

# A Gas Chromatography Procedure for Determining Milled Rice Surface Lipid Content

C. J. Bergman<sup>1,2</sup> and F. D. Goffman<sup>3</sup>

## ABSTRACT

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Rice (*Oryza sativa*, L.) degree of milling (DM) is a quantification of the amount of bran that has been removed from kernels during the milling process. Historically it has been determined using a time-consuming continuous solvent extraction (CSE) procedure to measure the surface lipid content. The objective of this research was to develop a gas chromatography (GC) method for rice surface lipids. The GC procedure was highly correlated with several other methods for DM including a commonly

used CSE procedure and it demonstrated repeatability within acceptable analytical limits. Other aspects in support of the utility of this procedure are that it requires a relatively small amount of sample, organic solvent, and technician time. Consequently, this GC method for rice surface lipids measurement is suitable for use in determining rice DM in cultivar development programs and studies that require the analysis of a large number of samples.

Rice (*Oryza sativa*, L.) degree of milling (DM) is a quantification of the amount of bran that has been removed from kernels during the milling process (Shams-Ud-Din and Bhattacharya 1978). The bran layer of rice kernels is darker in color compared with its endosperm and contains more lipids, vitamins, minerals, and other phytochemicals. Therefore, the more bran left on kernels after milling, the darker in color and the more nutrient dense they will be. When whiter colored kernels and longer shelf-life is desired, rice millers must remove relatively more bran. The trade-off in doing this is not just the reduction in nutrient content but the undesirable increase in broken kernels that is associated with a greater DM. All things being equal, a greater percentage of broken kernels will decrease the value of milled rice. Consequently, the amount of bran that has been removed during milling (i.e., DM) is an important measurement for the rice industry.

Rice samples that have been milled to a different degree reportedly have varying functional and sensory properties. For example, Champagne et al (1990) and Marshall (1992) reported that greater DM was associated with decreased pasting onset time and gelatinization temperature. Reduced cooking time and higher water binding capacity, swelling power, and peak viscosity were found for rice milled to a higher degree (Champagne et al 1990; Kim and Jeon 1996; Perdon et al 2001). Higher DM was reported to result in lower cooked rice corn flavor, raw rice flavor, wet cardboard flavor, hay-like flavor, and bitterness (Park et al 2001). This sensory report also found greater DM to be associated with increased cooked rice agglomeration, adhesiveness, cohesiveness of mass, inner moisture, and tooth packing while hardness and chewiness decreased. Therefore it is important that only rice samples that have been milled to the same degree be compared in end-use quality related studies. This, however, is seldom documented in published research.

The numerous methods that have been developed to determine rice DM have been reviewed (Barber and Benedito de Barber 1979; Bergman et al 2004). These methods can be categorized into several groups: those that visually assess the amount of bran remaining on milled rice; those that measure chemical components in rice kernel's outer layers; or those that determine the percentage of rice by mass remaining after milling.

Visual estimation of the bran remaining after milling is performed by comparing milled samples with established rice standards, as well as after staining kernels with various bran specific dyes (Tani et al 1952; USDA 1995). Visualization techniques rely on a person's subjective judgment and are thus likely to be susceptible to a relatively large amount of error.

Optical instruments have also been used to assess the amount of bran remaining on kernels after milling by quantifying the light that is reflected or absorbed when a sample is illuminated with light (Yadav and Jindal 2001) as in the Satake milling meter (Satake USA, Stafford, TX). Optical instruments allow for relatively rapid measures of milling degree and are not destructive but the values are affected by differences between samples other than bran content, such as chalk and inherently dark colored endosperm (Bergman and Xu 2003). These instruments also require up to 20 g of sample, which is a relatively large amount.

