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MICROBIOLOGICAL EVALUATION OF ASSIUT MARKET YOGHURT THROUGH THE SHELFLIFE TIME IN REFRIGERATOR

(With 4 Tables)

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(Received at 9/3/2004)

التقييم الميكروبيولوجى للزبادى المتداول بأسواق أسيوط
أثناء فترة صلاحيته داخل الثلاجة

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تــم تجميع عدد ١٠٥ عينة من الزبادى المنتج محليا فى مدينة أسيوط وكذلك المنتج بواسطة
المصانع الكبرى والذى يباع فى محلات منتجات الألبان والسوبرماركت. قسمت العينات إلى
ثلاث مجموعات, المجموعة الأولى (٤٥ عينة) وتمثل الزبادى المنتج محليا فى أسيوط ومدة
الصـلاحية ثلاث أيام, المجموعة الثانية (٣٠ عينة) وتمثل الزبادى المنتج بواسطة الشركات
الكبرى أمـا المجموعـة الثالثة (٣٠ عينة) فتمثل الزبادى المنتج بواسطة هذه الشركات
والمضاف اليه الفواكه (مانجو – فراولة – خوخ) والذى صلاحيته م ١٢-١٥ يوم عند الحفظ
فـى الـثلاجة على درجة ٥° ٢° م. وقد تم فحص جميع العينات ميكروبيولوجيا ووجد أن
٥٣,٣% , ٦,٧% , ٤٠% , ٤٠% , ٢٠% مـن العينـات موجبة فى اليوم الأول لصلاحية
الـزبادى للعيـنات من المجموعة الأولى والثانية والزبادى المطعم بالمانجو–الفراولة وكذلك
الـخـوخ على الترتيب. كانت نسبة العينـات الموجبـة للبكتريـا المتكورة المعوية ١٣,٣%
١٣,٣% , ٤٠% فـى اليوم الاخير للصلاحية للمجموعات ١, ٢ , والزبادى المطعم بالخوخ
علـى الترتيب. ٣٣,٣% , ٢٠% ,%٢٠ ,%٦٠,% و ٦٠% من العينات التى تم فحصها كانت
موجـبة للميكروبات القولونية فى العينات من المجموعة ١ ٢, والمطعمة بالمانجو , الفراولة
وكذلـك الخوخ على الترتيب. وقد كشفت النتائج أن عينات الزبادى التابعة للمجموعة الاولى
هــى الوحـيدة الموجـبة للميكروبات المتكورة فى أول يوم للصلاحية. أما بالنسبة للخمائر
والفطـريات فقد وصل المتوسط العددى إلى ٥٠٢× ١٠ ⁴ , ١×١٠ ⁴ فى اليوم الثالث للزبادى
المنـتج محليا بينما كانت النسبة والمتوسط العددى أقل فى الزبادى المنتج بواسطة الشركات
الكـبرى. وقـد وجـد أن إضافة السكر والفواكه للزبادى يجعلها وسط جيد لنمو الخمائر
والفطـريات وكذلـك ربما للتلوث بالميكروبات الأخرى ولكن كثير من الميكروبات الضارة
بالصـحة تمـوت نتـيجة للتـنافس المضاد بواسطة بكتريا حامض اللاكتيك وكذلك الوسط
الحمضى للزبادى ولذا يجب الإشارة الى ضرورة ان تكون الإضافات للزبادى من الفواكه أو

السكـــر مـــن مصـــادر جـــيدة. وأخيرا فأنه نتيجة للنتائج التى توصل لها هذا البحث ننصح
المستهلك بأن يتناول الزبادى المنتج محليا فى اليوم الثانى للصلاحية كما أن تناول الزبادى
المنتج بواسطة الشركات الكبرى يعد أكثر أمانا من الناحية الميكروبيولوجية.

