

## SANITARY CONDITION OF YOGHURT PRODUCED IN PORT SAID CITY

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### ABSTRACT

This study was designed to evaluate the sanitary conditions of yoghurt processed by local dairy shops in Port Said City, Egypt. Yoghurt samples were evaluated for assessment of the syneresis susceptibility, pH, titratable acidity and microbiological qualities. The microbiological study of yoghurt samples revealed that the percentages of positive samples for total coliforms were 66.7%, for *Escherichia coli* were 44% for Psychrotrophic bacteria were 13.3 % for *Staphylococcus* spp. were 10.7 % and for yeast and mold were 40% and 26.7%, respectively. The mean Coliforms, *Escherichia coli*, Psychrotrophic, *Staphylococcus* spp., yeast and mold counts were  $6.3 \times 10^3$ ,  $4.9 \times 10^2$ ,  $5 \times 10$ ,  $3.5 \times 10$ ,  $1.9 \times 10^5$  and  $2.7 \times 10^3$  cfu/g, respectively. The samples were also analyzed for the investigation of food-borne pathogens. Three *Staphylococcus aureus* positive samples were found. All examined samples were found to be free from *Salmonella* spp., *E. coli* O157:H7, and *Bacillus cereus*. The presences of microorganisms in yoghurt poses threat to food safety and give some indication of the relative exposure risk for consumers. The public health significance and the recommendations to ensure safety and quality of yoghurt were discussed.

### INTRODUCTION

Yoghurt is nutritiously balanced food containing almost all the nutrients present in milk but in a more assimilable form. Its production and consumption is growing continuously due to its therapeutic properties beside its high nutritive value. The manufacture of yoghurt today is mainly based upon traditional technologies. Usually 1 day old yoghurt is used as a starter culture for production of yoghurt. In principle, world wide, there is no any difference between manufacturing of home-made and factory-made yoghurt (White, 1995). The yoghurt culture is a system of two (or more) microbial populations with mutually complimenting metabolisms (Beshkova et al., 2002). The bacteria are incubated to facilitate the conversion of available lactose in the milk into lactic acid, which causes the milk to curdle. Once the yoghurt has reached the desired pH

(3.9). It is refrigerated to induce the bacteria into a dormant state to halt acid production (Marshall, 1993). The partial digestion of the milk when these bacteria ferment milk makes yoghurt easily digestible.

Consumption of yoghurt has been shown to have a wide range of beneficial effects on human health. The health promoting properties of live lactic acid bacteria in yoghurt include protection against gastrointestinal upsets, enhanced digestion of lactose, decreased risk of cancer, lower blood cholesterol, improved immune response and help the body assimilate protein, calcium and iron (Perdigon et al., 1998 and Maroña and Pedrigo, 2004). Several studies have also found that consumption of yoghurt during antibiotic treatment can reduce side-effects of antibiotics by reestablishing the "healthy" intestinal bacteria very quickly (Benwal et al., 2003) as well as decrease some of the other side effects associated with antibiotics such as abdominal distress, stomach pain and flatulence (Silitonen et al., 1990). Because lactic acid bacteria convert lactose into lactic acid, their ingestion may help lactose intolerant individuals tolerate more lactose than what they would have otherwise (Sanders, 2000).

The pH in lactic acid fermented foods is usually sufficient to suppress the growth of most foodborne pathogens (Kingamkono et al., 1994). Fermented dairy products are bactericidal to both pathogenic and spoilage microorganisms, with yoghurt being the most effective (Goelk et al., 1971 and Park and Marth, 1972). Poor hygiene, practiced by handlers of yoghurt may lead to introduction of pathogenic microorganisms into the products and these may pose risk to consumers.

Pathogenic *E. coli* are significantly more acid tolerance than non pathogenic strains (Arnold and Kaspar, 1995). Detection and enumeration of coliforms is one of the standard tests required by International Dairy Federation (Mossel et al., 1995). *Escherichia coli* is frequently contaminating organism, and is reliable indicator of fecal pollution generally in milk and other dairy products (Dillio, 1982).

Microbiological deterioration of refrigerated raw and pasteurized milk and dairy products is often caused by the growth of Psychotropic Gram-negative bacteria species, yeasts, and molds (Ternstrom et al., 1993; Jay, 2000; Boor and Murphy, 2002 and Chambers, 2002). The chemical composition and microbial quality of yoghurt were reported by several workers (Yaygin and Kılıç, 1980, and Ali et al., 2004).

