Australian Journal of Basic and Applied Sciences, 4(3): 532-536, 2010
ISSN 1991-8178

Chemical and Microbiological Evaluation of Mozzarella Cheese During Storage

Mohamed Osman Mohamed Abdalla and Nisreen Nourein Mohammed Ibrahim

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 carried out to evaluate the chemical composition and microbiological quality of traditionally made Mozzarella cheese by two manufacturers in Khartoum State, Sudan. Thirty six samples, eighteen from each manufacturer, were collected. The chemical composition (fat, protein, total solids, ash and titratable acidity) and microbial quality (total bacteria count, proteolytic bacteria count, lipolytic bacteria and psychrotrophic bacteria count) of the cheese were evaluated. The results showed that during the shelf life of 75 days the fat content of cheese gradually ($P<0.01$) increased from day one to a maximum at day 60, and then decreased thereafter. The protein content showed a gradual decrease from day one to day 60, and then slightly increased at the end of shelf life. The total solids content slightly increased at day 30 and decreased towards the end. The ash content increased to a maximum at day 30 and then decreased at day 45, followed by an increase at day 60 and decreased at the end. The titratable acidity showed an irregular pattern throughout the shelf life and decreased at the end. The total bacteria count decreased to a minimum at day 60, then slightly increased. The lipolytic and psychrotrophic bacteria counts showed a gradual increase from day one to the end. The proteolytic bacteria count increased from day one to the end.

Key words: Mozzarella cheese, storage period, chemical composition, microbiological quality

INTRODUCTION

Mozzarella cheese is a Mediterranean Pasta filata cheese originated in Italy. Its consumption is increased in Sudan due to its incorporation in pizza that is gaining popularity. When kept in brine this cheese has a short shelf life of 5-7 days (Altieri *et al.,* 2005; Conte *et al.,* 2007).

Owing to the variety of microorganisms found in this product, mozzarella has a rather short shelf life. The specific characters of mozzarella cheese mainly arise from the specific raw materials employed, the area of production, the environmental conditions, the traditional tools, and manufacturer (Mauriello *et al*., 2003).

Extending shelf life of mozzarella cheese is important issue to the dairy industry due to the interest in extending the distribution of the product beyond the market, and to keep good quality food product depends on the improvement of raw materials, process innovations, and use of suitable storage facilities (Farkye *et al.,* 1991; Kindstedt and Fox, 1993).

Numerous studies have characterized the presence of spoilage microorganisms that limit the shelf life of Mozzarella cheese. Microorganisms such as Klebsiella, Enterobacter, *Escherichia coli*, Enterococcus, Pseudomonas, yeasts and moulds were isolated from Mozzarella cheese (Massa *et al.,* 1992; Romano *et al.,* 2001; Duan *et al.,* 2007).

Mozzarella cheese is spoiled by *Pseudomonas spp*. coming from water or dairy manufacture, in addition to the fact that coliforms limit the shelf life of Mozzarella cheese (Altieri *et al.,* 2005).

The Sudanese standards states that the shelf life of Mozzarella cheese should not exceed eight weeks from the day of manufacture, provided cheese is stored at temperature not more than 10°C (SSMO, 2008)

Due to increasing consumption of Mozzarella cheese for pizza and other purposes, the technology of making this kind of cheese is gaining popularity although all manufacturers are producing it traditionally, and many small-scale manufacturers are involved in this industry.

Therefore the objectives of this study were to evaluate Mozzarella cheese chemically and microbiologically during the shelf life and to determine how the manufacturing conditions can affect the quality of the product during its shelf life.

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

MATERIALS AND METHODS

Collection of Samples:

Thirty six samples of mozzarella cheese were collected from two factories in Khartoum and Khartoum North (18 samples from each one) in the day of manufacture (day one). The samples were stored in the refrigerator at 4°C to determine the chemical composition and microbiogical quality.

Chemical Analyses:

Fat content was determined by Gerber method according to AOAC (1990). Protein content was determined by Kjeldahal method according to the method described in AOAC (2000). Total solids content was determined by oven drying method according to AOAC (2000). Ash content and titratable acidity were determined according to methods described in AOAC (2000).