SUMMARY

One hundred and five commercially produced yoghurt samples were collected randomly from Assiut Dairies and supermarkets in their containers representing 3 main groups. Group I: yoghurt produced in small dairies of 3 days shelf life. Group II and III yoghurt produced in large modern dairies of 12-15 days shelf life as recommended by their producers when refrigerated at $5 \pm 2°C$, samples were divided as plain yoghurt and fruit yoghurt. All yoghurt samples were subjected to microbiological examination for total psychrotrophic count, enterococci count, coliform count, staphyococcal count, yeast and mold count and anaerobes. The total psychrotrophic counts showed that 53.3, 6.7, 40, 40 and 20% of the samples were positive in the first day of validity for samples of group I, group II and yoghurt flavored with mango, strawberry and peach respectively. The percentages of enterococci were 13.3, 13.3 and 40% in the last day of validity in group I, II and yoghurt flavored with peach respectively. 33%, 20%, 20%, 60% and 60% of the samples tested for coliform in the last day of validity were positive in group I, II, mango, strawberry and peach flavored yoghurt respectively. In the first day of validity the only samples found to be positive for staphylococcal count belongs to group I. Regarding yeast and mold, the average counts reached to $5.2x\ 10^4$ and $1x10^4$ in the last day of validity in group I. Lower incidence and counts were obtained in group II, III. Also in the first day of validity the three groups were positive to anaerobes except that flavored with peach. Unfortunately the added sugar, fruit and flavor to yoghurt make it a good medium for growth of yeasts and molds. It may also contribute to microbial contamination even though most bacteria, particularly those of public health significance, soon die out because of the marked antagonism exerted by the lactic acid bacteria and acidic pH. For these reasons fruits and flavors added to yoghurt must be subjected to vigorous quality control program as well as control of cultures and sanitation during manufacture. From the stand point of safety, the authors highly recommend to consume yoghurt of group I in the second day of validity. Moreover, yoghurt produced in modern dairies excelled other samples.

Key words:Microbiological, Plain yoghurt, Fruit yoghurt, Shelflife.

INTRODUCTION

Yoghurt is the most popular fermented milk produced in Egypt and worldwide. The great popularity of yoghurt is due to its refreshing and thirst-quenching in hot weather. The value of yoghurt in human nutrition is based not only on the strict nutritive effect of milk from which it is made and increased digestibility due to changes of milk constituents during the fermentation period, but also on the beneficial effect of intestinal microflora, prophylactic and heeling effects (Rasic and Kurmann, 1978; Agerbeak *et al.*, 1995; Tvede, 1996; Buttriss, 1997; Hussein and Kebary, 1999 and Zedan *et al.*, 2001).

Fruit yoghurt usually have stabilizers incorporated to reduce whey separation during distribution. Many of the stabilizers are complex carbohydrates which providing "a bulking agent" so stimulating intestinal peristalsis and avoiding some of the risks of colonic malfunction . It also absorb some of the potentially toxic chemicals that may be formed in the large intestine as a result of bacterial action. This unavailable carbohydrates acting to further delay the diffusion of sugar to the intestinal wall that could help both lactose-intolerant patients and those prone-to-post prandial hyperglycaemia (Robinson and Khan, 1978 and Tamime and Robinson, 1985).

Long shelflife of dairy products is a very important aim for people working in dairy industry especially after changing in production trends, processing and distribution of dairy products. Shelflife is the period between packaging of the product till become unacceptable for consumers. (Smithwell and Kailasapthy, 1995)

The shelflife of a product is determined by its physical characteristics (smell, taste, feel, appearance) and safety. When a product shows signs of deterioration or if a pathogen is found, then the product is no longer fit for sale. Temperature has a great effect on both the growth and elimination of bacteria. As temperature gets farther from optimal, either higher or lower, bacteria will cease to grow although they may still exist. For many organisms there may be a decline over time but a high percentage will survive and flourish when the temperatures are right for their growth. Furthermore, wide temperature fluctuation of the refrigeration system during handling of yoghurt is inadequate to prevent the rise of temperature until it reaches to consumer (Moustafa *et al.*, 1988 and El-Baba, 1999).

