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EFFECT OF SOME CHEMICAL SANITIZERS ON MICROBIAL QUALITY OF DAIRY FARM MILK

(With 6 Tables and 3 Figures)

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تاثير استخدام بعض المطهرات على جودة الحليب فى المزارع

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لاشك ان تلوث اوانى الحليب بالميكروبات ظاهرة خطيرة ينتج عنها تلوث الالبان والذى يؤدى الى خسائر صحية واقتصادية فادحة. لذلك يهدف البحث إلى دراسة كفاءة المطهرات المستخدمة مثل الايودوفورو مركب رباعى الامونيوم والكالسيوم هيبوكلوريد على العدد الكلى للميكروبات (TMC) والعدد الكلى للميكروبات المحبة للبرودة (TPC) والمقاومة للحرارة (TTC) وميكروبات الكوليفورم وكذلك على عترات الكوليفورم والتى تم عزلها من عينات اللبن الخام التى تم فحصها. وقد أثبتت النتائج ان الميكروبات المحبة للبرودة اكثر مقاومة للمطهرات من الميكروبات المقاومة للحرارة. وبدراسة التأثير الأمثل للثلاث انواع من المطهرات التي تستخدم لاواني الحليب في المزارع، أثبتت التجارب أن استخدام مركب رباعي الأمونيوم هو الأكثر كفاءة ضد المجموعات البكتيرية المختلفة وذلك عند استخدامة لمدة ٢٠ دقيقة فى ماء دافئ يلية استخدام اللأيودوفور والكالسيوم هيبوكلورايت. وقد خلصت النتائج الى ان مقاومة الميكروبات للمطهرات المختلفة تجعلنا نخطط لاستخدام الاكثر كفاءة للقضاء عليها فى اوانى الحليب.

SUMMARY

Biofilm formation on milk equipment is a well-known phenomenon and it has caused pollution, safety hazards and substantial economic loss. This study was undertaken to examine the efficacy of the sanitizers including iodophore, quaternary ammonium compounds and calcium hypochlorite against total mesophilic count (TMC), total psychrophilic count (TPC), total thermoduric count (TTC) and total *coliform* count MPN/ ml of the examined raw milk samples. Additionally the sanitizers were also tested against *Escherichia coli* strains isolated from the

examined milk samples. The result shows that psychotrophic bacteria were more resistant on sanitizer used than thermoduric bacteria. Among the three commercially sanitizer tested, quaternary ammonium compound was the most potent against microorganisms after 20 min exposure time using warm water. However, calcium hypochlorite and iodophore showed a lower effect on the bacterial groups. The resistance of *Escherichia coli* strains to the sanitizer was strain dependent. It was concluded that the resistances of microbes to different sanitizers should be taken into account when planning the cleaning of milking equipment.

Key words: *Milk, dairy farms, iodophore, calcium hypochlorite, quaternary ammonium compounds*

INTRODUCTION

Bacterial contamination can adversely affect the quality, functionality and safety of milk and dairy products produced by the dairy industry. When contamination of milk occurs evidence suggests that biofilms on the surfaces of milk processing equipment are a major source. During milk storage undesirable deposits, consist mainly of protein and minerals, are formed on the milking equipment surface. These protein and mineral rich deposits provide a good ground for bacteria to settle and grow (Flint *et al.,* 1997; Peng, *et al.,* 2002; Taormina and Beuchat, 2006).

Over the last years, foodborne diseases caused millions of illnesses worldwide (Dontorou *et al.,* 2003; Castonguay *et al.,* 2006). One way in which milk can be contaminated with pathogens is by contacting contaminated milk-processing equipment. Therefore, it is of the utmost importance to clean and disinfect these milk equipment regularly and sufficiently to provide consumers with wholesome and safe product (Rombouts and Abee, 2002; Dunsmore *et al.,* 2006; Hagel *et al.,* 2008).

Efficient sanitation is required in food plants where wet surfaces provide favorable conditions for microbial growth (Peng *et al.,* 2002). Sanitizers used in the food processing industry include oxidizing agents, e.g. hypochlorite, hydrogen peroxide, ozone, iodophore and quaternary ammonium compounds (Kumar and Anand, 1998; Lang *et al.,* 2000; Lomander *et al.,* 2004; Myung *et al.,* 2007). The sanitizers must be effective, safe and rinsable, as well as easy to use, and they should not affect the sensory qualities of the product (Larson *et al.,* 1991).

