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BIOCHEMICAL CHANGES IN MILK BY *BACILLUS CEREUS* CELLS

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The trials were conducted by inoculating *Bacillus cereus* spores in low count milk and holding at 37°C and subjected to three treatments 1. Pasteurisation, 2. Pasteurisation and boiling 3. Boiling of milk. The spore cells of *B. cereus* grew in pasteurised milk upto two hours and thereafter formed spores after 8 hours The keeping quality of pasteurised milk and boiled milk was 8 hours whereas in the case of pasteurised and boiled milk it was only 6 hours The rate of proteolysis was found to be higher in the case of pasteurised and boiled milk. There was not much of change in the lipolysis in the treatments.

The prevelence of *B. cereus* in raw milk, pasteurised milk and indifferent milk products have been reported by Lausten (1978). This organism causes typical broken cream followed by sweet curdling (Barkeley and Delani, 1980). Hence the biochemical changes by added *B. cereus* spores in 1. Pasteurised milk. 2. Pasteurised and boiled milk, 3. Boiled milk were undertaken.

MATERIALS AND METHODS

Milk was collected aseptically from a clean healthy cow after taking due precautions to clean the udder with disinfectents. The milk was cooled immediately. It was distributed in sterilized test tubes in 5 ml quantities. The lab pasteurisation was done by immersing in a water bath and the temperatute was raised to 71° C for 15 sec. and the tubes were immediately cooled to 10° C. The milk was boiled for 5 min. The pasteurisation and boiling of milk was done to simulate the condition in house holds where usually the pasteurised milk is boiled. Spores of *B cereus* cells at a level of 1200 spores/ml was added. These samples were plated in yeastreal milk agar. The total count was done at 0, 1, 2, 4, 6, 8, and 10 hours depending upon the keeping quality. The milk was heated to 80° C for 10 min. and plated. The acidity and PH & C. O. B. test was done as described in I. S. 1479 (part I) 1960. The percentage of germination was calculated by deduction. The proteolytic activity was measured as described by Hull (1947).

RESULTS AND DISCUSSION

Table 1 shows the total bacteria, count spore count, acidity, pH proteolysis and lipolysis in pasteurised milk in which *B. cereus* cells were added. The total bacterial count has

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Table 1. Effect of boiling on growth of added spores of *B. cereus* and the biochemical changes in milk

Period in Storage in hours	Total bacterial count X10[illegible] Cfu ml	Spore count ml X 10[illegible]	No. of germinated spores ml	Acidity	pH	Proteolysis mg. tyrosine 5 ml	Lipolysis ml 0.025 N KOH 15 ml
0	1.4 X 10[illegible]	1.2 X 10[illegible]	...	0.14	6.65	0.072	0.054
1	3.10 X 10[illegible]	1.2 X 10[illegible]	1.07 X 10[illegible]	0.14	6.65	0.072	0.54
2	7.8 X 10[illegible]	6.8 X 10[illegible]	1.142 X 10[illegible]	0.14	6.60	0.110	0.54
3	1.05 X 10[illegible]	3.8 X 10[illegible]	1.154 X 10[illegible]	0.15	6.50	0.134	0.55
4	1.02 X 10[illegible]	3.2 X 10[illegible]	1.17 X 10[illegible]	0.16	6.45	0.158	0.55
5	6.20 X 10[illegible]	2.0 X 10[illegible]	1.18 X 10[illegible]	0.17	6.42	0.166	0.55

* *B. cereus* added at 1200 spores ml—COB positive at 5th hour.

shown an increase from 3.2X10[illegible] to 64X10[illegible] CFU ml, and the spore count has shown a decrease from 1.2X10[illegible] to 2.80X10[illegible] CFU ml at the end of one hour. The spore count increased in number till two hours, thereafter there was sudden sporulation followed by maintaining the dormancy. The milk showed C.O.B. positive. Increase in acidity was found to be favourable and the acidity at C.O.B. positive was found to be 0.165. The rate of proteolysis was found to increase to the extent of 0.1 mg. of tyrosine for 5 ml of milk at the end of 5 hours of incubation. The rate of lipolysis was almost the same.

The biochemical activity in pasteurised and boiled milk is shown in table 2. It can be seen that the total bacterial count which was 1.5X10[illegible] has shown a gradual increase to 1.32X10[illegible] CFU ml and spore cell count from 1.2X10[illegible] to 6.0X10[illegible] CFU ml contrary to the rate of growth of spore cells of *B. cereus* in pasteurised milk. The reason may be that in pasteurised milk there were other organism also present which may be competing for their growth into each other whereas in boiled milk there were only spores present and in the absence of other micro organism as present in pasteurised milk there was uninhibited growth in boiled milk. The rate of increase in acidity was low and the coagulation of milk was observed at 5 hours. The rate of proteolysis was also found to be

Table 2 Effect of pasteurization and boiling on germination and growth of added spores of *B. cereus* on the biochemical changes in milk (Average of 5 trials)

