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The survival and growth of *Bacillus cereus* in boiled and fried rice in relation to outbreaks of food poisoning

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SUMMARY

A number of outbreaks of food poisoning attributed to *Bacillus cereus* have been reported recently and all have been associated with cooked rice usually from Chinese restaurants and 'take-away' shops.

Tests were made to assess the heat resistance of *B. cereus* spores in aqueous suspension, the growth of the organism in boiled rice stored at temperatures in the range 4–55° C., and the effect of cooking and storage on the growth of the organism in boiled and fried rice. The spores of *B. cereus* survived cooking and were capable of germination and outgrowth. The optimum temperature for growth in boiled rice was between 30° and 37° C. and growth also occurred during storage at 15° and 43° C.

To prevent further outbreaks it is suggested that rice should be boiled in smaller quantities on several occasions during the day, thereby reducing the storage time before frying. After boiling the rice should either be kept hot (> 63° C.) or cooled quickly and transferred to a refrigerator within 2 hr. of cooking. Boiled or fried rice must not be stored under warm conditions especially in the range 15–50° C.

INTRODUCTION

Over 30 separate incidents of food poisoning associated with cooked rice (usually fried) and usually from Chinese restaurants or 'take-away' shops have been reported in Great Britain since 1971 (Public Health Laboratory Service, 1972, 1973, and unpublished information). Bacteriological examination of food remnants and faecal specimens has failed to yield any of the organisms usually associated with food poisoning, and staphylococcal enterotoxin was not detected in three samples of cooked rice from separate outbreaks. In most of the incidents large numbers of aerobic, spore-forming bacilli, identified as *Bacillus cereus*, have been isolated from remnants of cooked rice or from faecal specimens or from both. Plate counts on blood agar of *B. cereus* in cooked rice from 17 incidents have ranged from 3×10^5 to 2×10^9/g. with a median value of 5×10^7/g. (Gilbert & Taylor, to be published).

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Rice

Long grain rice was purchased for the work.

Diluent and colony plate count method

All dilutions were made in quarter-strength Ringer's solution.

Colony plate counts were made on blood agar containing 5 % defibrinated horse blood by means of a modified Miles & Misra (1938) technique with incubation for 18–40 hr. at 30° C. In experiments where low numbers of *B. cereus* were expected 0·5 ml. volumes were plated.

Determination of heat resistance of B. cereus *spores in aqueous suspension*

Samples (0·2 ml.) were distributed from a 1 ml. syringe into 2 ml. freeze-drying ampoules which were sealed under air. The ampoules were heated at 90°, 92·5° and 95° C. by total immersion in a thermostatically controlled water bath and at 100° C. in boiling water. Ampoules were removed at appropriate time intervals and immediately cooled in an ice–water mixture. They were opened, the contents washed out and tenfold dilutions prepared and plated.

Survivor curves of log percentage surviving organisms against time were constructed, using the mean count from 2 or 3 unheated ampoules as 100 %. Decimal reduction times (D), the time required to reduce the number of surviving organisms by 90 % at a constant temperature, were calculated from regression analyses of $\log_{10}$ colony plate counts for various intervals of time, using the digital computer program of Navani, Scholefield & Kibby (1970). Values for z, the number of degrees of temperature to bring about a tenfold change in D values, were also calculated.

Growth of B. cereus *in boiled rice*

Two-lb. (*ca.* 900 g.) quantities of rice were rinsed twice in cold water to remove superfluous starch. The washed rice was mixed with four pints (*ca.* 2270 ml.) of cold water, brought to the boil and allowed to simmer with occasional stirring until all the water was absorbed, *ca.* 20 min. The boiled rice was rinsed once in boiling water to facilitate separation of the grains and 10 g. samples were distributed into 1 lb. screw-capped jars.

Tenfold dilutions of spore suspensions of BC 2, 9 and 25 were prepared and 0·2 ml. volumes were distributed onto the surface of the rice with a 50 drop/ml. pipette to give an initial inoculum of *B. cereus* spores between *ca.* 10 and 2×10^4/g. of rice. Sets of jars were stored at 4°, 10°, 15°, 22°, 30°, 37°, 43° and 55° C. for periods of time up to 3 days. Jars were removed at various time intervals and 90 ml. of diluent were added to each to give a 1/10 dilution. After thorough mixing, further tenfold dilutions were prepared and plated on blood agar for counts.

