

- قسم : المراقبة الصحية على الأغذية •
- كلية : الطب البيطري - جامعة أسيوط •
- رئيس القسم : أ.د. • توفيق البسيوني •

مدى نمو وبقاء ميكروب الاشيرشياكولاي الممرض

في الجبن الدمياطي

مصطفى خليل ، أحمد عبدالحميد ، نجاح سعد

تعد الجبن الدمياطي من أكثر أنواع الجبن الطرية انتشارا في مصر ودول الشرق

الأوسط، ولما كانت هذه الجبن قد تكون مصدر للتسمم الغذائي وخاصة بميكروب
الاشيرشياكولاي الواسع انتشارا، لذلك تم تصنيع جبن دمياطي من اللبن المبستر مع
اضافة الملح بنسب ١، ٥، ١٠% •

وقد تم حقن اللبن بميكروب الاشيرشياكولاي 0125/B15 قبل اضافة الملح
والمنفحة ، وتم التخزين في شرش مملح عند درجة حرارة الغرفة العادية •

ثم اخذت عينات من الجبن مباشرة بعد التصنيع ثم اسبوعيا لمدة ٤ أسابيع •
تبين من الفحص أن الميكروب ينمو في المراحل الأولى من التصنيع وخاصة
في الجبن المعد من لبن مضاف اليه ملح بنسبة ١، ٥% مع أنه ظل متواجد لنهاية
المدة المذكورة في الجبن المعد من لبن مضاف اليه ملح بنسبة ١٠%، وقد ثبت عدم
وجود الميكروب في تركيز الملح ١، ٥% بعد اسبوعين •

وقد ناقش البحث الخطوات الصحية التي يجب اتباعها عند تصنيع وتسدادول
الجبن الدمياطي للحفاظ على صحة المستهلك •

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**FATE OF ENTEROPATHOGENIC ESCHERICHIA COLI DURING
MANUFACTURE AND STORAGE OF DOMIATI CHEESE**
(With 2 Tables)

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SUMMARY

3 lots of pasteurized milk artificially contaminated with approx. 10^5 Enteropathogenic Escherichia coli (EPEC)/ml, were used to manufacture Domiati cheese, after addition of sodium chloride (1,5,10%, respectively) and rennet. Cheese were stored in whey containing 15% sodium chloride at room temperature ($25 \pm 1^\circ\text{C}$) and examined periodically for EPEC count, aerobic plate count, pH value and moisture and salt contents. EPEC increased in numbers by about two log cycles during the initial stages of manufacture of lot 1 and lot 2. However, the organism was not detectable after 2 and 4 weeks in these cheeses, respectively. The EPEC inoculated into cheese milk with 10% added salt persisted at numbers in excess of 200/g by the end of the fourth week. Progressive reduction in EPEC counts in all cheeses was associated with a significant drop in pH. The public health significance of these findings are discussed.

INTRODUCTION

Domiati cheese is a white soft cheese, most popular in Egypt as well as some other countries. It is characterized by addition of an appreciable amount of salt to milk prior to renneting. It may be consumed either fresh or after maturation in salted whey, usually at high ambient temperatures.

Certain strains of Escherichia coli, designated as enteropathogens, can cause either a toxigenic or an invasive type of illness (OGAWA *et al.*, 1968).

Outbreaks of food-borne illness caused by E.coli have been associated with consumption of milk, ice cream, kefir, cheese and other dairy products (AGENJO, 1945; SHIFRIN and OSTILOVSKAYA, 1963; MANZULLA and ESTEVEZ, 1965 and BARNARD and CALLAHAN, 1971).

Presence of enteropathogenic Escherichia coli (EPEC) in cheese has become a matter of concern since 1971 when at least 387 persons in 107 separate episodes in the U.S. stricken with gastroenteritis after ingestion of EPEC-contaminated Camembert cheese imported from France (MARIER *et al.*, 1973). Counts on cheese samples manufactured on the production days in question revealed presence of 10^5 to 10^7 enteroinvasive E.coli O124 per g of cheese.

Since this outbreak occurred, several studies have been done to characterize survival of EPEC in fermented skim milk (FRANK and MARTH, 1977), Camembert cheese (FRANK *et al.*, 1977) and Brick cheese (FRANK *et al.*, 1978).

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GLATZ and BRUDVIG (1980) tested commercial cheese samples and found *E.coli* in 41% of intermediate-moisture cheeses. Also, several studies have been done to characterize the incidence of *E.coli* in Domiati cheese (FAHMY and YOUSSEF, 1974; SHELAIH, 1979 and AHMED *et al.*, 1987).

