Hydrolysis of Acylglycerols and Phospholipids of Milled Rice Surface Lipids During Storage

Henry S. Lam and Andrew Proctor*

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

ABSTRACT: The relative contribution of acylglycerols and phospholipids to the lipid hydrolysis in milled rice was investigated during storage at 37°C and 70% RH for 50 d. The MAG, DAG, and lysophospholipid contents of surface lipid were determined by reversed-phase HPLC. The MAG and DAG content of milled rice increased during storage from 0.06 to 0.18% (w/w milled rice), with the MAG content increasing more than that of the DAG. Lysophosphatidylcholine increased throughout the study from 0.013 to 0.034% (w/w), whereas lysophosphatidylinositol and lysophosphatidylethanolamine contents initially increased from 0.002 to 0.003% and from 0.005 to 0.009% (w/w), respectively, and then stabilized until day 50. The relative percentage of TAG and phospholipids hydrolyzed in milled rice increased rapidly during the first 3 d of storage from 12.3 to 37.6% and 25.0 to 62.5% (w/w), respectively, and steadily increased to 53.1 and 73.8% (w/w) on day 50. A higher percentage (62.5%) of phospholipids was hydrolyzed relative to TAG (37.6%) after 3 d and probably contributed significantly to milled rice lipid hydrolysis during early storage. However, TAG concentration (0.57%, w/w) was much higher relative to phospholipids (0.08%, w/w) in surface lipids, and therefore TAG hydrolysis was the major contributor to FFA development and the quality of stored milled rice.

Paper no. J10718 in *JAOCS 81*, 385–388 (April 2004).

KEY WORDS: Acylglycerols, free fatty acids, hydrolysis, lipids, milled rice, phospholipids.

Rice bran lipid is prone to hydrolysis due to the presence of endogenous lipases (1). Bran streaks on commercially milled rice contain rice bran lipid, 85% of which is TAG that readily hydrolyze to FFA. The TAG FA are mainly unsaturated and rapidly oxidize, compromising the milled rice flavor quality. Phospholipids constitute about 2% of the total rice bran lipid (2) and form the membranes of the spherosomes, which contain rice bran TAG (3). They decompose immediately after milling (4). Lipid hydrolysis and oxidation in rice bran streaks on milled rice plays an important role in milled rice quality. Brewers are a major rice user in the United States, and they are particularly concerned about lipid hydrolysis and oxidation that can cause flavor problems in beer.

We have determined that linoleic and oleic acids were the main FA released during milled rice surface lipid hydrolysis (5). However, it was not clear whether the FA originated primarily from acylglycerol or phospholipid hydrolysis. Takano (4) indicated that phospholipids decomposed rapidly at the beginning of rice bran storage, but no study has been conducted on milled rice. Other studies of triglycerol decomposition in rice bran (6–9) investigated FA composition, oxidative changes, and prevention of lipid hydrolysis. A study into the nature of triglycerol and phospholipid hydrolysis in milled rice would provide insight into their relative contributions to FFA formation.

The objective of this study was to determine the origin of FFA and acylglycerol products of TAG and phospholipid hydrolysis on milled rice, and the relative contributions of TAG and phospholipids to the total milled rice lipid hydrolysis.

EXPERIMENTAL PROCEDURES

Rice samples. Commercially milled long-grain rice (Riceland Foods, Stuttgart, AR) was obtained at the first-break milling stage, transported under dry ice to our laboratory, and stored at −10°C.

Rice storage and sampling. The milled rice was divided into three 2-kg portions to provide three replicates for each temperature used. These were placed on perforated trays and stored in a humidity chamber (Precise Humidity Control, PGC, Inc., Black Mountain, NC) at 37°C and 70% RH for 50 d. Rice samples (40 g) were drawn from storage after 0 (no storage), 0.3, 1.6, 2, 3, 5.5, 8, 11.6, 17.4, 20.5, 37.5, and 50 d. The drawn samples were analyzed for total surface lipids, neutral lipids, and phospholipid contents.

