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Clostridium perfringens IN FETA AND PROCESSED CHEESE AND ITS BEHAVIOUR WITH LACTIC ACID BACTERIA

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SUMMARY

The prevalence of Clostridium species in Feta and processed cheese was investigated. Respectively, four and three species of Clostridia were isolated from Feta and processed cheese samples showing frequency percentage of 54% and 40%. *Cl. perfringens* showed to be the most frequent species in both examined cheeses. The ability of isolated *Cl. perfringens* to produce haemolysin was also tested and considered as one of its virulence factors. Furthermore, the antibacterial activity of some strains of lactic acid bacteria (LAB) using spot-on-the-lawn and Agar well-diffusion methods was investigated. The results revealed descending inhibition potential from *Pediococcus acidilactici* followed by *Lactobacillus plantarum*, *Lb. rhamnosus* and *Lactococcus lactis*, while *Enterococcus faecium* was the least. *Leuconostoc mesenteroides* did not show any effect upon the pathogen. The behaviour of *Cl. perfringens* in Feta cheese with LAB was also studied. Results revealed the pathogen survival under refrigeration up to the end of cheese storage period (90 days). Meanwhile, lactic acid bacteria affected the growth of *Cl. perfringens* . *P. acidilactici*, in particular, showed the most potent in Feta cheese, since the pathogen counts dropped from 9 x 10^5 to 8.9 x 10 cfu/g during the cheese storage period (3 months).

Key Words: *Clostridium perfringens*, Feta cheese, Processed cheese, Lactic acid bacteria, Antimicrobial activity.

INTRODUCTION

Spore-forming bacteria make numerous problems for the dairy industry and the soft cheese making, in particular. This is because the heating process used is not effective to kill spores. *Clostridium perfringens* is one of the common spore-forming bacteria in nature, which exists in soil, water, milk, dust, sewage and intestinal tract of many animals and humans. The bacterial spores are long-lived and resistant to pasteurization treatment and many cleaning procedures, so that, their presence in food may be unavoidable (Brynestad and Granum, 2002).

The organism can grow between 15 and 50°C with an optimum temperature about 43-46°C. The generation time for most strains at temperatures

between 33 and 49°C is below 20 min, and generation time of 8 min has been reported (Labbé, 2000). So, the spore-forming ability and rapid growth rates at a range of temperatures are features, which allow the bacterium to multiply and survive in foods.

Moreover, the virulence of *Cl. perfringens* is determined by its prolific toxin-producing ability, including enterotoxins. Over 13 different toxins were detected due to this bacteria; only one strain may produce a subset of these toxins (Petit *et al.*, 1999). Also, the bacteria have been divided into five types (A-E) on the basis of production of four major lethal toxins: alpha, beta, epsilon and iota (Al-Kaldi, 2002). Type A food poisoning was reported due to confirmation Clostridium enterotoxin A, which was produced in the small intestine after ingestion of at least 10^7 *Cl. perfringens* cells. Usually 8-12 hr (6-24 hr) after eating contaminated food, the symptoms start with acute abdominal pain, nausea and diarrhea (Brynestad and Granum, 2002). Type C food poisoning is rare but very serious, the disease is often fatal and has a mortality rate of 15-25% even with treatment (Granum, 1990).

The Food and Drug Administration and the Center of Food Safety and Applied Nutrition reported prevalence of *Cl. perfringens* at 6.03% of the total estimated bacterial food borne outbreaks (Sabah *et al.*, 2003).

On the other hand, many investtigations have led to control microorganisms though the application of protective starter cultures such as some strains of lactic acid bacteria, which are commonly used in production of fermented milk products: cheese, sour cream, butter and yogurt. Besides their technological role in the process, they also increase the hygienic quality and safety of food (Klaenhammer, 1988 and Daeschel, 1989). The primary antimicrobial effect is due to production of lactic acid and subsequent reduction of pH (Daeschel, 1989). Furthermore, they also produce a number of inhibitory compounds include hydrogen peroxide, CO_2, diacetyl, acetaldehyde and bacteriocins (Klaenhammer, 1988; Stiles and Hastings, 1991 and Nettles and Barefoot, 1993).

