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MICROBIOLOGICAL INVESTIGATIONS OF SOME DRIED MIXES OF DAIRY DESSERTS SOLD IN ASSIUT CITY

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ABSTRACT

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The research work was aimed to evaluate the microbiological quality of dried mixes of dairy products highly consumed by the public in Egypt. To verify the quality and safety of these products, 90 random samples of whipped cream; chocolate pudding powder and sauce (all in powder form and main component is milk powder either full or skimmed milk powder and other components differ in each product) were collected from different manufactures. These products examined for total bacterial count (average counts were $8.72x10^4$; $9.29x10^4$ and $1.29x10^5$ cfu/g in examined samples of cream, chocolate pudding and sauce respectively). While, total thermophillic count were detected in examine samples of whipped cream and chocolate and failed to be counted in sauce samples. In addition, the total coliforms counts and total yeasts and molds counts were recorded. Also, the examination of these products for contamination by some pathogenic microorganisms were done and revealed that E. coli O157:H7 and coagulase positive S. aurous could not be isolated from all examined samples. While listeria monocytogenes could be isolated from chocolate pudding in percentage of 26.7 %. The microbial population detected in terms of numbers and types of microorganisms reflect the poor hygienic standard of production which constituting a public health hazard.

Key words: *Dairy desserts, Total coliform count, public health hazard.*

INTRODUCTION

Milk desserts are popular products worldwide, usually formulated with milk, sugar, modified starch, hydrocolloids such as carrageenans, flavorings and colorants (DeWijk *et al.*, 2003 and González-Tomás and Costell, 2006). Whipped toppings have become popular both for commercial and consumer use on puddings, sodas, cakes, ice cream, fruits, and pastries and for cream pie bases. The importance of dairy-based foods as vehicle for the transmission of various diseases has been documented; especially in countries where hygienic standards are not strictly enforced (Meyer-Broseta *et al.*, 2003). Contaminated milk and its by-products may harbor a variety of microorganisms which are responsible for many food-borne outbreaks (Danielsson-Tham *et al.*, 2004; MacDonald *et al.*, 2005; Makino *et al.*, 2005; Okwumabua *et al.*, 2005 and Oliver *et al.*, 2005).

Dried skim milk products stored in optimal conditions in proper packaging show essentially no change in color, even during two years of storage at 35°C. In commercial situations, most dried milk products are susceptible to reactions that can result in small changes in the physical properties of the product, its palatability and nutritive value. These changes, however, do not significantly impact the nutritional benefits of milk powders. Vitamin and protein quality losses during storage of milk powders, when stored in good conditions, are negligible (U.S. Dairy Export Council, 2001).

Likewise, the quality of food depends on the total number of viable organisms as revealed by the total bacterial count. However, the microbial load of food products is influenced by a number of factors such as the general environment from which the raw materials were obtained, the environment in which it was processed, the sanitary conditions under which the food was handled and processed, and the adequacy of processing procedures targeted at reducing contaminants during the packaging, handling and storage of the product (Osamwonyi, 2005).

The thermophilic bacilli are an important group of contaminants in the dairy industry. Although these bacilli are generally not pathogenic, their presence in dairy products is an indicator of poor hygiene and high numbers are unacceptable to customers. In addition, their growth may result in milk product

defects caused by the production of acids or enzymes, potentially leading to off-flavours. Dairy thermophiles are usually selected for the conditions during dairy manufacture. These bacteria are able to grow in sections of dairy manufacturing plants where temperatures reach 40–65 °C. Furthermore, because they are spore formers, they are difficult to eliminate. In addition, they exhibit a wide temperature growth range, exhibit a fast growth rate (generation time of approximately 15–20 min) and tend to readily form biofilms (Burgess *et al.*, 2010).

Coliforms being non-spore formers should be susceptible to pasteurization. Their post pasteurization presence in the examined samples may be due to either faulty heat process or to post pasteurization contamination by handlers with poor sanitary practices. The presence of these organisms in food had been described as an index of food hygiene (Frazier and Westhoff, 1978 and Jay, 1978).

