PREVALENCE AND BEHAVIOUR OFAEROMONAS HYDROPHILA IN RAW MILK AND REFRIGERATED SOFT CHEESE

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ABSTRACT

The prevalence of A.hydrophila in marketing raw milk, Kariesh 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 5xl0 to 3.2x10^4 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 6xl0^2 to 1.3xl0^5 and 4x10 to 2.1x10^3 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±1°C was studied Domiati cheese salted with 2% and stored at 5±1°C had a suitable condition for growth of bacterium for about two and half months. The last detection (60cells/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 4th day of cold storage (pH 4.1).

INTRODUCTION

Aeromonas hydrophila are gram-negative, motile bacilli and widely distributed in nature ever in chlorinated water. Ithas 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 foodpoisoning 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.

MATERLL AND METHODS

Sampling :

120 samples (50 raw milk, 35 domiati cheese and 35 kariesh 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 oftryptose 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 ef 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)**.

Domiafi cheese manufacfure:

Raw buffalo's milk samples (ca, 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 ($2x10^7$ 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±1°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 5x10 to $3.2x10^4$ 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-bome 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 $3.2x10^2$ and $3x10^2$ from both media, respectivelly, 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 $6x10^2$ to $1.3x10^5$ 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 4x 10 to 2.1 x 10^4 cells/g (table, 1). **El-Prince (1998)** isolated Aeromonas species from 14 and 16% of the examined Domiati cheese samples using MacCkonkey mannitol ampicillin agar (MMA) and trypticase soya ampicillin agar (TSA) with average count of $10x10^4$ and $1x10^4$/g, respectively. While, the percentages of positive samples in kareish cheese were 66 and 64%,with counts of $5x10^3$ to $9x10^4$/g. El-Dweny (2000), mentioned that the minimal counts of Aeromonas spp. in refrigerated cheese was $1.5x10^3$, the maximal count was $6x10^5$ with a mean $1x10^4$ cell/g.

Fig. (1&2) illustrate- the behaviour of A. hydrophila in refrigeration Domiati cheese with 2% salt and stored at 5–1°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 $1x10^8$ 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 $3x10^6$ cells/g (pH3.67), $9x10^4$ (pH 3.38), $7x10^2$ (pH 3.38), at 7th, 8th and 9th weeks of storage. The last detection (60 cells/g) was recorded at 10th 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.

Papagcorgiou 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.

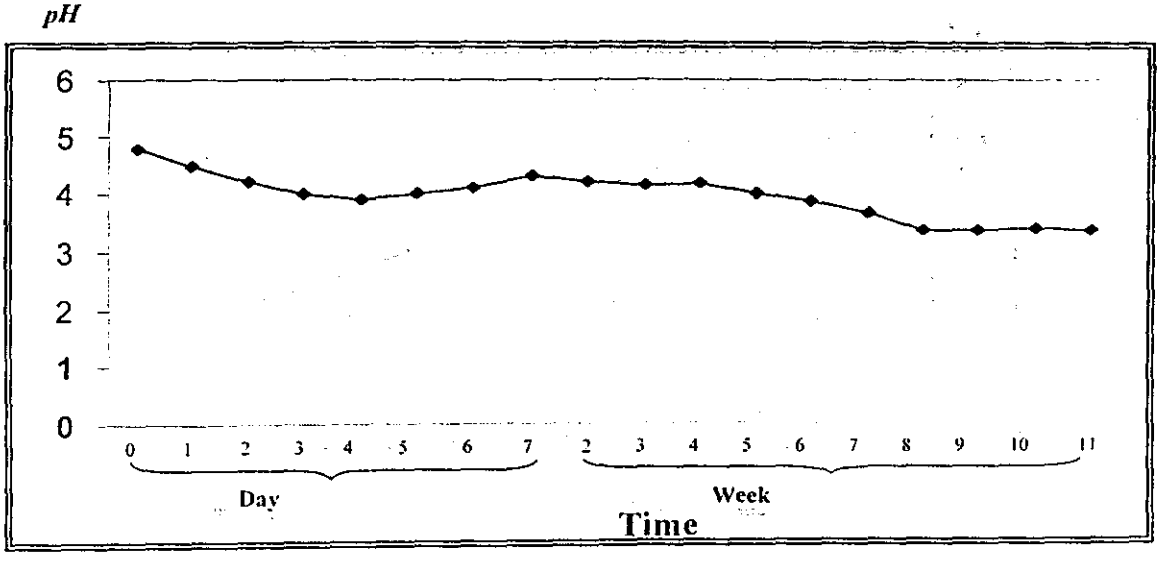
The fate of A.hydrophila in Domiati cheese containing 5% salt and stored at 5 ± 1^oC, were reported in Fig. (3&4). The results of experiment indicated that the high concentration of salt has destructive effect against the pathogen. At the first day of storage the population of A.hydrop hila was reduced to reach $1x10^3$ cells/g (pH 4.73), at the second day the count was $6x10^2$ 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 4th day of old storage, this may be 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	$5x10 - 3.2 x10^4$
Kareish cheese	35	20	57.1	$6x10^2 - 1.3x10^5$
Domiati cheese	35	7	20	$4x10 - 2.1x10^3$