Cheese is one of the oldest dairy products and it has a high nutritive value particularly when introduced in special diets. Feta and processed cheese could be estimated as microbiologically safe milk products due to the use of suitable heat treatment and preventive control measures during the manufacturing and packaging chain. Nevertheless, it has been reported that pathogen such as *Cl. perfringens* could survive (El-Bassiony, 1980; Ahmed, 1987; Ibrahim, 1986; Nazem and Aman, 1994). The heat treatment may activate spore germination, and the subsequent slow/ insufficient cooling or hot holding of food may then allow germination, outgrowth and multi-plication. Therefore, as a result of this rapid growth, high levels of vegetable cells can be reached within a short time (Andersen *et al.*, 2004).

Generally, the consumer sensation and awareness has played important role in emphasizing the need for production of microbiologically safe foods. Hence, the present work was carried out to study the incidence of *Cl. perfringens* in Feta and processed cheese in the local

market, the antagonistic activity developed by some strains of lactic acid bacteria against *Cl. perfringens* and their potential to inhibit the pathogen in LAB prepared Feta cheese.

MATERIALS AND METHODS

Samples Collection

A total of 80 random samples of locally made Feta cheese (50 samples) and processed cheese (30 samples) were collected from different markets in Cairo and Giza Govornorates, delivered directly to the laboratory according to American Public Health Association (A.P.H.A. 1992) and examined for incidence of clostridia spp.

Isolation, Enumeration and Identification of Clostridium

Anaerobic count was done using Rinforced Clostridium Medium (RCM) (Oxoid); plates were incubated anaerobically at 37°C for 48 hrs in gas pack anaerobic jar (Nazem and Aman, 1994). Presumptive colonies were sub-cultured for subsequent identification. All suspicious isolates were identified according to Varadaraj (1993) and Cato *et al.* (1986). The isolates were confirmed as *Cl. perfringens* if they are non-motile, reduce nitrate, ferment lactose, liquefy gelatin within 48 hr and produce acid from raffinose (FDA, 1978).

Source of Microbiological Cultures

Microbial cultures were obtained from the sources indicated in Table (1). Lactic acid bacteria cultures were activated by inoculation in 11% reconstituted skim milk (RSM) at 37°C/18 hr except *L. lactis* and *Lc. mesenteroides* incubated at 30°C/18 hr. *Cl. perfringens* strain was propagated anaerobically in RCM broth (Oxoid) at 37°C.

Haemolysin Detection

The isolates which have been identified as *Cl. perfringens* were chosen to be examined for haemolysin production. Strains were grown overnight in broth. Then broth cultures were centrifuged (10000 g, 4°C, 15 min). The supernatants were filtered through Millipore membrane filters (0.2 µm). Haemolysin activity was assayed on blood agar plates by pippetting the sterile culture filtrates into wells (5 mm diameter) punched in the agar plates. The plates were incubated at 37°C for 24 hr. Zones of haemolysin were measured and the titre was carried out according to FDA, 1978.

Inbibition of *Cl. perfringens* by Lactic Acid Bacteria

Spot-on-the-lawn Method

The lactic acid bacteria (LAB) were screened for potential inhibitory activity against *Cl. perfringens* by the spot-on-the-lawn method. Trypticase soy agar supplemented with 0.5% yeast extract (TSAYE) was used as the bottom agar. The plates were inoculated with 2 µl of overnight MRS broth cultures of the tested LAB and incubated for 48 hr at 10°C and 30°C. Perfringens soft agar was used to prepare the lawns of *Cl. perfringens* which was seeded at a final concentration ~10^4 cfu/ml, and poured over the colonized TSAYE plates. Plates were further incubated anaerobically for 24 hr at 35°C and then checked for inhibition zones (Okereke and Montville, 1991).

