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Studies on growth of certain micro-organisms in reconstituted infant foods

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Baby food, breast milk and infant formula are foods unique to the child care environment. Each must be handling safety to prevent food borne disease. Breast milk is the best source of nutrient, antimicrobials and other protective substance for infants. Like breast milk, infant formula is not a sterile product. During the drying process, pathogen can be sub lethally injured, meaning that the damage to the cell is minimal, so the cell can recover. Some bacteria can be multiply when the powdered infant formula is reconstitute. The present study was based on the microbiological examination of reconstituted infant foods and 100 samples were collected from different locations of Meerut district in the year 2009-10. There are eight brands of infant milk food and three brands of infant weaning food which were collected within a month of manufacture and subjected to microbiological examination and all collected samples were analyzed at 37°C and 7°C on holding time 01, 2, 3, 4 and 5 hours in the laboratory. The total bacterial count multiplied rapidly and there was a 2 folds increase in their counts at 37°C within 5 hours. Similar trend was noted in *B.cereus* while, indicated on almost 3 folds increase. The coliform multiplied most rapidly and there was a 2 folds increase in their count at 37°C within 5 hours. The growth pattern of staphylococci was, however, different. Although an appreciable increase was noted in the beginning a phase of decline was found after subsequent holding at 37°C. The population of staphylococci was static at 7°C. On the other hand no significant change was observed at 7°C within 5 hours. During the current study, representative samples of infant weaning foods were reconstituted kept at 37°C and 7°C. On examination, it was found that *B.cereus*, which indicated an almost 4 folds increase. Similar trend was noted in coliform, multiplied most rapidly and there was a 2 folds increase was observed at 37°C. However, no significant increase in their counts at 7°C within 5 hours. Although staphylococci counts increased appreciably in the beginning, a phase of decline was noted after subsequent holding at 37°C. The population of staphylococci was static at refrigeration temperature (7°C). The study suggests that prepared infant feed should not be stored even for small duration because holding of prepared feed will endanger the health of such delicate consumers. Only freshly prepared feed should be given to the infant to achieve healthy feeding practices

Key Words : Baby food, Time, Storage, Weaning foods, Temperature, Micro-organism

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INTRODUCTION

In recent years, realization of microbiological quality of infant food has been felt earnest by in our country and need to control microbial contamination in infant food and strict exclusion of pathogen from them. Milk powder is generally considered a good microbiological quality production however, several factors may contribute to changes in the physical and chemical properties that reduce the shelf-life and commercial value (Becker *et al.*, 1994). Various researchers agree that the health conditions under which raw milk production is the main

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factor affecting the quality of powder (Lück, 1987; Muir *et al.*,1986; Woodhall, 1989 and Griffiths *et al.*, 1988). The degree of storage and transport temperatures may also affect milk powder properties, especially solubility and pH indicator (Jayarao and Henning, 2001). Milk is a food complement, high nutritional value makes it an ideal way for rapid multiplication of bacteria, especially of unhealthy production and storage temperatures (Kim *et al.*, 1983 and OECD, 2005) milk powder quality to be free of disease-causing bacteria and toxic substances harmful, microbiological analyzes critical to assess the safety, quality, conformation with the standards and specifications, and regulatory compliance (Kim *et al.*, 1983). While one bacterial species may grow under certain conditions, other types may weaken. These conditions are interrelated and include the ability nutrient availability, and levels of oxygen and moisture and the level of other gases, having pH inhibitors, temperature (Eifert *et al.*, 1996). Coliforms can cause damage to the milk because they ferment lactose with the production of acid and gas and can also lead to a deterioration of the milk proteins (Shojaei and Yadollahi, 2008). Yeasts and fungi in the milk may create a risk to human health products (United States Department of Agriculture, Agricultural Marketing Service, "Commodity Areas," 2013 and ICMSF (1978), many of these Microbial can cause an imbalance in the formative characteristics of the milk and microbial growth is a major concern objects (Kaper *et al.*, 2004; Singh *et al.*, 2006; Lòpez-Malo *et al.*, 2005 and Rydlo *et al.*, 2006). The microbiological quality of infant milk food and weaning foods, storage duration at ambient temperature, the quality of the used for reconstituted were studied. At some occasions there is likelihood that the reconstituted infant food may be stored over a period of time and reject to the babies. The condition of holding may offer the necessary opportunities to pathogen contamination like *B.cereus,* coliform and staphylococcal to multiply and release enterotoxins in the stored fed. Relative humidity also plays a important role in infant food. At high humidity a rapid fall in the number bacteria was found followed by over growth of yeast and mould. Improper handling of reconstituted milk at consumer level offers opportunities for growth and multiplication of resident organisms. If full hygienic care is not taken, reconstituted infant food generally offers on ideal medium for the rapid multiplication of resident organisms present in milk foods. Such changed are more revealing, if reconstituted baby food is kept even for few hours at ambient temperature. At times associative action of the organisms either help them grow mutually or even retard the multiplication.