Even though food-borne *S. aureus* poisoning is a mild, generally self-limiting disease, with symptoms that include vomiting with or without diarrhea (Dinges *et al.*, 2000), hospitalization is required in approximately 10% of the cases (Holmberg and Blake, 1984).

Moreover Listeria is considered to be one of the most important causes of food-borne diseases. *L. monocytogenes*, a ubiquitous gram-positive microaerophilic bacterium, is capable of causing severe listeriosis infections in humans (encephalitis, meningitis and septicaemia especially in immunocompromised individuals) and animals (mastitis, diarrhea and gastroenteritis) (Herman *et al.*, 1995; Vela *et al.*, 2001; Siegman-Igra *et al.*, 2002; McLauchlin *et al.*, 2004 and Aygun and Pehlivanlar, 2006). *L. monocytogenes* has been involved in many outbreaks and sporadic cases of disease primarily associated with the consumption of pasteurized milk, cheeses made from unpasteurized milk and other dairy based products that serve as good medium for the growth and survival of many pathogenic organisms in both industrialized and developing countries (Kells and Gilmour, 2004; Makino *et al.*, 2005 and Manfreda *et al.*, 2005). Usually, the presence of any Listeria species in food is an indication of microbial contamination (Gilot and Content, 2002).

E. coli O157:H7 (designated by its somatic, O, and flagellar, H, antigens) was first recognized as a human pathogen following two hemorrhagic colitis outbreaks in 1982. Cross-contamination of foods can occur in food-processing plants and during subsequent handling and preparation, resulting in a wide range of foods being implicated in outbreaks of *E. coli O157:H7* infections. *E. coli* serotype *O157:H7* is a rare variety of *E. coli* but is a normal inhabitant of the intestines of all animals, including humans. The pathogen produces large quantities of one or more related potent toxins, called Shiga toxins, which cause severe damage to the lining of the intestine and to other target organs, such as the kidneys. The most severe outcome of Shiga toxin exposure among the general population is typically hemorrhagic colitis, a prominent symptom of which is bloody diarrhea. However, life-threatening complications sometimes occur. Some victims, particularly the very young, may develop hemolytic uremic syndrome (HUS). Overall, the Center for Disease Control and Prevention estimates that *E. coli O157:H7* is responsible for approximately 73,500 infections, 2150 hospitalizations, and 61 deaths in the United States each year (Saleh *et al.*, 2009; Burgess *et al.*, 2010 and CDC, 2012).

Economically, the presence of yeasts and molds in dairy products are undesirable even when found in few numbers as they rapidly grow in the product in a wide range of temperature, PH and humidity, resulting in objectionable changes that render the product of inferior quality or even unmarketable. Yeasts and moulds are used as an index of the proper sanitation and quality control for certain dairy products. The public health importance of moulds has been emphasized as certain species can produce mycotoxins at a temperature 2-10 °C, which is implicated in human cases of food poisoning and neoplastic diseases including leukemia and cancers as reported by (Bullerman, 1980 and Mossel, 1982).

The purpose of the present study is to investigate the presence of some bacteria such as E. *coli O157:H7*; *Staph. aureus* and *Listeria* spp in dried mixes of dairy desserts sold in Assiut city. Also, total aerobic plate count, total thermophillic count, total coliforms and total yeast and molds count will be estimated.

MATERIALS and METHODS

A) Collection, preparation and serial dilutions of samples:

A total of ninety random samples of whipped cream, chocolate pudding and sauce (all are in powder status, packaged, unpackaged and 30 samples of each product) were purchased from different shops and supermarkets in Assiut city. The samples were still valid for consumption more than one year from production time and they were transferred to the laboratory in their packages to be analyzed microbiologically to evaluate their quality. Eleven grams of the prepared samples were mixed with 99 ml of sterile 0.1 % peptone water and thoroughly mixed to give a dilution of 1/10, and then ten fold serial dilutions were carried out according to (A.P.H.A., 1992).

B) Experimental techniques:

1) *Enumeration of total bacterial count according* to A.P.H.A. (1992) by using standard plate count agar.

2) *Enumeration of total thermophillic count* at 55 °C for 48 h ***according* to Frank and Yousef (2004).**

3) *Enumeration of total yeasts and molds count according* to Harrigan and MacCance (1976) by using malt extract agar (containing 500 mg each of chlortetracycline and HCL chloramphenicol).

