase in FFA. Loeb and Mayne (11) previously demonstrated that mold growth leads to a simultaneous increase in FFA production in rice bran.

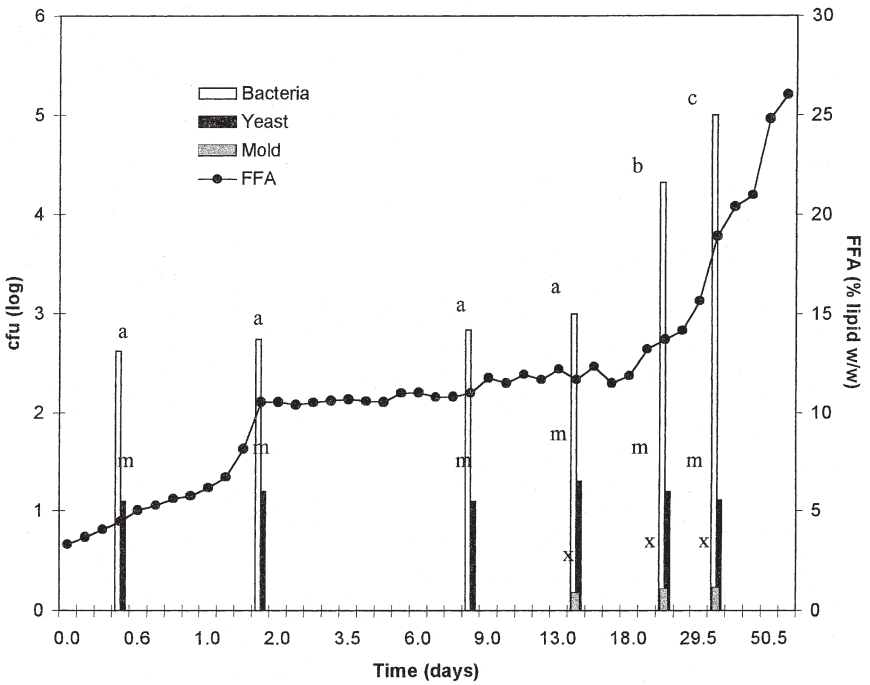

FIG. 4. Microbial count and FFA on the surface of milled rice stored at 37°C and 70% RH. Different letters indicate significant differences among bacteria counts (a,b,c). Yeast counts (m) and mold counts (x) were not statistically different. cfu, colony-forming units.

In conclusion, this study showed that rice lipid hydrolysis proceeded in three distinct phases and that the last stage was temperature-dependent. Rice lipid oxidation closely followed the same trend as hydrolysis, suggesting a strong relationship between the two processes. Linoleic and oleic acids provided the bulk of the total FFA produced during milled rice hydrolysis. Microbial growth correlated with FFA formation after 21 d of storage during which time FFA levels increased to >0.1%.

ACKNOWLEDGMENTS

The authors would like to thank the Arkansas Rice Research Board for supporting this work and Riceland Foods Inc., Stuttgart, Arkansas, for donating the milled rice.

