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MICROBIOLOGICAL EVALUATION OF SOME READY TO EAT EGG-BASED DESERTS SOLD IN ASSIUT CITY

(With 9 Tables)

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التقييم الميكروبيولوجى لبعض الحلاوىالمحتويه على البيض الجاهزة للاستهلاك والمباعة بمدينه اسيوط

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فى هذة الدراسه تم جمع عدد٤٥ عينة عشوائيه من منتجات الحلاوى المحتويه على البيض الجاهزة للاستهلاك، وتشمل الكريم كراميل والكيك والجاتوة (عدد ١٥ عينة لكل منتج) من العديد من المحلات والأسواق ومحلات الحلويات والمخابز فى مدينة اسيوط لفحصها ميكروبيولوجيا وتحديد الحالة الصحيه لأنتاجها. وقد تم تقييم كل من Aerobic plate count و Psychrotrophs و Enterococci و Coliforms و Fecal coliforms و *E. coli* و *B. cereus* و *S. aureus* و Anaerobes و Yeasts & molds. وقد اظهرت النتائج ان متوسط العدد الكلى لكل من Aerobic plate count و Psychrotrophs كالتالى $1x10^5$، $1x10^6$، $1.9x10^6$ و$<10^2$، $2.6x10^5$، $1.1x10^5$ لكل جرام من عينات الكريم كراميل والكيك والجاتوة، على التوالى. ومتوسط عدد Enterococci للعينات $<10^2$ و$2.5x10^2$ و $1.2x10^4$ لكل جرام، على التوالى. وبالفحص عن Coliforms تبين ان معظم عينات الكريم كراميل والكيك (93.3 و86.7%) لم تظهر وجودها (اقل من 3 للجرام)، بينما 60% من عينات الجاتوة تحتوى على عدد اقل من 10^2 للجرام حيث ان عينتان من الجاتوة (13.3%) تحتوى على عدد اكثر من 10^3 للجرام. ولم يتم عزل Fecal coliforms من عينات الكريم كراميل (اقل من 3 للجرام)، بينما معظم عينات الجاتوة (53.3%) كانت ملوثه حيث الأعداد تتراوح من 10> الى $<10^3$ للجرام وعينه واحدة فقط (6.66%) تحتوى على عدد اكثر من 10^3 للجرام. وبالبحث عن *E. Coli* لم يتم عزلها من جميع عينات منتجات الحلاوى المحتويه على البيض، ولكن تم عزل *B. cereus* من 20 و20 و46.7% بأعداد $6.7x10^3$ و$3.5x10^3$ و$5.3x10^3$ لكل جرام من عينات المنتجات، على التوالى. وبالنسبة *S. aureus* لم يتم عزلها من عينات الكريم كراميل بينما عزلت من 6.6 و13.3% من عينات الكيك

والجاتوة، على التوالى. أما Anaerobes فكانت فى 33.3 و73.3 و80 % من عينات المنتجات، على التوالى. وقد لوثت Yeasts & molds 46.7 و60 و100% من عينات المنتجات، على التوالى وذلك بأعداد $1.8x10^2$ و $6.7x10^2$ و $2.2x10^4$ لكل جرام. وقد تبين ان معظم منتجات الحلاوى المحتويه على البيض الجاهزة للاستهلاك والمباعة بمدينه اسيوط تعتبر جيدة من الحالة الميكروبيولوجيه والجودة الصحيه ماعدا الجاتوة. ومع ان الحمل الميكروبى تحت معدل الخطر الا ان الأهميه الصحيه لهذة الميكروبات مازالت موجودة وذلك لو اتيحت لها الفرصه لكى تنمو وتتكاثر. وقد تمت مناقشة الأهميه الصحيه ومدى خطورة هذة الميكروبات على صحة المستهلك وكذلك الشروط الواجب اتباعها.