4) *Enumeration of total coliform count according* to Ray and Speck (1978) by using violet red bile glucose agar

5) *Isolation and identification of S. aureus:*

S. aureus was isolated by using the technique given by Baird Parker (1962). Enriched samples (A Portion 10 g) from each sample was extracted aseptically and homogenized with 90 ml sterile mannitol salt broth) were streaked on Baird Parker Agar (BPA) and the plate was incubated at 37 °C for 24–48 hours. Appearances of jet black colonies surrounded by white halo were considered to be presumptive *S. aureus*. Identification of S. ***aureus*** by using coagulase test, catalase test, anaerobic utilization of glucose and mannitol and Gram stain.

6) Isolation and identification of *E. coli o157:H7*:

For detection of *E. coli O157:H7*, trypticase soy broth was supplemented with cefixime (0.05 mg/l), cefsulodin (10 mg/l) and vancomycin (8 mg/l) for pre enrichment (37 °C). After the addition of the samples into the modified trypticase soy broth, shake-incubation was performed at 37 °C the enriched samples were plated onto sorbitol Mac-Conkey agar (SMCA) supplemented with 0.05 mg/l cefixime and potassium tellurite (2.50 mg/l) after 4 and 24 hours. Presumptive *E.coli O157:H7* colonies (indole positive) were confirmed serologically using antibodies to the O157 antigen (*E.coli O157:H7* latex test, Oxoid DR 260). According to these results, agglutination and indole positive colonies were recognised as E.coli O157 (AOAC, 1998).

7) Isolation and identification of *L. monocytogenes*:

A 25 g portion of each sample was weighed aseptically into a sterile stomacher bag containing 225 ml of sterilized 1% (w/v) peptone water and macerated in a laboratory blender stomacher for 3 min (Peng and Shelef, 2000). A selective medium: Listeria Selective Agar (LSA) (Oxoid, Hampshire, UK) containing Listeria selective supplement (Oxford modified) (Oxoid, Hampshire, UK) was used for the isolation, enrichment and plating of Listeria (Gulmez and Guven, 2003). Bacteriological analyses were performed, by plating in duplicates (a volume of 0.1 ml of each dilution on agar plates containing appropriate selective media) (Gulmez and Guven, 2003). All analyses were conducted under aseptic conditions. Plated cultures were then incubated at 35 °C for 48 h (Gulmez and Guven, 2003). Colonies that exhibited the *L. monocytogenes* morphology were preserved for further analyses. All bacteriological analyses were done according to the Compendium of Methods for the Microbiological Examination of Foods (Downes and Ito, 2001 and Horwitz, 2001).

Biochemical identification of the suspected L. monocytogenes isolates:

Colonies appearing on LSA were first selected based on their morphology then identified by biochemical tests. Black to brown colonies surrounded by black halos was chosen (Hitchins, 1995 and Aygun and Pehlivanlar, 2006). Those colonies were Gram stained. Only Gram-positive short rods were further tested for their ability to produce acids from the fermentation of D-xylose and L-rhamnose sugars, and were also subjected to the β-haemolysis test (Cocolin *et al.*, 2002 and Zhou and Jiao, 2005).

RESULTS

Table 1: Statistical analytical results of aerobic plate count of the examined samples.

Type of sample	No. of examined samples	Positive samples		Counts / g		
		No.	%	Min.	Max.	Average
Whipped cream	30	29	96.7	>300	$7.36x10^5$	$8.72x10^4$
Chocolate pudding	30	28	93.3	>300	$6.88x10^5$	$9.29x10^4$
Sauce	30	29	96.7	>300	$7.8x10^5$	$1.29x10^5$

No.: number of samples

Table 2: Frequency distribution of positive samples based on their aerobic plate count.

