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EVALUATION OF THE HYGIENIC ASPECTS OF DRIED MILKS AND INFANT FORMULAS

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ABSTRACT

The purpose of the current study was to explore the hygienic aspects of different dried milks and infant formulas procured from different supermarkets and pharmacies in Assiut city, Egypt. All the samples were examined for some sensory, physical, chemical and microbiological parameters. The obtained results for the examined dried milks and infant formulas showed the mean values of the insolubility index as 3.24 & 2.17 mg, respectively; pH as 6.9 & 7.1, respectively. The chemical parameters showed the results of acidity%, moisture content, total solids%, fat% and SNF%. For the results of coliforms, fecal coliforms & *E. coli*, all the examined samples were acceptable according to the Egyptian Standards. In contrast, *B. cereus* was detected in 33.33 & 26.67% of the examined dried milk and infant formula samples, respectively. It was noticed that all the samples were free from *Cronobacter species*. Unfortunately, the unacceptable samples according to *Cl. perfringens* were 6.67% of the dried milk and 3.33% of the infant formula samples. The average yeasts & molds count was 1.09×10^5 and 1.44×10^5, respectively.

Keywords: Hygienic quality, Dried milks, Infant formulas.

INTRODUCTION

Dairy powders are used as a fortified ingredient in a broad variety of products, such as frozen desserts, baked goods, cheese, yoghurt, hot beverages, soups and various baby foods; therefore, they must be of perfect quality in sensory, nutritional and microbiological criteria for safety during their long shelf life with low storage and transportation costs (Lloyd *et al*., 2004).

One important requirement for the milk powder industry is that the products are assessed for biological hazards and foodborne pathogens, as contamination of these products is mainly due to defects in the processing steps (Abdelkhalek *et al*., 2016; Oyeyipo *et al*., 2017). 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 shelf life and commercial value (Cousins and Bramley, 1987). The quality of dried milk products greatly depends upon the microorganisms of liquid milk, while milk has a high nutritive value, not only for the newborn mammal and the human consumer, but also microbes (Wouters *et al.,* 2002). The degree of storage and transport temperatures may also affect milk powder properties, especially solubility and pH indicator (Jayaro and Henning, 2001).

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Infant formula is a synthetic version of mothers' milk that belongs to a class of materials known as dairy substitutes which are made by blending fats, proteins, and carbohydrates (Crawley and Westland, 2012). When breast-feeding is not possible, desirable, or sufficient; infant formulas are often used as substitutes for human milk and play an indispensable role in infant nutrition (Sola and Navarro, 2006).

According to the aforementioned, the present investigation was designed to assess the hygienic aspect of dried milk &infant formulas present in the local markets of Assiut city, Egypt.

MATERIALS AND METHODS

Sampling:

A total of 60 samples of dried milk and infant formula (30 each) were collected in their retail packages from different supermarkets and pharmacies in Assiut city, Egypt, from October 2022 to January 2023. The 30 dried milk samples were selected equally into packed and unpacked. Moreover, all the available data were recorded in designed sheets including batch no., shelf-life … etc.

Sensory examination:

The sensory evaluation of the powdered milk was done according to Bodyfelt *et al*. (1988). The powdered milk samples were sensory evaluated and scored by a regular score panel. The score was based on hedonic scale provided in a score card comprising the 9 points hedonic scale (from 1= dislike extremely to 9= like extremely). The sensory properties were evaluated depending on the status form of the powdered milk as designed in the following table.

The sensory parameters

Attributes													Resultant
For packaging (4 parameters)				For dried powder (5 parameters)					For reconstituted powder (5 parameters)				
Soiling absence	Sealing absence	Rust absence	Blowing absence	Taste	Odor	Color	Absence of dark particles	Appearance	Taste	Odor	Color	Absence of dark particles	Appearance*	Overall acceptability (OAA)

*The following defects in reconstituted powder should be observed in the estimation of the appearance parameter:

Grainy (visible insoluble particles in reconstituted milk) definition by USDA for reconstituted dry products (minute particles of undissolved powder appearing in a thin film on the surface of the glass)

Churned particles (masses of coalesced fat and/or coagulated protein that may float to the surface & eventually adhere to the side wall)

Pressure (dissolution of lumps as the product is rehydrated & blended)
- -very slight pressure (lumps fall apart with only light touch)
- -slight pressure (only sufficient pressure to disintegrate lumps readily)
- -moderate pressure (only sufficient pressure to disintegrate lumps easily)
- -undispersed lumps (masses of caked or lumpy powder that do not readily dissolve in water)

Physical examination:

1) Insolubility index was done according to IDF Standard 129A (1988) and was expressed as the weight of the sediment after drying (mg).

