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ISOLATION OF S. ENTERITIDIS AND OTHER SALMONELLAE FROM CREAM AND IT'S STABILITY AGAINST SORBATES AND HONEY

(With 2 Tables)

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عزل السالمونيلا المعوية والسالمونيلا الأخرى من الكريمة ومدى ثباتها ضد السوربات والعسل

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تعتبر السالمونيلا من اخطر الميكروبات المسببة للأمراض التي تنتقل عن طريق الغذاء في العالم الثالث خاصة السالمونيلا المعوية حيث أنها تعتبر من المسببات الأساسية لحدوث المرض والوفاة وان نسبة الوفيات بها تكون أعلى من غيرها من الميكروبات ووجد أنها تمثل 7% من الوبائيات التي حدثت عن طريق الغذاء. لذا تم تجميع 100 عينة من الكريمة الخام والكريمة المخفوقة (50 عينة لكل منها) للكشف عن تواجد ميكروبات السالمونيلا بها. تم عزل 15 ، 14 عزله مبدئيا على كل من bismuth sulphite agar & S.S. agar من الكريمة الخام ثم باستخدام Modification of FDA تم التعرف على عزلتين فقط على كل مستنبت على أنها ميكروب السالمونيلا. أما الكريمة المخفوقة فقد تم عزل 13,4عزلة مبدئية على كل من المستنبتين السابقين على التوالي. تم التعرف على 3و2عزلة على أنها ميكروبات السالمونيلا. وبالنسبة للسالمونيلا المعوية فقد أثبتت النتائج أن عينة واحدة فقط كانت ايجابية فى كل من الكريمة الخام والمخفوقة. كما تناولت الدراسة مقارنة بين استخدام سوربات البوتاسيوم والعسل كمادة حافظة للكريمة ومدى قدرة السالمونيلا المعوية على الثبات ضد تأثير كل منهم لذا تم إضافة سوربات البوتاسيوم بتركيزات 0.2,0.4,0.6 % وعسل الشمر بتركيزات 10,5,1,0.6,0.4,0.2 % إلى الكريمة بعد حقنها بميكروب السالمونيلا المعوية بتركيز 1× 10 ^{7} ميكروب / جم مع وضع عينات خالية تماما من سوربات البوتاسيوم أو العسل كضابط للتجربة. تم فحص العينات وقياس pH عند بداية التجربة وفى أول وثاني يوم ثم كل يومين حتى انتهاء فترة التخزين (15 يوم) داخل الثلاجة. وبإضافة سوربات البوتاسيوم إلى الكريمة حدث تناقص في عدد الميكروب حتى وصل إلى 1 × 10 ^{2} / جم بنهاية فترة التخزين داخل الثلاجة باستخدام تركيزات 0.2 & 0.4 في حين لم يتم عزل الميكروب بعد اليوم العاشر باستخدام تركيز 0.6 سوربات البوتاسيوم. هذا وقد لوحظ زيادة في عدد الميكروب في اليوم الأول في العينات الخالية من السوربات ثم بدا يتناقص تدريجيا حتى وصل إلى 2 ×10 ^{5}

بنهاية فترة التخزين. أظهرت النتائج فاعلية العسل بتركيز 10 % حيث تم القضاء عليها منذ اليوم الأول أما باستخدام تركيز 1 & 5 % فقد كانت النموات غير واضحة وحجمها صغير جدا (INJURED COLONIES) فى اليوم الأول والثاني ثم بدأت في الظهور بصورة طبيعية حتى اليوم العاشر. أما عينات الكريمة التي احتوت على تركيزات 0.2 & 0.4 & 0.6 % من العسل فقد استمر الميكروب في التناقص التدريجي حتى تلاشى في اليوم العاشر. ولم تكن هناك فروق واضحة بين التركيزات المختلفة. خلصت الدراسة بأفضلية استخدام عسل الشمر بتركيز 10% كمادة حافظة طبيعية للكريمة بدلا من سوربات البوتاسيوم نظرا لفاعليته في القضاء على السالمونيلا خلال 24 ساعة وقيمته الغذائية المرتفعة.

