

Rapid Methods for Milled Rice Surface Total Lipid and Free Fatty Acid Determination

H. S. Lam¹ and A. Proctor^{1,2}

ABSTRACT

Cereal Chem. 78(4):498–499

Total lipids and free fatty acid (FFA) determination is widely used in the food industry to assess the quality of milled rice. An improved rapid ambient temperature isopropanol (IPA) extraction method to determine milled rice surface lipid was more effective than Soxhlet solvent extraction. The improved method was probably due to better extraction of polar lipids and antioxidants by IPA. A colorimetric method to determine

FFA requiring only 30 μ L of sample is also described. The new technique provides results similar to those obtained using the slower, conventional acid-base titration method. The colorimetric FFA method requires a smaller sample size, has greater precision, and is more objective. The new methods are particularly suitable for industrial use in providing rapid results for large numbers of samples.

Rice millers and rice users use milled rice surface free fatty acid (FFA) content as an indicator of potential off-flavors and odor development. FFA is present because bran lipid and lipases are deposited together on the rice kernel during milling, resulting in FFA formation on the rice surface (Prabakar and Venkatesh 1986). Subsequent oxidation of FFA and other lipids (Galliard 1983) is probably responsible for off-flavor development.

Milled rice FFA is conventionally measured by first obtaining a surface lipid extract by Soxhlet extraction. The lipid extract is then air-dried and the FFA content determined by acid-base titration (AOCS 1993). The Soxhlet method is also used to measure total milled rice surface oil to determine degree of milling (Chen and Siebenmorgen 1997). However, the Soxhlet method for rice involves rigorous extraction with nonpolar solvents and is tedious and time-consuming. Such extraction conditions seem an excessive means to obtain surface nonbound rice lipids and could cause thermally induced lipid changes such as FFA and hydroperoxides breakdown with additional lipid oxidation. Furthermore, the Soxhlet extraction solvent petroleum ether is nonpolar and unlikely to extract polar bran lipids such as phospholipids from the rice surface. The use of a more polar solvent such as isopropanol (IPA) should extract polar and nonpolar lipids (Shahidi and Wanasundara 1989). Proctor and Bowen (1996) showed that a 5-min ambient temperature wash of rice bran with IPA could remove as much oil as an extensive hot solvent reflux extraction with petroleum ether.

The acid-base titration used for milled rice FFA determination (AOCS 1993) was designed for oilseed oil quality determination rather than milled rice lipid that usually contains <1% surface lipids. A more sensitive, less subjective method for FFA determination would be desirable. Walde and Nastruzzi (1991) described a simple colorimetric method for FFA determinations in oils that gave a comparable result with the acid-base titration method but required only 30 μ L of oil per replicate. This method could be adapted for milled rice evaluation where oil is limited.

The objectives of this study are to 1) develop a rapid improved ambient temperature extraction method for milled rice surface lipid determination, and 2) develop an improved milled rice lipid FFA analysis by adapting the colorimetric FFA method (Walde and Nastruzzi 1991).

MATERIALS AND METHODS

Rice preparation. Long-grain rice 'Drew' and medium-grain rice 'Bengal' with \approx 12.5% moisture content were dehulled with a Satake huller (Satake Engineering Co., Tokyo) and milled for 10, 20, and 30 sec with a McGill No. 2 mill (McGill, TX). Whole rice kernels were separated from broken kernels in a Grainman shaker table (Grain Machinery Mfg. Corp., Miami, FL) with 12/64 trays and recovered for use in the study. The milled head rice was divided into three portions.

One portion was subject to traditional FFA analysis by Soxhlet lipid extraction and subsequent FFA determination by acid-base titration. The second portion was also subjected to Soxhlet lipid extraction but the FFA determination was done colorimetrically. The third portion had surface lipid removed by an IPA wash and the FFA was determined colorimetrically. Three determinations were made for each method. Each determination was the mean of three replicates. Analysis of variance in the data between the conventional and new methods was calculated using JMP IN 3.2.1 software (SAS Institute Inc., Cary, NC).

