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ESTIMATION OF MICROBIAL LOAD AND CHEMICAL COMPOSITION OF PASTEURIZED MILK FROM MARKETS IN KHARTOUM NORTH, SUDAN

تقدير الحمل الميكروبي و المكونات الكيميائية للبن المبستر في أسواق الخرطوم بحري، السودان

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المستخلص

أجريت هذه الدراسة لتحديد تأثير ظروف التخزين على جودة اللبن المبستر والذي يوزع في أسواق الخرطوم بحري بواسطة ثلاث شركات. جمعت ستين عينة من منتج كل شركة واخضعت للتحليل الكيميائي والميكروبيولوجي في كل من اليوم الثالث و الخامس من التصنيع.

اظهرت نتائج تحليل عينات اللبن المبستر من الشركات الثلاث اختلافات معنوية ($P<0.05$) في نسبة الدهن والمواد الصلبة اللادهنية و الكثافة و البروتين والسكر. تبين من التحليل الميكروبيولوجي لعينات اللبن المبسترتأثر العد الكلي للبكتريا و باكتريا القولون والبكتريا المكونة للأبواغ تأثرا معنويا ($P<0.05$) بمصادر و فترة التخزين لعينات اللبن المبستر. خلصت الدراسة الي تدني نوعية اللبن المبستر في كل من الثلاث شركات وبالتالي هنلك حوجه عاجله لتطوير برامج الصحة ونوعية منتجات الألبان وضبط جودتها لضمان نوعية جيدة من اللبن المبستر المنتج والمستهلك في ولاية الخرطوم.

Abstract

This study was conducted to determine the effect of storage conditions on the quality of pasteurized milk distributed in Khartoum North by three companies. Sixty samples were collected from the product of each company and subjected to chemical analysis and microbiological examination in the third and fifth day of processing.

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The results revealed significant (P<0.05) differences between the products of the three companies in fat, solids not fat, density, protein and lactose. Microbiological analysis showed that total bacterial count, coliform count and aerobic spore formers count were significantly (P<0.05) affected by the sources of samples and storage period.
The present study concluded that the quality of pasteurized milk produced by the three dairy companies was low. 0Therefore, development of programs of health and quality of dairy products are urgently needed to ensure that good quality pasteurized milk is produced and consumed in Khartoum State.

Key words: Pasteurized milk, markets, storage period, microbial quality, chemical composition, Khartoum State

Introduction

Pasteurization aims to reduce the number of viable pathogens, so they are unlikely to cause disease assuming the pasteurization product is refrigerated and consumed before its expiration date (Montville and Matthews, 2005). Milk pasteurization has been subjected to increasing scrutiny in recent years, due to the discovery of pathogens that are widespread and heat resistant (Grant *et al*., 2002). Moreover, poor sanitation practices (unsatisfactory cleaning and sterilization of dairy equipment) and lack of refrigeration (raw milk in some developing countries is kept at room temperature for 3-4 hours before pasteurization), raw milk may contain over 2×10^6 cfu /ml before processing for liquid milk or cheese making (Kameni *et al.,* 2002).
Elmagli and El Zubeir (2006a) reported that the fat, protein and lactose contents of pasteurized milk ranged between 1 to 2.8%, 2.13, 3.6% and

2.13 to 4.8%, respectively. However Abd Elrahman *et al.* (2009) estimated the protein content of pasteurized milk as 3.21± 0.004, while lactose content was 4.32± 0.022% and the mean of density was 1.028± 0.014. Furthermore the fat content of pasteurized milk was affected significantly (P≤0.001) by the storage conditions (Elmagli and El Zubeir,

2006a and Abd Elrahman *et al.*, 2009). Improving the microbial safety of perishable food is currently a major preoccupation in the food industry (Vachon *et al.,* 2002). Coliforms bacteria are destroyed by pasteurization and that their presence requires urgent attention for the many reasons such as their relationship with faecal contamination (*E. coli*) and the 'off ' tastes they cause in milk (Van Kessel *et al*., 2004). The heat treatment of milk prior to packing for liquid consumption, or manufacture into milk based product, is an important critical control point to ensure that pathogenic organisms are killed, it also ensures that the spoilage organisms are eliminated, or at least reduced in number, for optimum keeping quality (IDF, 1994).

There has been interest in recent year in expanding the shelf life of the fluid milk because of potential advantages for both processor and consumer (El Zubeir *et al.,* 2007). They also reported that flavor changes of pasteurized milk are affected by processing condition, packing materials and bacterial growth, one of the principle factors associated with this concern about milk quality is its shelf-life. Gruetmacher and Bradley (1999) mentioned the factors that limit the shelf life of refrigerated pasteurized milk such as the microbial quality of raw milk, time and temperature of pasteurization, presence and activity of post pasteurization contaminants, types and activity of pasteurization resistant microorganisms, and the storage temperature of milk after pasteurization. Post pasteurization contamination has received most of the attention and is considered to be the factor, which limits shelf life in the majority of cases (IDF, 1993).

