IMPROVEMENT OF MICROBIOLOGICAL QUALITY of BALADY YOGHURT

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SUMMARY

Part I: A total of 40 random Samples of balady yoghurt collected from small milk and dairy products shops in Cairo and Giza Governments. Each sample was divided into four parts. The first part was examined microbiologically, and revealed that mean counts of, Enterobacteriacae, Staphylococcus aureus and (yeast & mold) were 10^5 , 10^2 and 3x 10^3 cfu/g respectively. The incidence of isolated Staphylococcus aureus was 75%, and all examined samples proved to be free from Bacillus cereus, Escherichia coli, Listeria monocytogens and Salmonellae spp. The second, third and forth parts were mixed with 0.05%, 0.06% and 0.07% potassium sorbate respectively to study its reduction effect on bacterial and fungal counts, then these samples were examined microbiologically and found that the mean counts of Enterobacteriacae were 10³,10² and 40 while for Staphylococcus aureus were 60, 21 and 11 and for yeast & mold were 21.0 and 0 respectively. These indicated that the reduction % for Enterobacteriacae counts, Staphylococcus aureus counts and (yeast & mold) counts after addition of potassium sorbate with concentration of 0.05% were 99.00%,40.00% and 99.30% respectively while in samples mixed with 0.06% potassium sorbate were 99.90%, 79.00% and 100.00 %respectively and in those mixed with 0.07% potassium sorbate were 99.96%.89.00% and 100.00% respectively.

Part II: Manufacture of yoghurt in the laboratory: Milk was divided into four parts, first part without any addition of potassium sorbate (control), second, third and fourth parts were mixed respectively with 0.05%, 0.06% and 0.07% potassium sorbate for determination of shelf life of prepared yoghurt. Yoghurt mixed with 0.05%,0.06% and 0.07% was subjected to sensory evaluation which revealed that yoghurt mixed with 0.06% and 0.07% was unaccepted organoleptically, but yoghurt mixed with 0.05% potassium sorbate was accepted (i.e. no change in color, taste and appearance). The shelf life of yoghurt without any addition(control) was 1 week and its pH started at 4.4 While yoghurt mixed with 0.05% potassium sorbate its shelf life extend to 45 days & its pH started from 4.6 till reached 4.2. While PH of yoghurt mixed with 0.06% and 0.07% potassium sorbate reached 5 and was unaccepted.

INTRODUCTION

Fermentation is one of the oldest methods practiced by human beings for the transformation of milk into products with an extended shelf life. The exact origin(s) of the making of fermented milks is difficult to establish, but it could

date from some 10-15 00 years ago as the way of life of human beings changed from being food gatherer to food producer (Pederson, 1979). It is likely that the origin of yoghurt was the Middle East, and the evolution of this fermented product through the ages can be attributed to the culinary skills of the nomadic people living in that part of the world. Today, fermented milk products are manufactured in many countries (Campbell-Platt, 1987).

The accepted homeland of yoghurt is the Balkan Peninsula and the Middle East region. To the communities living in those parts of the world, this type of fermented milk product is identified and known as natural/plain unsweetened yoghurt. It is evident that yoghurt plays an important role in the diets of these communities. The great popularity of yoghurt lies in the facts that yoghurt consumed not only as a refreshing drink and thirst quenching in hot weather but also for its unique nutritional and therapeutic attributes, furthermore it is a main ingredient during the preparation of a wide variety of dishes including salads and soups; such food habits and their ensuing consumer attitudes may well be a contributory factor to the high annual consumption. Incidentally, recipes for yoghurt dishes are increasingly being included in cookery books all over the world (Tamime and Robinson, 2000). Also yoghurt is an excellent source of high quality proteins, vitamins, minerals and considers a rich source of calcium. It could supply many lactic acid bacteria that may induce changes in the equilibrium and metabolism of the intestinal micro flora and thus excrete a healthful influence on the host and also improves the bioavailability of other nutrients and decrease yeast infection. (Weinsier and Krumdieck, 2000 and Piaia, 2001). Moreover, yoghurt exhibits significant anti-mutagenic activities against colon carcinogens, beneficial effects against osteoporosis, impaired lactose indigestion, in addition yoghurt can lower cholesterol, has positive

effect on immune defense mechanism and cardiovascular diseases. (Heyman, 2000).