Microbiological Examination:

The bacteria count was determined according to Houghtby *et al*. (1992) using a standard plate count agar. The plates were incubated at 32°C for 48 hrs and colonies were counted. Lipolytic bacteria count was determined as described by Zaki (1988). Nutrient agar was used and the plates were incubated at 37°C for 3 days. The lipolytic colonies were identified using copper sulphate (20%) flooded after incubation. Psychrotrophic bacteria count was determined according to Frank *et al*. (1992) using plate count agar. The plates were incubated at 7±1°C for 10 days. Proteolytic bacteria count was determined according to Frank *et al*. (1992) using plate count agar plus 10% sterile skim milk. The plates were incubated at 37°C for 3 days

Purification was done by sub-culturing of a well isolated typical colony on nutrient agar medium for 24 hrs and the plates were checked by Gram's stain, then the colonies were transferred to a plate containing a fresh solidified corresponding medium (Barrow and Feltham, 1993).

Identification of Organisms:

The purified isolates were identified according to the criteria described by Barrow and Feltham (1993).

Statistical Analyses:

Statistical analyses were performed using Statistical Package for Social Sciences (SPSS). The data were analyzed for fat, protein, ash, total solids, titratable acidity, total bacteria count, lipolytic count, proteolytic count and psychrotrophic count. Means were separated using Duncan Multiple Range Test with $P \leq 0.05$.

Results:

Table (1) shows the chemical composition of cheese from the two manufacturers during the storage period of 75 days. The fat content gradually ($P<0.01$) increased from day one (22.17±2.93) to a maximum at day 60 (26.0±1.29), and then decreased thereafter (24.5±2.46). The protein content showed a gradual ($P<0.05$) decrease from 37.67±2.18 at day one to 24.58±1.81 at day 60, and then slightly increased to 25.2±1.35 at day 75. However, the total solids content slightly increased to 57.05 ±1.28 at day 30 and decreased towards the end, although the fluctuation was not of a significant value ($P >0.05$).

The ash content increased to a maximum of 3.13±0.89 at day 30 and decreased to 2.62± 2.84 at day 45, followed by an increase at day 60 (3.73±2.72) and decreased to 2.45± 2.87 at the end ($P< 0.01$). The titratable acidity showed an irregular pattern throughout the storage period, with the highest value being reached at days 30 (0.36±0.13) and 45 (0.36±0.06), after which the acidity decreased to 0.29±0.08 at the end ($P<0.05$).

The fat content of cheese from manufacturer1 (M1) showed a gradual increase till day 60, the decreased thereafter. The protein and total solids contents decreased towards the end. The ash content increased to a maximum at day 60, and then decreased. The titratable acidity increased till day 30, then decreased. The fat content of manufacturer2 (M2) increased till days 45 and 60, and then decreased. The protein and total solids contents decreased till day 60, and then slightly increased thereafter. The ash content showed an irregular pattern throughout the period, while the titratable acidity showed a decrease towards the end (Table 2).

Table (3) showed that the total bacteria count decreased to a minimum of $\log_{10}$ 6.08 ±1.57 at day 60, then slightly increased to $\log_{10}$ 7.86±1.09 at day 75 ($P<0.05$) 8. The lipolytic bacteria count showed a regular increase from $\log_{10}$ 4.48 ±1.57 at day one to $\log_{10}$ 6.82±1.08 at the end ($P<0.01$).

The proteolytic and psychrotrophic counts increased from $\log_{10}$ 5.88±2.45 and $\log_{10}$ 4.07±0.82 respectively at day one to $\log_{10}$ 7.44±1.37 and $\log_{10}$ 6.10 ± 1.72 at the end.

Table 1: Chemical composition of mozzarella cheese during storage period

Storage period (days)	Fat content (%)	Protein content (%)	Total solids content (%)	Ash content (%)	Titratable acidity (% lactic acid)
1	22.17 ^{b} ± 2.93	37.67^{a} ± 2.18	56.57 ^{a} ± 1.56	2.58 ^{bc} ± 0.33	0.43 ^{a} ± 0.15
15	24.17 ^{ab} ±1.87	27.05 ^{b} ± 2.86	56.58 ^{a} ± 1.67	2.58 ^{bc} ± 0.24	0.24 ^{c} ± 0.04
30	24.33 ^{ab} ±1.78	24.40 ^{c} ± 1.41	57.05 ^{a} ± 1.28	3.13 ^{b} ± 0.89	0.36 ^{ab} ± 0.13
45	25.08 ^{a} ±1.0	24.63 ^{c} ± 1.65	55.08 ^{a} ± 1.28	2.62 ^{bc} ± 2.84	0.36 ^{ab} ± 0.06
60	26.00 ^{a} ±1.29	24.58 ^{c} ± 1.81	55.35 ^{a} ± 4.59	3.73 ^{a} ±2.72	0.28 ^{bc} ± 0.03
75	24.50 ^{a} ± 2.46	25.20 ^{c} ±1.35	55.58 ^{a} ± 0.87	2.45 ^{c} ± 2.87	0.29 ^{bc} ± 0.08
Grand mean	24.38	27.26	56.03	2.85	0.33
S.L	**	0	NS	**	*