Effect of cooking and storage on the growth of B. cereus *in boiled and fried rice*

A 5 lb. quantity of rice was rinsed twice in water and inoculated with low numbers of spores of BC 2, *ca.* 140 spores/g. or BC 9, *ca.* 680 spores/g. Ten pints of

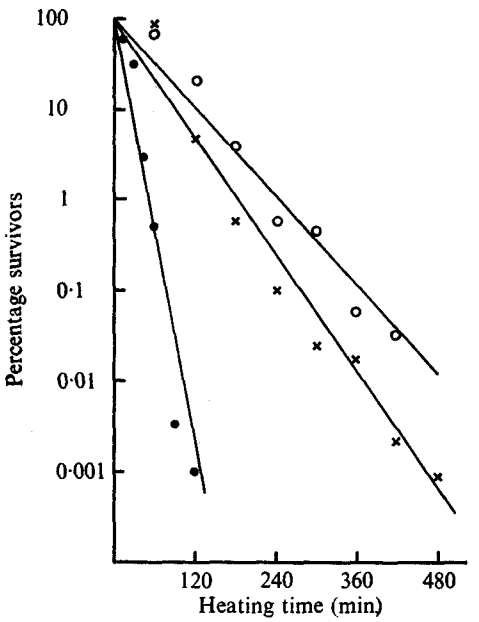

Fig. 1. Heat resistance of *B. cereus* spores in aqueous suspension at 90° C. ○, culture BC 8; ●, culture BC 9; ×, culture BC 25.

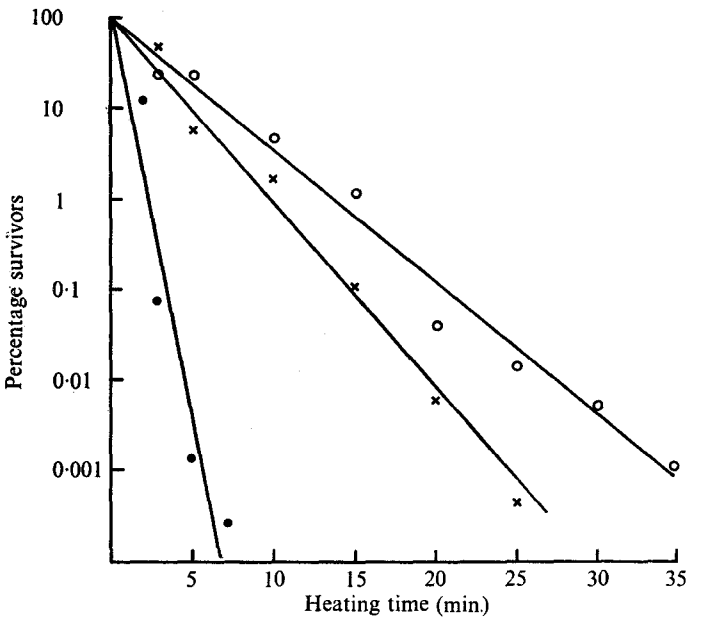

Fig. 2. Heat resistance of *B. cereus* spores in aqueous suspension at 100° C. ○, culture BC 8; ●, culture BC 9; ×, culture BC 25.

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Table 2. *Heat resistance of* B. cereus *spores in aqueous suspension*

Culture	Decimal reduction times (min) at °C. 90	92·5	95	100	*z* value (°C.)
BC 1	67	36	17	2·3	6·8
BC 2	100	62	21	6·0	7·7
BC 8	112	74	36	7·5	8·3
BC 9	21	13	5·0	1·2	7·9
BC 10	93	59	24	4·2	7·2
BC 23	104	71	33	5·0	7·4
BC 25	90	57	23	4·7	7·6
BC 40	35	23	7·2	1·6	7·1
BC Br	137	73	36	4·5	6·7

water were added and the mixture cooked as before. After rinsing once with boiling water three 10 g. samples were taken from different areas (surface, middle and base) and dilutions prepared and plated. Counts were also carried out on 10 g. portions of rice, sampled at three different areas within the bulk, at intervals up to 24 hr. after boiling. During this period the cooking vessel was left on the bench to cool slowly to room temperature (23–26° C.).

