

## PREVALENCE AND BEHAVIOUR OF *AEROMONAS HYDROPHILA* IN RAW MILK AND REFRIGERATED SOFT CHEESE

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### ABSTRACT

The prevalence of *A. hydrophila* in marketing raw milk, Kareish and Domiati cheese was investigated. A total of 120 samples (50 raw milk, 35 Kareish cheese and 35 Domiati cheese), collected from different localities in Cairo Governorate and examined for the presence and countable population of *A. hydrophila*. The results indicated that 38% of raw milk samples were contaminated with *A. hydrophila* and showing colony counts varying from  $5 \times 10^1$  to  $3.2 \times 10^4$  cfu/ml. while the contamination rates of *A. hydrophila* in Kareish and Domiati cheese samples were 57% and 20%, respectively and colony counts varying from  $6 \times 10^2$  to  $1.3 \times 10^5$  and  $4 \times 10^1$  to  $2.1 \times 10^3$  of Kareish cheese and Domiati cheese samples respectively. The survival of *A. hydrophila* in Domiati cheese salted with 2% and 5% NaCl and stored at  $5 \pm 1^\circ\text{C}$  was studied. Domiati cheese salted with 2% and stored at  $5 \pm 1^\circ\text{C}$  had a suitable condition for growth of bacterium for about two and half months. The last detection (60 cells/g) was observed at 10th week (pH 3.39). Also results indicated that the high concentration (5%) of salt has destructive effect against the *A. hydrophila* at the 4<sup>th</sup> day of cold storage (pH 4.1).

### INTRODUCTION

*Aeromonas hydrophila* are gram-negative, motile bacilli and widely distributed in nature even in chlorinated water. It has been reported as foodborne pathogen (Carnahan et al., 1991). The bacterium capable to induce intestinal and extraintestinal infection for human (Cahill, 1990).

Isolation of *Aeromonas* from milk and dairy products has been reported by Kirove et al., (1993b), Santos, et al., (1996) and Khalil, (1997). *A. hydrophila* could play an important role in spoilage of products stored at low temperature due to its psychrotrophic character and liberation of extracellular enzymes (Beuchat, 1991).

The relation between NaCl and pH values on *A. hydrophila* in dairy products still conflicting

additionally the obvious implications of food poisoning that can grow readily at refrigeration temperature increasing the necessity to secure the behaviour of pathogen in white soft cheese of different levels of salt during storage at refrigeration temperature.

## **MATERIALS AND METHODS**

### **Sampling :**

120 samples (50 raw milk, 35 domiati cheese and 35 karkash cheese) were collected from different localities in Cairo Governorate.

### **Quantitative detection of *A. hydrophila*;**

Twenty five ml/g of each sample were added to sterile container contained 225 ml of tryptose soya broth plus ampicillin (30 mg/l) to form tenth fold dilution from which decimal dilutions were prepared according to **A.P.H.A. (1985)**. Amount of 0.1 ml from each dilution was evenly spread onto duplicated Starch Ampicillin Agar (**Palumbo et al., 1985**). Inoculated plates were incubated at 30°C/ 24 h. The countable plates showing yellow with clear haloes (amylase positive) on addition of Lugol iodine solution were computed.

### **pH measurement (using pH meter Jenco-Model 609) :**

pH of milk containing contaminated rennet was measured directly by introducing electrode of pH into the sample. pH of cheese sample was measured by aseptically added 10 g. of the sample to 90 ml of distilled water to be homogenized by using a blender and the electrode of pH meter was immersed into the cheese emulsion. The results of pH values were recorded.

### **Survival of *A. hydrophila* in soft cheese:**

Strain: The type strain of *A. hydrophila* (NCTC 8049) was used. The strain was provided by QUB Food Science Center, UK. Preserved in semi solid medium. The strain was cultivated on brain heart infusion broth (**Santose et al, 1995**).