Counts / g	Whipped cream		Chocolate pudding		Sauce	
	(No. : 29)	%	(No. : 29)	%	(No. : 29)	%
10 - < 10^2	4	13.8	7	25	4	13.8
10^2 - < 10^3	7	24.2	3	10.7	7	24.2
10^3 - < 10^4	5	17.2	6	21.4	6	20.7
10^4 - < 10^5	5	17.2	4	14.3	4	13.7
10^5 - < 10^6	8	27.6	8	28.5	8	27.6
Total	29	100	28	100	29	100

Table 3: Statistical analytical results of total thermophillic count of the examined samples:

Type of samples	No. of examined samples	Positive samples		Count / g		
		No.	%	Min.	Max.	Average
Whipped cream	30	8	26.7	>10	$6.9x10^2$	8.27x10
Chocolate pudding	30	4	13.3	>10	$1.6x10^2$	1.27x10
Sauce	30	0	0	>10	>10	>10

Table 4: Frequency distribution of positive samples based on their total thermophillic count.

Counts / g	Whipped cream		Chocolate pudding		Sauce	
	(No. : 8)	%	(No. : 4)	%	No. : 0	%
10 - < 10^2	2	25	2	50	0	0
10^2 - < 10^3	6	75	2	50	0	0
Total	8	100	4	100	0	0

Table 5: Statistical analytical results of total coliforms count in the examined samples:

Type of samples	No. of examined samples	Positive samples		Count / g		
		No.	%	Min.	Max.	Average
Whipped cream	30	29	96.7	>100	$4.12x10^5$	$6.55x10^4$
Chocolate pudding	30	22	73.3	>100	$4.4x10^5$	$6.67x10^4$
Sauce	30	27	90	>100	$7x10^5$	$1.19x10^5$

Table 6: Frequency distribution of positive samples based on their total coliforms count.

Counts / g	whipped cream		Chocolate pudding		Sauce	
	(No.:29)	%	(No.:22)	%	(No.:27)	%
10^3 - < 10^4	4	13.8	4	18.2	4	14.8
10^4 - < 10^5	19	65.5	11	50	16	59.3
10^5 - < 10^6	6	20.7	7	31.8	7	25.9
Total	29	100	22	100	27	100

Table 7: Statistical analytical results of total yeasts and molds count in the examined samples.

Type of samples	No. of examined samples	Positive samples		Count / g		
		No.	%	Min.	Max.	Average
Whipped cream	30	20	66.7	>10	$1.16x10^4$	$1.41x10^3$
Chocolate pudding	30	17	56.7	>10	$1.1x10^4$	$1.11x10^3$
Sauce	30	21	70	>10	$6.2x10^3$	$7.55x10^2$

Table 8: Frequency distribution of positive samples based on their total yeasts and moulds count.

Counts / g	whipped cream		Chocolate pudding		Sauce	
	No. /20	%	No. /17	%	No. /21	%
10 - < 10^2	7	35	2	11.8	11	52.4
10^2 - < 10^3	8	40	7	41.2	4	19
10^3 - < 10^4	3	15	7	41.2	6	28.6
10^4 - < 10^5	2	10	1	5.8	0	0
Total	20	100	17	100	21	100

Table 9: Incidence of *S. aureus; E. coli O157:H7 and L. monocytogenes* in the examined samples.

Microorganisms	Whipped cream		Chocolate pudding		Sauce	
Products	(No.:30)	%	(No.:30)	%	(No.:30)	%
S. aureus	0	0	0	0	0	0
E. coli	2	6.7	0	0	1	3.3
L. monocytogenes	0	0	8	26.7	0	0

DISCUSSION

Data depicted in Table 1. revealed that total aerobic plate count were found in most of the examined whipped cream samples, with a minimum value of >10 cfu g^{-1}, maximum value of $7.36x10^5$ cfu g^{-1} and a mean value of $8.72x10^4$ cfu g^{-1}. Also, it is found that 96.7% of sauce samples had total aerobic plate count ranged from >10 to $7.8x10^5$ with an average count $1.29x10^5$. While, chocolate pudding had an average count $9.29x10^4$. The majority of the positive samples of three examined products occurred between 10^5 - < 10^6 (Table 2). The results obtained in our study showed that most of the samples tested positive for aerobic plate counts which are indicators of aerobic bacteria. This finding may reflect the absence of strict hygienic practices in the preparation of those products. A high aerobic plate count level, in general, is indicative of the possible presence of harmful microorganisms and makes the food unsatisfactory

for human consumption (Gilbert *et al.*, 2000 and Gillespie and Little, 2000).