A further three 250 g. portions of rice were sampled as before, and each was fried in a pan for *ca.* 1½ min. mixed with freshly beaten egg and a small amount of corn oil. Three 10 g. samples of each portion of fried rice were taken for plate counts immediately after frying and again after 24 hr. storage at room temperature. The fried rice was then fried a second time in small amounts of corn oil for *ca.* 1 min. Counts were carried out on three 10 g. samples of each portion immediately after refrying and again after 24 hr. storage at room temperature.

RESULTS

Nearly all the survivor curves for the nine *B. cereus* strains were linear on a plot of log percentage survivors against time of heating (Figs. 1, 2) with correlation coefficients greater than tabulated values at $P = 0{\cdot}05$ for the appropriate degrees of freedom. These curves are therefore exponential. Calculated D and z values are given in Table 2. The D values were in the range 21–137 min. at 90° C. and 1·2 to 7·5 min at 100° C. Of the nine strains spores of BC Br were the most resistant at 90° C. and those of BC 8 at 92·5° and 100° C. The z values were in the range 6·7–8·3° C., with a mean of 7·4° C.

Survivor curves for BC 23 and BC Br at 95° C. did not appear to be linear when plotted, and the curve for BC 2 at 95° C. showed an initial shoulder before it became exponential (Fig. 3). However, coefficients of linear correlation for these three curves were greater than tabulated values and the data could therefore be represented approximately by straight lines.

Table 3 shows D and z values for spores of BC 9 and BC 25 produced under varying conditions of growth and sporulation. For both strains the spores produced on soil-extract agar were the most resistant at 100° C.

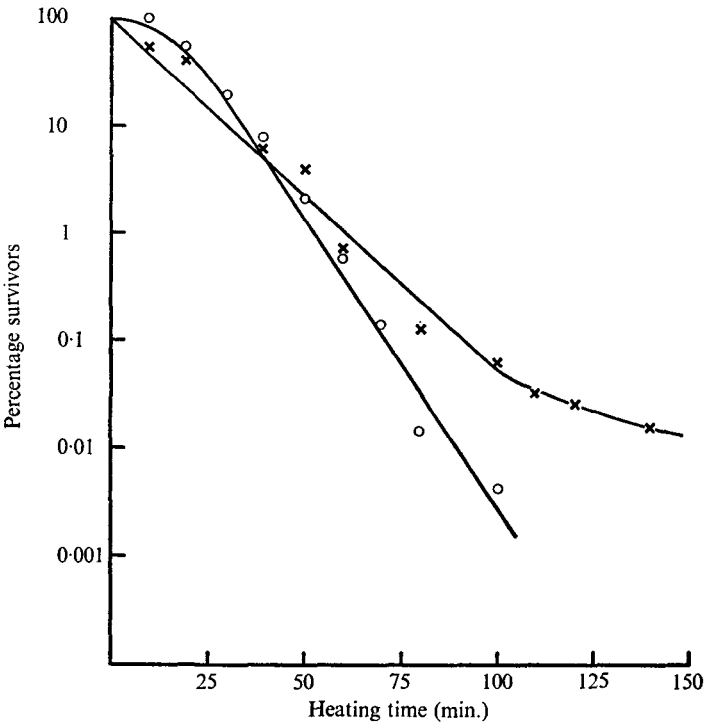

Fig. 3. Heat resistance of *B. cereus* spores in aqueous suspension at 95° C. ○, culture BC 2; ×, culture BC 23.

Table 3. *Heat resistance in aqueous suspension of* B. cereus *spores produced under different conditions*

Culture	Sporulation medium and conditions*	Decimal reduction times (min) at °C. 90	92·5	95	100	*z* value (°C.)
BC 9	1	21	13	5·0	1·2	7·9
	2	27	N.T.	4·8	0·6	6·1
	3	27	N.T.	5·2	0·9	6·8
BC 25	1	90	57	23	4·7	7·6
	2	64	N.T.	20	3·2	7·7
	3	77	N.T.	25	3·9	7·7

* See text. N.T., not tested.

Total colony counts of the uncooked rice were < 100/g. but *B. cereus* was isolated after enrichment in nutrient broth. *B. cereus* was not isolated, however, from *ca.* 2 g. of uninoculated rice taken from a large bulk which had been boiled for 20 min.

