

Dept. of Food Hygiene,
Fac. of Vet. Med. Assiut Univ.

FECAL CONTAMINATION OF ICE CREAM MIX POWDER SOLD IN ASSIUT CITY

(With 4 Tables)

By

**ENAS EL-PRINCE MOHAMED;
AMAL ALI ABDEL-HALEEM*; A.A. ABDEL-HAMEID
and MANAL MOHAMED AMIN ABDEL-RAHIM***

* Animal Health Institute, Assiut, Egypt

(Received at 16/12/2006)

التلوث القولوني البرازي لبودرة مخلوط الآيس كريم المباع في مدينة أسيوط

**إيناس البرنس محمد ، آمال على عبد الحليم ، أحمد عبد الحميد أحمد،
منال محمد أمين عبد الرحيم**

بالرغم من أن الآيس كريم يعتبر من منتجات الألبان الواسعة الانتشار، السهلة الهضم، ذات القيمة الغذائية العالية، والذي ينتشر تداوله بين فئات كثيرة وأعمار مختلفة من المستهلكين، إلا أنه قد يتعرض، تحت ظروف الإنتاج المختلفة وكذلك التصنيع والتوزيع، للتلوث الميكروبي بمختلف الأنواع الممرضة والتي تشكل خطورة لا يستهان بها على صحة المستهلك، ومنها الميكروبات القولونية البرازية، لذلك كانت هذه الدراسة على بودرة مخلوط الآيس كريم المباع في مدينة أسيوط. وقد تم جمع عدد ١٥٠ عينة عشوائية منها مختلفة الطعم مثل الشيكولاتة والمانجو والفراولة والفانيليا والبرتقال والموز وكذلك النيسكافيه (كل عبوة ٢٠٠ جم) وذلك من محلات مختلفة بمدينة أسيوط بتاريخ صلاحية عامان من تاريخ الإنتاج وقد تم فحص هذه العينات للوقوف على مدى تلوثها بالميكروبات القولونية البرازية. وقد أسفرت النتائج على أن الميكروبات المعوية قد تم عزلها بنسبة ٣٨.٧% من عينات بودرة الآيس كريم وكانت تحتوي على أعداد تراوحت بين ١٠^٢ و ١٠^٨×٨.٧ بمتوسط عددي قدره ١٠^٣×٣.٢ لكل جرام وكانت معظم العينات (٧٩.٣٢%) تحتوي على أعداد أقل من ١٠^٢ وأخرى بنسبة ٢٠.٦٨% تحتوي على أعداد تقع ما بين ١٠^٢ وأقل من ١٠^٣ لكل جرام وبواسطة طريقة MPN وجد أن ٣.٣٣% من العينات كانت ملوثة بكل من بكتريا القولون وكذلك بكتريا القولون البرازية، وقد كانت معظم العينات (٦٠%) تحتوي على عدد أقل من ١٠ والنسبة الباقية (٤٠%) تحتوي على أعداد من كل من هذه البكتريا تراوحت بين ١٠^٢ وأقل من ١٠^٣ لكل جرام. بينما لم يستدل على تواجد ميكروب الايشيريشيا كولاي في العينات التي تم فحصها. وتم عزل الميكروبات السبحية المعوية بنسبة ١٠.٧%، وكانت تحتوي على أعداد تقع بين أقل من ١٠٠ و ١٠^٦×١٠ بمتوسط عددي ١٠^٤×٤.٠٨ لكل جرام، وقد كانت ٥٦.٢٥، ٢٥ و ١٨.٧٥% من العينات المفحوصة تحتوي على أعداد من البكتريا تتراوح بين ١٠^٢ إلى أقل من ١٠^٣، أقل من ١٠^٢ و ١٠^٣ إلى أقل من ١٠^٤ لكل جرام على التوالي. وقد

أثبتت هذه النتائج أن عينات مخلوط بودرة الآيس كريم المختبرة لم تتعدى حدود المواصفات القياسية سواء العالمية أو المصرية. هذا وقد تم مناقشة الشروط الصحية الواجب اتخاذها لإنتاج مخلوط بودرة آيس كريم ذو جودة عالية وخالي من الميكروبات الممرضة التي قد تشكل خطورة على صحة المستهلك.

