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SAFETY AND QUALITY ASPECTS OF WHOLE AND SKIMMED MILK POWDERS

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ABSTRACT

Background. Nowadays, dried milk products are highly traded and consumed all over the world, so we aimed in this study to evaluate to what extent whole and skim milk powders are safe and comply with Egyptian standards.

Materials and methods. Eighty samples of dried milk (50 whole milk powder and 30 skim milk powder) were gathered from several retailers and supermarkets for evaluation of their differing quality and safety parameters.

Results. The most frequent off-flavors recovered from whole milk powder samples were cooked ones and, in the case of skim milk powder samples, flat ones. Five samples of whole milk powder were of fair quality and three samples of poor quality, according to the sensory evaluation. The compositional parameters, moisture, %, fat, %, protein, %, and acidity, %, were measured as mean values of 3.90 ±0.15, 26.90 ±0.19, 25.53 ±0.27, and 0.99 ±0.03% in the examined whole milk powder samples and 3.77 ±0.08, 1.11 ±0.05, 34.62 ±0.29, and 1.22 ±0.03% in the examined skimmed milk powder samples, respectively. These results were within the range of component requirements set by the Egyptian Standard (2014; ES: 1780/2014) for dried milk products. Also, the microbiological safety of the milk powder samples was analyzed by assessment of the total viable count, total yeast and mold count, Coliforms count, *Enterobacteriaceae*, *E. coli*, *C. sakazakii*, *Staphylococcus aureus*, *Salmonella*, and *Listeria monocytogenes*. *Staphylococcus aureus* was the most prevalent isolate (36.00% and 6.67%) followed by *Enterobacteriaceae* (20.00% and 3.33%), of whole and skim milk powder, respectively. *Enterobacteriaceae* isolates included *Enterobacter cloacae* ssp. *Cloacae*, and *Pantoea* spp., which were specified by traditional biochemical tests and Vitek2 system. All *Enterobacteriaceae* isolated spp. were resistant to cephalothin, neomycin, tobramycin and colistin sulphate, and sensitive to chloramphenicol, gentamycin and nalidixic acid. *E. coli*, *C. sakazakii*, *Salmonella*, and *Listeria monocytogenes* couldn't be isolated from all the tested samples. By using Inductive Coupled Plasma – Mass Spectrometer (ICP-MS), we could measure lead and mercury as mean values of 0.243 ±0.069 and 0.261 ±0.052 mg/kg for whole milk powder samples at a percentage of 68.00 and 34.00%, while for the skim milk powder samples they were 0.150 ±0.037, and 0.347 ±0.110 mg/kg at a percentage of 66.67 and 40.00%, respectively.

Conclusion. Finally, thirty-four whole milk powder and twelve skimmed milk powder samples didn't comply with Egyptian standards, so it is necessary for authorities to put more attention on this and regular monitor it.

Keywords: whole milk powder, skim milk powder, *Staphylococcus aureus*, spectrometer, *Enterobacteriaceae*, Vitek2 system

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INTRODUCTION

Milk and dairy products are considered to be an important widely consumed nutritional source for humans, especially children and elderly adults, all over the world, as they contain all the necessary nutrient components, which are milk fat, lactose, milk casein, whey proteins, fat-soluble and some water-soluble vitamins, as well as mineral elements. One of the highest primary and vital dairy products is dried milk products, which contain fundamental milk nutrients in addition to nutritional supplements required for young kid's growth and improvement (Abdelkhalek et al., 2015; Youssif et al., 2020).

Whole and skim milk powder are used principally as powdered dairy products for their nutritional, functional, and sensory properties in a wide range of beverages and foods. Their sensory properties are considered to be important criteria in the grading of these powders, and flavor has been the main thing used in evaluation acceptance, as has the shelf life of the powdered product (Drake et al., 2003; Park and Drake, 2014). Nowadays, these 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, so they must be of perfect quality in sensory and nutritional composition, and microbiological criteria for safety during their long shelf life with low storage and transportation costs (Lloyd et al., 2004). In addition to this, the quality of their chemical constituents, which include the proportion of moisture, total solids, fat, total protein, lactose, titratable acidity, and ash, must be high (Afrin and Shilpi, 2018).

One milk powder industry requirement 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). There are several microorganisms which can occur in dairy powders and adversely affect the human body, such as *E. coli*, *Salmonella*, *Staphylococcus aureus*, and *Listeria monocytogenes*. These pathogens have the ability and viability to remain in these dried products for an extended period after the powder has been rehydrated and stored at a temperature suitable for regrowth of these microbial pathogens, so these organisms must be nonexistent in powdered milk (Afrin and Shilpi, 2018; Pal et al., 2016). Powdered milk contains high concentrations of minerals and carbohydrates that may help in enterotoxin production and proliferation, especially when they are rehydrated and held at suitable temperatures for extended periods, causing food poisoning mainly in immune-compromised persons (Tunio et al., 2013).

