# Microbiological profile of dried milk products available in local markets

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## ABSTRACT

*A total of 50 random samples of dried milk powder (25) and milk cereal based weaning food (25) was purchased from different shops, supermarkets and pharmacies in El-Beida city and subjected to microbiological evaluation. Results revealed that the mean aerobic plate counts; psychrotrophic counts; Enterobacteriaceae counts and mould and yeast counts in examined samples were* $2.3\times 10^3\pm 5\times10^2$, $1.8 \times 10^3\pm 5\times10^2$, $3.7 \times 10 \pm 1.1\times10$, $1.3 \times10^3 \pm 3.1\times10^2$ *and* $9.6\times10^4 \pm4,1\times 10^3$, $3.9\times 10^3\pm1,1\times 10^3$, $7.3 \times 10^2\pm2.5\times 10^2$ *and* $1.8 \times 10^3\pm5.5\times 10^2$ *cfu/g, respectively. Among the examined samples; 44% and 80% were positive for anaerobes by using " Stormy fermentation test". The respective incidences of B. cereus were 52 and 44% .The effect of refrigerator and ambient temperatures on growth of B. cereus strain isolated from examined samples was studied. This strain was not psychrotrophic and did not grow at 7 $^0$C.*

**Keywords:** *B. cereus, dried milk powder, milk cereal based weaning food, mould and yeast.*

## INTRODUCTION

Milk is a basic source of protein, so it is widely consumed by all age groups of people particularly by children. Milk and milk products are supplied as fresh milk products, concentrates and dry products. Milk powders may be used as a substitute for fresh milk and concentrates, and can play many functional roles when incorporated into food products. It is known that milk powders are made from high quality milk and that their compositional and technical specifications relate to end-use requirements (Ramesh *et al.*, 2008).

Bovine milk is an essential ingredient of milk products and a potential source of bacteria that are pathogenic to humans (Breeuwer *et al.* 2003). All available data indicate the increased infection risk arising from multiplication of pathogenic bacteria in reconstituted formula kept at room or warmer temperatures for prolonged periods (Codex Committee on Food Hygiene, 2004). Psychrotrophs are ubiquitous; their natural habitats are soil, water, plants and animals. Psychrotrophs can grow at or below 7 $^0$C which are a problem resulting from prolonged refrigeration storage of dairy products (Swart *et.al.*, 1989). They were implicated in many defects in milk dairy products (Gary, 1990). The presence of Enterobacteriaceae in dairy products induces undesirable changes that render the product of inferior quality, unmarketable and unfit for human consumption. Moreover, their presence is frequently considered as reliable index of faecal contamination. Therefore, determinations of any or all members of the family Enterobacteriaceae have been used as a microbiological

guideline and indicator of food quality and sanitation (Thatcher and Clark, 1978).

There has been growing use of baby foods with dairy base as replacers of fresh milk or in addition to it. Milk cereal based weaning food constitutes a major category of baby foods, which are fed to infants above three months old. The rapid use of baby foods as substitute of or with mother milk has made its microbial quality of great concern. Due to low quality ingredients of baby food and unsatisfactory methods of preparation and handling, infant foods may at times, be responsible for some cases of food poisoning in children. Also, baby foods may not receive adequate heat treatment during their preparation, constituting a public health hazard as well as economic losses.

Since the first description of food borne illness from *Bacillus cereus* in the 1950's this microorganism has received much attention and has become a possible cause of food poisoning. *Bacillus cereus* is an aerobic, rod-shaped bacterium that is widely distributed in the environment and that produces spores, enterotoxins and lecithinase. Approximately 5% of all food borne illnesses reported to the World Health Organization Surveillance Programme in Europe in 1990 involved *B. cereus*. Generally, acceptable epidemiological evidence to incriminate *B. cereus* as the causative agent of food borne illness requires the presence of greater than $10^5$ cells/ g of food (Feijoo *et al.*, 1997). This study was conducted to monitor the microbiological status of dried milk and milk cereal based weaning foods.

