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Note

Decontamination Effect of Milling by a Jet Mill on Bacteria in Rice Flour

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The decontamination effect of milling by a jet mill was investigated by counting the number of bacteria in brown and white rice flour with mean particle diameters of 3, 20, and 40 μm prepared by the jet mill. In the jet mill, the particles are crushed and reduced in size by the mechanical impact caused by their collision. Although the brown and white rice grains were contaminated with approximately 10^6 and 10^5 CFU/g bacteria, the microbial load of the rice flour decreased as the mean particle diameter decreased, ultimately decreasing to approximately 10^4 and 10^3 CFU/g in the brown and white rice flour. The temperature and pressure changes of the sample were not considered to have an effect on reducing the bacterial count during the milling. Hence, it was thought that the rice flour was decontaminated by other effects.

Key words : Food powder/Particle size/Polished rice/Unpolished rice.

Disinfection of food powder is problematic since the quality of most kinds of food powder is seriously affected by heat (Kawashima, 1981). Radiation is a promising method for disinfecting food without significant quality deterioration; however, in Japan, the use of radiation for food processing is prohibited except for the inhibition of sprouting in potatoes (Hayashi, 1998). Hence, the food industry is still in need of an effective non-thermal disinfection method for food powder.

A jet mill is a milling machine that reduces the particle size by applying an extremely strong impact to the material with a high-speed current of air or gas, enabling the milled food powder particles to have a mean diameter of less than 20 μm (Hayakawa et al., 1996; Jarrard and Hung, 2007). Decreasing the particle size of food powder particles to a size as small as that of bacteria by using this kind of milling machine is expected to affect the survival of the bacteria due to physical effects on the bacteria that presumably accompany the production of such small flour particles. This concept of disinfection by milling was previously presented by Horiuchi (2004), who reported that the number of bacteria in the powder of the fruit body of *Agaricus blazei* Murril decreased from 10^5 CFU/g to 10^2 CFU/g when the mean diameter of the powder particles was decreased to 10.96 μm. Recent jet mills are expected to yield more effective decontamination of food powder since they are able to produce fine food powder particles with a mean diameter of less than 5 μm. However, it is quite difficult to find published data discussing the relation between particle size and the bacterial load of the food powder after milling. More data on particle size and the disinfection effect should be acquired to investigate the potential of the milling disinfection method.

Rice flour is used as an ingredient in an increasing number of food products including bread, confectionaries, beverages, and salad dressing (Kadan et al., 2008; Yoza et al., 2008). However, rice flour is frequently contaminated with bacteria, as are many kinds of food powder (Hayashi, 1998). If a fine milling process inactivates the bacteria in rice flour, it is expected that less contaminated rice flour of higher quality will be produced by improving the jet mill to

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reduce the flour particle size. In this study, rice flour with a mean particle diameter from 3 to 40 μm was prepared using a jet mill. The decontamination effect of the mean particle diameter by milling was subsequently investigated.

Brown rice (*Oryza sativa* L. cv. Koshihikari) harvested in Ibaraki Prefecture, Japan, in 2007 was purchased from a local rice distributor and stored at 5 ℃ before the experiments. White rice was obtained by polishing the purchased brown rice grains down to 90% weight. Preliminary experiments presented that the concentration of naturally contaminating bacterial cells in the rice was constant within a narrow range of 10^5-10^6 CFU/g. The decontamination of rice grains from the natural contaminants by the jet mill was consequently investigated in this study.

Figure 1 schematically depicts the jet mill (IDS-2, Nippon Pneumatic Mfg. Co., Ltd.) used in this study. This system generates a supersonic air current by blowing pressurized air supplied by a compressor through a supersonic nozzle. The ingredient particles injected into the air jet stream are accelerated and crushed by collision with an impact plate and with other particles. Particles are input from a feeder, and the crushed particles are sent to a classifier. The classifier separates the fine particles from the large ones, sending the fine particles to a cyclone separator by an air current. Finally, the fine particles are captured from the air current and sent to a sample vessel by the cyclone separator. The large particles are returned to the supersonic air jet stream from the classifier and pulverized again. The particles circulate inside the jet mill until they have been crushed to a size small enough for separation by the classifier.