Agar Well Diffusion Method

The cell-free supernatant fluids of 24-hr old lactic acid bacteria cultures were obtained by centrifuging at 4000 rpm for 15 min at 4°C. Supernatant fluids were filter-sterilized through 0.22 µm membrane (Millipore) and pH adjusted to 6 with 2 N NaOH to exclude the organic acid effects. The activity of resulting solutions were tested against the pathogen using agar well diffusion assay described by Lyon and Gletz (1993).

Table (1): Source of microbiological cultures.

Strain	Source
Lactobacillus plantarum DSA 20174	Cairo Mircen
Pediococcus acidilactici B-1153	Northern Regional Research Lab. (NRRL), Illinois, USA
Lactobacillus rhamnosus B-445	Northern Regional Research Lab. (NRRL), Illinois, USA
Lactobacillus gasseri B-14168 (Sub-group B_1 of the acidophilus group)	Northern Regional Research Lab. (NRRL), Illinois, USA
Leuconostoc mesenteroides spp *mesenteroides* B-118	Chr. Hansen's Lab., Denmark.
Lactococcus lactis	Chr. Hansen's Lab., Denmark
Lactobacillus acidophilus	Chr. Hansen's Lab., Denmark
Lactobacillus helviticus	Chr. Hansen's Lab., Denmark
Enterococcus faecium	Dairy Microbiology Lab., National Research Center
Clostridium perfringens ATCC3626 (EMCC11531).	Cairo Mircen.

Survival of *Cl. perfringens* in Feta Cheese

Cheese Manufacture

Feta cheese was manufactured as described by Robinson and Tamime (1991). The pasteurized milk was cooled and divided into five equal portions. Four portions were inoculated separately with either 2% *Lb. plantarum* or *P. acidilactici* or *L. lactis* or *Lb. rhamnosus*, respectively. The fifth portion served as control. All portions were inoculated in batches with proportional amounts of activated cultures of *Cl. perfringens* to bring out an initial inoculum size of ~10^5 cfu/ml. The inoculated cheese milk batches were incubated for 2 hr, then salted with 2% NaCl followed by rennet adding. The resultant cheese batches were wrapped in plastic closed containers and stored under refrigeration for 3 months and microbiologically examined: fresh and at time intervals 7, 15, 21, 30, 60 and 90 days.

Microbiological Analysis

Cheese samples (25 g) were homogenized for 1 min in 225 ml of a sterile solution (2% w/v) of sodium citrate. Analysis was carried out using the following procedures: *Lb. plantarum*, *Lb. rhamnosus* and *P. acidilactici* were counted on MRS agar (Oxoid). Plates were incubated at 37°C for 48 hr. *L. lactis* were counted on M17 agar (Oxoid) after incubation at 30°C/48 h. *Cl. perfringens* counts were carried out on perfringens agar (TSC) (Oxoid) according to Varadaraj (1993).

pH Measurement

The measurement of pH was performed during storage with pH meter model Hanna HT 4817.

RESULTS AND DISCUSSION

Clostridia in Feta and Processed Cheese

Results in Table (2) show that Clostridia spp. could be detected in 27 out of 50 samples of examined Feta cheese (54%). The range of counts varied between 40 to 3 x 10^4 cfu/g with a mean value of 9.6 x 10^2 cfu/g. The obtained results were relatively lower than those reported by Ibrahim (1986) and Ahmed (1987), which were 72% and 76% for Domiati and Kareish cheese, respectively. While nearly similar findings were reported by Sharaf *et al.* (1997) for Ras cheese (45%).

Table (2) also reveals that Clostridia spp. could be detected in 12 out of 30 (40%) of the examined processed cheese samples. The bacterial count ranged between 2 x 10 to 5 x 10^3 cfu/g with an average 2.8 x 10^2 cfu/g. Similar results were reported by Ibrahim (1986) who reported that 44% of the processed cheese contained Clostridia spp. A higher incidence was obtained by Abd Alla *et al.* (1996) who found that 75% of the processed cheese samples contain anaerobic spore-formers (Clostridia). Also, Nazem and Aman (1994) isolated Clostridia spp. from all of blowing processed cheese samples.

