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## GROWTH AND SURVIVAL OF AEROMONAS HYDROPHILA IN COOKING BUTTER AT DIFFERENT STORAGE TEMPERATURES

(With 2 Fig.)

By

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### نمو وبقاء ميكروب الأيروموناتس هيدروفيليا في الزبد الفلاحي

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ايروموناتس هيدروفيليا ميكروب واسع الانتشار في الطبيعة ، معدى للانسان حيث انه يفرز أنواعاً عديده من السموم التي تسبب النزلات المعويه والاسهال ، وقد تم عزله بكثرة من الأغذية في السنوات القليلة الماضية . وقد بدأ الميكروب يأخذ اهتماماً من جانب المشتغلين بعلوم الأغذية خاصة وان هذا النوع من البكتريا يستطيع النمو والتكاثر على درجات الحرارة المنخفضة . لذلك تم حقن زبد فلاحى مبستر معملياً بميكروب الايروموناتس هيدروفيليا ، وحفظ في درجة حراره التلاجه والفريزر لمدة ستة أسابيع . وقد تبين من الفحص أن الميكروب تناقص في العدد ولكنه ظل متواجدا في الزبد لنهاية فترة التخزين بالرغم من انخفاض الرقم الهيدروجيني . وقد تم مناقشة الأهمية الصحيه لوجود هذا الميكروب وسمومه على الصحة العامة .

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### SUMMARY

Two lots of laboratory pasteurized cooking butter artificially contaminated with approx.  $10^7$  *Aeromonas hydrophila*/g were prepared. The first lot was stored at refrigerator temperature while, the other at freezing temperature. Samples were taken and examined initially, after 24 h and then weekly up to six weeks for pH value and number of *A. hydrophila*/g. More reduction in the counts of the organism occurred during freezing storage than refrigerator storage. *A. hydrophila* survived 6 weeks at pH 4.6. The public health significance of these findings is discussed.

### INTRODUCTION

*Aeromonas hydrophila* was first described in 1890 by ZIMMERMAN. It is widely distributed in nature (ABEYTA and WEKELL, 1988), and has been associated with diarrhoea. The bacterium is psychrotrophic and can produce virulence factors including a relatively heat -stable cholera- like enterotoxin and a heat labile cytotoxic enterotoxin (LJUNGH, et al. 1981, 1982. a, 1982 b, LJUNGH and WADSTROM, 1983 and TURNBULL, et al. 1984). *A. hydrophila* has become recognized as a potential cause of food- associated outbreaks of gastroenteritis (DOYLE, 1989). It has been isolated from a variety of foods including raw milk, meat, poultry and sea-foods (AYRES, 1960, NAGEL, et al. 1960, JAY, 1967, KIELWEIN, et al. 1969, HOOD, et al. 1984 and SIMARD, et al. 1984). *A. hydrophila* also has been recognized as a spoiler (LERKE, et al. 1965 and ALUR, et al. 1971). In addition, it has been implicated in outbreaks of bovine abortion (WOHLEGE MUTH, et al. 1972). It was found in the mid-1950 that refrigerated holding of fresh foods slows but does not prevent the growth of *A. hydrophila*. POPOFF (1984) indicated that *A. hydrophila* can grow at temperature from 0°C to 41°C, including refrigeration temperature, and it has been associated with spoilage of refrigerated foods of animal origin other than raw milk (ENFORS, 1979 and PALUMBO, et al. 1985 a). Also freezing does not inactivate the pathogen since ABEYTA, et al. (1986) isolated the bacterium from oyster samples which had been held frozen at -27°C for 1.5 year. Also it was less able to tolerate extremes of Na Cl and pH as the temperature was decreased (PALUMBO, et al. 1985 b).

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The possibility that this organism may represent a potential psychrotrophic foodborne pathogen has stimulated our interest in determining if isolates of *A. hydrophila* are capable of growth under condition that would occur in food. The present study describes the effect of refrigeration and freezing of butter on the growth of *A. hydrophila*.

### MATERIAL and METHODS

#### The organism:

*A. hydrophila* strain used in this study was isolated from, raw milk, it was cultured in trypticase soy broth with 10 µg of ampicillin/ml.

#### Preparation of butter:

A sample of cooking butter was obtained from Assiut City market, and it was laboratory pasteurized. Broth culture of *A. hydrophila* was added to the warmed butter to yield approx.  $10^7$  cells/g. A sample was taken after inoculation to determine initial count of *A. hydrophila* and pH value of butter.

