PREVALENCE OF STAPHYLOCCUS AND AEROMONAS IN SOME SALTED DAIRY PRODUCTS

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ABSTRACT

120 samples of pickled white soft cheese (domiata cheese) and Mish were collected from local markets in Port Said governorate, Egypt. Samples were analyzed for sodium chloride level, determination of Staph. Spp at 3% and 10% Na CL, isolation of Staphylococcus aureus at 3% and 10% NaCL and Determination of Aeromonas spp. at 3% and 10% NaCL. Results obtained revealed that the mean values of the sodium chloride percentage were 3.7 ± 0.13 % in pickled domiata cheese samples and 6.1±0.14 % in mish samples. Incidence of Staph. spp in Pickled domiata cheese was 85% at 3% NaCL and 71.6% at10% NaCL. while in mish samples was 93% at3% NaCL and 83.3% at 10% NaCL. Incidence of Aeromonas spp at 3% NaCL was nil while at 10 % NaCL was 48.3% in pickled domiata cheese and in Mish 38.3% in. Incidence of A. hydrophila, A. caviea, A.trota and A.schubertii in Pickled domiata cheese were 25.7 %, 40%, 20% and 14.3%, respectively. While in Mish were 25%, 46.4%, 21.4% and 7.1% respectively.

Key words: Salted dairy products, halophilic bacteria, Staph. Spp., Aeromonas spp.

INTRODUCTION

Pickled white soft cheese (domiata cheese) and Mish are well-known as local types of cheese in Egypt. Handling of milk during cheese manufacture considered the main source of microbial contamination of cheese which affecting on cheese quality and render it unfit for human consumption (Yousef et al., 2001).

Staphylococcus spp. are considering one of halophilic bacteria which its presence in milk or milk products can cause a public health hazard. Staphylococcus aureus is responsible for food poisoning (ICMSF, 1986) S. aureus is consider as one of important food-borne pathogen. It is a versatile pathogen of humans and animals; it causes a wide variety of diseases ranged in severity from slight skin infection to more severe diseases such as pneumonia and septicaemia (Lowy, 1998). Staph.aureus is capable of producing several enterotoxins cause food poisoning in human with varying degree and generally characterized by nausea, diarrhea, abdominal cramps and emesis (Normanno et al., 2005) and (Brightwell et al., 2006). In the last few decades’ Staphylococcal food poisoning (SFP) has been reported as third cause of food-borne illnesses in the world (Zhang et al., 1998).

Aeromonas spp are considered one of the most important halophilic bacteria which are widely distributed in nature. Moreover it is considered as food borne pathogen (Palumbo et al., 1992). Aeromonas spp. is pathogens that cause food-borne gastroenteritis, extra intestinal symptoms such as septicemia, meningitis, endocarditis and osteomyelitis with a high mortality rate in immune-compromised person (Gold and Salit, 1993). The mechanisms by which Aeromonas spp cause diarrhea has been known that they produce enterotoxins, certain enzymes, they are able to adhere cell membranes and invade them (Kirov et al., 1994). The presence of such bacteria in milk products may be due to direct contact with contaminated sources in dairy farm environment, excretion from udder of an infected animal or during processing of cheese. (Oliver et al., 2005).

MATERIALS AND METHODS

1. Collection of samples
One hundred and twenty random samples of pickled white soft cheese and Mish (60 of each) were collected from different markets in Port Said city,
and then taken aseptically to the laboratory immediately, where it is subjected to chemical and microbiological examinations.


250 gm. of each sample were transferred aseptically in a sterilized polyethylene sac and thoroughly mashed in a sterile blender before being emulsified in the diluent solution under aseptic condition then divided into two subsamples for chemical examination and bacteriological examination.

0.1 ml from each dilution was inoculated in duplicated plates containing standard plate count agar following by spread plate technique and the plates were incubated at 37°C for 48 hours, then the total slight and moderate halophiles were counted and expressed as CFU/g.