The most practical hygienic program is the use of sanitizer to eliminate transient bacteria from the dairy equipment. The purpose of sanitizing is to kill residual microorganisms present on these surfaces immediately prior to milking. All milking equipment, lines, and utensil surfaces that come into contact with milk or dirt or manure must be thoroughly cleaned and sanitized before the next milking. Inadequate or improper cleaning or sanitizing or both allows bacteria to remain on equipment surfaces and to grow and multiply (McDdonnell, and Denver, 1999; Wirtanen *et al.,* 2001; Dufour *et al.,* 2004; Wiszniewska and Szteyn, 2006). Although chemical companies tend to push new and innovative cleaning sanitizers, there is little published research on the relative effectiveness of different chemical sanitizers and an apparent lack of techniques that can simply and effectively determine their effectiveness. Therefore the purpose of this study was to compare the relative effectiveness of iodophore, calcium hypochlorite and quaternary ammonium compound against total mesophilic, total psychrophilic, total thermoduric and coliform count as well as the efficacy of the sanitizers against *Escherichia coli* strains isolated from the examined raw milk under conditions typical of those encountered in dairy manufacturing plants.

MATERIALS and METHODS

Collection of samples

Fresh raw cow milk samples were collected from two different dairy farms herds at Giza governorate. Samples were collected manually using hand milking (100 ml each). The samples directly added to milking bottles as described by Helke *et al.* (1993). The bottles were soiled with raw milk on both sides and then air dried, untreated and served as (control), while the other bottles were treated as follows: sanitizer treatment with either cold water (with exposure time 10 minutes (T1); with exposure time 20 minutes (T2) and warm water at 40°C with exposure time 10 minutes (T3); with 20 minutes exposure time (T4). These steps were all performed at room temperature. All the experiments in this study were performed three times. The sanitizers used were: iodophor (50-70 mg / liter of free iodine); quaternary ammonium compounds, (150-250 mg / liter) and calcium hypochlorite (200-250 ppm available chlorine) a product intended for use in the dairy milk industry. They were made as commercially recommended by manufacturer and used immediately after preparation. The milk samples

were transferred directly to the laboratory with a minimum of delay for microbiological examination.

Microbiological analysis

Milk samples of all treatments were examined for some microbial groups including: Total mesophilic count (T.M.C.) according to APHA (1993); total psychrophilic count (T.P.C.) according to Cox and MacRae (1998); total thermoduric count (T.T.C.) according to Desmasures and Gueguen, (1997); *coliform* count "MPN" according to *ICMSF (1986)* as well as isolation and serological identification of the isolated *coliforms* strains according to Krieg and Holt (1986).

The efficiency of the detergents used on the growth of the isolated *Escherichia coli* strains

The bacteriocidal effect of the commercially available detergents on the isolated *E. coli* serotypes was applied by adding 1 ml of cell suspension to 10 ml disinfectant solution maintained at 20°C after 10 and 20 min of exposure time. Cell suspension was mixed with 10 ml of sterile distilled water and served as control. One ml of the mixture was spread onto a tryptone soy agar plate (TSA, Oxoid Ltd.) and incubated at 37°C. Colonies were counted after 48 hours according to Anonymous (1998).

Bacterial suspension preparation

Prior to the experiment loopfull of the each culture strain was inoculated in 10 ml of tryptone soy broth (Oxoid Ltd) and incubated at 37 C for 20 hours. After incubation, the broth was spread on a tryptone soy agar tube (Oxoid Ltd) at 37C for 20-24 h. Cells were suspended in tryptone sodium chloride solution (TSC) in order to obtain 1.0×10^9 to 3.0×10^9 CFU/ml. Concentration was determined by optical absorbance at 405 nm according to Anonymous (1998).

