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CONTROL OF FOOD POISONING BACTERIA DURING MANUFACTURING OF ACID CHEESE USING SOME ORGANIC ACIDS

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ABSTRACT

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Kariesh cheese is a soft acid cheese made from naturally fermented skimmed milk mainly at farmers' home. Although it is an excellent source of nutritive substances, it is unhygienically produced and pose public health risks due to raw contaminated milk. Efficacy of acetic and citric acids were evaluated as an alternative method for kariesh cheese production to reduce the risk of *S.* Typhimurium and *E. coli.* food poisoning. The yield of cheese did not significantly differ (P>0.05) between citric acid treatments and control, but acetic acid treatments were significantly (P<0.01) the lowest yield. For sensory evaluation, lemon juice treatment was significantly the lowest score, while other treatments did not differ significantly (P>0.05). Acetic acid 1% was the most effective antimicrobial, producing a significant (P<0.01) 3.5 log reduction in *S.* Typhimurium and 1.84 log reduction in *E. coli* followed by Acetic acid 0.75%; 1.9 and 0.9 log reduction in *S.* Typhimurium and *E. coli* levels, respectively. Meanwhile, citric acid (0.75% and 1%) resulted in non-significant (P> 0.05) reductions in both of *S.* Typhimurium and *E. coli* level*S.* Citric acid 2% achieved significant but low biological value reductions (0.59 and 0.58 log reduction) in *S.* Typhimurium and *E. coli* levels, respectively. But 3% citric acid treatment significantly (P<0.01) reduced *S.* Typhimurium and *E. coli* by 1.7 and 1.52 log reduction in comparison with the initial count. These results suggested that treatment of raw skimmed milk with acetic acid 1% or citric acid 3% is a good alternative for production of hygienic kariesh cheese.

Key words: *Kariesh cheese; acid cheese; organic acids; E. coli; Salmonella.*

INTRODUCTION

Cheese plays an important role in the Egyptian diet, and many people such as farmers eat a certain amount of cheese with at last one meal a day owing to its high protein content, low fat and price; most of the cheese is consumed either directly or with bread (Todaro *et al.*, 2013). Kariesh cheese is a soft acid cheese commonly made and consumed in Egypt. This cheese is an excellent source of protein, amino acids, calcium, phosphorus, vitamins and many micronutrients. Environmental conditions prevailing during processing and storage, combined with the composition of the cheese often, which reduces considerably its quality (Reps *et al.*, 2002). It is an acid coagulated fresh cheese, made from skimmed milk (cow milk, buffalo milk or buttermilk from sour cream) with soft composition, white curd and slightly salty (Francois *et al.*, 2004). Manufacture of such product is made at home from naturally fermented skimmed milk. Then it is ready to be consumed as fresh cheese. It is mainly manufactured by small holders and sold at local markets. Thus, the product is exposed to contamination with several types of microorganisms.

The quality and composition of kariesh cheese may vary considerable due to such factors as the quality and composition of the clotted skim milk, the method of manufacture, the time required to complete the drainage of whey, the quality of salt added and the method of handling the finished cheese. (Fahmi, 1950; El-Gendy, 1983; Abou-Donia, 1984, 1991, 1995, 1999[a, b]).

All microbiological quality parameters indicated low milk quality just before the milk is transferred into

cheese factories. These results suggest that milk is heavily contaminated right from the farm level (Hofi, 2011). Kariesh cheese is unhygienically produced and pose public health risks. Fadel and Jehan (2009) reported Enterobacteriaceae mean count of 6.78 log cfu/g. *E. coli* was detected in 56 % of the examined kariesh cheese with mean count of 2.97×10^4/g in Beni-Suef city and surrounding villages (Meshref, and Hassan, 2009). In Qena City Hassan and Elmalt (2008) recovered *E. coli* from 38 (76%) of raw milk and 11 (47.8%) of kariesh cheese samples with 6.1% of the isolates were VTEC.