SUMMARY

100 random samples of raw and whipped cream (50 samples each) were tested for the presence of S. enteritidis and other *Salmonella* spp. From raw cream 15 and 14 initial *Salmonella* isolates were recovered using S.S. and Bismuth sulphite agars, respectively. Only 2 isolates were identified as *Salmonella* spp. on both media on the basis of modefication of FDA. Regarding whipped cream, 13 and 4 presumptive *Salmonella* colonies were isolated on the same media, of these, 3 and 2 isolates were identified as *Salmonella* spp on both media respectively. The stability of *S. enteritidis* against potassium sorbate or honey in cream stored at refrigerator temperature was studied. Cream inoculated with $1x10^7$ *S. enteritidis*, devided into 10 parts to which potassium sorbate was added, in concentrations of 0.2, 0.4 and 0.6%. Fennel honey was added in concentrations of 0.2, 0.4, 0.6, 1, 5 and 10%. One part was kept as control. The samples were examined for *S. enteritidis* count and pH in the 1st and 2nd day then, every 2 days of storage. Lower decrease in count of *S. enteritidis* was noticed in cream containing 0.2, 0.4 and 0.6% pot .sorbate, stored at refrigerator temperature. Undetectable numbers of *S. enteritidis* were observed at 10th day in concentration of 0.6%. While, in control samples the count reached $8x10^7$ in the 1st day then, decraesed to be $2x10^5$ at the end of the storage time. In contrast, addition of honey at conc. of 10% inhibits the growth of *S. enteritidis* within 24 hours of storage at refrigerator temperature. Lower concentration of honey (1 and 5 %) led to appearance of injured colonies in the 1st and 2nd day, the colonies begin to recover at the 4th day, and no viable cells were noticed after the 10th day. Gradual reduction in the count of *S. enteritidis* using 0.2, 0.4 and 0.6% honey was observed till the 10th day. Our results showed which preservative is most active against *S. enteritidis*, thus, the safety of cream could be improved by addition of fennel honey in a concentration of 10%.

key words: *Cream, Salmonella, sorbates, honey*

INTRODUCTION

Despite of the extensive public health measures over the past century, *Salmonella* remains the second most commonly identified cause of bacterial foodborne disease in the developed countries and a signficant cause of morbidity and mortality in the developing world (Edward, 1999; Abdou *et al.* 2001; WHO, 2002). In Egypt salmonellae were found in 3% cases of children diarrhea in rural areas and 4% in urban areas. In Upper Egypt, salmonellae were detected in 14.8% of cases of children diarrhea (FAO, 1993)

S. enteritidis continued to be a major cause of illness and death. It is the most common serovar causing approximately 80% of foodborne salmonellosis cases (WHO, 1998). Moreover, it results in more deaths than any other pathogen (Olsen *et al.*, 2000).

Cream is a popular dairy variety; it was added as an ingredient to a large number of commercial food products. It could be a vehicle of transmitting *Salmonella* (Nasseib *et al.*, 2003). Cream is one of the perishable dairy products which has high moisture content and enjoys only a limited shelf life. Pasteurized cream is one of the slow moving goods in the Egyptian markets, so the recommended shelf life is considered short and may constitute an economic problem due to its spoilage on shelves and evidence of public health threat (Abdou *et al.* 2001).

Preservatives either chemical or natural are used to prevent or retard both chemical and biological deterioration in foods (Davidson and Branen 1993). At the moment sorbic acid and its salts especially potassium sorbate has been used extensively in food (Beek *et al.*, 2007). Many previous reports studied the inactivation of *Salmonella* spp, using pot. sorbate (Rice and Pierson 1982; Tuncan and Martin 1985; Larocco and Martin 1987 and Alvarez *et al.*, 2007).

Use of sorbic acid in foods is permitted in most countries which regulate their food supply (Luck 1980), the maximum permissible level other than in exceptional situations is between 0.1% and 0.2% (Code of Federal Regulations 1981). Environmental factors such as pH, water activity, temperature, microbial load, type of microbial flora and certain food components can influence the effectiveness of sorbic acid. All these factors should be considered when using sorbic acid and its salts as antimicrobial preservatives (Liewen and Marth 1985).