SUMMARY

A total of 45 random samples of ready to eat egg-based deserts including cream caramel, cake and jatooh products (15 samples each), were collected from different retailers, supermarkets, confectionery shops and bakeries in Assiut City. To assess their quality, the samples were examined microbiologically for the incidence and counts of aerobic plate count, psychrotrophs, enterococci, coliforms, fecal coliforms, *Eschericia coli*, *Bacillus cereus*, *Staphylococcus aureus*, anaerobes and yeasts & molds. The obtained results verify that the total bacterial and psychrotrophs counts averaged $1x10^5$ and $1x10^6$, $1.9x10^6$ and $<10^2$, $2.6x10^5$ and $1.1x10^5$/g of cream caramel, cake and jatooh samples, respectively. Enterococci averages were $<10^2$, $2.5x10^2$ and $1.2x10^4$/g of the examined samples, respectively. Most of the examined samples of cream caramel and cake products (93.3 and 86.7%) failed to yield coliforms (less than 3/g), while 60% of jatooh samples had counts below 10^2/g. Two samples (13.3%) of such product yielded counts over 10^3/g. Fecal coliforms could not recover from all of cream caramel samples (<3/g), while most of the jatooh samples (53.3%) were contaminated by numbers ranged from <10 up to $<10^3$/g. Only one sample (6.66%) of such product had fecal coliforms more than 10^3/g. *E. coli* could not be detected in any of the examined egg-based deserts samples. *Bacillus cereus* could be isolated from 20, 20 and 46.7% in numbers averaged $6.7x10^3$, $3.5x10^3$ and $5.3x10^3$/g of the examined samples, respectively. *S. aureus* could not be detected in any of cream caramel samples. The organism recovered from 6.6 and 13.3% of the examined cake and jatooh samples, respectively. The incidence of anaerobes was 33.3, 73.3 and 80% in the examined products, respectively. Yeasts & molds contaminated 46.7, 60 and 100% of the examined products, respectively. They existed in numbers averaged $1.8x10^2$, $6.7x10^2$ and $2.2x10^4$/g of the samples, respectively. The results prove that most of the examined egg-

based deserts products are of quite good quality, except jatooh product that needs to be improved. However, the microbial loads are below the hazard point, the health hazard of such microorganisms still exists, if they are allowed to grow and multiply. Suggestive measures for improving these products were discussed.

Key words: *Egg-based deserts - Microbiological quality.*

INTRODUCTION

The main and natural function of eggs is to provide for protein and development of chick embryo. However, eggs have also been used as a staple item in the diet of many peoples for thousands of years. They provide a unique well balanced source of nutrients of high quality.

Fresh eggs are generally devoid of bacteria (Frazier and Westhoff, 1978). However, if the ovary is infected with pathogens, the egg may become infected before it is laid. On the other hand, the outside egg is not sterile and the shells soon become contaminated by fecal matters from hen, by lining the nest, by wash if the eggs are to be washed, by handling and perhaps by the material in which eggs are packed (Board and Fuller, 1994 and Cox *et al.*, 2000).

Molds and bacteria from these sources can grow through a moistened shell into egg content. It has been recognized that fresh eggs usually contain less than 10 bacteria/g and seldom 10^2 bacteria/g (Speck, 1976). While, the shell of fresh egg carries from 10^2 to 10^7 bacteria (Longree, 1980). Some pathogenic bacteria such as *Listeria monocytogens*, *S. aureus*, salmonella, shigella, *E. coli* and other enterobacteriaceae could be isolated from the shells and content of marketable eggs at variable percentages (Wieneke *et al.*, 1993; Henzler *et al.*, 1994; Brooks *et al.*, 1995 and ICMSF, 1996). However, the risk of getting illness from eggs is very low, the nutrients that make egg a high quality food for human, are also a good growth medium for bacteria. These bacteria may thrive into egg products or any egg-based food, and when find moisture, favorable temperature and time, they will multiply and increase risk of illness.