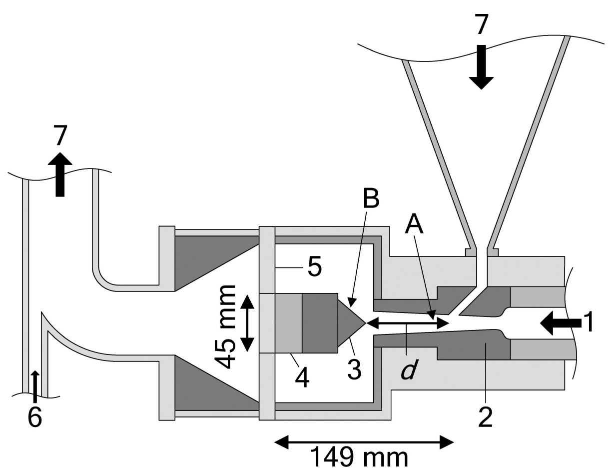

FIG. 1. Schematic drawing of the jet mill. 1, Compressor; 2, Supersonic nozzle; 3, Impact plate; 4, Adjustment ring for the impact plate; 5, Impact plate holder; 6, Feeder; 7, Classifier.

Table 1 presents the milling conditions of the jet mill for preparing rice flour particles with mean diameters of 3, 20, and 40 μm. The produced particle size usually decreases when the nozzle stagnation pressure (i.e., pressure upstream from the nozzle throat) is increased, the distance from the injection point to the impact plate (*d* in Fig. 1) is decreased, or the feed rate is decreased. To mill 40μm-flour, the impact plate was not attached to the jet mill. In this condition, the rice particles were crushed by collision with other particles and with the impact plate holders. The settings of the classifier were adjusted for each milling condition. The temperatures at 10 mm downstream from the injection point (Position A in Fig. 1) and at the surface of the impact plate or the impact plate holder (Position B in Fig. 1) were measured by a thermocouple for each condition. 1000 g of rice grains was milled for each condition.

The jet mill was disassembled and all the parts of the mill were cleaned by an air jet cleaner before the experiments; however it was difficult to sterilize all the parts of jet mill due to their sizes and materials. Hence cross contamination between the samples was presumably caused through the mill structure. In this study, the cross contaminants in the rice flour were evaluated by milling sterilized rice grain as follows. First, the jet mill was disassembled and cleaned by an air jet cleaner after milling the white rice to 20μm-flour particles. Second, the white rice sterilized by 25 kGy gamma-ray irradiation was milled with the jet mill to 20μm-flour particles. Finally, the cross contaminants were evaluated by counting the microbial load of the rice flour.

The size distribution of the rice flour particles was determined by a laser diffraction particle analyzer (SALD-2100, Shimadzu Corporation). Mass median

TABLE 1. Milling conditions.

Target mean diameter (μm)	Nozzle stagnation pressure (MPa)	Distance to the impact plate *d* (mm)	Feed rate (kg/h)
3	0.65	62	6
20	0.42	92	12
40	0.30	–[a]	12

[a]The impact plate was not used for milling the 40μm-flour.

TABLE 2. Temperature and collected amounts of sample mass.

Target mean diameter (μm)	Temperature (℃)		Collected sample mass (g)	
	Position A	Position B	White	Brown
3	0.7	36.0	836	781
20	1.7	23.6	886	794
40	3.5	15.4	867	804

diameter D_{50} of the flour was calculated from the particle size distribution curves obtained with the particle analyzer. The mass median diameter D_{50} is the diameter that 50% of the particles by mass in the flour are smaller than and the other 50% of the particles are larger.

The population of bacteria was determined by the direct-plating method. Rice flour homogenized in 0.1% peptone water was serially diluted in 0.1% peptone water. The sample solution was surface-plated in duplicate on plate-count agar (Merck). The number of colonies was subsequently counted after incubating the plate at 37℃ for 48 hours.

The air jet temperatures inside the mill at each experimental condition are presented in Table 2. The average air temperature at 10 mm from the nozzle injection point decreased after the pressure of the air supplied by the compressor decreased to almost atmospheric pressure by adiabatic expansion inside the nozzle diffuser. The average air temperature increased at the impact plate surface by comparison with the air temperature inside the diffuser. It was because the high velocity air current was compressed by collision with the impact plate and the kinetic energy of the air current was converted into thermal energy by the formation of a boundary layer on the surface of impact plate.

The amounts of sample mass collected in the vessel under each condition are presented in Table 2. The collected sample mass was smaller than the sample mass supplied to the mill due to the moisture evaporation from the sample (40～50 g/batch) and sample loss. Some fraction of the lost sample remained inside the jet mill adhering on the inner surface of the mill, and another fraction of the lost sample was exhausted outside the mill. The yields of brown rice flour were lower than that of white rice. More of the brown rice flour presumably remained inside the mill since such flour contains bran oil and is more adhesive. The yield tended to decrease as the mean diameter of the flour particle decreased. It was because the flour became cohesive and adhesive as the particle size decreased (Fitzpatric et al., 2004; Sotome et al., 2009). In addition, the separative power of the cyclone separator generally decreases as the particle size of the flour decreases. Hence, the sample fraction that was not captured by the cyclone separator and exhausted outside of the mill presumably increased as the particle size decreased.