METHODOLOGY

The study was conducted in Meerut district of western Uttar Pradesh during the year 2009-10. The study was based on the microbiological examination of infant milk foods and 100 samples of infant foods were procured from different location (*i.e.* local/market/urban/rural) of Meerut district. These comprised of different batches of 8 brands (Lactodex, Lactogen, Amul Spray, Glaxo, Liver Spray, Sapan, Raptakos and Parag) of infant milk food and 3 brands (Cerelac, Farex and Nestum) of infant cereal weaning foods. The samples were collected within a month of manufacture and subject to microbiological examination for total bacterial count, coliform count, *B.cereus* and staphylococcal. All the samples were kept at two different temperature *i.e.* (4°C to 7°C and at room temperature). In order to see the effect to temperature on shelf-life and microbiological changes in the samples in two different sealed packets was stored at 7°C and room temperature and analysed their total bacterial count, *B.cereus* and staphylococcal and coliform count etc. was done at duration of 0, 1, 2, 3, 4 and 5 hours.

All the samples were analysed for total bacterial count using nutrient agar, by serial dilution. The samples Petri plates was incubated at 37°C ± 1°C for 24 hours and number of colonies were counted. The enumeration of coliform counts was done using on violet red bile agar (VRBA) plate. The enrichment procedure was also followed using 2% brilliant green lactose bile broth (BGLB) to recover the low population in the product. The incubation was carried out for 24 to 48 hours at 37°C. The samples were also plated in eosine methylene blue agar. The suspected colonies was carried through indole, methyle red,vogesproskauer and citrate utilization etc. To differentiate between faecal and non faecal coliform Elijkans test was employed by incubating at 44°C ± 1°C (Harrigan and McCance, 1976). To detected *Bacillus cereus* counts colonies were picked up at random from Mannitol egg yolk Polymoysin Agar (MYPA) and were examined microscopically (Mossel *et al.*, 1967). Approximate dilution of the sample were plated in incubation was carried out at 37°C for 48 hours.

Conformation of *B.cereus* was done by biochemical reactions - sugar fermentation, gelatinase production and citrate utilization.

To analysis of staphylococcal counts was spread over prepared plate of Blood agar (Mishra *et al.*,1987). The plate was incubated for 24°C to 48 hours at 37°C. A none selective enriched procedure was also followed using Tryplic Soy Broth Himedia (1979) to enumerate low population stressed cell of staphylococcal. These were also examined by gram's staining reaction and the suspected colonies were transfer on yeast extract glucose agar slant for further test. Confirmation of staphylococcal was done by these biochemical reaction fermentation of sugar, catalase production, phosphatase production, gelatinase production, haemolysins salts tolerance and coagulose production etc. All samples was taken 10 g of samples + 90 ml of sterile saline water and then it is serially diluted to 10^4 from that 0.1 ml sample is taken in agar plates following spread plate technique and it incubated at 37°C. for 48 hour which colonies formed were counted and expressed as colony forming unit per g [cfu/g]

