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## MICROBIOLOGICAL QUALITY OF MOZZARELLA CHEESE (With 6 Tables)

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### التقييم الميكروبيولوجي للجبن الموزاريلا

إيناس البرنس ، ماضى إسماعيل

تعتبر الجبن الموزاريلا نوعاً جديداً فى الأسواق المصرية وتتميز عن مثيلاتها من أنواع الجبن الأخرى بخاصيتها المطاطية المرغوبة لاضفاء الطعم والشكل المطلوب فى عمل البيتزا ، لذلك أجريت هذه الدراسة لمعرفة مدى تلوثها بالعديد من الميكروبات ، ولهذا الغرض تم جمع ٤٠ عينة عشوائية من السوبر ماركت بمدينة أسيوط . وقد أسفرت النتائج على أن متوسط العدد الكلى لكل من البكتريا الهوائية ، والمحبة للبرودة، والقولونية ، والباسيلس سيربوس والمكورات العنقودية الذهبية كان  $10 \times 9$  ،  $10 \times 9$  ،  $10 \times 2$  ،  $10 \times 2$  ،  $10 \times 1$  و  $10 \times 1$  لكل جرام من الجبن على التوالي . أما البكتريا السبحية القولونية فلم توجد فى العينات المفحوصة ، بينما عزلت البكتريا اللاهوائية بنسبة ٦٢,٥٪ والميكروبات المعوية العصوية بنسبة ٦٠٪ ولم يتمكن من عزل الليستريا مونوسيتو جينس . ومن الفحص الميكولوجى أوضحت النتائج أن ٢٠ عينة ( ٥٠٪ ) ، ٢٣ عينة ( ٥٧,٥٪ ) من العينات تحوى على mesophilic molds على كل من مستنبتى malt extract and dicloran agar and psychrotrophic molds على قدره  $10 \times 5$  ،  $10 \times 5$  ، بينما كان متوسط عدد psychrotrophic molds على dicloran agar  $10 \times 3$  /جم . وبتصنيف الفطريات المعزولة أمكن التوصل الى أن أكثر الفطريات وجوداً كانت Aspergillus, Penicillium, Cladosporium and Alternaria وذلك بالنسبة للفطريات التى تنمو فى درجة حرارة ٢٥م أما الفطريات المحبة للبرودة كانت ممثلة فى Cladosporium, Aspergillus, Penicillium and Penicillium . وقد كان مستنبت dicloran أفضل من مستنبت malt extract فى عد وعزل الفطريات الموجودة . هذا وقد تم مناقشة الاشتراطات الصحية التى يجب أن تتبع لمنع تلوث الجبن الموزاريلا بالميكروبات المختلفة لما لها من خطورة على الصحة العامة وفساد المنتج، وكذلك الاقتراحات المطلوب توافرها لتحسين جودة المنتج.

## SUMMARY

Fourty random samples of locally manufactured Mozzarella cheese were purchased from different supermarkets in Assiut Province and examined microbiologically to evaluate its quality. The obtained results revealed that the average counts of aerobic plate, psychrotrophes, coliforms, *B. cereus* and *Staph. aureus* were  $9 \times 10^6$ ,  $9 \times 10^4$ ,  $2 \times 10^2$ ,  $2 \times 10$  and  $1 \times 10^2$  cfu/g, respectively. While, enterococci failed detection. Also, the incidence percentages of anaerobes, Enterobacteriaceae and *Listeria monocytogenes* were 62.5, 60.0 and 0.0%, respectively. The mycological examination of Mozzarella cheese showed that the average counts of mesophiles on malt extract agar and dicloran agar were  $5 \times 10$  and  $5 \times 10^2$  cfu/g, respectively, as well as, the psychrotrophic count on dicloran agar ranged from  $1 \times 10$  to  $1 \times 10^3$  cfu/g in a percentage of 17.5%. Moreover, various species of mesophilic molds were recovered from 20 examined samples on malt extract agar. *Aspergillus* was the most prevalent genus (55.0%), *Penicillium* was ranked secondly (32.5%), while *Cladosporium* spp., *Emericella nidulans* and *Rhizopus stolonifer* were infrequently encountered (2.5 - 5.0%). Furthermore, 184 species belonging to 16 genera of mesophilic molds were isolated on dicloran agar. Percentage of 25.57% of isolates were for *Penicillium*, 24.99% for *Aspergillus*, 20.11% for *Cladosporium* and 11.42% for *Alternaria*, while the other isolates existed in rare frequency of occurrence (0.54 - 5.44%). In addition, *Cladosporium*, *Aspergillus* and *Penicillium* species were found to be common psychrotrophic contaminants of examined cheese samples. Suggestive measures for improving the microbiological quality of Mozzarella cheese are discussed.