Rice surface lipid extraction, neutral lipids, and phospholipid estimation. Rice kernel surface lipids were extracted, and total oil was determined in triplicate according to the method of Lam and Proctor (10). Briefly, 10 g of milled rice was extracted twice with 4 mL of 2-propanol for a total of 4 min at room temperature (22°C). The 2-propanol was separated from rice by decanting the extract into a preweighed round-bottomed flask. Lipids from the extract were recovered by evaporating the solvent under vacuum on a rotary evaporator. Lipids were weighed, and lipid content was expressed in g/100 g milled rice, wet basis. The phospholipid content was calculated (P × 25) after phosphorus was determined in the total lipid extract by the method of Bartlett (11). The TAG (neutral lipid) content was estimated to be the difference between total lipid and phospholipid contents. Phospholipid and TAG contents were also expressed as g/100 g milled rice, wet basis.

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

Lipid fractionation. The total lipid extracts were redissolved in 3 mL of chloroform. Neutral and phospholipids were separated by using amino-propyl-bonded phase solid-phase extraction (SPE) columns (Supelclean LC-NH_2; Supelco, Bellefonte, PA) with a 500 mg/3 mL capacity following the procedure of Kaluzny *et al.* (12). This method involves conditioning SPE columns with 3 mL of hexane before loading the lipid extract in chloroform onto the column. Neutral lipids were eluted with 4 mL of chloroform/2-propanol (2:1, vol/vol), and phospholipids were then eluted with 4 mL of methanol.

Neutral lipid fraction. The neutral lipid fraction was loaded onto a conditioned SPE column, and DAG and MAG were separated. DAG were eluted with 4 mL of 15% (vol/vol) ethyl acetate in hexane, and MAG were then eluted with 4 mL of chloroform/methanol (2:1, vol/vol).

Acylglycerol and phospholipid hydrolysis products. (i) MAG. The MAG fraction was separated and quantified according to the method described by Maruyama and Yanese (13) by isocratic reversed-phase HPLC. MAG in chloroform/methanol (2:1, vol/vol) were filtered through a 0.45 μm polytetrafluoroethylene membrane (Whatman Inc., Ann Arbor, MI), and 10 μL was injected. Acetonitrile/water (8:2, vol/vol) was used as the mobile phase at a flow rate of 1.0 mL/min. The HPLC system was a Spectra System (Spectra-Physics Analytical, San Jose, CA) equipped with a P2000 binary gradient pump, an AS1000 fixed-loop autosampler, and a Spectra Focus forward optical scanning detector operated at 210 nm. The absorbance data were collected and analyzed 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 interfaced with a Supelcosil LC-18 5-μm (2 cm × 4.6 mm) guard column (Supelco) was used at ambient temperature. Pure 1-monooleoyl glycerol, 1-monolin-oleoyl glycerol, and 1-monolinolenoyl glycerol (Sigma, St. Louis, MO) were used as standards.

(ii) DAG. The DAG fraction was separated and quantified by reversed-phase HPLC according to a modified method of Lin *et al.* (14). The eluting solvent was methanol/isopropanol (1:1, vol/vol), and the DAG were eluted isocratically at 1.0 mL/min. The elution profiles were obtained by measuring UV absorbance at 205 nm. Pure 1,3-diolein and 1,3-dilinolein (Sigma) were used for calibration.

(iii) Lysophospholipid components of the phospholipid fraction. The lysophospholipids were separated and quantified according to the method described by Creer and Gross (15) by reversed-phase HPLC run isocratically using methanol/water/acetonitrile (57:23:20) as mobile phase at a flow rate of 1.0 mL/min. The lysophospholipid species were detected at 203 nm. Lysophosphatidylcholine (LPC), lysophosphatidylinositol (LPI), and lysophosphatidylethanolamine (LPE) were quantified as products of phospholipid hydrolysis. Pure L-α-LPC, L-α-LPI, and L-α-LPE (Sigma) were used as standards.

Contribution of TAG and phospholipids to lipid hydrolysis on milled rice. (i) Relative contribution of TAG to lipid hydrolysis. The contribution of TAG to lipid hydrolysis was determined by dividing the sum of the MAG and DAG mass by that of neutral lipids. The contribution was then expressed as a percentage (w/w) of total TAG.

