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Evaluation of Microbiological Quality of Sudanese Fermented Dairy Product 'Mish' During Storage

M.O.M. Abdalla and S.Z. Abdel Nabi Ahmed
Department of Dairy Production, Faculty of Animal Production, University of Khartoum, Shambat, P.O. Box 32, Postal Code 13314, Khartoum North, Sudan

Abstract: This study was conducted to microbiologically evaluate the Sudanese fermented dairy product 'mish' during storage. Ninety samples were collected on the first day from three dairy plants (P1, P2, P3), transported to the laboratory in icebox and stored at 7ºC. The samples were examined for total viable bacteria, coliform bacteria, *Staphylococcus aureus*, psychrotrophic bacteria and yeasts and molds counts at 1, 7, 14, 21 and 28 days. The results showed that coliform bacteria, *S.aureus*, psychrotrophic bacteria and yeasts and molds counts were high in P2, while total viable bacteria count was high in P1. During storage, total viable bacteria and coliform bacteria counts increased till day 14, followed by a slight decrease at day 21 and then increased towards the end. *S.aureus* count decreased at day 14, increased at day 21 and then decreased. Yeasts and molds count steadily increased towards the end, while psychrotrophic bacteria count increased to a maximum at day 14, then decreased at the end.

Key words: Manufacturing plants, microbiological quality, mish, storage period

INTRODUCTION

Fermented milk products are cultured dairy products made from skim, whole or slightly concentrated milk that require specific lactic acid bacteria to develop their characteristic flavor and texture. Many traditional fermented milk products are made in Asia, Africa, the Middle East and Northern and Eastern Europe, and the microbiological characteristics of several of these products have been studied in the world. The nature of fermented products is different from one region to another, and depends mainly on the local indigenous microflora, which in turn reflects the climatic conditions of the area (Savadogo *et al.,* 2004).

Recent evidence, from *in vitro* systems in clinical studies, suggests that lactic acid bacteria can enhance both specific and non-specific immune responses possibly by activating macrophages, altering cytokine expression, increasing natural killer cell activity and/or increasing levels of immunoglobulins (Vitini *et al.*, 2000). There are claims that consuming fermented milk food can boost the immune system, alleviate symptoms of diarrhea and chronic constipation, lower the risk of colon cancer and lower blood sugar levels, blood lipid levels and blood pressure (Maeda *et al.*, 2004). One of the most accepted ways to extend the shelf life of perishable food products is the use of bio-preservatives. It has long been recognized that some volatile essential oils have antimicrobial properties and they can be used as food flavoring agents or preservatives and for medicinal purposes. The effects of different concentrations of volatile oils on different microorganisms present on food have been varied, ranging from partial to complete inhibition (Burt, 2004; Draughon, 2004).

'Mish', as a fermented dairy product manufactured in the Sudan, is known to all regions with different names, and is now available in the markets of urban areas in Sudan. Traditionally, 'mish' is made by boiling the milk, and after cooling, is inoculated with a small quantity of soured 'laben-rayeb' or 'mish', followed by the addition of seeds of black cumin, fenugreek and perhaps a few pods of green or red pepper and the product is fermented for two or more days before consumption. In modern dairy industry, it is made from whole cow's or skimmilk by adding starter culture, and after curdling spices such as black cumin, fenugreek, garlic and sometimes hot or green pepper are added, then is packaged and left for 24 hours to ripen and develop a curd (Ahmed, 2007; Dirar, 1993).

This study was carried out to evaluate the microbiological load of the Sudanese fermented dairy product 'mish' produced by some factories and to check the validity of shelf life shown on the label.

MATERIALS AND METHODS

Collection of samples: During the period February-June 2009, thirty samples of 'mish' from each of three

Corresponding Author: M.O.M. Abdalla, Department of Dairy Production, Faculty of Animal Production, University of Khartoum, Shambat P.O. Box 32, Postal Code 13314, Khartoum North, Sudan

Table 1: Microbiological quality of 'mish' from the three manufacturing dairy plants (mean±SE)

Parameter	Manufacturing dairy plant			SL
	P1	P2	P3	
Total viable bacteria	9.25^{a}±0.088	9.06^{a}±0.089	9.02^{a}±0.091	NS
Coliform bacteria	6.14^{a}±0.069	6.34^{a}±0.070	6.23^{a}±0.072	NS
Staphylococcus aureus	7.22^{b}±0.069	7.49^{a}±0.070	6.11^{c}±0.072	***
Psychrotrophic bacteria	8.30^{c}±0.052	9.31^{a}±0.053	9.03^{b}±0.054	***
Yeasts and molds	5.01^{b}±0.045	5.17a±0.046	4.97^{b}±0.047	***
Titratable accidity (% lactic acid)	3.96^{a}±0.035	2.48^{c}±0.035	3.23^{b}±0.035	***

Means in a row bearing the same superscripts are not significantly different (p>0.05),
P1, P2, P3 = Manufacturing dairy plants 1, 2 and 3 respectively,
***: p<0.001; NS = Not significant; SL = Significance level