Statistical analysis

Analysis of variance (ANOVA) was performed using the General Lineal Models Procedure of SAS- System (Statistical Analysis Systems Institute, 1999) were conducted on the log transformed data to determine if any significant differences ($p < 0.05$) lay between the treatments and the control. Significant differences between means were determined using least square means (LS means). The Means were calculated by using a Statistical Analysis System (SAS Institute, 1999).

RESULTS

Table 1: Statistical analytical results of bacteriological examination in the examined milk samples mean log_{10} cfu/ml using Iodophore

Bact.exam.	Control	T1		T2		T3		T4	
		Log	%	Log	%	log	%	log	%
T.M.C.	9.9	4.8	51.52	3.8	61.62	3.7	62.63	3.6	63.64
T.P.C.	7.7	3.7	51.95	3.6	53.25	3.4	55.84	3.5	54.55
T.T.C.	7.9	3.7	53.16	3.7	53.16	3.2	49.59	3.0	62.03
MPN	7.7	4.4	42.86	2.4	68.83	2.3	70.13	1.7	77.92

Table 2: Statistical analytical results of bacteriological examination in the examined milk samples mean log_{10} cfu/ml using quaternary ammonium compounds

Bact.exam	Control	T1		T2		T3		T4	
		Log	%	Log	%	log	%	log	%
T.M.C.	9.8	3.60	63.27	3.59	63.38	3.38	65.51	3.46	64.69
T.P.C.	8.6	3.70	56.98	3.64	57.67	3.39	60.58	3.44	60.00
T.T.C.	8.9	3.56	60.00	3.49	60.79	2.98	66.52	2.94	66.97
MPN	6.9	3.30	52.17	2.54	63.19	1.30	81.52	1.11	83.91

Table 3: Statistical analytical results of bacteriological examination in the examined milk samples mean log_{10} cfu/ml using calcium hypochlorite

Bact.exam	Control	T1		T2		T3		T4	
		Log	%	log	%	log	%	log	%
T.M.C.	8.88	3.7	58.33	3.63	59.12	3.57	59.98	3.36	62.16
T.P.C.	7.69	3.6	53.19	3.53	54.1	3.38	56.05	3.52	54.23
T.T.C.	7.9	4.68	40.76	3.62	54.18	3.39	57.09	3.45	56.33
MPN	7.24	2.8	61.33	2.78	61.6	1.87	74.17	1.38	80.94

Table 4: Bactericidal effect of iodophor on the isolated *Escherichi coli* serotypes.

E. coli serotypes	Control	T1		T2	
	Log	log	%	Log	%
O_{26}	9	5.52	71.1	0	100
O_{128}	9	5.22	74.4	0	100
O_{148}	9	5.60	86.7	0	100
O_{166}	9	4.7	75.9	0	100

Table 5: Bactericidal effect of Q.A.C. on the isolated *Escherichia coli* serotypes.

E. coli serotypes	Control log	T1		T2	
		log	%	Log	%
O_{26}	9	0	100	0	100
O_{128}	9	0	100	0	100
O_{148}	9	0	100	0	100
O_{166}	9	0	100	0	100

Table 6: Bactericidal effect of Ca. hypochlorite on the isolated *Escherichia coli* serotypes.

E. coli serotypes	Control *log*	T1		T2	
		Log	%	Log	%
O_{26}	9	3.9	43.3	0	100
O_{128}	9	4.56	50.7	0	100
O_{148}	9	5	55.6	0	100
O_{166}	9	3.7	41.1	0	100

Fig. 1: Statistical analytical results of bacteriological examination after using iodophor.

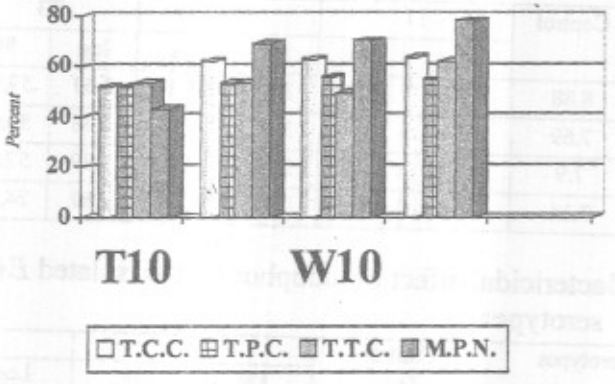

Fig. 2: Statistical analytical results of bacteriological examination after using Q.A.C