Dried milk products can be contaminated with different toxic metals through the exposure of milking cows to pollution or contaminated water, feed, or feeding materials, leading to raw milk contamination. Other sources that where elements can enter directly into products are the water and equipment used in manufacturing, as well as the packaging materials used. These toxic metals are known to accumulate destructive effects, leading to chronic diseases, particularly in the nervous system and vital organs, like the kidneys and liver. In addition, some of them may have carcinogenicity and teratogenicity implications depending on their role in enzymatic restriction, and antioxidant impairment with free radical production (Abdelkhalek et al., 2015). To achieve our aim of evaluating the safety and quality parameters of whole and skim dried milk, we investigated sensory, compositional, and microbiological criteria, measuring the degree of antibiotic susceptibility of *Enterobacteriaceae* isolates and determining some toxic elements (Pb, Hg, Cd, and As) as well as the acceptability degree of these powdered samples based on Egyptian standards.

MATERIALS AND METHODS

50 whole milk powder and 30 skim milk powder samples were collected as common trade goods from different shops and supermarkets in the Cairo and Giza Governorates, which were imported and repacked by Egyptian companies.

Sensory examination

It was done according to Clark et al. (2009), Indian Standard (IS: 10030-1982), after opening, each sample was reconstituted immediately by weighing 10 g of skim dry milk and 13 g of whole dry milk and adding it to 100 ml of distilled water in a glass container, before being covered and thoroughly mixed and allowed to stand for about an hour at 24°C before evaluation.

Finally, the reconstituted milk samples were examined for color, flavor (taste and odor), appearance and package. The grading of samples was done in the following groups: excellent (90 and above), good (80–89), fair (60–79), and poor (59 or less).

Chemical composition analysis

All whole and skim milk powders were assessed for the percentage of fat (IS 1224-2/1977), moisture and total solids (AOAC, 2000), and protein using the formol method (Kumar and Seth, 2004). In addition, titratable acidity was determined according to AOAC (2000).

Preparation of samples for microbiological examination

10 g of milk powder was transferred directly into 90 ml of sterile peptone water 0.1% as a diluent and homogenized for 1 minute as preparation for decimal serial dilution, according to APHA (2004).

Total Viable Count. One ml from each of the previously prepared decimal dilutions was carefully transferred to a sterile petridish, into which standard plate count agar (Oxoid, CM0463) was poured. The inoculated plates were incubated in an inverted position at 32°C for 72 hours (APHA, 2004).

Total Yeast and Mold Count. Yeast Extract Dextrose Chloramphenicol Agar (HiMedia, M1590) was poured onto the plates with the inoculums. Then, the plates were put into an incubator in an upright position for 5 days at 25°C (ISO, 2012).

Total Staphylococci Count. 1 ml of all dilutions to be plated was distributed and spread over the surface of 3 plates of Baird-Parker agar (Oxoid, CM1127) at 0.4 ml, 0.3 ml, and 0.3 ml, with the plates being retained in an upright position until the inoculum was completely absorbed by the agar (about 10 min). Finally, the plates were inverted and incubated for 45–48 h at 35–37°C. All the purified isolates were identified morphologically using Gram staining and biochemically by catalase, coagulase tests, and anaerobic utilization of mannitol (BAM, 2016).

Coliforms Count, MPN/g, and isolation of *E. coli*. 1 ml of appropriately prepared dilutions was inoculated in 3 tubes of Lauryl Sulphate Tryptose (LST) broth (Oxoid, CM0451) and incubated for 48 hours at 35°C with observation for gas production. Then, only the positive tubes were subcultured into Brilliant Green Lactose Bile Broth 2% (Oxoid, CM0031) after 48 h of incubation at 35°C. The confirmed MPN/g was calculated from the results of the positive gas tubes and a loopful was streaked onto Eosin Methylene Blue (EMB) agar plates (Oxoid, CM0069), then incubated at 35°C for 48 h (APHA, 2004).

Cronobacter spp. and other *Enterobacteriaceae* isolation. 25 grams of each powdered milk sample was mixed with 225 ml of buffered peptone water (Oxoid, CM0509) and kept in an incubator at 37°C for 18 ±2 h. Only 0.1 ml of each pre-enriched buffered peptone water was transferred into 10 ml of modified Lauryl Sulfate Tryptone broth (mLST; Oxoid CM1133) with a vancomycin supplement (10 mg/L; Oxoid, SR0247E); these broths were incubated at 44 ±0.5°C/24 h. Then, from all the enriched cultures, a loopful was streaked onto Violet Red Bile Glucose Agar (Oxoid, CM0485) and the plates were incubated at 37°C for 24 h. Finally, five red and/or purple colonies circled by a halo of purple color were picked up and streaked onto plates of Tryptic Soya Agar (TSA; Oxoid, CM0876), which were incubated for 72 h at 25°C. Each yellow and non-yellow pigmented colony on the TSA was picked off for further identification (ISO/IDF, 2006; El-Zamkan and Mohamed, 2018).