(ii) Relative contribution of phospholipids to lipid hydrolysis. The contribution of phospholipids to lipid hydrolysis was calculated by dividing the sum of LPC, LPI, and LPE contents by the total phospholipid content. The contribution was expressed as a percentage of total phospholipids.

Statistical analysis. Data were subjected to one-way ANOVA, and means were separated using the least significant difference test at a 5% probability level. Analysis was carried out using JMP IN program (Version 5, SAS Inc., Cary, NC).

RESULTS AND DISCUSSION

Changes in acylglycerol and phospholipid hydrolysis products. Changes in total lipids, TAG, and phospholipids in milled rice during storage are presented in Figure 1. TAG constituted 0.49% of the milled rice with phospholipids being only 0.08% milled rice. Total surface lipids and TAG changed slightly but significantly ($P > 0.05$) during the study, but phospholipids did not change. Total lipid content decreased from 0.57% on day 0 to 0.41% on day 20 but did not change significantly until the end of the study on day 50. Likewise, the TAG content decreased from 0.49% on day 0 of storage to 0.35% after 20 d but did not change significantly for the rest of the study period. Shin *et al.* (16) also observed decreases in the lipid content of brown rice from 1.75 to 1.65% and in the neutral lipid fraction from 89 to 82% during storage at 35°C. The change in TAG content can be attributed to the activities of lipases. Similar losses in TAG and total lipids during storage were expected since the TAG content was calculated as a difference between total lipid and phospholipid contents. The total phospholipid

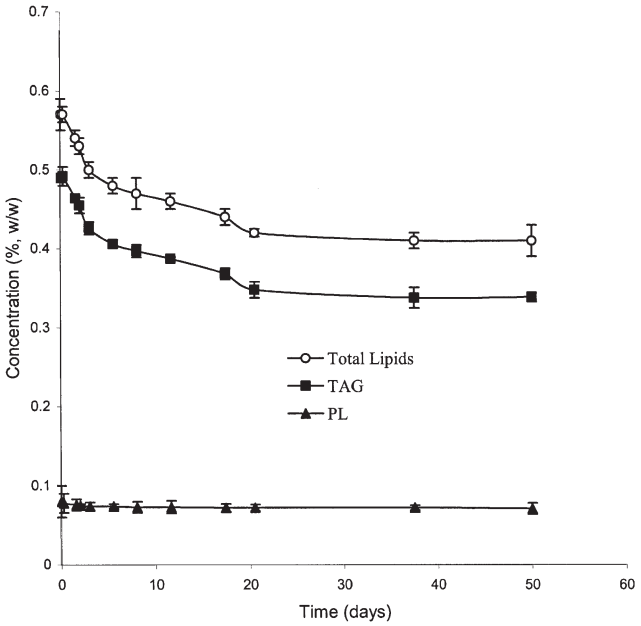

FIG. 1. Total surface lipid, TAG, and phospholipid (PL) contents (%, w/w) of milled rice stored at 37°C and 70% RH.

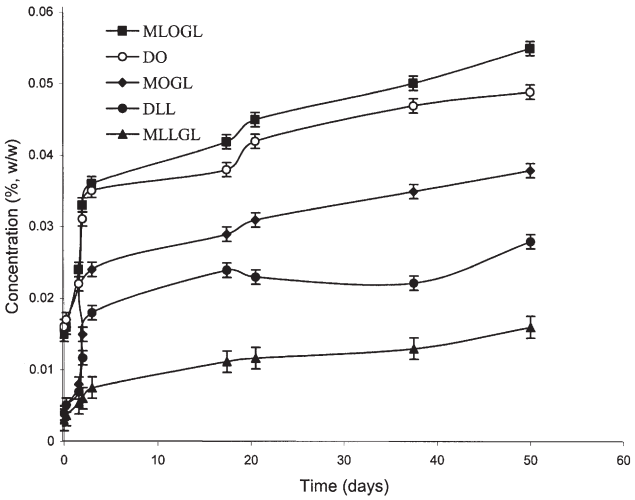

FIG. 2. Changes in glycerol hydrolysis products of TAG (%, w/w) of rice surface lipids stored at 37°C and 70% RH. Mono-oleoyl glycerol (MOGL), monolinoleoyl glycerol (MLOGL), monolinoleneoyl glycerol (MLLGL), diolein (DO), and dilinolein (DLL).