A possible shift to the use of naturally occurring antimicrobials increased in the future. Since, these compounds have been in the food supply and consumed for a number of years. They appear to be safe and not require a new synthetic compound (Davidson and Branen 1993).

The antibacterial property and preservative nature of honey had been studied (Badway *et al.,* 2004; Mundo *et al.,* 2004; Ali *et al.,* 2005 and Krushna *et al.,* 2007). Honey which is - chiefly a combination of various sugars and hydrogen peroxide - can be used as a preservative of milk due to firstly the bactericidal property of hydrogen peroxide (Krushna *et al.,* 2007), and secondly, it contains syringic, methyle syringate and other aromatic acids in honey that are structurally simillar to benzoic acids which are typically used in foods as preservatives (Russel *et al.,* 1990).

Although honey is a safe natural product prevents growth of G-ve, G+ve and C. albicans (Al-Waili *et al.,* 2005). The ability of honey to inhibit the growth of microorganisms varies widely and the bacteria were not uniformly affected by honey (Mundo *et al.,* 2004).

So, the present work firstly aimed to isolate *Salmonella* spp from raw and whipped cream and secondly to study the stability of *S. enteritidis* against pot. sorbate and honey in cream.

MATERIALS and METHODS

Isolation of *Salmonella* spp from cream samples

100 random samples of raw and whipped cream (50 samples each) were collected from different localities in Assiut City. Cream samples were thawed in water both adjusted at 40ºC / 10 minutes according to Al Ashmawy *et al.* (2002).

Cream samples were preenriched on lactose broth and selectively enriched for Salmonella spp. in Selenite F broth at 37ºC for 24 hours. S.S. agar and Bismuth sulphite agars were used to isolate Salmonella spp. according to Janda and Abott (1998) and Andrews and Hammack (2001). Pink colonies with black center on S.S. agar as well as the black colonies on Bismuth sulphite agar were identified as Salmonella spp. by Gram stain and various biochemical tests as described by Benson (1994) and Stephen and Caren (1997). Modification of the confirmatory process indicated by FDA (1995, 2001) protocols was done.

The stability of *S. enteritidis* against sorbates and honey in cream

Pasteurized cream (23% milk fat free from preservatives) was inoculated with a suspension of 24 hours incubation of *S. enteritidis* strain at a concentration of 1×10^7 cfu /ml. *S. enteritidis* strain was identified serologically by the Central Administration for Laboratories of Health Ministry, Cairo. Egypt and obtained from Dept. of Food Hygiene Fac. Vet. Med. Assiut Univ.

Cream samples were divided into 10 parts. Pot. sorbates was added to 3 parts at concentrations of 0.2, 0.4 and 0.6%. Fennel honey was added to 6 parts to achieve final concentrations of 0.2, 0.4, 0.6, 1, 5 and 10 %. The last part was kept as a control. Sample was taken to determine the initial count and pH.

The inoculated parts as well as the control were kept in the refrigerator (4±ºC) for a period of 15 days which is the shelf life of the pasteurized cream as recommended by the manufacturer. Samples were taken from each part to determine the count of *S. enteritidis* and pH at time zero, first and second day and every 2 days untill the end of the storage period.

RESULTS

The results were shown in Tables 1 and 2

Table 1: Incidence of *Salmonella* spp. in the examined cream samples:

Samples	No.of examined samples	Media used	Presumptive *Salmonella* colonies		Positive *Salmonella* spp.		*S. enteritidis*	
			No.	%	No.	%	No.	%
Raw cream	50	S.S.agar Bismuth sulphite agar	15 14	30 28	2 2	4 4	1 1	2 2
Whipped cream	50	S.S.agar Bismuth sulphite agar	13 4	26 8	3 2	6 4	1 1	2 2
Total	100		46	46	9	9	4	4

DISCUSSION

Isolation of *Salmonella* spp. from cream

Foodborne salmonellosis continues to be a major health concern world wide (kiessling *et al.,* 2007) thus, detection of *salmonella* isolates is of interest. The literature dealing with the prevalence of salmonella in cream is very scanty However, salmonella spp. were isolated from 3.32% of food samples by Wang *et al.* (2004) and 5.9% by Kiessling *et al.* (2007). In the present study, (15&14) and (13&4) presumptive salmonella colonies recovered from raw and whipped cream using S. S and Bismuth sulphite agars, respectively (Table1). On the basis of modification of FDA 2 isolates (4%) were identified as *Salmonella* spp. From raw cream on both media. However 3 (6%) and 2 (4%) isolates were proved to be *Salmonella* spp. from whipped cream on S.S and Bismuth sulphite agars, respectively. Only one sample from each of raw and whipped cream contain *S. enteritidis* (Table1).