Middle East has a subtropical climate and summer temperatures can reach as high as 40°C. In such climate, milk turns sour and coagulates within a short time of milking, particularly as the milk is produced under primitive conditions. Thus, the animals are hand milked, no cooling of the milk is possible, and the risk of contamination by micro-organisms from the air, the animal, the feeding stuffs or the hands of the milker is extremely high, (Tamime and Robinson, 2000). Under these conditions the possibility of transporting or even keeping milk for long length of time is non-existent, and as a result the tendency of milk manufacture appear to be of great importance.

In Egypt, small producers manufacture Zabadi by boiling buffalo's milk for 30 min, cooling it to 40-42°C, inoculating with a starter (i.e. previous day zabadi) and incubating in the retail container, finally the milk is fermented in the retail container (Mahran, 1996and Iniguez et al., 1997). This type of locally produced zabadi (balady yoghurt) is so much available for risk of contamination by different micro organisms, yeast and molds beside that its way of manufacture not decrease but on contrary it increases the sources of contamination, and we find that its expire date is 3 day as a maximum period for consumption during which its physical and sensory attributes changes. Although yoghurt has many desirable properties, it is still prone to deterioration, especially at ambient temperature, within a matter of days, and one discernible trend in the Middle East has been the search for simple techniques to extend its keeping quality. (Tamime and Robinson, 2000).

Sorbic acid and its potassium salt, commonly named as sorbates are generally recognized as safe compounds and are some of the most widely used food additives in the world. They are extensively used in the dairy industry, as they are effective preservatives due to their inhibitory action on yeast and mold growth and their ability to selectively depress bacterial growth including pathogenic strains. The usefulness of sorbates to the dairy industry is acute due to the fact that, it is a successful antimicrobial against pathogens of concern, but does not destroy fermentative and desirable bacteria (Roberts, 2002).

Sorbate is an approved preservative and is added in Brazil at up to 300µg-1 (Moreira et al., 2001). A maximum concentration of 0.3%by weight is permitted in Federal Definitions and Standards of identity as a preservative for many cheeses and cheese products (Collins and Moustafa, 1968). Egyptian Ministry of Health (1992) low No., 32 allowed the presence of 20 ppm sorbic acid in white cheese, in 1995 this law was modified by law No.,478 which raises this concentration up to 1000ppm sorbic acid in unripened fresh cheese (ElShazly et al.,1998).

The purpose of the present study is to provide information about microorganisms which may indicate contamination and have public health significance occurring in balady yoghurt, also a trial to improve microbiological and keeping quality by addition of potassium sorbate during manufacture of balady yoghurt.

MATERIALS AND METHODS

Part 1:

A total of forty random samples of balady yoghurt were collected from Cairo and Giza Governments., transferred to the laboratory with minimum of delay (

Each sample was divided into four parts, first part was subjected directly to microbiological examination, while second, third and fourth parts were thoroughly mixed under aseptic conditions with potassium sorbate 0.05%, 0.06% and 0.07% respectively, after 15 min the samples were subjected to the following microbiological examinations.

Microbiological examinations:-

1-Determination of microbial counts:-

- 1.1-Enterobacteriaceae count: was performed according to APHA (1992)
- 1.2-Coliforms and Fecal coliforms (MPN): Carried out according to, Fenget al. (1998).
- 1.3-Staph aureus count: Was adopted by the technique described by FAO (1992)
- 1.4-Mould and yeast count: Were applied according to Koneman et al. (1994)

2-Isolation and identification of food borne illness bacteria:

- 2.1-Bacillus cereus: According to the procedures of Oxoid (1996).
- 2.2- Staph aureus: Were done according to APHA (1992).
- 2.3 Listeria monocytogenes: According to FAO (1992).
- 2.4- E. coli: According to FAO (1992).
- 2.5- Salmonella spp.: According to Andrews and Hammack (1998).

Part II:

Manufacture of yoghurt in the laboratory .According to Hui (1993). The experiment was repeated 3 times. Before fermentation, milk was divided into four parts, first part without any additions (control), second, third and fourth parts were mixed separately with potassium sorbate in concentrations of

0.05%, 0.06% and 0.07% respectively, after fermentation (yoghurt manufacture) samples were stored refrigerated at 4°C. Then subjected to the following:

- 3.1- Sensory evaluation: According to Hui (1993). Samples were evaluated for their sensory attributes by 5 panelists; the panelist evaluated overall acceptability of yoghurt by giving scores for flavor& body and texture & appearance of manufactured yoghurt which was mixed with 0.05%, 0.06% and 0.07% potassium sorbate.
- 3.2- Determination of pH: According to O'Connor (1995) and Tamime and Robinson (2000).
- 3.3- Determination of shelf-life: According to Kilcast and Subramaniam (2000):

The shelf-life was defined as the no., of days in which yoghurt could be stored without change in its pH and its appearance, flavor and /or taste.