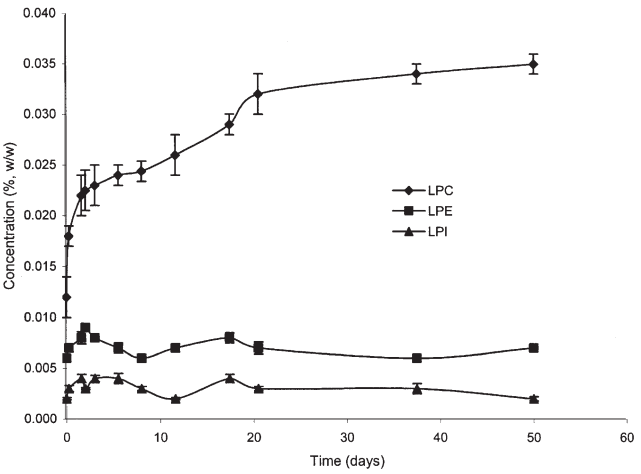

FIG. 3. Changes in phospholipid hydrolysis products of rice surface lipids stored at 37°C and 70% RH. Lysophosphatidylcholine (LPC), lysophosphatidylethanolamine (LPE), and lysophosphatidylinositol (LPI).

content did not change significantly because the phospholipid content was calculated based on the phosphorus content of rice lipid extract, which would not be expected to change during storage.

Figure 2 represents changes in the MAG and DAG contents of milled rice. All MAG and DAG increased rapidly during the first 3 d of storage and then increased gradually for the remaining study period. The rapid initial increases may be attributed to the activity of rice bran lipases, whereas the decrease in rate was probably due to FFA inhibition of lipase activity (17) or enzyme degradation. The trends in changes in MAG and DAG were similar to those observed for total FA on milled rice in storage by Lam and Proctor (5), who observed similar phases of an initial short-lived rapid increase (1–3 d) and, thereafter, a steady increase beyond 3 d. The linoleoyl glycerols increased to higher concentrations than the oleoyl glycerols, which in turn increased more than the linoleneoyl glycerols for both DAG and MAG after 3 d of storage. The observed levels of increase in the glycerol species reflect the FA composition of rice lipids (7).

Monolinoleoyl glycerol and diolein had the highest concentrations throughout the study, with the monolinoleoyl glycerol content being generally higher than that of diolein. Hemavathy and Prabhakar (18) found a higher content of MAG than DAG in rice bran, and Yoshizawa *et al.* (19) reported that the MAG of milled rice lipids formed by hydrolysis had higher levels of linoleic acid and lower levels of oleic acids. The high levels of monolinoleoyl glycerol and diolein could be due to the *sn*-positions of the linoleic and oleic acids on the TAG molecule. Rice bran lipase is known to hydrolyze fatty ester bonds preferentially at the 1,3-position (20).

All three lysophospholipids increased significantly ($P > 0.05$) during the first 3 d of storage but only LPC continued to increase until the end of the study period (Fig. 3). This study demonstrates that PC, whose hydrolysis product is LPC, was the main component of phospholipid hydrolysis during storage. PC is the main phospholipid (35%) in rice bran lipids and also the main membrane component of rice bran spherosomes (4). This study suggests that rice bran spherosomes are decomposed during hydrolysis of rice bran lipids.