The growth of BC 2, 9 and 25 in boiled rice stored at different temperatures in the range 4–55° C. is shown in Tables 4 and 5. At 4° and 10° C. there was no growth with any of the three strains after 3 days storage. There was slight growth at 15° C., much more at 22° C. and the optimum was reached at 30° to 37° C. Growth at 43° C. was more rapid than at 15° C. but less than that at 22° C. At

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Table 4. *Growth of* B. cereus *spores in boiled rice stored at* 4°, 10°, 15°, 43° *and* 55° *C.*

Culture	Storage temperature (°C.)	Log count of *B. cereus* in boiled rice after storage (hr.) 0	16	24	48	72
BC 2	4	3·74	N.T.	3·70	3·74	3·60
	10	3·74	N.T.	3·78	3·85	3·74
	15	2·74	2·78	2·88	3·18	3·30
	43	2·54	3·70	4·40	4·54	N.T.
	55	3·54	2·90	2·30	< 1·30	N.T.
BC 9	4	3·81	N.T.	3·78	3·85	3·78
	10	3·81	N.T.	3·85	3·78	3·78
	15	2·81	3·08	3·18	3·30	3·60
	43	3·30	4·18	4·65	4·74	N.T.
	55	4·30	3·48	3·24	2·30	N.T.
BC 25	4	3·60	N.T.	3·60	3·48	3·54
	10	3·60	N.T.	3·60	3·54	3·70
	15	2·60	2·78	2·88	3·18	3·30
	43	2·93	3·48	3·66	4·18	N.T.
	55	3·93	2·90	2·60	1·90	N.T.

N.T., not tested.

Table 5. *Growth of* B. cereus *in boiled rice stored at* 22°, 30° *and* 37° *C.*

Culture	Storage temperature (°C.)	Log count of *B. cereus* in boiled rice after storage (hr.) 0	4	9	18	23	28	33
BC 2	22	1·00	N.T.	3·18	5·40	5·93	6·24	6·30
	30	1·00	2·35	5·70	6·81	7·18	7·60	7·81
	37	1·00	3·40	5·40	7·30	7·54	7·78	7·85
BC 9	22	1·60	2·40	3·65	5·60	6·54	6·74	6·88
	30	1·60	2·70	6·18	7·51	7·93	8·18	8·48
	37	1·60	3·90	6·90	7·93	8·24	8·48	8·60
BC 25	22	1·30	N.T.	3·30	5·48	6·40	6·60	6·74
	30	1·30	2·70	5·65	6·70	7·00	7·48	7·60
	37	1·30	3·52	5·18	7·24	7·81	7·93	8·00

55° C. there was no growth and the number of organisms recovered decreased with storage time. Mean generation times in the logarithmic phase of growth, calculated (Rose, 1968) from plate counts of *B. cereus* in boiled rice after storage for 4 and 9 hr., were in the range 26–31 min. at 30° C. and 30–54 min. at 37° C. Total colony counts on uninoculated freshly boiled rice stored at 30° and 37° C. for 24 hr. were $2{\cdot}4 \times 10^3$/g. and $3{\cdot}5 \times 10^3$/g. respectively.

The effects of the boiling, storage, frying and storage sequence on the survival and growth of BC 2 and 9 are shown in Figs. 4–5. Fig. 4 shows the range of counts of *B. cereus* from 3 sampling sites within the rice and indicates an uneven distribution of organisms which most probably occurred when the rice was inoculated. In general, plate counts on boiled rice were greater for samples taken from the surface than for those taken from the middle or base of the rice bulk.