SUMMARY

One hundred and fifty random samples of ice cream mix powder with chocolate, mango, strawberry, vanilla, orange, banana and Nescafe flavors (200 grams weigh each) were collected from different groceries and supermarkets in Assiut City over a period of one year and examined for fecal contamination. Our current results showed that, *Enterobacteriaceae* were isolated from 38.7% of samples with a minimum of 10^2 , a maximum of 8.7×10^2 and an average of $3.2 \times 10/g$. The highest frequency distribution 46 (79.32%) had numbers of less than 10^2 cfu/g and 12 (20.68%) had counts ranged from 10^2 - $<10^3$ cfu/g. *Enterococci* could be isolated from 10.7% of the examined samples with a minimum, a maximum and an average count of <100 , 6×10^3 and $4.08 \times 10^3/g$, respectively. The frequency distribution of positive samples was descending from 9 (56.25%), 4 (25.0%) and 3 (18.75%) containing *Enterococci* in counts ranged from 10^2 - $<10^3$, $<10^2$ and 10^3 - $<10^4/g$, respectively. Otherwise, 5 (3.33%) samples were contaminated by both *coliforms* and *fecal coliforms* with frequency distribution of 3 (60%) that had counts of less than $10/g$ and 2 (40%) had counts ranged from 10 - $<10^2/g$. *E. coli* could not be detected. Recommendations were suggested to control the presence of such microorganisms in ice cream mix powder samples to avoid their undesirable changes that resulted in economic losses as well as public health hazard.

Key words: *Enterobacteriaceac, coliforms, fecal coliforms, Enterococci, contamination, ice cream mix powder.*

INTRODUCTION

Ice cream is the most palatable, nutritious, healthful and relatively inexpensive dairy food. It is highly appreciable by all ages because it is considered a safe, enjoyable, energy giving and refreshing food because levels of water soluble vitamins and minerals are two to three times higher than those of full cream milk. Also, it is a valuable source of vitamin B, and contains twice vitamin A content as that of

milk (Varnam and Sutherland, 1994). Nowadays, ice cream mix powder produced and sold in increasing quantities for manufacturing of ice cream on a large scale, in markets, and it is also purchased for preparation of ice cream at home. Ice cream mix is often stored (aging) for few hours or even overnight; so there is opportunity for bacterial growth and multiplication, rendering the product to be of public health hazard. Because ice cream is consumed without any treatment that might reduce its microbial load, it is necessary to maintain a high level of microbial quality. For this purpose, many countries have adopted mandatory manufacturing practices and standards to ensure an adequate and wholesome supply of such product.

In spite of the high temperature attained in preparation of ice cream mix powder and its low moisture content, its packaging process, addition of additives and flavoring agents, methods of preservation and transportation it may, at times, be responsible for transmitting some pathogenic and food poisoning bacteria to consumers. Their presence in dried milk products provides a useful index in determining the hygienic quality of the products. The presence of *coliform* bacteria in milk powder has been reported by Leitao *et al.* (1973); Aleksieva (1974); Jhodekeer and Nambudripad (1975); Jarchovská and Hartmanova (1975); Arun *et al.* (1980); and Lück *et al.* (1980). *Enterococci* organisms have a distinctive role as an indicator of poor factory sanitation; so that, they were enumerated and isolated from milk powder by many workers (Stark, 1970; Leitao *et al.*, 1973; Aleksieva, 1974 and Lück *et al.*, 1980).

