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MICROBIOLOGICAL QUALITY OF DRIED MILK-BASED INFANT FOODS IN ASSIUT CITY

(With 6 Tables)

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التقييم الميكروبيولوجي لبعض أغذية الأطفال اللبنية الجافة في مدينة أسيوط

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يتزايد الإقبال على استعمال ألبان الأطفال كبدائل للبن الأم نتيجة لعدم قدرة بعضهن على إشباع أطفالهن , لذلك كان لابد من استعمال بدائل مناسبة للأعمار المختلفة ومتوافر بها الشروط الصحية لضمان سلامة الأطفال. لذا تم في هذه الدراسة تجميع ٩٠ عينة عشوائية من أغذية الأطفال اللبنية الجافة ، بواقع ٣٠ عينة من كل من ٤ أنواع من اللبن البودرة للأطفال حديثي الولادة (أ)، ٣ أنواع من أغذية الفطام الجافة المحتوية على خلاصة الحبوب (ب) ونوع واحد من الألبان الجافة كاملة الدسم والتي تعطى للأطفال من عمر سنة (ج) وذلك من المحلات والصيدليات المختلفة بمدينة أسيوط والتي تم إنتاجها خلال عامي ٢٠٠١-٢٠٠٢ م وكانت صالحة للاستهلاك خلال عام من تاريخ الإنتاج . وقد تم فحص هذه العينات ميكروبيولوجيا، ودلت النتائج على أن متوسط العدد الكلى للميكروبات والميكروبات اللاهوائية ، الباسيللس سيريوس والفطريات والخمائر في العينات (أ) كـــان٩,٥٠ × ١٠^{٣} ، ١٠×٠,٤ ، ١٠×٢,١ و ١٠×٣,٦^{٣} في الجرام على التوالي ، أما في حالة النوع (ب) وجد أن ١١(٣٦,٦٦%) ، ٢(٦,٦٦%) و١٣(٤٣,٣٣ %) من العينات المفحوصة تحتوى على ميكروبات هوائية ، ميكروبات لا هوائية والفطريات والخمائر على التوالي، هذا وقد لوحظ أن متوسط العدد الكلى للميكروبات ، الباسيللس سيريوس والميكروبات المحبة للبرودة، الميكروبـــات المقاومـــــة للحرارة و الفطريات والخمائـــر كان على التوالى٦,٥٠× ١٠^{٤}، ١٠×٦^{٢} ، ١٠×٩,٥٠ ، ١٠ ×١,٣^{٢} و ١٠×٣^{٣} في الجرام من عينات (ج). وأوضحت النتائج أيضا عدم وجود الميكروبات القولونية والمكور العنقودي الذهبي في كل العينات (أ) و (ب) و (ج) بالإضافة إلى عدم عد وعزل كل من الميكروبات المحبة للبرودة والمقاومة للحرارة في عينات (أ) ، ومثلها علاوة على الباسيللس سيريوس في عينات (ب) والميكروبات اللاهوائية في عينات (ج). وقد ناقش البحث الآثار المترتبة على تواجد هذه الميكروبات وتأثيرها على صحة الأطفال ، واسترشادا بالمواصفات القياسية المصرية والعالمية للألبان الجافة نوصى بتحليل هذه المنتجات بصفة دورية للتأكد من عدم تلوثها بالميكروبات الممرضة بأنواعها المختلفة.