**Keywords:** *Microbiological quality, Mozzarella cheese.*

## INTRODUCTION

Cheese is one of the most popular milk products all over the world. Recently, a new commodity of soft cheese has been introduced to the Egyptian supermarkets known as Mozzarella cheese. It is a soft, elastic perishable Italian cheese, having a brief commercial shelflife of 20-30 days according to the extent and degree of refrigerated storage.

Mozzarella cheese differs from other types in that, it is usually consumed in the melted state as in pizza (Kindstedt *et al.*, 1989).

Mozzarella cheese like any dairy product is subjected to the risk of microbial contamination by various microorganisms as psychrotrophes like *Pseudomonas*, *Achromobacter*, *Acinetobacter*, *Citrobacter*, *Enterobacter*, *Escherichia* and *Serratia* from its production until consumption (Rondinini and Garzaroli, 1990 and El-Shinawy *et al.*, 1994). Also, the product has been infrequently implicated as a vector in an outbreak of salmonellosis caused by *Sal. javiana* (Eckner *et al.*, 1990). While, Massa *et al.* (1992) found that *Klebsiella pneumoniae* was the most frequently isolated species from spoiled Mozzarella cheese. Moreover, yeast and mold could be isolated with varying percentages from cheeses which lead to musty off-flavors (El-Shinawy and Ragheb, 1995). The presence of these microorganisms affects the health of the consumers as they have been implicated as a causal agents of food borne infection or food poisoning. Furthermore, may be responsible for deterioration of Mozzarella cheese which dramatically affects its suitability as a pizza ingredient and rendering it unmarketable resulting in economic losses (Pilcher and Kindstedt, 1991 and Massa *et al.*, 1992). Additionally, manufacturing operation as well as the condition of raw ingredients may influence the keeping quality of the product.

According to the scanty information about the microbiological status of Mozzarella cheese produced in Egypt, the present investigation was undertaken to ascertain the level of microbial contamination.

## **MATERIAL and METHODS**

### **I - Collection of samples:**

Fourty random samples of locally manufactured Mozzarella cheese were purchased from different supermarkets in Assiut province. These samples were transferred directly to the laboratory without delay in their plastic bags to be examined microbiologically to evaluate their quality.

### **II - Preparation of samples:**

Eleven grams of each sample were diluted in 99 ml of 2% sodium citrate solution in sterile plastic bags, then blended in Stomacher (Lab. blender Model 400) for 2 minutes. Decimal dilutions were then prepared from every sample for bacteriological and mycological analysis according to A.P.H.A. (1985).

### **III- Bacteriological examination:**

*The prepared samples were subjected to the following examination:*

- 1 - Aerobic plate count (APC): using Standard plate agar (A.P.H.A., 1985).
- 2 - Psychrotrophic count: using Standard plate agar (A.P.H.A., 1985).
- 3 - Coliforms count: using violet red bile agar (A.P.H.A., 1985).
- 4 - Bacillus cereus count: using direct plating technique on Mannitol egg yolk polymyxin agar (MYP) (Lancette and Harmon, 1980).
- 5 - Staphylococcus aureus count: using Baird-Parker agar (Baird-Parker, 1962).
- 6 - Enterococci count: using Pfizer selective agar (PSA) (Geldreich, 1975).
- 7 - Anaerobes detection: by Stormy fermentation test (Schönberg, 1956).
- 8 - Isolation of Enterobacteriaceae: performed according to Speck (1985).
- 9 - Isolation of Listeria species: The warm enrichment procedures (McClain and Lee, 1988) was carried out. Subculture the broth onto McBride listeria agar (Biolife Code, 1602).