## MATERIALS AND METHODS

### Collection of samples

A total of 50 random samples of dried milk powder (25) and milk cereal based weaning food (25) was purchased from different shops, supermarkets and pharmacies in El-Beida city. Samples were transferred to the laboratory in their packages to be examined microbiologically to evaluate their quality.

### Preparation of samples

Cartons of samples were shaked, thoroughly mixed and aseptically opened. Eleven grams of each sample was mixed with 89 ml of sterile 0.1% peptone water in 250 ml sterile flask and thoroughly mixed to give a dilution of $10^{-1}$, then ten-fold serial dilutions were prepared.

### Microbiological examinations

The prepared samples were subjected to:
Aerobic plate count (APC): using standard plate agar (A.P.H.A., 1992).
Psychrotrophic count: *for* psychrotrophic bacterial count, the technique described by Gilliland *et al.* (1976) was adopted.
Enterobacteriaceae count: according to Harrigan (1998).
Mould and yeast count: all mould isolates were identified according to Pitt and Hocking (1997), while yeast isolates were identified according to Kriger Van Rij (1984).
Detection of anaerobic spore formers (Stormy fermentation test): the technique described by Cruickshank *et al.* (1969) was carried out.
Isolation and identification of Bacillus cereus: according to Kim and Goepfort (1971).

### Effect of temperature on growth of isolated Bacillus cereus

*Bacillus cereus* strain used in the present study was previously isolated and identified from the examined samples of dried milk and milk cereal based weaning food. The organism was propagated in *Bacillus cereus* enrichment broth overnight at $32^0$C until a steady state of growth was reached. The *B. cereus* was subsequently enumerated on plate of KG agar. Bags of dried milk obtained from market

(previously examined and found to be free from *B. cereus*) was reconstituted with sterile water as 10 g dried milk with 100 ml of sterile water. Prepared samples were inoculated with 1 ml of a diluted culture of *B. cereus* to yield final populations of approximately 30 cfu/ml. Control samples were prepared , each of inoculated samples and controls were divided into two equal portions, one was stored at room temperature ( 25± $1^0$C) and the other was stored at refrigeration temperature (5±$1^0$C) with control. From inoculated samples and control, ten-fold serial dilutions were prepared using 0.1% peptone water for determination of *Bacillus cereus* count according to Kim and Geopfort (1971). The samples and their control were stored at room temperature and examined for *Bacillus cereus* at 0, 1,2,4,8 and 24 hr, while those stored at refrigeration temperature were tested at 0, 2, 4,8 and 24 hr.

## RESULTS AND DISCUSSION

Results recorded in Table (1) revealed that 84% of the dried milk powder and 80% of the milk cereal based weaning food samples contained aerobic bacteria. The aerobic bacterial counts in dried milk powder sample ranged from <20 to $6.2\times10^3$ cfu/gm with a mean value of $2.3\times10^3\pm 5\times10^2$ and in milk cereal based weaning food ranged from $5\times10^2$ to $7.7\times10^3$ cfu/gm with a mean value $9.6\times10^4\pm 4.1\times10^3$. Salahudin and Anwar (2006) and Ahmed *et al.* (2008) recorded that the mean counts in dried milk powder and milk cereal based weaning food samples were $2.9\times10^2$ and $2.9\times10^3$, respectively. According to the Codex Alimentarius Commission Guideline (Codex, 1999), the dried milk powder should contain less than $5.0\times10^4$ cfu/gm, the results of APC are considered acceptable. According to the Egyptian Standards (2001) for dried milk , it is found that all of examined samples comply with these standards (Total plate counts reflect the microbial content of raw material and ingredient, effectiveness of manufacture technique, sanitary care of equipments and utensils and the time –temperature profile of storage and distribution ).