Yoghurt quality and consumer satisfaction are essential for the continued successful growth of the yoghurt market. Therefore, this study was designed to assess yoghurt commercially produced and sold by local dairy shops in Port Said City for physical, chemical and microbiological characteristics.

## MATERIALS AND METHODS

### Yogurt samples:

A total of 75 plain yoghurt samples, packed in plastic containers that had a shelf life up to 3 days under refrigeration, were collected randomly from big different processing dairies in Port Said City. Yoghurt samples were brought in an icebox to the laboratory for analysis.

### 1. Physico-chemical Analysis

#### 1.1. Syneresis

One hundred grams of yoghurt sample was placed on a filter paper resting on a top of a funnel. After 2 h of drainage at 7°C, the quantity of whey collected in a 50 ml graduated cylinder was used as an index of syneresis (Farooq and Haque, 1992).

#### 1.2. pH and Titratable acidity measurements

Yoghurt pH values were measured by using pH-Meter standardized with pH 4.00 and 7.00 standard buffer solutions. Before measuring pH, yogurts were tempered to approximately 15°C (Aryana, 2009).

Titratable acidity was determined as lactic acid by titrating with 0.1 N NaOH using phenolphthalein as an indicator and its recalculation as percentage lactic acid according to the accepted formula : % lactic acid =  $0.009 \times \text{OT}$ . (Karleskind et al., 1993).

### 2. Microbiological analysis

#### 2. 1. Microbiological Counts:

Decimal dilutions (up to  $10^{-6}$ ) of yoghurt samples in Butterfield's phosphate buffer were prepared. All samples were subjected to the following estimations according to the relevant methods.

Coliforms counting (MPN) using LAuryl tryptose broth and confirmed by culture on Brilliant green bile broth according to FENG et al., (2002). E. coli counting using LAuryl tryptose broth and confirmed by culture on EC medium .Presence of E. coli was confirmed by applying IMVIC tests to the typical metallic green colored, smooth sided colonies on EMB .Those resulting +, +, -, - from IMVIC tests at 44.5°C and were evaluated as E .coli (FENG et al., 2002). Standard Psychrotrophic count using plate count agar according to Frank et al. (1992). Staphylococcus spp count using Baird Parker tellurite egg yolk agar (BPA) according to Bennett et al., (2001). Yeast and mould count using malt extract agar (Difco) and potato dextrose agar (Difco) plates. To prevent bacterial growth 100 ppm Chloramphenicol was added (Tournas et al., 2001).

## 2.2. Isolation of pathogens:

All samples were subjected to the following examination according to the relevant methods. The isolation and identification of *Staphylococcus aureus* using Baird Parker tellurite egg yolk agar (BPA). The characteristic black colonies of *S. aureus* with peripheral clearance zone on BPA were counted and typical isolates were tested for gram reaction, catalase and coagulase activity (Bennett and Lancette, 2001). *E. coli* O157:H7 using MacConkey Sorbitol agar according to (Feng and Weagant, 2002), *Bacillus cereus* by streaking on plates of Mannitol-egg yolk-polymyxin agar plates (Rhodehamel and Harmon, 2001) and *Salmonella* detection using Lactose broth and Rappaport-Vassiliadis medium as enrichment broth and xanthine lysine decarboxylase agar (XLD) as selective plating agars (Andrews and Hammack, 2007).

## RESULTS & DISCUSSION

### 1- Physico-chemical Analysis :

The values of syneresis, titratable acidity and pH of examined yoghurt samples were summarized in Table (1).

The values for syneresis (whey separation) in examined samples ranged from 23.3% - 35.2% with a mean value of 25.23%. These results are in line with the findings of Ozera et al. (2007). Syneresis values ranging from 12.5% -29.5% were obtained by Hassan et al. (1996) for ropy yoghurt and yoghurt made with encapsulated non ropy cultures. Variations in syneresis between yoghurt samples may be due to variations in quality of milk. Harwalkar and Kalab (1986) found that an increase in total solid increased the density of yoghurt matrices which resulted in decreased syneresis. Increasing storage temperatures and high rates of acid production have been reported to increase the degree of syneresis in yoghurt (Richmond et al., 1985). Also Gassem and Frank (1991) recorded that during cold storage of milk that has been contaminated by psychrotrophic bacteria,  $\kappa$ -casein is subjected to break down by proteolysis. This causes the yoghurt made from such milk to be firmer, more viscous and sensitive to syneresis. Adequate firmness without syneresis is essential for a top-quality product. The most frequent defects related to yoghurt texture that may lead to consumer rejection, is the occurrence of syneresis (Kroger, 1975). Wheying-off may be indicative of faulty fermentation and off-flavors. To reduce syneresis, thickening agents such as gelatin, starch, cellulose derivatives, alginates and carrageenan can be legally added (FDA, 1996).