Salmonella spp. was previously isolated from cream samples by El- Kosi (2001) and Nasseib *et al.* (2003) while, it failed detection by El Saied (1985). The use of the modification of FDA to confirm *Salmonella* spp. was recommended; out of 46 presumptive *Salmonella* colonies, 9 isolates were proved to be *Salmonella* spp. (Table1). Also Nasseib *et al.* (2003) recommended the modification of the confirmatory process indicated by FDA (1995, 2002) as they found that only 7 out of 247 initial isolates were identified as *Salmonella* spp. using this modification.

Salmonellae are frequently isolated from dairy cattle and from various dairy farm environments such as water, feed, and manure. Moreover, asymptomatic shedding of *Salmonella* in feces also occurs, it follows that there is a risk of the pathogen entering the bulk tank through fecal contamination (Troutt *et al.* 2001 and Huston *et al.* 2002). Using raw milk in the preparation of cream could serve as a contaminating point. *Salmonella* spp. were isolated from raw milk in variable percentages 8% (Rohrbach *et al.,* 1992), 6.6% (Sayed 2002), 28% (El Said 2002), 6% (Amin 2004) while, failed detection by Sharma *et al.* (1995).

Our results suggest negligence such as poor sanitation during preparation of raw and whipped crèam. Additionally, the possibility of contamination by carrier food handlers and the temperature abuse considered a major contributary factors (Varnam and Sutherland, 1994).

Although, there are relatively low numbers of positive samples in this study, the pathogen represent a potential risk to consumers on the

basis that all salmonellae are potentially pathogenic (Zansky *et al.*, 2002).

The stability of *S. enteritidis* against sorbates and honey in cream

The weak organic acid, sorbic acid, and its salts are commonly used as food preservatives, as they inhibit the growth of bacteria, yeasts and molds (Beek *et al.*, 2007). In general, two theories have been postulated to explain the inhibitory mechanism of sorbate. One of these is carried out by the inhibition of one or more vital microbial enzymes (Sofos and Busta, 1981); the other mechanism is carried out by the inhibition of nutrient uptake (Tuncan and Martin, 1985 and Beek *et al.*, 2007).

Our results demonstrated a slow decrcase in the count of S.enteritidis in cream containing various concentrations of pot. sorbate. The organism was still detected well till the end of the storage time at 0.2% and 0.4 % pot. sorbate. There was no death or injury observed in these samples throughout the storage. On the other hand, Rice and Pierson (1982) found that concentration of 0.26 and 0.39 % pot. sorbate were effective in inhibiting *Salmonella*.

As the storage time increased, cells of *Salmonella* were stressed at a faster rate (Larocco and Martin 1987). The numbers of bacteria diminished gradually till the 6^{th} day then, decreased in a faster rate reaching 1×10^2 at the end of 15^{th} day in cream samples containing 0.2 and 0.4% pot. sorbates (Table 2). Inhibition of growth of microorganisms was increased by increasing sorbate concentration (Sofos and Busta 1981). By increasing the concentration to 0.6% no viable cells were detected after 10 days (Table 2). This may be referred to the effect of pH, samples that contained pot. sorbate had pH ranged from 7.4-6.1 this pH may contribute the action of pot. sorbate, Environmental factors such as pH can influence effectiveness of sorbate (Liewen and Marth 1985). Furthermore Park and Marth (1972) found that 0.3 % sorbic acid at pH 5 inactivated *Salmonella* in 12 hours. As noted earlier, the maximum permissible level of sorbates, other than in exceptional situation is between 0.1% and 0.2% (Code of Federal Regulations 1981).