The CFU/g of the sample was calculated by using the following formula:

$$\text{CFU/g} = \frac{\text{Number of colonies x Dilution factor}}{\text{Dry weight of the sample}}$$

OBSERVATIONS AND ASSESSMENT

The results obtained from the present investigation as well as relevant discussion have been summarized under following heads :

Behaviour of micro-organism in reconstituted infant milk foods :

During the current study, representative samples reconstituted of infant milk food kept at 37°C and 7°C. Research findings recorded in Table 1 exhibit the build up of organisms such as staphylococci, coliform and

Table 1 : Behaviour of micro-organisms in reconstituted infant milk foods (37°C)

Period (Hours)	Log counts/g			
	Total bacterial counts	Coliform counts	*B. cereus* counts	Staphylococcal counts
0	3.8	2.4	1.9	2.6
1	3.9	2.6	2.4	2.9
2	4.1	3.4	2.8	3.2
3	4.9	3.8	3.3	3.4
4	4.9	4.2	3.6	2.3
5	5.6	5.0	4.2	1.8

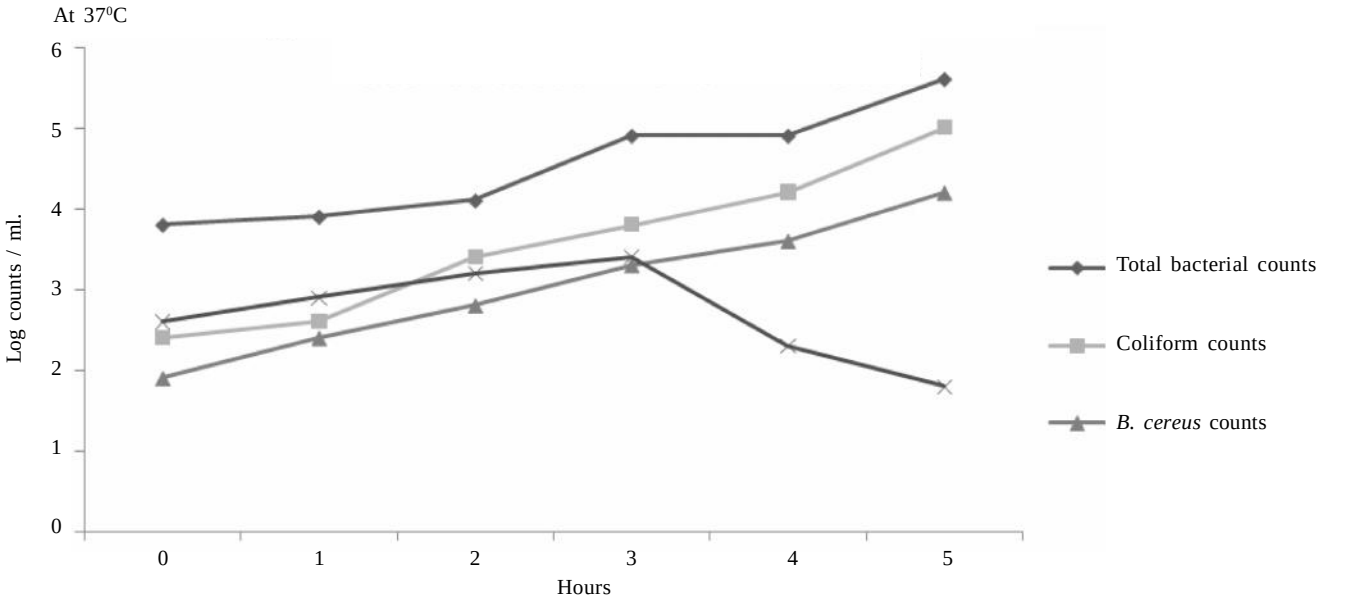

Fig. 1 : Behaviour of micro-organisms in reconstituted infant milk foods

B.cereus when incubated at 37°C. As expected, reconstituted milk foods kept at 7°C did not indicated much change in microbial load.