Fig. 3: Statistical analytical results of bacteriological examination after using calcium hypochlorite

DISCUSSION

The effectiveness of our sanitizing program was determined against total mesophilic count (TMC), total psychrophilic count (TPC), total thermoduric count (TTC) and *coliform* count (MPN) in the examined raw milk samples. The use of iodophore achieved a mean log reduction of 4.8, 3.7, 3.7& 4.4; 3.8, 3.6, 3.7 & 2.4; 3.7, 3.4, 3.2 &2.3 and

3.6, 3.5, 3 and 1.7 cfu/ml milk using T1, T2, T3 and T4 respectively as compared to the control samples (Table 1 & Fig. 1). There was a significant difference (P> 0.05) between the control and the treated milk samples but no significant difference (P< 0.05) between T1, T2, T3 and T4 respectively. Warm water appeared to have little additional impact on cell numbers. Nearly similar results were reported by Suárez and Ferreirós (1991) and Boulange *et al.,* (2004). Higher results were recorded by Magrex-Debar *et al.,* (2000); Wirtanen *et al.,* (2001); Orden *et al.,* (2002). and Peng *et al.,* (2002) and microorganisms become more resistant to sanitizers and other antimicrobial agents when they are attached to milking surface (Stone and Zottola, 1999; Wirtanen *et al.,* 2001). The bacterial cells were very resistant and even 20 minutes exposure time with warm water was not able to effectively eliminate the microbial population. The different antimicrobial efficacies of iodophore among the bacterial groups may be due to its limited penetration into the biofilms (Myung *et al.,* 2007).

It is clear from the results recorded in Table (2) & Fig (2) that use of quaternary ammonium compounds gave a mean log reduction of TMC, TPC, TTC and MPN count 3.6, 3.7, 3.56 & 3.3; 3.59, 3.64, 3.49 & 2.54; 3.38, 3.39, 2.98 & 1.30 and 3.46, 3.44, 2.94 and 1.11 cfu/ml milk for T1; T2; T3 and T4 respectively. There was a significant difference (P > 0.05) between the control and the treated samples. It was clear that on increasing exposure time for 20 min leading to increase the reduction of MPN count. Nearly similar finding were recorded by Suárez and Ferreirós (1991).

Results illustrated in Table (3) and Fig. (3) proved that use of Calcium hypochlorite gave a mean log reduction in the microbial groups TMC, TPC, TTC and MPN count 3.7, 3.6, 4.68 & 2.8 ; 3.63, 3.53, 3.62 &2.78 ; 3.57, 3.38, 3.39 &1.87 and 3.36, 3.52, 3.45 & 1.38 cfu/ml milk sample for T1; T2; T3 and T4 respectively. There was significant difference between the control samples and all the treatment (P>0.05). The *coliform* count was highly decreased on increasing the exposure time with warm water. The results agree, to a certain extent, with those reported by Girgis *et al.,* (1999) and Wirtanen, *et al.,* (2001). The obtained results declared that the use of quaternary ammonium compound was the most effective in reduction of total mesophilic, total psychrophilic, total thermoduric and *coliform* count, followed by calcium hypochlorite and iodophor. The use of iodophor and calcium hypochlorite had a lower effect than quaternary ammonium compound on lowering the total thermoduric count. The reduced accessibility of

the bacterial cells to the different sanitizers may be due to the interfering action of the substances in contact with the bacteria (Ntsama-Essomba, *et al.,* 1997; Sundheim *et al.,* 1998). The cells associated with the biofilms have the ability to form exopolysaccharide (EPS) matrix which surrounds the biofilm and protect the bacteria from sanitizers (James *et al.,* 1995; Gibson *et al.,* 1999). The variability in effectiveness with the used sanitizers in eliminating bacteria is not surprising as a large number of factors can influence their effectiveness including the: composition, concentration, nature and age of the contaminated surface; temperature, time of exposure, degree of water hardness and the characteristics of the surface being cleaned (Austin and Bergeron, 1995; Stewart and Seiberling, 1996; Changani I 1997; Wong, 1998; Storgards *et al.,* 1999; Faille *et al.,* 2001; Lelievre *et al.,* 2001, Lelievre *et al.,* 2002a; Lelievre *et al.,* 2002b; Boulange *et al.,* 2004; Dufour *et al.,* 2004). In general while sanitizers such as chlorine, iodophores and quaternary ammonium compounds have proven to be effective against bacteria their effectiveness against bacterial biofilms is variable (Mosteller and Bishop, 1993, Rossoni and Gaylarde, 2000; Bremer *et al.,* 2002).

