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Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com

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Cholesterol Content of Mozzarella Cheese During Storage as Affected by Level of Milk Fat

Amal Mohammed Ali[1] and Kamal Awad Abdel-Razig[2]
[1]Department of Food Sciences and Technology, School of Health Sciences,
Ahfad Centre of Sciences and Technology, Ahfad University for Women, Sudan
[2]Department of Food Science and Technology, Faculty of Agriculture,
Al-Zaeim Al-Azhari University, P.O. Box 1432, Khartoum North, 13311, Sudan

Abstract: Four Mozzarella cheese samples were prepared from cow's milk, standardized at four different fat levels (0, 3, 5 and 7% respectively) and stored at 4°C for 30 days. Stored cheese was examined at 0, 10, 20 and 30 days intervals. The total solids of cheese increased significantly ($p \leq 0.05$) due to the loss of moisture content. The increase was 58.12% when 7% fat milk used compared to 57.75, 51.24 and 48.78%, for 5, 3 and 0% cheese, respectively. Fat, protein content showed increasing trend as the storage period progressed. The pH value decreased significantly ($p \leq 0.05$) in sample with 7% fat milk than those with 5%, 3% and 0% fat milk. The Total Volatile Fatty Acids (TVFA) increased during storage period. Cholesterol level of Mozzarella cheese increased significantly ($p \leq 0.05$) with storage period. The cholesterol content of samples 7% fat milk at the end of storage period (106 mg/100 g) was significantly higher than respective values of 5% (87.51 mg/100 g), 3% (61.72 mg/100 g) and 0% fat milk (17.10 mg/100). 5% fat milk was significantly ($p \leq 0.05$) the best in appearance, texture, flavour and overall acceptability compared to the other cheese product.

Key words: Mozzarella cheese, cholesterol level, level of fat, storage

INTRODUCTION

Cheese is among the main sources of animal fat and dietary cholesterol. Even though cholesterol is essential for membrane structure, hormone and steroid biosynthesis (Mahann and Escott-Stump, 1996), it has been recognized that it is at an elevated level in plasma and directly correlated with the increase the incidence of cardiovascular heart disease.

Cholesterol is found almost exclusively in foods of animal origin especially in meat and dairy products. No correlation of cholesterol with fat and other cheese constituents has been searched. In addition, no detailed data are available for Sudanese of cheese varieties in the majority of cheese producing areas. Since cheese manufacture in Sudan is concerned mainly with white cheese processing employing traditional technology, other cheese varieties were also produced but in a limited amounts. These include Mozzarella cheese and Mudaffara cheese.

The manufacture of Mozzarella was first practiced in Khartoum dairy product company in 1992 and then practiced by Koko dairy and other few individual (small laboratories). There is a high demand for Mozzarella cheese by reputable senior class hotels and pizza center, because of public awareness of the positive correlation between serum cholesterol concentration and the risk of developing coronary heart disease. This point motivated search for processing Mozzarella cheese from different fat level to lower the effect of fat on cholesterol to meet the consumer demand and at the same time to choose the safest level of fat that minimize the risk of heart disease.

The objective of this work is to study the cholesterol level of Mozzarella cheese during storage period as affected by level of fat.

MATERIALS AND METHODS

Fresh cow's milk was obtained from a private farm in Shambat, Khartoum North. Salt (sodium chloride) was obtained from local market Khartoum North and Rennet powder was obtained from Chr-Hansen's laboratory, Denmark.

Preparation and manufacture of mozzarella cheese: Four Mozzarella cheese samples were prepared from cow milk which were standardized at four different fat levels (0, 3, 5 and 7% respectively) heated to 45°C and cool to 37°C. After standardization, the rennet powder was introduced at rate 0.05% (for each 5 liter of milk) and left for 30-40 min for coagulation. After coagulation, the curd was cut into about 3 centimeter cubes with stainless steel knives and healed for 10 min before

