INCIDENCE OF ENTEROTOXIGENIC STAPHYLOCOCCI AND YEASTS IN RAS CHEESE LAYERS

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Abstract

Forty-five samples of Ras cheese layers were collected from supermarkets, representing the surface, the middle and the core layers of Ras cheese. The mean count values of *Staphylococcus aureus* from each layer were 4.544±4.342, 2.230±1.602 and 0 respectively; only, two enterotoxigenic strains could produce enterotoxin type A, while, the mean values of yeasts were 4.462±3.204 , 2.255±0.380 and 2.322±0.919. The most common isolated yeast species were *Candida, Rhodotorula, Torulopsis,* and *Saccharomyces.* These results indicate that the core layers of Ras cheese were more safe microbiologically than the surface layers. The chemical examination of Ras cheese showed that pH, acidity % and moisture % were 4.30±0.100 , 1.62 ±0.033 and 32.3 ±0.014, respectively. The public health of examined Ras cheese and the control measures to safeguard the consumers were discussed.

INTRODUCTION

Ras cheese is one of the most common types of hard cheese in Egypt. The product is liable to contamination with bacteria and fungi from various sources during manufacturing, storage and marketing which ultimately contribute to a potential health problems (Girgis *et al.*, 1992). Nowadays, there is an increase in the consumption of various cheese types due to their favourable and stable properties, quality acceptability by children, and long shelf-life. On the other hand, cheese which is excessively contaminated with microorganisms be undesirable from the stand point of public health and storage quality. During ripening period, yeasts may find the opportunity to grow and multiply on cheese producing undesirable changes that render the product unmarketable causing an economical losses and may be potentially hazardous to the health of consumers. The public health importance of yeasts that has been emphasized as pathogenic types was implicated in cases of gastrointestinal disturbances, endocarditis and thrush in particular for immuno compromised patients (Elein *et al.*,1999).Also, the economic losses of cheese due to spoilage by yeasts have been increasing because of the reduced use of preservatives, packaging in modified atmospheres, or new formulations that do not strictly control the growth of these organisms (Abdel- Hady,2002).

Presence of *S.aureus* in milk and its products is usually taken as an index for contamination, either from bovine udder or human sources during production, processing or handling of such products. Staphylococcal food poisoning is an intoxication resulting from ingestion of one or more of enterotoxins produced by enterotoxigenic strains of *S.aureus* during their growth and multiplication in food infections; as food borne illnesses result from the consumption of foods contaminated with pathogenic bacteria and / or their toxins, or other substances hazardous to human health. Presence of *S.aureus* in milk and its products is usually taken as an index for contamination either from bovine udder or human sources during production, processing or handling of such products (El- Soda and Abou-Donia,1978).

Hard cheeses are usually ripened within several months at a suitable temperature and relative humidity. The microbiological quality and safety of Ras cheese is one of the major areas of concern for producers and consumers(Amer, 2002). Microbiological criteria can provide a tool for evaluating the acceptability of the products or as a process designed to control the growth of microorganisms. The value of setting microbiological criteria can ultimately enhance the safety and evaluate consumer confidence in the commercially produced food supply (Zottola and Smith, 1991).

In Egypt, the information about the involvement of Ras cheese in human illness and economic losses are unknown(Girgis *et al.*, 1992 and Elein *et al.*,1999). Therefore, this investigation was conducted to determine the microbiological quality of locally produced Ras cheese with spotting light on the prevalence of enterotoxigenic *Staphylococci*, as well as the yeast contamination of Ras cheese layers, and suggesting the control measures for microbial hazard to safe-guard the consumer health.

MATERIALS AND METHODS

Forty- five of Ras cheese samples (fifteen from the surface, the middle and the core) of the cheese layers were collected in sterile bottle from supermarkets in Cairo governorate, and were transferred rapidly to the Lab. The collected samples were subjected to :

a- Microbiological examination: The cheese samples were prepared for microbiological examination to *S. aureus* and total yeast count/g, according to APHA (1992).

- Isolation and identification of organisms were carried out according to Buchanan and Gibbons (1975). The isolated *S. aureus* strains were examined for enterotoxin production using the sac culture method recommended by ICMSF (1986).
-Yeast isolates were identified according to the technique recommended by Lodder and Kreger (1967) and Deak & Beuchate (1996).

b- Chemical analysis:All cheese samples were chemically examined for pH using pH meter and titratable acidity according to AOAC (1990), while moisture % according to APHA (1985).