The keeping quality of yoghurt therefore, depends upon the number and types of microflora present in it. So, the present study was

undertaken to provide information on the safety of Assiut market yoghurt throughout the shelflife time in refrigerator to protect the consumer from purchasing of poor quality product, or in the extreme cases, product that might constitute a health hazard.

MATERIAL and METHODS

A-Collection of samples:

A total of 105 random yoghurt samples were collected from Assiut dairies and supermarkets in their containers representing 3 main groups:

Group I: 45 samples of plain yoghurt produced in small dairies of 3 days shelflife. These samples (15 each) were examined in the first, second and third day of production

Group II and III: yoghurt produced in large modern dairies of 12-15 days shelflife as recommended by their producers when refrigerated at 5 ± 2°C, samples were divided as plain yoghurt (30 samples) and fruity yoghurt with mango, strawberry and peach (10 samples, each). These samples were examined as fresh and at the last day of validity.

B-Preparation of samples:

Samples were prepared following the procedures described by American Public Health Association (APHA), (1992).

C- Examination of samples:

Each sample was subjected to the following examinations:

1- Determination of titratable acidity percentage as described by AOAC, (1975).

2- Total psychrotrophic count:

Standard plate count technique was performed as recommended by Frank *et al.*, (1992).

3- Enterococci count:

Using KF streptococcal agar as described by Deibel and Hartman, (1982).

4- Coliform count (MPN).

Using lauryl sulfate tryptose broth and confirmed by culture on brilliant green bile broth , AOAC, (1975) and ICMSF (1978).

5- Staphylococcal count:

Surface spread plate method recommended by ICMSF (1978) was used.

6- Yeast and molds count:

Carried out according to Harrigan and McCance, (1976).

7- Detection of anaerobes (Cruickshank *et al.*, 1969).

RESULTS

The obtained results were summarized in Tables 1- 4.

DISCUSSION

The results recorded in Table 1 showed that the acidity percentage of the examined group I yoghurt samples obtained from small dairies ranged from 0.65-1.0% with an average of 0.86% in the first day of validity. It reached 0.96 and 0.94% in the second and third day of validity respectively.

In group II and III, the average percentage of acidity ranged from 0.98-1.17 during its shelflife time. The maximum titratable acidity was 1.55 (Tables 2-4), similar results were recorded by Abdel-Hakeim (1986) and El-Bessery (2001).

From the data obtained in Table 1, both psychrotrophs and enterococci organisms were detected in 53.3 and 26.7% in group I samples in the first day of validity. The maximum count reached $7x \ 10^3$ and $1x10^3$ /g, it should be noted that the average count was decreased in the second day and reached $1.5x10^2$ and $5x \ 10$/g, respectively.

In group II, only 6.7% of the samples contained psychrotrophs with a minimum number of only 50 cfu/g in the first day of validity and completely disappeared from all the samples tested in this group in the last day of validity. Opposite results were recorded with fruit yoghurt with mango, the maximum numbers of the organisms were 10 and 50 cfu/g in the first and last day of validity, respectively. While in case of strawberries flavored yoghurt the psycrotrophic count were higher than in case of mango flavored samples where it reached maximum numbers of 3×10^2 and 4×10^2 /g in the first and last day of validity respectively. The maximum numbers of psychrotrophs in peaches flavored yoghurt were near to that in yoghurt flavored with strawberries. This finding is approximately similar to Moustafa *et al.,* (1988), they isolated psychrotrophic organisms with a level ranging from 10^2- 10^3/g. A higher maximum numbers were recorded by Arnott *et al.,* (1974), they found that 18 out of 152 samples of commercially produced yoghurt in Ontario, Canada had psychrotrophic counts $> 10^3$/g and 15 of the 18 samples registered counts in excess of 10^5/gm. Higher results of psychrotrphic counts were also recorded by Abdel-Hakeim (1986) and El-Bessery (2001), they recorded a maximum numbers of psychrotrophic organisms of 6.9×10^4 and 8×10^8 /g respectively. The psychrotrophic organisms may produce proteolytic or lipolytic enzymes

leading to decrease the keeping quality of the product. Furthermore, individual members of these bacteria have been implicated as a causal agents of food poisoning (Hobbs, 1975).