Shiga toxin producing *E. coli* (STEC) was first recognized as a human pathogen in 1982 in the USA when strains of serotype O157:H7 caused two outbreaks of hemorrhagic colitis (Wells *et al.*, 1983). Cattle are the reservoir of the pathogen and consumption of raw milk of bovine origin are considered to be the main cause of several outbreaks of *E. coli* O157:H7 (Chapman *et al.*, 1993).

Contaminated unpasteurized dairy products such as raw milk and raw milk cheese have been incriminated in recent foodborne STEC outbreaks (CDC, 2007). Fermented dairy products manufactured using raw milk contaminated with *E. coli* O157:H7 can pose a threat to human health. It has been shown that if present in raw milk, the pathogen can survive during the manufacturing and ripening stages of dairy products that do not undergo a sufficient heating step, like soft cheeses, aged cheddar cheese, feta cheese and even yoghurt (Govaris *et al.*, 2002). In Egypt, the incidence of *E. coli* is widely ranged from 6% to 86% in raw milk and kariesh cheese collected from different farmers houses and shops (El-Hady *et al.*, 1995; Abdul-Raouf *et al.*, 1996; Hassan and Afify, 2007; Basha and Zayed, 2012) with count more than 10^4 cfu/g (Ghada *et al.*, 2004; Meshref, and Hassan, 2009). It is worthily to note that presence of *E. coli* in milk and milk products is an indication of direct or indirect fecal contamination. Toxigenic *E. coli* is found in soft cheeses made from raw cow's milk and can pass to the milk destined to make cheese, and survive and that could make soft cheese to be considered as a possible vehicle of infection (Quinto and Cepeda, 1997).

Salmonella is consistently the most common bacterial pathogen in laboratory confirmed foodborne illness cases in USA with *S.* Typhimurium the most serotype representing 19% (Mani-López *et al.*, 2012). *Salmonella* species is the causative agent on several outbreaks of foodborne diseases particularly in dairy products. Raw milk is an important vehicle for salmonellae causing human infection (Kasrazadeh and Genigeorgis, 1994). Soft cheese from unpasteurized cows' milk was the cause of outbreaks occurred in France due to *S.* Typhimurium (De Valk *et al.*, 2000) and England and Wales due to *S.* Dublin (Maguire *et al.*, 1992).

Consumers are increasingly avoiding the consumption of foods treated with chemicals. There is an increasing interest in applying natural antimicrobial compounds in the food industry. Natural alternatives are needed to achieve a high level of safety with respect to foodborne pathogenic microorganisms (Rauha *et al.*, 2000). Organic acids offer several advantages as antimicrobials because they are generally recognized as safe (GRAS), have no limited acceptable daily intake, are low-cost, easy to manipulate, and effect minor sensory changes on the product. However, it is important to use these acids according to good manufacture practices in order to avoid the development of strains resistant to acidic conditions (Mani-López *et al.*, 2012).

Organic acids such as citric and acetic acids have been used for years for decontamination of bacteria on beef, pork, and poultry (Mani-Lopez *et al.*, 2012). Using of lemon juice or vinegar in food (as salads) provide a harsh environment for foodborne pathogens such as *Salmonella* and *E. coli* to survive because of the acetic or citric acids (Beuchat *et al.*, 2006).

The aim of this study was to investigate the use of citric and acetic acids from natural sources to produce kariesh cheese as alternative method for home traditional method without affecting the traditional taste.

MATERIALS and METHODS

1. Fresh cow's skimmed milk was obtained from Dairy processing unit, Animal Production Research Institute (8.98% total solids, 0.48% fat, 3.5% protein, 4.9% lactose, 0.69% ash and pH 6.7).

2. Yoghurt starter culture, lactic yoghurt culture (Yo-Mix 495 LYO 100 DCU) was obtained from Danisco, France.

3. Natural lemon juice (4.48% acidity as citric acid) Vinegar (5%) from local market.

4. Citric acid (Merck) and commercial fine food grade salt (El-Nasr Chemicals Salinas Company).

5. Lemon juice was used as citric acid source for final concentrations of 0.75% and 1%, while citric acid crystals was used for 2% and 3%. On the other hand, vinegar was used for acetic acid treatments for final concentrations of 0.75% and 1%.