Total bacterial counts:

All of the reconstituted infant milk foods favoured the growth of different type of bacteria, when held at 37°C and 7°C. The average total bacterial counts multiplied rapidly and attained log counts of 3.8 to 5.6 in infant milk foods within 5 hours when reconstituted milk foods kept at 37°C However, 3.9, 4.1, 4.9 and 4.9 log was found in reconstituted infant milk foods with in 1, 2, 3 and 4 hours, respectively. On the other hand, reconstituted milk kept at 7°C did not found change in total bacterial counts.

Incidence of coliform:

Coliform multiplied rapidly and found 2.4 to 5.0 log in reconstituted infant milk foods within 5 hours when incubated at 37°C. However, a negligible, 2.6, 3.4, 3.8 and 4.2 log in 1, 2, 3, and 4 hours, respectively multiplied rapidly at 37°C. On the other hand, no significant change in the density of organisms was seen at 7°C (Table 1 to 2).

Incidence of *B.cereus*:

In the case of *B.cereus* the multiplied rate was found a increase number from 1.9 to 4.2 log after 5 hours at 37°C. Average counts of *B.cereus* in reconstituted infant milk foods was found negligible 2.4, 2.8, 3.3 and 3.6 log in 1, 2, 3 and 4 hours when held at 37°C. On the other hand, 1.9 to 2.1 log counts was found in 1 hour, but no significant change in the density of organisms was seen at 7°C after 1 to 5 hours (Table 1 to 2 and Fig. 1 and 2). Becker *et al.* (1994) also found 100 *B.cereus*/g were reconstituted and incubated at a temperature of 27°C. Levels of 10^5 *B.cereus*/g were reached after 7.9 hours. Singh *et al.* (1980) observed that one of the baby food

Table 2 : Behaviour of micro-organisms in reconstituted infant milk foods (7°C)

Period (Hours)	Log counts/g			
	Total bacterial counts	Coliform counts	*B. cereus* counts	Staphylococcal counts
0	3.8	2.4	1.9	2.6
1	3.8	2.4	2.1	2.6
2	3.8	2.4	2.1	2.6
3	3.8	2.4	2.1	2.6
4	3.8	2.4	2.1	2.6
5	3.8	2.4	2.1	2.6