Counts of BC 2 and 9 were < 20/g. in inoculated boiled rice sampled immediately

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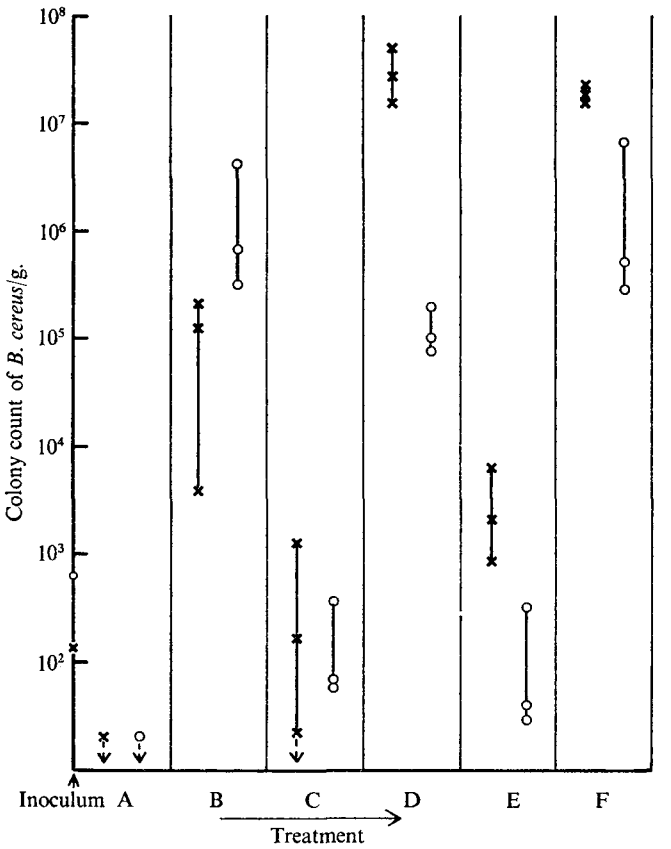

Fig. 4. Range of counts of *B. cereus* in boiled and fried rice after cooking and storage. ×, culture BC 2; ○, culture BC 9.

Key	Plate count after	Cumulative time (hr.)
A	Boiling (25 min.)	0
B	Storage	24
C	Frying (1½ min.)	24
D	Storage	48
E	Frying (1 min.)	48
F	Storage	72

after preparation and after storage for 2 hr. Viable spores were present in the rice, however, because vegetative growth occurred on subsequent storage (Fig. 5). After 24 hr. storage large numbers of *B. cereus* were present in the boiled rice and microscopic examination showed that many of the vegetative cells contained spores. Rapid frying reduced the number of organisms but those that survived, presumably heat-resistant spores, were capable of germination and outgrowth. Growth and spore formation occurred when the fried rice was stored again at room temperature. There was a second rapid fall in bacterial numbers when the rice was refried and subsequent storage for a third time again encouraged bacterial growth.

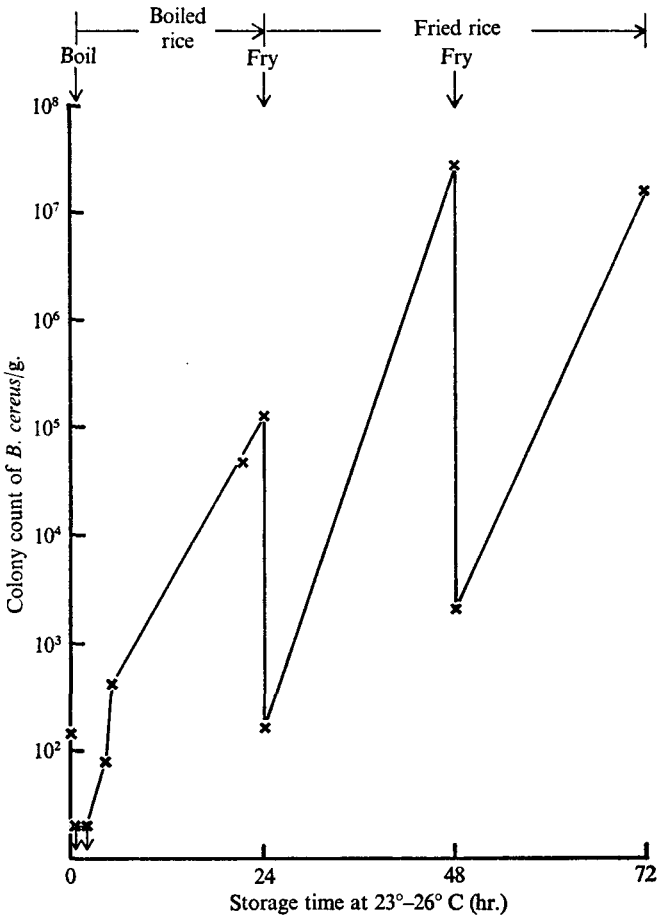

Fig. 5. Effect of cooking and storage on the growth of *B. cereus* (BC 2) in boiled and fried rice. Points plotted are median values of counts on 3 samples of rice.

DISCUSSION

The experiments described in this paper were designed to simulate the times and methods of cooking and the storage conditions used by some Chinese restaurateurs (Perry, 1974; Mortimer & McCann, 1974).