### **IV - Mycological examination:**

The dilution-plate method was used for detection of viable mold propagules in examined Mozzarella cheese samples. Two types of media were used. Malt extract agar (Harrigan and McCance, 1976) was used for enumeration and identification of mesophilic molds at 25°C and dicloran-rose bengal medium (King *et al.*, 1979) was used for enumeration and identification of mesophiles at 25°C as well as psychrotrophes at 7°C. Triplicate plates for each medium were incubated for 7 - 10 days. Then molds were counted and identified according to Raper and Fennell (1965), Ellis (1976), Booth (1977), Pitt (1979), Samson and van Reeneh-Hoekstra (1988) and Kozakiewicz (1989).

## **RESULTS**

The obtained results were recorded in Tables 1 - 6.

**Table 1:** Statistical analytical results of different microbial counts/g of examined Mozzarella cheese samples.

Types	Positive samples		**cfu / g		
	No. /40	%	Min.	Max.	Average
* APC	40	100.0%	$1 \times 10^4$	$5 \times 10^7$	$9 \times 10^6$
Psychrotrophic count	22	55.0%	$1 \times 10^2$	$7 \times 10^5$	$9 \times 10^4$
Coliforms count	13	32.5%	$1 \times 10$	$1 \times 10^3$	$2 \times 10^2$
Bacillus cereus count	13	32.5%	$1 \times 10$	$5 \times 10$	$2 \times 10$
Staphylococcus aureus count	12	30.0%	$2 \times 10$	$4 \times 10^2$	$1 \times 10^2$
Enterococci count	-	-	-	-	-

\* APC : Aerobic plate count.

\*\* cfu : Colony forming unit.

**Table 2:** Incidence of some microorganisms recovered from examined Mozzarella cheese samples.

Isolated microorganisms	Positive samples	
	No. / 40	%
- Anaerobes	25	62.5%
- Enterobacteriaceae		
Lactose fermenters	13	32.5%
Non-lactose fermenters	11	27.5%
- Listeria species	-	-

**Table 3:** Statistical analytical results of total molds recovered from examined Mozzarella cheese samples.

Molds	Media	Positive samples		cfu / g		
		No. / 40	%	Min.	Max.	Average
Mesophiles on malt extract agar		20	50.0%	$1 \times 10$	$3 \times 10^2$	$5 \times 10$
Mesophiles on dicloran agar		23	57.5%	$1 \times 10^2$	$3 \times 10^3$	$5 \times 10^2$
Psychrophiles on dicloran agar		7	17.5%	$1 \times 10$	$1 \times 10^3$	$3 \times 10^2$

**Table 4:** Frequency distribution of mesophilic molds recovered from examined Mozzarella cheese samples on malt extract agar at 25°C.

Mold species		Isolated molds	
		No. of cases	%
<i>Aspergillus flavus</i>	Link	6	15.0
<i>A. fumigatus</i>	Fres.	1	2.5
<i>A. niger</i>	van Tieghem	15	37.5
<i>Cladosporium cladosporioides</i>	(Fres.) de Vries	1	2.5
<i>C. sphaerospermum</i>	Penzig	2	5.0
<i>Emericella nidulans</i>	(Eidam) Vuillemin	1	2.5
<i>Penicillium aurantiogriseum</i>	Dierckx	3	7.5
<i>P. chrysogenum</i>	Thom	3	7.5
<i>P. citrinum</i>	Thom	2	5.0
<i>P. restrictum</i>		3	7.5
<i>P. viridicatum</i>	Westling	2	5.0
<i>Rhizopus stolonifer</i>	(Ehrenb.) Lind.	1	2.5
<b>Total</b>		<b>40</b>	<b>100.00%</b>

**Table 5:** Frequency distribution of mesophilic molds recovered from examined Mozzarella cheese samples on dicloran agar at 25°C.