Contribution of TAG and phospholipids to lipid hydrolysis on milled rice. The presence of partial esters such as MAG and DAG in oil reflects the extent of lipid hydrolysis (2). However, phospholipase C, which releases DAG from phospholipids, has an optimal temperature of 70°C (4) and is most unlikely to be responsible for the DAG and/or MAG determined at the temperature (37°C) used during this study. The percentage of TAG and phospholipids hydrolyzed increased during the first 3 d of storage, followed by a period of decline and finally a significant ($P > 0.05$) increase in the percentage hydrolyzed after day 17 until the end of the study (Table 1). The changes in relative percentage of hydrolyzed TAG and phospholipids were similar to those of FFA formation on milled rice (5). The initial increase in percentage of TAG and phospholipids hydrolyzed was due to lipases that become active after rice milling. The period of minimal change or decline in hydrolysis after 3 d of storage was probably due to lipase inhibition by FFA. The observed increase after day 17 may be attributed to bacterial lipases. Lam and Proctor (5) observed a significant increase in bacterial growth in milled rice after 20 d of storage under the same conditions.

Although more TAG hydrolysis products were formed than phospholipid products, a higher proportion of total phospholipids was hydrolyzed than total TAG. The higher percentage of hydrolysis observed in phospholipids may be due to the phospholipids forming the spherosomes membrane in rice bran (3). Lipases act only at the oil–water interface (21), and because the phospholipids are more polar than their TAG counterparts (22), they are probably present at the interface as emulsifiers and hence will be more readily exposed to lipase hydrolysis.

MAG and DAG contents of milled rice increased during storage with MAG content being higher than that of DAG. LPC

TABLE 1
Means of the Relative Percentage of TAG and Phospholipids Hydrolyzed in Milled Rice Stored at 37°C and 70% RH[a]

	Time (d)											
	0.0	0.3	1.6	2.0	3.0	5.5	8.0	11.6	17.4	20.5	37.5	50.0
%TAG hydrolyzed[b]	12.3[a]	14.2[a]	21.6[b]	19.8[b]	37.6[c]	37.0[c]	32.8[c]	36.2[c]	35.3[c]	46.0[d]	53.3[e]	53.1[e]
%PL hydrolyzed[c]	25.0[a]	35.8[b]	57.6[c]	63.1[d]	62.5[d]	57.8[c]	52.4[c]	55.6[c]	66.1[d]	66.1[d]	69.4[e]	73.8[e]

[a]Means of triplicate analyses. Values in the same row followed by different roman superscript letters are significantly different ($P < 0.05$).
[b]TAG–TG; %TAG = (MOGL + MLOGL + MLLGL + DLL + DO)/TAG × 100.
[c]PL–phospholipids; %PL = (LPC + LPE + LPI)/PL × 100. MOGL, mono-oleoyl glycerol; MLOGL, monolinoleoyl glycerol; MLLGL, monolinolenoyl glycerol; DLL, dilinolein; DO, diolein; LPC, lysophosphatidylcholine; LPE, lysophosphatidylethanolamine; LPI, lysophosphatidylinositol.

increased throughout the duration of the study, whereas LPI and LPE contents initially increased until day 3 and then leveled off until day 50. The hydrolyses of TAG and phospholipids in milled rice proceeded rapidly in the first 3 d of storage and gradually increased until day 50. Phospholipids were hydrolyzed at a larger relative percentage than TAG, which indicates that phospholipases were apparently more active. However, the importance of the contribution of phospholipids to the total FFA may be significant only during the early part of storage rather than toward the end because of less total phospholipids relative to the total TG content in rice lipids.

ACKNOWLEDGMENTS

The authors wish to thank the Arkansas Rice Research Board and Anheuser Busch Inc. for supporting this work and Riceland Foods Inc. for their donation of milled rice.

