تقيم ميكروبيولوجى للجبن المطبوخ المصرى

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الملخص العربى

تم جمع أربعون عينة من الجبن المطبوخ المصرى من مصادر مختلفة فى القاهرة والجيزة وفحصت بكتريولوجيا لتقيم الميكروبات الموجودة بها ٠

تبين من الفحص أن متوسط العد الكلى للميكروبات فى الجرام الواحد من الجبن المطبوخ هو ٢٨٧ × ٣١٠ ومتوسط العد الكلى للفطريات هو ٥٢ ولقد ثبت وجود الميكروبات اللاهوائية بجميع العينات التى فحصت بينما عزلت الميكروبات القولونية من ٥ر١٢٪ فقط ٠

تم عزل الميكروبات الآتية :

(أ) الميكروبات العصوية الهوائية : سركيولانس (٥ر٥٢٪) ، سبتلس (٥ر٤٢٪) ، كوأجيولانس (٥ر٣٧٪) ، سيرس (٥ر٣٢٪) ، يلفيفسيانس (٣٠٪) ، بريفس (٥ر٢٧٪) ، لنتس (٢٥٪) ، فيرمس (٥ر١٧) ، فيجاتيرم (١٥٪) ، استياريثير موفيلس (٥ر١٢) ، وليشينبفورمس (١٠٪) ٠

(ب) الميكروبات القولونية : اشيرشياكولاى (٥٪) ، انتيروباكتركولواكا (١٠٪) ، وانتيروباكتر ليكويفسيانس (٥٪) ٠

(ج) الميكروبات العنقودية : الميكروب العنقودى الذهبى (٥٪) ، الميكروب العنقودى ابيدرمس (٣٠٪) ، وميكروكوكاى (٥ر٤٢٪) ٠

(د) الفطريات : فطر جوتريكم (٤٥٪) ، بنسيليم (٥ر٣٧٪) ، كلادوسبوريم «٢٥٪» ، واسبرجلس (١٥٪)٠

اقترح بالبحث عدة توصيات بشأن الاشتراطات الصحية الواجب توافرها فى تصنيع الجبن المطبوخ المصرى وذلك على ضوء النتائج التى تم الحصول عليها ٠

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MICROBIOLOGICAL EVALUATION OF PROCESSED CHEESE

(With 5 Tables)

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SUMMARY

Microbiological studies have been conducted on forty samples of processed cheese.

The mean total colony count of examined samples was $287 \times 10^3 \pm 69$, while the count of moulds was 52 ± 7. Anaerobic bacteria were present in all examined samples and Coliforms were detected in 12.5% of samples.

Qualitative analysis of organisms isolated revealed the presence of:

a) Aerobic spore-formers: B. cerculans (52.5%), B. subtilus (42.5%), B. coagulans (37.5%), B. cereus (32.5%), B. pulvifaciens (30%), B. brevis (27.5%), B. lentus (25%), B. fermus (17.5%), B. megaterium (15%), B. stearithermophilus (12.5%) and B. licheniforms (10%).

b) Coliforms : E. coli (5%) Enterobacter coloaca (10%) and Enterobacter liquefaciens (5%).

c) Gram-positive cocci : Staphylococcus aureus (5%) Staph. epidermidis (30%) and Micrococci (42.5%).

d) Moulds : Geotrichum (45%), penicillium (37.5%), Cladosporium (25%) and Aspergillus (15%).

Suggestive control measures are discussed.

INTRODUCTION

The organisms present in processed cheese are those that survive the heat treatment used in processing as well as post- processing contaminants. The microflora, however, varies in cheeses of the same type, and even more in different types.

Few bacteria other than spore-formers survive the heat treatment. Aerobic spore-formers (Bacillus mesentricos and B. subtilus) may be present, but the anaerobic species are more important as a cause of spoilage (LAMPERT 1965).

Defective processed cheese having a bleached appearance, crumbly texture, and a penetrating putrefactive odour caused by Cl. coagulans like organism was studied by GRIFFITHS (1939). The organism was believed to have come from milk of low quality. Cl. sporogenes, Cl. pasteuranium and othe species heve been reported in cases of gas fermentation (HOOD& SMITH, 1951).

Although moulds are killed during processing, they may recontaminate the product during packaging and will grow if oxygen is available (FOSTER *et al.*, 1958).