***Table (1): Statistical analytical results of aerobic plate counts (APC) in the examined samples.***

| Type of sample | No. of examined samples | Positive samples | | Colony counts / g | | |
|---|---|---|---|---|---|---|
| | | No. | % | Min. | Max. | Mean ±S.E |
| Dried milk powder | 25 | 21 | 84 | <20 | $6.2\times10^3$ | $2.3\times10^3\pm 5\times10^2$ |
| Milk-cereal based weaning food | 25 | 20 | 80 | $5\times10^2$ | $7.7\times10^4$ | $9.6\times10^3\pm4,1\times10^3$ |

***Table (2): Statistical analytical results of psychrotrophic counts in the examined samples.***

| Type of sample | No. of examined samples | Positive samples | | Colony counts / g | | |
|---|---|---|---|---|---|---|
| | | No. | % | Min. | Max. | Mean ±S.E |
| Dried milk powder | 25 | 17 | 68 | $1\times10^2$ | $7.1\times10^3$ | $1.8\times10^3\pm 5\times10^2$ |
| Milk-cereal based weaning food | 25 | 19 | 76 | $1\times10^2$ | $1.7\times10^4$ | $3.9\times10^3\pm1,1\times10^3$ |

According to the data presented in Table (2), psychrotrophs were detected in 68 and 76% of dried milk samples and milk-cereal based weaning food samples respectively, at levels ranged from $1\times10^2$ to $7.1\times 10^3$ with a mean value of $1.8 \times 10^3\pm 5\times10^2$ and $1\times10^2$ to$1.7\times 10^4$ with mean values of $3.9\times 10^3\pm1$ and $1\times 10^3$, respectively. Lower counts and percentages were detected by Ahmed *et al.* (2008) who reported that the mean counts of psychro-trophic bacteria in milk based cereal weaning food and dried milk powder were $2.8\times10^2$ and $3\times10$, respectively. Some species of psychrotrophic organisms are heat resistant which cause problems in heat-treated milk products, as they produce heat resistant proteolytic and lipolytic enzymes, which cause spoilage of refrigerated dairy products, and the degree of enzymes production generally depends on the storage conditions, temperature and pH. Several thermoduric bacteria can grow at $5^0$C and induce spoilage of the product.

Psychrotrophs are capable of producing food poisoning when ingested in large number. High psychrotrophic count may be referred to contamination of raw materials used in the manufacturing and bad processing as well as contamination from the different sources (Celestino *et al.*, 1996).

***Table (3): Statistical analytical results of Enterobacteriaceae counts in the examined samples.***

| Type of sample | No. of examined samples | Positive samples | | Colony counts / g | | |
|---|---|---|---|---|---|---|
| | | No. | % | Min. | Max. | Mean ±S.E |
| Dried milk powder | 25 | 6 | 24 | <10 | $1\times10^2$ | $3.7 \times 10\pm 1.1\times10$ |
| Milk-cereal based weaning food | 25 | 18 | 72 | $3.3\times10$ | $3.7\times 10^3$ | $7.3 \times 10^2\pm2.5\times 10^2$ |

Results given in Table (3) revealed that Enterobacteriaceae could be detected in 24% of dried milk powder and 72% of milk cereal based weaning food. Counts ranged from < 10 to $1\times10^2$ with a mean of $3.7 \times 10 \pm 1.1\times10$ cfu/gm in dried milk powder and in case of milk- cereal based weaning food ranged from$3.3\times10$ to $3.7\times 10^3$ with a mean of $7.3 \times 10^2\pm2.5\times 10^2$ cfu/gm. These results agree with Ahmed *et al.* (2008) who recorded that 40 and 14 % of milk cereal based weaning food and dried milk powdered were positive for Enterobactericeae, respectively.

Enterobacteriaceae sometimes are contaminants of pasteurized dairy products. Their presence is an indicative of unsanitary methods of manufacture, in adequate pasteurization or post- pasteurization contamination. This indicates a need for more concern on the part of the dairy industry to prevent contamination with Enterobacteriaceae and thus additional out breaks of food-borne illness which caused by their members. It is apparent from the results recorded in Table (4) that 11 out of 25 (44%) and 13 out of 25 (52%) of dried milk powder and milk cereal based weaning food samples were contaminated with yeasts and moulds in counts ranged from $2\times10$ to $2.5\times10^3$ with a mean of $1.3 \times10^3 \pm 3.1\times10^2$ and $2\times10$ to $5.7\times 10^3$ with a mean of $1.8 \times 10^3\pm5.5\times 10^2$, respectively. The obtained findings were coincident with those reported by Ahmed *et al.* (2008) who recorded that total mould and yeast counts ranged from < 10 to $1.0 \times 10^3$ and< 10 to $1.5 \times 10^3$ in dried milk powder and milk cereal based weaning food sample. Lower counts were recorded by Salahudin and Anwar (2006) who reported that