SUMMARY

Ninety random samples of dried milk-based foods (30 samples each) comprising 4 brands of infant's milk powder (A) (for babies after birth), 3 brands of milk-cereal based weaning foods (B) (from up 4 months) and dried milk powder (C) (from up one year) were collected from different shops and pharmacies in Assiut City during 2001-2002. These samples were examined for incidence of different types of microorganisms, to evaluate their quality. The obtained results revealed that the average counts of APC, anaerobes, B. cereus and yeasts and molds were 9.5×10^3, 0.4×10, 2.1×10 and 3.6×10^3/g of examined samples of type A, respectively. However, coliforms, psychrotrophes, Staph. aureus and thermodurics failed to be detected. In case of type B, 11(36.66%), 2(6.66%) and 13(43.33%) of the examined samples contained aerobic bacteria, anaerobes and yeasts and molds, respectively, while no B. cereus, coliforms, psychrotrophes, Staph. aureus and thermodurics could be isolated. Moreover, it was noticed that the average values of APC, B. cereus, psychrotrophes, thermodurics and yeasts and molds were respectively, 6.5×10^4, 6.0×10^2, 9.5×10, 1.3×10^2 and 3×10^3/g of the examined samples of type C. On the contrary, anaerobes, coliforms and Staph. aureus could not be detected. The public health and economic significance as well as suggestive measures for improving the microbiological quality of these products were discussed.

Key words: *Microbiological quality, dried milk-based infant foods.*

INTRODUCTION

Fresh milk and its products have made a major contribution to infants and adults diet in all countries allover the world. In recent years, there has been a growing use of baby foods with dairy base as replacers of fresh milk or in addition to it. In Egypt, dried milks are utilized in many food industries as baking, confectionery and dairy industry. In addition, 8 to 10 thousand tons of standardized fortified milk powder is imported for infant milk food, infant formulae and milk-based cereal weaning foods (Abdel-Mottaleb, 1988). Undoubtedly, the ideal food for babies is the breast milk, however, there are occasions when a substitute must be found. Dried milk is still the most ingredient of breast milk substitute formulation, because many mothers cannot nurse their infants (El-Shinawy *et al.*, 1995). Moreover, milk powder has long shelf life, consequently lowers the cost of transportation and stored for long

periods without significant deterioration (Edgar and Axel, 1998 and Ranken and Baker, 1999).

The manufacture of baby foods based on dried milk involving mixing of various ingredients so, serious health hazards may be found if good manufacturing and hygienic practices are not followed (Anon, 1986). Furthermore, the shelf life, storage stability, flavor quality and nutritional value of milk powder are impaired when stored at high temperatures over a long period (Renner, 1988). Mettler (1989) reported that numerous incidents of salmonellosis associated with the use of milk powders, while Hobbs and Roberts (1993) found that contamination of milk powder with Staph. aureus causes many cases of food poisoning. Normal powder quality can be judged by subjective tests, which include physical (taste, odor and texture), chemical properties and both quantitative and qualitative estimation of microbial flora (Hayes, 1992). Milk powder microbial quality is of primary concern due to the susceptibility of children to foodborne diseases (Ismail and Saad, 1995). Furthermore, reconstituted infant formulas are considered a food class of high risk because of the occurrence of enteric bacterial pathogens, severe response to enterotoxins and increased mortality of the infant population (Rowan and Anderson, 1998).

Many reports have been published earlier worldwide on the microbiological quality of baby foods by several investigators (Jarchovska *et al.*, 1980; Singh *et al.*, 1980; Schwab *et al.*, 1982; Moustafa *et al.*, 1984; Saad, 1985; Alvarez Marante *et al.*, 1986; Sabreen, 1986; Muytjens *et al.*, 1988; Al-Ashmawy *et al.*, 1993; Ismail and Saad, 1995; Saad, 1995 and Rowan and Anderson, 1998).

The most important index of microbiological quality is T.B.C, coliforms, yeasts and molds, thermoduric counts and detection of specific pathogens and their toxins as recorded by Kwee *et al.* (1986), Robinson (1990) and A.P.H.A. (1992). The microbial quality of infant foods reflects the care with which milk was produced and the sanitary conditions prevailing during its manufacture, thus baby foods must be examined at regular intervals. Therefore, this work was accomplished to secure information regarding the sanitary conditions as well as the existing pathogens in baby foods and some dried milk-based infant foods currently available at the retail level.