Fig. (1 & 2)

Survival of A. Hydrophila in Domiati cheese salted with 2% NaCI and stored at 5+1oC.



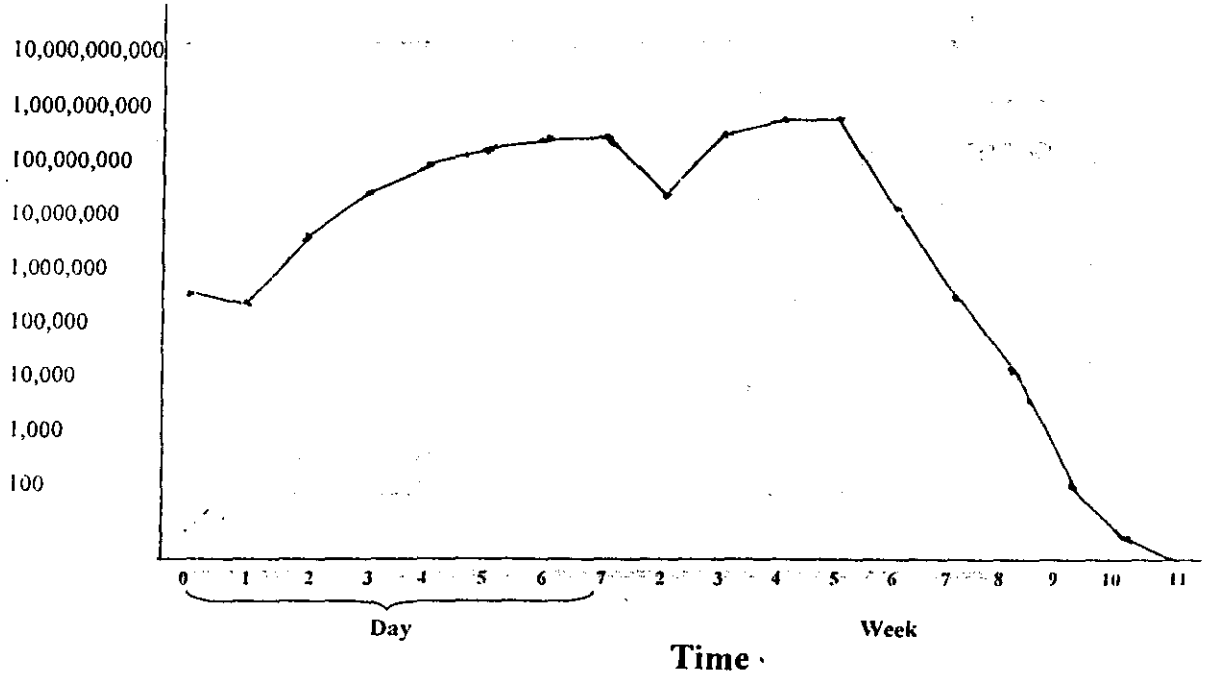
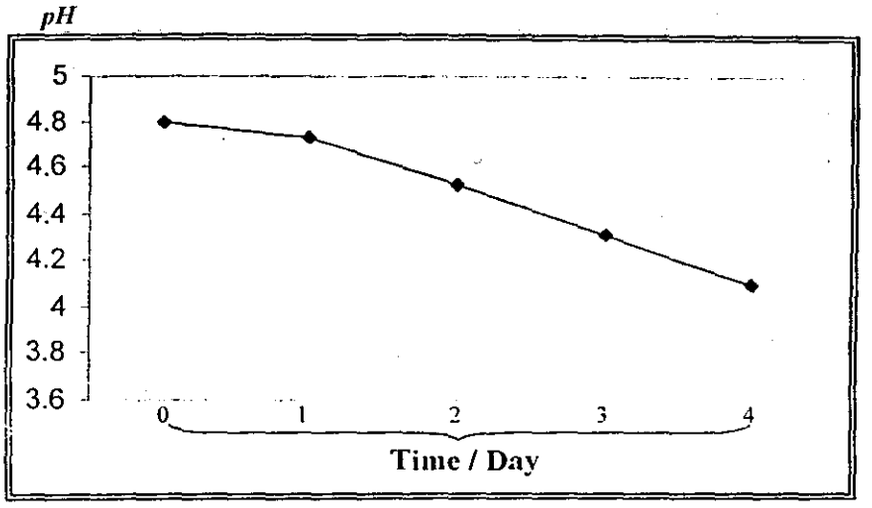
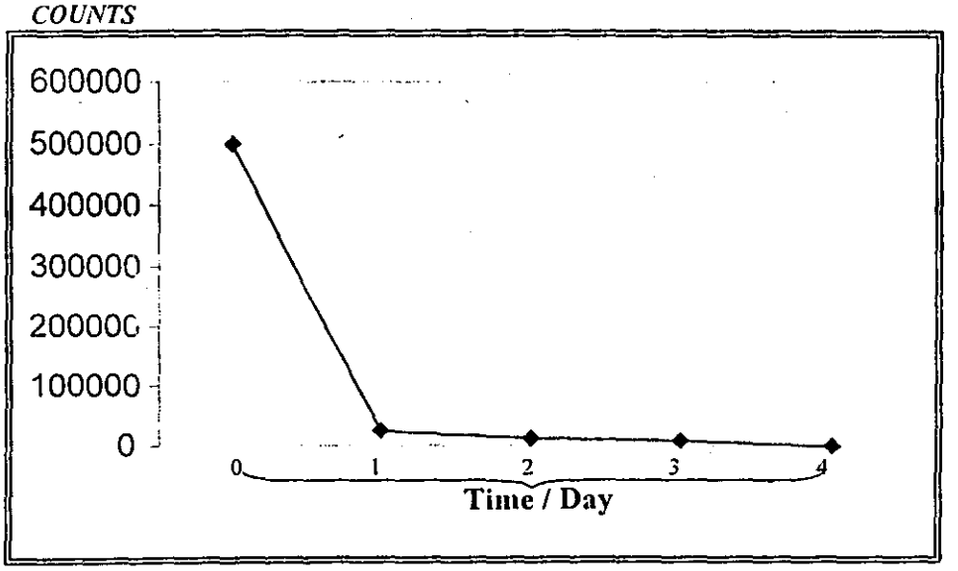