The common occurrence of *E.coli* in dairy products, the implication of soft cheeses in at least two outbreaks of gastroenteritis caused by *E.coli* (MARIER, *et al.*, 1973 & MacDONALD *et al.*, 1985) together suggest that Domiati cheese might be a potential vehicle for transmission of EPEC.

Hence, this study was undertaken to determine whether the traditional preparation of Domiati cheese without addition of lactic culture might enhance survival of EPEC and to assess the fate of these organisms during storage of finished cheese.

MATERIAL and METHODS**The organism :**

EPEC O125:B15 (obtained from the department of Microbiology, Faculty of Medicine, Assiut Univ.), originally isolated from Domiati cheese, was cultured at 37°C in brain heart infusion broth (Oxoid) for 24 h.

Preparation of Domiati cheese :

Raw milk from the farm of the Faculty of Agric., Assiut Univ., was Laboratory pasteurized at 63°C for 30 min. Enough of the broth culture was added to the warmed (37°C) pasteurized milk, previously screened for coliforms and found negative, to yield approx. 10^5 *E.coli*/ml. A sample was taken after inoculation to determine the initial EPEC count, aerobic plate count and pH value. The inoculated milk was divided into three portions, which were salted by addition of sodium chloride to give concentrations of 1, 5, and 10% (lot 1, lot 2 and lot 3, respectively). The procedure described by FAHMY and SHARARA (1950) was used to manufacture Domiati cheese. Three control blocks of Domiati cheese were prepared, containing the test amounts of salt but EPEC was not added. Samples of the curd and finished product were tested for EPEC count, aerobic plate count and pH value. Finished fresh cheeses were examined for moisture and salt contents. Cheeses with their controls were stored at room temperature ($25 \pm 1^\circ\text{C}$) in whey containing 15% salt, and were tested weekly for EPEC count, aerobic plate count, pH value and for contents of moisture and salt.

Preparation of cheese samples for examination :

Cheese samples were prepared according to the "alternative" method described in Standard Methods for the Examination of Dairy Products (MARTH, 1978).

Enumeration of *E.coli* :

The method suggested by SPECK *et al.* (1975) was employed. Samples were surface plated onto Trypticase Soy Agar (Oxoid). Plates were held for 1 h. at room temperature followed by adding a layer of Violet Red Bile Agar (Oxoid). Plates were then incubated at 37°C for 24 h.

Aerobic plate count :

Standard plate counts were determined as described in Standard Methods (MARTH, 1978).

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Measurement of pH, moisture and salt :

The pH of cheese was determined by using an Orion pH meter, Model 201, equipped with standard combination electrode. For moisture analysis, cheese was dried for 5 h. at 100 ± 2 C (MARTH, 1978). The ATHERTON and NEWLANDER method (1977) was used to determine NaCl in the cheese.

RESULTS

The obtained results are recorded in Tables 1 and 2.

DISCUSSION

Table (1) shows growth and survival of EPEC during manufacture and storage of different lots of cheeses. This table also compare changes in pH and aerobic plate counts of cheeses.

EPEC growth occurred in lot 1 and 2 (prepared from pasteurized milk with 1 and 5% added salt, respectively) during preparation by about two log cycles. A further increase occurred in EPEC in these cheeses during storage, especially in lot 1 and achieved its maximum population by the end of the first week. Then, E.coli decreased in numbers in both lots of cheeses and the rate of decrease was greater in lot 1 than lot 2. The organism was not detectable after 2 weeks in lot 1 and 4 weeks in lot 2.

In the curd of lot 3 (prepared from pasteurized milk with 10% added salt), substantial increase in numbers of EPEC during manufacture did not occur, and there was a decrease in population with increasing time of storage.

From data in table 1., it is evident that E.coli rapidly lost its viability in cheese made from milk containing 1% salt. This may be the result of the rapid decrease of pH to 3.9 by the end of the second week, and of competition from the large number of bacteria other than E.coli.

In cheese of lot 3, addition of 10% salt to milk induced inhibition of growth of micro-organisms other than E.coli and minimize the decrease in pH value during cheese preparation and storage (Table 1). This inhibition of the competitive microflora, the resultant favorable pH of the cheese medium and the physical properties of the final curd (high moisture content-Table 1) provided an opportunity for E.coli to persist at numbers in excess of 200/g by the end of the fourth week. The survival of E.coli in cheese of lot 3 lends support to LEE and RIEMANN (1971). They reported that typically, enteric organisms may survive in foods with pH values above 4.5 for extended time intervals.