3. Chemical Examination


3 grams of prepared sample were weighted in 200 ml Erlyn – Meyer flask. 25 ml of N/10 silver nitrate solution, 10 ml of halogen free nitric acid and 50 ml of distilled water were added and the mixture was boiled. 15 ml of 5% potassium permanganate solution in 5 ml portion were added during boiling (till the solution become yellowish and clear). Then the solution was allowed to cool then filtered into 200 ml volumetric flask, the filter paper was washed thoroughly with distilled water at 20°C, and the filtrate was made up to standard volume. The excess of silver nitrate in 100 ml of the clear solution was titrated against 0.1 N potassium thiocyanate solution (9.71 g / liter) using 2 ml of saturated solution of iron alum as indicator. The salt content was calculated according to the following equation:

\[
Na CL \% = 2 \times (25-R) \times 0.00584 \times 100 \\
Weight (3g)
\]

4. Microbiological Examination

4.1. Determination of total Halophilic bacterial counts (A.P.H.A.2004)

Preparation of serial dilution:

10 gm of each sample was transferred in sterile stomacher bag with 90 ml synthetic sea water solution (3% NaCL for Slight or10% NaCL for moderate Halophilic counts). Apply in a stomacher lab-blender (2000 rpm) used to homogenize the specimen for 2 minutes to make a1:10 dilution (wt/vol) then decimal dilution of 10⁻², 10⁻³, 10⁻⁴ and 10⁻⁵ were prepared.

4.2. Identification of isolated Staphylococci organisms according to (FDA, 2001). Suspected colonies of Staphylococcus organisms were examined morphologically, microscopically according to (Ryan and Ray 2004)

4.3 Biochemical examination: according to (FDA, 2001)
The biochemical tests were Coagulase test, Catalase test, DNase test, Acetoin production, Oxidase test and D-mannitol fermentation.

4.4. Isolation of Aeromonas spp.

10 gm of each sample were homogenized with 90 ml alkaline peptone water (with 3% or 10% NaCL) for 2 min, and then incubated for 24 hr at 30°C (Villari et al., 2000). A loopful from alkaline peptone water was subsequently plated on the surface of starch ampicillin agar plate and incubated for 48 hr. at 30°C. Typical yellow colonies of Aeromonas species were purified on tryptone soya agar then stained by Gram's stain (A.P.H.A., 2004) and confirmed on the basis of the following test: Oxidase test, resistance to vibriostatic agent o/129, esculin hydrolysis, sugar fermentation and gas production, indole production and voges- proskauer test.

4.5. Differentiation of common motile Aeromonas isolated.

From clinical specimens according to Carnhan et al. (1991) as modified by Joseph and Carnahan (1994). All bacterial isolates would be short G -ve bacilli, oxidase positive that ferment glucose.

RESULTS

Sodium chloride content

Table 1: Statistical analytical results of salt conc. % in Pickled domiati cheese samples (n=60) and Mish samples (n=60) comparison with Egyptian Standards 2005.

<table>
<thead>
<tr>
<th>samples</th>
<th>Min.</th>
<th>Max.</th>
<th>Mean ± SE</th>
<th>E.S 2005</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pickled domiati cheese</td>
<td>2.9%</td>
<td>5.3%</td>
<td>3.7±0.135</td>
<td>Not more than 7.0%</td>
</tr>
<tr>
<td>Mish</td>
<td>4.2%</td>
<td>8.3%</td>
<td>6.1 ± 0.142</td>
<td>Not more than 15%</td>
</tr>
</tbody>
</table>
Determination of total Slight & moderate Halophilic counts

Table 2: Statistical analytical results of Total Slight and moderate Halophilic count in examined samples.

<table>
<thead>
<tr>
<th>Examined samples</th>
<th>Positive samples</th>
<th>Count (cfu/g.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Pickled domiati cheese</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3% NaCl</td>
<td>49</td>
<td>81.6%</td>
</tr>
<tr>
<td>10% NaCl</td>
<td>46</td>
<td>76.6%</td>
</tr>
<tr>
<td>Mish</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3% NaCl</td>
<td>56</td>
<td>93.3%</td>
</tr>
<tr>
<td>10% NaCl</td>
<td>58</td>
<td>96.6%</td>
</tr>
</tbody>
</table>

Detection of *Staphylococcus* organisms at 3% and 10% NaCl:

Table 3: Prevalence of *Staphylococci* isolated from examined samples.