As the microbial quality of ice cream mix powder with various flavoring agents, reflects the care with which milk was produced and the sanitary conditions prevailing during its manufactures, therefore, this work was planned to detect the fecal pollution by determination of *Enterobacteriaceae*, total *coliforms*, *fecal coliforms*, *E.coli* and *Enterococci* counts.

MATERIALS and METHODS

A- Collection of the samples:

150 random samples of ice cream mix powder with chocolate, mango, strawberry, vanilla, orange, banana and Nescafe flavors (200 grams weigh each) in retail packages ready for sale were collected from different groceries and supermarkets in Assiut City over a period of one year. The expired date was after 2 years, from the production time. To avoid contamination, the samples were dispatched directly to the

laboratory where they were examined microbiologically to evaluate their quality.

B- Preparation and serial dilution of samples:

Packages of ice cream mix powder were cleaned and being aseptically opened. 11 grams of the ice cream mix powder samples were mixed with 99 ml of sterile 0.1% peptone water and thoroughly mixed to have a dilution of 1/10 from which ten fold serial dilutions were prepared as recommended by A.P.H.A. (1992).

C- Experimental techniques:

Included:

- 1- Enumeration and isolation of *Enterobacteriaceae* using Violet Red Bile Glucose Agar (VRBGA). (Mercuri and Cox, 1979).
- 2- Total *coliforms*, *fecal coliforms* and *E.coli* counts using (MPN). (A.O.A.C., 1975).
- 3- Enumeration and isolation of *Enterococci* using KF streptococcal agar. (Deibel and Hartman, 1976).

D- Biochemical reactions for *Enterobacteriaceae* organisms:

Included:

- 1- Indol production, Methyl red, Voges-Proskauer, Citrate utilization, H₂S production tests: (Finegold and Martin, 1982)
- 2- Urease test: (Edwards and Ewing, 1972)
- 3- Glucose and sugar fermentation tests: (Speck, 1976)

RESULTS

The obtained results are recorded in Tables 1-4.

Table 1: Statistical analytical results of *Enterobacteriaceae* and *Enterococci* in the examined ice cream mix powder samples.

Types of M.os	Positive samples		Count/g		
	No./150	%	Min.	Max.	Average
Enterobacteriaceae	58	38.7	*<100	8.7X10 ²	3.2X10
Enterococci	16	10.7	*<100	6X10 ³	4.08X10 ³

*Colonies could not be detected on the plates.

Table 2: Frequency distribution of the positive ice cream mix powder samples based on their *Enterobacteriaceae* and *Enterococci* count/g.

Count/g	Distribution			
	Enterobacteriaceae		Enterococci	
	No.	%	No.	%
$<10^2$	46	79.32	4	25.00
$10^2-<10^3$	12	20.68	9	56.25
$10^3-<10^4$	-	-	3	18.75
Total	58	100.00	16	100.00

Table 3: Incidence of *coliforms* and fecal *coliforms* in the examined ice cream mix powder samples.

Types of M.os	Positive samples	
	No. /150	%
coliforms	5	3.33%
fecal coliforms	5	3.33%

Table 4: Frequency distribution of positive ice cream mix powder samples based on their *coliforms* and *fecal coliforms* count (MPN/g).

Count/g	Distribution			
	coliforms		fecal coliforms	
	No.	%	No.	%
3-<10	3	60.00	3	60.00
$10-<10^2$	2	40.00	2	40.00
Total	5	100.00	5	100.00

DISCUSSION

The results recorded in Table 1 revealed that, 58 (38.7%) of the examined ice cream mix powder samples were contaminated by *Enterobacteriaceae* in counts ranged from <100 to 8.7×10^2 with an average count of 3.2×10 cfu/g. Table 2 showed that, most of positive

samples 46 (79.32%) contained numbers less than 10^2 cfu/g, while, 12 (20.68%) had counts ranged from 10^2 to $<10^3$ cfu/g. These counts are in fair agreement with those recorded by Abdel-Haleem (1998) in ice cream mix powder. But in milk powder, Sprang (1969) recorded lower incidence. While, higher counts were conducted by Dardir (2005).