Regarding enterococci, the average counts in group I were 3.5 x 10^2 and 0.6 x10^2 cfu/g in the first and last day validity, respectively. Other wise, 20 and 40% of strawberry and peach flavored yoghurt were contaminated with enterococci in the first day, while, it failed detection in yoghurt samples falvored with mango. These results are lower than that obtained by Abdel-Hakeim (1986) 60% and El-Bessery (2001) 68%.

The presence of enterococci in yoghurt even in few numbers is considered as an index of fecal contamination. Enterococci are comparatively heat resistant, salt tolerant, can grow at a wide range of temperature and could induce certain undesirable changes. Furthermore, their presence in large numbers could be implicated with outbreak of food borne gastroenteritis (Slantez, et al., 1963 and ICMSF, 1980).

Table 1 showed rapid decrease of staphylococcal organisms (in group I) in the second day of validity with an average of 2.6x 10 cfu/g and completely disappeared in the third day. On the other hand, these organisms could not be detected in group II and III samples except one sample of yoghurt flavored with peach which was found to be positive with a minimum count of 2x10/g in the last day of validity (Tables 2-4). The obtained findings are coincided with those obtained by El-Bessery (2001) who found that all the examined yoghurt samples were free from *Staphylococcus aureus*. Arnott et al., (1974) detected staphylococci with a count ranging from <1 to 940/g in the examined samples. The relatively high acidity and/or the greatest inhibitory effect of yoghurt starter culture of this product on undesirable organisms should explain the absence or low number of staphylococci count in the examined samples.

Coliforms and fecal coliform bacteria still continue to be considered as indicator organisms of choice in examining milk and milk products for pin pointing the unhygienic conditions during milking, handling and distribution.

Realizing the results recorded in Table 1, 66.6% of group I yoghurt samples was found to be positive for coliforms, with a maximum number of more than 2400/g in the first day. While, 46.7 and 33.3% of the samples contained coliforms in the second and third day of validity, respectively.

Concerning yoghurt produced in large modern dairies, 46.7% and 20% of group II samples were contaminated with coliforms, with

maximum numbers of 1.1×10^3 and 2.3×10 cfu/g in the first and last day of validity, respectively (Table 2).

Data illustrated in Tables 3&4 revealed that only one sample of strawberry and mango flavored yoghurt contained coliforms in the first and last day of validity, respectively. A larger percentage (60%) of strawberry and peach flavored samples had at least 9 cfu/g coliforms when tested in the last day. Similar results were recorded by El-Baba (1999) who found that yoghurt of modern dairies recorded the least coliforms count. On the other hand, higher percentages of coliforms 75 and 70% were detected by Abdel-Hakeim (1986) and El-Bessery (2001).

It is noteworthy from this study that the drastic reduction in number of coliforms in yoghurt throughout the shelflife time may be due to the increased acidity, also, it is worthy to state that the combination of *Lactobacillus bulgaricus* and *Strep. thermophilus* in yoghurt having strong effect on the growth and survival of the organisms.

Yeasts and molds may grow over an extremely wide range of temperature, therefore, they can be present on practically all food at almost any temperature under which food are held. Various species of fungi play an important role in spoilage and discoloration of food. Also, they are considered undesirable organisms because they affect the flavor, producing musty odor and bitter taste. It is commonly accepted that the presence of yeasts or mold in yoghurt is also indicative of poor sanitary practices in manufacturing or packaging yoghurt with added sugar or fruits are especially susceptible to yeast growth. Data in Tables 1-4 indicated a problem area for manufactures of yoghurt in Egypt.

The results pinpointed that 60 and 86.7% of the group I samples were spoiled by yeasts and molds in the first day of validity with maximum counts of 1.4×10^3 and 5×10^3 cfu/g, respectively. The average count of yeasts and molds decreased in the second day to be 2.2×10^2 and 6.7×10^2/g, it reached its maximum (2.5×10^5 and 3×10^4/g) during the third day of validity. Lower incidence and counts were observed in samples of group II and III (Tables 2-4).