Chemical components that decrease with greater DM and that have been used to quantify this grain characteristic include proteins, lipids, pigments, ash, silica, protein, thiamin, and fiber (Bhattacharya and Sowbhagya 1972; Barber and Benedito de Barber 1979). These methods may suffer from error stemming from variance in the concentration gradient of these compounds between rice cultivars. But this hypothesis needs to be studied. These techniques are also relatively time-consuming and technically challenging, but most are highly repeatable.

During the last few decades, determining the amount of lipid in the outer layers of milled rice (i.e., surface lipids) using continuous solvent extraction techniques has become the most common method for assessing the degree to which bran has been removed from rice kernels. The instruments historically used and studied for continuous solvent extraction of rice include the Goldfisch, Soxhlet, and Soxtec (Hogan and Deobald 1961; Shams-Ud-Din and Bhattacharya 1979; Rohrer et al 2004; Matsler and Siebenmorgen 2005). The former instrument along with petroleum ether is used by the USDA Grain Inspection, Packers and Stockyard Administration for their official measurements of rice total lipids and free fatty acids (USDA Handbook 1997). Although highly repeatable, the measurement of surface lipids using the instruments mentioned above requires  $\geq 5$  g of sample.

Attempts to develop predictive infrared spectroscopy (IR) techniques for determining rice SLC have been reported. For example, Chen et al (1997) developed near-infrared reflectance calibration equations to predict milled rice SLC using three rice cultivars. The best equation they reported utilized visible and near-infrared wavelengths, modified partial least squares, and pretreatments of standard normal variate and first derivative. Gangidi et al (2002) reported the use of diffuse reflectance Fourier transform infrared

<sup>1</sup> Department of Food and Beverage, University of Nevada-Las Vegas, Las Vegas, NV 89154 U.S.A.

<sup>2</sup> Corresponding author. E-mail: christine.bergman@unlv.edu

<sup>3</sup> Philip Morris International, R&D Department, Quai Jeanrenaud 56, 2000 Neuchâtel, Switzerland.

spectroscopy to predict DM as measured by solvent extraction of surface lipids. Analysis showed a high degree of correlation ( $R^2 = 0.96$ ) between SLC and the spectra in the 4,000 to 400  $\text{cm}^{-1}$  range for one long-grain and one medium-grain cultivar. These studies indicate that predictive IR methods for rice SLC are robust when genotypes used to develop the calibration equation are similar to those being evaluated. An example of such an application is milling organizations that are able to develop predictive equations using samples of cultivars grown in their region during the season they will be evaluating.

The robustness of IR methods to predict rice surface lipid data for use in cultivar development and genetic studies has yet to be determined. However, reports for other rice end-use quality traits other than moisture and protein content have been not been constant (Bao et al 2001; Bergman et al 2004).

The quantity of each rice sample available for research projects is often limited (<2 g), while the number of samples to be analyzed is relatively large, reaching into the hundreds and sometimes thousands. These needs are frequently found in research designed to understand the genetics controlling kernel traits or when scientists desire to identify genotypes of interest from a mutated population. Consequently, analytical methods are needed that are rapid, repeatable, and require a small amount of sample. The objectives of the research reported here were 1) develop a rapid analytical procedure for determining milled rice SLC that requires a small sample size; and 2) evaluate the association between values found using the new method and other measures of degree of milling.

## MATERIALS AND METHODS

Long-, medium-, and short-grain rice milling standards were obtained from the USDA, GIPSA, Federal Grain Inspection Service, Technical Services Division (10383 North Ambassador Drive, Kansas City, MO). These samples are used by Federal Grain Inspection Service inspectors to grade rice using visual inspection. For each conventional rice grain type, samples including hard-milled, well-milled, and reasonably well-milled samples were used for analysis.

