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Note

Inactivation of *Bacillus cereus* Spores during Rice Cooking

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The *D*-values at 80, 90 and 100°C of *Bacillus cereus* spores (Philippine strain 1061) in 2% broth of Philippine rice cultivar PSB Rc72H were 38, 12 and 5 min, respectively. The *Z*-value of the test spore was 20°C. The mathematically calculated lethality for the *Bacillus* spores in rice cooked at 100°C was established to be 25 min, which could be adequately attained during normal rice cooking times of ≥25 min by boiling.

Keywords: heat resistance, *B. cereus* spores, lethality, cooking time

Bacillus cereus has been reported to be part of the normal microflora of raw rice (Chung & Sun, 1986). The organism is considered ubiquitous to nearly all plant foods and its dehydrated counterparts. Generally, the reported cases of raw rice contamination of *B. cereus* were minimal, however, pending concerns still prevail considering that the endospores of the organism were reported to survive normal cooking procedures of rice (Gilbert *et al.*, 1974; Hobbs & Roberts, 1993). The organism in rice has been implicated with emetic food poisoning outbreaks. The symptoms of human intoxication include nausea, vomiting, and malaise similar to *Staphylococcal* poisoning starting 1–5 h after ingestion of contaminated food (Kramer & Gilbert, 1989; Notermans & Batt, 1998).

Surviving heat resistant spores have been reported to germinate and produce emetic toxins in cooked rice when stored for a long time under warm conditions (15–50°C) (Kramer & Gilbert, 1989; Nichols *et al.*, 1999). This phenomenon has grave implications on how cooked rice is handled in households and food service institutions in the country. The practice of preparing large amount of cooked rice in advance and storing it for long periods (≥2 h) at ambient condition would favor the growth of *B. cereus* spores and induce the risk of food poisoning outbreaks (Kramer & Gilbert, 1989). In the advent that the Philippine government is pushing for the cultivation and use of Philippine Seedboard Rice cultivars more specifically PSB Rc72H (*Mestizo*), concern with regards to the potential hazard of the organism in cooked form of the hybrid rice arises.

The objective of the study was to evaluate the efficacy of boiling as a rice cooking process in inactivating *B. cereus* spores (Philippine strain 1061) in Philippine rice cultivar PSB Rc72H. The heat resistance parameters of *B. cereus* spores in 2% rice broth and the heat penetration data were used in the mathematical calculation of the cooking schedule for cooked rice.

Materials and Methods

Bacillus cereus spores *Bacillus cereus* (Philippine strain 1061) was obtained from the culture collection of the Natural Sciences Research Institute (NSRI), University of the Philippines, Diliman, Philippines. The organism was locally isolated from the Dampalit River, Malabon, Philippines. Working cultures grown on Nutrient Agar (NA) slants were maintained at 37°C for a period of 1 mo. The propagation of *B. cereus* spores was based on the method of Johnson *et al.* (1982). Aliquots (0.1 ml) of 18–24 h cultures of *B. cereus* grown in nutrient broth (NB) at 37°C were spread plated on the surface of prepoured plates with NA medium. The inoculated NA plates were initially incubated in an upright position at 37°C for 24 h and then inverted for an additional 24 h. The harvesting of spores was done by flooding the surface of the incubated NA plates with 5 ml cold (approximately 15°C) sterile 0.1% peptone (BBL, USA) and the surface was scraped with a sterile bent glass rod. The harvested spores suspended in 0.1% peptone were centrifuged at least 8× for 15 min at 2,000 rpm. Between each centrifugation, the supernatant was discarded and the pellets were resuspended in 100 ml cold sterile 0.1% peptone. The final pellets were stored in 100 ml sterile 0.1% peptone at 4±2°C.

The Breed's Smear Method for Direct Microscopic Counts (Harrigan & McCance, 1964) was used in the quantification of *B. cereus* spores. Briefly, the method was used by counting spores in 20 microscopic fields in a heat-fixed smear of known volume and area. The smear was stained with malachite green and viewed using 400× magnification under a light microscope (Zeiss Axioskop II, Germany). The number of spores per ml in the original sample was determined as the product of the following parameters: mean number of spores in 20 microscopic fields, area of smear over area of microscopic field of view and unit of volume for reported spore count over the volume of spore suspension.