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Results

Table (1) Statistical analytical results of micro biological counts/ gm of purchased yoghurt samples:

Microbiological Count/g	yoghurt											
	Without addition of p.sorb(control)			Second part (mixed with 0.05%p.sorb)			Third part (mixed with 0.06%p.sorb)			Fourth part (mixed with 0.07% p.sorb)		
	Min	Max	Mean ±SE	Min	Max	Mean ±SE	Min	Max	Mean ±SE	Min	Max	Mean ±SE
Enterobacteriacae	10 ²	4x10 ⁵	$10^{5} \pm 3 \times 10^{4}$	12	5x10 ³	$10^3 \pm 3 \times 10^2$	0	3x10 ²	10 ² ±20	0	10 ²	40±10
Staph. aureus	0	7×10^2	$10^2 \pm 5 \times 10$	0	3×10^2	60±19	0	10 ²	21±9	0	9±5	11±4
Yeast &Mold	10 ²	3×10 ⁴	3x10 ³ ±10 ³	0	10 ²	21±9	0	0	0	0	0	0

Table (2): Reduction % of potassium Sorbate for microbial counts

Concentration of p.sorbate Microbial counts	0.05 %	0.06%	0.07 %
Enterobacteriacae	99.00	99.90	99.96
Staph. aures	40.00	79.00	89.00
Yeast & Mold	99.30	100.00	100.00

Table (3): Incidence of food borne illness bacteria isolated from examined yoghurt.

	Number	Positive samples				
Microorganisms	of examined samples	No.,	%			
Bacillus cereus	40	0	0			
E.coli	40	0	0			
L. monocytogenes	40	0	0 -			
Salmonella. spp	40	0	0			
Staph. aureus	40	30	75 %			

Table (4): Statistical analytical results of sensory evaluation of (score card) examined yoghurt samples (No.,=15)

Samples Sensory evaluation	,	urt mix 06%p.:	ed with	yoghurt mixed with 0.07%p.sorb							
Flavour criticism		Score	•	Numb	er of s	amples	%				
No criticisms		10		5			33.33				
		Intensity of defect									
		Sligh	:	1	Definit	<u>—</u> —	Pronounced				
	Score	No.	%	Score	No.	%	Score	No.	%		
Bitter	9	5	33.33	7	2	13.33	5	3	20		
Low acid	9	5	33.33	8	1	6.67	6	4	26.67		
Body and texture		Score		Number of samples			%				
No criticisms		5		5			33.33				
		Intensity of defect									
		Slight		Definite			Pronounced				
	Score	No.	%	Score	No.	%	Score	No.	%		
Too thin	4	5	33.33		2	13.33	2	3	20		
Appearance		Score		Number of samples			%				
No criticisms		5		5 33.33							
	Intensity of defect										
		Slight		Definite			Pronounced				
	Score	No.	%	Score	No.	%	Score	No.	%		
Free whey	4	5	33.33	3	1	6.67	2	4	26.67		
Atypical color	4	5	33.33	3	5	33.33	-	-	-		

No criticisms: In yoghurt samples mixed with 0.05% potassium. sorbate

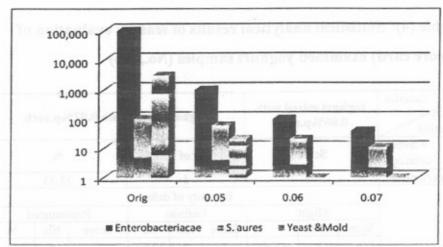


Fig (1): Reduction % of pot. Sorbate.

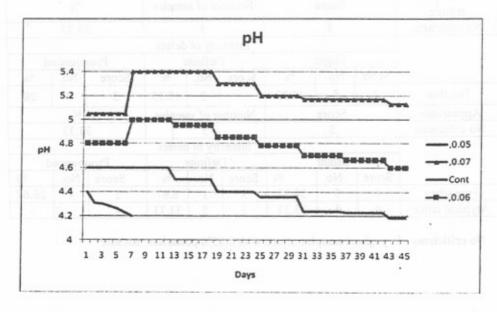


Fig (2): pH measurement and shelf life.