The pH values of examined yoghurt samples ranging from 4.3 to 4.7 with a mean value of 4.5. The optimum pH growth range of lactic acid bacteria has been reported to be 4.5. (Holt et al.,

1994). The titratable acidity values ranged from 0.89 % to 1.6 % with a mean value of 1.13 %. The present results are in line with the findings of **Salji et al. (1985)** and **Gueimonde et al. (2003)**. On the other hand, **Laye et al., (1999)** reported lower titratable acidity values than the present result. However, the pH values were almost similar. Bulgarian yoghurt has acidity up to 1.48%, Netherland standards maximum of 1.17% lactic acid (**Tamime and Robinson, 1985**). **FDA (2003)** proposed a requirement of 0.5% minimum titratable acidity expressed as lactic acid and acidity of pH 4.6 or lower.

## 2. Microbiological examination

### 2-1. Microbiological Counts

The Microbiological counts of examined yoghurt samples were present in Table (2).

The summarized results in Table (2) pinpointed that coliforms were present in 50 (86.7 %) of examined yoghurt samples with a mean count of  $6.3 \times 10^3$  cfu/g. The obtained findings are coincided with those recorded by **All et al., (2004)** in yoghurt produced in Assiut City. In this concern, **Dayısoylu (1993)** reported that yoghurt sold in the markets in Van, Turkey had average coliforms bacteria count of  $5.0 \times 10^2$  cfu/g.

Coliforms numbers in food samples are used as an index of sanitation (**Doyle and Erickson, 2006**). Coliforms are killed by pasteurization. The detection of any coliform bacteria suggests that some point in processing has been neglected in regard to effective cleaning, sanitation procedures, production, processing or packaging. A common problem with yoghurt is contamination by coliform organisms. Coliforms along with other bacteria, may produce off flavors in milk and reduce shelf life of dairy products (**Tamime and Robinson, 1999**). Most coliforms originate from the intestines of warm-blooded animals, including people. Due to the significance of the faecal-oral route transmission for many bacterial food-borne diseases, basic hygiene measures assume a decisive importance in food safety management (**Untermann, 1998**).

The present results showed that *Escherichia coli* was present in 33 (44%) of yoghurt samples. The mean value of *Escherichia coli* count in positive samples was  $4.9 \times 10^2$  cfu/g. *Escherichia coli* can grow actively at pH 4.4 to 9.0 and also *E. coli* is resistant to low pH (**Jay, 1992**). The presence of *Escherichia coli* is of interest since when present it indicates that recent fecal contamination has occurred with the possibility of accompanying enteric pathogens (**Jay, 1996**).

The present results revealed that in most of yoghurt samples, psychrotrophic bacteria were absent this may be due to heating of milk prior to its incubation as most psychrotrophic bacteria do not survive pasteurization (**Cousin, 1982**). Psychrotrophic bacteria was present in 10

(13.3%) of 75 examined yoghurt samples. Post-pasteurization contamination may happen at the filling operation. The average number of psychrotrophic bacteria in positive samples was  $5 \times 10$  cfu/g. The longer raw milk is held before processing (legally up to five days), the greater the chance that psychrotrophs will increase in number (Eneroth et al., 1998). The contamination of yoghurt by psychrotrophic has been reported by Abdel-Haklem (1986) who recorded psychrotrophic organisms with a level of  $6.9 \times 10^4$  /g. In this concern, Lopez et al. (1998) studied the incidence of psychrotrophic bacteria in two batches of yoghurt ice-cream manufactured in Spain and freezed at  $-23^\circ\text{C}$  for 60 weeks. Psychrotrophic bacteria were detected in the both batches.

Psychrotrophic bacteria are generally the cause of most shelf-life problems in fluid milk. *Pseudomonas* species produce heat stable extracellular proteases, lipases and phospholipases. These enzymes can survive pasteurization and even UHT treatment (Braun et al., 1999). Psychrotrophic microorganisms can enter the milk from soiled cows, dirty equipment and the environment. Minimizing the level of milk contamination from these sources will help prevent psychrotrophs from growing to significant levels in the bulk tank during the storage period on the farm or at the dairy plant.