Preparation of rice broth The rice cultivar PSB Rc72H (*Mestizo* hybrid) was obtained from the 2001 wet season harvest of Philippine Rice Research Institute (PhilRice) Maligaya in Muñoz, Nueva Ecija, Philippines. The preparation of rice broth was based on the method utilized by Chung and Sun (1986). Twenty grams of raw milled rice were suspended in

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1,000 ml distilled water and then autoclaved at 121°C for 15 min. After sterilization, the suspension was filtered using Whatman filter paper No. 1. The filtrate was dispensed in 9-ml portions into 10-ml screw-capped test tubes and autoclaved again at 121°C for 15 min.

Heat resistance studies Heat resistance was determined using the test tube method described by Stumbo (1973). The experiments were carried out in 150×15 mm screw-capped test tubes containing 9-ml 2% rice broth. In each test tube, 1 ml of *B. cereus* spore suspension was inoculated to obtain 10^7 spores/ml. The inoculated test tubes were heated in a thermostatically controlled water bath (Memmert GmbH+, West Germany). Thermal resistance was determined at 80, 90 and 100°C. At predetermined intervals, 3 replicate tubes were removed and immersed in ice water bath. After cooling, 0.1 ml of appropriate dilutions were spread plated on pre-poured NA medium. The plates were incubated at 35°C for 24 h and the number of survivors was determined by counting colony-forming units on NA plates.

Survivor curves were constructed by plotting the logarithm of the number of survivors against the time of heating, on semi-log paper. The *D*-values of *B. cereus* spores in 2% rice broth were based on the time required for the survivor curves to traverse 1 logarithmic cycle (Jay, 1992; Mossel *et al.*, 1995). This *D*-value is numerically equal to the time required to effect 90% destruction on the treated bacterial population.

In the same way, thermal resistance curves were constructed by plotting the logarithm of *D*-values against the exposure temperature. The *Z*-value is the number of unit temperature required for the curve to traverse 1 logarithmic cycle (Stumbo, 1973; Frazier & Westhoff, 1988). It is numerically equal to the temperature increase required to decrease *D*-values by 90%.

Heat penetration profile of cooked Mestizo rice Heat penetration characteristics of cooked rice by boiling were determined using raw-milled PSB Rc7H (*Mestizo*) rice grains. The rice grains were washed twice with water 2× the volume of rice. Maintaining 1 : 2 rice to water ratio (w/v), rice was cooked over medium heat. A thermocouple was used to measure temperature at the heating lag point located at the center of the rice mixture. Temperature was recorded at 1-min intervals during the cooking process. The time-temperature profile of the cooked rice was recorded using the Honeywell Potentiometer (York, PA, USA).

The cooking lethality was calculated using Ball's Mathematical Method (Ball & Olson, 1957) and was evaluated against the required cooking time. The adequacy of the rice cooking process was determined by comparing the cooking lethality with the required cooking time for the reference organism (Alabastro, 1987).

Results and Discussion

Heat resistance studies The thermal resistance characteristics of an organism in a food system are defined by the *D*- and *Z*-values. The established *D*- and *Z*-values of *B. cereus* spores (Philippine strain 1061) in 2% rice broth using PSB Rc72H (*Mestizo*) are shown in Table 1. The $D_{90°C}$ and $D_{100°C}$ values of the spore in rice broth were 12 and 5 min, respectively. The *Z*-value of the test spore was 20°C. These calculated values were proximal to other reported thermal resistance values for *B. cereus* spores in rice broths and buffered systems. Chung & Sun (1986) established that the $D_{92°C}$ and $D_{100°C}$ values of 6 *B. cereus* spore isolates in rice broths ranged from 16–36 min and 4.2–6.5 min, respectively. Johnson *et al.* (1982) reported that the calculated $D_{95°C}$ values for several strains of *B. cereus* spores in 0.25 M phosphate buffer medium ranged from 1.2 to 20.2 min with *Z*-values ranging from 6.8 to 13.9°C. The *Z*-values indicate the temperature range necessary to bring about 10-fold change in the *D*-value.

Table 1. Heat resistance characteristics of *B. cereus* spores (Philippine Strain 1061) in 2% rice broth using PSB Rc72H (*Mestizo*) with a Z-value of 20°C.