MATERIALS and METHODS

Collection of samples:

Ninety random samples of dried milk-based foods (30 samples each) constituting 4 brands of infant's milk powder (for babies after birth), 3 brands of milk-cereal based weaning foods (from up 4 months) and dried milk powder (from up 1 year) were purchased from different shops and pharmacies in Assiut City. These samples were still valid for consumption as shelf life is at least to be one year from production time (2001-2002). They were transferred to the laboratory in their packages to be examined microbiologically to evaluate their quality.

Preparation of samples:

Cartons or cans of samples were cleaned, thoroughly mixed and aseptically opened. Eleven grams of each sample were diluted in 99 ml of peptone water 0.1%, from which decimal dilutions were prepared according to A.P.H.A. (1992).

Microbiological examination:

The prepared samples were subjected to the following examinations:

1- Aerobic plate count (APC), psychrotrophic and thermoduric counts: using Standard plate agar (A.P.H.A., 1992).
2- Anaerobic count: by applying Lactose-Sulphite broth (LS) using MPN technique and incubation at 46°C for 18-24 h (Beerens *et al.*, 1982).
3- Bacillus cereus count: using direct plating technique on Mannitol egg-yolk polymyxin agar (MYP) (Lancette and Harmon, 1980). Isolates were identified morphologically and biochemically according to the methods adopted by MacFaddian (1976).
4- Coliforms count: using Violet red bile agar (A.P.H.A., 1992).
5- Staphylococcus aureus count: using Baird-Parker agar (Baird-Parker, 1962).
6- Total yeasts and molds count: using Sabouraud's dextrose agar with chloramphenicol to adopt the technique recommended by Mislivec *et al.* (1992).

RESULTS

The obtained results were recorded in Tables 1 - 6.

Table 1: Statistical analytical results of microbiological examination of infant milk powder samples.

Types of microorganisms	Positive samples		Count / g		
	No./30	%	Min.	Max.	Average
1- Aerobic plate count	16	53.33	< 10	1×10^5	9.5×10^3
2- Anaerobic count	1	3.33			0.4×10
3- Bacillus cereus count	5	16.66	< 100	2×10^2	2.1×10
4- Coliforms count	-	-	-	-	-*
5- Psychrotrophic count	-	-	-	-	-*
6- Staph. aureus count	-	-	-	-	-*
7- Thermoduric count	-	-	-	-	-*
8- Yeasts and molds count	7	23.33	< 10	9×10^4	3.6×10^3

*Could not be detected on the plate (<10 or <100)

Table 2: Frequency distribution of microbiological examination of infant milk powder samples.

Intervals	Aerobic plate count		Anaerobes		Bacillus cereus		Yeasts & molds Count	
	No.	%	No.	%	No.	%	No.	%
$< 10^2$					1	20.0		
10^2 -< 10^3	3	18.75	1	100.0	4	80.0		
10^3 -< 10^4	5	31.25					5	71.4
10^4 - <10^5	7	43.75					2	28.6
*10^5 -< 10^6	*1	6.25						
Total	**16**	**100.0**	**1**	**100.0**	**5**	**100.0**	**7**	**100.0**

*Unacceptable quality

Table 3: Statistical analytical results of microbiological examination of milk-cereal based weaning food samples.

Types of microorganisms	Positive samples		Count / g		
	No. / 30	%	Min.	Max.	Average
1- Aerobic plate count	11	36.66	< 10	5×10^5	6.2×10^4
2- Anaerobic count	2	6.66	1	3.6×10	0.9×10
3- Bacillus cereus count	-	-	-	-	-*
4- Coliforms count	-	-	-	-	-*
5- Psychrotrophic count	-	-	-	-	-*
6- Staph. aureus count	-	-	-	-	-*
7- Thermoduric count	-	-	-	-	-*
8- Yeasts and molds count	13	43.33	< 10	2×10^5	1.1×10^4

*Could not be detected on the plate (<10 or <100)

Table 4: Frequency distribution of microbiological examination of milk-cereal based weaning food samples.