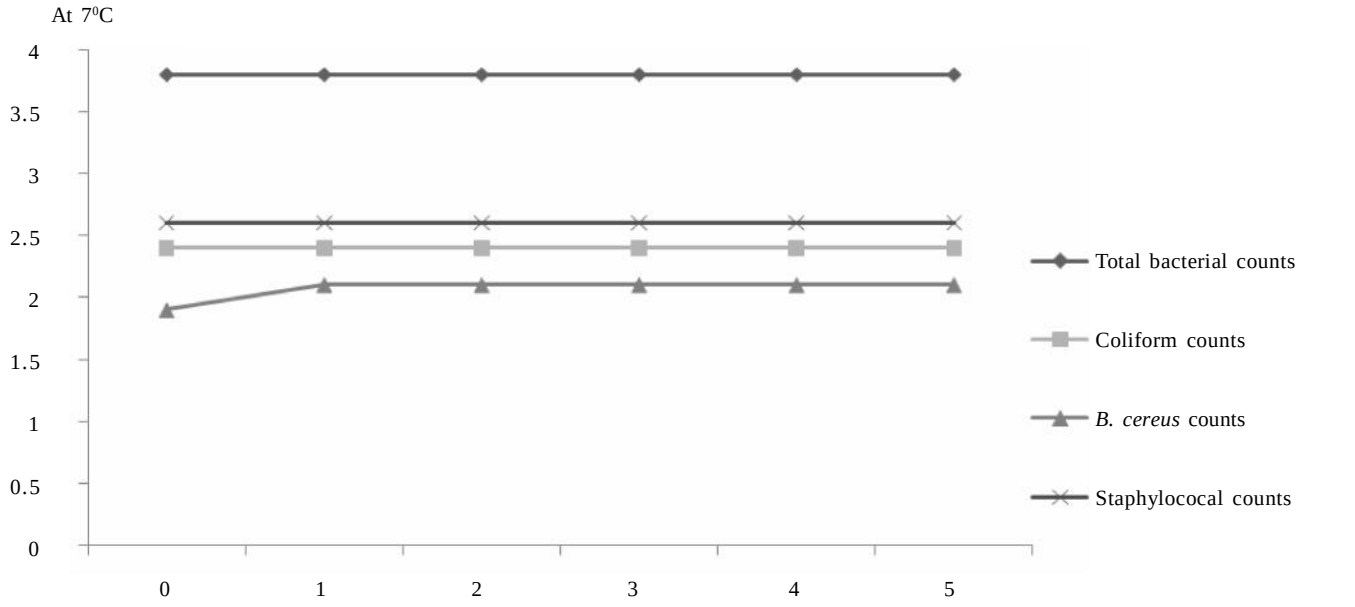

Fig. 2 : Behaviour of micro-organisms in reconstituted infant milk foods

samples when held at ambient temperature (37.5°C). *B.cereus* increased 10 fold in 3 hours. However, at 7°C little or no change in count occured in the same reconstituted baby food samples held for 3 hours.

Incidence of staphylococci:

Although the initial multiplication of staphylococci 2.6 to 3.4 log was appreciable during first 3 hours of holding at 37°C, a slow decline in growth pattern was observed during subsequent incubation. On the other hand, no significant change in the density of staphylococci was seen at 7°C. It can be concluded from the table that in the reconstituted infant milk foods the growth of different types of bacteria such as coliform, staphylococci and *B.cereus* was rapidly multiplied when held at 37°C, while, no significant change in the density of organisms was seen at 7°C. Singh *et al*. (1980) In one of the reconstituted baby food samples when held at a ambient temperature (37.5°C) the Staphylococcus count increased 10 folds in 3 hours. However, at 7°C little or no change in count occured in the same reconstituted baby food samples held for 3 hours. Ghodekar and Nambudripad (1975) observe survival of staphylococci and enterococci in milk powder even after prolonged storage.

Behaviour of micro-organisms in reconstituted infant weaning foods:

Similar observation were made in the reconstituted samples belonging to the infant weaning food on holding at 37°C and 7°C with in 5 hours.

Total bacterial counts:

The average bacterial counts was multiplied rapidly and attained log count of 4.6 to 7.9 log in infant weaning foods in 5 hours at 37°C. However, 5.2, 5.7, 6.5 and 7.4 log was found in 1, 2, 3 and 4 hours when infant weaning

Table 3 : Behaviour of micro-organisms in reconstituted infant weaning foods (37°C)

Period (Hours)	Log counts/g			
	Total bacterial counts	Coliform counts	*B. cereus* counts	Staphylococcal counts
0	4.6	3.4	1.1	2.6
1	5.2	3.9	2.5	2.8
2	5.7	4.6	2.7	3.9
3	6.5	4.9	3.4	4.4
4	7.4	5.4	3.8	3.7
5	7.9	6.2	4.4	3.2

