MICROBIOLOGICAL EVALUATION OF MOZZARELLA CHEESE

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ABSTRACT

Mozzarella cheese is a good source of nutrients namely protein, fat, minerals and vitamins. The study was performed to evaluate the microbiological quality of Mozzarella cheese and it's correlation with Egyptian and International standards. A total of 50 mozzarella cheese samples were collected from supermarkets in Assiut City, Egypt. All samples were investigated to determine the total aerobic plate, yeasts, molds and coliforms counts also, for detection of *Staph. aureus*, *E. coli* and *Salmonella* spp. In the examined samples the incidence of yeasts, molds and coliforms were 94, 32 and 96 %, respectively while, *Staph. aureus*, *E. coli* and *Salmonella* were detected in 24, 20 and 12% of the examined samples. The microbiological results in this study were compared with the limits of Egyptian Organization Standards and International Standards.

***Key words**: Mozzarella cheese, Microorganism, Egyptian Organization Standards.*

INTRODUCTION

Mozzarella cheese is asemi hard, white, un-ripened has very lively surface sheen and has unique property of stretch-ability. It owes its characteristics mainly to the action of lactic acid on dicalcium-para-caseinate that may be consumed shortly after manufacture. Its melting and stretching characteristics are highly appreciated in the manufacture of pizza, where it serves as a key ingredient (Jana and Mandal, 2011).

Mozzarella has been the ubiquitous increase in the popularity of pizza, in which mozzarella is the main cheese used. The functional attributes of importance for pizza include the desired degrees of flow and stringiness on baking. The cheeses best endowed with these characteristics, especially stretch-ability, are members of the pasta-filata group (Fox *et al.*, 2000).

Milk used for cheese manufacture is required to be pasteurized at 72°C for 15 s or its equivalent, according to US Food and Drug Administration (FDA) regulations (FDA, 2011), which destroys most common pathogenic or spoilage bacteria. However, heat-resistant pathogenic and spoilage bacteria may be present in raw milk or equipment surfaces, and even non-heat resistant bacteria may gain access during cheese manufacture and storage (Kikuchi *et al.*, 1996). The presence of these bacteria is detrimental to cheese shelf life and quality (El-Gazzar and Marth, 1992; Schlesser *et al.*, 2006). Although Mozzarella cheese might be contaminated with pathogens, such as *Salmonella serovars* (De Felip and Toti 1984), *Staphylococcus aureus* (Bowen and Henning, 1994) and *Listeria monocytogenes* (Buazzi *et al.*, 1992). Similarly, the Stx2 shiga toxin produced by enterohemorrhagic Escherichia coli O157:H7 is resistant to milk pasteurization and other equivalent heat treatments and is destroyed only by 100°C for 5 min (Rasooly and Do, 2010).

Microbial contamination, causing approximately one-fourth of the world's food supply loss, has become an enormous economic and ethical problem worldwide (Huis in't Veld, 1998). Dairy products are an excellent growth medium for a wide range of microorganisms and, thus, display a reduced shelf life (Ruegg, 2003). The microbiological quality of dairy products is influenced by the initial flora of raw milk, the processing conditions, and post-heat treatments. Spoilage bacteria and various bacteria of public health concern can be found in these products and their concentrations should be kept as low as possible (Varga, 2007).

Therefore, the aim of this work was to evaluate the quality of mozzarella cheese and compare the results with Egyptian Organization Standards and International Standards.

MATERIALS and METHODS

A total of 50 mozzarella cheese samples were taken for analyses aseptically after few hours of collection from supermarkets in Assiut City, Egypt. The samples were collected in clean plastic bags as

marketed to the consumers and transported, as soon as, possible to be examined for:-

1- Total viable count according to A.P.H.A. (1992).

2- Yeasts and molds counts according to Harrigan and MeCance (1976).

3- Total coliform counts (MPN) according to FAO (1992).

4- Isolation of *Staph. aureus* according to A.O.A.C. (2000).

5- Isolation of *E.coli*: Samples were prepared to isolate the E. coli according to FAO (1992).

6- Isolation of Salmonella according to Wallace *et al.* (2009).

A- Serodiagnosis of *E.coli*:
This part has been done in the Food Hygiene Lab in the Faculty of Veterinary Medicine of Moshtohor, Banha Univ., Egypt.

The isolates were serologically identified according to Kok *et al.* (1996) by using rapid diagnostic *E. coli* antisera sets for diagnosis of the Enteropathogenic types. The technique applied as recommended by the manufacture (DENKA SEIKEN Co., Japan).

B. Serological identification of Salmonellae*:*
Serological identification of Salmonellae was carried out according to Kauffman – White scheme *(Kauffman,* 1974) for the determination of Somatic (O) and flagellar (H) antigens using Salmonella antiserum (DENKA SEIKEN Co., Japan).