Means within each column bearing the same superscripts are not significantly different (P > 0.05).
**=P≤0.01
*=P≤0.05
N.S= Not significant (P≥0.05)

Table 2: The chemical composition of mozzarella cheese during storage period from two manufacturers under study.

Storage period (days)	Fat content (%)	Protein content (%)	Total solids content (%)	Ash content (%)	Titratable acidity (% lactic acid)
			Manufacturer (M1)		
1	20.67	39.17	57.37	2.87	0.29
15	22.67	29.40	57.23	2.77	0.26
30	23.33	25.40	58.47	2.93	0.39
45	23.17	25.77	55.13	3.10	0.32
60	25.00	26.37	57.73	3.63	0.28
75	22.67	26.40	55.77	2.93	0.24
			Manufacturer (M2)		
1	23.67	36.17	55.77	2.30	0.56
15	25.67	24.70	55.93	2.40	0.22
30	25.33	23.40	55.63	3.33	0.33
45	27.00	23.50	55.00	2.13	0.40
60	27.00	22.80	52.97	3.83	0.28
75	26.33	26.33	55.40	1.97	0.35

Table 3: Microbiological quality (Log10 cfu/gm) of mozzarella cheese during storage period (days):

Count (Log10 cfu/gm)	Storage period (days)							
	1	15	30	45	60	75	Grand mean	S.L
Total bacteria	7.72 ^{ab} ± 1.59	7.13^{abc} ±2.11	7.66 ^{ab} ±1.05	6.50 ^{bc} ±3.23	6.08 ^{c} ± 1.57	7.86 ^{a} ±1.09	7.15	*
Lipolytic bacteria	4.48 ^{d} ± 1.57	4.92^{cd} ± 1.24	5.73 ^{bc} ± 0.42	5.85 ^{b} ± 1.52	5.90 ^{b} ± 0.47	6.82 ^{a} ± 1.08	6.78	**
Porteolytic bacteria	5.88 ^{d} ± 2.45	6.56^{cd} ±0.50	6.82 ^{bc} ± 1.24	7.72 ^{a} ± 1.26	6.29 ^{cd} ±1.19	7.44 ^{ab} ±1.37	6.78	*
Psychrotrophic bacteria	4.07 ^{c} ±0.82	4.83 ^{c} ± 0.45	5.74^{bc} ± 1.41	6.94 ^{a} ±1.59	6.52 ^{ab} ±1.14	6.10 ^{ab} ±1.72	5.69	**

Means within each row bearing the same superscripts are not significantly different (P > 0.05).
*= P<0.05
**=P<0.01

The total bacteria count of cheese from M1 increased till day 30 and then decreased at day 45 and then gradually increased towards the end, while the proteolytic bacteria count increased at day 15, followed by a decrease at day 30, and then increased at the end. The lipolytic bacteria count steadily increased towards the end of storage period. The psychrotrophic bacteria count increased till day 60 then decreased thereafter. Cheese from M2 showed that total bacteria count showed an irregular pattern throughout the storage period. However the proteolytic, lipolytioc and psychrotrophic bacteria count gradually and regularly increased with increasing storage period (Table 4).

Discussion:

This study was carried out to evaluate Mozzarella cheese manufactured by two manufacturers located in two different cities in Khartoum State. The manufacturing method of cheese from both manufacturers was traditional, where M1 used vacuum packaging under aseptic conditions with shelf life of the product being 75 days as stated in the label, while M2 used no packaging and no labeling therefore the shelf life of the product was not stated. It was observed that both manufacturers used no hygiene system of production and the milk might have come from different sources of varying hygienic conditions and the milk was not subjected to heat treatment.

