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Studies of *Bacillus cereus* Resistance to Fermentation and Drying of Four Milk Based Cereals Dough

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Abstract: The resistance of *Bacillus cereus* (*B. cereus*) cells to fermentation and drying in 4 different dough based on cereal flours (malted and unmalted sorghum, malted and unmalted maize) was studied. *B. cereus* vegetative cells in order of 10^7 cfu g^{-1} inoculated in the dough before the onset of fermentation increased to 10^8 cfu g^{-1} after fermentation for 48 h at 30°C inspite of the low pH recorded at the end of fermentation (pH<4,7). Similarly, the cells present in the fermented dough at the start of drying reduced to only 4×10^4-$4,71\times10^4$ cfu g^{-1} after drying in an oven at either of the three different temperatures (50, 65 and 80°C) to a final moisture content of less than 7%. These results reveal that once the cells of *B. cereus* have established themselves in dough based on cereal flours and milk, they can withstand fermentation and drying at temperatures between 50 and 80°C.

Key words: *Bacillus cereus*, milk, maize, sorghum, fermentation, drying

INTRODUCTION

B. cereus is a spore forming ubiquitous bacterium contaminating different types of foods (Johnson, 1984; Etoa *et al*., 1989). It is a causative agent of diarrhoeal and emetic types of diseases following the consumption of cereal-based food (Schiemman, 1978; Bryan *et al*., 1981) as well as milk and milk products (Davies and Wilkinson, 1973).

Kindirmu is a fermented flours based on cereal and milk produced traditionally by the rural population of the Central African Region specially in the northern parts of Cameroon where cattle rearing and milk production is the principal preoccupation of the people. In the method used for the manufacture of this foodstuff, cow milk obtained after treatment is fermented spontaneously and mixed manually with cereal flour (millet, maize, sorghum, rice). The dough obtained is sun-dried and the resulting flour used for the preparation of gruel.

In general, the traditional method for *Kindirmu* production is time-consuming and exposed to contamination (Toukour, 1989). Recent studies in our laboratory have been focusing on reducing the production time as well improving its hygienic quality.

Although earlier studies by Toukour (1989) had revealed that *B. cereus* is a contaminant of *Kindirmu* product, its occurrence and rate of growth in the product have not been documented.

To evaluate the role of a micro-organism as a contaminant and pathogen in a foodstuff, it is important to know the rate of occurrence and growth of the micro-organism in the foodstuff in question (Hin *et al*., 1988). The aim of the present research was to evaluate the effect of the fermentation of mixtures of cereal flours and milk, as well as the drying temperature on the survival of *B. cereus*.

MATERIALS AND METHODS

Sorghum and maize grains used for this study were procured from the local market. Milk was obtained from a nearby rural household. To minimise variations in its consistency and composition, the milk was always obtained from the Canadian-run pilot dairy farm located close to the University of Ngaoundere-Cameroon. *B. cereus* used was obtained from Microbiology Laboratory of the National School of Agro-Industrial Sciences, University of Ngaoundrere. The inoculum was prepared by cultivating the cells in Tryptone Soya Broth (TSB Oxoid) for 24 h at 30°C under moderate agitation.

To ensure similar microbial culture, the source of inoculum for fermentation consisted of a dried starter (Rhône Poulenc, France) containing a strain of *Lactobacillus bulgaricus* and one of *Streptococcus thermophilus*.

***Kindirmu* fermentation:** Sorghum and maize grains were cleaned of dust and other foreign material, washed and divided into two lots, one of which was germinated for 72 h in the dark. The ungerminated as well as germinated

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cereals were each dried to constant weight in a laboratory oven at 50°C and separately grind into a powder using a 0.5 mm sieve size in a hammer mill type (Monto, France). The flours obtained were sieved through a 150 μm sieve.

The cereal flours were partitioned into 1000 mL erlenmeyer flask (100 g flask^{-1}) and sterilised. Three hundred gram of sterilised milk and 1 g of starter were added aseptically to each flask. No *B. cereus* was detected in this culture. Ten mililiter of inoculum containing vegetative cells of *B. cereus* was also introduced to the mixture. In the control, the inoculum of *B. cereus* was replaced with 10 mL of sterile distilled water. All the flasks were incubated at 30°C and samples withdrawn, respectively after 0, 4, 6, 8, 12, 24 and 48 h for pH measurement and *B. cereus* counts.

Drying of fermented dough: After 48 h the fermented dough was spread aseptically with the aid of sterile spatula on sterile aluminium plates of dimension 30×30×0.5 cm. The plates thus prepared were dried in a laboratory oven (Memmert, Germany) at 50, 65 and 80°C, respectively until constant weight.

***Bacillus cereus* and pH during fermentation:** pH was measured according to the method of Cook *et al.* (1991) while the enumeration of *B. cereus* was done according to a modified method described by Hin *et al.* (1988). Briefly, 10 g of sample were homogenised in 90 mL of physiological solution. Serial dilutions were made and the dilutions (0.1 mL) were spread on mannitol-egg yolk-polymyxin B agar and incubated at 30°C for 24 h. Typical *B. cereus* colonies were counted.

Statistical analysis: The experiment was carried out in triplicate and data obtained were analysed by analysis of variance in Statistica software (Statsoft, 1995). Differences between means were tested using the Duncan Multiple Range Test.