Numbers of bacteria in processed cheese are not generally related to public health. Although comparatively few chesee- borne disease outbreaks have been reported (HENDRICKS *et al.*, 1959 and ALLEN & STOVALL, 1960). FOSTER *et al.* (1958) stated that 22 disease outbreaks were attributed to milk products including processed cheese. GRECZ *et al.* (1965) and KARIM & GRECZ (1972) stated that processed cheese is a favourable medium for Cl. botulinum as well as for spore and toxin stability over long storage periods. Staphylococcal food-poisoning outbreaks, traced to cheese, has been reported by TAKAHASHI & JONES, 1959 : HASULER ET *et al.*, 1960; Mickelsen *et al.*, 1961 ; GULLOTTI & SPANO, 1962; EPSOM 1964; RIVAS *et al.* 1965; DONNELLY *et al.*, 1967 and ZEHREN & ZEHREN, 1968. HOBBS (1970) stated that some unsual outbreaks of staphylococcal food poisoning from cheese drew attention to the fact that milk contaminated with staphylococci and insuffiently heated could introduce staphylococci at the start of cheese making

The aforementioned studies showed that processed cheese may contain considerable numbers of different types of microorganisms. Some of which are of publis health importance and others may lead to spoilage of cheese. Therefore, the present study was planned to investigate the microflora of Egyptian processed cheese.

MATERIAL AND METHODS

Forty retail samples of processed cheese were collected from different shops in Cairo and Giza. Samples in foil-lined cartons were transferred to the laboratory to be examined bacteriologically according to the STANDARD METHODS FOR THE EXAMINATION OF DAIRY PRODUCTS (1972) as follows :

1) *Total colony count* :

Eleven grams of the sample were aseptically weighed and thoroughly emulsified in 99 ml. of sterile 2% sodim citrate sol at 40°C in a sterile mortar containing sterile sand to make a dilution 1/10, from which 10- fold serial dilutions were prepared. The serial dilutions were plated in duplicates using plate count agar medium. Inoculated plates were incubated at 32 °C for 3 days. The total colony count per gram of sample was calculated and recorded. Colonies from countable plates were picked and isolated in pure culture for further identification according to BREED *et al.* (1957) and Cowan & STEEL (1970)

2) *Mould count* :

Sabouraud's maltose agar containing 20 units of penicillin and 40 ug of streptomycin per ml. was used as a selective medium. Duplicate plates were inoculated from each serial dilutions before being incubated at 25°C for 5 days. The average number of colonies per gram of sample was calculated. Separate mould colonies were inoculated on sabouraud slope medium to be used for further identification according to BESSEY (1950).

3) *Coliform test* :

Macconkey's bile salt lactose broth was inoculated with one ml. from each of the serial dilutions previously prepared. Inoculated and control tubes were incubated at 37°C for 48 hours. Tubes showing acid and gas were considered positive and the titre was recorded. Loopfuls from positive tubes were streaked on Macconkey's agar plates and incubated for 24 hours at 37°C. Typical lactose fermenters were picked and cultured on slope agar for further identification according to COWAN & STEEL (1970).

4) *Gram- positive cocci* :

Loopfuls from the dilution 1/10 were streaked on sodium azide crystal violet blood agar plates (MERCHANT & PACKER, 1967) and incubated at 37°C for 48 hours. Suspected colonies were picked and cultured in nutrient broth for further identification according to COWAN & STEEL (1970).

5) Detection of anaerobes

Stormy fermentation test : One gram of the sample was transferred to each of 3 sterile test tubes containing 10 ml. of sterile skim-milk, then a layer of melted paraffin was added to the tubes to a depth of one cm. Inoculated tubes were heated in a water bath adjusted at 85°C for 15 minutes, cooled and then incubated at 37°C for 5 days. A stormy clot (torn of paraffin layer) is considered positive.

RESULTS AND DISCUSSION

1) *Total colony count*

Results given in table (1) show that the total colony count per gram of the examined samples ranged between 40×10^2 and 140×10^4 with a mean value of $287 \times 10^3 \pm 69$. The highest percentage distribution (72.5%) lies withen the range 10^2-10^4 (Table 2).

Nearly similar findings were reported by ITO & EBINE (1966), MARENZI & SALVADORI (1969) and MLADENOV et al. (1972).

TABLE 1. **Statistical analysis of bacterial and mould counts in examined samples/**

	Maximum	Minimum	Mean ± S.E.M.
Total colony count	140×10^4	40×10^2	$287 \times 10^3 \pm 69$
Mould count	40	0	52 ± 7

TABLE 2. **Frequency distribution of samples based on their total colony count/g.**

Range	Frequency	
	No. of samples	Percentage
10^2—10^3	13	32.5 %
10^3—10^4	16	40.0 %
10^4—10^5	11	27.5 %

2) *Mould count* :

It is evident from table (1) that the mould count /g of samples ranged between 0 and 140 with a mean value of 52 ± 7. Inspection of table 3 reveals that the highest percentage distribution (52.5%) lies withen the range of 1-100, while 35% of samples showed negative results. These findings lead to conclude that during processing moulds are killed, but only recontamination of the poroduct may occur during packing.

3) *Coliforms* :

Table (4) reveals that coliforms were present in 12.5% of the samples. The organism failed detection in 0.01g cheese.

The presence of coliforms is indicative of insanitary conditions practiced during processing and handling. Efforts should be practiced to ensure the effeciency of hygienic measures adopted in manufacturing and handling of the product .