The chemical composition of Mozzarella cheese indicated that fat content increased at first and then decreased at the end, and this was the trend for protein and ash contents. The milk used for cheese making

Table 4: The microbiological quality (Log10 cfu/gm) of mozzarella cheese during storage period from two manufactures under study

Storage period (days)	total bacteria count	Porteolytic bacteria count	Lipolytic bacteria count	Psychrotrophic bacteria count
		Manufacturer (M2)		
1	6.37	4.87	3.23	3.87
15	5.30	6.12	3.97	4.47
30	6.77	5.75	5.75	4.55
45	3.99	6.62	4.80	5.35
60	4.67	5.92	5.60	5.65
75	7.03	6.19	5.92	4.70
		Manufacturer (M2)		
1	9.07	6.88	5.73	4.27
15	8.97	7.00	5.87	5.18
30	8.55	7.88	5.70	6.93
45	9.00	8.82	6.90	8.53
60	7.35	6.65	6.20	7.38
75	8.68	8.68	7.72	7.50

was mainly cow milk. These results are within the limits reported by Imm *et al.* (2003) and Guinee *et al.* (2000). However, the results are higher than those of Everett *et al.* (2004) and de Candia *et al.* (2007). During shelf life major components (fat, protein, total solids, and ash) showed a gradual decrease in cheese from both manufacturers, although the fat content showed a slight increase at the end compared to day one. The protein content decreased, while the total solids content showed no change. The titratable acidity slightly decreased towards the end, and this might be due to the traditional method of manufacture in which no starter cultures were used.

Generally, the fluctuation in chemical composition of cheese samples may be due to different manufacturing conditions between manufacturers and different sources of milk used, method of manufacture in terms of temperature and use of starter cultures.

From microbiological point of view, cheese from M2 was more contaminated compared to that from M1. All bacteria examined were found to increase during shelf life in a regular behaviour indicating that both manufacturing conditions and raw material were responsible for the high number of bacteria and a consequent increase during storage period. Massa *et al*. (1992) isolated *Klebsiella pneumoniae*, *K. oxytoca*, and *Enterobacter aerogenes* from Mozzarella cheese as spoilage organisms, while Romano *et al.* (2001) isolated yeasts from traditional water buffalo Mozzarella.

The shelf life of Mozzarella cheese was prolonged using different packaging systems where *Pseudomonas spp*. were reduced from Log 7.77 cfu/g to Log 5.14-6.08 cfu/g and therefore the shelf life was prolonged from 1.58 days to 2.73-3.26 days using three different innovative active packaging systems (Conte *et al.*, 2007). Duan *et al.* (2007) reported that chitosan-lysozyme films and coating resulted in a drastic reduction in the population of *Listeria monocytogenes*, *E. coli*, *Pseudomonas fluorescens* and yeasts and moulds in Mozzarella cheese stored for 14 days. In another study it was found no significant variation in lactic acid bacteria and coliforms between cheese treated with chitosan and chitosan-free cheese (Altieri *et al*., 2005).

The increase in number of bacteria isolated with advancement of shelf life might in part be due to absence of commercial starter cultures which if were present might have resulted in decreasing the number of bacteria during shelf life (Duan *et al.,* 2007).

Psychrotrophic bacteria such as *Pseudomonas fluorescens* cause bitter taste and bad odour through lipolytic and proteolytic reactions resulting in spoilage of cheese (Sandrou and Arvantitoyannis, 2000). Constable *et al.* (2007) reported that changes in cheese making process were insufficient to produce a significant difference in proteolysis of cheeses.

Hasan *et al*. (2006) reported that psychrotrophic bacteria cause a significant spoilage problem in refrigerated dairy products due to secretion of hydrolytic enzymes especially lipases and proteases.

Conclusions:

There was a significant increase in fat content, and a decrease in protein and total solids contents and titratable acidity, while an irregular pattern was observed during shelf life. However, except for fat content of cheese from M1 which increased with time, all other cheese components studied showed a decreasing pattern throughoutt shelf life. Total bacteria, lipolytic bacteria, proteolytic bacteria and psychrotrophic bacteria counts increased with time in cheese from both manufacturers.

REFERENCES

Altieri, C., C. Scrocco, M. Sinigaglia and M.A. Del Nobile, 2005. Use of Chitosan to prolong Mozzarella cheese shelf life. Journal of Dairy Science, 88: 2683-2688.

AOAC, 1990. Official Methods of Analysis. Association of Official Analytical Chemists, Washington, DC, USA.

AOAC, 2000. Official Methods of Analysis of AOAC International, 17th ed., AOAC International, Gaitherburg, MD, USA, Official Methods, 920.124, 926.08, 955.30, 2001.14.

