Distribution of Total Aerobic and Coliform Bacterial Counts Among Rice Kernel Components

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ABSTRACT

Nine lots of long-grain rice were milled, and the basic rice components (hulls, bran, brokens, and head rice) were segregated. Serial dilutions of ground samples were conducted to determine aerobic plate counts (APC) and coliform counts. Significant differences ($p<0.05$) in bacterial counts were found between the components for both APCs and coliform counts. The mean APCs in the basic rice components were ranked in the following order from the highest counts to lowest: hulls, bran, brokens, and head rice. Additionally, the mean coliform counts in the rice components were ranked in the following order from the highest counts to lowest: bran, hulls, brokens, and head rice.

Key Words: rice, grain, milling, microbiology, bacteria

INTRODUCTION

THERE IS AN INCREASING DEMAND THAT RICE processors meet microbial specifications for aerobic plate counts (APC) (e.g., <10,000 CFU/g) and for coliform counts (e.g., <100 CFU/g) in rice flour. Anecdotal evidence shared with the authors has indicated a consistent annual trend, in which bacterial counts in rice flour are often outside the microbial specification from harvest to late spring (September through April), but are within the microbial specification during the summer months. One hypothesis for the cause of this trend might be that colder weather inhibits adequate separation of bran from milled rice products in the process stream. An additional hypothesis might be that the drop in bacterial counts during the summer months results from bacterial senescence caused by stress in the low water activity environment of dry rice storage.

The processing of rough rice to rice flour consists of four main operations: hulling, milling, separation, and flouring. In general terms, the rough rice kernel has three primary parts: the hull (outside shell), the bran (lipid-rich layers under the hull), and the endosperm. During the hulling operation, the hull is removed from the rough rice kernel, resulting in two distinct products, hulls and brown rice. Brown rice consists of the endosperm and the bran layers. The brown rice is then milled in abrasive or friction type milling systems to remove the bran layers from the endosperm. The milling process also results in two distinct products, bran and endosperm (white rice). White rice is further separated into head rice and broken kernels (i.e., brokens). Head rice is defined as a milled rice kernel that is three-quarters or more of the original length of the whole kernel (USDA, 1979). A percentage of the brokens, depending on market demand, is then further processed into flour by hammer or roller type flour mills. Overall, the milling of rough rice therefore results in four basic rice components (hulls, bran, brokens, and head rice) and involves three intermediate or combination components (rough rice, brown rice, and white rice).

Rice flour is processed from milled white rice brokens, which can typically be 15% of the initial rough rice weight. Rice flour has been used in pancake and waffle mixes, in baby foods as a cereal and as a thickening agent, in extruded products as an ingredient, and in refrigerated biscuit dough as a dusting or anticaking agent (James and McCaskill, 1983). Because rice flour is typically used as an ingredient in value-added food products, both rice processors and end-users are increasingly concerned with the microbial counts in this ingredient.

Previous research directed at cereal microbiology has focused primarily on control of the microflora, mainly fungi, during storage (e.g. Cahagnier et al., 1993; Magan and Lacy, 1988). It is fairly well known that the main factor contributing to spoilage of high moisture content (MC) rice is fungi developing from spores inherent in the rice production and harvesting systems. Drying of rough rice below the MC needed for fungi to grow (i.e., ~ 13% wet basis) is the most effective and widely used method to preserve the microbial quality of rice. However, little research has focused on other components of the microflora (e.g., bacteria) within stored, low-MC cereal grains.

Ueda and Kuwabara (1988) reported on levels of *Bacillus cereus*, the leading cause of bacterial food poisoning associated with rice (Beuchat, 1978), and the APCs for rough rice, hulls, bran, brown rice, and white rice throughout the milling process. They showed significant differences in the APCs between rough rice and white rice as well as between brown and white rice. Currently, rice processors are interested in reducing the APCs and coliform counts in milled rice, specifically milled brokens, which are processed into rice flour. However, previously reported research has not included coliform counts, nor has it determined the bacterial counts specifically in brokens.