Clostridia spores in cheeses used for processing can survive heat process of 85-105°C, which is achieved during the melt process (Broughton, 1990).

However, the obtained results reveal that Clostridium species were found in Feta cheese with a relatively high incidence compared with processed cheese. Probable explanation for this difference in counts are the differences in pH, salt content, moisture content, method of manufacturing, handling and temperature abuse during transport and storage.

Frequency Distribution of Different Clostridium Species from Examined Cheese Samples

The isolates of Clostridium spp. were classified to: *Clostridium perfringens*, *Cl. sporogenes*, *Cl. butyricum* and *Cl. tyrobutyricum*. They were isolated at percentages of 28, 18, 6 and 2% from the examined Feta cheese samples, respectively. The same isolates were also recovered from processed cheese samples at varying percentages *Cl. perfringens* (20%), *Cl. sporogenes* (6.6%) and *Cl. butyricum* (13.3%) except *Cl. tyrobutyricum*, which failed to be detected in processed cheese samples (Table 3).

Similar species were recovered from Kareish cheese by Ahmed (1987) who reported that *Cl. perfringens* and *Cl. sporogenes* were isolated from 30% and 10% of the examined samples, respectively. While Nazem and Aman (1994) isolated *Cl: perfringens* from 62.5% of the blown processed cheese. They also reported that *Cl. perfringens* was

responsible for outbreak of food poisoning, the other species, *Cl. Sporogens*, *Cl. butyricum*, Cl. *bifermentans* and *Cl. tyrobutyrium* were responsible for late blowing, putrefaction and spoilage of the processed cheese. However, Ibrahim (1986) isolated different types with different percentages of Clostridia spp. from Domiati, Romy and processed cheese samples.

Table (3) also revealed that *Cl. perfringens* was the most frequent Clostridia in the examined cheese of both Feta and processed. In this respect, Sharaf (1997) reported that *Cl. perfringens* was the most predominant Clostridia species isolated from Ras cheese.

Hereupon, the increasing incidence of *Cl. perfringens* in the cheeses emphasizes that such products may constitute, a public health hazard and need much attention.

Haemolysin Production by the Isolates

Results in Table (4) reveal that most of the isolates of *Cl. perfringens* show haemolysin production, which is one of the virulence factors of the organism. All of the isolates from Feta cheese (18 isolates) were found to produce haemolysin (100%). Out of 11 isolates of *Cl. perfringens* from processed cheese samples, 9 strains (81.8%) showed haemolytic activity. Colonies usually show a double zone of haemolysis on blood agar plates with a clear inner theta-toxin zone and a hazy outer zone caused by alphatoxin production (Brynestad and Granum, 2002).

Concerning the haemolytic titres of the isolates, Table (4) reveal that haemolytic activity was slightly higher in the supernatant fluids obtained from the strains of *Cl. perfringens* isolated from Feta cheese than those from processed cheese.

Antagonistic Activity of Some Lactic Acid Bacteria Against *Cl. perfringens*

(a) By Spot-on-the-lawn Antagonism Method

Results of bacteriocin like inhibition activity and the potential of anticlostridial effect of nine different strains of lactic acid bacteria upon *Cl. perfringens* using spot-on-the-lawn method at 10 and 30°C is shown in Table (5). The results show that *Lc. Mesenteroides* did not produce inhibition zones at 10°C and 30°C. Meanwhile, *Enterococcus faecium* was also inactive and did not produce inhibition zone at 10°C, but the bacterium was positive and active at 30°C. It produced the least inhibition zone activity within the tested LAB strains. Controversially, *Lb. plantarum* was more active and show larger inhibition zone at 30°C than at 10°C. In contrast, *P. acidilactici* followed by *Lb. rhamnosus* and *L. lactis* were more active at 10°C and show higher anticlostridial activity than the other tested strains at this temperature. Moreover, *Lb. grassei* and *Lb. acidophilus* did not show any difference in their antibacterial activity when incubated at 10°C or 30°C. In this concern, Okereke and Montville (1991) noted that *Lb. plantarum* BN, *L. lactis* and *P. pentosaceus* ATCC 43200 were bacteriocinogenic at 4, 10 and 15°C against *Cl. botulinum*.