Barrow, G.H. and P.K.A. Feltham, 1993. Cowan and Steel's Manual for the Identification of Medical Bacteria. Third edition, Cambridge University Press, Cambridge, pp: 331.

de Candia, S., M. De Agelis, E. Dunlea, F. Minervini, P.L.H. McSweeney, M. Faccia and M. Gobbetti, 2007. Molecular identification and typing of natural whey starter cultures and microbiological and compositional properties of related traditional Mozzarella cheeses. International Journal of Food Microbiology, 119: 182-191.

Conte, A., C. Scrocco, M. Sinigalia and M.A. Del Nobile, 2007. Innovative active packaging systems to prolong the shelf life of mozzarella cheese. Journal of Dairy Science, 90: 2126-2131.

Constabel, L., M.S. Paulettims and E. Hynes, 2007. proteolysis in Mozzarella Cheeses manufactured by different industrial processes. Journal of Dairy Science, 90(5): 2103-2112.

Duan, J., S.I. Park, M.A. Daeschel and Y. Zhao, 2007. Antimicrobial chitosan-lysozyme (CL) films and coatings for enhancing microbial safety of mozzarella cheese. Journal of Food Science, 72(9): M355-M362.

Everett, W.D., M.K. Rowney, M.W. Hickey and P. Roupas, 2004. Salt-induced structural changes in Mozzarella cheese and the impact upon free oil formation in ripening cheese. Lait, 84: 539-549.

Farkye, N.Y., L. Kiely, R.D. Allshouse and P.S. Kindstedt, 1991. Proteolysis in Mozzarella cheese during refrigerated storage. Journal of Dairy Science, 74: 1433-1438.

Frank, F.J., L.G. Christen and L.B. Bullerman, 1992. Tests for groups of microorganisms. In Standard Methods for the Microbiological Examination of Dairy Products, 16th edition., Ed., R.T. Marshal. Washington, DC: American Public Health Association, pp: 271-286.

Guinee, P.T., D. Harrington, M.O. Corcoran, E.O. Mulholland and C. Mullins, 2000. The compositional and functional properties of commercial Mozzarella, Cheddar and analogue pizza cheeses. International Journal of Dairy Technology, 53: 51-56.

Hasan, F., A. Alishah and Abdul Hameed, 2006. Industrial applications of microbial lipases. Enzyme and Microbial Technology, 39: 235-251.

Houghtby, A.G., L.J. Maturin and K.E. Koenig, 1992. Microbiological count methods. In Standard Methods for the Examination of Dairy Products, 16th edition, Ed., R.T. Marshal. Washington, DC: American Public Health Association, pp: 213-246.

Imm, J.Y., E.J. Oh, K.S. Han, S. Oh, Y.W. Park and S.H. Kim, 2003. Functionality and physico-chemical characteristics of bovine and caprine Mozzarella cheeses during refrigerated storage. Journal of Dairy Science, 86: 2790-2798c

Kindstedt, P.S. and P.F. Fox, 1993. Effect of manufacturing factors, composition and proteolysis on the functional characteristics of Mozzarella cheese. Critical Reviews in Food Science and Nutrition, 33: 167-187.

Massa, S., F. Gardini, M. Sinigaglia and M.E. Guerzoni, 1992. *Klebsiella pneumoniae* as a spoilage organism in mozzarella cheese. Journal of Dairy Science, 75: 1411-1414.

Mauriello, G., L. Moio. A. Genovese and D. Ercolini, 2003. Relationships between flavoring capabilities,bacterial composition, and geographical origin of natural whey cultures used for traditionalwater buffalo Mozzarella cheese manufacture. Journal of Dairy Science, 86: 486-497.

Romano, P., A. Ricciardi, G. Salzano and G. Suzzi, 2001. Yeasts from water buffalo mozzarella, a traditional cheese of the Mediterranean area. International Journal of Food Microbiology, 69: 45-51.

Sandrou, D.K. and I.S. Arvanitoyannis, 2000. Application of hazard analysis critical control point (HACCP) system to the cheese-making industry: a review. Food Reviews International, 16: 327-368.

SSMO, 2008. SSMO Standard No. 3869 for Mozzarella cheese. Sudanese Standards and Metrology Organization, Khartoum, Sudan.

Zaki, S.A., 1988. Application of Practical Microbiology. DarELmaarif (inArabic), Egypt.