yeast and mould counts were found in samples of four companies as 2- 20 cfu/ g. Their presence in milk or milk products is usually considered as spoilage agents. The Egyptian Standards (2001) mentioned that moulds and yeasts must not exceed 10/g. Moulds and yeasts may grow over a wide range of temperatures and gain entrance to milk powder either from the milk used, air contamination or utensils. So, their presence is an indicative of unsatisfactory sanitation during processing and handling of the product. The high level of these microorganisms may be due to post drying contamination.

***Table (4): Statistical analytical results of total mould and yeast counts in the examined samples.***

| Type of sample | No. of examined samples | Positive samples | | Colony counts / g | | |
|---|---|---|---|---|---|---|
| | | No. | % | Min. | Max. | Mean ±S.E |
| Dried milk powder | 25 | 11 | 44 | $2\times10$ | $2.5\times10^3$ | $1.3\times10^3 \pm 3.1\times10^2$ |
| Milk-cereal based weaning food | 25 | 13 | 52 | $2\times10$ | $5.7\times 10^3$ | $1.8\times 10^3 \pm 5.5\times 10^2$ |

It could be seen from Table (5) that *Penicillium corylophilum Dierckx* and *Candida parapsilosis* were the most prevalent fungi in dried milk powder and milk cereal based weaning food. These results agree with Abdel Mohsen and El-Prince (2002). Pitt and Hocking (1997) reported that dried cereals often show high levels of field fungi as well as the more xerophilic fungi capable of causing spoilage. *Aspergillus penicillioides* is probably the pioneer species in the development of fungal population in stored grains. In wheat stored for 12 months, the development of *A. penicillioides*, followed by Aspergillus spp. was observed. *Penicillium citrinum* is the most common *Penicillium* spp. encountered in rice samples from Thailand, Indonesia and Philippines. Ten species of yeasts are responsible for spoilage of foods, which have been processed and packaged according to normal standards of good manufacturing practice. It is important to note that if good manufacturing practice is neglected i.e. if factory hygiene is poor, if pasteurizing temperatures are inadequate or filling machinery or factory premises are unsanitary, raw materials are poor quality, etc, many others adventitions yeast contaminants can develop in the product. *Rhodotorula* species produce extracellular lipases and proteases and *Candida parapsilosis* is lipolytic and strongly proteolytic at 37-42 $^0$C (Pitt and Hocking, 1997). Results recorded in Table (6), reveal that out of 25 examined samples from each dried milk powder and milk cereal based weaning foods, 44% and 80% were positive for anaerobes by using " Stormy fermentation test". It is apparent that the obtained results are lower than those of Wahba (1997) who recorded that 65.7 % of dried milk powder samples contained these organisms. Lower incidences were recorded by Sayed (2004). Wiese (1992) reported that higher incidences of anaerobes in dried milk powder and milk cereal based weaning foods indicated bad sanitation, although, *Cl. perferingns* must be absent from dried foods for infants. The incidence of *B. cereus* was 52 and 44% in dried milk powder and milk cereal based weaning food, respectively. Nearly, similar findings were obtained by Reyes *et al.* (2006) and Ahmed *et al.* (2008). Dried milk powder and milk-cereal based weaning food contaminated with *B. cereus* should be considered as potential vehicles for food borne *B. cereus* disease. These products contain an elevated level of carbohydrates and minerals, which can promote proliferation and

enterotoxin production when they are reconstituted and held at ambient temperature for extended periods, potentially even at refrigeration temperature (Rowan and Anderson, 1998).