RESULTS

Table 1. Statistical analysis results of *S. aureus* count of examined Ras cheese layers Log10 CFU/g.

Types of samples	Total number	Positive samples		Min	Max.	mean ± S.E
		No	%			
The surface layers	15	8	53.3	4.30	5.77	4.54 ± 4.34
The middle layers	15	5	33.3	2.69	3.47	2.23 ± 1.60
The core layers	15	0	0	0	0	0

Table 2. Entertoxigenic *S. aureus* strains isolated from Ras cheese layers.

Types of samples	No. of strain tested	No. of strain Producing enterotoxin	Type of enterotoxin
The surface layers	8	1	A
The middle layers	5	1	A
The core layers	0	0	0

Table 3. Statistical analysis results of yeasts count of examined Ras cheese layers Log10 CFU/g.

Types of samples	Total number	Positive samples		Min	Max.	mean ± S.E
		No	%			
The surface layers	15	12	80.0	4.84	6.47	4.46 ± 3.20
The middle layers	15	7	46.6	2.69	3.47	2.25 ± 0.38
The core layers	15	2	13.3	1	3.95	2.32 ± 0.91

Table 4. Frequency distribution of yeasts of examined Ras cheese layers.

Isolated strains	surface layers		middle layers		core layers	
	frequency	%	frequency	%	frequency	%
Candida tropicalis	2	13.33	0	0	0	0
C. lipolytica	4	26.66	0	0	2	13.33
C.pseudotropicalis	3	20	1	0	0	0
C. kefyr	0	0	4	6.66	5	33.33
C. rugsa	3	20	1	26.66	0	0
Cryptococcus diffluens	2	13.33	0	6.66	0	0
Rhodotorulapallida	3	20	2	0	3	20
R. rubra	1	6.66	2	13.33	4	26.66
Torulopsis holmi	7	46.66	5	13.33	1	6.66
Torulopsis sake	4	26.66	1	33.33	0	0
Saccharomyces bailli	6	40	4	6.66	0	0
S.miso	3	20	1	26.66	0	0
S.cerevisiae	2	13.33	0	6.66	0	0

Table 5. Chemical analysis of Ras cheese samples.

Parameter	Minimum	Maximum	Mean ± S.E
pH	4.10	5.70	4.30 ± 0.100
Acidity %	1.00	2.15	1.62 ± 0.033
Moisture %	28.6	35.0	32.3 ± 0.14

DISCUSSION

Milk products can harbour a variety of microorganisms and can be important sources of food borne pathogens. This pathways pose a risk to the consumer from direct exposure to food borne pathogens present in unpasteurized dairy products, as well as, dairy products that become re-contaminated after pasteurization (Oliver *et al.*, 2005). The results recorded in Tables 1 and 2 showed that 53.3and 33.3 % from the surface and middle layers of positive cheese samples respectively, had high levels of *S.aureus*. Nearly similar results were obtained with Halawa (1987) who found Enterotoxins A, B and C single or combined produced by 3.8% of the tested *S.aureus* strains. *S. aureus* counts decreased in middle layers and not detected in the core layer. This may be due to absence of aerobic conditions required for their growth, due to decreasing the pH value and the relatively high salt content. Also, the ripening process had the greatest influence upon survival of *Staphylococcus aureus* .

Thus, the presence of *S. aureus* potentially represents the risk of intoxication (Nazem and Saleh, 1994). So, it is of special importance to follow the presence of enterotoxigenic *S.aureus* strains in food, especially for protecting the consumers from food poisoning, as the International Microbial Legislation recommends that hard cheese should not exceed 10^2-10^3 cfu/g with their freedom from all pathogenic microorganisms (Law, 1999).

Yeast microflora were found in 80.0, 46.6 and 13.3% of surface, middle and core layers of Ras cheese, respectively as shown in Tables 3 & 4, indicating poor hygiene during handling. The results especially of the surface layers agree with El-Leboudy *et al.*(1992) who examined random samples of Ras cheese and found that yeasts were present in 80% of the examined samples. Shelaih *et al.*(1986) concluded that yeasts were found in 78% in (roumy) cheese and the obtained yeasts were *Candida, Torulpsis, Rhodotorula* and *Saccharomyces* species.