Cow's skimmed milk was divided into 4 groups; the first (control) was used for making of kariesh cheese as described by Fahmi (1950). Milk was pasteurized at 80ºC for 15 s, thereafter cooled to 40ºC then inoculated with 3% (v/v) of yoghurt starter culture. The other three groups were used for making kariesh cheese by adding natural lemon juice (T_2) (as citric acid 0.75% and 1% final concentration), citric acid (T_3) (2% and 3%) and vinegar (T_4) (as acetic acid 0.75% and 1% final concentration). While adding of acids milk was shaken every 5 min until the pH reached to 4.6. All treatments were incubated at 37 ºC up to curding. Salt at 1% was added between cheese layers and the curd was left to whey drain into small cheese molds at room temperature overnight. Three replications were performed.

Sensory analyses: Organoleptic evaluation was carried out according to the scheme of (Bodyfelt and Potter, 2009). Kariesh cheese samples were subjected to organoleptic analyses by Staff Members of the Dairy Technology Department (Animal Production Research Institute, Agriculture Research Center, Cairo, Egypt). The sensory attributes evaluated were the flavour (1-10 points), body and texture (1-5 points) and appearance and colour (1-5 points).

Bacteriological analysis

Culture preparation: *Salmonella* Typhimurium (ATCC 14028) and *Escherichia coli* (ATCC 8739) strains (acquired from the Department of Food Hygiene, Animal Health Research Institute) from frozen cultures were activated with two successive passes in 9 ml of tryptic soy broth (TSB) (Oxoid) and incubated at 37^{o}C for 18 h. For each individual strain, 1 ml of the stock inoculum was added to 100 ml of TSB and incubated with shaking at 37^{o}C for 18 - 24 h to reach a final concentration of approximately 10^8 CFU/mL (determined by plating serial dilutions on XLD and EMB agar, Oxoid). Then, 2.5 ml of the stock inoculum was added to 250 ml of milk to give final concentration of approximately 10^6 CFU/mL. The inoculated milk was counted on selective media for each strain (XLD, for *S.* Typhimurium and EMB, for *E. coli)* in duplicate to determine the initial count before treatment with the organic acids.

Bacterial count: After treatments, 25 g of cheese were placed in a stomacher bag with 225 ml of 0.1% peptone water and stomached for 1 min. Serial dilutions were prepared, spread plated in duplicate on XLD, for *S.* Typhimurium and EMB, for *E. coli* and incubated at 35^{o}C for 24 h. Colonies were enumerated, and the CFU/g of the cheese was calculated.

Statistical analysis: A completely randomized design was selected. The experiment was conducted in three repetitions. Data were analyzed by using the mixed procedure from SPSS software (release 20, IBM CO) after logarithmic transformation for bacteriological count. Means were separated by Fisher's least significant difference test, and significance was tested at $\alpha = 0.05$.

RESULTS

Yield of cheese: The yield of kariesh cheese was affected by the kind of acidulant. The yield of cheese made by citric acid (T_3) (19.05%) was the highest, followed by that made by natural lemon juice (T_2) (18.89%) and the lowest yield was recorded by acetic acid (T_4) (16.94%) compared to that made by the control (18.9%). There was no significant difference ($P>0.05$) between the yield between control and citric acid treatments (T2 and T3). On the contrary, acetic acid treatments were significantly ($P<0.01$) the lowest.

Sensory properties: The scores for sensory evaluation of fresh kariesh cheese manufactured by various ways are presented in Table (1). The type of milk coagulation was the principle factor influencing the sensory properties of cheeses. Kariesh cheese made with yoghurt starter (control), the citric acid and the acetic acid were more accepted by the panelists (reach flavour and creamy body and texture) as compared with that made by the natural lemon juice ($P<0.01$), which characterized by lemon flavour and crudeness body and texture. Moreover, control, acetic acid (T_3) and citric acid (T_4) cheeses were characterized by whiter colour than natural lemon juice (T_2).