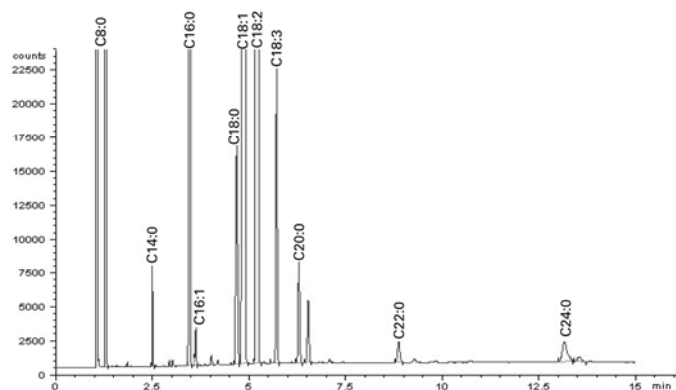
Another group of accessions included conventional U.S. long-grain cultivars (Cocodrie, Lemont, and Newbonnet); conventional U.S. medium-grain cultivars (Bengal and Mars); a Japanese premium quality cultivar (Koshihikari); waxy cultivars (Shinchiku Mochi No. 39 and Rikuto Norin 4); and others (Emanaye Carambak, IR66-103-2, CA 497/V/7, Phudugey, WC 758, Iari 6626, and Luks). These accessions were grown in Beaumont, TX, during the 2000 season using cultural management practices common to the region. At maturity, the plants were harvested by hand and threshed. The samples were hulled on a rice huller (Satake model

THO35A) and broken, diseased, and immature kernels were removed. Hulled samples (50 g each) were then milled for 30 sec using a mill (McGill #1, Rapsco, Brookshire TX) with an 858-g weight in position 12 or 6 for long- and medium-grains, respectively. Samples were defined as long- or medium-grains using the length-to-width ratio definitions described in Adair et al (1973).

Milled rice SLC was determined by refluxing 5 g of milled rice with petroleum ether in a Goldfish extraction apparatus for 30 min. The solvent was collected and evaporated; percent SLC was calculated as the mass of the extracted lipid divided by the beginning total milled rice mass. A milling meter (Satake model MM1-B) was used to determine the whiteness of all the FGIS samples. Protein content of milled rice was determined using a nitrogen/protein analyzer (Leco FP-528, St. Joseph, MI). With each use of this instrument, EDTA was used as a check. Moisture content was determined based on ground rice sample weight loss during 2 hr in a forced air oven at 120°C.

The mass percentage SLC of the samples was also determined by fatty acid methyl esters (FAME) quantification through gas-liquid chromatography (Christie 1982). FAME were prepared with ~500 mg of milled rice extracted and transmethylated while shaking for 30 min at RT in 1 mL of a 0.5M solution of sodium methylate (Fluka, Milwaukee, WI) in methanol. This methanol solution also contained an internal standard, tricaprylin (C8:0). Isooctane (1 mL) was added and the samples were vortexed; then 0.5 mL of 5% (w/v) of  $\text{NaHSO}_4$  (Fluka) in water was added and the sample was vortexed again. The tubes were then centrifuged for 5 min at  $3,400 \times g$  (Jouan CR 412) and a portion of the isooctane layer was pipetted into GC vials. Aliquots of the isooctane phase (2  $\mu\text{L}$ ) were injected into the gas chromatograph. Analysis was performed in a gas chromatograph (model 5890 Series II, Hewlett-Packard, Palo Alto, CA) equipped with a flame ionization detector (FID) and a DB-Wax 30 m  $\times$  0.25 mm, i.d.  $\times$  0.25  $\mu\text{m}$  thickness column (122-7032, Agilent Technologies, Wilmington, DE). The carrier gas was helium at a pressure of 30.6 psi. The oven temperature program was initial temperature (160°C) linearly increased to 167°C at 2°C/min, then to 210°C at 15°C/min, then to 250°C at 10°C/min; final temperature was held for 14 min. The injector and detector temperatures were 230 and 280°C, respectively. The samples were injected at a split rate of 1:8.8.

GC fatty acid peaks were identified by comparing retention times with those found for a set of reference standards (Sigma-Aldrich, St. Louis, MO, cat. no. 189-18). Response factors were determined for all of the fatty acids found in milled rice using tricaprylin (Bannon et al 1986). The factors were developed from three GC analyses run on different days and used to determine individual fatty acid levels that were then summed and reported as total surface lipids.