DISCUSSION

Part I

From the results recorded in table (1) it was evident that the mean value of Enterobacteriacea \pm S.E in examined yoghurt samples was $10^5 \pm 3 \times 10^4$.

The reason for the existence of enterobacteriacea with high incidence in the above samples may be attributed to either absence of any kind of heat treatment to milk or due to post contamination and presence of poor sanitary packaging and storage conditions at the production area While the mean value of Staph aureus ±S.E in yoghurt samples was $10^2\pm50$, nearly similar results were recorded by Abd Elhafiez and Mervat-Ragab (2004), while were lower than (Azza, 1999) and higher than (Al-Tahiri, 2005), the incidence of Staph. aureus was 75%, this result was higher than (Aisha, 1996).

Most literature refers that Staph aureus appears in milk from lactating animals affected with mastitis, or it may be transmitted directly to milk or other dairy products by human through nasal and throat discharges. Raw milk was often implicated in outbreaks of staphylococcal intoxication due to slow or inefficient cooling or imperfect pasteurization. As well as staph aureus favors a high protein and high salt environment, so it is found in many dairy products, acidity and heat can limit the number of outbreaks due to staph aureus but this limitation occurs in heat treated or pasteurized yoghurt and when high acidity due to milk fermentation during yoghurt manufacturing (Bryan,1983 and Al-Tahiri,2005). On the other hand, the mean values of Yeast & Mold were $3\times10^3\pm10^3$. This result was similar to Moustafa et al., (1988), Azza(1999), El Bagory and Mosaad (2002), and Susan and Hammad (2002), but lower than those obtained by Aisha(1996) and Al-Tahiri (2005). Although bacteria can be spoilage organisms, low PH value of yoghurt and its high lactic acid

concentration act as a high selective environment favoring growth of yeasts and filamentous fungi which are often involved in the deterioration of yoghurt (Fleet, 1990and 1992) yeast and molds are responsible for off-flavors, loss of texture quality due to gas production, package swelling and shrinkage (Foschino et al., 1993) causing economic loss due to rejection of the product.

The achieved results in table (2), Fig(1) revealed that the reduction % for Enterobacteriacae, Staph aureus and Yeast & Mold counts were (99.00%, 40.00% and 99.30%), (99.90%, 79.00% and 100.00%) and (99.96%, 89.00% and 100.00%) in samples containing 0.05%, 0.06% and 0.07% potassium Sorbate respectively. Potassium sorbate have antimicrobial activity, and is effective against yeasts, molds, and select bacteria, it is widely used at 0.025 to 0.10 % levels in yoghurt Tamime and Robinson (2000).

Part II

From figure (2) the results showed that shelf life of manufactured yoghurt without addition of potassium Sorbate (control) was one week and the pH started at 4.4 within normal level then started to decrease till reached 4.2 at the end of the week beside appearance of mold growth .While the pH of yoghurt mixed with potassium Sorbate 0.06% and 0.07% started at 4.8 and 5 then began to elevate till reached 5 and 5.4 after which declination occurred till reached 4.6 and 5.1 respectively ,these results were higher than normal level so they were not acceptable. Yoghurt mixed with potassium Sorbate 0.05% was examined daily for appearance and measurement of pH. The pH started from 4.6 till reached 4.2 within 45 days, beside that normal appearance up to 60 days after which mold growth appears and pH decreases.

The achieved results in table (4) declare that 5 samples which contain 0.05% potassium Sorbate had no criticisms for (flavor& body and texture & potassium Sorbate had no criticisms for (flavor& body and texture & potassium Sorbate and 2 samples with slight bitter flavor, which contained 0.06% potassium Sorbate and 2 samples with definite bitter flavor and 3 samples with pronounced bitter flavor defect resulted from addition of 0.07% potassium Sorbate), this result agreed with that reported by **Kristoffer and Chakraborty** (1964) who mentioned a slight bitterness in cottage cheese with 0.065 % potassium Sorbate, but contrasted with **Geminder** (1959) who Observed that sorbic acid or potassium Sorbate contributed a bitter flavor at 0.10% but not at 0.075%. Also slight low acid flavor was reported in 5 yoghurt samples mixed with 0.06% potassium Sorbate, but definite and pronounced low acid flavor was reported in 2&3 yoghurt samples mixed with 0.07% potassium Sorbate.