The results obtained by Abdel-hakeim (1986) are in agreement to these results, while Arnott *et al.*,(1974) recorded better results, they found that only one quarter of the samples analysed was unsatisfactory owing to yeast contamination and almost one fifth was unsatisfactory owing to mold contamination in yoghurt commercially produced in Canada. El-Baba (1999) found that mold could not be detected in the first day of validity tell the 5th day of the storage, but yoghurt with loose covers recorded higher counts.

The present work recorded that all of the samples were with in the scope of Tables 1&2 . Anaerobic organisms could be detected in 26.7 and 53.3% of group I and II, respectively. 40% of yoghurt samples flavored with mango and strawberry were positive while, it failed detection in peach flavored yoghurt samples (Table 3).

Fruit yoghurt are very popular types of milk products and pasteurization in flavored yoghurt represent an extremely important stage in the pre-treatment of fruit additives to inactivate all vegetative microorganisms, but without impairing the taste and structure of fruits (Alfa-Laval , 1983).

The overall picture of yoghurt quality in Assiut markets as measured by microbiological evaluation appears to indicate a need for emphasis on quality control within processing plants. The level of coliforms, enterococci, psychrotrophs, yeasts, molds and anaerobes indicated neglected sanitary measures applied during production, handling, storage and distribution of yoghurt. Therefore application of good hygienic measures during production, storage and distribution of such products are essential to safe yoghurt quality, consequently prevent the risk of human hazard. Like wise, rapid development of lactic acid by good starter culture and use of clean milk are essential for making the product unfavorable for growth and survival of these organisms.

From the stand point of safety, the authors highly recommend consuming yoghurt of group I in the second day of validity. Moreover, yoghurt produced in large modern dairies excelled other samples.

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Table 1 : Statistical analytical results of group I samples based on their acidity percentage and microbial aspects.

Acidity percentage And Microbial aspects cfu/ml	During the 1st day of validity (n=15)					During the 2nd day of validity (n=15)					During the 3rd day of validity (n=15)				
	+ve samples		Min.	Max.	Average	+ve samples		Min.	Max.	Average	+ve samples		Min.	Max.	Average
	No.	%				No.	%				No.	%			
Acidity	-	-	0.65	1.0	0.86	-	- -	0.9	1.05	0.96	-	-	0.7	1.15	0.94
Total psychrotrophic count	8	53.3	8×10	7×10^3	1.2×10^3	3	20	4×10	2×10^3	1.5×10^2	4	26.7	8×10	1×10^3	1×10^2
Enterococci count	4	26.7	1×10	1×10^3	3.5×10^2	3	20	2×10^2	3×10^3	5×10	2	13.3	1×10^2	8×10^2	0.6×10^2
Coliforms count	10	66.6	15	>2400	-	7	46.7	3.6	>2400	-	5	33.3	3.6	>2400	-
Staphylococcal count	5	33.3	3×10	4×10^3	1.2×10^2	2	13.3	1×10	3×10^2	2.6×10	-	-	-	-	-
Yeast count	9	60	1×10^2	1.4×10^4	3.8×10^3	11	73.3	3×10	1×10^3	2.2×10^2	9	60	1.2×10^3	2.5×10^5	5.2×10^4
Mold count	13	86.7	3×10	5×10^3	2.3×10^3	12	80	3×10	7×10^3	6.7×10^2	14	93.3	1×10^2	3×10^4	1×10^4
Anaerobes	4	26.7	—	—	—										

Table 2 : Statistical analytical results of group II samples based on their acidity percentage and microbial aspects.