Intervals	Aerobic plate count		Anaerobes		Yeasts & molds count	
	No.	%	No.	%	No.	%
$< 10^2$			2	100.0		
10^2 -$< 10^3$	1	9.1			2	15.4
10^3 - $< 10^4$					8	61.5
10^4 - $< 10^5$	6	54.5			1	7.7
*10^5 - $< 10^6$	*4	36.4			2	15.4
Total	11	100.0	2	100.0	13	100.0

* Unacceptable quality

Table 5: Statistical analytical results of microbiological examination of dried milk powder samples.

Types of microorganisms	Positive samples		Count / g		
	No. / 30	%	Min.	Max.	Average
1- Aerobic plate count	16	53.33	< 10	8×10^5	6.5×10^4
2- Anaerobic count	-	-	-	-	-*
3- Bacillus cereus count	6	20	< 100	1×10^4	6.0×10^2
4- Coliforms count	-	-	-	-	-*
5- Psychrotrophic count	8	26.66	< 10	9×10^2	9.5×10
6- Staph. aureus count	-	-	-	-	-*
7- Thermoduric count	6	20	< 100	3×10^3	1.3×10^2
8- Yeasts and molds count	18	60	< 10	5×10^4	3.0×10^3

*Could not be detected on the plate (<10 or <100)

Table 6: Frequency distribution of microbiological examination of dried milk powder samples.

Intervals	APC		B.cereus count		Psychrotrophes		Thermoduric		Yeasts & molds count	
	No.	%	No.	%	No.	%	No.	%	No.	%
$< 10^2$			1	16.67	2	25.0	1	16.67		
10^2 - $< 10^3$	2	12.5			6	75.0	4	66.66	6	33.33
*10^3 - $< 10^4$			*4	66.66			1	16.67	10	55.56
*10^4 - $< 10^5$	10	62.5	*1	16.67					2	11.11
*10^5 - $< 10^6$	*4	25.0								
Total	**16**	**100.0**	**6**	**100.0**	**8**	**100.0**	**6**	**100.0**	**18**	**100.0**

* Unacceptable quality.

DISCUSSION

Milk powders are used in many food industries as manufacture of infant formula, ice cream, chocolate, bakery products, sweets as well as supplies to emergency areas in catastrophes and calf milk replacers (Tetra Pak, 1995 and Edgar and Axel, 1998).

The results recorded in Tables 1- 6 showed the min., max. and average values and the frequency distribution of APC, anaerobes, B. cereus, coliforms, psychrotrophes, Staph. aureus, thermodurics and yeasts and molds counts/g of the examined infant milk powder (A), milk-cereal based weaning food (B) and dried milk powder (C) samples.

From the results which given in Tables 1, 3 and 5, it is evident that the APC/g ranged from <10 to 1×10^5, 5×10^5 and 8×10^5 with average values of 9.5×10^3, 6.2×10^4 and 6.5×10^4 of the examined A, B and C samples, respectively. The highest frequency distribution of the corresponding positive samples (43.75, 54.5 and 62.5%, respectively) lie within the range of 10^4 - $<10^5$ (Tables 2, 4 and 6). Relatively similar findings were stated by Jarchovska *et al.* (1980); Saudi *et al.* (1984); Bhatt *et al.* (1992); Al-Ashmawy *et al.* (1993) and El-Shinawy *et al.* (1995). However, lower counts were estimated by Singh *et al.* (1980) (9×10^3), Schwab *et al.* (1982) (52/g), Alvarez Marante *et al.* (1986) and Sabreen (1986) (4.8×10^4), while, higher results were recorded by Moustafa *et al.* (1984). According to the limits proposed by Egyptian Standards (1988), A. P. H. A. (1992) and U. S. Dairy Export Council (1996-2002) of dried milks that, APC must not exceed 5×10^4/g, only one (6.25%), 4 (36.4%) and 4 (25.0%) of examined A, B and C samples, respectively are considered unsatisfactory as recorded in Tables 2, 4 and 6.