On the other hand the two concentrations 0.06% and 0.07% declared defects in body and texture of manufactured yoghurt as 5 samples were slight too thin and 2 &3 samples showed definite and pronounced too thin (weak) body and texture. And for appearance, slight free whey appeared in 5 samples (which were mixed with 0.06% potassium Sorbate), but definite and pronounced free whey appeared in 1&4 samples (which were mixed with 0.07% potassium Sorbate), while slight atypical color appeared in 5 samples (which were mixed with 0.06% potassium Sorbate) and definite atypical color appeared in 5 samples (which were mixed with 0.06% potassium Sorbate).

Although the higher reduction % obtain from samples mixed with potassium Sorbate 0.06% and 0.07% but unaccepted organoleptically, while samples mixed with potassium Sorbate 0.05% were accepted organoleptically. So, the

results suggest that concentration of potassium Sorbate of 0.05% by weight might be used advantageously as a way of increasing the shelf life of balady yoghurt.

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تحسين الجوده الميكروبيولوجية للزبادى البلدى

ملوى رجب سليمان حجازى و غادة سعد الدين سالم

قسم صحة الاغدية معهد بحوث صحة الحيوان الدقى

أجرى البحث على 40 عينة عشوائية من الزبادي البلدي المصنع في معامل محلات الألبان الموجودة في مناطق القاهرة و الجيزة قسمت كل عينه الى أربع أجزاء : الجزء الأول تم فحصه ميكروبيولوجيا و كان متوسط العد البكتيري المبكروبا ت المعوية و الميكروب المكور العنقودي الدهبي و الخمائر و الفطريات هو 510, التوالى. وكانت نسبة الميكروب المكور العنقودي الذهبي75 % ولم يتم المكور العنقودي الذهبي التوالى. عزل أي من ميكروب الباسيلس سيرس أو الميكروب القولوني البرازي أو ميكروب الليستيريا مونوسيتوجينز أوالسالمونيلا. و تم ,إضافة البوتاسيوم سوربات الى الجزء الثاني و الثالث والرابع بالنسب التالية، 0.05%، 0.06%، 0.07% على التوالي لدراسة تأثير البوتاسيوم سوربات على العد البكتيري والفطري وكان متوسط العد البكتيري للمبكروبات المعويه $^{2}10$ و 2 و 2 البكتيري للمبكروب النعبة للمبكروب المكور العنقودي الذهبي كان متوسط العد الكلي 60و 21 و 11 على التوالي بينما متوسط العد للخمائر والفطريات كان 21و0و0 على التوالي وبذلك كانت نسبة الانخفاض في متوسط العد البكتيري للميكروبا ت المعوية والميكروب المكور العنقودي الذهبي والخمائر والفطريات بعد أضافه سوربات البوتاسيوم بتركيز 0.05% (99.00% و 40.00% و99.30%) على التوالي. بينما للعينات المضاف لها سوربات البوتاسيوم بنسبه 0.06% كانت (99.90%و 79.00% و100.00%) على التوالي وبالنسبة للعينات المضاف اليها سور بات البوتاسيوم بنسبه 0.07% كانت (99.96% و 89.00% و100.00%) على التوالي.

الجزء الثاني:

تم تصنيع الزبادى داخل المعمل وقسم اللبن الى أربع أجزاء . الجزء الأول بدون أى الصافات. والجزء الثانى والثالث والرابع تم أضافه سوربات البوتاسيوم لها بتركيزات 0.05%، 0.06%، 0.07% على التوالى لتعيين مده صلاحيتها وأجراء الاختبارات الحسيه عليها .وقد اظهر ت النتائج أن العينات المضاف إليها البوتاسيوم سوربات بنسبة،

، 0.06%، 0.07% غير مقبولة حسيا بينما المضاف اليها0,0% كانت مقبولة حسيا (لا يوجد تغيير في اللون أو الطعم أو الشكل) وكانت مدة صلاحية الجزء الأول من عينات الزبادي المصنعه معمليا (بدون أي إضافات) 7ايام ودرجة الحموضة بدأت من 4.4 . بينما امتدت مده الصلاحيه للزبادي المضاف له سوربات البوتاسيوم بنسبه 0.05% إلى 45 يوم و درجة حموضة بدأت من 4.6 ووصلت إلى 4.2 بينما درجه الحموضه في عينات الزبادي المضاف لها سوربات البوتاسيوم بنسبتي 0.06%، وصلت إلى 5 وكانت غير مقبوله حسيا.