Our specific objective was to quantify the distribution of both APC and coliform counts among the basic rice components (hulls, bran, brokens, and head rice) derived from the milling of long-grain rice, and among the intermediate products representing steps in the milling process: rough rice (entire kernel), brown rice (bran and endosperm), and white rice (head rice and brokens).

MATERIALS & METHODS

NINE 23 KG LOTS OF LONG-GRAIN RICE WERE randomly sampled from incoming trucks at a commercial rice processing facility in Mississippi starting March 18, 1996 and ending April 5, 1996 (3 trucks/wk for 3 wk). Two cultivars of rice were tested; lots 5 and 7 were cv. Cypress, and the remaining lots were cv. Lemont. All 9 lots were commercially dried and stored from harvest (September, 1995) until shipment to the processing facility in March and April. The rice lots were shipped each week to the Rice Processing Laboratory at the Univ. of Arkansas and stored at 4°C for up to 3 wk prior to testing. Each lot was allowed to equilibrate to room temperature for 24h before separation into 150g sub-samples via a No. 34 Boerner divider (Seedburo Co., Chicago, IL).

Oven MC, head rice yield (HRY), degree of milling (DOM), and water activity (a_w) were determined for each lot. The oven tests were conducted in duplicate by drying 20-25g of rice at 130°C for 24h (Jindal and Siebenmorgen, 1987). The HRYs for each lot were determined by hulling and milling one of the 150g sub-samples of rough rice in a laboratory huller (Satake Rice Machine; Satake Inc., Houston, TX) and a McGill No. 2 laboratory mill. During milling, a 1500g weight was placed on the lever arm of the McGill No. 2, 15 cm from the center of the milling chamber. The milling time was 30s. A Seedbouro shaker-sizer (4.76 mm holes) was used to separate

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the broken kernels from the head rice. HRY is the weight basis percentage of rough rice that remained as head rice after the milling process. The DOMs were measured by a Satake Milling Meter (model MM-1B, Satake Inc., Houston, TX). This meter determines a numeric value between 0 (for brown rice) and 200 (for extremely well-milled white rice), based on the transmittance and reflectance properties of a 50g sample. Water activities were measured at 25°C in a water activity meter (Rotronic Hygroskop DT, Rotronic Instrument Corp., Huntington, NY), which was calibrated with a saturated solution of NaCl, which had a corresponding a_w of 0.753 at 25°C (Rahman, 1995).

To generate the materials for the microbial assays, two 150g sub-samples from each lot were hulled and separated into head rice and brokens as described. After hulling, a 15-20g sample of hulls and a 10-15g sample of brown rice were placed in sterile bags for microbial assays. During milling, all of the bran was collected in a sterile bag that was attached to the cover plate of the McGill #2 mill. After milling, a 10-15g sample of white rice was placed in a sterile bag. After separating the brokens from the head rice, a 10-15g sample of head rice and a 10-15g sample of brokens were placed in sterile bags for the microbial assays. The rough rice samples (30 g) used for the microbial assays were divided from the remaining sub-sample. Between millings, the surfaces of the milling equipment and trays were sanitized by rinsing and wiping with 70% ethanol to minimize any cross contamination between lots.

After milling and sample collection, 10g of each component sample were mixed with 90 mL of Butterfield phosphate buffer (Vanderzant and Splittstoesser, 1992) and processed in a blender (Osterizer model 869-18; Sunbeam-Oster Co., Inc., Wood-Ridge, NJ) at the highest speed for 30s. Successive dilutions were made by transferring 10 mL of the suspension medium to 90 mL of Butterfield phosphate buffer. Because the microbial populations were initially unknown, the components were plated in duplicate using dilutions from 10^{-1} to 10^{-6} on Petri-Film™ (3M, Minneapolis, MN) specific for APC and coliform determinations. Plated samples were incubated at 37°C; the coliform films were counted at 24h, and APC films were counted at 48h.