**Fig. 1.** GC chromatogram of fatty acid methyl esters obtained after extraction and derivitization of lipids from the surface of a milled sample of Lemont with C8:0 used as an internal standard.

**TABLE I**  
Response Factors of Esters Relative to Tricaprylin

Fatty Acid Methyl Ester	Response Factor	Standard Deviation <sup>a</sup>
C8:0	1.0000	0.0000
C10:0	0.9187	0.0001
C12:0	0.8613	0.0003
C14:0	0.7980	0.0007
C16:0	0.7580	0.0013
C16:1	0.7684	0.0011
C18:0	0.7257	0.0017
C18:1	0.7282	0.0015
C18:2	0.7300	0.0020
C18:3	0.7351	0.0021
C20:0	0.7302	0.0022
C22:0	0.6966	0.0023
C22:1	0.7096	0.0025
C24:0	0.6939	0.0018

<sup>a</sup> Based on three replicates.

All solvents used were HPLC grade from Fisher Scientific (Fair Lawn, NJ). The analytical data is expressed on a 12% moisture basis. Three replicates of each assay were performed on each sample. Data analyses were performed using statistical software (v.8, SAS Institute, Cary, NC).

## RESULTS AND DISCUSSION

The sample set for the study reported here was chosen in an effort to study samples with conventional U.S. length-to-width ratios, as well as with varying kernel morphology ratios as is typically found in landrace material and breeding populations. The Goldfish SLC of these samples ranged from 0.16 to 0.58%. Approximately 0.3% SLC is often quoted within the U.S. rice industry and research community as signifying a well-milled sample. These results indicate that some of the samples studied were within what is termed in the U.S. rice processing industry as over-milled, well-milled, or under-milled. Therefore, this set of samples was suited for study because they covered the full range in DM.

The samples for this study were milled using standard methods as specified for different grain length-to-width ratios. In spite of the use of standard milling methods, a great deal of variation in SLC was found. The variation is not surprising because slightly different kernel length-to-width ratios between cultivars and within samples have been reported to mill to different degrees when using the same milling protocol (Chen et al 1998; Gujral et al 2002). The different DM, however, are generally overlooked by those studying aspects of rice end-use quality. The data then supports the need for reporting sample DM in rice end-use quality research. Without doing so, scientists cannot be sure differences are due to treatments rather than to the variation in DM between samples, even when samples were milled using the same method.

The GC method for FAME was linear when using five levels of rice bran oil (data not shown). The chromatogram shown in Fig. 1 is an example of the fatty acids identified by gas chromatography in the rice samples used for this study. The identified fatty acids correspond to those reported previously for milled rice (Juliano 1985). Tricaprylin (C8:0) was used as an internal standard for our method because its peak is completely resolved with no interference of any rice fatty acids. The repeatability of the GC method

was evaluated using the response factors of multiple runs of the standard FAME (Table I). The standard deviation values for those analyses were low (0.0001–0.0025). Precision was also evaluated by multiple measurements of each rice sample. The mean of the relative standard deviation across all samples for the GC measurement of surface lipids was 2.64% (Table II). By comparison, the GC method was less precise than the percent protein and Satake milling meter methods, but more precise than the Goldfish method. Analytical method precision is generally considered acceptable when the relative standard deviation is <5%, thus the GC method for surface lipids reported here has acceptable repeatability.

A high correlation ( $r = 0.94$ ) was found between the Goldfish and GC surface lipid method when analyzed across the FGIS standard samples, U.S. cultivars, and international cultivars (Table III). Across all samples studied, the mean Goldfish SLC (0.45%) was greater than that found using the GC method (0.36%). The two extraction solvents used for these methods, iso-octane and petroleum ether, have the same polarity index (0.1); thus solubility differences were not likely to be the cause of the different results. It is more likely that the different results are due to the crude measure of lipids using the Goldfish method compared with the GC. That is, the values obtained using the Goldfish method would include nonsaponifiable materials such as sterol esters. Although some of these compounds are transesterified in the process of FAME production, they were not quantified in the method reported here (Christie 1993).