Isolation of *Salmonella*. From each previous homogenized sample, only one decimal ml was inoculated in a sterile tube of 10 ml Rappaport Vassiliadis Broth (Oxoid, CM0669), which was incubated at 42°C for 24 h. After incubation, a loopful was streaked onto Xylose Lysine Deoxycholate Agar (Oxoid, CM0469) and all the plates were incubated at 37°C for 24 h (ISO, 2002).

Identification of *Enterobacteriaceae* isolates. All isolates were identified conventionally according to (Whitman et al., 2015) using the following tests: Gram's stain, oxidase, catalase, motility, indole, methyl red, voges proskauer, citrate production, Triple

Sugar Iron (TSI), dulcitol and sorbitol fermentation, arginine and lysine decarboxylase and yellow pigmentation on TSA. These were followed by the identification of isolates using the Vitek2 compact system according to (BioMérieux, 2013). The results were gained and printed automatically within 8 h.

Measuring of antimicrobial sensitivity for isolated *Enterobacteriaceae* spp. by the Kirby-Bauer disc diffusion procedure according to NCCLS (2002). The following antimicrobial disks and its concentrations were used: Streptomycin – S (10 µg), Chloramphenicol – C (30 µg), Cephalothin – KF (30 µg), Gentamycin – GEN (10 µg), Neomycin – N (10 µg), Tobramycin – TOB (10 µg), Colistin sulphate – CT (10 µg), Piperacillin – PRL (100 µg) and Nalidixic acid – NA (30 µg). The assessment of findings was accomplished according to CLSI standards (2017).

Isolation of *Listeria monocytogenes*. 25 g of sample was added to 225 ml of pre-warmed half Fraser broth and incubated at 30°C for 25 ±1 h. 0.1 ml of each incubated half Fraser broth was cultured into 10 ml of Fraser broth at 37°C for 48 ±2 h in an incubator. Then, all of these were sub-cultured onto Oxford agar plates at 37°C for up to 52 h and examined every 24 h (ISO, 2017).

Analysis of samples for their content of trace metals (lead – Pb, arsenic – As, cadmium – Cd and mercury – Hg) according to AOAC (2012)

The technique of Inductive Coupled Plasma-Mass Spectrometer (ICP-MS) "Perkin-Elmer model optima 2000DV, Waltham, USA" was applied in the analytical lab at the Regional Center for Food and Feed, Agriculture Research Center, Giza, Egypt. Accounting of the estimated daily intake of toxic metals was done using the following equation:

$$\text{Estimated daily intake, mg/kg body weight/day} = \frac{\text{concentration of analyzed metals, mg/kg} \times \text{concentration of daily intake of dried milk products, g/day}}{\text{average body weight of adult person (70 kg)}}$$

The average daily dosages of whole and skim milk powder (milk consumption) recommended on instruction labels and from the Nutrition Institute (1996) was 200 ml of reconstituted milk powder/day; with 26 g/day and 20 g for whole and skim milk powders, respectively. We compared the EDI with PTDI set by JECFA (2018).

RESULTS AND DISCUSSION

Sensory evaluation of the examined whole and skim milk powder samples

Dried milk sensory examination 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). Therefore, we decided to determine the sensory quality of the powdered samples and the results of sample grading are shown in Figure 1; most of the samples were graded as excellent with a percentage of 60.00 and 56.67 for whole and skim milk powder, respectively. Only 10.00% and 6.00% of whole milk powder were of a fair and bad grade, respectively.

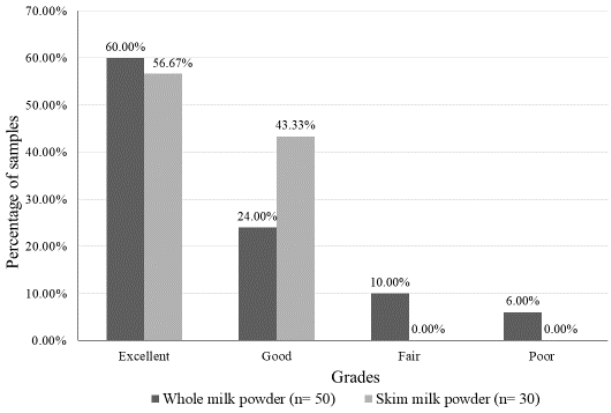

Fig. 1. Grading of the examined whole and skim milk powder samples based on their sensory properties: n – number of examined samples

The findings exhibited in Figure 2 revealed that the highest off-flavor observed in the examined samples was the cooked flavor in 48.00% of the whole powder samples and the flat flavor in 53.33% of the skim milk powder samples. The ideal characteristic flavor of rehydrated dried whole milk is to be rich, fresh, sweet, pleasant, and clean. The American Dairy Products