Table 2: Microbiological quality of mish during storage (data are the mean of P1, P2 and P3) (mean±SE)

Parameter	Storage period (days)					SL
	1	7	14	21	28	
Total viable bacteria	9.47^{b}±0.121	8.03^{d}±0.115	9.26^{b}±0.113	8.89^{c}±0.113	9.91^{a}±0.113	***
Coliform bacteria	6.22^{ab}±0.096	6.36^{a}±0.091	6.22^{ab}±0.090	5.99^{b}±0.090	6.39^{a}±0.090	*
Staphylococcus aureus	6.22^{c}±0.096	7.33^{b}±0.091	6.57^{d}±0.090	7.59^{a}±0.090	6.99^{c}±0.090	***
Psychrotrophic bacteria	8.59^{c}±0.072	9.06^{b}±0.069	9.30^{a}±0.067	8.79^{c}±0.067	8.66^{c}±0.067	***
Yeasts and molds	4.72^{c}±0.062	4.84^{c}±0.059	5.05^{b}±0.058	5.29^{a}±0.058	5.34^{a}±0.058	***
Titratable accidity (% lactic acid)	2.70^{d}±0.046	3.04^{c}±0.046	3.14^{c}±0.046	3.39^{b}±0.046	3.84^{a}±0.046	***

Means in a row bearing the same superscripts are not significantly different (p>0.05),
*: p<0.05; ***: p<0.001; SL = Significance level

local manufacturing dairy plants (P1, P2, P3) were collected in 250 g size packaged plastic cups, transported to the laboratory in icebox and kept in the refrigerator (7ºC) for 28 days. Microbiological examination was carried out at 1, 7, 14, 21 and 28-day intervals.

Microbiological examination: Samples were serially diluted using sterile distilled water as diluent. Eleven grams from a homogeneous 'mish' sample were added to 99 ml of sterile distilled water in a clean sterile flask, shaken to make 10^{-1} dilution, and then serial dilutions were prepared. From appropriate dilutions, 1 ml each was plated in duplicate using pour plate method. Plate count agar medium was used for the determination of total viable bacteria (Houghtby *et al.*, 1992) and psychrotrophic bacteria (Frank *et al.*, 1992). The plates for total viable bacteria count were incubated at 32ºC for 48 hours and for psychrotrophic bacteria count at 7ºC for 10 days. MacConkey agar medium was used for the determination of coliform bacteria count (Christen *et al.*, 1992) and the plates were incubated at 32ºC for 48 h. Baird Parker agar base medium was used for the determination of *Staphylococcus aureus* count (Flowers *et al.*, 1992) after incubation at 35ºC for 48 h. Potato dextrose agar (PDA) was used for the determination of yeasts and molds count (Frank *et al.*, 1992) followed by incubation at 25ºC for 5 days. After incubation, developed colonies were counted and the suspected ones were further isolated and identified (Barrow and Feltham, 1993).

Statistical analysis: The data were statistically analyzed using Statistical Package for Social Sciences (SPSS, 2004). Completely randomized design was used for statistical analysis, and mean separation was done by Duncan's multiple range tests at $p \leq 0.05$.

RESULTS

No significant differences were obtained in total viable bacteria and coliform bacteria counts of the three plants (Table 1), and there were significant differences in *S. aureus* count, psychrotropic bacteria count and acidity percentage. Yeasts and molds counts were significantly higher in P2 than in P1 and P3 (Table 1).

Table 2 shows that total viable bacteria count fluctuated during storage decreasing at day 7 followed by increase at day 14 before decreasing at day 21 and finally increased at the end. Coliform bacteria increased at day 7, decreased to the minimum at day 21 followed by an increase at the end of storage. *S. aureus* count increased at day 7, decreased at day 14 and increased at day 21 before decreasing towards the end. Psychrotrophic bacteria count increased to the maximum at day 14 then gradually decreased at the end of storage period. Yeasts and molds count and acidity percentage showed a gradual increase towards the end.

During storage of samples from the three plans, total viable bacteria count showed a fluctuating pattern in P1, P2 and P3. Coliform bacteria count increased in P1 to the maximum at day 21 then decreased thereafter, while in P2 the count gradually decreased to the end and in P3 the count showed fluctuating pattern towards the end. *S. aureus* count fluctuated till day 21 then decreased at the end in P1 and P3, while in P2 the count fluctuated till day 21 then increased at the end. Psychrotrophic bacteria count increased till day 14 then gradually decreased in all the three plants. Yeasts and molds count increased to the maximum at day 21 then decreased in P1, while in P2 and P3 the count gradually increased towards the end. Titratable acidity gradually increased towards the end in all plants (Table 3).