Egg products refer to eggs that are removed from their shell, for processing (FSIS, 2001). The processing of eggs includes breaking eggs, filtering, mixing, stabilizing, blending, pasteurizing, cooling, freezing or drying and packing (FSIS, 2001). Egg products include whole liquid egg, white, yolks and various blends with or without non-egg ingredient that processed or pasteurized and may be available in liquid, frozen and

dried form for commercial service and home use. Although the eggs are marketed primarily for many years as shell eggs, in recent years egg consumption in the form of egg products or egg-based food products has increased dramatically. Because they provide certain desirable functional attributes, egg and egg products are widely used as ingredients in many food products (FSIS, 2001). Many new forms of convenience products such as different varieties of cakes, pudding mixes, pasta, ice cream, candies, cream caramel, mayonnaise, cokies and many other bakery goods utilize egg manufacturers and food service industry. Fueled by increasing consumer's demands for more convenience food products, growth of egg products industry is expected to continue.

It needs no provability that all foods have the ability to carry microorganisms or toxins that can cause illness, if these bacteria are allowed to grow in or on food without being killed before eating. Although, there are no data dealt with quality of egg-based products, many of these products need to be checked out for safeteness and quality, as they are liable to be contaminated from various sources such as infected egg, contaminated egg products, processing and handling. Therefore, this work was conducted to secure the microbiological picture of some popular egg-based deserts produced widely in Assiut City and consumed by a wide rage of peoples.

MATERIALS and METHODS

Collection of samples:

A total of 45 random samples of egg-based deserts representing cream caramel, cake and jatooh products (15 samples each) were collected from different retailers, supermarkets, confectionary shops and bakeries in Assiut City. All samples were aseptically packaged and dispatched directly to the laboratory with a minimum of delay.

Preparation of samples:

Cartons or cans of samples were opened aseptically and then thoroughly mixed by sterile spoon in a sterile mortar. Approximately 10 g of the sample were aseptically weighed and then added to 90 ml of 0.1% sterile peptone water to obtain a dilution of 10^{-1} (APHA, 1992), and then decimal dilutions were prepared and followed by microbiological analysis.

Microbiological examinations:

1- Aerobic plate count (APC), (APHA, 1992).
2- Psychrotrophic count, (APHA, 1985).

3- Enterococci count, (Deibel and Hartman, 1982).
4- Coliforms count (MPN/g), (AOAC, 1980).
5- Enumeration of fecal coliforms (MPN/g), (AOAC, 1980).
6- Enumeration of *E. coli* (MPN/g), (AOAC, 1980).
7- Enumeration and isolation of *B. cereus*, (Kim and Goepfert, 1971) followed by:
   a) Nitrate reduction test, (Speck, 1976).
   b) Gelatin liquefaction test, (Cowan and Steel, 1974).
   c) Voges-Proskauer test, (Speck, 1976).
8- Isolation of *S. aureus*, (Finegold and Martin, 1982) followed by:
   a) Gram staining, (Speck, 1976).
   b) Anaerobic mannitol fermentation, (Baired-Parker, 1962).
   c) Coagulase test, (Cruickshank *et al.*, 1969).
9- Detection of anaerobes by stormy fermentation test, (Cruickshank *et al.*, 1969).
10- Yeasts & molds count, (Harrigan and MeCance, 1976).

RESULTS

Table 1. Aerobic plate count of the examined egg-based deserts samples.

Products	Positive samples		Counts/g		
	No./15	%	Minimum	Maximum	Average
Cream caramel	12	80	$1x10^2$	$9.2x10^5$	$1x10^5$
Cake	14	93.3	$4x10^2$	$1x10^7$	$1x10^6$
Jatooh	15	100	$1.7x10^3$	$1.3x10^7$	$1.9x10^6$

Table 2. Psychrotrophic count of the examined egg-based deserts samples.