### **Domiati cheese manufacture:**

Raw buffalo's milk samples (ea, 10kg) were obtained from the herd of the Faculty of Agriculture Al-Azhar Univ. The milk samples were Laboratory pasteurized at 63°C/30 m., then tempered at 38°C. Calcium chloride (0.1%) was added. *A. hydrophila* ( $2 \times 10^7$  cells/ml) was artificially inoculated into calf rennet. The contaminated rennet was added to pasteurized milk. Salt was added with two levels 2% and 5% of inoculated milk. Salted curds were pickled in its whey and stored at refrigeration temperature ( $5 \pm 1^\circ\text{C}$ ). Samples were taken directly from artificially contaminated milk, the curd at 0 time, daily and weekly during the storage period. Cheese samples were

examined for *A. hydrophila* count and pH values.

*Quantitative detection of bacteria as mentioned before:*

## RESULTS AND DISCUSSION

The results given in table (1) showed that, 38% of raw milk samples were contaminated with *A. hydrophila*, with colony counts ranged from  $5 \times 10$  to  $3.2 \times 10^4$  cfu/ml.

The potential importance of raw milk as a source of *Aeromonas* spp. has been demonstrated by **Varnam & Evans (1991)**; **Hafez & Halawa (1993)** and **Kirov et al., (1993b)**. These microorganisms are commonly present in farm feed, water, soil, faeces and equipment used thus contaminate the surface of udder, teats and get into milk. Venice the role of raw milk as a vehicle of transmission causing milk-borne disease is well documented (**Robinson et al., 1984**). The overall incidence of *Aeromonas* spp. was nearly similar to those reported by **Saad (1991)**, who mentioned that *Aeromonas hydrophila* could be isolated from 30% and 28% of 100 raw milk samples examined, using MacConkey and Rimler shoot's agar, the average count of  $3.2 \times 10^2$  and  $3 \times 10^2$  from both media, respectively, while 38% of the examined samples were positives using MPN technique, while lower incidences were reported by **El-Gamal (1997)** who tested 150 samples of raw milk and pasteurized milk for the presence of motile *Aeromonas* spp. and found that the motile *Aeromonas* were occurred in 5% and 3% of examined samples using direct plating methods, respectively.

From the foregoing results it was observed that the contamination rate of *Aeromonas* in kareish cheese samples examined was 57.1% (20 of the 35 samples were positive) with counts varying from  $6 \times 10^2$  to  $1.3 \times 10^5$  cfu/g (table, 1). While in Domiati cheese the contamination rate of *Aeromonas* was 20 %. Only 7 of the 35 samples were positive. The bacterial counts ranged from  $4 \times 10$  to  $2.1 \times 10^4$  cells/g (table, 1). **El-Prince (1996)** isolated *Aeromonas* species from 14 and 16% of the examined Domiati cheese samples using MacConkey mannitol ampicillin agar (MMA) and trypticase soya ampicillin agar (TSA) with average count of  $10 \times 10^4$  and  $1 \times 10^4$ /g, respectively. While, the percentages of positive samples in kareish cheese were 66 and 64%, with counts of  $5 \times 10^3$  to  $9 \times 10^4$ /g. **El-Dweny (2000)**, mentioned that the minimal counts of *Aeromonas* spp. in refrigerated cheese was  $1.5 \times 10^3$ , the maximal count was  $6 \times 10^5$  with a mean  $1 \times 10^4$  cell/g.

Fig. (1&2) illustrate- the behaviour of *A. hydrophila* in refrigeration Domiati cheese with 2% salt and stored at 5–10°C. It was observed that the low temperature of storage is suitable for growth of bacterium in Domiati cheese of low salt content (2%), for about one and half month to reach  $1 \times 10^8$  cells/g (pH 3.88) due to its psychrophilic nature and tolerate the low level of salt.

On long storage the population of *A. hydrophila* was reduced gradually to  $3 \times 10^5$  cells/g (pH 3.67),  $9 \times 10^4$  (pH 3.38),  $7 \times 10^2$  (pH 3.38), at 7<sup>th</sup>, 8<sup>th</sup> and 9<sup>th</sup> weeks of storage. The last detection (60 cells/g) was recorded at 10<sup>th</sup> week (pH 3.39). **Hafez (1993)** reported that *A. hydrophila* remained viable in Domiati cheese stored in refrigerator for ten weeks. He attributed the viability of the organisms in the cheese to absence of the starter culture which play an important role in inhibition of some pathogens. **Palumbo et al., (1985)** reported that at refrigerated temperature, *A. hydrophila* tended to be more sensitive to lowering of pH than at higher temperatures.