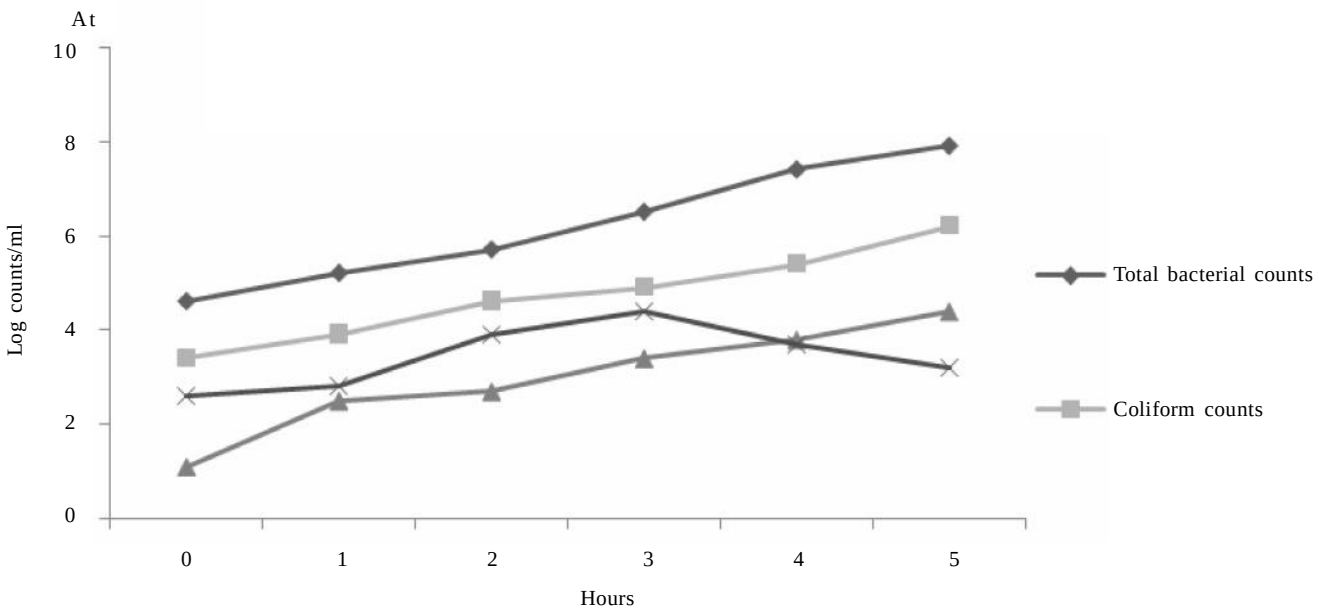

Fig. 3 : Behaviour of micro-organisms in reconstituted infant weaning foods

Table 4 : Behaviour of micro-organisms in reconstituted infant weaning foods (7°C)

Period (Hours)	Log counts/gm.			
	Total bacterial counts	Coliform counts	*B. cereus* counts	Staphylococcal counts
0	4.6	3.4	1.1	2.6
1	4.8	3.4	1.1	2.6
2	4.8	3.4	1.1	2.6
3	4.8	3.4	1.1	2.6
4	4.8	3.4	1.1	2.6
5	4.8	3.4	1.1	2.6