Table (2): Incidence of clostridia in examined cheese samples.

Type of tested samples	Number of samples	Positive sampes		Clostridia count (cfu/g)		
		No.	%	Min.	Max.	Average
Feta cheese	50	27	54	4x10	$3x10^4$	$9.6x10^2$
Processed cheese	30	12	40	2x10	$5x10^3$	$2.8x10^2$

Table (3): Distribution of clostridium species from examined samples.

Isolates	Type of tested samples			
	Feta cheese		Processed cheese	
	No. of samples	%	No. of samples	%
Cl. perfringens	14	28	6	20
Cl. sporogenes	9	18	2	6.6
Cl. butyricum	3	6	4	13.3
Cl. tyrobutricum	1	2	-	-

Table (4): Haemolysin production and activity of the positive strains isolated from Feta and processed cheese.

Type of tested samples	Number of isolates	No. of positive strains		Haemolytic titre				
		No.	%	1:2	1:4	1:8	1:6	1:32
Feta cheese	18	18	100	4	6	5	2	1
Processed cheese	11	9	81.8	4	2	2	1	-

Table (5): Inhibition of *Cl. Perfringens* by some strains of lactic acid bacteria.

Tested strains	Spot-on-the-lawn Antagonism Method		Agar well-diffusion Method
	Incubation temperature		Inhibition zone diameter (mm)
	10°C	30°C	
Lb. plantarum	+	++	16
P. acidilactici	++	+	18
L. lactis	++	+	15
Lb. rhamnosus	++	+	16
Lb. helviticus	-	+	5
Lb. gasseri	+	+	12
Lb. acidophilus	+	+	4
Ent. faecium	-	+	2
Lc. mesenteroides	-	-	-

- no inhibition
+ inhibition.
++ more inhibition.

b) By Agar Well-Diffusion Method

The results of antibacterial activity of the supernatants of LAB tested strains against *Cl. perfringens* via agar well-diffusion test are shown in Table (5). Supernatants of *Lb. plantarum*, *P. acidilactici*, *Lb. rhamnosus* or *L. lactis* inhibited the growth of the *Cl. perfringens* and this appeared by the difference in growth inhibition zones; 16, 18, 16 and 15 mm, respectively. In this respect, Barefoot and Nettles (1993), also, Nettles and Barefoot (1993) reported that *Lb. plantarum* produce the bacteriocins plantaricin A and plantain. Furthermore, Okereke and Montville (1991) suggested that low levels of *Lb. plantarum* BN or *L. lactis* in the presence of 3 or 4% NaCl, could be formulated into minimally processed refrigerated food products for protection against *Cl. botulinum* hazards. Also, these results confirm the observation of Rodgers *et al.* (2003) who reported the anticlostridial properties of *Lb. rhamnosus* and some other selected strains of LAB. Also, Bhunia *et al.* (1988) and Nettles and Barefoot (1993) reported that pediocin ACH, produced by *P. acidilactici*, is active against *Staphylococcus aureus*, *Listeria monocytogenes* and *Cl. Perfringens*. These findings are also in agreement with the current results and those found by Cintas *et al.* (1998) who mentioned that supernatants of either *P. acidilactici*, *L. lactis* or *Lb. sake* inhibited the growth of the food borne pathogens *Cl. perfringens* and *Cl. botulinum*.