Products	Positive samples		Counts/g		
	No./15	%	Minimum	Maximum	Average
Cream caramel	0	0	$<10^{2*}$	$<10^{2*}$	$<10^{2*}$
Cake	3	20	$3x10^4$	$6.6x10^5$	$2.6x10^5$
Jatooh	14	93.3	$4x10^2$	$8.6x10^5$	$1.1x10^5$

*No colonies could be counted on the plates.

Table 3. Enterococci count of the examined egg-based deserts samples.

Products	Positive samples		Counts/g		
	No./15	%	Minimum	Maximum	Average
Cream caramel	0	0	$<10^{2*}$	$<10^{2*}$	$<10^{2*}$
Cake	2	13.3	$2x10^2$	$3x10^2$	$2.5x10^2$
Jatooh	6	40	$1x10^2$	$7x10^4$	$1.2x10^4$

*No colonies could be counted on the plates.

Table 4: Frequency distribution of the examined samples of egg-based deserts based on their coliforms count (MPN technique).

Products	Counts/g									
	<3		3 - <10		10 - $<10^2$		10^2 - $<10^3$		>10^3	
	No./15	%	No./15	%	No./15	%	No./15	%	No./15	%
Cream caramel	14	93.3	0	0	1	6.7	0	0	0	0
Cake	13	86.7	0	0	2	13.3	0	0	0	0
Jatooh	2	13.3	0	0	9	60	2	13.3	2	13.3

Table 5: Frequency distribution of positive samples of egg-based deserts based on their fecal coliforms count (MPN technique).

Products	Counts/g									
	<3		3 - <10		10 - $<10^2$		10^2 - $<10^3$		>10^3	
	No./15	%	No./15	%	No./15	%	No./15	%	No./15	%
Cream caramel	15	100	0	0	0	0	0	0	0	0
Cake	15	100	0	0	0	0	0	0	0	0
Jatooh	6	40	2	13.3	5	33.3	1	6.66	1	6.66

Table 6: Incidence and count of *B. cereus* in the examined samples of egg-based deserts.

Products	Positive samples		Counts/g		
	No./15	%	Minimum	Maximum	Average
Cream caramel	3	20	$<10^{2*}$	$1x10^4$	$6.7x10^3$
Cake	3	20	$1x10^2$	$1x10^4$	$3.5x10^3$
Jatooh	7	46.7	$1x10^2$	$2x10^4$	$5.3x10^3$

*No colonies could be counted on the plates.

Table 7: Incidence of *S. aureus* in the examined samples of egg-based deserts.

Products	No. of samples examined	Positive samples	
		No.	%
Cream caramel	15	0	0
Cake	15	1	6.6
Jatooh	15	2	13.3

Table 8: Incidence of anaerobes in the examined samples of egg-based deserts.

Products	No. of samples examined	Positive samples	
		No.	%
Cream caramel	15	5	33.3
Cake	15	11	73.3
Jatooh	15	12	80

Table 9: Yeasts & molds count of the examined egg-based deserts samples.

Products	Positive samples		Counts/g		
	No./15	%	Minimum	Maximum	Average
Cream caramel	7	46.7	10	$1x10^3$	$1.8x10^2$
Cake	9	60	10	$3x10^3$	$6.7x10^2$
Jatooh	15	100	20	$2.8x10^5$	$2.2x10^4$

DISCUSSION

The summarized results presented in Table 1 pinpoint that 80, 93.3 and 100% of the examined cream caramel, cake and jatooh samples had countable numbers of total bacteria, ranged from $1x10^2$ to $9.2x10^5$, $4x10^2$ to $1x10^7$ and $1.7x10^3$ to $1.3x10^7$ with averages $1x10^5$, $1x10^6$ and $1.9x10^6$/g, respectively. It is worth to mention that higher total bacterial counts in the examined egg-based deserts did not prove the presence of pathogens but they reflect the unhygienic status of such products during processing methods, post manufacturing or during handling process.