It appears to be the practice in many Chinese restaurants and 'take-away' shops to save portions of boiled rice from bulk cooking until required for frying. The boiled rice is allowed to 'dry off' at room temperature for varying periods of time from a few hours to about 3 days, but usually overnight. The rice is then either reheated or more usually fried for a very short time with beaten egg and a small amount of oil before serving: the beaten egg is not always freshly prepared and may itself be highly contaminated with a variety of bacteria. The Chinese are reluctant to store boiled rice in a refrigerator because they say the rice grains stick together and it becomes difficult to 'toss them' in beaten egg during frying. In some instances the fried rice is stored at room temperature and 'flash' fried again before serving.

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The situation is made worse by the preparation of large bulks of boiled rice which take several hours to cool down, and there are reports (Mortimer & McCann, 1974) of the practice of adding fresh batches of boiled rice to the remains of old, which are sometimes left over from the previous day. Whether the boiled rice is allowed to dry off for varying periods of time at kitchen temperature or is left in or near a warm oven, conditions may be ideal for the germination and outgrowth of spores which have survived the boiling process.

Bacillus cereus is common in soil and on vegetation and has been isolated in several countries from a wide variety of routine samples of food (Nygren, 1962; Jantea, Milosescu, Bistriceanu & Bad-Oprisescu, 1965; Mossel, Koopman & Jongerius, 1967; Kim & Goepfert, 1971). Nygren (1962), for example, reported an isolation rate of 47·8% after examination of 3,888 samples of food and food ingredients in Sweden. In food poisoning outbreaks in this country the most likely source of *B. cereus* is the uncooked rice. The heat resistance of *B. cereus* during the boiling, frying or reheating of rice is important and the data presented show that organisms, presumably spores, survive cooking and are capable of germination and outgrowth.

The D100° values of the nine spore suspensions of *B. cereus* studied were in the range 1·2–7·5 min., similar to those reported by Murrell & Warth (1965) 0·8–14·2 min., Briggs (1966) 5·5 min. and Molin & Snygg (1967) 8·0 min., for spores heated in aqueous suspension or phosphate buffer. In the present study variations in sporulation medium and incubation conditions had little effect on the heat resistance of the spores produced and none of the spore suspensions showed any evidence of exceptional resistance to heat.

Results from growth experiments in boiled rice inoculated with spore suspensions of *B. cereus* (BC 2, 9 or 25) showed that the optimum temperature for vegetative cell growth was between 30° and 37° C. The minimum temperature for vegetative growth was between 10° and 15° C. Mol (1957) reported that *B. cereus* would grow in yeast extract phosphate broth when stored at 12° C. for a few days but not at 8° C. when held as long as 4 months.

The outbreaks of food poisoning attributed to *B. cereus* in Great Britain since 1971 differ in a number of respects such as incubation period, symptoms and food vehicles, from outbreaks hitherto reported in several other countries (Hauge, 1950, 1955; Goepfert, Spira & Kim, 1972). The short incubation period (between 1 and 5 hr.) for the episodes in this country is of particular interest and it suggests that the illness is caused by a toxin produced in the rice. The large numbers of *B. cereus* isolated from samples of fried or boiled rice implicated in outbreaks indicates that neither the organisms (spores) nor the toxin are destroyed in the process of frying or reheating. Work is in progress on the typing of *B. cereus* using antisera produced in rabbits against H antigens.

Until the methods described in this paper for the preparation and in particular the storage of cooked rice are discontinued, outbreaks of food poisoning will occur. Long, slow cooling and non-refrigerated storage of cooked rice, indeed of all cooked foods, provide ideal conditions for bacterial growth particularly from surviving spores. To prevent further outbreaks:

(1) Rice should be boiled in smaller quantities on several occasions during the day, thereby reducing the storage time before frying.

(2) After boiling the rice should either be kept hot, at not less than 63 °C. (145° F.), or cooled quickly and transferred to a refrigerator within 2 hr. of cooking. The cooling of rice, especially large bulks of boiled rice, will be hastened by dividing the product into separate portions or by spreading the bulk in clean shallow containers.

(3) Boiled or fried rice must not be stored under warm conditions and never at a temperature between 15° and 50° C. Under no circumstances, therefore, should cooked rice be stored at kitchen temperature for more than 2 hr.

(4) The beaten egg used in the preparation of fried rice should be freshly prepared.

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