The results in Table (5) also reveal that the inhibition zones produced by *Lb. helveticus*, *Lb. gasseri* and *Lb. acidophilus* extracts showed lower measures (5, 12 and 4 mm, respectively) than those obtained by extracts of the other tested strains. Although they reported to produce bacteriocins or bacteriocin like inhibitors (Barefoot and Nettles, 1993), they are not found to be suitable inhibitors of *Cl. perfringens*. Hereupon, L. plantarum, *Lb. acidilactici*, *Lb. rhamnosus* and *L. lactis* appeared to be promising cultures for further investigation for their effectiveness as a biopreservative agents against *Cl. Perfringens* during cheese storage at refrigerated temperature as they are employed throughout Feta cheese making.

Growth Behavior of *Cl. perfringens* with Lactic Acid Bacteria in Refrigeration Feta Cheese

(a) *Cl. perfringens*

The inhibitory effect of four selected LAB strains against *Cl. perfringens* in Feta cheese was determined and shown in Fig. (1). Cheese without LAB (control) showed continuous growth of *Cl. perfringens*, which was detected during storage period and reached a count estimated to be sufficient to produce enterotoxin (5×10^7 cfu/g) at the end of the refrigerated period. In contrast, in the presence of LAB strains, a continuous decrease of the viable counts of *Cl. perfringens* was observed. The population level of the pathogen at the end of storage was lower than the initial count with all the tested LAB strains. This coincides with the importance of starter cultures in cheese making that render the growth of clostridium spp. (Rilla *et al.*, 2003). Meanwhile, *Cl. perfringens* was eliminated similarly in cheeses manufactured with the four different LAB strains. But, *Pediococcus acidilactici* followed by *Lb. rhamnosus* showed higher inhibition effect than *Lb. plantarum* and *L. lactis* to control the pathogen

(Fig. 1-a,b,c and d). At the end of the refrigerated storage period (3 months), *P. acidilactici* showed the highest activity against *Cl. perfringens* as the count decreased from 9 x 10^5 to 8.9 x 10 cfu/g (Fig. 1-b) while counts were recorded 6.8 x 10^2, 4 x 10^2 and 8 x 10^2 cfu/g when using *Lb. plantarum*, *Lb. rhamnosus* and *L. lactis*, respectively (Fig. 1-a,c and d). The obtained results are in agreement with those found by Cintas *et al.* (1998) who reported that *P. acidilactici* and *L. lactis* were very active against Clostridia spp; this effect was attributed to pediocin PA and nisin A, respectively. Also, Ziemer and Gibson (1989) reported that *Lb. rhamnosus* was effective in prevention of *Cl. difficili diarrhoea*. Moreover, *Lb. rhamnosus* was found to produce substance having potent inhibitory effect upon a wide range of bacterial species (Silva *et al.*, 1987). In addition, Hurst (1981), Delves-Broughton (1990) and Mishra *et al.* (1996) recorded that nisin, which is produced by *L. lactis* spp lactis was considered as a bacteriocin accepted as food preservative with a broad spectrum of antibacterial effect against Gram-positive organism including Clostridium spp.

(b) Lactic Acid Bacteria

Growth of the four lactic acid bacterial strains as counts in Feta cheese during storage is shown in Fig. (1). Similar behaviour (increasing counts) were recorded from the first week of storage and up to 21-days old cheese. Then, a progressive count decrease was recorded for all of LAB up to the end of the storage period. The obtained results coincide with those obtained for Kareish cheese and Feta cheese by Effat *et al.* (2001) and El-Kholy *et al.* (2003).

Respectively, *Lb. rhamnosus* showed the highest counts all over the storage period (Fig. 1). This could be attributed to the ability of *Lb. rhamnosus* to grow at low temperatures (Marshall and Tamime, 1996).

The pH determined during storage period indicated that there was no difference between the treatments, pH (Fig. 2), and the pH values were not reached the range to affect the growth of *Cl. perfringens*. Therefore, it is likely to neglect this parameter from the side of microbial interactions.