C. Identification of total Staphylococci species:
Morphological examination (Cruickshank *et al.,* 1975).

Biochemical identification:
Catalase activity test (MacFaddin, 1976).
Detection of haemolysis (Collins and Lyne, 1984).
Mannitol fermentation test (Bailey and Scott, 1978).
Coagulase test (Baron *et al.*, 1994).

Thermostable nuclease test "D-Nase activity" (Lachia *et al.,* 1971).

D-Nase agar plates were inoculated with loopfuls of suspected colonies by spotting them on small areas of the plates which incubated at 37°C for 18 hours. Moreover, the incubated plates were flooded with normal hydrochloric acid which precipitated DNA resulting in cloudiness of the plates. Accordingly, Appearance of a clear zone around the colony indicated the production of D Nase and recorded as positive result.

RESULTS

Table 1: Statistical analytical results of microbiological examination of Mozzarella cheese samples.

Microbial Examination	Results of mozzarella counts (cfu/g)				
	Positive Samples		Min.	Max.	Average
	No./50	%			
SPC	50	100	$4x10^3$	$1.8X10^7$	$3.84X10^6$
Coliform count	48	96	<3	$1 x10^2$	8.5x10
Yeasts	47	94	0	$9x10^6$	$3.2x10^6$
Molds	16	32	0	$6x10^5$	$6.8x10^4$

Table 2: Incidence of *Staph.aureus, E.coli and Salmonellae* recovered from Mozzarella cheese samples.

Types of M.Os	**Positive samples**	
	No./50	**%**
Staph. aureus	12	24
E.coli	10	20
Salmonellae	6	12

Table 3: Serological identification of *E.coli* strains isolated from Mozzarella.

Serodiagnosis	Positive samples		Strain characterization
	No./50	%	
O157 : H7	**1**	**2**	**EHEC***
O111 : H4	**4**	**8**	**EHEC**
O55 : H7	**2**	**4**	**EPEC****
O26	**2**	**4**	**EHEC**
O128 : H2	**1**	**2**	**ETEC*****

● EHEC: - Enterohaemorrhagic *E. coli*, ** EPEC: - Enteropathogenic *E.coli*, *** ETEC: - Enterotoxigenic *E. coli*.

Table 4: Serological identification of *Salmonellae* strains isolated from Mozzarella.

Identified strains	Group	Antigenic structure		Positive strains	
		O	H	No./50	%
Salmonella Typhimurium	**B**	1,4,5,12	i : 1,2	3	6
Salmonella Virchow	**C2**	1,9,12	g,m : -	1	2
Salmonella Haifa	**B**	1,4,5,12	Z10: 1,2	1	2
Salmonella Enteritidis	**D1**	1,9,12	g,m : 1,7	1	2

Table 5: Identification of *S.aureus* strains isolated from Mozzarella.

Identified bacterium	Further Identification	Positive strains	
		No./50	%
Staph. aureus	**Coagulase +ve / DNase -ve**	**5**	**10**
Staph. aureus	**Coagulase +ve / DNase +ve**	**4**	**8**
Staph. aureus	**Coagulase -ve / DNase -ve**	**3**	**6**

Table 6: Summarized results of microbiological examination of Mozzarella cheese samples compared with the International Standards (IDF, 1984).

Organisms	Requirements	Mozzarella cheese samples examined			
		Acceptable		Unacceptable	
		No./50	%	No./50	%
Total plate count/g	Max. 50.000	1	2	49	98
Coliform count	Absent in 0.1 g	2	4	48	96
E.coli	Absent in 1 g	40	80	10	20
Salmonella	Absent in 25 g	44	88	6	12
Staph.aureus	Absent in 1 g	38	76	12	24
Yeast count	Absent in 1 g	3	6	47	94
Mold count	Absent in 1 g	34	68	16	32

Table 7: Summarized results of microbiological examination of Mozzarella cheese samples compared with the Egyptian Standards (E.O.S.Q.C., 2005).

Organisms	Requirements	Mozzarella cheese samples examined			
		Acceptable		Unacceptable	
		No./50	%	No./50	%
Coliform count	10 cells/g	5	10	45	90
E.coli	Absent	40	80	10	20
Salmonella	Absent	44	88	6	12
Staph.aureus	Absent	38	76	12	24
Yeast count	400 cells/g	3	6	47	94
Mold count	10 cells/g	34	68	16	32