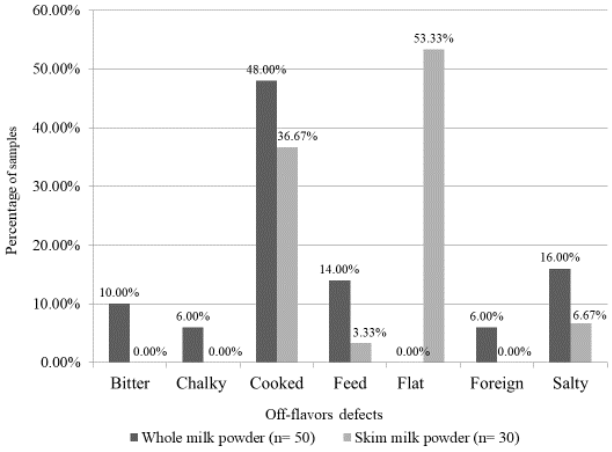

Fig. 2. Occurrence of off-flavors in the examined milk powder samples: n – number of examined samples

Institute (2002) announced that extra Grade whole milk powder could retain a definite cooked flavor with a slight feed flavor but should be free from other unfavorable flavors. High quality skimmed milk powder has to be like that of fresh skim fluid milk after its reconstitution, with a mainly flat, clean, and pleasant flavor and a slight cooked flavor, as according to US standards reconstituted low fat dried milk may have a chalky, feed, cooked, or flat flavor to a certain extent (Drake et al., 2003; USDA, 2001).

Different reconstituted milk powder off-flavors, such as cooked, flat, salty, chalky, bitter, feed, lipolytic, oxidized, acidic, metallic, and buttery flavors, have been reported in various studies (Abdalla et al., 2017; Drake et al., 2003; Kobayashi and Nishimura, 2014). Mainly, these sensory defects may be attributed to the use of poor-quality raw milk, bad hygienic handling, and manufacturing with bad and incorrect product storage (Hough et al., 2002). In addition, the type of heat treatment used during dried milk production could raise the possibility and intensity of cooked flavors developing in the final powder (Abdalla et al., 2017; Drake et al., 2003).

Statistical analysis of the compositional parameters for the examined milk powder samples

Data summarized in Table 1 show: the minimum to maximum moisture, %, TS, %, fat, %, protein, %, and titratable acidity, %, of dry whole milk samples, which were 2.20–5.00, 95.00–97.80, 26.00–28.70, 25.33–27.84 and 0.75–1.20 respectively. The levels of moisture, fat, and protein determined in the study are in agreement with those found by Sabah El Khier and Yagoub (2009). The mean values of moisture, %, TS, %, fat, %, protein, %, and titratable acidity, %, were 3.77 ±0.08, 96.25 ±0.08, 1.11 ±0.05, 34.62 ±0.29 and 1.22 ±0.03 for the examined dry skim milk samples, respectively. The compositional parameters of the examined skim milk powders were almost identical to those reported by Patil et al. (2016).

According to the statistical analysis of the data presented in Table 1, and the results of the milk powder

Table 1. Statistical analytical results of the compositional parameters in the examined milk powder samples

Parameters %	Values	Type of sample	
		whole milk powder (n = 50)	skim milk powder (n = 30)
Moisture	minimum	2.20	3.00
	maximum	5.00	4.33
	mean ±SEM	3.90 ±0.15	3.77 ±0.08
Total solids	minimum	95.00	95.67
	maximum	97.80	97.00
	mean ±SEM	96.05 ±0.15	96.25 ±0.08
Fat	minimum	26.00	0.75
	maximum	28.70	1.40
	mean ±SEM	26.90 ±0.19	1.11 ±0.05
Protein	minimum	25.33	32.20
	maximum	27.84	36.70
	mean ±SEM	25.53 ±0.27	34.62 ±0.29
Titratable acidity	minimum	0.75	1.00
	maximum	1.20	1.40
	mean ±SEM	0.99 ±0.03	1.22 ±0.03

n – number of samples examined, SEM – standard error mean.

analysis showed its characteristics are higher protein, lactose, mineral contents and low moisture content. The high protein values of the skimmed milk powder samples were attributed to their high solid contents, not fat contents, when compared with values from the full-fat powder samples. Consequently, all the samples of dried milk were within the limit of the Egyptian standards (ES: 1780/2014), as in all samples, the moisture content was not more than 5.00%, fat content ranged from 26.00% to less than 42.00% for whole milk powder and not more than 1.5% for skim milk powder, and the titratable acidity percentage was not more than 1.20% and 1.50% for whole and skimmed milk powder, respectively. These results agreed with the findings of Aly and Elewa (2014). The slight variation in milk and the composition of its products is mainly attributed to seasonal changes, lactation stage, diet, herd calving pattern, animal health, and weather (O'Callaghan et al., 2016).