In conclusion, the results of this study indicate that the presence of *Cl. perfringens* in cheese must be regarded as a highly public health hazard, because it has been well-established that this pathogen grow likely and produces a number of potential virulence factors in such products. Therefore, it could be suggest to use the anticlostridial starter, besides, their technological roles in cheese processing will also increase the cheese quality and safety due to the antimicrobial activity of the cultures specially against the danger of *Cl. perfringens*.

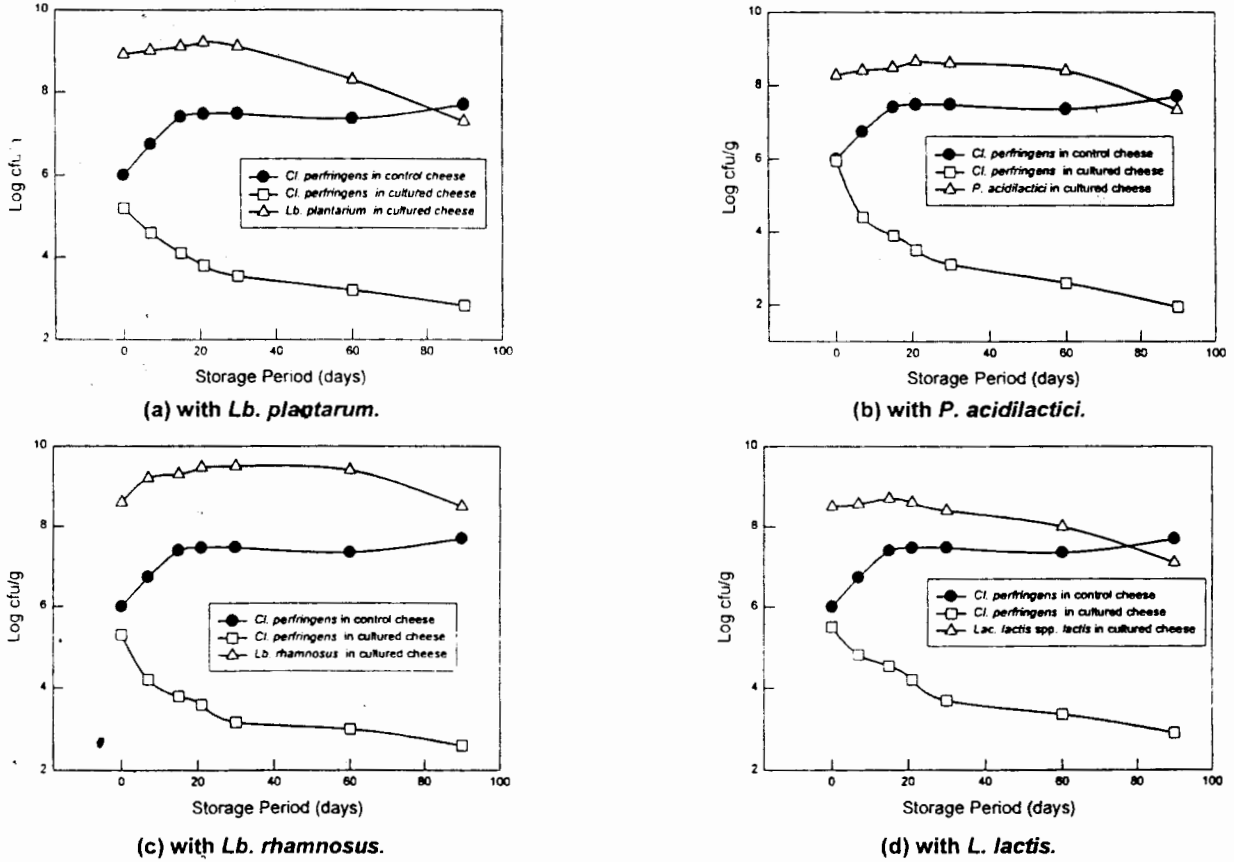

Fig. 1. Evaluation of selected anticlostridial starter cultures upon *Cl. perfringens* throughout storage of Feta cheese.