The general pattern of the obtained growth and survival results for E.coli are different from those of EL-BASSIONY (1977) who reported that the survival periods of E.coli were 22, 49 and 54 days at room temperature in stored Kareish cheese containing 1, 5 and 7.5% sodium chloride, respectively. The difference observed between the two studies might have been due to the lower pH and the lower level of initial inoculum of the present cheese and or due to using a different strain of E.coli.

Data in Table 2. reveal an increase in salt content and a decrease in moisture content of cheese (water phase) occurred during storage of cheese. This was comparable to results obtained by AMER *et al.* (1979). The pH of the cheese appeared to have a greater effect on the counts than did moisture or salt. FRANK *et al.* (1977) suggested that the lower pH of unripened Camembert cheese associated for the lower survival of EPEC in it. This is also likely to be true for Domiati cheese.

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The number of bacteria necessary to cause illness, as estimated through feeding studies is 10^6 to 10^8 for invasive *E. coli* and 10^8 to 10^9 for toxigenic *E. coli* (MEHLMAN *et al.*, 1976). The present results indicate that *E. coli* may reach infective levels in fresh Domiati cheese within few days at room temperature. The presence of these bacteria in Domiati cheese has been reported by several authors (FAHMY and YOUSSEF, 1974; SHELAIH, 1979 and AHMED *et al.*, 1987). It is clear from the foregoing that the present investigation support the suggestion that Domiati cheese-borne infection with *E. coli* may be a potential public health problem.

Moreover, it was suggested by FRANK *et al.* (1978) that enterotoxigenic *E. coli* may produce enterotoxins during growth, which would be left behind in the cheese after the numbers of enterotoxigenic *E. coli* had decreased. Conceivably this cheese, if consumed, could cause gastrointestinal illness.

In conclusion, the increased amount of salt added during preparation of Domiati cheese may allow survival of *E. coli*. Salt may retard growth of *E. coli* and reduce its survival only when accompanied by a pH value lower than 4.5. Effective measures that should be taken to insure the safety of Domiati below room temperature to slow the growth of *E. coli*, and the use of stringent sanitation measures during manufacture, package and storage of cheese. Nevertheless, testing laboratories must consider routinely that presence of even small numbers of *E. coli* in domiati cheese presents a potential health hazard.

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M.K. MOUSTAFA *et al.*Table 1 : Counts^a of EPEC and changes in pH and aerobic plate counts (APC) during manufacture and storage of different lots^b of Domiati cheese.

Sample	Lot # 1			Lot # 2			Lot # 3		
	EPEC	pH	APC	EPEC	pH	APC	EPEC	pH	APC
Milk, just after inoculation	2x10 ⁵	6.4	3x10 ⁶	2x10 ⁵	6.4	3x10 ⁶	2x10 ⁵	6.4	3x10 ⁶
Curd	1x10 ⁶	6.0	1x10 ⁹	3x10 ⁵	5.9	1x10 ⁷	1x10 ⁵	5.9	9x10 ⁶
Finished cheese	4x10 ⁷	5.2	3x10 ¹⁰	1x10 ⁷	5.8	2x10 ⁸	2x10 ⁵	5.9	2x10 ⁷
Cheese after 1 week	2x10 ¹⁰	4.9	3x10 ¹²	2x10 ⁷	5.3	3x10 ¹⁰	6x10 ⁴	5.4	3x10 ⁸
Cheese after 2 weeks	--	3.9	1x10 ¹²	5x10 ⁶	4.7	2x10 ¹⁰	1x10 ³	5.1	3x10 ⁸
Cheese after 3 weeks				3x10 ³	4.2	1x10 ¹⁰	4x10 ²	4.7	2x10 ⁷
Cheese after 4 weeks				--	3.9	1x10 ⁸	2x10 ²	4.5	1x10 ⁷

^aCounts per ml (milk) or g (curd, cheese)

^bLot # 1 = cheese prepared from milk containing 1% salt,
 Lot # 2 = " " " " " 5% salt,
 Lot # 3 = " " " " " 10% salt.

Table 2 : Moisture and salt^a contents of different cheeses during storage.

Time of storage(weeks)	Lot # 1		Lot # 2		Lot # 3	
	Moisture(%)	Salt(%)	Moisture(%)	Salt(%)	Moisture(%)	Salt(%)
0	51.6	0.6	55.2	2.5	60.6	5.1
1	47.5	2.1	53.8	5.4	54.1	5.5
2	45.4	6.1	49.6	6.4	52.3	7.2
3	44.1	6.7	47.2	6.7	48.2	7.1
4	43.5	6.6	46.7	7.0	47.8	7.6

^aSalt content in water phase of the cheese.