Total thermophillic counts were detected in 8 and 4 samples of whipped cream and chocolate pudding in percentage of 26.7% and 13.3%, respectively. Sauce samples found to be uncontaminated with thermophillic bacteria (Table 3). Table, 4 revealed that the 75% of the positive samples of filling cream occurred between 10^2 to $< 10^3$. In addition, 50 % of the positive samples of chocolate pudding harbor thermphillic bacteria between 10^2 - $< 10^3$ cfu g^{-1}.

The presence of total coliforms is an indicator of faecal as well as post processing contamination and is reflective of unsanitary conditions practiced during the different stages of food production (Van Kessel *et al.*, 2004). The assumption is that there is an association between the detection of faecal coliforms and pathogenic organisms (Van Kessel *et al.*, 2004). Prevalence of coliforms contamination were 96.7%, 73.3% and 90%, while the average coliform counts were $6.55x10^4$; $6.67x10^4$ and $1.19x10^5$ for whipped cream; chocolate pudding and sauce samples, respectively (Table 5). 19 (65.5%); 11(50%) and 16 (59.3) of the positive examined samples of whipped cream; chocolate pudding and sauce had frequency distribution between 10^4 - $< 10^5$ cfu g^{-1}, respectively (Table 6).

Table 7 and 8 showed that yeasts and molds were counted in 66.7%; 56.7% and 70% with average values / g of $1.41x10^3$; $1.11x10^3$ and $7.55x10^2$ of whipped cream; chocolate pudding and sauce samples, respectively. Out of 20 positive examined whipped cream samples, 8 (40%) had counts ranged from 10^2 to $<10^3$. Yeasts and molds may grow over a wide range of temperature and gain entrance to milk 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 high level of these microorganisms may be due to post pasteurization contamination.

None of examined whipped cream, chocolate pudding and sauce samples were positive for coagulase positive *S. aureus* (Table 9). *S. aureus* is considered the third most important cause of disease in the world amongst the reported food-borne illnesses (Zhang *et al.*, 1998). This Gram-positive bacterium has no particular nutritional and environmental requirement for its growth and it can grow at an pH above 4.8 and its minimum growth temperature is 18 - 45°C (Scott, 1953 and Martin and Iandolo, 2000).

Three isolates of *E. coli* could be detected in the examined samples of whipped cream and sauce (2 and 1 isolates, respectively, Table 9), but Presumptive *E. coli O157:H7* colonies (indole positive) did not confirmed serologically using antibodies to the O157 antigen. Since its identification as a human pathogen in1982 (*Riley et al., 1983*), *E. coli O157:H7* has become a pathogen of major concern for the food and dairy industries because of its ability to cause severe illness such as haemorrhagic colitis (HC), haemolytic uremic syndrome (HUS), and thrombotic thrombocytopenic purpura (TTP). The diseases affect all age groups and the pathogen is exceptional in its severe consequences of infection, its low infectious dose and unusual acid resistance (*Buchanan and Doyle, 1997*). *E. coli O157:H7* serotypes are identified as enterohemoragic *E. coli* and categorized in Shiga-like toxin producing *E. coli* (STEC) (*Oksuz et al., 2004*). It causes haemorrhagic colitis, hemolytic-ureamic syndrom (HUS) and thrombotic thrombocytopenic purpura (TTP) (*Zhao et al., 1998*).

In addition to the previous indicator bacteria, investigation of *L. monocytogenes* was undertaken to examine the safety of dairy products and to determine the possibility of any potential health hazard. *L. monocytogenes* could be isolated from chocolate pudding in percentage of 26.7 % (Table 9). The sources of *L. monocytogenes* in such products may be faecal and environmental contamination during milking, storage and transport, infected cows in dairy farms, poor silage quality and improper handling of these products at the points of sale (Sanaa *et al.*, 1993 and Van Kessel *et al.*, 2004). Lack of hygienic practices during the processing and production of these dairy-based products may also contribute to high levels of contamination. For example, cross-contamination may occur after heat treatment. Furthermore, various studies have indicated that certain strains of *L. monocytogenes* survive within the food processing environment (Unnerstad *et al.*, 1996 and Senczek *et al.*, 2000). The ability of *L. monocytogenes* to form biofilms (Harvey *et al.*, 2007) may contribute to its persistence in food processing plants (Thimothe *et al.*, 2004). The milk processing environment and handling practices change may vary among the processing plants. About 47% surface of hand of the food handlers and 16% on the processing tables were found to carry *L. monocytogenes* (Kerr *et al.*, 1993 and Jeyasekaran *et al.*, 1996).