Acidity percentage And Microbial aspects cfu/ml	During the 1st day of validity (n=15)					During the last day of validity (n=15)				
	+ve samples		Min.	Max.	Average	+ ve samples		Min.	Max.	Average
	No.	%				No.	%			
Acidity	-	-	0.8	1.4	0.98	-	-	0.8	1.55	1.16
Total psychrotrophic count	1	6.7	$5x10$	-	-	-	-	-	-	-
Enterococci count	5	33.3	$7x10$	$8x10^2$	$1.1x10^2$	2	13.3	$2x10$	$3x10^2$	$2.1x10$
Coliforms count	7	46.7	21	$1.1x10^3$	$1.2x10^2$	3	20	9.1	$2.3x10$	2.8
Staphylococcal count	-	-	-	-	-	-	-	-	-	-
Yeast count	3	20	$4x10$	$6x10^2$	$4.7x10$	6	40	$1x10$	$7x10^2$	$5.5x10$
Mold count	6	40	$1x10$	$7x10$	$1.5x10$	8	53.3	$1x10$	$4x10$	$1.4x10$
Anaerobes	8	53.3	-	-	-	-	-	-	-	-

Table 3 : Statistical analytical results of group III samples based on their acidity percentage and microbial aspects during the first day of validity.

Acidity percentage And Microbial aspects cfu/ml	Fruit yoghurt with mango (n=5)					Fruit yoghurt with strawberry (n=5)					Fruit yoghurt with peach (n=5)				
	+ve samples		Min.	Max.	Average	+ ve samples		Min.	Max.	Average	+ve samples		Min.	Max.	Average
	No.	%				No.	%				No.	%			
Acidity	-	-	0.90	1.2	1.0	-	-	0.95	1.4	1.1	-	-	0.9	1.3	1.1
Total psychrotrophic count	2	40	$1x10$	$1x10$	-	2	40	$1x10$	$3x10^2$	$6.2x10$	1	20	$1x10$	-	-
Enterococci count	-	-	-	-	-	1	20	$1.6x10^2$	-	-	2	40	$3x10$	$2x10^2$	$4.6x10$
Coliforms count	-	-	-	-	-	1	20	4	-	-	—	-	-	-	-
Staphylococcal count	-	-	-	-	-	-	-	-	-	-	—	-	-	-	-
Yeast count	3	60	$1x10$	$3x10^2$	$6.8x10$	3	60	$8x10$	$4x10^2$	$1.1x10^2$	3	60	$1x10$	$1.9x10^2$	$5x10$
Mold count	1	20	$1x10$	-	-	3	60	$1x10$	$7x10^2$	$1.4x10^2$	3	60	$1x10$	$3x10^2$	$6.6x10$
Anaerobes	2	40	-	-	-	2	40	-	-	-	—	-	-	-	-

Table 4 : Statistical analytical results of group III samples based on their acidity percentage and microbial aspects during the last day of validity.

Acidity percentage And Microbial aspects cfu/ml	Fruit yoghurt with mango (n=5)					Fruit yoghurt with strawberry (n=5)					Fruit yoghurt with peach (n=5)				
	+ve samples		Min.	Max.	Average	+ve samples		Min.	Max.	Average	+ve samples		Min.	Max.	Average
	No.	%				No.	%				No.	%			
Acidity	-	-	1.1	1.25	1.13	-	-	0.85	1.15	1.0	—	-	1.0	1.55	1.17
Total psychrotrophic count	2	40	$2x10$	$5x10$	$3x10$	2	40	$2x10$	$4x10^2$	$8.4x10$	2	40	$3x10$	$8x10^2$	$1.6x10^2$
Enterococci count	-	-	-	-	-	-	-	-	-	-	2	40	$1x10$	$2x10$	$0.6x10$
Coliforms count	1	20	23	-	-	3	60	9	43	15	3	60	$1x10$	$2x10$	$0.6x10$
Staphylococcal count	-	-	-	-	-	-	-	-	-	-	1	20	$2x10$	-	-
Yeast count	3	60	$5x10$	$2.5x10^2$	$7.1x10$	3	60	$1x10^2$	$4x10^2$	$1.3x10^2$	5	100	$1x10$	$5x10^1$	$2.7x10^1$
Mold count	3	60	$3x10$	$5x10$	$2.2x10$	2	40	$2x10$	$3x10$	$1.2x10$	4	80	$1x10$	$1x10^2$	$3.4x10$
Anaerobes	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-