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ABSTRACT

The prevalence of A. hydrophila in marketing raw milk, Kariesh and Domiat cheese was investigated. A total of 120 samples (50 raw milk, 35 kariesh cheese and 35 Domiat 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 5x10 to 3.2x10^4 cfu/ml, while the contamination rates of A. hydrophila in kariesh and Domiat cheese samples were 57% and 20%, respectively and colony counts varying from 6x10^2 to 1.3x10^5 and 4x10 to 2.1x10^3 of kariesh cheese and Domiat cheese samples respectively. The survival of A. hydrophila in Domiat cheese salted with 2% and 5% NaCl and stored at 5±1°C was studied. Domiat 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 (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 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. 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.

**MATERIAL AND METHODS**

**Sampling:**

120 samples (50 raw milk, 35 domiati cheese and 35 kartesh 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 (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 min, 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 5x10^3 to 3.2x10^4 cfu/ml.

The potential importance of raw milk as a source of Aeromonas spp. has been demonstrated by Varanam & 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 Ringer shoot’s agar, the average count of 3.2x10^2 and 3x10^2 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 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 4x10 to 2.1x10^4 cells/g (table, 1). El-Prince (1996) isolated Aeromonas species from 14 and 16% of the examined Domiati cheese samples using MacCkonkey mannitol ampicillin agar (MMA) and tryptiase 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 5-10°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 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 $3 \times 10^5$ cells/g (pH 3.67), $9 \times 10^4$ (pH 3.38), $7 \times 10^2$ (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 Domia 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±1°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 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.

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

<table>
<thead>
<tr>
<th>Type of samples</th>
<th>No. of examined samples</th>
<th>+ ve samples</th>
<th>Range (cfu/g. or ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>%</td>
<td></td>
</tr>
<tr>
<td>raw milk</td>
<td>50</td>
<td>19</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5x10^4 - 3.2x10^4</td>
</tr>
<tr>
<td>Kareish cheese</td>
<td>35</td>
<td>20</td>
<td>57.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>6x10^2 - 1.3x10^5</td>
</tr>
<tr>
<td>Domiati cheese</td>
<td>35</td>
<td>7</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4x10^1 - 2.1x10^3</td>
</tr>
</tbody>
</table>
Survival of A. Hydrophila in Domiatı cheese salted with 2% NaCl and stored at 5±1°C.
Fig. (3 & 4)
Survival of A. Hydrophila in Domiati cheese salted with 5% NaCl and stored at 5+1 °C.

**pH**

![pH graph]

**COUNTS**

![Counts graph]


الالثاني

كلية الزراعة - جامعات العمر - مدينة نصر - القاهرة

لمعرفة مدى انتشار ميكروب إبروموناس هيدرفيليا في اللبن الم sok و في البقر والليمون وكذلك الجين الدمياطي، فقد تم
تجميع 12 عينة كاثانى 50 عينة من اللبن الم sok و 34 عينة من البقر و 34 عينة من الجين الدمياطي من
مناطق مختلفة من محافظة القاهرة. و لقد أظهرت التحليلات أن 38% من عينات اللبن الم sok ملوثة بـ ميكروب إبروموناس
هيدرفيليا وكانت تتراوح أعدادها من 0.1 إلى 2.6 × 10⁶ خلية/مل بينما كان معدل التلوث بنفس الميكروب في
الليمون البقر و 1.75% وكان عددنا يتراوح بين
6 × 10⁶ إلى 3 × 10⁷ خلية/مل و في الجين الدمياطي كان معدل التلوث 2% بأعداد تتراوح بين 100 × 10⁴ إلى 1 × 10⁶ خلية/مل.

تم تتبع نمو ميكروب ابروموناس هيدروفيليا في الجين الدمياطي المصنع من لبن ملوث (2 × 10⁶ خلية/مل)
ابوموناس هيدروفيليا معضو إلغاء 20% مل ح والمخزن على درجة حرارة 5 ± 1° C وكذلك قياس ال pH طوال فترة التخزين.

وقد أظهرت النتائج أن الجين الدمياطي المضاد إلغاء 20% مل ح كان مناسب لنمو و نشاط هذا الميكروب حيث ظل الجين
لعدة أشهر ونصف الشهر (11 × 10⁶ خلية/جم) عند 
pH 3.9 (1). 8

و أظهرت النتائج أيضًا أن الجين الدمياطي المصنع (5% مل ح) قد انخفضت أعداد بكتيريا الإبروموناس هيدروفيليا
بسرعة شديدة حيث لم يمكن الكشف عنها في اليوم الرابع من التصنيع والتخزين على درجة حرارة التخزين
(pH 4.1)