Microbiological safety of the examined milk powder samples and evaluation of their compatibility with Egyptian standards

Powdered milks are mainly contaminated with different pathogens from water, air and processing, storage equipment and containers, and raw milk is one of the important sources of microorganisms in dried products through poor handling, uncleaned dairy equipment and bulk tank, feed, and bedding (Afrin and Shilpi, 2018; Faille et al., 2014).

The data illustrated in Table 2 shows that the minimum Total Viable Count was 100 CFU/g and the maximum was 5.00×10^3 CFU/g, with a mean value of $4.97 \times 10^2 \pm 1.21 \times 10^2$ CFU/ g, and the highest frequency distributed between 10^2 to $<10^3$ for the examined whole milk powder samples. These results were lower than data reported by Oyeyipo et al. (2017) and Afrin and Shilpi (2018), and nearly similar to those reported by Ahmed and Anwar (2006) and Sabah El

Table 2. Statistical analytical results of the microbiological parameters of milk powder samples

Type of sample	Positive samples		Parameters	Values		Mean ±SEM
	no.	%		minimum	maximum	
Whole milk powder (n = 50)	50	100.00	Total Viable Count, CFU/g	10^2	5.00×10^3	$4.97 \times 10^2 \pm 1.21 \times 10^2$
	2	4.00	Total Yeast Count, g	0.60×10^2	0.70×10^2	$0.65 \times 10^2 \pm 0.05 \times 10^2$
	0	0.00	Total Mold Count, g		<10	
	0	0.00	Coliforms MPN, g		<3	
	33	66.00	Total Staphylococci Count, CFU/g	<10	5.00×10^2	$0.94 \times 10^2 \pm 0.21 \times 10^2$
	18	36.00	Coagulase Positive Staphylococci Count, CFU/g	10	10^2	$0.27 \times 10^2 \pm 0.06 \times 10^2$
Skim milk powder (n = 30)	30	100.00	Total Viable Count, CFU/g	20	7.00×10^2	$2.25 \times 10^2 \pm 0.40 \times 10^2$
	0	0.00	Total Yeast and Mold Count, g		<10	
	0	0.00	Coliforms, MPN/g		<3	
	6	20.00	Total Staphylococci Count, CFU/g	<10	30	$0.03 \times 10^2 \pm 0.02 \times 10^2$
	2	6.67	Coagulase Positive Staphylococci Count, CFU/g	10	10	10 ±0.00

n – number of examined samples, no. – number of positive samples, SEM – standard error mean.

Khier and Yagoub (2009). Only two of the samples were positive for yeast microorganisms, with a mean value of $0.65 \times 10^2 \pm 0.05 \times 10^2$ CFU/g. While $0.94 \times 10^2 \pm 0.21 \times 10^2$ CFU/g is the mean value of thirty-three positive whole milk powder samples for Total Staphylococci Count and 18/50 samples were positive for Coagulase in a count range of $10–10^2$ CFU/g at a percentage of 32.00. Oyeyipo et al. (2017) recorded findings higher than our Staphylococci count but Ahmed and Anwar (2006) had results almost similar to our reported count. All the whole powder samples had a coliforms count <3 MPN/g, which is in disagreement with Oyeyipo et al. (2017), whose examined samples had a coliforms count ranging from 35 ±0.23 MPN/g to 92 ±0.05 MPN/g, which agreed with data obtained by Ahmed and Anwar (2006) and Sabah El Khier and Yagoub (2009).

However, all the skim milk powder samples were positive for Total Viable bacteria with counts of a minimum of 20 CFU/g and a maximum of 7.00×10^2 CFU/g. This finding was nearly similar to data obtained by Yacoub et al. (2017). Only 20.00% of the samples were positive for Total Staphylococci Count with a mean value of $0.03 \times 10^2 \pm 0.02 \times 10^2$ CFU/g. All thirty samples of dried skim milk had a count of less than 10 CFU/g of Total Yeast and Mold Count and <3 MPN/g for Coliforms. *S. aureus* is well recognized as causing food intoxication, especially at a high growth rate ($\geq 10^6$) in several types of food, as in this case, it is able to generate heat tolerant enterotoxins. Besides this, it has the capability to survive in dried milk products for a long time with the absence of competition from microorganisms, so, without growth limitation, *S. aureus* could subsequently produce enterotoxins after reconstitution (Tunio et al., 2013).

Faille et al. (2014) reported that biofilm formation during dairy processing was the major cause of the recurrence of microbial contamination in final products, due to improper cleaning and sanitation. The amount of heat treatment exposure in the spray-drying method wasn't enough to make milk powder pathogen-free or to reduce post-manufacturing contamination from food processing environments. So, raw milk must be pasteurized first (Afrin and Shilpi, 2018; Pal et al., 2016).