4) *Anaerobes* (stormy fermentation test)

Results given in table 4 show that all the samples examined proved to contain clostridium organisms.

TABLE 3. Frequency distribution of samples based on their mould count / g.

Range	Frequency	
	No. of samples	Percengate
0	14	35.0 %
1 — 50	6	15.0 %
51 — 100	15	37.5 %
101 — 140	5	12.5 %

TABLE 4. Incidence of Coliforms and anaerobes in processed cheese

	No. of samples	Positive samples	
		No.	%
Coliforms .	40	5	12.5% (Coliforms failed detection in 0.01 g).
Anaerobes .	40	40	100.0% (Stormy fementation test).

Presence of anaerobic bacteria in processed cheese was reported by GRIFFITHS (1939), HOOD & SMITH (1951), LAMPERT (1965), GUDKOV (1968) and MLADENOV *et al.* (1972).

Even though these products are stored at room temperature, the anaerobes normally cannot grow because of the relatively hight acidity and salt content ; yet if the acidity is too low or if unusually high numbers of spores are present spoilage is likely to result. Excessive numbers of these organisms may be attributed to the processing mixture (defective cheese or other ingredients), FOSTER *et al.* (1958). TANNER (1944) reported that one possible defect of processed cheese is gaseous spoilage resulting from development of anaerobic microorganisms originating in the milk. GRECZ *et al.* (1965) and KARIM & CRECZ (1972) reported that toxin and spores resulting from the growth of Cl. botulinum in processed cheese remained stable for twelve years.

5) *Microflora :*

Results recorded in table 5 show that the isolated bacteria from examined samples were aerobic spore-formers, coliforms, gram-positive cocci and moulds.

TABLE 5. **Frequency distribution of isolated organisms.**

Organism	Frequency	
	No. of samples	%
B. circulans	21	52.5
B. subtilus	17	42.5
B. coagulans	15	37.5
B. cereus	13	32.5
B. pulvifaciens	12	30.0
B. brevis	11	27.5
B. lentus	10	25.0
B. fermus	7	17.5
B. megaterium	6	15.0
B. stearithermophilus	5	12.5
B. licheniformis	4	10.0
Enterobacter coloaca	4	10.0
Enterobacter liquefaciens	2	5.0
Escherichia coli	2	5.0
Micrococci	17	42.5
Staphylococcus epidermedis	12	30.0
Staphylococcus aureus	2	5.0
Geotrichum species	18	45.0
Penicillium species	15	37.5
Cladosporium species	10	25.0
Aspergillus species	6	15.0

a) Aerobic spore-formers. The following species of aerobic spore-formers in a descending manner were isolated : B. circulans (52.5%), B. subtilus (42.5%) B. coagulans (37.5%), B cereus (32.5), B. pulvifaciens (30%), B. brevis (27.5%), B. lentus (25%), B. fermus (17.5%), B. megaterium (15%), B. stearithermophilus (12.5%) and B. licheniformis (10%).

Nearly similar findings were reported by LAMPERT (1965), MARENZI & SALVADORI (1969) and MLADENOV *et al.* (1972).

b) coilforms : E. coli could be isolated from 5% of examined samples, while Enterobacter coloaca and Enter. Liqufaciens were isolated from 10% and 5% of samples respectively.

These results agree with those obtained by HALL *et al.* (1967) and MLADENOV *et al.* (1972).

c) Gram- positive cocci : The isolated species were Staphylococcus aureus (5%), Staph. epidermidis (30%) and Micrococci (42.5%).

Isolation of enterotoxigenic staphylococci from cheese were reported by TAKAHASHI & JOHNS (1959) ;, GULLOTTI & SPANO (1962), BALLOZOV *et al.* (1963), EPSOM (1964), RIVAS *et al*, (1965) DONNELLY *et al.* (1967) and ZEHREN & ZEHREN (1968). The staphylococci might have get into the product from the mill, cotaminated ingredients or employee.

Isolated micrococci simulate the findings of GRUEV (1969).

Staphylococcal food poisoning can be controlled by proper manuf acturing and thorugh sanitation to prevent contamination.

d) Moulds : Geotrichum species were isolated from 45 % of examined samples while Penicillium, Cladosporium and Aspergillus species from 37.5%, 25% and 15% respectively.

Moulds may recontaminate the product during packing. This problem is most serious with packaged sliced process cheese because of the greater surface involved. Mould spoilage can be controlled by rigid precautions to minimize contamination of cheese with mould spores.

From the results achieved one may safely conclude that processed cheese samples examined proved to contain different types of microorganisms, some of which may be responsible for deterioration of the product, while others are of public health importance. Therefore, it is highly recommended that strict hygienic measures should be adopted during processing and handling of the product. Periodical inspection of processing plants should be conducted by specialists.

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