In control samples the count increased to 8×10^7 in the 1^{st} and 2^{nd} days then, decreased to reach 2×10^5 at the end of the storage time (Table 2). Since the pH of control samples ranged from 6.8-6.1, this reduction may attributed to the effect of refrigerator temperature through the storage period (15 days)

In sharp contrast to cream without preservatives or cream with pot. sorbate, addition of honey at a conentration of l0% inhibit the growth of *S. enteritidis* within 24 hours of storage at refrigerator temperature (Table 2). High concentration of honey proved more effective as antibacterial agents (Badawy *et al.,* 2004). Furthermore, honey combind with low temperature increased the inhibitory effect of honey (Russel *et al.,* 1990)

Cream with honey had pH ranged from 6.8-5 (Table 2). Honey pH between 3-5 acts as inhibitory media to most pathogen that requires pH 7.2-7.4 for thier bioactivity (Molan, 1992).

The potency of honey as antimicrobial agent is thought due to its acedic pH, its hyperosmolarity property and to hydrogen peroxide which is the main and principle bacterial growth inhibin in honey (Molan, 1992, and Al-Waili 2001). Fennel honey was the most potent and had the highest H2O2 level among the different tested honey batches (Ali *et al.,* 2005)

Low concentration of honey (1and 5%) led to appearance of injured colonies in the 1st and 2nd days, the colonies begin to recover at the 4th day. No viable cells were noticed after the 10th day (Table 2). It was found that bacteria could overcome the antibacterial activity of honey after a period of inhibition (Molan, 1992). However, the appearance of microbial growth after initial inhibition by a single dose of honey might be a result of inability of such dose to kill all the growing isolates (Al- Waili *et al.,* 2005).

Gradual reduction in the count of *S. enteritidis* till the 10th day was observed using 0.2, 0.4 and 0.6 % honey, the present findings coincided with Lusby *et al.* (2005) who found that little or no antibacterial activity was seen at honey concentrations <1%, with minimal inhibition at 5 %.

Not only honey acts as antibacterial agent, but also it reduced *S. enteritidis* adhering to the intestinal epithial cells in vitro (Al naqdy *et al.,* 2005). Moreover, honey has been gaining interest as a substitute sweetener in foods due to its high nutritive value, palatability and inhibitory properties against pathogens (Somal *et al.,* 1994).

Our results show which preservative is most active against *S. enteritidis* thus, the safety of cream could be improved by the addition of Fennel honey at a concentration of 10%.

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Table 2: Stability of *S. enteritidis* against pot. sorbate and honey in cream held at refrigeration temperature.

Time	Control	Pot.sorbate concentrations			Honey concentrations					
		0.2%	0.4%	0.6%	0.2%	0.4%	0.6%	1%	5%	10%
pH range	6.8 - 6.1	6.8 – 6.1	6.7-6.8	6.7-7.4	6.8 – 5.50	6.7-5.50	6.7-5.50	6.7-5.50	6.7-5.50	6.80-5
1st day	8×10^7	1×10^7	1×10^7	9×10^6	4×10^4	5×10^3	6×10^3	Injured colonies	Injured colonies	—
2nd day	8×10^7	6×10^6	2×10^6	1×10^5	1×10^4	4×10^2	2×10^3			—
4th day	2×10^7	9×10^5		8×10^3	2×10^3	3×10^2	2×10^2	Injured colonies	Injured colonies	—
6th day	1.1×10^7	2×10^5	7×10^5	6×10^3	3×10^2	1×10^2	1×10^2	3×10^2	2×10^2	—
8th day	4×10^6	2×10^4	9×10^4	3×10^2	3×10	1×10	1×10	2×10^2	1×10^2	—
10th day	2×10^6	7×10^2		1×10^2	—	—	—	1×10	1×10	—
12th day	6×10^5	3×10^2	1.5×10^4	—	—	—	—	—	—	—
15th day	2×10^5	1×10^2	6×10^2	—	—	—	—	—	—	—
			4×10^2					—	—	
			1×10^2						—	

- Initial count = 1×10^7 cfu / g