Contamination of large numbers of ice cream mix powder samples with *Enterobacteriaceae* could be taken as an index of fecal contamination and also could be attributed to the unsanitary practices, poor hygienic quality of ingredients used and/or may be due to the contamination during packaging.

Regarding the *Enterococci* counts, it is evident that 16 (10.7%) samples contaminated with *Enterococci*. The level of contamination varied from <100 to 6×10^3 with an average of 4.08×10^3 /g. The frequency distribution arranged descending from 9 (56.25%), 4 (25.0%) and 3 (18.75%) samples ranged from 10^2 - $<10^3$, $<10^2$ and 10^3 - $<10^4$ /g, respectively (Table 1 and 2). Higher counts and incidences were estimated by El-Bassiony and Aboul-Khier (1983) and Abdel-Haleem (1998) in ice cream mix powder samples. While, Aleksieva (1974) recorded higher counts in milk powder samples.

The occurrence of *Enterococci* in ice cream mix powder samples seems to be illogic, because no acceptable level of these microorganisms could be present, and may be attributed to post manufacture contamination, the heat resistant character of the organisms, and contamination during packaging and/or improper methods of distribution. The public health significance can not be denied, especially, when the organisms found in tremendous number in the product as they have been implicated in several food poisoning outbreaks (International Committee on Microbiological Specification for Foods (ICMSF), 1978).

It is clearly evident from the results illustrated in Table 3, that both *coliforms* and *fecal coliforms* could be isolated from 5 out of 150 samples (3.33%). Most of positive samples 3 (60%) contained *coliforms* and *fecal coliforms* less than 10 cfu/g. While, 2 (40%) had counts varied from 10 - $<10^2$ cfu/g (Table 4). Higher incidence of total *coliforms* was established by El-Bassiony and Aboul-Khier (1983). World Health Organization Standards (1981) reported that *coliforms* should fail to be detected in the examined ice cream mix powder samples. By comparing these counts with Egyptian Standard (1988), Varnam and Sutherland (1994) and U.S. Dairy Export Council (1996-2002) standards, *coliforms* counts did not exceed these standards.

In case of *fecal coliforms*, nearly similar results were recorded by Aleksieva (1974) in infant dried milk samples. In the contrary, El-Prince and Korashy (2003) could not isolate these organisms from the examined milk powder samples.

Fortunately, *E. coli* could not be detected in all of examined ice cream mix powder samples. *Coliforms* contamination could be attributed to poor quality ingredients, careless hygiene during packaging. Moreover, *coliforms* and *fecal coliforms* still continue to be considered as indicator organisms of choice in examining milk and milk products (Marier *et al.*, 1973).

As good sanitation and strict hygienic measures during processing, packaging, preservation and transportation of these desserts is fundamental, therefore, suggestive measures for control of microorganisms are to be considered to ensure safety and high quality ice cream mix.

REFERENCES

- A.O.A.C. (1975): Association of Official Analytical Chemists Official Methods for Analysis, 21st Ed., Washington, D.C.
- A.P.H.A. (1992): Standard Methods for the Examination of Dairy Products. 16th Ed., American Public Health Association, New York.
- Abdel-Haleem, Amal, A. (1998): Bacterial contamination of ice cream mix powder in Assiut City. Alex. J. Vet. Sci., 14: 53-61.
- Aleksieva, V. (1974): *Enterococci* in dried milk. Veterinarmeditsiinski Nauki, 11, 72. Dairy Sci. Abst., 37: 36 (1975).
- Arun, A.P.S.; Prasad, C.R.; Basant, K.S. and Prasad, B.N. (1980): Occurrence of *coliform* bacteria in skim milk powder. Indian J. Dairy Sci., 33(1): 119-122.
- Dardir, H.A. (2005): Health threatening of *Enterobacter sakazakii* associated with infant food. J. Egypt. Vet. Med. Assoc., 65(2): 7-13.
- Deibel, R.H. and Hartman, P.A. (1976): The *Enterococci*. In: Compendium of Methods for the Microbiological Examination of Foods. M. L. Speck 2nd Ed., American Public Health Association, Inc.
- Edwards, P.R. and Ewing, W.H. (1972): Identification of *Enterobacteriaceae*. 3rd Ed., Burgess Pub. Co., Minneapolis, Minnesota.