The GC procedure was also highly correlated ( $r = -0.84$ ) with Satake milling meter values for whiteness (Table II), but not with protein content ( $r = -0.38$ ). Goldfish surface lipid values and Satake milling meter values were also not highly correlated with protein content. These results then indicate that the GC method, Goldfish procedure, and Satake milling meter were correlated with each other. Of these methods, the Satake milling meter method is the most repeatable and requires the least amount of time. However, as mentioned previously, its use is limited by the large sample size required and by the error than can result when samples contain chalky or inherently dark-colored kernels. In comparison, the gas chromatography procedure requires less sample for analysis than the continuous solvent extraction method and the Satake milling meter.

TABLE II  
Degree of Milling of USDA GIPSA FGIS Standard Rice Samples

Sample	GC Surface Lipids (%) <sup>a</sup>	Goldfish Surface Lipids (%)	Protein (%)	Milling Meter Whiteness
Long grain				
Hard milled	0.511 ± 0.009	0.480 ± 0.026	7.667 ± 0.158	39.3 ± 0.265
Well milled	0.584 ± 0.042	0.683 ± 0.006	7.893 ± 0.051	36.9 ± 0.346
Reasonably well milled	1.030 ± 0.056	1.367 ± 0.025	7.830 ± 0.035	27.3 ± 0.231
Medium grain				
Hard milled	0.335 ± 0.004	0.360 ± 0.026	7.830 ± 0.214	35.3 ± 0.416
Well milled	0.379 ± 0.004	0.490 ± 0.017	7.933 ± 0.072	34.9 ± 0.709
Reasonably well milled	0.902 ± 0.003	0.930 ± 0.017	8.463 ± 0.012	27.0 ± 0.300
Short grain				
Hard milled	0.360 ± 0.011	0.453 ± 0.034	6.400 ± 0.100	38.5 ± 0.321
Well milled	0.377 ± 0.019	0.496 ± 0.069	6.267 ± 0.115	36.3 ± 0.100
Reasonably well milled	0.445 ± 0.003	0.656 ± 0.004	6.600 ± 0.100	31.5 ± 0.289
Relative error (%) <sup>b</sup>	2.64	4.79	1.32	0.98

<sup>a</sup> Means and standard deviations based on two replicate analyses for all measurements.

<sup>b</sup> Mean coefficient of variation of individual samples; lower values denote better reproducibility.

TABLE III  
Correlation Coefficients Between Different Measures of Rice Milling Degree<sup>a</sup>

	GC Surface Lipids	Goldfish Surface Lipids	Milling Meter Whiteness	Protein
GC surface lipids	1.000			
Goldfish surface lipids	0.935	1.000		
Milling meter whiteness	-0.854	-0.756	1.000	
Protein	0.377	0.418	-0.401	1.000

<sup>a</sup> Pearson Product Moment correlation coefficients significant at  $P \leq 0.05$ .

With 2 hr of technician time, this GC method can be used to analyze  $\approx 50$  samples in 24 hr. This number could be increased if more than one GC instrument were available for use by one technician. Throughput could also be increased if ultra-fast GC techniques were used. But for this, additional research evaluating the use of a short narrow-bore capillary column, high carrier gas velocities, and rapid temperature programming would be required.

## CONCLUSIONS

The GC method for rice SLC was highly correlated with the commonly used continuous solvent extraction procedure and it demonstrated greater repeatability. Thus, the GC method will result in values similar to those obtained using a conventional method. In addition, the GC method offers several advantages compared with the older method. Specifically, it requires less organic solvent, technician time, and sample size.

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