<table>
<thead>
<tr>
<th>Examined samples</th>
<th>Positive samples</th>
<th>3%NaCL</th>
<th>10%NaCL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Pickled domiati cheese</td>
<td>60</td>
<td>51 85</td>
<td>43 71.6</td>
</tr>
<tr>
<td>Mish</td>
<td>60</td>
<td>57 93</td>
<td>50 83.3</td>
</tr>
</tbody>
</table>

4. Isolation of *Aeromonas spp.* at 3% and 10% NaCl.

Table 4: Incidence of *Aeromonas spp.* in examined samples at 10% NaCl.

<table>
<thead>
<tr>
<th>Types of Examined samples</th>
<th>No. of Examined samples</th>
<th>Positive samples</th>
<th>Aeromonas spp</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>A. hydrophila</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>No.</td>
</tr>
<tr>
<td>Pickled domiati cheese</td>
<td>60</td>
<td>29 48.3%</td>
<td>35 58.3%</td>
</tr>
<tr>
<td>Mish</td>
<td>60</td>
<td>23 38.3%</td>
<td>28 46.6%</td>
</tr>
</tbody>
</table>

DISCUSSION

Sodium chloride content

The use of salting is one of the classical methods of food preservation. Salting is used to extend the shelf-life of foods throughout civilization. In this study, it is evident in table (1) that salt percentage of examined pickled domiati samples ranged from 2.90% to 5.30%. While in mish samples ranged from 4.20% to 8.30% all results obtained were within the normal range of (Egyptian Standards, 2005). Nearly similar results were reported by Ceylan et al. (2003), Hassan and Afify, (2007), Nawar, (2007), El-Ansary et al. (2011), El Bakry, (2012), Yasser, (2015) and EL-Refaay, (2016), while higher result were obtained by Mohamed, (2004), Patrick et al., (2004), Hayaloglu and Kirbag, (2007) and EL Zahar,
(2010). While lower result were obtained by Riad, (1996) and Sadek, (2009).

**Detection of total Slight & moderate Halophilic counts**

Slight, moderate, and extreme halophiles as those bacteria that grow best in media containing 2 to 5%, 5 to 20% and 20 to 30% salt respectively. (Kushner 1978). in table (2) Samples examination refer that maximum value of slight halophilic count in Pickled domiati cheese samples and Mish samples were 1x 10⁶ and 2.6 x 10⁹ respectively while minimum value was 1x10³ and 1.6 x10³ respectively where salt conc. was 3%. This results are nearly similar to Freitas et al. (1993), and Saad and Moawad (1999). While higher results are obtained by Riad (1996) and Omar et al. (2007).

The maximum value of moderate halophilic count in Pickled domiati cheese samples and Mish were 3.2 x 10⁶ and 9. 0x10⁵cfu/g, respectively, while minimum value was 1.0 x10² and 5.0 x10² cfu/g. Respectively with mean value 0.76 x 10⁴± 0.14 x10⁵ in pickled domiati cheese samples. And with mean value 2.09 x10³± 3.6 x10² in Mish samples (Where NaCL concentration is 10%). These results are nearly similar to El-prince (1994) and Riad (1996), while lower results are obtained by Omar et al. (2007).

**Detection of Staphylococcal organisms at 3% and 10% NaCL:**

The presence of *Staphylococcus* is an index of contamination from operators or workers. It has a potential significance to public health due to its ability to produce enterotoxin leading to food poisoning. The results in Table (3) show that incidence of *Staphylococcus* in Pickled domiati cheese was 85% at 3% NaCL and 71.6% at 10% NaCL. While in Mish samples was 93% at 3% NaCL and 83.3% at 10% NaCL. These results are nearly similar to those obtained by Abou El-Makarem, (2009). Higher results are obtained by Sheleby(2008) and Elshafey (2011).

**Isolation of Aeromonas spp. at 3% and 10% NaCL:**

*Aeromonas* species are widely distributed in the aquatic environment, including raw and processed drinking water (Holmes et al., 1996), and have been frequently isolated from various food products such as fish and shellfish, raw meat, vegetables and raw milk Palumbo, (1996). It is associated with travellers’ diarrhea (Hänninen et al., 1995 and Yamada et al., 1997). In this study, *Aeromonas* species was not isolated from any examined samples either Mish or Pickled domiati cheese using 3% salt concentration while at 10% Na CL, as shown in table (4) that the incidence of *Aeromonas spp.* was 25.7% A.hydrophila, 40.0% A.caviae, 20.0% A. trota and 14.3% A. schuberti in Pickled domiati cheese samples. These results are nearly similar to those obtained by to Freitas et al. (1993), Khalil (1997), Effat et al. (2000), Ahmed et al. (2014) Higher results are obtained by Yasser (2008), Nazem et al. (2010) and Alhazmi (2015).