- Egyptian Standard* (1988): Dried Milk. Egyptian Organization for Standardization and Quality Control (1648-988).
- El-Bassiony, T.A. and Aboul-Khier, F.A.* (1983): Bacteriological evaluation of dried milk products produced in Sakha processing dairy plant. *Assiut Vet. Med. J.*, 11(21): 159-163.
- El-Prince, Enas, M. and Korashy, Eman, A.* (2003): Microbiological quality of dried milk based infant foods in Assiut city. *Assiut Vet. Med. J.*, 49(97): 190-203.
- Finegold, S.M., and Martin, W.J.* (1982): *Bailley and Scott Diagnostic Microbiology*. 6th Ed., C.V. Mosby Co., St. Louis, Toronto, London.
- International Committee on Microbiological Specification for Foods (*ICMSF*) (1978): *Microorganisms in Food. Their significance and methods of enumeration*. 2nd Ed., Univ. of Toronto Press, Toronto, Buffalo London.
- Jarchovská and Hartmanová* (1975): Incidences of Gram-negative organisms in dried milk product. *Veterinativstvi*, 25: 399. *Dairy Sci. Abst.*, 38: 693 (1976).
- Jhondekeer, D.R. and Nambudripad, V.K.N.* (1975): *Indian J. Dairy Sci.*, 28: 215 (Cited after Arun *et al.*, 1980).
- Leitao, M.F.D.; Delazari, I. and Mazzoni, H.* (1973): Microbiology of dehydrated foods. *Coletanea do Instotute Tecnologia do Alimentos*, 5: 223. *Dairy Sci. Abst.* 37: 590 (1975).
- Lück, H.; Gordaam, I. and Dunkeld, M.* (1980): Incidence of pathogenic and other undesirable bacteria in milk powder. *South African J. Dairy Technol.*, 12(2): 51-56.
- Marier, R.; Wells, J.G; Swanson, R.C.; Callahan, W. and Mehlman, L.J.* (1973): An outbreak of enteropathogenic *E.coli* food borne disease traced to imported French cheeses. *Lancet*, 2:1376.
- Mercuri, A.J. and Cox, N.A.* (1979): *Coliforms and Enterobacteriaceae* isolates from selected foods. *J. Food Prot.*, 42(9): 712.
- Speck, M.L.* (1976): *Compendium of Methods for the Microbiological Examination of Foods*. 2nd Ed., Printing. American Public Health Association, Inc.
- Sprang, F.J. Van* (1969): Bacteriological requirements for dried infant and weaning foods. (In” the microbiology of dried food. *Proceedings of the sixth International Symposium on Food Microbiology*”) pp.: 496-510. *Dairy Sci. Abst.*, 32: 2563 (1970).
- Stark, E.* (1970): Fecal *Streptococci* on barley, malt kernels and instant milk powder. *J. Appl. Microbiol.*, 20: 2000.

- U.S. Dairy Export Council (1996-2002):* U.S. Standards for milk powders. American Dairy Products Institute, Bulletin 916.
- Varnam, A.H. and Sutherland, J.P. (1994):* Milk and Milk Products, Technology, Chemistry and Microbiology. 1st Ed., Chapman and Hall Pub., London.
- World Health Organization Standards (1981):* Nutrition and Food Safety Spain. Int. Digest of Health Legislation, 32(4): 781-783. Dairy Sci. Abst., 45: 186 (1983).