Period of storage in hours	Total bacterial count/ml	Spore count/ml	No. of germination spore ml	Acidity record	pH	Proteolysis mg/tyrosine/5 ml	Lipolysis/ml 0 025 N KOH/15 ml
0	1.6 X 10^5	1.2 X 10^5	...	0.15	6.55	0.072	0.67
1	4.6 X 10^5	2.8 X 10^5	9.2 X 10^5	0.15	6.55	0.080	0.67
2	9.4 X 10^5	1.06 X 10^5	1.094 X 10^5	0.16	6.50	0.086	0.68
4	9.84 X 10^7	2.3 X 10^6	1.17 X 10^5	0.165	6 50	0.106	0.68
+6	1.32 X 10^8	6.1 X 10^6	1.19 X 10^5	0.17	6 50	0.212	0.68

* COB Positive in 6th hour; + *B. cereus* at 1200 spores/ml.

higher but there was no increase in lipolysis.

The effect of addition of *B. cereus* spores and boiling of milk and the accompanying biochemical changes when the milk was incubated for 8 hours is shown in table 3. The increase in total bacterial count was from 1.4X10^3 to 8.2X10^3 CFU/ml. while the spore count showed an increase from 1 2X10^3 to 2.0X10^4. The coagulation of milk at low acidity was due to the extra cellular enzymes liberated due to free growth of *B cereus* in boiled milk. The rate of proteolysis has shown a very high increase.

In the biochemical changes in the pasteurised milk it was seen that the shelf life of milk was 8 hours at 37° C. Both total and spore count recorded a gradual increase in their increase. The proteolytic action was found to be more pronounced. It is therefore deduced that if the cells of *B. cereus* are highly predominant in pasteurised milk it is likely to affect the quality in respect of low shelf life and development of off flavour due to proteolysis. Similar studies carried out by following usual household method of subjecting pasteurised milk to boiling after addition of spore *B. cereus* showed that the rate of biochemical change was very high with respect to proteolysic action and higher growth rate of cells of *B. cereus*. Koka (1968) also observed that vegetative cell prolification was much faster in heated than unheated raw, pasteurised, high

Table 3 Effect of pasteurisation on germination and growth of added spores of *B. cereus* *on the biochemical changes in milk held at 37°C. (Average of 5 trials)

	Total bacterial count/ ml Cfu/ml	Spore count/ ml Cfu/ml	No. of germination spores/ml Cfu/ml	Acidity	pH	Proteolysis/ mg tyrosine/ 5 ml N	Lipolysis/ ml/0.025 KOH/15 ml
0	3.2 X 10^{3}	1.2 X 10^{3}	...	0.15	6.55	0.072	0.66
1	6.4 X 10^{3}	2.32 X 10^{2}	8.8 X 10^{2}	0.15	6.55	0.076	0.67
2	9.6 X 10^{3}	2.12 X 10^{3}	1.08 X 10^{4}	0.17	6.50	0.078	0.67
4	1.124 X 10^{5}	1.86 X 10^{3}	1.64 X 10^{3}	0.18	6.50	0.080	0.69
6	2.124 X 10^{6}	1.64 X 10^{3}	1.16 X 10^{3}	0.185	6.50	0.088	0.70
+8	2.88 X 10^{9}	1.82 X 10^{3}	1.18 X 10^{3}	0.195	6.45	0.100	0.70

+ Inoculum level : 1200 spore cells of *B. cereus* per ml of milk

+ COB positive in 8th hour.

heat treated and autoclaved skim milk sample to which *B. cereus* spores were added. It is evident that the boiling of pasteurised milk results in rapid spoilage of milk. Boiling of milk contaning spore cells of *B. cereus* has shown moderate changes with respect to increase in total number as well as spore count accompanied with gradual changes in proteolysis The shelf life of milk appears to be 8 hours at 37°C which is slightly more compared to the treatment of pasteurisation followed by boiling.

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REFERENCES

BARKELY, M. B. and DELANEY, P. 1980. An occurrence of bitty cream in Australian pasteurized milk. Neth. Milk Dairy J., *34*: *191*.

HULL, M. E. 1947. Studies on milk proteins "Colorimetric determination of the partial hydrolysis of the proteins in milk". J. Dairy Sci., *30* : 881.

INDIAN STANDARD - METHODS OF TEST FOR DAIRY INDUSTRY, 1960. No. 1479, Part I. Rapid examination of milk. Indian Standard Institution, New Delhi.

KOKA, M. 1968. Factors influencing post dormant acidity of *Bacillus cereus* spores in milk and model systems. Diss. Abstr., Sect. B. *28* : 4863 Columbus, Ohio (Dairy Sci. Abstr., 1968, *30* : 4194.)

LAUSTSEN, K. 1978. Development of aerobic psychrotrophs in whole and skim milk. Dansar veterinaertidsskrift. *61* : 1098 (Dairy Sci. Abstr., 1881, *43* : 1638.