**REFERENCES**


group in food. In The Genus Aeromonas ed.
Austin, B., Altwegg, M., Gosling, P.J. and
Joseph. S., pp.287-310. Chichester, UKWiley
and Sons.

Patrick, F.; Fox, M.; Cogan, T.M. and Timothy, G.
(2004): Cheese Chemistry, Physics and
Microbiology: Major Cheese Groups: Major
Cheese Groups v. 2.

Medical Microbiology 4th ed. McGraw Hill,
New York.

dairy products as indices of sanitary quality,

Saad, A.H. and Maawad, A. (1999): Incidence of
slight and moderate Halophiles in some
selected food. Beni-Suef Veterinary Medical

manufactured milk products. M.V.Sc. Thesis,

Street-Vended Dairy Products. M.V.Sc.

Villiari, P.; Crispino, M.; Montuori, P. and Stazione,
S. (2000): Prevalence and Molecular
characterization of Aeromonas spp in Ready-to-Eat foods in Italy. Department of Health
and Preventive Science. University Federico
II. Journal of food protection. 63(12): 1754-
1757.

Yamada, S.; Matsushita, S.; Dejsirilet, S. and
symptoms of Aeromonas-associated Traveller’s diarrhea in Tokyo. Epidemic
infect. 199, 121-126.

hydrophila group in milk and milk products,
Cairo university, faculty of Veterinary
Medicine, department of food hygiene.

Yasser, S.I.S. (2015): Incidence and Biocontrol of
Staph. Aureus in some milk products. M.V.
SC. Thesis. faculty of Vet. Med. Suez Canal
University.

Microbial status of Domiati cheese at Al
Gassiem area, Saudi Arabia., 8th sci. Cong.,
The fac. vet. Med. Assiut University, pp: 91-
97.

The enterotoxin D plasmid of Staphylococcus
aureus encodes a second enterotoxin
determinant (sej). FEMS Microbiology

وتواجد انتشار الاستفتيلوكوس أوريس والأيروموناس في الجبن الدمياعي المخزن والمش

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باید اجراعت این الدراسه على 100 عينة من الجبن الدمياعي المخزن والمش 10 عينة من كل نوع تم تجميع العينات عشوائيا من بعض

المحلات والأسواق والباعه الجائلين في كل من محافظة بورسعيد وتم تقييم هذه العينات.

وذد الاظهرت النتائج ميلي:

الفحص الكيميائي:

متوسط نسبة ملح الطعام في عينات الجبن الدمياعي المخزن 14.6±0.3، بينما كانت في المش 14.1±0.3.

الفحص الميكروبيولوجي:

1- متوسط العدد الكلي للبكتيريا طفيفة درجة الملحوة في عينات الجبن الدمياعي المخزن والمش كان 1.99±0.042، حالياً 1×10⁸+1×10⁷ و

2- متوسط العدد الكلي للبكتيريا متوسطة درجة الملحوة في عينات الجبن الدمياعي المخزن والمش كان 1.14±0.76، حالياً 1×10⁷+1×10⁶ و

3- مدى تواجد ميكروب المكورات العنقودية في عينات الجبن الدمياعي المخزن عند 3% حالياً 68% بينما عدد متوسط ملمح كانت.

4- مدى تواجد ميكروب التورطا في عينات الجبن الدمياعي المخزن عند 3% ملمح كانت 36% بينما عدد متوسط ملمح كانت 32%.

5- لا يوجد على الأطلاق لميكروب الأيروموناس في اي من عينات الجبن الدمياعي المخزن او المش عند تراکم ملمح 3% بينما عدد

تركيز ملمح 1% كان مدى تواجد ميكروب الأيروموناس كما يلي: 35% أتروتانا، 14% أتروتانا و

4% أتروتانا، و

0.3% أيكيرتيونى في عينات الجبن الدمياعي المخزن أما عينات المش كانت 36% أتروتانا، 44% أتروتانا و

21% أتروتانا و

3% أيكيرتيونى.