The inoculated butter was divided into 2 portions, which were kept at refrigerator ( $4 \pm 1^\circ\text{C}$ ) and freezer (zero:  $-2^\circ\text{C}$ ). Also control samples were prepared. Inoculated butter and the control were analyzed initially, after 24 h, and then weekly up to 6 weeks to determine the pH value and *A. hydrophila* count.

#### Preparation of butter samples for examination:

Butter samples were prepared according to the method described in Standard Method for the Examination of Dairy Products (MARTH, 1978).

#### Enumeration of *A. hydrophila*:

The method suggested by OKRENED, *et al.* (1987) was employed. Samples were surface plated onto MacConkey's mannitol ampicillin agar containing 1% mannitol and 30 µg of ampicillin/ml. Plates were incubated at  $37^\circ\text{C}$  for 24 h. Typical colonies were picked up and confirmed as *A. hydrophila* according to POPOFF AND VERON (1976).

#### Meassurment of pH:

The pH of butter was determined by using an Orion pH meter, Model 201, equipped with standard combination electrodes.

## RESULTS

The obtained results are recorded in Fig. 1 and 2.

## DISCUSSION

It is evident from Fig. 1 that the number of *A. hydrophila* decreased gradually during storage at refrigerator temperature and reached  $9 \times 10^3$ /g by the end of 6 th week of storage.

Results in Fig. 2 show that substantial increase in number of *A. hydrophila* did not occur during storage of butter at freezing temperature with increasing the time of storage and the count reached  $1 \times 10^3$ /g by the end of storage period.

Generally, storage of butter at freezing temperature is highly recommended than refrigerator temperature.

Although, the obtained results proved that there are parallel statistical correlation between pH and detection of *A. hydrophila* yet, the organism still viable in butter during storage period. Similar correlations were found by PALUMBO, et al. (1985 a) who indicated that the organism tended to be more sensitive to lowering of pH at low temperature than at higher temperature.

The data presented here indicated that *A. hydrophila* can grow readily under condition at which food is held and this confirms the work of MYERS, et al. (1982) and PALUMBO, et al. (1985 a & b). Moreover, it was suggested by ABEYTA, et al. (1986) that the organism may produce enterotoxin during growth and it presents a potential health hazard. Necessary precautions should be taken to prevent transmission of the bacterium by food handlers into the food chain, also appropriate hygienic measures should be practiced since the bacterium sometimes occurs in faeces.

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Fig.,1:Count of *A.hydrophila* and changes in pH during storage of butter at refrigerator temp. ( $4 \pm 1^{\circ}\text{C}$ ).

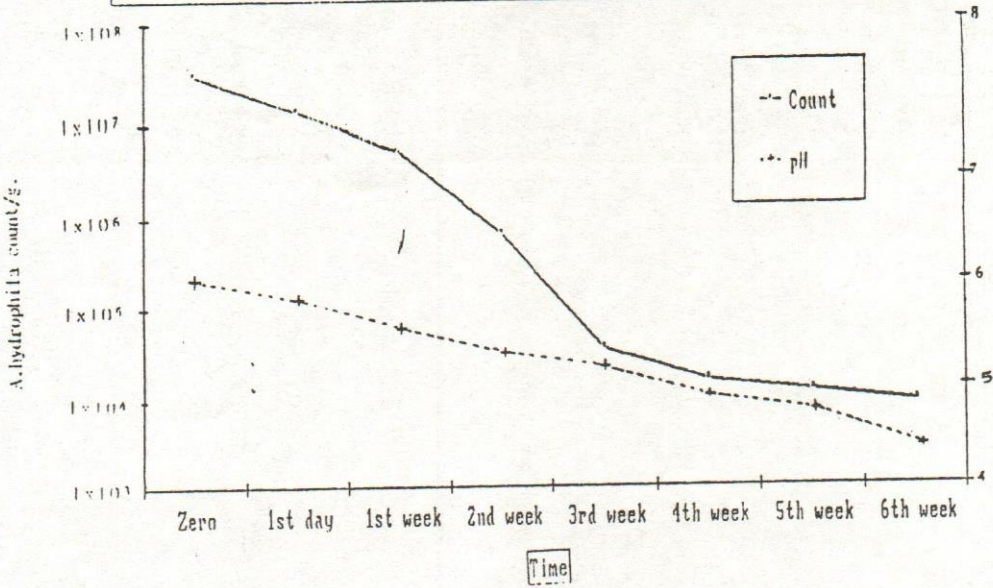


Fig.,2:Count of *A.hydrophila* and changes in pH during storage of butter at freezer temp. ( $0 - 2^{\circ}\text{C}$ ).

