

MICROBIOLOGICAL STATUS OF CHOCOLATE AND FRUITY MILKS

BY

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INTRODUCTION

Chocolate and fruit milks are delicious and nutritious drinks that makes it suitable and palatable for consumers. Chocolate milk is made by blending cocoa powder with sucrose and stabilizer then mixing with whole milk or reconstituted skimmilk. The mix is homogenized, heat-treated, bottled and autoclaved, while fruit milk is made by mixing nectar (Fruit juice and pulp blended with sugar syrup) with milk that has been pasteurized. Sugar syrup with disodium phosphate is then added. After homogenization, the drink was bottled while still hot. The inforcement of milk with sugar, fruits cocoa and other additives improve its flavour and food value. Although the product is a good medium for growth of organisms, yet such additives may add new contaminants to the products.

While locally inforced chocolate and fruit milks find the readiest sale on the market, its microbiological status has not yet been enough tackled. Therefore, this work has been planned to fulfil this gap.

Microbiological status of chocolate and fruity milks.

MATERIAL AND METHODS

Fifty random samples of chocolate and fruit milks (25 each), collected from different shops in Giza city, were microbiologically examined for determination of total colony count (TCC), aerobic spore formers count (ASFC), coliform content (MPN/100 ml), total yeast count (TYC), total mould count (TMC), as well as detection of anaerobes and staphylococci. ASF were counted on dextrose tryptone agar (Oxoid, 1982) and identified according to Sneath et al., (1986), Total yeast & mould counts as well as identification of isolates were conducted according to Samson et al., (1981). Anaerobic spore formers were detected by using Stormy fermentation test (Cruickshank et al., 1969). Isolated micrococci were identified according to Cowan & Steel (1965).

RESULTS AND DISCUSSION

Total colony count (TCC/ml):

Results given in Table (1) reveal that the maximum TCC/ml of chocolate and fruit milks was 9.9×10^3 & 4.2×10^3 , while the minimum was 160 & 180 with a mean value of $3.12 \times 10^3 \pm 0.56 \times 10^3$ & $1.64 \times 10^3 \pm 0.25 \times 10^3$ respectively. The highest frequency distribution (60% and 52%) of examined chocolate and fruit milk samples lies within the range $10^2 - 10^3$ & $10 - 10^2$ respectively (Table 2).

Aerobic spore formers (ASF):

Results presented in Table (1) show that the count of ASF organisms/ml of chocolate & fruit milks ranged from 200 to 1.3×10^4 & 270 to 6.4×10^3 respectively. The highest frequency distribution of examined samples (48 % & 68%) lies within the range $10^2 - 10^3$ (Table 2).

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Bacillus brevis, *B. stearothermophilus*, *B. subtilis*, *B. cereus*, *B. coagulans* and *B. mycoides* could be isolated from both types of milks at varying percentages ranging from 14% to 30% (Table 3).

The presence of ASF thermotolerant organisms in chocolate and fruit milks depends upon their initial load in raw milk, certain additives, heat treatment, and the extent of post-processing contamination. Those organisms proved to induce certain objectionable changes in milk and some dairy products (Ingram & Luthi, 1961, Mossel et al., 1974 & Pesic et al., 1986), while some strains have been implicated in cases of food poisoning (Frazier & Westhoff, 1983 and Smykal & Rokoszewska, 1976).

Yeast count (TYC):

Inspection of Table (1) indicates that 15 chocolate milk and 18 fruit milk samples contained yeasts, with a mean value of $0.45 \times 10^2 \pm 0.15 \times 10^2$ & $0.51 \times 10^2 \pm 0.11 \times 10^2$ respectively. The highest frequency distribution of examined chocolate & fruit milk samples (73.33% & 77.78%) lies within the range $10-10^2$ & $0-10$ respectively (Table 2).

Mould count (TMC):

Results presented in Table (1) shows that 80% of chocolate and 60% of fruit milk samples contained moulds with a mean count of $0.90 \times 10^2 \pm 0.50 \times 10^2$ & $0.32 \times 10^2 \pm 0.04 \times 10^2$ respectively. The highest frequency distribution of examined chocolate and fruit milk samples (70% & 100%), lies within the range $10-10^2$ & $0-10$ respectively (Table 2).

Isolated yeasts and moulds:

It is evident from Table (3) that *Saccharomyces ellegons*, *Sacch. cerevisiae*, *Trichosporon fermentans*, *Torulopsis stellata* and *Cryptococcus fermentans* could be isolated at varying percentages ranging from 9.09% to

Table (1): Statistical analytical results of total microbial counts in examined chocolate and fruit milk samples

	Chocolate milk						Fruits milk					
	+ve No.	Sam- ples %	Min.	Max.	Mean	SEM ±	+ve No.	Sam- ples %	Min.	Max.	Mean	SEM ±
TCC/ml	25	100	160	9.9×10^3	3.12×10^3	0.56×10^3	25	100	180	4.2×10^3	1.64×10^3	0.25×10^3
ASFC/ml	25	100	200	1.3×10^4	3.79×10^3	0.79×10^3	25	100	270	6.4×10^3	2.88×10^3	0.47×10^3
TYC/ml	15	60	20	2×10^2	0.45×10^2	0.15×10^2	18	72	10	100	0.51×10^2	0.11×10^2
TMC/ml	20	80	20	8×10^2	0.90×10^2	0.50×10^2	15	60	10	60	0.32×10^2	0.04×10^2