The research results of the middle layers of Ras cheese were nearly similar to Abdel- Hady (2002) who found that 56.6% of examined samples were contaminated with yeasts and molds with mean value of 9500 cfu/g in hard cheese and the most predominant yeasts were *Candida, Debaryomyces and Rhodotorula* spp. The obtained

results disagree with the data obtained by El-Komy (2002) who examined samples of hard cheese and found that yeasts were present in 45 and 65% of examined surface and core layers, respectively.

It is evident from Tables 3 & 4 that examined samples were above the permissible limit of yeast and mold counts, stipulated by the Egyptian Standards (2001) which stipulate that yeast and mold should be less than 10^2 and 10 cfu/g, respectively, with no visible growth.

The previous results led to the conclusion that counts in surface layers were higher than those in the middle and the core layers of Ras cheese. These results may be attributed to the high storage temperature with relative humidity which lead to abundant growth of yeasts widely distributed in nature, and the surface of hard cheese is liable to be contaminated specially during ageing in retail markets. Ras cheese present in the markets may represent a health risk for the consumers and act as an important vehicle of transmitting pathogens because the values obtained were close to the number of bacteria able to produce enough enterotoxins which cause the food-borne disease outbreak (Nazem & Saleh 1994).

Data reported in Table 5 agree with the results obtained by El-Soda & Abou-Donia (1978), but, disagree with those of Girgis *et al.* (1992) who found that in the examined Ras cheese, the average pH value, and moisture content were 5.13, and 28.81%, respectively. Most of the food articles in Egypt, especially cheese were left at room temperature between 18.5 and 28 degrees °C, with a relative humidity of 65 to 70%, thus, help the microorganisms to cause spoilage.

The growth of both yeasts and bacteria were monitored during the maturation of cheeses, and exhibited various degrees of extracellular proteolytic and lipolytic activities. Many problems of fat and protein breakdown in foods are microbial in origin, thus, the suitable growth of yeast strains were able to develop within the pH 2.5-8 range, at temperatures of 4 to 44 °C, thus, low pH value plays an important role in descending growth of microorganism count (APHA, 1992).

The results obtained during this study allow to conclude that the sanitary measures adopted during all phases of cheese making such as processing, handling and distribution of Ras cheese are improper and hazardous before they were consumed.

Physical touching of products, holding cheese at room temperature after preparation would allow germination of yeasts and multiplication of microbes. So, health promoters must be aware of these hazards and need to educate food workers, administrators, and the public will assure the cheese industry of a safe and ma‹etable product.

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مدى تواجد المكور العنقودي السمي والخمائر في طبقات الجبن الراس

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تم جمع عدد ٤٥ عينة من الجبن الرومي المحلية من السوبر ماركت وتم فحصها ميكروبيا من على السطح ومن الوسط ومن الداخل ، ووجد أن متوسط عدد الخمائر كانت ٤٦٢. ٤ ± ٣,٢٠٤ ، ٢,٢٥٥ ± ٠,٣٨٠ ، ٢,٣٢٢ ± ٩١٩. على التوالي وكانت العترات المعزولة من الخمائر هي الكانديدا والرودوتوريولا والسكارومايسس . بينما كان عدد ميكروب العنقودي الذهبي هو ٥٤٤. ٤ ± ٣٤٢. ٤ ، ٢٣٠. ٢ ± ٦٠٢. ١ ، 0 من كل من السطح، الوسط، ومن الداخل . وأنتجت عترتان فقط من هذا الميكروب السم من النوع A ، مما يشير إلى أن داخل الجبن الرومي أكثر أمانا من الناحية الميكروبية مقارنة بسطح الجبن، وبالفحص الكيميائي للجبن الراس كان متوسط تركيز الأس الهيدروجيني ونسبة الحموضة ٤,٣٠ ±١٠٠,٠ ، ١,٦٢ ±٠٣٣,٠ في حين أن نسبة الرطوبة ٣٢,٣± ٠١٤،٠ وتم مناقشة الأهمية الصحية لهذا المنتج والميكروبات المعزولة منه.