CONCLUSION

Cross-contamination of foods is one of the major concerns in the food industry, and if microorganisms are not completely removed from food-contact surfaces, they may go on to form biofilms and also increase the bio-transfer potential. The presence of *L. monocytogenes* in a processing plant could lead to post-processing contamination, which also draws attention to the need to reduce the level of contamination of milk that will eventually be transported to a milk processing plant. The present study indicated the incidence of *L. monocytogenes*

examined products processing plants which may possess a potential threat to public health.

In conclusion, this study demonstrates the presence of some pathogens including *L. monocytogenes*, and *E. coli* in dessert. Therefore, these foods are serious risk to the public health. Likewise, the presence of these organisms indicated that there were poor hygienic conditions during the manufacturing, storage and sales process of these two traditional foods. Manufacturing procedures within the scope of the HACCP, appropriate hygienic measures to avoid processing and post processing cross contamination and the use of pasteurized milk are critical for control of these pathogens in these foods.

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الفحوصات الميكروبيولوجية لبعض الخلطات المجففة من الحلويات اللبنية المباعة في مدينة أسيوط

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يهدف هذا البحث تقييم الجودة الصحية للخلطات المجففة من الحلويات اللبنية التي انتشرت بين المستهلكين لسهولة تحضيرها وكثرة استخدامها لتزيين المخبوزات والحلويات. تم تجميع ٩٠ عينة من كريمة الحشو وبودينج الشيكولاتة والصوص (بواقع ٣٠ عينة لكل منتج). اللبن المجفف البودرة كامل او منزوع الدسم هو المكون الاساسي في هذه المنتجات بالاضافة الى مكونات اخري تختلف من منتج الي اخر. تم تجميع العينات من المحلات التجارية المختلفة ومحلات العطارة وذلك لفحصها وتقدير العدد الكلي للبكتريا التي يمكن ان توجد بها والتى تنمو في درجات حراره مختلفة وكذلك المجموعة القولونية، هذا بالاضافة الى العدد الكلي للخمائر والفطريات بها. اظهرت نتائج الفحص ان متوسط العدد الكلي للبكتريا $8.72x10^4$، $9.29x10^4$ و$1.29x10^5$ في عينات كريمة الحشو وبودينج الشيكولاتة و الصوص علي التوالى، وكانت نسبة تواجد البكتريا التي تنمو في درجة حرارة عالية هي علي التوالي 26.7 %، 13.3و0 % بينما $6.55x10^4$، $6.67x10^4$ و$1.19x10^5$ كانت هى متوسطات العدد الكلي للبكتريا القولونية في العينات المفحوصة علي التوالي. اما متوسط عدد الخمائر والفطريات كان $1.41x10^3$ في كريمة الحشو، $1.11x10^3$ في بودينج الشيكولاتة و$7.55x10^2$ في الصوص. من ناحية اخري لم يتم تأكيد عزل بعض الميكروبات الممرضة مثل الميكروب القولوني النموذجي وذلك بعد عمل الاختبارات السيرولوجية للعترات المعزولة وكذلك الميكروب المكور العنقودي الايجابي لاختبار البلازما من عينات كريمة الحشو وبودينج الشيكولاتة والصوص. هذا بالاضافة الى انه تم عزل الليستريا بنسبة 26.7 % في عينات بودينج الشيكولاته. وجود هذه الميكروبات يدل علي وجود تلوث اثناء التصنيع مما يشكل خطرا علي صحة المستهلكين ويتطلب وجود اشتراطات صحية لتصنيع هذه المنتجات.