Soxhlet lipid extraction. Surface lipids of 10 g of milled rice were extracted with petroleum-ether by the Soxhlet method (Chen and Siebenmorgen 1997).

FFA determination by titration. FFA of the extracted oil was then determined by titration with 0.01*N* sodium hydroxide solution using phenolphthalein as indicator (AOCS 1993).

Rapid lipid extraction. Rice surface lipids were extracted by vortexing 10 g of milled rice for 2 min with 4 mL of IPA. An additional 4 mL of IPA was added and the sample was vortexed again for 2 min. The extract was centrifuged at 2,500 rpm for 10 min to remove bran particles. The weight of extracted lipids was determined after evaporating the solvent on a hotplate at the lowest setting.

Colorimetric determination of FFA. FFA was analyzed by the method of Walde and Nastruzzi (1991). An assay solution containing 0.375 mL of solution A (0.1*M* tris/HCl, pH 9.0), 0.125 mL of solution B (2 *mM* phenol red in 0.1*M* tris/HCl, pH 9.0) and 50 mL of solution C (50 *mM* Bis (2-ethylhexyl) sodium sulfosuccinate in isoctane) was prepared. Assay solution (1 mL) was placed in a 1-cm cuvette with 30 μ L of IPA extract and shaken for 1 min before measuring absorbance at 560 nm. The FFA content of each extract was obtained from a calibration curve. Oleic acid was dissolved in IPA to produce oleic acid solutions of 0.001–0.02% (w/w). The standard solutions were subjected to colorimetric analysis.

¹ Department of Food Science, University of Arkansas, Fayetteville, AR 72704.

² Corresponding author. E-mail: aproctor@mail.uark.edu Phone: 501 575-2980. Fax: 501 575-6936.

TABLE I
Surface Lipids (% rice) Extracted from Milled Rice^a

Method	Milling Time (sec)					
	Long Grain			Medium Grain		
	10	20	30	10	20	30
Isopropanol (IPA) extract	0.86 ± 0.03a	0.57 ± 0.01a	0.38 ± 0.02a	0.89 ± 0.08a	0.64 ± 0.05a	0.44 ± 0.02a
Soxhlet extract	0.83 ± 0.01a	0.52 ± 0.04a	0.36 ± 0.01a	0.88 ± 0.09a	0.58 ± 0.11a	0.43 ± 0.02a

^a Values followed by the same letter in the same column are not significantly different ($P < 0.05$).

TABLE II
Milled Rice Free Fatty Acid (% rice) Content^a

Method	Milling Time (sec)					
	Long Grain			Medium Grain		
	10	20	30	10	20	30
Isopropanol (IPA) and colorimetry	0.045 ± 0.008a	0.036 ± 0.004a	0.026 ± 0.006a	0.047 ± 0.004a	0.037 ± 0.003a	0.027 ± 0.004a
Soxhlet and colorimetry	0.046 ± 0.002a	0.036 ± 0.004a	0.026 ± 0.005a	0.047 ± 0.003a	0.037 ± 0.009a	0.027 ± 0.010a
Soxhlet and titration	0.043 ± 0.012a	0.037 ± 0.009a	0.028 ± 0.011a	0.048 ± 0.010a	0.035 ± 0.013a	0.026 ± 0.007a

^a Values followed by the same letter in the same column are not significantly different ($P < 0.05$).