Mold species		Isolated molds	
		No. of cases	%
<i>Acremonium strictum</i>	W. Gams	4	2.17
<i>Alternaria alternata</i>	(Fres.) Keissler	7	3.80
<i>A. chlamydospora</i>	Mouchacca	9	4.89
<i>A. egyptiacus</i>		2	1.09
<i>A. tenuissima</i>	(Kunze : Pers) Wiltshire	3	1.64
<i>Aspergillus flavus</i>	Link	13	7.07
<i>A. glaucus</i>		1	0.54
<i>A. niger</i>	van Tieghem	26	14.13
<i>A. Parasiticus</i>	Speare	1	0.54
<i>A. Sydowii</i>	(Bainier & Sartory) Thom & Church.	4	2.17
<i>A. terreus</i>	Thom	1	0.54
<i>Cladosporium cladosporioides</i>	(Fres.) de Vries	9	4.89
<i>C. sphaerospermum</i>	Penzig	28	15.22
<i>Emericella nidulans</i>	(Eidam) Vuillemin	1	0.54
<i>E. quadrilineata</i>	(Thom & Raper): Benjamin	3	1.64
<i>Fusarium moniliforme</i>	Sheldon	4	2.17
<i>Geotrichum candidum</i>	Link ex leman	10	5.44
<i>Mucor racemosum</i>	Fres.	1	0.54
<i>Paecilomyces variotii</i>	Bainier	2	1.09
<i>Penicillium aurantiogriseum</i>	Dierckx	5	2.72
<i>P. chrysogenum</i>	Thom	18	9.79
<i>P. duclauxii</i>	Delacroix	3	1.64
<i>P. funiculosum</i>	Thom	2	1.09
<i>P. roquefortii</i>	Thom	19	10.33
<i>Rhizopus stolonifer</i>	(Ehrenb.) Lind	1	0.54
<i>Scopulariopsis brumptii</i>		1	0.54
<i>S. candida</i>	(Gueguen) Vuill	1	0.54
<i>Stachybotrys chartarum</i>	(Ehrenb.: Lind) Hughes	2	1.09
<i>Stemphylium spp.</i>		1	0.54
<i>Torula herborum</i>		1	0.54
<i>Trichosporon cutaneum</i>		1	0.54
<b>Total</b>		<b>184</b>	<b>100.00%</b>

**Table 6:** Frequency distribution of psychrotrophic molds recovered from examined Mozzarella cheese samples on dicloran agar at 7°C.

Mold species		Isolated molds	
		No. of cases	%
<i>Acremonium strictum</i>	W. Gams	8	4.88
<i>Alternaria alternata</i>	(Fres.) Keissler	3	1.84
<i>A. chlamydospora</i>	Mouchacca	5	3.05
<i>A. egyptiacus</i>		1	0.60
<i>Aspergillus glaucus</i>		1	0.60
<i>A. flavus</i>	Link	7	4.27
<i>A. niger</i>	van Tieghem	16	9.76
<i>A. sydowii</i>	Thom & Church (Bainier & Sartory)	7	4.27
<i>A. versicolor</i>	(Vuill.) Tiraboschi	1	0.60
<i>Botryotrichum piluliferum</i>	Saccardo & Marchal	3	1.84
<i>Chaetomium globosum</i>		1	0.60
<i>Cladosporium cladosporioides</i>	(Fres.) de Vries	13	7.93
<i>C. sphaerospermum</i>	Penzig	21	12.80
<i>Emericella nidulans</i>	(Eidam) Vuillemin	1	0.60
<i>E. varicolor</i>	Berk. & Br.	1	0.60
<i>Fusarium moniliforme</i>	Sheldon	2	1.22
<i>Geotrichum candidum</i>	Link ex leman	9	5.49
<i>Hypomyces chrysosporium</i>		2	1.22
<i>Paecilomyces variotii</i>	Bainier	3	1.84
<i>Penicillium chrysogenum</i>	Thom	5	3.05
<i>P. citrinum</i>	Thom	1	0.60
<i>P. duclauxii</i>	Delacroix	3	1.84
<i>P. roquefortii</i>	Thom	8	4.98
<i>P. spinulosum</i>	Thom	2	1.22
<i>Rhizopus stolonifer</i>	(Ehrenb.) Lind.	3	1.84
<i>Scolecobasidium</i> spp.		2	1.22
<i>Scopulariopsis candida</i>	(Gueguen) Vuill.	2	1.22
<i>Trichosporon cutaneum</i>		2	1.22
<i>Torula</i> spp.		1	0.60
Yeast spp.		20	12.20
<b>Total</b>		<b>164</b>	<b>100.00</b>