REFERENCES

1. 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).
2. Nicolosi, R.J., E.J. Rogers, L.M. Ausman, and F.T. Orthoefer, Rice Bran Oil and Its Health Benefits, in *Rice Science and Technology*, edited by W.E. Marshall and J.I. Wadsworth, Marcel Dekker, New York, 1994, pp. 421–437.
3. Bechtel, D.B., and Y. Pomeranz, Ultrastructure of the Mature Ungerminated Rice Caryopsis. The Caryopsis Coat and the Aleurone Cell, *Am. J. Bot. 64*:966–973 (1977).
4. Takano, K., Mechanism of Lipid Hydrolysis in Rice Bran, *Cereal Foods World 38*:695–698 (1993).
5. Lam, S.H., and A. Proctor, Lipid Hydrolysis and Oxidation on the Surface of Milled Rice, *J. Am. Oil Chem. Soc. 80*:563–567. (2003).
6. Loeb, J.R., N.J. Morris, and F.G. Dollear, Rice Bran Oil. IV. Storage of the Bran as It Affects Hydrolysis of the Oil, *Ibid. 26*: 738–740 (1949).
7. Yasumatsu, K., and S. Moritaka, Fatty Acid Composition of Rice Lipid and Changes During Storage, *Agric. Biol. Chem. 28*:257–260 (1964).
8. Tsuzuki, W., H. Kasumimoto, S. Kobayashi, and T. Suzuki, Esterase Activity and Free Fatty Acid Accumulation in the Bran of Selected Rice Cultivars, *Cereal Chem. 71*:162–165 (1994).
9. Ramezanzadeh, F.M., R.M. Rao, M. Windhauser, W. Prinyawiwatkul, R. Tulley, and W.E. Marshall, Prevention of Hydrolytic Rancidity in Rice Bran During Storage, *J. Agric. Food Chem. 47*:3050–3052 (1999).
10. Lam, H.S., and A. Proctor, Rapid Methods for Milled Rice Total Lipid and Free Fatty Acid Determinations, *Cereal Chem. 78*: 488–489 (2001).
11. Bartlett, G.R., Phosphorus Assay in Column Chromatography, *J. Biol. Chem. 234*:466–468 (1959).
12. Kaluzny, M.A., L.A. Duncan, M.V. Merritt, and D.E. Epps, Rapid Separation of Lipid Classes in High Yield and Purity Using Bonded Phase Columns, *J. Lipid Res. 26*:135–140 (1985).
13. Maruyama, K., and C. Yanese, Separation and Quantitative Determination of Monoacylglycerol Mixtures by Reversed Phase HPLC, *J. Am. Oil Chem. Soc. 63*:902–905 (1986).
14. Lin, J.-T., C.L. Woodruff, and T.A. McKeon, Non-aqueous Reversed-Phase High-Performance Liquid Chromatography of Synthetic Triacylglycerols and Diacylglycerols, *J. Chromatogr. A 782*:41–48 (1997).
15. Creer, M.H., and R.W. Gross, Separation of Isomeric Lysophospholipids by Reversed Phase HPLC, *Lipids 20*:922–928 (1985).
16. Shin, M.G., S.H. Yoon, J.H. Rhee, and T.W. Kwon, Correlation Between Oxidative Deterioration of Unsaturated Lipid and *n*-Hexanal During Storage of Brown Rice, *J. Food Sci. 51*:460–463 (1986).
17. Smith, J.L., and J.A. Alford, Inhibition of Microbial Lipases by Fatty Acids, *Appl. Microbiol. 14*:699–702 (1966).
18. Hemavathy, J., and J.V. Prabhakar, Lipid Composition of Rice (*Oryza sativa* L.) Bran, *J. Am. Oil Chem. Soc. 67*:1016–1019 (1987).
19. Yoshizawa, K., T. Ishikawa, and K. Noshiro, Changes in Fatty Acid Composition of Lipids by the Change in Polishing Rate of Rice Grains, *Nippon Nogeikagaku Kaishi 47*:713–716 (1973).
20. Aizono, Y., M. Funatsu, M. Sugano, K. Hayashi, and Y. Fujiki, Enzymatic Properties of Rice Bran Lipase, *Agr. Biol. Chem. 37*: 2031–2036 (1973).
21. Verger, R., C.E. Mieras, and G.H. de Haas, Action of Phospholipase A at Interfaces, *J. Biol. Chem. 248*:4023–4034 (1973).
22. Morrison, W.R., Cereal Lipids, in *Advances in Cereal Science and Technology*, edited by Y. Pomeranz, American Association of Cereal Chemists, St. Paul, MN, 1978, pp. 221–348.

[Received August 19, 2003; accepted February 20, 2004]