Papageorgiou and **Marth (1989)** reported that *A. hydrophila* survived in Fetta cheese of pH 4.3 for 10 weeks. During initial stage of ripening the pathogen will liberate enzymes and toxins at acid pH (4.3) to generate a big problem to the health of susceptible consumer, especially those consumed fresh white soft cheese.

The fate of *A. hydrophila* in Domiati cheese containing 5% salt and stored at  $5 \pm 1^\circ\text{C}$ , were reported in Fig. (3&4). The results of experiment indicated that the high concentration of salt has destructive effect against the pathogen. At the first day of storage the population of *A. hydrophila* was reduced to reach  $1 \times 10^3$  cells/g (pH 4.73), at the second day the count was  $6 \times 10^2$  cells/g (pH 4.52) and at the third day the number was  $10^2$  cells/g (pH 4.31). Whereas artificially inoculated bacterium failed to be detected in the product (pH 4.1) at the 4<sup>th</sup> day of old storage, this may explain the increasing salt concentration in white soft cheese combined with acidic pH will eliminate the pathogen from the product.

**Table 1 :** Prevalence of *Aeromonas hydrophila* in examined samples collected from different localities in Cairo Governorate.

Type of samples	No. of examined samples	+ ve samples		Range (cfu/g. or ml)
		No	%	
raw milk	50	19	38	$5 \times 10^1 - 3.2 \times 10^4$
Kareish cheese	35	20	57.1	$6 \times 10^2 - 1.3 \times 10^5$
Domiati cheese	35	7	20	$4 \times 10^1 - 2.1 \times 10^3$

Fig. (1 & 2)  
Survival of *A. Hydrophila* in Domiati cheese salted with 2% NaCl and stored at 5+10C.

