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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 unfits 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 septicemia (Lowy, 1998). *Staph.aureus* is capable of producing several enterotoxin 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,

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and then taken aseptically to the laboratory immediately, where it is subjected to chemical and microbiological examinations.

2. Preparation of samples (A.P.H.A. 2004)
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.

3. Chemical Examination

3.1. Determination of sodium chloride content (A.O.A.C, 2000)
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 20ºc, 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:

$$\text{Na CL \%} = \frac{2\,(25-R)\ X0.00584\ X100}{\text{Weight (3g)}}$$

4. Microbiological Examination

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

Preparation of serial dilution:
10gm of each sample was transferred in sterile stomacher bag with 90ml 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}, $10^{-5}$and 10^{-6} were prepared.

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 37ºC for 48hours, then the total slight and moderate halophiles were counted and expressed as CFU/g.

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 *Aeronomas spp.*
10gm of each sample were homogenized with 90 ml alkaline peptone water (with 3% or 10% NaCL) for 2 min, and then incubated for 24hr at 30ºC (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 30ºc. 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- proskaur 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.

samples	Min.	Max.	Mean ± SE	E.S 2005
Pickled domiati cheese	2.9%	5.3%	3.7±0.135	Not more than 7.0%
Mish	4.2%	8.3%	6.1 ± 0.142	Not more than 15%

Determination of total Slight & moderate Halophilic counts

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

Examined samples (n= 60)		Positive samples		Count (cfu/g.)		
		No.	%	Min.	Max.	Mean ± SE
Pickled domiati cheese	3%Nacl	49	81.6	1 x10^3	1 x10^6	3.31 x10^5±0.81 x10^5
	10% Nacl	46	76.6	1 x10^2	3.2x10^5	0.76 x10^5±0.14 x10^5
Mish	3%Nacl	56	93.3	1.6 x10^3	2.6 x10^6	1.99 x10^5±0.42 x10^5
	10% Nacl	58	96.6	5 x10^2	9 x10^5	2.09 x10^5±0.36 x10^5

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

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

Examined samples	No.	Positive samples			
		3%NaCL		10%NaCL	
		No.	%	No.	%
Pickled domiati cheese	60	51	85	43	71.6
Mish	60	57	93	50	83.3

4. Isolation of *Aeronomas spp.* at 3% and 10% Na CL

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

Types of Examined samples	No. of Examined samples	Positive samples		isolates		*Aeromonas spp*							
						A. hydrophila		*A. caviae*		*A. trota*		*A. schubertti*	
		No	%	No.	%	No.	%	No	%	No	%	No	%
Pickled domiati cheese	60	29	48.31%	35	58.3%	9	25.7%	14	40%	7	20%	5	14.3%
Mish	60	23	38.3%	28	46.6%	7	25 %	13	46.4%	6	21.4%	2	7.1%

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).

Determination 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 10^6 and 2.6 x10^6 respectively while minimum value were 1x10^3 and 1.6 x10^3 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 Omer *et al.* (2007).

The maximum value of moderate halophilic count in Pickled domiati cheese samples and Mish were 3.2 x 10^5 and 9. 0x10^5cfu/g. respectively, while minimum value was 1.0 x10^2 and 5.0 x10^2 cfu/g. Respectively with mean value 0.76 x $10^5\pm$ 0.14 x10^5 in pickled domiati cheese samples. And with mean value 2.09 x$10^5\pm$ 3.6 x10^4 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 *Aeronomas spp.* at 3% and 10% Na CL:

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% *A.hydrophila*, 46.4% *A.caviae,* 21.4% *A .trota* and 7.1% *A. schubertti* in Mish cheese samples while the incidence of *Aeromonas* spp. was 25.7% *A.hydrophila*, 40% *A.caviae*, 20% *A. trota* and 14.3% *A. schubertti* in Pickled domiati cheese samples. This 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).

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تواجد انتشار الاستافيلوكوكس اوريس والايروموناس في الجبن الدمياطى المخزن والمش

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اجريت هذه الدراسه على ١٢٠ عينه من الجبن الدمياطى المخزن والمش ٦٠ عينه من كل نوع. تم تجميع العينات عشوائيا من بعض المحلات والاسواق والباعه الجائلين في كل من محافظة بورسعيد وتم تقييم هذه العينات.

وقد اظهرت النتائج مايلي:

الفحص الكيميائي:

متوسط نسبة ملح الطعام في عينات الجبن الدمياطى المخزن ٠,١٣ ± ٣,٧ بينما كانت في المش كانت ٦,١ ± ٠,١٤

الفحص الميكروبيولوجي:

١- متوسط العدد الكلي للبكتيريا طفيفة درجة الملوحة فى عينات الجبن الدمياطى المخزن والمش كان ١,٩٩ x ١٠ ^٥ ± ٠,٤٢ x ١٠ ^٥ و ٣,٣١ x ١٠ ^٥ ± ٠,٨١ x ١٠ ^٥ علي التوالى

٢- متوسط العدد الكلى للبكتيريا متوسطة درجة الملوحة في عينات الجبن الدمياطى المخزن والمش كان ١,٤ x ١٠ ^٤ ± ٧,٦ x ١٠ ^٤ و ٠,٣٦ x ١٠ ^٥ ± ٢,٠٩ x ١٠ ^٥ على التوالى

٣- مدى تواجد ميكروب المكورات العنقودية في عينات الجبن الدمياطى المخزن عند ٣% ملح كانت ٨٥% بينما عند ١٠% ملح كانت ٧١،٦%.

٤- مدى تواجد ميكروب المكورات العنقودية في عينات المش عند ٣% ملح كانت ٩٣% بينما عند ١٠% ملح كانت٨٣،٣%

٥- لا وجود على الاطلاق لميكروب الايروموناس في اى من عينات الجبن الدمياطى المخزن او المش عند تركيز ملح ٣% بينما عند تركيز ملح ١٠% كان مدى تواجد انواع ميكروب الايروموناس كما يلى: ٢٥,٧% أ.هيدروفيلا، ٤٠% أ.كافيه ، ٢٠% أ.تروتا ، و ١٤,٣% أ.اسكوبرتى في عينات الجبن الدمياطى المخزن اما عينات المش فكانت ٢٥% أ.هيدروفيلا ، ٤٦،٤ % أ.كافيه ، ٢١،٤% أ.تروتا و ٧,١% أ.اسكوبرتى.