The quality of raw milk has been considerably improved by the refrigeration on farms, and in processing plants. Unfortunately, the current sanitizing practices for the raw milk favored the growth of *psychrotrophic* bacteria, which are able to grow below 7° C during cold storage (Munsch and Alatossava, 2006). It was reported that *psychrotorophs* can grow and produce heat stable protease enzyme which breakdown casein, and in turn cause milk spoilage (Plock, 1994; Esther *et al.,* 2004; Maurilio *et al.,* 2006). Presence of thermophilic bacteria in the milk after cleaning and sanitizing milking equipment become a serious problem because the spores of this organism often exhibit a high resistance to heat treatment. The vegetative cells is capable of growth at 65°C and produce proteolytic enzymes which reduce the shelf life time of heat treated milk and other dairy products (Jeurnink, 1991; Zottola, and Sasahara, 1994; Peng, *et al.,* 2002; Jin- Ah Yoo, *et al.,* 2006).

Table (4) shows the reduction of *Escherichia coli* strains O26, O128, O148 and O166 in the suspension after 10 and 20 minutes exposure time to iodophore. The population reduction was 61.3, 58, 62.2 & 52.2% and 100, 100, 100 & 100 respectively. The use of quaternary ammonium compounds was completely effective to inactivate *Escherichia coli* strains in both 10 and 20 minutes exposure time (Table 5). Calcium hypochlorite was unable to inactivate *Escherichia coli*

serotypes after 10 minutes exposure time while completely inactivate them after 20 minutes exposure time (Table 6). The lesser effectiveness of iodophore and hypochlorite against the *Escherichia coli* strains in the present study, was in agreement with Mattila *et al.,* (1990) Gibson, *et al.,* (1999); Pompermayer, and Gaylarde, (2000) and Augustin *et al.,* (2004). On the other hands, Mosteller and Bishop (1993) found that the efficacy of sanitizers including iodophor, hyphohlorite, and quaternary ammonium compound versus the suspensions of *Escherichia coli* strains resulted in a >5.0 log-cycle reduction while the same concentrations were relatively ineffective against the attached cells. Frank and Koffi (1998) have observed that planktonic cells of *E. coli* decrease by more than 6.0 cycles after 30-s exposure to iodophore. Mustapha and Liewen (1989) have indicated that *E. coli* is more resistant to the lethal action of sodium hypochlorite on a stainless steel surface than in vitro. From the results recorded, it is clear that the use of Quaternary ammonium compound was the most effective and significant ($P>0.05$) in the reduction of different *Escherichia coli* serotypes, followed by Iodophor and Calcium hypochlorite. Sodium hypochlorite and quaternary ammonium compound (QAC) are used widely as sanitizers in food industries. As a strong oxidizing agent, hypochlorite is known to be very active in killing most bacteria, fungi and viruses. QAC are hydrophilic, cationic molecules. It was reported that QAC readily adsorb to bacterial surface, which is hydrophilic and negatively charged, penetrate the cell wall, and disrupt the cytoplasmic membrane (Natero and Levine, 1995; Peng *et al.,* 2002; Wilfido *et al.,* 2006). The choice of sanitizer or cleaning agent along with the time of action is very important when destroying microbes.

Escherichia coli serotypes have a great public health importance. It is capable of inducing gastro-intestinal illness among young children with severe cholera-like syndrome and shigella-like symptoms. Therefore their presence in milk represent a public health hazard for consumers (Natero and Levine, 1995; Ntsama-Essomba, *et al.,* 1997; Faille *et al.,* 2003; Bremer *et al.,* 2006).

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