The results obtained from the examined samples in Table 2 revealed that no countable numbers of psychrotrophs could recover from

the examined cream caramel samples ($<10^2$), while 20 and 93.3% of cake and jatooh samples had numbers of psychrotrophs averaged $2.6x10^5$ and $1.1x10^5$/g, respectively. It is precisely evident from these results that the high incidence and counts of psychrotrophic bacteria in jatooh samples could be attributed to ineffective processing and sanitizing methods, as well as the whipped cream (usually unpasteurized) which is added as layers on and in between cake layers after processing. Most of psychrotrophic bacteria are destroyed by a mild heat treatment. However, presence of these bacteria in cake and jatooh implies post processing contamination and existence of heat resistant types such as bacillus and clostridium (Bhadsavle *et al.*, 1972). Generally, in foods that are kept refrigerated till reach consumers or served, large numbers of psychrotrophic bacteria may indicate a history of unsanitary handling. Furthermore, some species of psychrotrophic bacteria are heat resistant or spore forming which cause problems in heat treated products, as they produce heat resistant proteolytic and lipolytic enzymes that may cause spoilage of refrigerated products (Stevenson and Rowe, 1994 and Celestino *et al.*, 1996).

Enterococci as recorded in Table 3 existed in 13.3 and 40% of the examined cake and jatooh samples in numbers varied from $2x10^2$ and $1x10^2$ as minimum to $3x10^2$ and $7x10^4$ as maximum, with an average count of $2.5x10^2$ and $1.2x10^4$/g of the samples, respectively. While, enterococci could not be counted ($<10^2$) in the examined cream caramel samples. No acceptable level of these bacteria can be stated because enterococci counts vary with the holding condition, time of storage and other factors. The presence of the bacteria in the examined samples of cake and jatooh may be due to post processing contamination, the heat resistant character of the organism, bad quality ingredients, from cream layer or from contaminated egg shells during breaking of eggs or blending of egg content, besides, the ability of enterococci to grow in food processing plant and possibly other environment, long after their introduction. Also, it is closely precised from the aforementioned results that contamination of cake and jatooh samples by enterococci, should be overlooked because their occurrence is considered to indicate contamination from fecal matter and unsanitary condition of production (Rao *et al.*, 1986). Nevertheless, the public health significance cannot be denied, especially when the organism is allowed to multiply and exist in tremendous numbers in the product, as they have been implicated in several food poisoning outbreaks (ICMSF, 1978).

Presence of coliform group of bacteria in eggs, egg products or in egg-based food products account a significant indicator for pinpointing the unhygienic conditions during production, processing, handling and distribution. In recent years much attention has been paid towards *E. coli*, because of its importance as an organism of true fecal origin with possible existence of associated enteric pathogens. However, coliforms and fecal coliforms still continue to be considered as indicator organisms of choice in examining foods. The results recorded in Table 4 prove that coliforms existed in numbers below 3/g in most (93.3 and 86.7%) of the examined cream caramel and cake samples, while the rest of the samples (6.7 and 13.3%) contained insignificant numbers of coliforms ($<10^2$/g). Most of jatooh samples (60%) had coliforms in numbers ranged from 10 to $<10^2$/g, while, 26.6% of the samples were contaminated by numbers ranged from 10^2 to more than 10^3 coliforms/g.

Furthermore, fecal coliforms as shown in Table 5 could not recovered from all of the examined cream caramel and cake samples (below 3/g) as well as could not be detected in 40% of jatooh samples (<3/g). However, considerable numbers of jatooh samples (46.6%) revealed fecal coliforms in numbers less than 10^2/g. Only one sample had numbers exceed 10^3/g. These data prove that most of the examined cream caramel and cake samples had insignificant numbers of coliforms and all of the examined samples failed to recover fecal coliforms. On the other hand, presence of coliforms and fecal coliforms in jatooh samples could be attributed to post processing contamination or from contaminated egg shells during breaking and blending egg contents, as well as the unpasteurized cream layer added after processing. Fortunately, no *E. coli* could be detected in all of the examined egg-based deserts. However, it is worthwhile to state that contamination of jatooh samples by coliforms beyond certain level should be considered a public health hazard as they may cause dreadful diarrhea disease (Shore *et al.*, 1974 and Robert *et al.*, 1977). Besides, existence of fecal coliforms may be a real index of fecal pollution and possible existence of associated pathogens.