Many authors recommended the aerobic plate count as an index of hygienic measures, organoleptic quality, safety and utility of infant foods. It reflects the microbial content of raw materials and ingredients, effectiveness of manufacture techniques and sanitary care of equipments and utensils.

The results outlined in Tables 1 and 3 indicated that 1(3.33%) and 2(6.66%) of examined A and B samples, respectively, contained anaerobes. The highest frequency distribution of the corresponding samples had counts within the range of 10^2- $<10^3$ (Table 2) and $<10^2$ (Table 4). Higher incidences were postulated with Sabreen (1986) (5-20%) of tested local and imported baby foods and infant's milk powder. Moreover, Saad (1995) could detect Clostridium perfringens in 14% of

examined milk powder samples in count ranged from <10-10^2 with an average of 50.48/g. It is worth to mention that, the probability of foodborne illness may occur to children due to the consumption of contaminated products with anaerobes which is indicative of careless methods of productions (Bouer-Hertzberger, 1982). The anaerobic endosporeformer Cl. perfringens has also been associated with food poisoning due to spray dried milk and growth in made-up products again appears to have been a major contributory factor (Varnam and Sutherland, 1994). On the contrary, dried milk powder (C) samples were free from anaerobic microorganisms. Although, these bacteria must be absent from dried instant foods for infants as reported by Wiese (1992), Cl. perfringens was previously isolated from milk and some milk products by El-Bassiony (1980); El-Bassiony and Aboul-Khier (1983) and Amer *et al.* (1986).

Data recorded in Table 1, show that B. cereus was present in 5 (16.66%) with counts varying from <100 - 2×10^2 with an average value of 2.1×10/g of examined A samples. The highest frequency distribution (80.0%) lies between 10^2 - <10^3 (Table 2). Similar finding was recorded by Sabreen (1986) while, higher results were postulated by Singh *et al.* (1980); Moustafa *et al.* (1984); Saad (1985) and El-Shinawy *et al.* (1995). Concerning C samples, 6 (20%) out of 30 found to contain the organism in a min. count of <100 and a max. of 1×10^4 with an average of 6.0×10^2/g as noted in Table 5. These results agree to a certain extent with that obtained by Saad (1985). It is worth to detect that 5 (83.33%) of positive samples, which lie in the range of 10^3- <10^5/g are considered of unacceptable quality (Table 6) which at levels above the Association of Dietetic Food Industries of the European Communities (IDAEC) proposed safety limit of 10^3cfu/g (Rowan and Anderson, 1998). It was suggested that food industry should be concerned with level as low as 10^3-10^4 of food because food intoxication may be caused by ingestion of B. cereus cells or spores which subsequently form enterotoxins in the ileum (Granum *et al.*, 1993). Furthermore, Becker *et al.* (1994) recorded that 54% of infant food samples distributed in 17 countries were contaminated with diarrheagenic B. cereus at level of 0.3-600/g. Thus, the high count indicates lack of adequate precautions taken either during production and processing of raw milk or during handling and distribution of milk powders.

All brands of infant foods including A, B and C samples were free from coliforms. Similar findings were obtained by Jarchovska *et al.* (1980); Singh *et al.* (1980) and El-Shinawy *et al.* (1995). Moreover,

these results were in accordance with Egyptian Standards (1988), A. P. H. A. (1992) and U. S. Dairy Export Council (1996-2002). On the other hand, Schwab *et al.* (1982); Moustafa *et al.* (1984); Saudi *et al.* (1984); Sabreen (1986); Bhatt *et al.* (1992) and Al-Ashmawy *et al.* (1993) pointed out that infant milk powder and other baby foods based on dried milk contained coliform bacteria.

Coliforms can reach the food processing plant and food service establishment from faeces, soil, and water or plant environment. Therefore, they are useful as part of microbiological criteria to indicate the hygienic status during and after processing.