Fig. (3 & 4)

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





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الملخص العربى

سلوك بكتريا الإيروموناس هيدروفيلا فى اللبن الخام وفى الجبن الطرى المخزن على درجة حرارة الثلاجة

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لمعرفة مدى إنتشار ميكروب إيروموناس هيدروفيلا فى اللبن المسوق والجبن القريش وكذلك الجبن الدمياطى، فقد تم تجميع ١٢٠ عينة كالآتى 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 إيروموناس هيدروفيلا وكانت تتراوح أعدادها من ٥×١٠ إلى ٢ر٣ × ١٠ ٤ خلية/ مل بينما كان معدل التلوث بنفس الميكروب فى الجبن القريش هو ١ر٥٧٪ وكان عددها يتراوح بين

٦ × ١٠ ٢ إلى ٣ر١ × ١٠ ٥ حلية/جم وفى الجبن الدمياطى كان معدل التلوث ٢٠٪ بأعداد تتراوح بين ٤ × ١٠ إلى ١ر٢ × ١٠ ٣ خلية/جم على التوالى .

تم تتبع نمو ميكروب الأيرومونـاس هيدروفيلا فى الجبن الدمياطى المصنع من لبن ملوث (٢ × ١٠ ٧ خلية/مل ايروموناس هيدروفيلا والمضاف إلية ٢ ، ٥٪ ملح و المخزن على درجة حرارة ٥+١م وكذلك قياس الـ 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 × ١٠ ٨ خلية/ جم) عندpH (٣ر٣٩).

وأظهرت النتائج أيضاً أن الجبن الدمياطى المصنع (٥٪ ملح) قد إنخفضت أعداد بكتيريا الايروموناس هيدروموناس هيدروفيلا بسرعة شديدة حيث لم يتمكن الكشف عنها فى اليوم الرابع من التصنيع والتخزين على درجة حرارة الثلاجة (PH 4.1).