It is obvious from the achieved results (Table 6) that 20% of cream caramel and cake samples were contaminated by *B. cereus* in numbers ranged from less than 10^2 and $1x10^2$ as minimum to $1x10^4$ and $1x10^4$ as maximum with average counts of $6.7x10^3$ and $3.5x10^3$/g, respectively. While, the organism existed in 46.7% of jatooh samples in numbers varied from $1x10^2$ to $2x10^4$ with an average count of $5.3x10^3$/g. Although, *B. cereus* existed in low percentage with non-significant

numbers in cream caramel and cake, and comparatively in higher incidence (46.7%) and counts in jatooh samples, its public health hazard should not be neglected. It has been well documented that high numbers of *B. cereus* is needed to elicit symptoms of food poisoning. Therefore, the few numbers of *B. cereus* contaminating the samples should not be overlooked, as they can resist heat processing (Thermoduric) and can grow and multiply during refrigeration storage to reach numbers able to produce sufficient toxins to induce symptoms of food poisoning or even induce spoilage of the product (Richter *et al.*, 1992 and Meer *et al.*, 1993).

Regarding the examined samples of cream caramel, cake and jatooh (Table 7), *S. aureus* could not be detected in all of the examined cream caramel samples. Only one sample of cake (6.6%) was positive for *S. aureus*, while two samples of jatooh (13.3%) yielded *S. aureus*. Such contamination could be introduced after processing directly into the product by process line workers from careless handling. However, the low incidence of *S. aureus* recorded in the examined products, the result of experimental researches of various investigators proved that *S. aureus* organisms have found to multiply under refrigeration temperature when cooling process does not processed fast enough and temperature is conductive for their growth and multiplication.

As recorded in Table 8 a high incidence of anaerobes was detected in the examined cake and jatooh samples (73.3 and 80%, respectively). While, 33.3% of cream caramel samples yielded anaerobes bacteria. The source of anaerobes could be the raw materials used, contaminated egg content from shells during preparation or from the unpasteurized cream. Awareness should be directed toward the incidence of such type of bacteria, due to their thermoduric nature, enabling them to escape the heat processing, and multiply slowly at refrigeration temperature enough to induce health hazard or spoilage of the product.

The data presented in Table 9 point out that 46.7, 60 and 100% of the examined cream caramel, cake and jatooh samples were contaminated by yeasts and molds in numbers averaged $1.8x10^2$, $6.7x10^2$ and $2.2x10^4$/g, respectively. Unfortunately, the high incidence of yeasts and molds in the examined samples even in low numbers reflect the improper plant sanitation and neglected hygiene after processing or during holding till selling or serving. From the viewpoint of public health, certain strains of yeasts and molds when grow and multiply could be implicated in food poisoning outbreaks (Leistner and Ayres, 1967 and Mossel, 1982).

It is clearly evident from the obtained results that jatooh samples are comparatively highly contaminated by different types of microorganisms, even the levels of contamination are still beyond the hazard point. However, existence of some types of these microorganisms could be of public health concern, since they can grow and multiply sufficiently, if the conditions are right to produce their toxins.

Fortunately, due to the methods of processing and refrigerated storage till selling or serving, most of cream caramel and cake samples are of quite good quality even there are no available data to compare with. The quality of jatooh needs to be improved through the use of pasteurized ingredients especially cream that added to the product after its preparation.

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