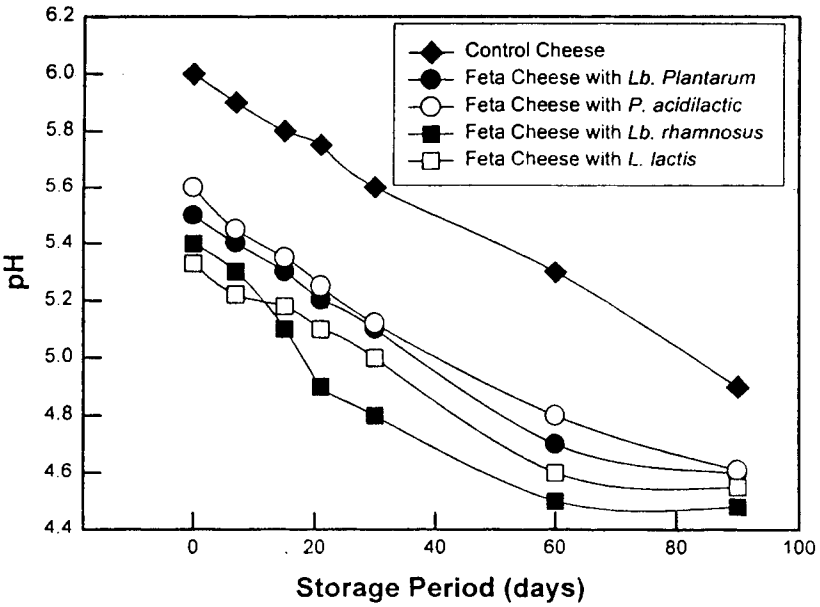

Fig. 2. pH values of Feta cheese during storage at refrigerator temperature.

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بكتريا *Clostridium perfringens* فى الجبن الفيتا والمطبوخ ونشاط الميكروب فى وجود بكتريا حمض اللاكتيك

تم دراسة مدى تواجد أنواع من البكتريا اللاهوائية من جنس Clostridium فى الجبن الفيتا والمطبوخ وقد تم عزل أربعة أنواع من ميكروب Clostridium وذلك من الجبن الفيتا وثلاثة أنواع من الجبن المطبوخ بنسبة ٥٤ ، ٤٠% على التوالى . وقد لوحظ عند التصنيف أن النوع *Cl. perfringens* قد تواجد بنسبة أعلى من الأنواع الأخرى فى كلا من النوعين من الجبن وكذلك أظهرت الدراسة قدرته الكبيرة على إنتاج بعض العوامل المرضية مثل إنتاجه للهيموليسين. أما الأنواع الأخرى فقد وجدت بنسب مختلفة باختلاف نوع الجبن . وقد درست أيضا قدرة بعض أنواع بكتريا حامض اللاكتيك على إنتاج مواد ذات تأثير مثبط على نمو *Cl. perfringens* وذلك بطريقتين . فقد أظهرت النتائج القدرة التثبيطية العالية لـ *Pediococcus acidilactici* يليها *Lactobacillus plantarum* و *Lb. rhamnosus* ثم *Lactococcus lactis* بينما كانت بكتريا *Enterococcus faecium* أقل الأنواع تأثيرا ولم تظهر السلالة المستخدمة من *Leuconostoc mesenteroides* أى تأثير على هذا الميكروب المرضى .

وأيضا تمت دراسة سلوك ميكروب *Cl. perfringens* مع وجود أنواع من بكتريا حامض اللاكتيك عند صناعة الجبن الفيتا . وقد أظهرت الدراسة على أن التلقيح ببكتريا *P. acidilactici* فى صناعة الجبن الفيتا والتخزين فى الثلاجة أظهرت أفضل النتائج فقد انخفضت أعداد بكتريا *Cl. perfringens* من ٩ × ١٠ ° إلى ٨,٩ × ١٠ خلية / جم من بداية التصنيع وحتى ثلاثة أشهر من التخزين.