## DISCUSSION

The results recorded in Table 1 show the min., max. and average values of aerobic plate, psychrotrophic, coliforms, *B. cereus*, *Staph. aureus* and Enterococci counts. The total aerobic plate count/g ranged from  $1 \times 10^4$  to  $5 \times 10^7$  with an average value of  $9 \times 10^6$  cfu/g. The obtained results substantiate what were stated by Cortesi *et al.* (1997) in which APC was  $2.3 \times 10^5$  cfu/g of Mozzarella cheese after 7 days storage at 6°C in brine solution. Psychrotrophes were detected in 55% of the examined samples with an average count of  $9 \times 10^4$  cfu/g. Somewhat, higher count was found by Rondinini and Garzaroli (1990), Massa *et al.* (1992), El-Shinawy *et al.* (1994) and Nazem and El-Hawary (1997). The contamination of Mozzarella cheese with psychrotrophes induces undesirable changes which rendering the product unmarketable resulting in a serious economic losses during extended periods of cold storage (Rondinini and Garzaroli, 1990 and Massa *et al.*, 1992). Nowadays, psychrotrophic food borne organisms have new concerns in the safety of refrigerated foods (Cousin *et al.*, 1992). Coliforms and *B. cereus* have been estimated but in lower counts than that of Idris and Ibrahim (1995), Cortesi *et al.* (1997) and Silva *et al.* (1997). The presence of coliforms may be implicated in food borne illness and gives an indication about the unhygienic measures during manufacturing, handling and storage of the product. Moreover, *B. cereus* was associated with food poisoning and has the ability to cause various infections which documented by Gilbert *et al.* (1974) and Turnbull *et al.* (1979). As regarding *Staph. aureus* count, it was noticed that these results were lower than that reported by Idris and Ibrahim (1995), Nazem and El-Hawary (1997) and Silva *et al.* (1997), on the other hand, *Staph. aureus* never detected by Cortesi *et al.* (1997) in Mozzarella cheese after 7 days storage at 6°C in brine. *Staph. aureus* is still among the microorganisms commonly involved in dairy products food poisoning. In the absence of proper cooling, the growth of *Staph. aureus* in cheese produces thermostable enterotoxins resulting in serious manifestations (Hekneby and Gondrosen, 1981). Lastly, in Table 1, Enterococci failed detection in the examined samples, however, Cortesi *et al.* (1997) recorded an average count of  $1.8 \times 10^4$ /g recovered from Mozzarella cheese samples. The presence

of enterococci reflects the unhygienic measures during handling and processing (Varnam and Evans, 1991).

It is obvious from the results presented in Table 2, that the incidence percentages of Anaerobes, Enterobacteriaceae and *Listeria* species were 62.5, 60.0 and 0%, respectively. Cortesi *et al.* (1997) stated that sulfite-reducing Clostridia were detected in Mozzarella cheese after 7 days storage at 6°C. The findings for Enterobacteriaceae organisms go parallel with the results recorded by Nazem and El-Hawary (1997). Also, Cortesi *et al.* (1997) and Silva *et al.* (1997) could isolate Salmonellae from the examined Mozzarella cheese samples. The prevalence of Enterobacteriaceae in milk and milk products is properly taken as an index of faecal pollution (Thatcher and Clark, 1978), also, it has been implicated in human infections and food poisoning outbreaks. In addition to the previous results, *Listeria* species could not be isolated from the examined samples. Similar findings were achieved by Silva *et al.* (1997).