RESULTS AND DISCUSSIONS

Oil extraction. The oil extracted by each method is shown in Table I. There were no significant differences between the two methods ($P < 0.05$). However, the mean values for Soxhlet extraction were consistently lower than those obtained by IPA extraction. This may be due to oil thermal decomposition during Soxhlet extraction and drying the extract (Chan et al 1976) and incomplete extraction of polar lipids (Shahidi and Wanasundara 1989). Proctor and Bowen (1996) showed that IPA extracted rice bran oil was oxidatively more stable than hexane extracted rice bran oil. Oil weight of IPA extracted oil was stable for 17 days at 64°C whereas the weight of hexane extracted oil increased after six days under the same conditions. This was probably because IPA extracted bran antioxidants with the oil, whereas Soxhlet petroleum ether extraction did not. Furthermore, IPA is a better lipid solvent for rice bran than either petroleum ether or hexane (Proctor and Bowen 1996) as it extracts both polar and nonpolar lipids.

FFA determination. The FFA contents of the extracted rice oil are shown in Table II. FFA levels determined by both the titration and colorimetric methods were not statistically significantly different ($P < 0.05$). However, the titration results showed more variability than the colorimetric results, as shown by the standard deviations. The higher variability in the titration results was probably due to difficulty in determining the titration end-point because of the low FFA levels in the oil. The rice FFA content obtained by either method was lower than that found in previous studies (Piggott et al 1991) where a level of 0.08% was reported. Rice aging and the commercial storage condition can be responsible for the higher levels of FFA in the extracted oil. Rice lipids breakdown rapidly into FFA during storage, particularly at high temperature (Morrison 1978).

CONCLUSIONS

The new rapid, rice surface lipid extraction method gave higher oil yields than conventional Soxhlet extraction. This was probably due to the low temperature extraction and the use of IPA solvent

that better extracted both polar lipids and antioxidants, thus providing a more efficient extraction and avoiding lipid degradation. Despite no statistical difference in the FFA data, the colorimetric method for FFA determination was faster, more sensitive and precise than the titration method. It would be an ideal method to rapidly evaluate large sample numbers where only a small amount of lipid is available.

LITERATURE CITED

- AOCS. 1993. Official Methods and Recommended Practices of the American Oil Chemists Society. 4th Ed. Method Aa 6-38. The Society: Champaign, IL.
- Chan, H. W. S., Prescott, F. A. A., and Swoboda, P. A. T. 1976. Thermal decomposition of individual positional isomers of methyl linoleate hydroperoxide: Evidence of carbon oxygen bond scission. *J. Am. Oil Chem. Soc.* 53:572-576.
- Chen, H., and Siebenmorgen, T. J. 1997. Effect of rice kernel thickness on degree of milling and associated optical measurements. *Cereal Chem.* 74:821-825.
- Galliard, T. 1983. Enzymic degradation. Pages 111-147 in: *Lipids in Cereal Technology*. P. J. Barnes, ed. Academic Press: London.
- Morrison, W. R. 1978. Cereal lipids, changes in lipids during harvesting, drying, storage, and processing of cereals. Pages 297-304 in: *Advances in Cereal Science and Technol.* Vol. 2. Y. Pomeranz, ed. Am. Assoc. Cereal Chem.: St. Paul, MN.
- Piggott, J. R., Morrison, W. R., and Clyne, J. 1991. Changes in lipids and in sensory attributes on storage of rice milled to different degrees. *Int. J. Food Sci. Technol.* 26:615-628.
- Prabhakar, J. V., and Venkatesh, K. V. I. 1986. A simple chemical method for stabilization of rice bran. *J. Am. Oil Chem. Soc.* 63:644-646.
- Proctor, A., and Bowen, D. J. 1996. Ambient-temperature extraction of rice bran oil with hexane and isopropanol. *J. Am. Oil Chem. Soc.* 73:811-813.
- Shahidi, F., and Wanasundara, J. P. D. 1989. Extraction and analysis of lipids. Pages 115-136 in: *Lipids*. C. C. Akoh and D. B. Min, eds. Marcel Dekker: New York.
- Walde, P., and Nastruzzi, C. 1991. Application of a new, simple and economic colorimetric method for the determination of non-esterified fatty acids in vegetable oils. *Food Chem.* 39:249-256.

[Received September 8, 2000. Accepted March 26, 2001.]