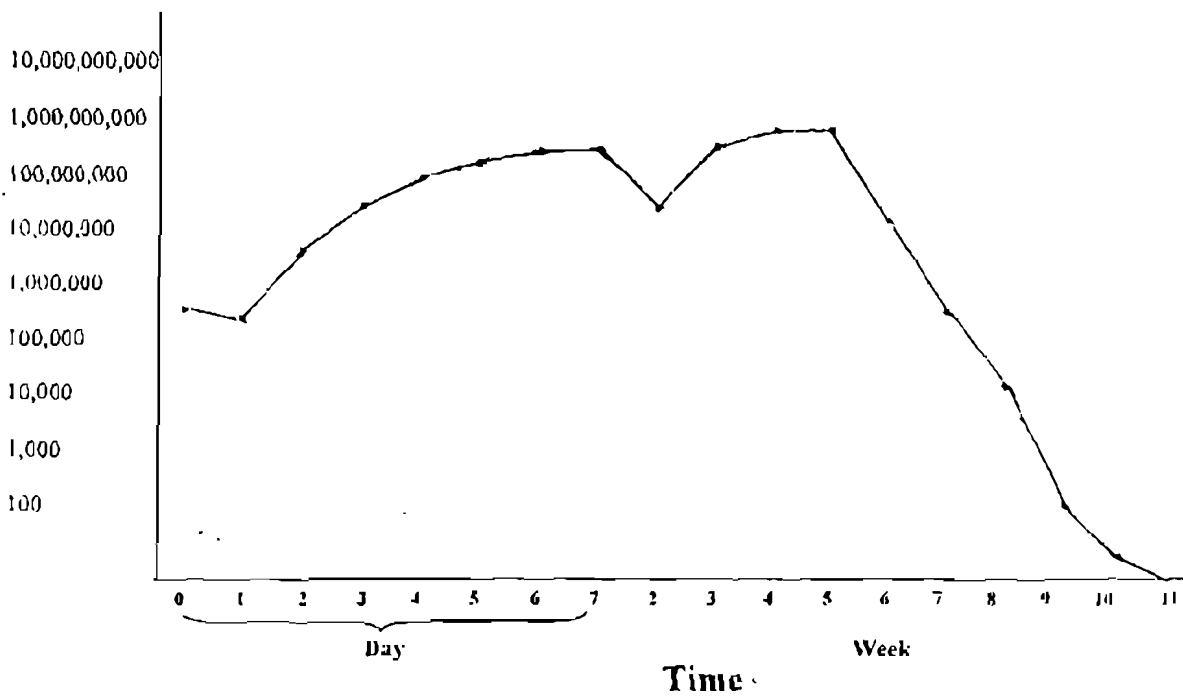
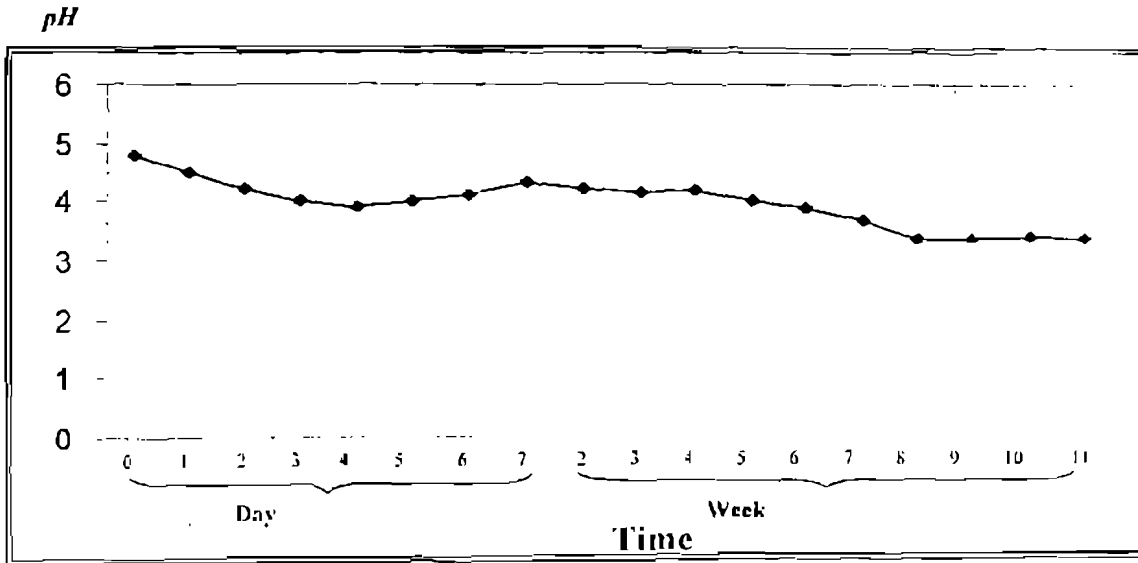
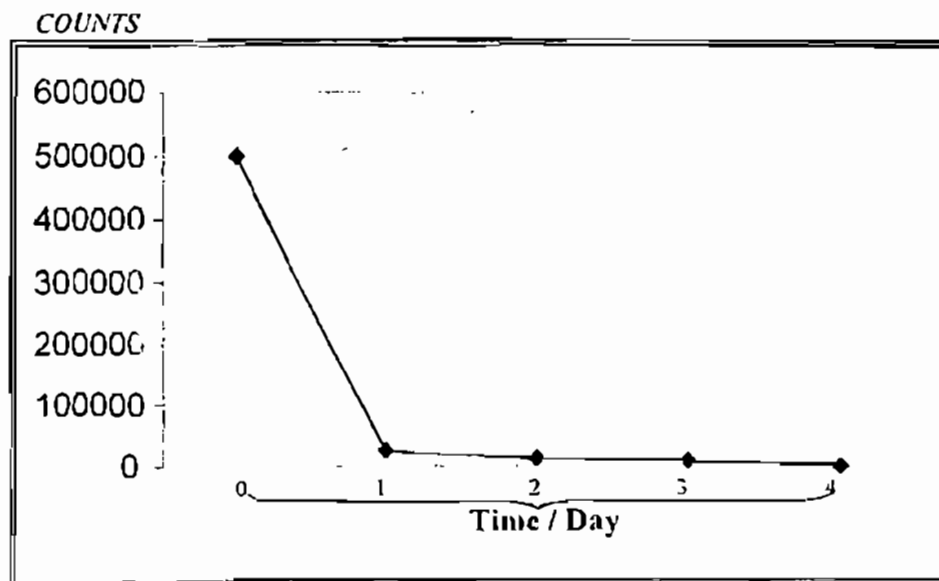
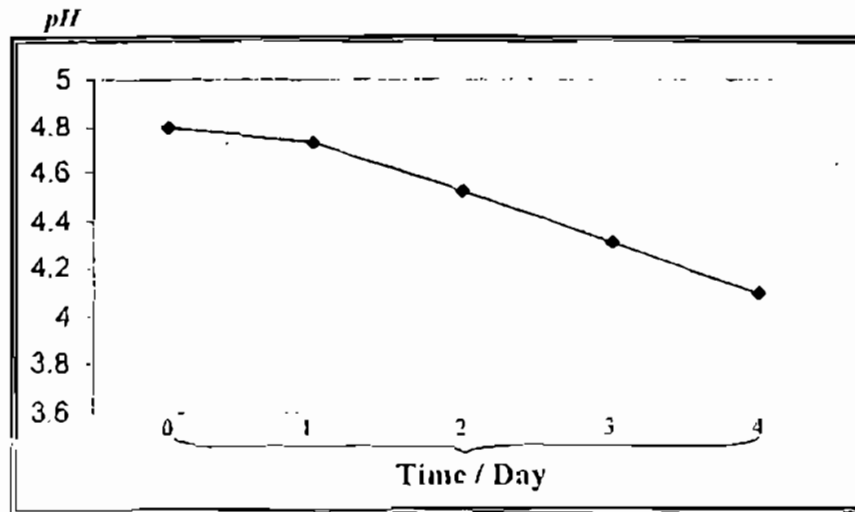