***Table (5): Prevalence of individual members of fungi in examined samples.***

| Individual members of fungi | Dried milk powder | | Milk-cereal based weaning food | |
|---|---|---|---|---|
| | No. of +ve sample | % | No. of +ve sample | % |
| Aspergillus spp. | | | | |
| *Asp. flavus* | 3/25 | 12 | 8/25 | 32 |
| *Asp. penicillioides* | 2/25 | 8 | ---- | ---- |
| *Asp. terreus* | 8/25 | 32 | 3/25 | 12 |
| *Asp. candidus* | 2/25 | 8 | 4/25 | 16 |
| Penicillium spp. | | | | |
| *P. corylophilum Dierckx* | 11/25 | 44 | 12/25 | 48 |
| *P. chrysogenum thom* | 9/25 | 36 | 3/25 | 12 |
| *P. citrinum Thom* | ----- | ---- | 5/25 | 20 |
| Cladosporium spp. | 7/25 | 28 | 10/25 | 40 |
| Yeasts spp. | | | | |
| *Candida parapsilosis* | 11/25 | 44 | 13/25 | 52 |
| *Rhodotorula mucilaginosa* | 6/25 | 24 | 8/25 | 32 |

***Table (6): Incidence of different microorganisms in the examined samples (N=25).***

| Microorganism | Dried milk powder | | Milk-cereal based weaning food | |
|---|---|---|---|---|
| | No. | % | No. | % |
| Anaerobes | 11 | 44 | 20 | 8o |
| *B. cereus* | 13 | 52 | 11 | 44 |

Regarding the results in Fig. (1), using a (3×10) inoculums, it is evident that the numbers of *B. cereus* increased to $3.9 \times 10^2$, $6.2 \times 10^2$, $5,2 \times 10^3$, $3.1 \times 10^4$ and $5.1 \times 10^5$ cfu /ml after 1,2,4,8 and 24 hr. Although the isolated strain did not grow at refrigeration temperatures after 2, 4, 8 and 24 hr. No growth was observed in the product stored at refrigeration temperature; therefore, this strain of *B. cereus* was not psychrotrophic and did not grow at 7 $^0$C.This result agrees with, Becker *et al.* (1994) and Feijoo *et al.* (1997).On the contrary, Jaquette and Beuchat (1998) reported that *B. cereus* strain survives, grows and even produces toxins at low temperatures. Ronner *et al.* (1999) examined five *B. cereus* strains; one was isolated from dairy products tested individually. It was reported that inoculated *B. cereus* strains with low level of spores (5.9 cfu/ g) and incubated at 25 $^0$C, growth to $10^5$/ ml and higher occurred by 24 hr. We considered that possibility that consumers might rehydrate dried milk powdered and milk cereal-weaning food but not use immediately, or that unused portions of formula might be left unrefrigerated and outgrowth of any *B. cereus* spores present in powder could occur.

Therefore, it could be assumed that the manufacturers of powder milk products are maintaining good personal hygiene and sanitation conditions in their processing units. Since, mainly children consume powder milk, a standard sanitation operating procedure (SSOP) should be maintained, which is a

prerequisite program of hazard analysis and critical control point (HACCP) in order to minimize the risk of contamination for safety purpose.