REFERENCES

1. Shibuya, N., T. Iwasaki, H. Yanase, and S. Chikubu, Studies on Deterioration of Rice During Storage. I. Changes of Brown Rice and Milled Rice During Storage, *J. Jpn. Soc. Food Sci. Technol. 21*:597–603 (1974).
2. Takano, K., Mechanism of Lipid Hydrolysis in Rice Bran, *Cereal Foods World 38*:695–698 (1993).
3. Champagne, E.T., R.J. Hron, Sr., and G. Abraham, Utilizing Ethanol to Produce Stabilized Brown Rice Products, *J. Am. Oil Chem. Soc. 69*:205–208 (1992).
4. Galliard, T., Rancidity in Cereal Products, in *Rancidity in Foods*, edited by J.C. Allen and R.J. Hamilton, Elsevier, London, 1989, pp. 141–157.
5. Yasumatsu, K., and S. Moritaka, Fatty Acid Compositions of Rice Lipid and Their Changes During Storage, *J. Biol. Chem. 28*:257–264 (1964).
6. Nishiba, Y., T. Sato, and I. Suda, Convenient Method to Determine Free Fatty Acid of Rice Using Thin-Layer Chromatography and Flame-Ionization Detection System, *Cereal Chem. 77*: 223–229 (2000).
7. Nawar, W.W., Lipids, in *Food Chemistry*, edited by O.R. Fennema, Marcel Dekker, New York, 1996, pp. 225–319.
8. Champagne, E.T., and R.J. Horn, Sr., Stabilizing Brown Rice to Lipolytic Hydrolysis by Ethanol Vapors, *Cereal Chem. 69*: 152–156 (1992).
9. Lam, H.S., A. Proctor, and J.-F. Meullenet, Free Fatty Acid Formation and Lipid Oxidation on Milled Rice, *J. Am. Oil Chem. Soc. 78*:1271–1275 (2001).
10. Lubert, D.J., L.M. Smith, and H.R. Thornton, Estimation of Lipase in Dairy Products. III. Lipases Activity in Cultures of Microorganisms and in Cheese, *Can. J. Res. F27*:499–503 (1949).
11. Loeb, J.R., and R.Y. Mayne, Effect of Moisture on the Microflora and Formation of Free Fatty Acids in Rice Bran, *Cereal Chem. 29*:163–175 (1952).
12. Lam, H.S., and A. Proctor, Rapid Methods for Milled Rice Total Lipid and Free Fatty Acid Determinations, *Ibid. 78*:488–489 (2001).
13. Puttmann, M., H. Krug, E. Ochsenstein, and R. Kattermann, Fast HPLC Determination of Serum Free Fatty Acids in Picomole Range, *Clin. Chem. 39*:825–832 (1993).
14. Nnanna, I.A., D.O. Ukuku, K.B. McVann, and L.A. Shelef, Antioxidant Activity of Sodium Lactate in Meat and Model Systems, *Lebensm. Wiss. Technol. 27*:78–85 (1994).
15. Jonsson, A., F. Winquist, J. Schnurer, H. Sundgren, and I. Lundstrom, Electronic Nose for Microbial Quality Classification of Grains, *Int. J. Food Microbiol. 35*:187–193 (1997).
16. Aizono, Y., Y. Funatsu, M. Sugano, K. Hayashi, and Y. Fujiki, Enzymatic Properties of Rice Bran Lipase, *Agri. Biol. Chem. 37*: 2031–2036 (1973).
17. Garcia, H.S., C.H. Amundson, and G.G. Hill, Partial Characterization of the Action of an *A. niger* Lipase on Butteroil Emulsions, *J. Food Sci. 56*:1233–1237 (1991).
18. Hemavathy, J., and J.V. Prabhakar, Lipid Composition of Rice (*Oryza sativa* L.) Bran, *J. Am. Oil Chem. Soc. 64*:1016–1019 (1987).
19. St. Angelo, A.J., R.L. Ory, and L.E. Brown, A Comparison of Minor Constituents in Peanut Butter as Possible Sources of Fatty Acid Peroxidation, *J. Am. Peanut Res. Educ. Assoc. 4*:186–189 (1972).
20. Chan, H.W.-S., and D.T. Coxon, Lipid Hydroperoxides, in *Autoxidation of Unsaturated Lipids*, edited by H.W.-S. Chan, Academic Press, London, 1987, pp. 17–48.
21. Ingraham, J.L., Growth of Psychrophilic Bacteria, *J. Bacteriol. 76*:75–80 (1958).
22. DeLucca, A.J., II, S.J. Plating, and R.L. Ory, Isolation and Identification of Lipolytic Microorganisms Found on Rough Rice from Two Growing Areas, *J. Plant Prot. 14*:28–30 (1978).

[Received November 14, 2002; accepted March 3, 2003]