2) pH value was done according to Wehr and Frank (2004), using a pH meter (AD11, Adwa, waterproof pH-Temp pocket tester with replaceable probe, Romania) on a 10% reconstituted solution of nonfat dry milk powder or a 13% reconstituted solution of dry whole milk powder.

Chemical examination:

1) Titratable acidity was done according to ISO 6091 (1980), in which the method was based on the titration of the sample with sodium hydroxide to the phenolphthalein end point.

The obtained total acidity was expressed as the percentage of lactic acid and was calculated according to the equation:

Lactic acid% = (Vg × 0.009 × 100) / Vm

Vg is the added volume of NaOH solution (ml)

Vm is the reconstituted milk volume used for titration (ml)

0.009 is equivalent to lactic acid normality

2) Moisture content was done according to IS 16072 (2012), in which the sample was dried to constant weight at 102±2° C and the loss in weight was reported as moisture.

$$\text{Moisture\% by mass} = \frac{100(M1-M2)}{M1-M}$$

M = weight of empty covered dish (g)
M_1 = initial weight of the covered dish with the sample before drying (g)
M_2 = final weight of the covered dish with the sample after drying (g)

3) Total solids% = 100 - moisture%

4) Fat% was done according to IS 1224-2 (1977) and was recorded by multiplying the butyrometer reading with 20/3.

5) SNF% = TS% - fat%

6) Starch test was done according to Kumar *et al*. (1998) by using 1 % iodine solution.

Microbiological examination:

Samples preparation (FDA, 2002)**:**
An amount of 10 g was diluted in 90 ml sterile diluent peptone water solution to make a primary solution (10^{-1}), then serial dilutions were prepared.

1) Coliforms count (MPN/g) was done according to AOAC (1980).

2) Fecal coliforms count (MPN/g) was done according to AOAC (1980).

3) *E. coli* count (MPN/g) was done according to AOAC (1980).

4) Isolation of *B. cereus* was done according to Kim and Geopfert (1971).

5) Isolation of *Cronobacter species* was done according to Iversen *et al*. (2008).

6) Detection of anaerobic spore-former was done according to Cruickshank *et al*. (1969).

7) Enumeration of *Cl. perfringens* (MPN) was done according to Beerens *et al*. (1980).

8) Total yeasts & molds count was done according to Houghtby *et al*. (1992).

RESULTS

Table 1: Sensory evaluation of the examined dried milk samples (no. = 30).

Score	Attributes					Resultant	
	Taste	Odor	Color	Absence of dark particles	Appearance	Overall acceptability (OAA)	
	Samples No.	Samples No.	Samples No.	Samples No.	Samples No.	Samples No.	Samples %
1	0	0	0	0	0	0	0.00
2	0	0	0	0	0	0	0.00
3	0	0	0	0	0	0	0.00
4	0	0	0	0	0	0	0.00
5	0	0	0	0	0	0	0.00
6	0	0	0	0	0	0	0.00
7	6	5	0	0	0	11	7.33
8	17	17	4	0	0	38	25.33
9	7	8	26	30	30	101	67.33
Total	30	30	30	30	30	150	100.00

Table 2: Sensory evaluation of the examined infant formula samples (no. = 30).

Score	Attributes					Resultant	
	Taste	Odor	Color	Absence of dark particles	Appearance	Overall acceptability (OAA)	
	Samples No.	Samples No.	Samples No.	Samples No.	Samples No.	Samples No.	Samples %
1	0	0	0	0	0	0	0.00
2	0	0	0	0	0	0	0.00
3	0	0	0	0	0	0	0.00
4	0	0	0	0	0	0	0.00
5	0	0	0	0	0	0	0.00
6	1	2	0	0	0	3	2
7	3	3	0	0	1	7	4.67
8	21	10	5	0	0	36	24
9	5	15	25	30	29	104	69.33
Total	30	30	30	30	30	150	100.00

Table 3: Physical properties of the examined samples.

Parameter	Dried milks (no. = 30)			Infant formulas (no. = 30)		
	Min.	Max.	Avg.	Min.	Max.	Avg.
Insolubility index (mg)	0	11	3.24	0.1	4	2.17
pH	6.3	7.2	6.9	6.8	7.3	7.1

Table 4: Chemical properties of the examined samples.

Parameter	Dried milks (no. = 30)									Infant formulas (no. = 30)		
	Unpacked dried milk (no. = 15)			Packed dried milk (no. = 15)								
				Whole dried milk (no. = 10)			Skim-dried milk (no. = 5)					
	Min.	Max.	Avg.	Min.	Max.	Avg.	Min.	Max.	Avg.	Min.	Max.	Avg.
Titratable acidity%	0.09	0.17	0.14	0.11	0.17	0.15	0.1	0.15	0.13	0.05	0.17	0.07
Moisture%	2	6	4.2	2	4	3.1	2	4	3.4	1	7	3.57
Total solids%	94	98	95.8	96	98	96.9	96	98	96.9	93	99	96.43
Fat%	13.33	45.33	36.4	26.67	46.66	32	4.67	5.33	4.8	2.67	10	4.57
SNF%	49.67	83.67	59.4	51.34	70.33	65	91.33	93.33	91.8	87.4	95	91.86

Table 5: Incidence of coliforms, fecal coliforms & *E. coli* in the examined samples.