Corresponding Author: Amal Mohammed Ali, Department of Food Sciences and Technology, School of Health Sciences, Ahfad Centre of Sciences and Technology, Ahfad University for Women, Sudan

stirring and the whey is drained. The curds were formed into blocks and left in open areas to drain off the remaining whey. The drained curd exposed to warm temperature until required acidity reached at critical pH (5.2-5.3). The curd was then put in hot water at 75-80°C and mixed properly for 5 min until a smooth elastic mass was obtained, stretched into proper forms using stainless steel container for 3 h, slightly salted in 5% cold brine solution (sodium chloride) for 2 h. The cheese removed from the brine, dried lightly, weighted and package in polyethylene. The resultant cheese was analyzed for physicochemical, biochemical analysis and organoleptic properties, when fresh and during storage periods of 0, 10, 20 and 30 days interval at 4°C.

Chemical analysis of cheese: Cheese samples were analyzed for total solids, fat (Gerber) and protein (semi-microKjeldahl) according to AOAC (1990) method. The pH value was measured by using pH meter (Model L. Pusl Müchen 15-1260/7, Germany) as described by Newlander and Atherton (1964). Total volatile fatty acids were determined by the direct distillation methods of Koiskowski (1982).

Cholesterol determination: Cholesterol content was determined by using Gas Chromatography (GC) according to Fletouris *et al.* (1998).

Preparation of saponification solution: A 0.2M methanolic KOH solution was prepared by dissolving 14 g of KOH in methanol on a magnetic stirrer plate and diluting to 500 ml volume with the solvent.

Preparation of cholesterol standards: For the preparation of cholesterol standards, the stock solution (2 mg/ml) was prepared by dissolving 20 mg of the reference standard (Philip Harris Ltd. England) in hexane in a 10 ml volumetric flask. The working solutions were prepared by appropriate dilution of aliquots from the stock solution with hexane to obtain solutions in the range of 10-80 µg/ml.

Gas chromatography conditions: A fused silica capillary column (15 m x 0.32 mm id) coated with SPB-1 (Supelco Inc, Bellefonte, PA) with 1.0- µm film thickness, was used in this study. Oven temperature was set at 285°C, injection port temperature at 300°C. The flow rates were 2 ml/min for helium, 30 ml/min for hydrogen and 300 ml/min for air. The injection volume was 1 µl with a split ratio of 20:1.

Sample preparation: 0.2 gram well ground cheese was accurately weighed into sample preparation tube. 5 ml of methanolic KOH solution), 0-5M KOH) were added. The tube was capped tightly and its contents were vortexed for 15s. The lower half of the tube was then immersed in an 80°C water bath and kept there for 15 min, removing the tube every 5 min to vortex for 10 s. Following heating the tube was cooled with tap water. 1 ml of water and 5 ml of hexane were added and the contents were vortexes vigorous/g for 1 min and then centrifuge for 1 min at 2000. An aliquot of the upper phase was transferred into clean dried container for the analysis using GC method. The concentration of Cholesterol (C) in the analysis samples was calculated according to the equation:

$$C = M \times V \times 2.5$$

Where:

M = Computed mass (nonograms) of the analytic in the injected extracted (IML).
V = Dilution factor if any that was applied.

Organoleptic properties of cheese: The sensory evaluation of Mozzarella cheese was evaluated by scoring procedures described by Ihekoronye and Ngoddy (1985).

Statistical analysis: Statistical analysis were done using the statistical analysis system SAS (1997).

RESULTS AND DISCUSSION

Table 1 shows changes in Total Solids (TS) of Mozzarella cheese during storage period as affected by levels of fat. The TS of cheese increased significantly ($p \leq 0.05$) with progress in storage period. Samples 7% fat milk recorded the highest TS (58.12%), compared to values 5% (57.75%), 3% (51.44%) and 0% fat milk (48.78%). The increase in TS of Mozzarella cheese during storage could be attributed to the decrease in moisture content of/or difference in milk fat (Jelen, 1990; El-Sheikh, 1997; Siber, 1998; Abdel-Razig *et al.*, 2002).