Table 3 explains that the average counts of mesophilic molds at 25°C were  $5 \times 10$  and  $5 \times 10^2$  cfu/g on malt extract agar and dicloran agar, respectively, as well as the average value of psychrotrophic molds at 7°C on dicloran agar was  $3 \times 10^2$  cfu/g of the examined Mozzarella cheese. These results revealed that, dicloran rose bengal medium allows the isolation of the highest number of different species and genera of molds. The high effectiveness of dicloran rose bengal agar is confirmed by King *et al.* (1979), Saad and Hemida (1995) and Weidenbörner *et al.* (1995) who recommended this medium for isolation of molds from food. In addition, higher mold counts were recovered from different products as processed cheese, pizza and cream cheese-like products by El-Shinawy and Ragheb (1994), Idris and Ibrahim (1995) and Nazem and El-Hawary (1997), respectively. The high counts of mycoflora in cheese may be contributed to the unhygienic measures during handling by attendants or failure to sterilize processing equipments (Aran and Eke, 1987).

Inspection of results in Table 4, declared that various species of mesophilic molds were recovered from 20 examined samples of Mozzarella cheese (50%) on malt extract agar. *Aspergillus* was the most prevalent genus encountered in the examined samples comprising 55.0% of total molds.

The genus is represented mainly by *A. niger* (37.5%) and *A. flavus* (15.0%), while, *A. fumigatus* was recorded in rare frequency of occurrence (2.5%). Similar results were reported by Saad and Hemida (1995), while, El-Shinawy and Ragheb (1994) demonstrated higher incidence of Aspergilli. *Penicillium* was represented by 5 species, *P. aurantiogriseum*, *P. chrysogenum*, *P. citrinum*, *P. restrictum* and *P. viridicatum* and ranked second in the percentage of isolation constituting 32.5% of total molds. *Penicillium* species were the most prevalent in Egyptian (Abdel-Rahman and El-Bassiony, 1984 and Ibrahim, 1987), Swiss (Bullerman, 1976 and 1980), Australian (King *et al.*, 1981), Greece (Zerfiridis, 1985), Turkish (Aran and Eke, 1987) and Poland cheeses (Weidenbörner *et al.*, 1995). Moreover, *Cladosporium cladosporioides*, *C. sphaerospermum*, *Emericella nidulans* and *Rhizopus stolonifer* were infrequently encountered (2.5 - 5.0%).

It is apparent from the results in Table 5 that, 184 species belonging to 16 genera were presented in samples of Mozzarella cheese. Percentage of 25.57% of the isolates were for *Penicillium*, 24.99% for *Aspergillus*, 20.11% for *Cladosporium* and 11.42% for *Alternaria*. While the other isolates existed in rare frequency of occurrence (0.54 - 5.44%). Similar findings were estimated by El-Sayed (1981). It was reported that, 82.2% of molds found on refrigerated cheddar cheese were belonged to *Penicillium* spp., 6.6% to *Aspergillus* spp. and 1.1% to *Fusarium* spp. Furthermore, some species of these genera are mycotoxin producing molds which possess potential hazards to food safety and human health (Van Walbeek, 1973 and Bullerman and Olivigni, 1974).

*Cladosporium*, *Aspergillus* and *Penicillium* species were found to be common psychrotrophic contaminants of Mozzarella samples in percentages of 20.73, 19.50 and 17.69%, respectively (Table 6). Moreover, yeast species comprising 12.20% and both *Alternaria* spp. and *Geotrichum candidum* were encountered in 5.49% of total isolates. The incidence and species of molds isolated from cheese samples were reported in the same trends by several investigators as Bullerman (1981), Fadd *et al.* (1989), Kivance (1990). In fact, the importance of mold lies in that cheese may be contaminated by some toxigenic fungal strains either directly or indirectly during ripening, curing as well as during refrigerated storage. Therefore, the hazard for

natural occurrence of mycotoxins in Mozzarella cheese is real (Tricksess and Page, 1986).

From the above achieved results, it is noted that Mozzarella cheese is liable to contamination by some pathogenic microorganisms. Moreover, many species of molds encountered are known to be mycotoxin - producers which threaten the consumers health.

Therefore, to safe guard consumers from being infected and to obtain a finished product of good keeping quality, strict hygienic measures should be adopted during manufacturing and storage. This requires the use of fresh raw ingredients, sterilized utensils and personal hygiene of the individuals. Also, it is very important to keep Mozzarella cheese under deep freezing (-18°C) at all times.

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