Table 3: Microbiological quality of mish from each plant during storage (Log10 cfu/gm) (mean±SE)

Parameter	Storage period (days)				
	1	7	14	21	28
		P1			
Total viable bacteria	9.99±0.196	7.53±0.196	8.70±0.196	9.97±0.196	10.07±0.196
Coliform bacteria	5.77±0.155	6.38±0.155	6.37±0.155	6.38±0.155	5.80±0.155
Staphylococcus aureus	6.85±0.155	7.90±0.155	6.66±0.155	7.99±0.155	6.70±0.155
Psychrotrophic bacteria	7.87±0.117	8.40±0.117	8.60±0.117	8.54±0.117	8.10±0.117
Yeasts and molds	4.77±0.101	4.67±0.101	5.09±0.101	5.30±0.101	5.23±0.101
Titratable accidity (% lactic acid)	3.63±0.079	3.72±0.079	3.48±0.079	4.20±0.079	4.75±0.079
		P2			
Total viable bacteria	9.64±0.196	8.24±0.206	8.96±0.196	8.90±0.196	9.57±0.196
Coliform bacteria	6.78±0.155	6.75±0.164	6.25±0.155	5.96±0.155	5.94±0.155
Staphylococcus aureus	6.04±0.155	7.60±0.164	7.37±0.155	7.93±0.155	8.52±0.155
Psychrotrophic bacteria	9.67±0.117	9.66±0.123	9.99±0.117	8.67±0.117	8.56±0.117
Yeasts and molds	4.97±0.101	4.95±0.106	4.94±0.101	5.37±0.101	5.59±0.101
Titratable accidity (% lactic acid)	1.93±0.079	2.26±0.079	2.58±0.079	2.66±0.079	2.97±0.079
		P3			
Total viable bacteria	8.79±0.235	8.31±0.196	10.12±0.196	7.78±0.196	10.09±0.196
Coliform bacteria	6.15±0.155	5.94±0.155	6.05±0.155	5.62±0.155	7.42±0.155
Staphylococcus aureus	5.77±0.187	6.50±0.155	5.69±0.155	6.83±0.155	5.76±0.155
Psychrotrophic bacteria	8.23±0.140	9.14±0.117	9.31±0.117	9.17±0.117	9.17±0.117
Yeasts and molds	4.14±0.101	4.91±0.101	5.11±0.101	5.20±0.101	5.20±0.101
Titratable accidity (% lactic acid)	2.53±0.079	3.16±0.079	3.38±0.079	3.30±0.079	3.79±0.079

DISCUSSION

The present study was carried out to determine the shelf life of 'mish' samples collected from three different dairy plants, two in Khartoum State and the third in the White Nile State about 300 km south of Khartoum. The purpose of this investigation was to determine the possibility that the product keeps its quality during the shelf life of 20 days, shown on the labels, in order to assist the local authorities (Sudanese Standards and Metrology Organization) to set a standard for this product.

The high total viable bacteria count in P1 explains the highest acidity of mish from this plant (Table 1), and that most of total viable bacteria count may be lactic acid bacteria. From microbiological point of view, P1 was better in terms of coliform bacteria, yeasts and molds and psychrotrophic bacteria counts (showed low count), but *S. aureus* count was slightly higher. Total viable bacteria count was higher than the reports of Abdel Hafiz (2001), Savadogo *et al.* (2004), Uzeh *et al.* (2006) and Hassan *et al*. (2008), and likewise coliform count was higher than these of Abdel Hafiz (2001), Benkerroum and Tamime (2004), Al-Tahiri (2005), Lore *et al.* (2005) and El-Baradei *et al.* (2008), and lower than those reported by Uzeh *et al.* (2006). The high number of coliforms is a sign of unsanitary conditions and/or post-processing contamination. *S. aureus* count was higher than in the reports of Benkerroum and Tamime (2004), Al-Tahiri (2005), El-Zubeir *et al.* (2005) and El-Baradei *et al.* (2008).

The high *S. aureus* count is indicative of poor hygienic conditions during production/processing of the product or contamination after processing. The results of yeasts and molds count are higher than the values reported by Lore *et al.* (2005) and lower than these of Mathara *et al.* (2004), Al-Tahiri (2005) and Hassan *et al.* (2008) who reported yeast and mold counts of as low as Log 2.18 cfu and as high as Log 5.70 from yogurt and Lebnah, respectively, produced by farmers. These high numbers reflect unsanitary hygienic conditions during processing and distribution of the product, despite the fact that the product under investigation was superior to all similar fermented products except that it had high acidity compared to these products. The present results are expected since the product chemically deteriorated towards the end of storage (Samolada *et al.,* 1998). The increase in the acidity of 'mish' towards the end of storage might be due to increase in number of lactic acid bacteria which converted lactose into lactic acid (Bozamic and Tratnik, 2001; Cais-Sokolinska *et al.*, 2008). However, the increase in titratable acidity did not adversely affect the growth and multiplication of microorganisms during storage.

CONCLUSION

The study revealed high total viable bacteria, coliform bacteria, *S. aureus,* psychrotrophic bacteria and yeasts and molds counts of mish. The dairy plants need to improve the production conditions in terms of processing, packaging, storage, handling and distribution in order to produce a safe and sound product for consumption. The product kept good quality from microbiological point of view for up to 21 days.

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