The present study is designed with the objective of evaluation of the chemical properties and microbial load of pasteurized milk and to estimate the effect of storage period on its keeping properties.

Materials and methods

Sources of milk samples

This Study was done during December 2008 to January 2009 on pasteurized milk samples from three factories distributing their products in Khartoum North (A, B and C). The numbers of samples were 60 samples from each factory (30 sample were examined during the third and 30 during the fifth day of processing).

Collection of samples

The pasteurized milk samples were collected from two supermarkets located in Khartoum North government and the samples were stored at the laboratory of Dairy Production Department in control temperature at 5°C.

Analysis of samples

Chemical analysis

Analysis of fat, protein, lactose, SNF and density of pasteurized milk samples were determined using milk analyzer Lactoscan 90 according to the manufacturer' instructions (Aple Industries services–La Roche Sur Foron, France).

Milk samples were mixed gently 4-5 times to avoid any air enclosure in the milk. The 25 ml of the samples; one at time were put in the sample-holder with the analyzer in the recess position. Then the starting button inactivated, the analyzer sucks the milk, makes the measurement, returns the milk in the sample- holder and the digital indicator (IED display) shows the specified results.

Microbial examination

One ml of pasteurized milk samples was transferred with sterile pipette to 9.0 ml sterile distilled water into a test tube and mixed thoroughly. The dilution was repeated to make serial dilution (Richardson, 1985). One ml of the pasteurized milk was transferred into the Petri dishes, and then 10-12 ml of sterilized media at 45-46 ºC was poured into the inoculated Petri dishes. The plates were rotated to mix the sample and the media were allowed to solidify. Then the plates were inverted and incubated at the prescribed temperature and time (Christen *et al.*, 1992).

Total bacterial count was determined on standard plate count agar according to Richardson (1985). The plates were incubated at 37 °C for 24-48 hours. MacConkey agar was used to determine the coliforms count. The plates were incubated at 37 ºC for 18-24 hours (Christen *et al.*, 1992). The aerobic spore former bacterial count (ASC) was determined by heating milk samples at 80ºC for 12 minutes and rapidly cooled down to 10 ºC. Then one ml was poured into sterile Petri dish and the standard plate count agar was added. The plates were incubated at 37 ºC for 48 hours (Ravanis and Lewis, 1995). The developed colonies were counted using manual colony counter. The plates countering 25-250 colonies were

selected (Christen *et al.*,1992). The number reciprocal of the dilution factor was recorded as colony forming unit/ ml.

Statistical analysis

Statistical Packages for Social Studies (SPSS 13.00) were used to analyze the data using ANOVA test. Duncan multiple range test were used to determine the difference between means.

Results

Chemical composition of pasteurized milk samples

Fat content

The minimum and maximum values of fat content of pasteurized milk samples from the three companies were shown in Table 1 and Table 2. The means of fat content of pasteurized milk samples from A, B and C companies at the 3rd day of processing were 3.93±0.06%, 3.47±0.06% and 4.14±0.06% (Table 1). They were 3.90±0.05%, 3.32±0.05% and 4.18±0.05% at the 5th day of processing (Table 2). Moreover the fat content of pasteurized milk was significantly different ($P<0.05$) during the storage period (Table 3).

SNF content

Minimum and maximum values of SNF content of the pasteurized milk collected from the three companies were presented in Table 1 and Table 2. The means of SNF content of pasteurized milk samples revealed 8.18±0.05%, 8.37±0.05% and 8.25±0.05%, and 8.30±0.05%, 8.30±0.05% and 8.27±0.05% respectively for A, B and C companies at day 3 and day 5 of storage. The data showed non significant variations (Table 3)

Density

Table 1 and Table 2 show that mean values of density of pasteurized milk samples were 1.029±0.162 gm/ ml, 1.029±0.162 gm/ ml and 1.028±0.168 gm/ ml during the 3rd day of the storage period. They were 1.028±0.135 gm/ ml, 1.029±0.135 gm/ ml and 1.028±0.135 gm/ ml during the 5th day of the storage period respectively, for A, B and C companies. Their ranges were shown in the same tables.