In a separate set of tests, surface disinfected (Tuite, 1986) brokens were plated for APC and coliform counts. The brokens from lots 1-3 were mixed together, and sub-samples were subjected to a 30s wash at room temperature in either water or a 5%, 2.5%, or 1% NaOCl solution. After the samples were treated, they were plated in duplicate on Petri-Film specifically for coliform counts and APC determinations, as mentioned. An untreated control sample was plated without a rinse treatment.

The statistical analyses of the resulting data were conducted via SAS/STAT™ (SAS Institute, Inc., Cary, NC). The effects of rice component, MC, HRY, DOM, and a_w on APC and coliform counts were evaluated by analysis of variance for a five-way classification. Duncan's multiple range test was used to determine any significant differences among the kernel components for both APC and coliform counts.

RESULTS & DISCUSSION

THE MC, HRY, DOM, AND A_W FOR EACH LOT were compared (Table 1). The 9 lots had typical characteristics for incoming rice at a commercial rice processing facility. Rice component was the only significant variable related ($p<0.05$) to either APCs or coliform counts. The R^2 values for the analysis of variance were 0.84 and 0.61 for APC and coliform counts, respectively.

Distribution of the means, and corresponding standard deviations, for APC (Fig. 1) and coliform counts (Fig. 2) for each of the basic and combined components were compared. The mean APCs and coliform counts for the rough rice and the brown rice were greater ($p<0.05$) than the counts for the white rice. These results confirmed the report of Ueda and Kuwabara (1988), which showed a reduction in bacterial counts with milling from rough rice to brown rice to white rice. Regarding the basic components, the mean APC for hulls was greater ($p<0.05$) than that in the bran, which was greater ($p<0.05$) than that in the brokens; the mean APC for the brokens was greater ($p<0.05$) than that in the head rice. Additionally, the mean coliform counts in the bran was greater ($p<0.05$) than those in the brokens, head rice, and white rice.

Because the bran had more coliforms and APCs than did the milled rice components (brokens, head rice, and white rice), better separation of the bran, particularly from brokens, should result in less contaminated rice flour. However, even with the careful separation of the bran from brokens in the laboratory milling treatments, none of the nine lots would have satisfied an APC specification of <10,000 CFU/g, and only seven of the lots would have satisfied a coliform specification of <100 CFU/g.

Results from the assays of the surface dis-

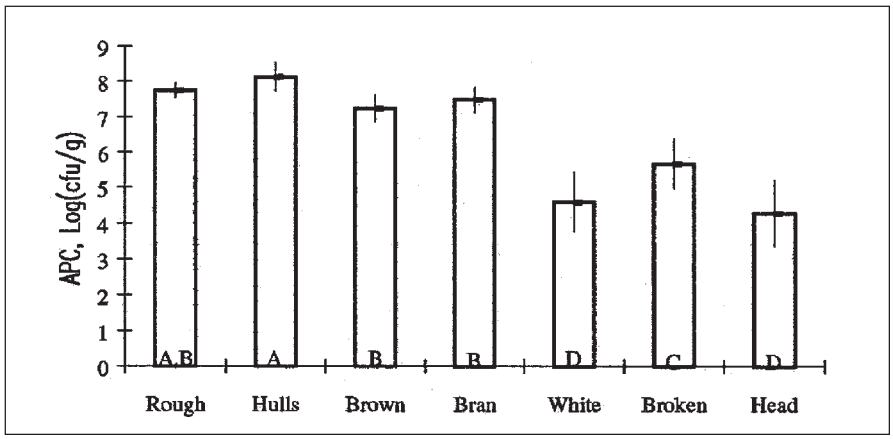

Fig. 1—Distribution of aerobic plate counts (APC) among milled rice components. Bars show ± standard deviation (n=9, with duplicate platings). Means with same letters not significantly different at $\alpha = 0.05$.