*Staphylococcus* spp. were detected in 8 (10.7%) of 75 examined yoghurt samples. The average number of *Staphylococcus* spp. in the examined samples was  $3.5 \times 10$  cfu/g (Table 2). *Staphylococcus* spp. was previously isolated from yoghurt by Arnott et al. (1974) and Ahmed and El-Bassiony (1978). *Staphylococci* may be enter into milk product from food handlers either suffering from acute pyogenic infections or being at a state of healthy carriers harboring the organisms in their nose or throat (Pelczar et al., 1985).

The present results in Table 2 showed that yeast was detected 30 (40%) of examined samples while mold was present in 20 (26.7%). The mean values of the counts of yeast and mold were  $1.9 \times 10^5$  and  $2.7 \times 10^3$  cfu/g, respectively. The high yeast count could be attributed to contamination from air and the culture used for yoghurt manufacture and gives some indication of the exposure risk for consumers. Dayisoğlu (1993) reported that yoghurt sold in the markets in Van, Turkey had average yeast and mold count of  $2.2 \times 10^5$  cfu/g. Counts of yeasts and molds  $\geq 10^6$  cfu/ml were considered as failure criteria for yoghurt as deterioration in the product's quality takes place at these levels (Yamanl and Abu-Jaber, 1994). The presence of mold and yeast in yoghurt was recorded by other researchers (Arnott et al., 1974 ; Alekaleva and Mirkov , 1978 and Ali et al., 2004).

Because of their low pH, yoghurts are a selective environment for the growth of yeasts, and the literature contains general references to the spoilage of yogurts by yeasts (AL-Tahiri, 2005). The spoilage of yoghurt by yeasts has been generally characterized by yeasty off flavors, loss of

textural quality due to gas production, swelling and occasional rupturing of the product containers (Davis, 1974). In this concern, Bennie et al., (2003) reported that yeasts play a substantial role in the spoilage of commercial yoghurts as yoghurt blowing especially when cold storage practices are neglected. The mold can easily contaminate the dairy products. Their growth can result in the production and accumulation of mycotoxins which are of public health and economic importance.

## 2.2. Isolation of pathogens :

The data present in Table (3) revealed that *Staphylococcus aureus* was present in 3 (4%) of samples. *Salmonella* failed to be detected in all examined samples. *Bacillus cereus* and *E.coli* O157:H7 were not isolated from any of the samples analyzed. Acid may be responsible for most of yoghurt's bactericidal abilities (Gilliland and Speck, 1972). Lactic acid bacteria are capable of inhibiting or inactivating food-borne and other pathogenic microorganisms (Daly et al., 1972 and Smith and Samuel, 1981). It has been reported that lactic acid bacteria inhibit the growth of the vegetative cells of *B. cereus* (Wong and Chen, 1988). In this concern, Oscar et al. (2007) found that there is a clear inhibitory effect of yoghurt cultures over potentially pathogenic bacteria for human beings. Although *E.coli* is frequently occurring organisms in milk and its products, the incidence of the species of *E.coli* itself in milk and milk products as a possible cause of food borne disease is insignificant because *E.coli* normally is a ubiquitous organism (Hahn, 1996). Important, however, is the occurrence of pathogenic strains of *E.coli* in milk products which could be hazardous for consumers. *E.coli* O157:H7 was not isolated from any of the samples analyzed.

The detection of *Staphylococcus aureus* in yoghurt is of public health importance because of its ability to cause food-borne intoxication (Le Lohr et al., 2003). The unclean worker's hands, poor quality of milk, unhygienic conditions of manufacturing unit, inferior quality of milk used and water supplied for washing the utensils and the post manufacturing contamination could be the source of accelerating the bacterial contamination of yoghurt with *Staphylococcus aureus*. Tondo et al. (2000) reported that 35.2% of food handlers were asymptomatic carriers of *Staphylococcus aureus*, and that 90.4% of raw milk samples among more than 3200 investigated dairy products. In this respect, Oscar et al. (2007) evaluated the effect of *Lactobacillus rhamnosus* probiotic culture added to yoghurt over *Staphylococcus aureus*, *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Salmonella enteritidis* populations. They found beneficial effects of probiotic cultures in yoghurt over bacteria. They added that the importance of keeping hygienic practices in order to avoid the contamination of yoghurt with *S. aureus* and the eventual production

of its enterotoxin, since it is not affected by probiotics. However, **Dahiya and Speck (1968)** found that *L. bulgaricus* produced amounts of hydrogen peroxide that was inhibitory to *Staphylococcus aureus*.