DISCUSSION

The total bacterial count gives a quantitative idea of the presence of mesophilic aerobic microorganisms of animal origin. It serves as an important criterion to evaluate the microbial quality of various foods and also the degree of freshness of food (Nanu *et al.*, 2007). Data presented in Table 1 illustrated that total bacterial count in examined mozzarella cheese samples ranged from $4x10^3$ to$1.8X10^7$ with an average count of $3.84X10^6$ bacteria /g, Francesca *et al.* (2014) reported nearly similar results which indicated that the TBC in mozzarella cheese was in the range of 10^3 to 10^5 organisms/g, similar results were also obtained by Asperger (1991) who reported that the total bacterial count in mozzarella cheese stored at 4 °C increased and was $>10^7$ cfu/g after 1 week of storage. Also, Tanweer (2011) detected the initial TBC in mozzarella cheese samples 1 $x10^6$ cfu/g and increased to 8 $x10^6$ cfu/g after 5 weeks of storage and reported that presence of atmospheric air and O_2 affect the overall quality of mozzarella cheese during storage, Also the highly nutritious nature of dairy products makes them especially good media for the growth of microorganisms. Milk contains abundant water and nutrients and has a nearly neutral pH. The major sugar, lactose, is not utilized by many types of bacteria, and the proteins and lipids must be broken down by enzymes to allow sustained microbial growth (Loralyn and Robert, 2009).

Also, in Table 1 coliforms were present in 96% of samples with an average count of 8.5×10 cfu/g. Higher results were obtained by Tanweer (2011) who found that the initial coliform count was $6x10^2$ cfu/g in Mozzarella cheese increased to 1000 cfu/g after 5 weeks of storage. The presence of coliforms or yeasts is indicative of low processing temperature, especially at filling or negligent sanitation. The major microbiological problem with these products is growth of yeasts and molds, especially if free moisture is available at the surface (Marth and Steele, 2001). Moreover some cheese defects may be caused by poor milk quality (late lactation milk, milk from mastitic animals, high in enzymes of animal origin, i.e. lipase and protease), inappropriate rate of acid development by the starter, or poor manufacturing and storage regimens (Fawaz *et al.*, 2011).

In cheese production, slow lactic acid production by starter cultures favors the growth and production of gas by coliform bacteria, with coliforms having short generation times under such conditions. In soft, mold-ripened cheeses, the pH increases during ripening, which increases the growth potential of coliform bacteria (Frank, 2001).

Yeasts were presented in 94% of samples with an average count of $3.2x10^6$ cells/g. while, molds were present in smaller amount only in 32 % of samples with an average count of $6.8x10^4$ cells/g (Table 1). Our results were slightly similar to (Tanweer, 2011) who reported that the initial count of yeast and mold counts of mozzarella cheese increased from $5x10^2$ to $2x10^5$ and $5x10^5$ cfu/g, respectively after 3 weeks of storage at 7±1 °C.

Molds can grow well on the surfaces of cheeses when oxygen is present, with the low pH being selective for them. In packaged cheeses, mold growth is limited by oxygen availability, but some molds can grow under low oxygen tension. Molds commonly found growing in vacuum-packaged cheeses include *Penicillium* spp. and *Cladosporium* spp. (Hocking and Faedo, 1992). *Penicillium* is the mold genus most frequently occurring on cheeses.

The results listed in Table 2 indicated that *Staph.aureus* was existed in 24%, of all examined samples. *Staph. aureus* is a ubiquitous bacterium, both human and animal commensal (Jay, 2000). Consequently, many foods can be contaminated by this species thus representing hazard for human health.

It is interesting to observe that the heating phase of pasteurization (until milk reached 65ºC) produced an

important lethal effect on *Listeria* sp., *Staphylococcus* sp. and especially *Salmonella* sp. but not on *Mycobacterium spp.* (Raimundo *et al.,* 2013), While contamination occurs mainly post pasteurization contamination or because of insufficient pasteurization.

Despite of the extensive public health measures over the past century, *Salmonella* remains the second most commonly identified cause of bacterial foodborne disease in the developed countries and a signficant cause of morbidity and mortality in the developing world (WHO, 2002 and Amin, 2004). 12% of examined samples were contaminated with *Salmonella* (Table 2) Although, there are relatively low numbers of positive samples in this study, the pathogen represent a potential risk to consumers on the basis that all salmonellae are potentially pathogenic (Zansky *et al.,* 2002).

Also, Jay (2000) said that the greater efficiency of stretching could be explained by the fact that microorganisms were already injured by curd acidity but this explanation does not elucidate the behavior of *Salmonella* sp. and *Staphylococcus* sp. under the same conditions. It is possible that these microorganisms are less sensitive to acid injury than the others. *Staph. aureus* showing more resistance to stretching than the other microorganisms analyzed.

Escherichia coli are commensal organisms that reside within the host gut, but some pathogenic strains are recognized as a cause of gastroenteritis (Callaway *et al.,* 2003). Table 2 represents the occurrence of *E. coli* in 20% of samples. Contamination from human and animal waste is traditionally indicated by the presence of commensal *E. coli*. Although these organisms are essentially nonpathogenic, their presence warns of the possible concurrent existence of pathogenic microbes (Sherfi *et al.,* 2006).