Table (2): Frequency distribution of total microbial counts in examined chocolate and fruit milk samples

Inter- vals	Chocolate milk						Frequency								Fruits milk			
	TCC/ml		ASFC/ml		TYC/ml		TMC/ml		TCC/ml		ASFC/ml		TYC/ml		TMC/ml			
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%		
0-10	-	-	-	-	4	26.67	6	30	-	-	-	-	14	77.78	15	100		
$10-10^2$	10	40	9	36	11	73.33	14	70	13	52	8	32	4	22.22	-	-		
10^2-10^3	15	60	12	48	-	-	-	-	12	48	17	68	-	-	-	-		
10^3-10^4	-	-	4	16	-	-	-	-	-	-	-	-	-	-	-	-		
Total	25	100	25	100	15	100	20	100	25	100	25	100	18	100	15	100		

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15.15%, while moulds that belonged to the genera *Penicillium*, *Scopulariopsis*, *Verticillium*, *Aspergillus*, *Fusarium*, and *Mucor* could be isolated at varying percentages ranging from 5.7% to 22.86% (Table 3.).

As chocolate and fruit milks contain fruits, sugars Coccoa or other additives, hence such nutrients favour the growth of yeasts & moulds. Yeast growth usually appears by their characteristic yeasty odour which may, at times, be masked by the present fruits, while presence of moulds causes economic losses due to discoloration, poor appearance and off flavour (Jantea et al., 1972 and Frazier & Westhoff, 1983). Some moulds are capable of producing mycotoxin as aflatoxin which is carcinogenic (Jacquet & Teherani, 1976, Bullerman, 1980 and ICMSF, 1982).

Results presented in Table (3) show that 11 out of 50 chocolate and fruit milk samples examined (22%) contained micrococcus spp., while *Staphylococcus aureus* and *S. epidermidis* failed detection.

Anaerobic spore forming organisms were detected in 10 chocolate milk and 5 of fruit milk samples (Table 4). Some strains of *Clostridium* were saccharolytic, others were proteolytic and most of them were also gas formers (Frazier & Westhoff, 1983, and Pesic et al., 1986).

The conclusion derived from this study indicates that chocolate and fruit milks produced in our country proved to contain different types of organisms at various rates which may be attributed to low quality ingredients and unsatisfactory methods of production. Therefore best quality ingredients and satisfactory hygienic measures during production and packaging should be adopted to improve the quality of the products.

Table (3): Incidence of isolated organisms from chocolate and Fruits examined milk samples

Isolates	Positive samples		Isolates	Positive samples	
	No.	%		No.	%
Bacteria			Trichosporon fermentans	5	15.15
Bacillus brevis	15	30	Torulopsis stellata	3	9.09
B. stearothermophilus	12	24	Cryptococcus fermentans	3	9.09
B. subtilis	10	20	Moulds		
B. cereus	8	16	Penicillium spp.	8	22.86
B. coagulans	8	16	Scopulariopsis spp.	6	17.14
B. mycoides	7	14	Verticillium spp.	6	17.14
Micrococcus spp.	11	22	Asperigillus flavus	3	8.57
Yeast			Asp. fumigatus	3	8.57
Saccharomyces ellegans	5	15.15	Fusarium spp.	2	5.7
Sacch. cerevisiae	4	12.12	Mucor spp.	2	5.7

Table (4): Incidence of anaerobes in examined chocolate and fruits milk samples (Stormy fermentation test).

	No. of examined samples	Positive samples	
		No.	%
Chocolate milk	25	10	40
Fruits milk	25	5	20

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SUMMARY

Fifty random samples of chocolate and fruit milks (25 each) collected from different shops at Giza city, were examined. The mean TCC, ASFC, TYC and TMC found/ml in chocolate and fruit milks were 3.12×10^3 & 1.64×10^3 , 3.79×10^3 & 2.88×10^3 , 0.45×10^2 and 0.51×10^2 and 0.90×10^2 & 0.32×10^2 respectively. Anaerobes were detected in 40% & 20 of chocolate and fruit milk samples respectively. *Bacillus brevis*, *B. Stearothermophilus*, *B. subtilis*, *B. cereus*, *B. coagulans* and *B. mycoides* could be isolated at varying percentages. Out of 50 samples examined, 11 samples proved to contain *Micrococcus* spp., while *Staphylococcus aureus* and *Staph. epidermidis* failed detection. Coliforms could not be detected in all examined samples. Yeasts belonging to the genera *Saccharomyces*, *Trichosporon*, *Torulopsis*, and *Cryptococcus* could be isolated at varying percentages. While moulds belonging to the genera *Penicillium*, *Scopulariopsis*, *Verticillium*, *Aspergillus*, *Fusarium* and *Mucor* were isolated. The public health importance of existing microorganisms, their effect on the products as well as suggested control measures were discussed.

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