As shown in Table 5, it was evident that 8 (26.66%) of C samples found to harbor psychrotrophes with an average count of 9.5×10/g and 75% of positive samples lie in the range of 10^2-< 10^3 (Table 6). While, none of tested A and B samples contained these microorganisms. Cooling of milk and controlling time and temperature during holding on farm and transportation to the factory is an essential mean for monitoring the hazard associated with psychrotrophes growth (ICMSF, 1988).

None of the evaluated samples showed the presence of Staph. aureus and this result substantiates what have been recorded by El-Bassiony (1984); Moustafa *et al.* (1984) and Sabreen (1986). In contrast, it was isolated from infant food samples examined by Singh *et al.* (1980); Schwab *et al.* (1982); Saudi *et al.* (1984) and El-Shinawy *et al.* (1995). Many incidents of Staph. aureus food poisoning associated with milk powders was traced to direct contamination of powder itself (Mettler, 1989 and Hobbs and Roberts, 1993). Furthermore, intoxication with staphylococcal enterotoxins, which survive subsequent processing, was identified as a major problem of spray dried milk (Varnam and Sutherland, 1994).

Infant milk powder and milk-cereal based weaning food samples (A and B) were free from thermoduric organisms as illustrated in Tables 1 and 3. While, 6 (20%) of dried milk powder samples (C) were contaminated with these organisms in a count ranged from <100 to 3×10^3 with an average of 1.3×10^2/g (Table 5). The highest frequency distribution (66.66%) lies in the range of 10^2 - $<10^3$/g as noticed in Table 6.

A lower frequency distribution (27%) was established by Asperger (1990) who examined milk powder samples for thermophilic organisms. A wide range of thermodurics has been isolated from dried milk powder and their occurrence depends on numbers and types of

bacteria in raw milk, preheating temperature, plant hygiene and dryer operating conditions (Varnam and Sutherland, 1994).

Inspection of Table 1 revealed that 7(23.33%) of A samples were contaminated with yeasts and molds in a count ranged from <10 - 9×10^4 with an average of 3.6×10^3/g. The highest frequency distribution 71.4% lies within the range of 10^3-$<10^4$ as recorded in Table 2. Nearly similar counts were obtained by El-Shinawy *et al.* (1995).

In contrast, higher percentages were outlined by Jesenska and Hardinova (1982) and Sabreen (1986). Moreover, Bhatt *et al.* (1992) stated that Aspergillus fungus was present in 5 out of 6 tested infant formulae samples.

In case of milk-cereal based weaning food (B), 13 out of 30 samples (43.33%) found to contain these organisms in a count ranged from <10 -2×10^5 with an average 1.1×10^4/g (Table 3). As showed in Table 4, it was found that 8 (61.5%) of positive samples existed in numbers ranged from 10^3 - $<10^4$/g.

The obtained findings were coincident with those reported by Singh *et al.* (1980) while higher incidences were postulated by Moustafa *et al.* (1984) (93.33%); Aboul-Khier *et al.* (1985) and Bhatt *et al.* (1992) (66.67%). Al-Ashmawy *et al.* (1993) stated that 100% of all examined local and imported baby food samples were contaminated with molds.

Results recorded in Table 5 verify that 18 (60%) of examined C samples harbor fungi with a min. <10, a max. 5×10^4 and an average 3×10^3/g. 55.56% of samples had yeasts and molds count within the range of 10^3-$<10^4$ as noticed in Table 6. Higher percentages (87.5 and 88%) were estimated by Bhatt *et al.* (1992) and Ismail and Saad (1995), respectively. Yeasts and molds may grow over a wide range of temperature and gain entrance to milk powder either from the milk used, air contamination or utensils. So, their presence is indicative of unsatisfactory sanitation during processing and handling of the product.

The above achieved results declared that milk powder and dried milk-based food are liable to contamination by some pathogenic microorganisms constitute public health hazard. Therefore, scrupulous hygienic measures must be maintained from milking, manufacturing, storage and distribution of finished product. Also, application of HACCP system along the line of dried milk production.

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