Protein content

Minimum and maximum values of the protein content of pasteurized milk from the three companies were presented in Table 1 and Table 3. The means of protein content of pasteurized milk samples from A, B and C companies were 3.20±0.32%, 3.28±0.32% and 3.38±0.32% respectively, at the 3rd day of storage (Table 1). They revealed 3.25±0.03%, 3.38±0.03% and 3.28±0.03% respectively, during the 5th day of storage (Table 2). Moreover significant (P< 0.05) variations were estimated for protein content of the pasteurized milk samples during storage from the different producing companies (Table 3). .

Lactose content

The maximum and minimum of lactose contents of the pasteurized milk samples from the three companies were shown Table 1 and Table 2. Lactose content of pasteurized milk samples for A, B and C companies at the 3rd day of processing revealed means of 4.21±0.05%, 4.40±0.05% and 4.27±0.05%, respectively (Table 1). The values were 4.36±0.02%, 4.35±0.02% and 4.30±0.02% respectively, during the 5th day of the storage (Table 2). Lactose content of the pasteurized milk samples were affected significantly (P< 0.5) during the storage period (Table 3)

Microbial content of milk samples

Total bacterial count

The means log of total bacterial counts of pasteurized milk samples for the three companies were log 6.44±0.02 and 6.81±0.02 and 6.71±0.02 during the 3rd day of storage (Table 1) in the 5th day of storage 6.37±0.02 and 6.76±0.02 and 6.72±0.02 (Table 2). The minimum and maximum means of total bacterial count of pasteurized milk samples collected from A, B and C companies were shown in Table 1 and Table 2. Also Table 3 showed highly significant variations in the level of total bacterial count of pasteurized milk samples.

Coliform count

Table 1 and 2 showed that the mean log of coliform count for pasteurized milk samples from A, B and C companies during the 3rd and 5th days of storage (4.62±0.04, 4.85±0.04 and 4.48±0.04, and 6.37±0.03 and 4.72±0.03 and 4.29±0.03, respectively). The minimum and maximum

values of coliform log count of pasteurized milk samples were presented in Table 1 and Table 2.

Aerobic spore formers counts

The mean log of aerobic spore bacterial count of pasteurized milk samples for the three companies in the third day of the storage were 4.44±04, 4.75±04 and 4.60±05 (Table 1). They revealed 4.38±0.04, 4.63±04 and 4.60±04 during the 5th day of storage (Table 2). The minimum and maximum means of the aerobic spore formers bacterial count of pasteurized milk samples from A, B and C companies were shown in Table 1 and Table 2.

Discussion

The present study revealed higher mean values of fat content in pasteurized milk (Table 2) for the three companies than that reported by the factories in the products which were 3%. Moreover, the fat percent obtained during the 3rd day of storage were higher than those of the 5th day of storage and both were higher than that reported by the factory in its product. The higher content of fat might be due to improper skimming of raw milk. The results of pasteurized milk fat content of B and C companies agreed with El Zubeir *et al* (2008).

The mean value obtained for the solids not fat of pasteurized milk samples from company C revealed higher values than that labeled by the company. The decrease and increase in the protein content of the three factories during the storage period might be due to proteolysis of protein by psychotropic bacteria (Harding, 1999).0

The proportion of lactose in the pasteurized milk samples from B company was lower than that reported by the company in their label which was 4.5%; there was significant change in the level of company C which was 4.7%. During the 5th and 3rd day of processing of pasteurized milk samples from the three companies revealed decrease in the production of lactose compared to that reported by the companies. The decrease during 5th day agreed with Abd Elrahman *et al.* (2009) and disagrees with Elmagli and El Zubeir (2006a). The variations of pasteurized milk composition might be due to method of processing and the initial raw milk composition.

Result of total bacterial counts for pasteurized milk samples obtained during the 3rd day of storage period from A, B and C companies agreed with Elmagli and El Zubeir (2006b). It might be due to improper pasteurization which could be due to high initial counts of microorganism or post-pasteurization contamination and disagreed with Abd Elrahman *et al.* (2009).

Coliform count obtained for pasteurized milk samples disagreed with Elmagli and El Zubeir (2006b) and Abd Elrahman *et al.* (2009). High count of coliform might be due to faecal contamination (Van Kessel *et al*., 2004).

Aerobic spore bacterial count of pasteurized milk samples obtained during the present results disagree with Abd Elrahman *et al.* (2009) who reported an aerobic spores forming bacteria of pasteurized milk was 1.492± 0.044 cfu/ml.

From the present study it can be concluded that the chemical components of pasteurized milk were not uniform among the samples of pasteurized milk were distributed in Khartoum North. Moreover the samples contain high bacterial count and high number of coliform bacteria, which might indicates either lack of efficient pasteurization or post pasteurization contamination. Hence monitoring of all stages of processing of pasteurized milk in addition to improvement of storage conditions in the markets should be implemented to ensure good quality of the final product by implementation of HACCP plans.