Temperature (°C)	*D*-values (min)*			Mean*
	1	2	3	
80	38	38	39	38
90	11	15	10	12
100	5	4	5	5

*Values are expressed in whole numbers.

Table 2. Heating parameters and cooking schedule of cooked PSB Rc72H (*Mestizo*).

Parameter	Value***
B: Heating time (min)	28
Pt: Processing time (min)	26
CUT: Come-up-time (min)	4
f_h: *f* of heating curve (min)	9×10^{-1}
j_{ch}: lag factor for heating	1×10^{-1}
T_{ih}: Actual initial temperature (°C)	30
g_c: difference between cooking temperature and temperature of product	2×10^{-30}
Cooking Lethality* (min)	25
F: Required cooking time** (min)	25
Cooking lethality=*F*; cooking process is adequate	

*Based on a $D_{100°C}$ of 5 min and a *Z* value of 20°C. **Based on a 5D process at 100°C. ***Values are expressed in whole numbers.

It is fortunate that *B. cereus* spores are only moderately heat resistant (Jenson & Moir, 1997; Notermans & Batt, 1998) considering that rice cooking by boiling may only attain a temperature of about 100°C. Based on the calculated D-values, heating at 100°C for 5 min could attain 90% reduction of *B. cereus* spores. Normal *B. cereus* spore loads in raw rice has been reported to be in the range of <10 to a maximum of 10^5 CFU/g (Lee & Chang, 1980; Chung & Sun, 1986; Kamat *et al.*, 1989; Lee *et al.*, 1995). Even with a maximum spore load of 10^5 CFU/g, cooking times of ≥25 min would be adequate to attain a spore level of 10^0 CFU/g using $D_{100°C}$. The thermal resistance data of *B. cereus* spores in rice broth generated in this phase of the study were used in the proceeding mathematical calculation of the cooking schedule for ordinary rice cooked by boiling.

Process schedule Data on thermal resistance combined with heat penetration studies can be employed to calculate a safe heating procedure for any food to be processed with heat (Stumbo, 1973). The calculated cooking schedule based on heat penetration data of cooked PSB Rc72H (*Mestizo*) rice by boiling and thermal resistance parameters of *B. cereus* spores (Philippine strain 1061) as reference microorganism for evaluation of cooking process are shown in Table 2. Juliano & Sakurai (1985) reported that the normal cooking times for rice in the Philippines ranged from 15–25 min while the study established a heating time of 28 min with a come-up-time of 4 min.

When establishing a thermal process for a particular food based on a microorganism, considerations must be given to the heat resistance parameters of that organism and the likely level of contamination of the raw material. Lee *et al.* (1995) cited maximum level of *B. cereus* spores in rice to be at 10^5 CFU/g while Goepfert *et al.* (1972) reported that a population of at least 10^5 CFU/g of *B. cereus* spore is required for food poisoning to occur. For rice cooking process where *B. cereus* is the reference organism, a cooking process sufficient to destroy 5 log units of the organism, i.e., a 5D process should therefore be adequate. A 5D cooking time of 25 min for boiled rice was calculated from the $D_{100°C}$ of 5 min for *B. cereus* spore (Philippine strain 1061). This calculated cooking time is equal to the cooking lethality of 25 min computed using Ball's Mathematical Method (Table 2). Therefore, the study was able to establish that the normal cooking times of ≥25 min would be adequate in inactivating *B. cereus* spores even in cases of excessive contamination of raw rice at levels of about 10^5 CFU/g *B. cereus* spores.

Conclusions

This study determined that the *D*-values of *B. cereus* spores (Philippine strain 1061) in 2% rice broth using PSB Rc72H (*Mestizo*) at 80, 90 and 100°C were 38, 12 and 5 min, respectively. The *Z*-value was established to be 20°C. Based on the thermal resistance characteristics of *B. cereus* spores and the heat penetration profile of cooked *Mestizo* rice, a cooking lethality of 25 min was established at 100°C, which is adequate relative to the calculated required cooking time of 25 min with the same temperature. Further investigations could be undertaken to test the heat resistance of *B. cereus* spores utilizing different strains and concentrations of the test organism in various Philippine rice cultivars.

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