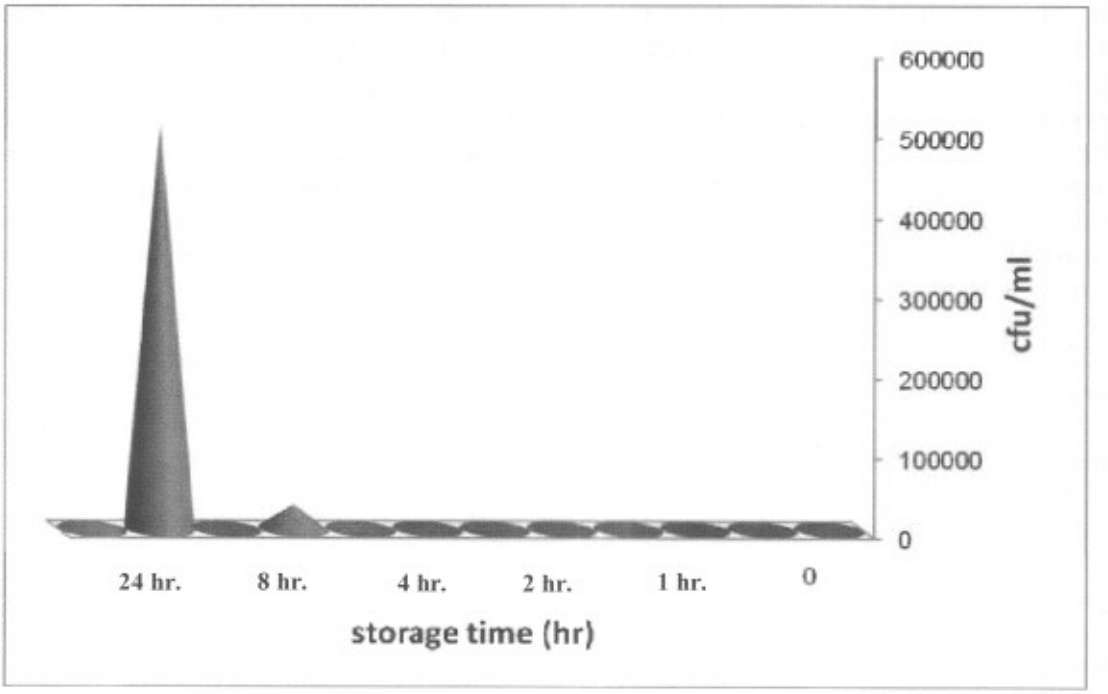


***Fig. (1): Growth of B. cereus vegetative cells in reconstituted dried milk powder at 7 and 25 ± 1 $^0$C.***

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## الملخص العربي

### الصورة الميكروبيولوجية لمنتجات الحليب المجفف المتوفرة في الاسواق المحلية



***منال حسين عبدالحميد منيسى و *ايمان محمود الدياسطى و*رمضان مصطفى تاج الدين و*رافع مصطفى الكاسح**

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تم فحص عدد خمسون عينة مجمعة بطريقة عشوائية عبارة عن مسحوق الحليب المجفف (25) وأغذية الفطام الجافة المحتوية على خلاصة الحبوب (25). وقد جمعت هذه العينات من المحلات المختلفة والأسواق المركزية والصيدليات في مدينة البيضاء Beida. وقد أخضعت تلك العينات للفحص والتقييم الميكروبيولوجى. وقد أوضحت النتائج أن العد الكلى للبكتيريا, العد الكلى للبكتيريا المحبة للبرودة, العد الكلى للبكتيريا القولونية المعوية, العد الكلى للفطريات والخمائر هو2.3× 310 ±5 ×210، 1.8 ± 310× 5×3.7،210 ± 10× 1.1×1.3،10 ± 310× 3.1 ×210 و9.6×310 ± 4.1 ×3.9،210 ± 310× 1.1×7.3،310 ×210 ± 2.5 ×210 و1.8 ×310 ± 5.5 ×210 على التوالي .وقد وجد أن نسبة 44% , 80 % من كل من عينات مسحوق الحليب المجفف (25) وأغذية الفطام الجافة المحتوية على خلاصة الحبوب (25) كانت ايجابية لفحص الميكروبات اللاهوائية وذلك عند استخدام (اختبار التخمر العاصفي ) . وقد كان معدل تواجد ميكروب الباسيلس سيريس هو 52% , 44 % في كل من عينات مسحوق الحليب المجفف (25) وأغذية الفطام الجافة المحتوية على خلاصة الحبوب (25) على الترتيب .وعند دراسة تأثير درجة حرارة التبريد ودرجة حرارة الغرفة على نمو أحد عترات ميكروب الباسيلس سيريس المعزولة من العينات المفحوصة وجد أن هذه العترة المعزولة ليست من الميكروبات المحبة للبرودة حيث لم تنمو عند درجة 7 درجة منوية .