Table 2 shows changes in fat content of Mozzarella cheese during storage as affected by level of fat. The fat content of cheese increased significantly ($p \leq 0.05$) with storage time. Samples 7% fat milk had the highest fat content (48.00%) significantly ($p \leq 0.05$) higher than respective values of 5% (36.00%), 3% (21.00) and 0% fat milk (6.00%). The fat content increased in all cheese samples due to the loss of moisture content during storage. The same trend of these results was reported by Kumar and Jha, (1997) and Abd El-Hamid *et al.*, (2001).

Table 3 shows changes in protein content of Mozzarella cheese during storage as affected by levels of fat. The protein content of cheese increased significantly ($p \leq 0.05$) during storage. The protein content of 0% fat milk at the end of the storage (22.91%) was higher than 3% (21.51%), 5% (21.08%) and 7% fat milk (20.86%). El-Koussy *et al.* (1995) found the protein content of Mozzarella cheese increased during storage. Kim *et al.* (1992) mentioned that, the protein content of cheddar cheese showed a tendency to increase during storage due to the rapid decrease of moisture.

Table 1: Change in total solid content* of mozzarella cheese during storage as affected by the level of milk fat

Storage period (days)	Level of fat (%)			
	0	3	5	7
0	42.98^{i}	44.95^{h}	51.00^{e}	53.50^{dc}
10	45.47^{gh}	45.92^{gh}	52.15^{d}	54.23^{c}
20	46.53^{g}	48.15^{f}	53.44^{dc}	57.64^{b}
30	48.78^{f}	51.44^{de}	57.75^{ab}	58.12^{a}

*Mean value within the rows and columns having different superscript letters are significantly different (p<0.05)

Table 2: Change in fat content* of mozzarella cheese during storage as affected by the level of milk fat

Storage period (days)	Level of fat (%)			
	0	3	5	7
0	0.40^{m}	17.00^{h}	31.00^{e}	45.00^{b}
10	0.50^{u}	17.50^{hg}	35.00^{d}	45.50^{ab}
20	5.00^{i}	18.00^{g}	35.50^{cd}	46.00^{ab}
30	6.00^{i}	21.00^{f}	36.00^{c}	48.00^{a}

*Mean value within the rows and columns having different superscript letters are significantly different (p<0.05)

Table 3: Change in protein content* of mozzarella cheese during storage as affected by the level of milk fat

Storage period (days)	Level of fat (%)			
	0	3	5	7
0	21.51^{b}	20.43^{g}	20.32^{h}	19.20^{i}
10	22.10^{ab}	20.99^{d}	20.50^{fg}	20.35^{h}
20	22.33^{ab}	21.08^{c}	20.70^{e}	20.58^{f}
30	22.91^{a}	21.51^{b}	21.08^{c}	20.86^{de}

*Mean value within the rows and columns having different superscript letters are significantly different (p<0.05)

Table 4: Change in pH value* of mozzarella cheese during storage as affected by the level of milk fat

Storage period (days)	Level of fat (%)			
	0	3	5	7
0	5.72^{a}	5.50^{b}	5.35^{c}	5.21^{e}
10	5.46^{b}	5.25^{ed}	5.30^{d}	5.18^{f}
20	5.33^{cd}	5.20^{e}	4.95^{gh}	4.85^{h}
30	5.12^{fg}	5.01^{j}	4.73^{i}	4.60^{i}

*Mean value within the rows and columns having different superscript letters are significantly different (p<0.05)

Table 4 shows changes in the pH value of Mozzarella cheese during storage period as affected by level of fat. The pH of cheese decreased significantly ($p \leq 0.05$) within storage time. Sample 7% fat milk had the lowest pH (4.60) significantly ($p \leq 0.05$) lower than respective values of 0% (5.12), 3% (5.01) and 5% fat milk (4.73). The decrease in pH value of samples may be due to an increase in titratable acidity (Koiskowski, 1982; Pintario *et al*., 2000; Ghosh and Singh, 1992; El-Koussy *et al*., 1995; Kebary *et al*., 1998; Mahran *et al*., 2000 and El-Safty *et al*., 2004).