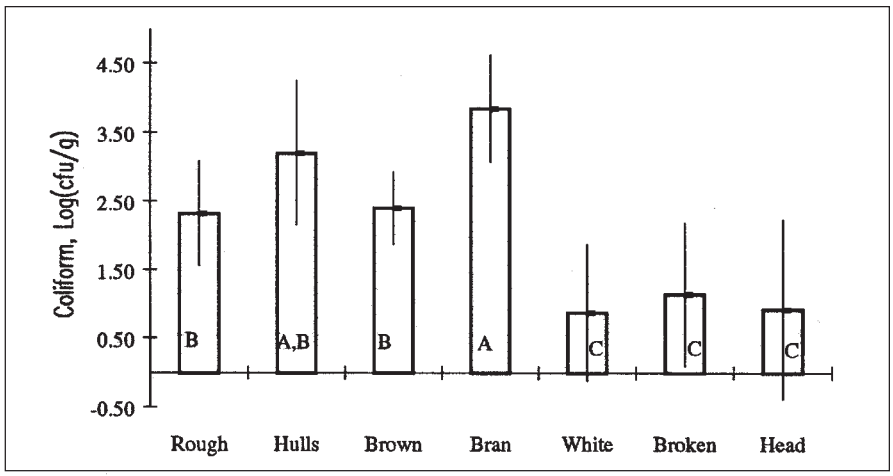

Fig. 2—Distribution of coliform counts among milled rice components. Bars show ± standard deviation (n=9, with duplicate platings). Means with same letters not significantly different at $\alpha = 0.05$.

Table 1—Some physicochemical properties for each lot of long grain rice

Lot #	Moisture content [a] (% w.b.)	Head rice yield (%)	Degree of Milling	a_w at 25°C
1	13.5	58.5	99	0.628
2	12.5	53.5	97	0.618
3	14.9	52.1	108	0.760
4	13.5	55.3	102	0.628
5	13.7	48.9	97	0.606
6	13.3	53.5	106	0.641
7	11.1	58.7	84	0.403
8	12.1	49.7	103	0.512
9	12.2	53.3	97	0.547

[a] Means of two determinations. (standard deviations < 0.06 for all lots).

Table 2—Effects of chlorine rinses on total aerobic plate counts (APC) and coliform counts of broken rice from lots 1–3 (Table 1)

Sample treatment	APC log (CFU)/g)	Coliforms log (CFU/g)
Control	5.85(0.03)	2.11(0.07)
30s wash w/H_2O	5.87(0.02)	<1.0 (0.00)[a]
30s wash w/1% NaClO	4.90(0.01)	<1.0 (0.00)[a]
30s wash w/2.5% NaClO	5.46(0.17)	<1.0 (0.00)[a]
30s wash w/5% NaClO	5.57(0.02)	<1.0 (0.00)[a]

[a]No viable colonies on 10^{-1} dilution. Counts are means of two replicates; standard deviations are given in parentheses.

infected brokens were compared (Table 2). If the bacterial population was entirely on the surface of the brokens, the mean APCs of the samples rinsed in NaOCl solutions should approach zero. Although the mean APCs were lower ($p<0.05$) than those from the control or water-rinsed samples, the counts did not decrease below <10,000 CFU/g. This implies that a major portion of the aerobic bacteria were probably present inside the kernel structure, and therefore were not affected by the surface rinse. With respect to coliforms, both the water and NaOCl washes resulted in decreases ($p<0.05$)in counts; after the washes, no viable colonies were detected at 10^{-1} dilutions. A population inside the kernels appeared to exist (by APCs), which could help guide possible treatments that rice processors might investigate to control bacterial counts in rice flour. In contrast, these limited data indicate that the coliform populations may be primarily on the surface of the kernels.

Results seem to indicate that viable bacteria were present throughout the entire rice kernel, even within the endosperm itself. This is important, because rice flour is predominantly milled from brokens, for value-added consumer food products. In order to ensure that the market for broken rice continues to expand, and the value of broken rice subsequently increases, processors need to minimize bacterial counts, to satisfy end- user requirements.

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