Samples	Samples No.	Coliforms		Fecal coliforms		*E. coli*	
		Positives samples*		Positives samples**		Positives samples***	
		No.	%	No.	%	No.	%
Dried milks	30	1	3.33	0	0	0	0
Infant formulas	30	1	3.33	0	0	0	0
Total	60	2	3.33	0	0	0	0

*Positive means gas production in BGLB broth
**Positive means gas production in EC broth
***Positive means EMB plates showing colonies

Table 6: Frequency distribution of coliforms count in the examined samples.

Samples	Coliforms count	Samples No.	Acceptability upon Egyptian Standards (2014)*	Acceptability %
Dried milks (no. = 30)	<3	29	Acceptable	100% acceptable
	3 - 10	1		
Infant formulas (no. = 30)	<3	29	Acceptable	100% acceptable
	3 - 10	1		

*Coliforms count is not >10 CFU/g

Table 7: Incidence of *B. cereus* & *Cronobacter sp.* in the examined samples.

Isolates	Positive samples			
	Dried milks		Infant formulas	
	No./30	%	No./30	%
B. cereus	10	33.33	8	26.67
Cronobacter sp.	0	0	0	0

Table 8: Incidence of *Cl. perfringens* in the examined samples.

Samples	Samples No.	Positives samples*	
		No.	%
Dried milks	30	2	6.67
Infant formulas	30	1	3.33
Total	60	3	5

*Positive means black precipitation & gas production in lactose sulphate broth

Table 9: Frequency distribution of *Cl. perfringens* count in the examined samples.

Samples	*Cl. perfringens* count	Samples No.
Dried milks (no. = 30)	<3	28
	3 - 10	1
	>10 - 100	1
Infant formulas (no. = 30)	<3	29
	3-10	1

Table 10: Incidence of anaerobic spore formers in the examined samples.

Samples	Samples No.	Positives samples*	
		No.	%
Dried milks	30	14	46.67
Infant formulas	30	7	23.33
Total	60	21	35

Table 11. Statistical analytical results of yeasts & molds count.

Samples	Positive samples*		Count/g			Acceptability% upon Egyptian Standards (2014)**	
	No. / 30	%	Min.	Max.	Avg.	Acceptable%	Unacceptable%
Dried milks	23	76.67	1×10^2	1.02×10^6	1.09×10^5	23.33	76.67
Infant formulas	22	73.33	1×10^2	2×10^6	1.44×10^5	26.67	73.33

*Positive means plates showing colonies
**Yeasts & molds count is not >10 CFU/g

Table 12: Frequency distribution of the positive* examined samples based on their yeasts & molds count.

Samples	Dried milks		Infant formulas	
	No.	%	No.	%
10^2 - $<10^3$	8	34.78	8	36.36
10^3 - $<10^4$	4	17.39	4	18.18
10^4 - $<10^5$	8	34.78	7	31.82
10^5 - $<10^6$	1	4.35	2	9.09
$\geq 10^6$	2	8.7	1	4.55
Total	23	100	22	100

*Positive means plates showing colonies

DISCUSSION

The sensory examination of dried milks is one of the most useful and powerful tools in the determination of the validity period of this product and the main method used in the identifying and evaluating of flavors in various dairy products is descriptive evaluation (Drake *et al*., 2003). The obtained sensory results in the present study were recorded in Tables 1 & 2 and revealed that most of the examined samples were graded as like extremely with percentages of 67.33 and 69.33% for the dried milk and infant formula samples, respectively.

The recorded results in Table 3 showed that average values of pH were 6.9 & 7.1 in the examined dried milk and infant formula samples, respectively. These values were higher than that detected by Elrofaei *et al.*

(2021) in dried milks and Sunarić *et al*. (2016) in infant formulas.

The expression of the insolubility index using the weight of the sediment has been found to be a better method in this study to compare different categories of milk powders and is in agreement with the classical differentiation from high to extra low heat milk powders, based on the whey nitrogen protein index (Bylund, 1995). It was found that the average value of the insolubility index for the examined dried milk samples was 3.24 mg and for the examined infant formula samples was 2.17 mg (Table 3). These values were lower than that detected by Pugliese *et al*. (2017) in whole milk powder.