Table 1: Chemical composition and microbial properties of pasteurized milk marketed in Khartoum North during day 3 of storage

Measurements	Day 3								
	A			B			C		
	Means±std. error	Maximum	Minimum	Means±std .error	Maximum	Minimum	Means±std. error	Maximum	Minimum
Fat (%)	3.93±0.06	4.04	3.81	3.47±0.06	3.59	3.35	4.14±0.06	4.26	4.02
SNF (%)	8.18±0.05	8.29	8.07	8.37±0.05	8.48	8.26	8.25±0.05	8.35	8.14
Density (gm/ ml)	1.029±0.162	1.029	1.028	1.029±0.162	1.03	1.029	1.028±0.168	1.028	1.027
Protein (%)	3.20±0.32	3.26	3.13	3.28±0.32	3.34	3.21	3.38±0.32	3.44	3.32
Lactose (%)	4.21±.05	4.31	4.12	4.40±.05	4.49	4.30	4.27±.05	4.36	4.17
Total bacterial count (cfu/ ml)	6.44±0.02	6.48	6.40	6.81±0.02	6.84	6.77	6.72±0.02	6.75	6.67
Coliform count (cfu/ ml)	4.62±0.04	4.69	4.54	4.85±0.04	4.92	4.77	4.48±0.04	4.55	4.41

Aerobic spore forming bacterial count (cfu/ ml)	4.44±0.04	4.48	4.41	4.75±0.04	4.79	4.72	4.60±0.05	4.63	4.56

A, B and C = Companies processing milk
Day3= 3 rd day of processing milk

Table 2: Chemical composition and microbial properties of pasteurized milk marketed in Khartoum North during day 5 of storage

Measurement	Day 5								
	A			B			C		
	Means±std.error	Maximum	Minimum	Means±std.error	Maximum	Minimum	Means±std.error	Maximum	Minimum
Fat (%)	3.90±0.05	3.40	3.81	3.32±0.05	3.41	3.22	4.18±0.05	4.27	4.08
SNF (%)	8.30±0.05	8.40	8.20	8.30±0.05	8.40	8.20	8.27±0.05	8.37	8.27
Density (gm/ ml)	1.028±0.135	1.03	1.028	1.029±0.135	1.029	1.029	1.028±0.135	1.028	1.028
Protein (%)	3.25±0.03	3.31	3.19	3.38±0.03	3.44	3.32	3.28±0.03	3.34	3.22
Lactose (%)	4.36±0.02	4.41	4.31	4.35±0.02	4.40	4.31	4.30±0.02	4.35	4.25
Total bacterial count (cfu/ ml)	6.37±0.02	6.41	6.32	6.76±0.02	6.80	6.72	6.72±0.02	6.76	6.67
Coliform	6.37±0.03	6.43	6.30	4.72±0.03	4.79	4.66	4.29±0.03	4.35	4.22

count (cfu/ ml)									
Aerobic spore forming bacterial count (cfu/ ml)	4.38±0.04	4.47	4.31	4.63±0.04	4.72	4.56	4.60±0.04	4.67	4.52

A, B and C = Companies producing milk
Day 5= 5th day of producing milk

Table 3: Effect of storage period on the chemical composition and microbial properties of pasteurized milk marketed in Khartoum North

Measurements	Day 3			Day 5		
	A	B	C	A	B	C
Fat (%)	3.92^{c}	3.47^{b}	4.13^{a}	3.90^{c}	3.31^{b}	4.17^{a}
SNF (%)	8.18^{b}	8.37^{a}	8.24^{ab}	8.29^{a}	8.30^{a}	8.27^{a}
Density (gm/ ml)	1.029^{a}	1.029^{a}	1.028^{b}	1.028^{b}	1.029^{a}	1.028^{c}
Protein (%)	3.19^{c}	3.27^{b}	3.37^{a}	3.25^{b}	3.38^{a}	3.27^{b}
Lactose (%)	4.12^{b}	4.39^{a}	4.26^{ab}	4.36^{a}	4.35^{b}	4.30^{c}
Total bacterial count (cfu/ ml)	6.71^{a}	6.75^{a}	6.36^{b}	6.70^{b}	6.80^{c}	6.44^{a}
Coliform count (cfu/ ml)	4.28^{c}	4.72^{b}	4.36^{b}	4.47^{c}	4.84^{a}	4.61^{b}
Aerobic spore forming bacterial count (cfu/ ml)	4.59^{a}	4.63^{a}	4.38^{b}	4.59^{b}	4.75^{a}	4.44^{c}

A, B and C = Companies producing milk

Means with the same raw bearing the same superscripts are not significantly different ($p>0.05$).

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