Table 3, verified serological phenotypic identification of different *E.coli* isolated from all examined samples. The result represented that O157:H7, O111:H4 and O26 were identified as EHEC. The ETEC strain recognized in serogroups O128:H2 while, EPEC represented in O55:H7.

Foodborne outbreaks of *Escherichia coli* O157:H7 infection have been associated with a wide range of food products, including raw and pasteurized milk and milk products, such as cheese (Honish *et al.,* 2005 and Strachan *et al.,* 2005). In the late 1990s, both the U.S. Food and Drug Administration (FDA) and Health Canada proposed bans on the use of raw milk in cheese making.

E. coli O157:H7 can readily contaminate raw milk on the farm because dairy cattle are a known reservoir of Shiga toxin– producing *E. coli*, including enterohemorrhagic strains such as serotype O157:H7 (Wells *et al.,* 1991). Similarly, the Stx2 shiga toxin produced by enterohemorrhagic *E.coli* O157:H7 is resistant to milk pasteurization and other equivalent heat treatments and is destroyed only by 100°C for 5 min (Rasooly and Do, 2010). Although the presence of Stx2 in foods is not known to cause illness upon direct consumption, Stx2 directly fed to mice caused mortality (Rasooly *et al.,* 2010), and may hence be a human health risk.

Salmonellosis is a foodborne infection of major economic importance. According to information gathered from 84 countries responding to a global survey conducted by the World Health Organization (WHO), *S. enteritidis* and *S. typhimurium* accounted for 70% of all human and nonhuman isolates of *salmonella* reported worldwide between 1995 and 2008 (CDC, 2009). Corresponding to Table 4, it is persisted that the different identified strains of *salmonella* via sero-typing technique were 6% *S. typhimurium* and 2% *S. virchow, S.Haifa* and *S.enteritidis*, respectively.

Some strains of *staph.aureus* are capable of producing many kinds of enterotoxins (SEs) which are currently being studied deeply and get much attention by the scientific community (Balaban and Rasooly 2000). Enterotoxigenic strains of *Staph. aureus* have been reported to cause a number of diseases or food poisoning outbreaks because of the ingestion of contaminated dairy products or milk (Asao *et al.,* 2003). Also, Coagulase Negative Staph (CNS) is emerging as important minor mastitis pathogens in Egyptian dairy animals. Such species can cause substantial economic losses resulting in decreased milk production. This reflects the environmental hazard and therefore, udder health must be followed up and control of intra-mammary infections is consequently of the greatest importance for dairy farms (Jakeen *et al.,* 2013). So, further identification was done to *Staph. aureus* strains, 10, 8 and 6% of samples were Coagulase +ve / DNase –ve, Coagulase +ve / DNase +ve and Coagulase -ve / DNase –ve, respectively (Table 5).

Comparing the obtained results of examined mozzarella cheese samples with the International Standards (IDF, 1984) (Table 6), 98, 96, 94, 32, 20, 12 and 24% of samples failed to comply with the limits of the standards which indicated the poorer sanitary practices during cheese production according to TBC, coliforms, yeast, mold count, *E.coli*, *Salmonella* and *Staph.aureus* isolates, respectively.

According to the limits proposed by the E.O.S.Q.C. (2005), only 10, 6, 68, 80, 88 and 76% of samples were acceptable according to yeast, mold, coliforms count, *E.coli. Salmonella* and *Staph.aureus* isolates, respectively (Table 7). This result indicated the

negligible sanitary control measures during production and handling of the products.

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التقييم الميكروبيولوجى لجبن الموتزاريلا

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جبن الموتزاريلا ايطالى المنشأ وقد دخل حديثا على مصر من قبل صناعة منتجات الالبان المختلفة. وتعد مصدرا جيدا للبروتينات والدهون والمعادن والفيتامينات لذا تم إجراء دراسة لتقييم الجودة الميكروبيولوجية للجبن الموتزاريلا ومقارنتها بالمعايير المصرية والدولية. تم جمع ٥٠ عينة من الجبن الموتزاريلا عشوائيا من مختلف المحلات فى مدينة اسيوط. وقد اظهرت النتائج ان متوسط العد الكلي لكل من البكتريا الهوائية ، البكترية السبحية القولونية ، والخمائر والفطريات كما يلي $3.84X10^6$ ، $8.5x10^6$ ، $3.2x10^6$ ، $6.8x10^4$ وعلي التوالي اما عن الكشف عن المكورات العنقودية الذهبية ، الاى كولاي والسالمونيلا فقد تواجدت في ٢٤ و ٢٠ و ١٢٪ من العينات التي تم فحصها على التوالى. وتمت مقارنة النتائج الميكروبيولوجية في هذه الدراسة مع حدود معايير المنظمة المصرية والمعايير الدولية.