According to the data displayed in Table 3, the most prevalent isolated microorganism from powdered milk was *Staphylococcus aureus* (total 25 isolates), which was isolated from 18 whole milk powder samples and

Table 3. Prevalence of *Enterobacteriaceae* species*, Staphylococcus aureus,* coliforms and *Listeria monocytogenes* in both examined types of milk powder

Organisms	Sample type	Positive samples		Number of isolates
		no.	%	
Enterobacteriaceae	whole milk powder	10	20.00	11 (8 *Enterobacter cloacae* ssp. *cloacae* and 3 *Pantoea* spp.)
	skim milk powder	1	3.33	1 (*Enterobacter cloacae* ssp. *cloacae*)
Staphylococcus aureus	whole milk powder	18	36.00	23
	skim milk powder	2	6.67	2
Coliforms	whole milk powder	8	16.00	8
	skim milk powder	1	3.33	1
C. sakazakii, *Salmonella*, *E. coli*	whole milk powder	0	0.00	0
	skim milk powder	0	0.00	0
Listeria monocytogenes	whole milk powder	0	0.00	0
	skim milk powder	0	0.00	0

n – number of examined samples, no. – number of positive samples.

2 skim milk powder samples at a percentage of 36.00 and 6.67 respectively. These results were similar to Oyeyipo et al. (2017), who isolated *Staphylococcus aureus* as the most prevalent isolate (50.20%). In addition, a total of 12 *Enterobacteriaceae* isolates were contaminated: ten (20.00%) of the whole milk powder samples, and one (3.33%) of the skimmed milk powder samples.

Exactly nine of the isolated *Enterobacteriaceae* spp. belonged to the coliforms group. All the studied samples were uncontaminated by *Listeria monocytogenes.* The *Enterobacteriaceae* species were identified as follows: only one *Enterobacter cloacae* ssp. *cloacae* from the skimmed milk powder samples and eight for the whole milk powder, with three *Pantoea* spp., unaccompanied by *C. sakazakii*, *Salmonella* and *E. coli*. The aforementioned findings were agreeable with those mentioned by Zakaria et al. (2018), as they didn't find *Salmonella*, *E. coli*, or *Listeria monocytogenes* in any of their examined samples. In addition, the data were acceptable to a survey by Iversen and Forsythe (2004), in which they isolated 13 *Enterobacter cloacae* and 4 *Pantoea* spp. from milk powder samples. *Pantoea* spp. was found to cause septicemia, pneumonia, urinary, respiratory, and nervous system infections, as well as abscesses with septic arthritis, while this opportunistic organism is mainly invasive, toxic, and infectious to persons with a weak immune system (Mardaneh and Dallal, 2013). Yan et al. (2012) explained that *Enterobacteriaceae* species are mainly distributed in a dried product environment and can be judged as the microorganism indicator for the sanitary status of powder milk.

Enterobacter spp. and *Pantoea* spp. have been found to be highly desiccation resistant, with the ability to sustain and remain alive in powdered milk and its processing environment (Iversen, 2014). *E. cloacae* is a predominantly opportunistic pathogen (in the animal environment), frequently causing pneumonia, abscesses, necrotizing enterocolitis and gastrointestinal conditions in immuno-compromised adults (Iversen, 2014; Iversen and Forsythe, 2004; Nesma et al., 2020). All distinguished species were susceptible to chloramphenicol, gentamycin, and nalidixic acid antimicrobials, but were highly resistant to cephalothin, neomycin, tobramycin, and colistin sulphate antibiotics. Streptomycin antibiotics didn't succeed in inhibiting the growth of 100.00% of *Pantoea* spp. and 77.80% of *Enterobacter cloacae* ssp. *cloacae* isolates, with intermediate inhibition for 2 *Enterobacter cloacae* ssp. *cloacae* growth. Only four *Enterobacter cloacae* ssp. *cloacae* and two *Pantoea* spp. were resistant to piperacillin (Table 4). This concurs with the research of Mardaneh and Dallal (2013), who were able to isolate highly resistant *Pantoea* spp. and Iversen (2014), who reported that *Enterobacter* spp. has a major resistance to antibiotics, which is considered to be a globally serious problem. The multiple antibiotic resistance observed in isolated species is an indication that these strains, found in contaminated

Table 4. Degree of antimicrobial resistance pattern of *Enterobacteriaceae* species from the examined powdered milk samples

Species		Degree of antibiotic resistance pattern								
		S	C	KF	GEN	N	TOB	CT	PRL	NA
Enterobacter cloacae ssp. *cloacae* (no. = 9)	S	0	9 (100%)	0	9 (100%)	0	0	0	0	9 (100%)
	I	2 (22.2%)	0	0	0	0	0	0	5 (70%)	0
	R	7 (77.8%)	0	9 (100%)	0	9 (100%)	9 (100%)	9 (100%)	4 (30%)	0
Pantoea spp. (no. = 3)	S	0	3 (100%)	0	3 (100%)	0	0	0	0	3 (100%)
	I	0	0	0	0	0	0	0	1 (33.3%)	0
	R	3 (100%)	0	3 (100%)	0	3 (100%)	3 (100%)	3 (100%)	2 (66.7%)	0

no. – number of isolates, S – susceptible, I – intermediate resistant, R – resistant, S – streptomycin, C – chloramphenicol, KF – cephalothin, GEN – gentamycin, N – neomycin, TOB – tobramycin, CT – colistin sulphate, PRL – piperacillin, NA – nalidixic acid.