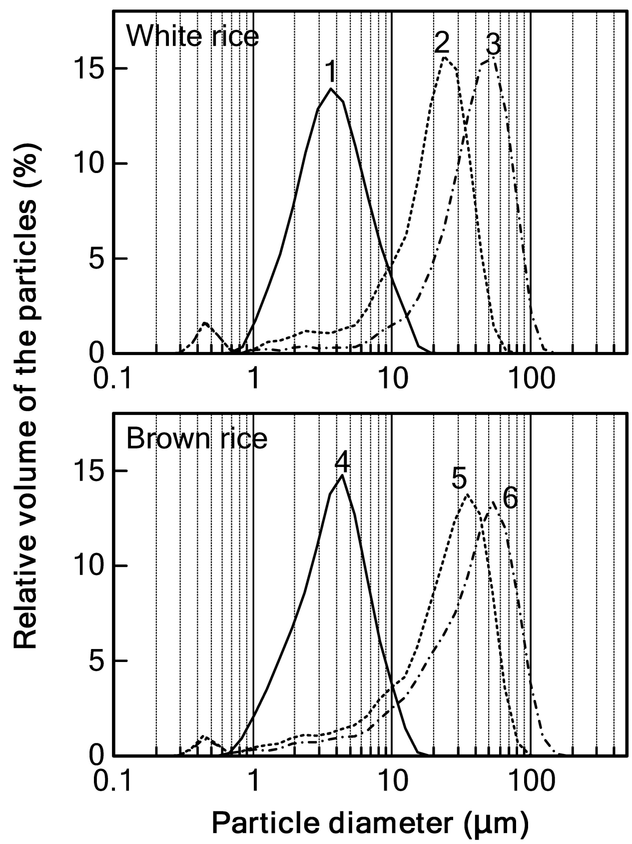

FIG. 2. Particle size distribution of the rice flour. The mass median diameter of the flour: (1) 3.3 μm; (2) 18.6 μm; (3) 37.2 μm; (4) 3.5 μm; (5) 22.1 μm; (6) 35.9 μm.

Figure 2 presents the particle size distribution of rice flour. In all samples, a narrow particle distribution with a single peak was observed. The mass median diameters of all of the samples were within the range of the target mean diameter ± 20%. In the 20 μm and 40 μm samples, a few particles with diameters much smaller than the mean diameter were found. These small particles were mainly produced when the edges of relatively large particles were removed by successive collisions with other particles. In contrast, very few 3 μm-sample particles had a diameter much smaller than the mean diameter. This was presumably because the smaller particles produced in milling the 3 μm-samples were not captured by the cyclone separator.

Figure 3 presents the changes in bacterial count in the rice flour caused by milling. The bacterial counts in the rice flour decreased as the mean particle diameter decreased. The bacterial count for the white rice flour was smaller than that for the brown rice flour. This was presumably because most of the bacteria contaminating the brown rice existed on the grain surfaces; hence polishing removed some of the bacteria. The microbial load of the flour prepared from the irradiated rice was 22 ± 12 CFU/g ($n = 5$), though microorganisms were not detected from the irradiated rice grains. Hence, it was considered that the bacteria counts of the flour presented in Fig. 3 contained 1～2 log CFU/g cross contaminants.

It was supposed that the bacterial counts in the rice flour decreased as the flour particle size decreased since the bacteria in the flour were influenced by some physical effects during the milling. The temperature of the sample changed during the milling since there was a temperature change in the air current inside the mill caused by compression and expansion. However, the bacteria in the rice flour samples were not considered to have been affected by the heat during milling since the increases in air temperature inside the mill, which were highest on the surface of the impact plate (Table 2), were not high

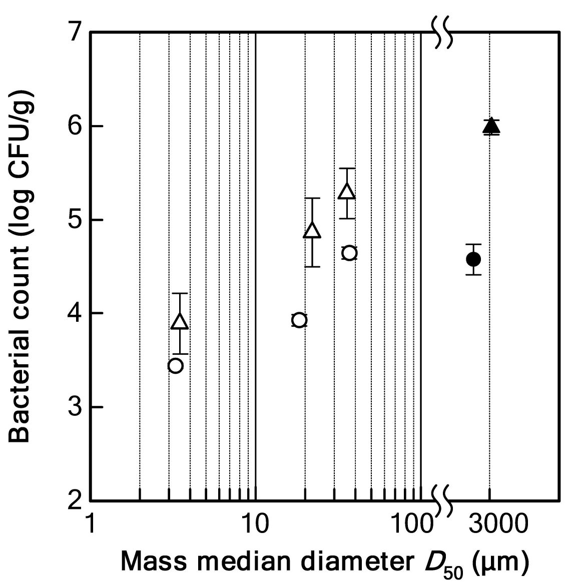

FIG. 3. Bacterial counts of the rice grains (●, white; ▲, brown) and the rice flour (○, white; △, brown). The mean diameter of the rice grains was defined as the mean diameter of the voluminal equivalent sphere of the grains. Bars represent the standard error of the means ($n = 3$).