According to the legal requirements of the Egyptian Standards (2014), the recorded results in Table 4 showed the obtained results of both titratable acidity% and moisture% were acceptable, while, the fat% of the skimmed milk samples was higher than the normal range. The Egyptian Standards (2014) stated that the moisture content was not more than 5%, fat content ranged from 26% to less than 42% for whole milk powder, not more than 1.5% for skim milk powder and ranged from 1.5% to less than 26% for partially skimmed milk powder and the titratable acidity% was not more than 1.2% and 1.5% for whole and skimmed milk powder, respectively.

It is known that the addition of starch to dried milks is a type of adulteration. Therefore, all examined samples were checked for the presence of starch, and fortunately, all the samples were starch free.

According to the data presented in Tables 5 & 6, it was apparent that coliforms, fecal coliforms & *E. coli* count in all the examined samples were within the permissible limit of the Egyptian Standards (2014), which means, they were of good quality and sanitary measure during manufacturing.

In the dairy industry, *B. cereus* is traditionally considered the most problematic member of the genus *Bacillus* (Mekillip, 2000). Rapid detection of *B. cereus* in food is important to facilitate the application of quality control measures to eliminate *B. cereus* from food and enhance the diagnosis of food poisoning outbreaks (Rambabu and Kaiser, 2005). The obtained results in Table 7 showed that 33.33% of the examined dried milk samples were contaminated with *B. cereus*. A higher result was obtained by Ibrahim *et al*. (2022). Moreover, 26.67% of the infant formula samples were contaminated with *B. cereus* (Table 7). A similar result was obtained by Ibrahim *et al*. (2022).

When throwing the light towards the isolation of *Cronobacter*, it was found that all the examined samples were free from *Cronobacter species* (Table 7). On the other hand, El-Shall (2013) isolated *Cronobacter species* from infant formula.

Table 8 revealed that 6.67% of the dried milk samples & 3.33% of the infant formula samples contained *Clostridium perfringens*. The Egyptian Standards stated that powdered milks (Egyptian Standards, 2014) and infant formulas (Egyptian Standards, 2006) must be free from pathogenic microorganisms and their toxins, so, all the contaminated samples with *B. cereus* and/or *Cl. perfringens* were considered incompatible with the Egyptian Standards.

The given data in Table 10 showed that the stormy fermenter microorganisms were present in 46.67% of the examined dried milk samples, while in the case of the infant formula they were found in 23.33% of the examined samples. It was rational that all the positive samples for *Cl. perfringens* were positive in the stormy fermentation test (Tables 8 & 10).

For yeasts & molds, Table 11 cleared that 76.67% and 73.33% of the present examined dried milk and infant formula samples, respectively were fungal contaminated and unacceptable according to the legal requirement of the Egyptian Standards (2014).

In conclusion, the overall view of the examined dried milks and infant formulas was of a good keeping quality; in which most of the examined samples were of good sensory evaluation; moreover, all the examined samples were free from starch and also acceptable for coliforms, fecal coliforms & *E. coli*; and free from *Cronobacter species*.

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تقييم الجوانب الصحية للألبان الجافة وتوليفات الأطفال

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هدفت هذه الدراسة إلى تقييم الجوانب الصحية لمختلف أنواع الألبان الجافة وتوليفات الأطفال التي تم جمعها من محلات السوبر ماركت والصيدليات فى مدينة أسيوط, مصر، وقد تم فحص جميع العينات لبعض الخواص الحسية والفيزيائية والكيميائية والميكروبيولوجية، وأظهرت النتائج أن متوسط قيم مؤشر عدم الذوبان للألبان الجافة وتوليفات الأطفال هي ٣,٢٤ و ٢,١٧ مجم على التوالى, والأس الهيدروجيني ٦,٩ و ٧,١ على التوالى. وقد أظهر الفحص الكيميائي نتائج نسبة الحموضة ومحتوى الرطوبة ونسبة المواد الصلبة الكلية ونسبة الدهون ونسبة المواد الصلبة غير الدهنية. وقد كان عدد القولونيات والقولونيات البرازية والإيشيريشيا كولاي في جميع العينات المفحوصة مقبول مقارنة بالمواصفات القياسية المصرية، وعلى العكس، فقد تواجدت الباسيلس سيريس بنسب ٣٣,٣٣ و ٢٦,٦٧% من عينات الألبان الجافة وتوليفات الأطفال، على التوالى. وقد لوحظ أن جميع العينات كانت خالية من ميكروبات الكرونوباكتر. وللأسف، كانت نسب العينات الغير مقبولة وفقا لوجود الكلوستريديم برفرنجنز هى٦,٦٧% من الألبان الجافة و ٣,٣٣% من توليفات الأطفال. وقد تواجدت الخمائر والأعفان بمتوسط أعداد ١,٠٩×١٠°, ١,٤٤×١٠° على التوالى.

الكلمات المفتاحية: الجودة الصحية، الألبان الجافة، توليفات الأطفال