RESULTS AND DISCUSSION

Figure 1 a-d shows the changes in pH and the growth pattern of *B. cereus* cells during fermentation of the different dough. In general, the pH of the dough dropped with increase in fermentation time. In agreement with similar studies on cereals (Nout and Rombouts, 1992; Hounhouigan *et al.*, 1993) the pH of the dough fell (from about 6 to 4.5) with increase in fermentation time. Such fall in pH has been explained to be partially due to the formation of organic acids (lactic acid, acetic acid, propionic acid and isobutyric acid) by the lactic bacteria (Mikolajcik *et al.*, 1973; Smith *et al.*, 1989). The decline of

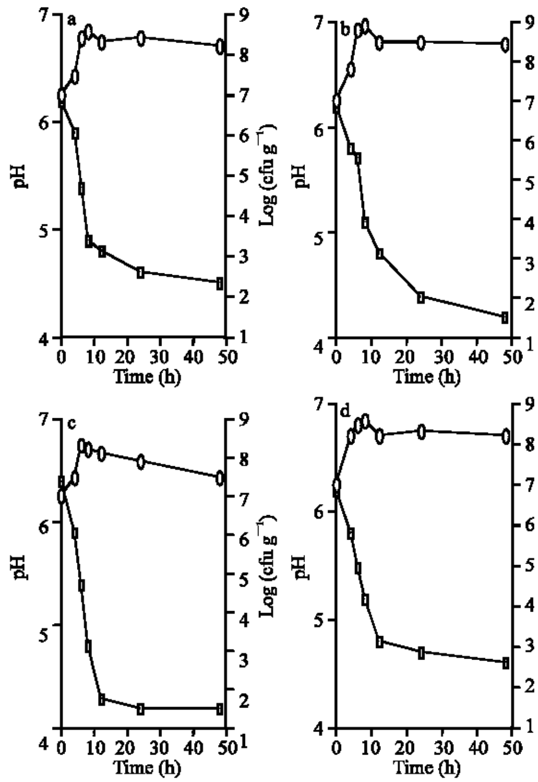

Fig. 1: Evolution of (0) *Bacillus cereus* and (`) pH during the fermentation of the different dough. (a) dough with non germinated maize, (b) dough with germinated maize, (c) dough with non germinated sorghum, (d) dough with germinated sorghum

the pH was also accompanied by an increase in the number of vegetative cells of *B. cereus* during the first 10 h. After this time, there was a gradual decrease in growth especially when the pH fell under 5. Thereafter, the population remained relatively static throughout the fermentation process. This pattern of growth has also been observed by Cook *et al.* (1991) when they studied growth of vegetative cells of *B. cereus* during the fermentation of a traditional Indonesian dish made from rice. The gradual decrease observed for pH<5 may be due to the creation of unfavourable conditions for the development of *B. cereus*. Raevuori *et al.* (1975) found that the minimum pH necessary for the growth of *B. cereus* in foodstuff is between 4.5 and 5.2. Above this decrease, it was observed that the number of *B. cereus* remained relatively constant in all the dough. It is likely that due to the creation of unfavourable conditions in the dough, *B. cereus* develops a micro - cycle for survival.

The dough obtained from non-germinated sorghum was less supportive of *B. cereus* growth than that obtained from non germinated maize. This difference may

Table 1: No. of *B. cereus* (Log cfu g^{-1}) after drying at the three temperatures

Flour type	No. of *B. cereus* after 48 h fermentation	No. of *B. cereus* after drying — Drying temperatures 50°C	65°C	80°C
Dough with non germinated maize	8.2 (8.2-8.4)	4.65 (4.59-4.70)	4.62 (4.65-4.68)	4.63 (4.59-4.63)
Dough with germinated maize	8.4 (8.3-8.6)	4.64 (4.62-4.67)	4.58 (4.53-4.62)	4.58 (4.55-4.61)
Dough with non germinated sorghum	7.5 (7.1-7.7)	4.62 (4.55-4.69)	4.65 (4.62-4.67)	4.64 (4.55-4.63)
Dough with germinated sorghum	8.2 (8.0-8.4)	4.69 (4.68-4.71)	4.68 (4.66-4.70)	4.60 (4.55-4.62)

Value are means of three replicates. Ranges are shown in parentheses

be partially due to the difference in the chemical composition of the two cereals. Sorghum contains more phenolic compound, especially tannins, than maize (Vernon, 1981). These substances liberated during fermentation have an inhibiting effect on the growth of micro-organisms (Yoshizawa *et al*., 1970; Cook *et al*., 1991). In general, *B. cereus* growth on dough made from non germinated cereals was significantly ($p<0.05$) slower compared to that made from germinated cereals. One reason that explains the observed pattern may be the fact that during germination, the hydrolysis of carbohydrates to simple sugars (Osho and Adenekan, 1995) provide simple sugars that are readily utilisable by *B. cereus*.

Table 1 presents the residual number of *B. cereus* at the end of drying the various dough at three different temperatures (50, 65 and 80°C). Neither the type of the flour used for the making of the dough nor its drying temperature, influenced the cell counts. On the whole, *B. cereus* counts ranged between 4×10^4 and $4,71\times10^4$ cfu g^{-1}. It is likely that these residual *B. cereus* count are present in the form of spores. According to Dufresne *et al*. (1994) the vegetative cells of *B. cereus* can easily inactivated by heating while spores are considerably more resistant. Given that these values are much lower than the level 10^7 cfu g^{-1} known to induce toxinogenesis of *B. cereus* (Gilbert *et al*., 1974) it is concluded that the temperature of 50, 65 and 80°C used in the study are appropriate for reducing *B. cereus* counts below toxic levels. These residual counts could lead to food spoilage and food poisoning after subsequent germination.

CONCLUSION

Inoculated *B. cereus* is resistant to the fermentation and drying conditions used in this study production of *Kindirmu*. Appropriate care must therefore be taken to avoid contamination during normal processing of the food. While it may be necessary to dry the dough at the temperatures higher than 80°C and this could affected the functional and nutritional qualities of the end products. The fabrication of fermented flours based on local cereals and milk at the present stage requires rigorous control to avoid contamination by *B. cereus*.

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