Fig. (3 & 4)

Survival of *A. Hydrophila* in Domiati cheese salted with 5% NaCl and stored at 5+1oC.



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## الملخص العربى

سلوك بكتريا الايرومونات هيدروفيليا فى اللبن الخام وفى الجبن الطرى  
المخزن على درجة حرارة الشلاجة

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لمعرفة مدى إنتشار ميكروب إيرومونات هيدروفيليا فى اللبن المسوق والجبن القريش وكذلك الجبن الدمياطى، فقد تم تجميع ١٢٠ عينة كالاتى ٥٠ عينة من اللبن الخام المسوق و٣٥ عينة من الجبن القريش و ٣٥ عينة من الجبن الدمياطى من مناطق مختلفة من محافظة القاهرة. ولقد أظهرت التحليلات أن ٣٨٪ من عينات اللبن الخام ملوثة بميكروب إيرومونات هيدروفيليا وكانت تتراوح أعدادها من ١٠×٥ إلى ٢٢×٤١٠ خلية/مل بينما كان معدل التلوث بنفس الميكروب فى الجبن القريش هو ٥٧٪ وكان عددها يتراوح بين

٦ × ٢١٠ إلى ٣ × ١٠٠ خلية/جم وفى الجبن الدمياطى كان معدل التلوث ٢٠٪ بأعداد تتراوح بين ٤ × ١٠ إلى ٢١ × ٢٠٠ خلية/جم على التوالى .

تم تتبع نمو ميكروب الأيرومونات هيدروفيليا فى الجبن الدمياطى المصنع من لبن ملوث (٢ × ٧١٠ خلية/مل ايرومونات هيدروفيليا والمضاف إليه ٢ ، ٥٪ ملح و المخزن على درجة حرارة ٥+١م وكذلك قياس ال pH طوال فترات التخزين.

وقد أظهرت النتائج أن الجبن الدمياطى المضاف إليه ٢٪ ملح كان مناسب لنمو و نشاط هذا الميكروب حيث ظل بالجبن لمدة شهرين ونصف الشهر (١ × ٨١٠ خلية/جم) عند pH (٣.٣٩).

وأظهرت النتائج أيضاً أن الجبن الدمياطى المصنع (٥٪ ملح) قد إنخفضت أعداد بكتريا الايرومونات هيدرومونات هيدروفيليا بسرعة شديدة حيث لم يتمكن الكشف عنها فى اليوم الرابع من التصنيع والتخزين على درجة حرارة الشلاجة (PH 4.1).