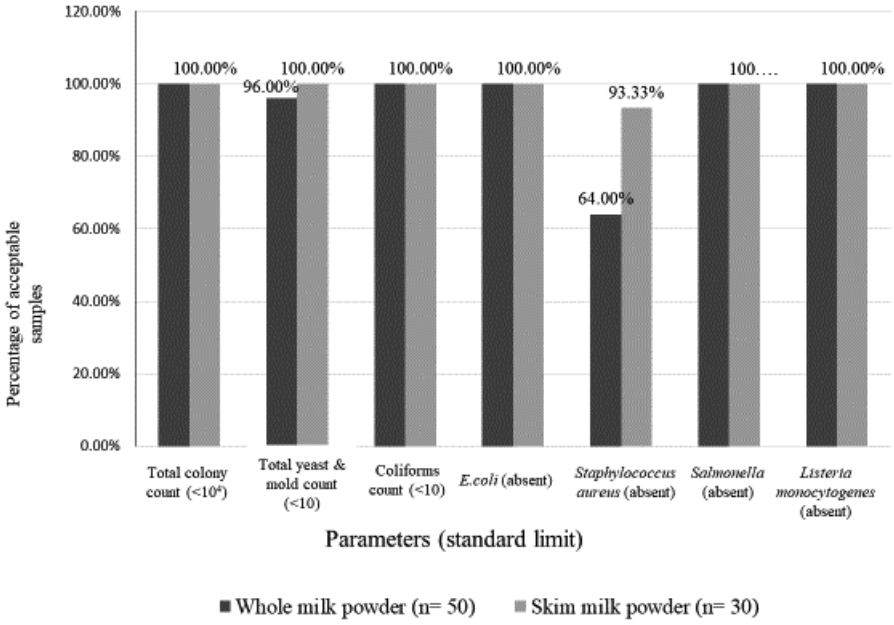

Fig. 3. Degree of microbiological acceptability of whole and skim milk powder samples vs. Egyptian standards ES: 1780/2014: n – number of examined samples

powdered milk, originated from the surroundings, where numerous antimicrobials were applied. This data agrees with Oyeyipo et al. (2017). The occurrence of resistance to antimicrobial agents is considered to be a global health issue, causing increased morbidity and death rates as it frequently makes curing microbial infections difficult (Falegan and Oluwaniyi, 2015).

According to Egyptian standards (ES: 1780/2014), only 32 samples of whole milk powder were accepted in a percentage of 64.00, and other 18 samples were contaminated by Staphylococcus aureus, also 2 samples of skimmed milk powder were unacceptable due to contamination by *Staphylococcus aureus* as illustrated in Figure 3.

In addition, the results of this analysis of dried milk were similar to data obtained by Abdelkhalek et al. (2016), who concluded that samples contaminated with *Staphylococcus aureus* were unacceptable when compared to legal Egyptian limits. Suitable manufacturing practices and the implementation of Hazard Analysis and Critical Control Points (HACCP) systems are required for products with the highest levels of safety (Hafiz et al., 2016; Ibrahim et al., 2020).

Analysis of toxic metal concentrations and degree of acceptability of the examined powdered milk samples

As illustrated in Table 5, both whole and skim milk powders were contaminated with lead at a mean value of 0.243 ±0.069 and 0.150 ±0.037 mg/kg and a percentage of 68.00% and 66.67%, respectively. These lead concentrations in the examined whole milk powder samples were higher than data obtained by Abdelkhalek et al. (2015), and lower than Elbarbary and Hamouda (2015), while the results of the skim milk powder lead concentrations were higher than those found by Abdelkhalek et al. (2015) and Zakaria et al. (2018). Navas-Acien et al. (2009) stated that the toxic metal, lead, has different serious teratogenicity effects, causing kidney dysfunction, reproductive system impairments and inhibition of hemoglobin synthesis with acute and chronic destruction of nervous and cardiovascular systems.