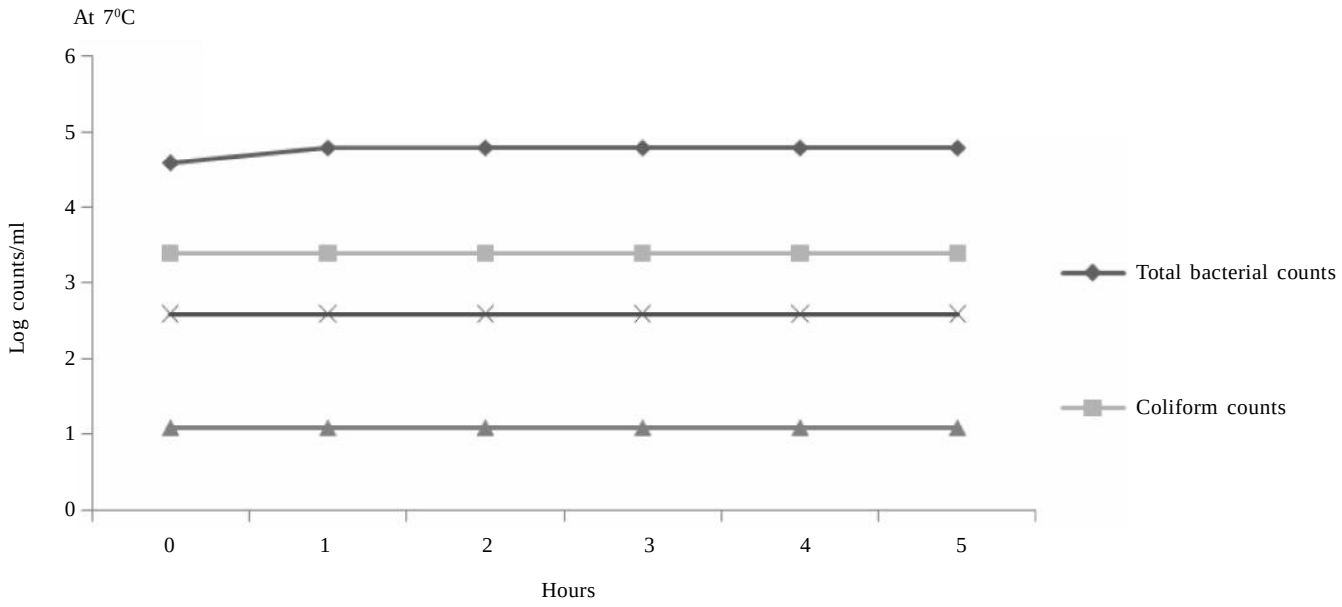

Fig. 4 : Behaviour of micro-organisms in reconstituted infant weaning foods

foods was incubated at 37°C. As expected, reconstituted infant weaning foods kept at 7°C did not indicate much, change in microbial load. The average count 4.6 to 4.8 is found in 5 hours at 7°C (Table 3 and 4 and Fig. 3 and 4).

Incidence of coliform:

These were followed by coliform which after a slow initial multiplication went upto 3.4 to 6.2 log counts during the period of 5 hours and at 37°C as stated above. Approximately. 3.9, 4.6, 4.9 and 5.4 log was found in 1, 2, 3 and 4 hours, respectively at same temperature. On the other hand, no significant change was found in reconstituted infant weaning foods when stored at 7°C (Table 3 to 4).

Incidence of *B.cereus*:

Although the initial multiplication of *B.cereus* in reconstituted infant weaning foods was found 1.1 to 4.4 log within 5 hours at 37°C. About 2.5, 2.7, 3.4 and 3.8 log counts was found during the period of 1, 2, 3 and 4 hours, respectively at same temperature. On the other hand, no significant change was found in 7°C within 5 hours in reconstituted infant weaning foods (Table 3 to 4).

Incidence of staphylococci:

Staphylococci multiplied rapidly in reconstituted infant weaning foods and the initial 2.6 to 4.4 log was found during first 3 hours of holding at 37°C, a slow decline in growth pattern was observed during subsequent incubation. As expect, reconstituted infant weaning foods kept at 7°C did not indicate much change in microbial load (Table 3 to 4). It can be concluded from the table that in the reconstituted infant weaning foods the initial multiplication of such organism as *B.cereus* and coliform went upto log counts per ml during the period of 5 hours where stored at 37°C while, reconstituted weaning foods

did not indicate much change in microbial load at 7°C. It can further be concluded that the initial multiplication was found during first 3 hours of holding at 37°C, a slow decline in growth was observed during subsequent incubation while, these foods kepts at 7°C did not indicate much change in microbial load.

Conclusion :

The study suggests for infant food users the almost care should be taken during storage of reconstitution of infant foods. This fact has been also suggested that there is a need for developing such infant formulae which are reasonable resistant to bacterial over growth for the at least 1 to 2 hours after reconstitution of infant food.

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