Table 5: Change in total volatile fatty acids* of mozzarella cheese during storage as affected by the level of milk fat

Storage period (days)	Level of fat (%)			
	0	3	5	7
0	3.00^{k}	8.50^{h}	10.81^{e}	11.70^{cd}
10	3.50^{j}	10.04^{f}	11.16^{ed}	12.03^{c}
20	3.99^{j}	10.29^{ef}	11.29^{d}	13.65^{ab}
30	4.03^{j}	10.66^{ef}	12.80^{b}	14.70^{a}

*Mean value within the rows and columns having different superscript letters are significantly different (p<0.05)

Table 6: Change in cholesterol content* of mozzarella cheese during storage as affected by the level of milk fat

Storage period (days)	Level of fat (%)			
	0	3	5	7
0	12.60^{l}	46.25^{h}	53.67^{f}	73.80^{d}
10	15.37^{m}	49.01^{g}	60.67^{e}	82.06^{c}
20	17.00^{j}	53.80^{f}	75.30^{d}	95.51^{b}
30	17.10^{l}	61.72^{e}	87.51^{c}	106.00^{a}

*Mean value within the rows and columns having different superscript letters are significantly different (p<0.05)

Table 5 shows changes in Total Volatile Fatty Acids (TVFA) of Mozzarella cheese during storage as affected by level of milk fat. The TVFA of Mozzarella cheese increased significantly ($p \leq 0.05$) with storage time. Sample 7% fat milk produced the highest (14.70 mls 0.1N NaOH) which was significantly higher than respective values of 5% (12.80 mls 0.1N NaOH), 3% (10.66 mls 0.1N NaOH) and 0% fat milk (4.03 mls 0.1N NaOH). Badawi *et al*. (2006) mentioned that, the total volatile fatty acid of Mozzarella cheese increased during storage. The volatile free fatty acids of chain length C_2-C_8 were reported to contribute to the cheese flavour (Harper and Gould, 1952; Abdel-Razig, 1996; Mahran *et al*., 2000). The improvement of cheese flavour, was mainly attributed to the production of acid by lactic acid bacteria (Koiskowski, 1982; Elowni and Hamid, 2008 and Law, 1992). Abdel-Razig *et al*., 2002), reported that, the TVFA of Sudanese semi-hard cheese increased during storage.

Table 6 shows changes in cholesterol content of Mozzarella cheese during storage. The cholesterol level of Mozzarella cheese increased significantly ($p \leq 0.05$) with storage time. The cholesterol content of Sample 7% fat milk at the end of the storage (106.00 mg/100 g) was significantly ($p \leq 0.05$) higher than respective values of 5% (87.51 mg/100 g), 3% (61.72 mg/100 g) and 0% fat milk 17.10 mg/100 g). It's apparent that cholesterol content increase with increasing fat content (Souci *et al*., 1994 and AndriKopoulos *et al*., 2003). The differences observed for the fat and cholesterol content between the examined samples could not be attributed only to the physiological fluctuations of cholesterol content of milk but could be related to processes and treatment applied during production of different levels of fat.

Table 7: Changes in the acceptability of Mozzarella cheese during storage as affected by the level of fat

Storage period (days)	Appearance				Texture				Flavour				Overall acceptability			
	Level of fat (%)															
	0	3	5	7	0	3	5	7	0	3	5	7	0	3	5	7
0	1.0^{h}	3.5^{f}	4.5^{b}	4.0^{de}	1.0^{i}	3.3^{g}	4.4^{c}	4.0^{e}	1.2^{ij}	3.6^{g}	4.3^{d}	4.2^{de}	1.0^{ij}	3.1^{g}	4.1^{cd}	4.0^{d}
10	1.1^{h}	3.6^{f}	4.6^{b}	4.1^{d}	1.2^{i}	3.4^{g}	4.6^{b}	4.1^{de}	1.3^{i}	3.7^{g}	4.5^{c}	4.1^{e}	1.1^{i}	3.5^{f}	4.3^{b}	4.2^{c}
20	1.4^{g}	4.1^{d}	4.9^{a}	4.5^{bc}	1.4^{h}	3.6^{f}	4.9^{a}	4.4^{c}	1.5^{h}	4.2^{de}	4.9^{a}	4.7^{b}	1.3^{h}	3.9^{e}	4.6^{a}	4.5^{ab}
30	1.3^{g}	3.9^{e}	4.8^{a}	4.4^{c}	1.3^{h}	3.5^{fg}	4.7^{b}	4.2^{d}	1.4^{hi}	3.9^{f}	4.7^{b}	4.5^{c}	1.2^{ih}	3.6^{f}	4.5^{ab}	4.5^{ab}