enough for bacterial inactivation.

The pressure change of the air current was another physical effect on the sample. In the jet mill used in this study, the location where the sample was most pressurized was the surface of impact plate. Based on some assumptions, the air pressure on the impact plate surface P_B was predicted as follows. First, the ideal adiabatic compression of the air current was assumed on the impact plate surface. Second, the air pressure at position A in Fig. 1 (P_A) was assumed to be atmospheric (0.101 MPa), though the P_A actually should be lower than the atmospheric pressure since the air pressure at the injection point (i.e. upstream from the position A) was almost atmospheric pressure. The P_B under the 3 μm-flour milling condition was calculated from the temperatures presented in Table 2 as

$$P_B = \left(\frac{T_A}{T_B}\right)^{\frac{\gamma}{1-\gamma}} P_A = \left(\frac{273.15+0.7}{273.15+36.0}\right)^{\frac{1.403}{1-1.403}} 0.101 = 0.144\,[\mathrm{MPa}] \qquad (1)$$

where γ is the specific heat ratio of air. On the other hand, it has been noted in the literature that a hydrostatic pressure greater than 200 or 300 MPa was required for the inactivation of several species of microorganisms (Koseki and Yamamoto, 2006; O'Reilly et al., 2000; Patterson et al., 1995). The predicted pressure P_B was much lower than these values and not high enough for bacterial inactivation.

Destruction of the bacteria cells was thought to be one of the possible factors of the decease in microbial load of the flour. When the rice particles were processed by the milling, there was a probability that the bacteria cells were destroyed at the same time. There is an extremely low probability of this if the flour particle size is remarkably larger than the bacteria cell size, but the probability increases as the flour particle size decreases to become comparable with the bacterial cell size. In this study, however, the microbial load of flour already had decreased when the mean diameter of the rice particles was approximately 20 or 40 μm, at which most of the rice particles were larger than the size of common bacteria (0.5～5 μm). This result suggested that an effect other than cell destruction was involved in the decontamination of rice flour during the milling.

Separation of bacteria cells from the rice particles was another possible reason for the decrease in the microbial load of the flour by the milling. It was supposed that some bacteria cells were detached from the rice particle when the particles were processed by the milling. This probability would also increase as the rice particles are milled into smaller sizes. Since the size of bacteria cell was presumably smaller than the particle size of most fractions of rice flour (Fig. 2), it was possible that the remaining and exhausted fractions of the bacteria cells detached from the rice particle were larger than that of the rice particles. In the case of brown rice, it was supposed that the adhesion of bran, in which the microbial load is higher than the rice core, on the inner surfaces of the mill contributed more to the decrease in microbial load because the microbial load of the 40μm-flour already decreased while the microbial load of 40μm-particles from the white rice flour was almost same level with that of white rice grains (Fig.3).

The results obtained in this study suggested that milling by the jet mill tended to remove the bacteria in the rice flour. However, the decontamination effect was smaller than the result presented by Horiuchi (2004), even though the mean particle diameter of the powder prepared by Horiuchi exceeded the mean diameter of the finest rice flour particles prepared in this study. This was presumably because the decontamination effect of the jet mill was affected by the physical properties of the powder ingredient as well as the size and morphology of the contaminating bacteria. Therefore, it will be necessary in future studies to clarify in more detail the relation between the particle size and the decontamination effect for each food material and each bacterial species to determine the full potential use of milling for disinfection.

In addition, it is supposed that the quality of fine food powder may change easily because the oxidation of the powder as well as the evaporation of some kind of components will be accelerated due to the large specific surface area. It is also supposed that some of the bacteria cells damaged by the milling become viable but non-cultural (VNC). For the development of a practical disinfection technology by milling, the research from various points of view is required to solve these problems.

Nevertheless, it can be concluded that the current jet milling technology is already practically useful since the reduction of the microbial load to 1/10～1/100 and the production of the flour (i.e original purpose of the milling) are simultaneously achieved.

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