Product quality and satisfaction of consumer are essential for the continued successful growth of the yoghurt market. Investigation of the microbiological quality of yoghurt sold in Port Said City revealed some undesirable contamination. Some yoghurt commercially sold showed a viable count of bacteria, yeast and mould. All above microbes can have a hazardous effect on human, beside their effect on the organoleptic properties of the final products. Several types of control methods may be effective in preventing or minimizing microbial contamination of yoghurt and inhibiting the growth of or destroying microbial contaminants. Processors need to select high-quality raw milk with low levels of microorganisms. Manufacturing of milk into yoghurt under hygienic conditions is also important. Processing equipment must be designed and constructed so that it is readily accessible for cleaning and inspection, is self-emptying or self-draining has covers to prevent external contamination and has readily cleanable surface. Regular sanitation of dairy equipment, washing of utensils, milker's hands, and udders, eradication of diseased animals and pasteurization /boiling of milk is required before yoghurt making.



Table (1): Summarized results of syneresis (%), pH and titratable acidity (%) of examined yoghurt samples (n=75).

parameters	Min	Max	Mean
Syneresis (%)	23.3	35.2	25.23
pH	4.3	4.7	4.5
Titratable acidity (%)	0.89	1.6	1.13

Table (2): Summarized results of viable counts (cfu/g) of examined yoghurt samples (n=75).

Microbial count	Positive samples		Minimum	Maximum	Mean
	No.	%			
<i>Coliforms</i>	50	66.7	$9.4 \times 10^2$	$9.4 \times 10^4$	$6.3 \times 10^3$
<i>E. coli</i>	33	44	50	$2.7 \times 10^3$	$4.9 \times 10^2$
<i>Psychrotrophic</i>	10	13.3	$2 \times 10$	$2 \times 10^2$	$5 \times 10$
<i>Staphylococcus spp.</i>	8	10.7	$2 \times 10$	$8 \times 10$	$3.5 \times 10$
<i>yeast</i>	30	40	$2.3 \times 10^3$	$2.8 \times 10^7$	$1.9 \times 10^5$
<i>Mold</i>	20	26.7	$1.3 \times 10^2$	$2.8 \times 10^3$	$2.7 \times 10^3$

NB: Mean is calculated out of positive results.

Table (3): Incidence of isolated organisms from examined yoghurt samples (n=75)

Organism	No. of positive samples	Percent of positive samples
<i>Staph aureus</i>	3	4
<i>Salmonella</i>	0	0
<i>E. coli</i> O157:H7	0	0
<i>Bacillus cereus</i>	0	0

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## الملخص العربي

### الحالة الصحية للزبادى المنتج فى مدينة بورسعيد

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بعثير الزبادى من منتجات الألبان التى تجمع بين القيمة الغذائية والفائدة الصحية وعلى الرغم من ذلك فقد تعتبر مصدراً لبعض الميكروبات إذا تم تصنيعها تحت ظروف غير صحية، فى دراسة للوقوف على الحالة الصحية للزبادى المنتج محلياً فى مدينة بورسعيد تم فحص ٧٥ عينة من الزبادى التى جمعت عشوائياً من محلات ألبان كبيرة مختلفة فى مدينة بورسعيد، تم تقييم العينات من حيث بعض الفحوص الطبيعية والكيميائية والميكروبيولوجية، وقد أظهرت النتائج أن نسبة العينات الإيجابية لميكروبات الكوليفورم كانت ٦٦.٧٪ وميكروب الإشيرشيا كولاي ٤٤٪ والميكروبات المحبة للبرودة ١٣.٣٪ والميكروبات المكورة العنقودية ١٠.٧٪ والخمائر ٤.٠٪ والفطريات ٢٦.٧٪ من العينات التى تم فحصها، وكان متوسط العدد الكلى فى العينات الإيجابية لميكروبات الكوليفورم الكلية وميكروب الإشيرشيا كولاي والميكروبات المحبة للبرودة والميكروبات المكورة العنقودية والخمائر والفطريات  $10^3 \times 6.3$  ،  $10^2 \times 4.9$  ،  $10 \times 1.5$  ،  $10^5 \times 1.9$  ،  $10^4 \times 2.7$  خلية / جم على التوالى، هذا وقد تم عزل الميكروب المكور العنقودى الذهبى من ثلاث عينات من إجمالى عدد العينات التى تم فحصها ولم يتم عزل ميكروب السالمونيلا وميكروب الباسيلس سبرس وكذلك ميكروب الإشيرشيا كولاي العنقودى H7 : O157 من أى من العينات، هذا وقد تم مناقشة تلوث الزبادى بتلك الميكروبات وأثرها على الصحة كما نوقشت الشروط الصحية الواجب توافرها لإنتاج الزبادى.