Only seventeen samples of dried whole milk were contaminated with mercury in concentrations in the range 0.310–0.315 mg/kg, and a mean value of 0.261 ±0.052 mg/kg. These results were higher than the data found by Elbarbary and Hamouda (2015). For the

Table 5. Toxic metal levels in the examined powdered milk samples

Type of samples	Positive samples		Metal	Values		Mean ±SEM mg/kg	EDI
	no.	%		minimum	maximum		
Whole milk powder (n = 50)	34	68.00	Pb	0.150	0.393	0.243 ±0.069	0.0001
	17	34.00	Hg	0.310	0.315	0.261 ±0.052	0.0001
	16	32.00	Cd	0.007	0.010	0.008 ±0.002	0.000003
	0	0.00	As	<0.008			
Skim milk powder (n = 30)	20	66.67	Pb	0.136	0.246	0.150 ±0.037	
	12	40.00	Hg	0.490	0.540	0.347 ±0.110	
	0	0.00	Cd	<0.001			
	0	0.00	As	<0.008			

n – number of examined samples, no. – number of positive samples, SEM – standard error mean, Pb – lead, Hg – mercury, Cd – cadmium, As – arsenic, EDI – estimated daily intake.

same metal, 12 samples of examined dried skim milk were contaminated at a maximum level of 0.540 mg/kg, with contamination values higher (Table 5) than those recorded by Zakaria et al. (2018). The existence of mercury in all milk products might be imputed to the serving of impure water and feed with metal pollution to dairy cows with the usage of a huge amount of pesticides and fungicides, which may hold this toxic element (Elbarbary and Hamouda, 2015).

Cadmium could only be detected in 16/50 of the whole milk powder samples with a mean value of 0.008 ±0.002 mg/kg. These results were below data reported by Abdelkhalek et al. (2015). This metal couldn't be found in all the examined skim milk samples, contrary to the results produced by Zakaria et al. (2018), who detected cadmium in a minimum concentration of 0.013 mg/kg (Table 5). The exposure to Cd in food has been stated as the reason for hazardous health sequelae, such as kidney damage with the possibility of bone defects and fractures (Järup, 2003). The tested 50 whole and 30 skim milk powder samples weren't contaminated with toxic metal arsenic, unlike in the data found by Carrera et al. (2016), who discovered arsenic in eleven whole and ten skimmed milk powder samples at mean values of 0.016 and 0.026 mg/kg, respectively (Table 5).

The existence of lead and cadmium is reported to cause several health complications, like heart and kidney failure, and cancer; this has also been observed at minimum levels of contamination (Hu, 2002). In Table 6, it is obvious that the lead concentrations of the examined powder samples exceeded the permissible limits (0.02 mg/kg) of Egyptian standards (ES: 7136/2010) with a percentage of 68.00 for 34 whole milk powder samples and 66.67 for 20 skim milk powder ones. In addition, thirty-three whole and eighteen skim powdered milk samples surpassed the limit for mercury (0.02 mg/kg) set by Egyptian standards (ES: 2360/1993). All the evaluated whole milk powder samples had cadmium concentrations within the allowable limits permitted by Egyptian standards (ES: 2360/1993).

All samples had an EDI (Estimated Daily Intake) for all measurable metals lower than the PTDI (Provisional Tolerable Daily Intake) set by JECFA (2018), as presented in Table 6. The high values and varieties in toxic metal contents in the milk powders are mainly due to species variation and contamination during handling and manufacturing procedures. Our results proved the possible pollution of dairy powders with toxic metals through the equipment used, so, control during all steps of the manufacture to improve the quality of the final product and to prevent contamination, is necessary (Abdelkhalek et al., 2015).

Table 6. Degree of acceptability of the examined powder milk samples based on toxic metal concentrations

Metal	Limits mg/kg	Acceptable samples			
		whole milk powder (n = 50)		skim milk powder (n = 30)	
		no.	%	no.	%
Pb	ML = 0.02**	16	32.00	10	33.33
	PTDI = 0.004*	50	100.00	30	100.00
Hg	ML = 0.02***	33	66.00	18	60.00
	PTDI = 0.0005*	50	100.00	30	100.00
Cd	ML = 0.05***	50	100.00	30	100.00
	PTDI = 0.0009*	50	100.00	30	100.00

n – number of examined samples, no. – number of acceptable samples, PTDI – provisional tolerable daily intake, EDI – estimated daily intake, ML – maximum limit.
*JECFA (2018).
**Egyptian standards (2010) (ES: 7136/2010).
***Egyptian standards (1993) (ES: 2360/1993).

CONCLUSION

Our results proved that the sensory and microbiological quality of skimmed milk powder is more satisfactory than that of whole milk powder. Despite this, some of the examined samples didn't comply with Egyptian standards due to high lead and mercury contamination levels, as well as the presence of *Staphylococcus aureus* and multiple antibiotic resistant *Enterobacteriaceae* species. This indicates the need for more restrictions in handling and processing practices, sanitary measures for utensils, and proper storage conditions. Application of the HACCP is considered a necessary tool for avoiding contamination during and post-processing, with appropriate pasteurization of milk before drying for efficient pathogen control. In addition, there is a strong need for more regulated monitoring and more limited values for these imported powdered milks.

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