*Mean value within the rows and columns having different superscript letters are significantly different ($p<0.05$)

It's known that cholesterol in milk was found-together with phospholipids-in the hydrophilic surface layer of fat globules (Patton and Keenan, 1975). As consequence, the cholesterol/fat ratio varies tremendously according to globule size, being highest in the smallest globules (with the highest free surface values), which are the main components of the low amounts of fat in skim milk. According to all the available data (Souci *et al*., 1994 and Park, 1999), the cholesterol to fat ratio in butter milk ranging between 6-20 mg/100 g. Kayser and Tachkova (1957) found that, the casein separated from milk and precipitated acid casein contained cholesterol. Jennes and Patton (1959), Huang and Kuksis (1967), Kitchen (1974), Antila and Antila (1976) and Bachman and Wilcox (1976), are amongst others who reported on the occurrence of cholesterol in skim milk. A high level Low Density Lipoprotein (LDL) cholesterol (160 mg/dl and above reflects an increased risk of heart disease. If you have heart disease, your LDL cholesterol should be less than 100 mg/dl (Stryer, 1995). Cholesterol is found almost in food of animal origin, especially in meat and dairy product.

The cholesterol content of Mozzarella cheese was found to be lower than those of (Fletouris *et al*., 1998; Piironen *et al*., 2002; Andrikopoulos *et al*., 2003 and Kinik *et al*., 2005).

Table 7 shows changes in appearance of Mozzarella cheese during storage as affected by level of fat. The score of appearance improved with storage time. Samples 5% fat milk gave the best score (4.9), which is significantly better than 7% (4.6) 3% (4.1) and 0% fat milk (1.4). Abd El-Rafee *et al*. (2004) concluded that, the appearance score of Mozzarella cheese improved with homogenization and during storage. Sameen *et al*. (2008) observed that, the appearance of low-fat Mozzarella cheese improved in high fat cheese.

Table 7 shows changes in texture of Mozzaerlla cheese during storage as affected by level of fat. The score of texture improved with storage time, then decreased to the end of storage period. Sample 5% fat milk gave the best score (4.9), which was significantly better ($p\leq0.05$) than 7% (4.4), 3% (3.6) and 0% fat milk cheese (1.4). Abd El-Hamid *et al*. (2001) and Sameen *et al*. (2008) found that, the texture of Mozzarella cheese improved during storage period.

Table 7 shows changes in flavour of Mozzarella cheese during storage as affected by level of fat. The score of flavour of Mozzarella cheese improved with storage time. 5% fat milk cheese gave best score (4.9), which was significantly better ($p\leq0.05$) than 7% (4.7), 3% (4.2) and 0% fat milk cheese (1.5). Sameen *et al*. (2008) stated that, flavour of Mozzarella cheese improved during storage.

Table 7 shows changes in overall acceptability of Mozzarella cheese during storage as affected by level of fat. The score of acceptability of Mozzarella cheese improved with storage period. 5% fat milk gave the best score (4.6), which is significantly better ($p\leq0.05$) than 7% (4.5), 3% (3.6) and 0% fat milk cheese at day 20. Abd El-Rafee *et al*. (2004) observed that, the flavor and appearance score of Mozzarella cheese improved during storage, while body and texture score decreased. Sameen *et al*. (2008) concluded that, the sensory attribute of Mozzarella cheese (appearance, texture and overall acceptability) were improved during storage period.

Conclusion: Total solids, fat, protein and TVFA increased during storage, while the pH value decreased. Cholesterol level of cheese increased significantly ($p\leq0.05$) with levels of milk fat and storage. Overall acceptability of cheese containing 5%fat milk was the best than the other samples followed by 7, 3 and 0% fat milk. 20 days storage is quite satisfactory for Mozzarella cheese to obtain good quality.

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