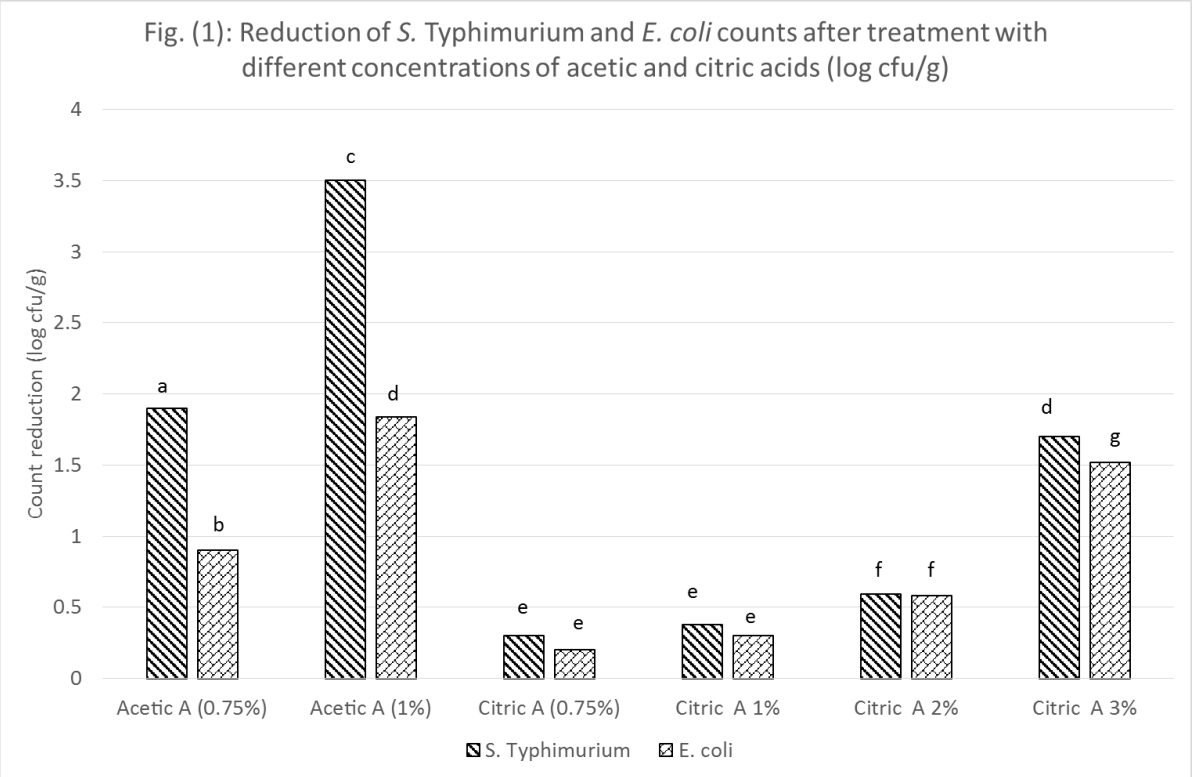
Bacteriological analysis: Fig. (1) represented the results for reduction in counts of *S.* Typhimurium and *E. coli* for different treatments during manufacturing of kariesh cheese. Acetic acid 0.75% achieved a significant ($P<0.01$) reduction in *S.* Typhimurium and *E. coli* levels (1.9 and 0.9 log reduction, respectively) compared with the initial counts. Acetic acid 1% was the most effective treatment in this study, producing a significant ($P<0.01$) 3.5 log reduction in *S.* Typhimurium and 1.84 log reduction in *E. coli* levels in comparison with the initial counts.

The treatment with citric acid (0.75% and 1%) resulted in non-significant ($P> 0.05$) reductions in *S.* Typhimurium (0.3 and 0.38 log reduction, respectively) and *E. coli* levels (0.2 and 0.3 log reduction, respectively). Although citric acid 2% achieved significant reductions in *S.* Typhimurium and *E. coli* levels (0.59 and 0.58 log reduction, respectively) in comparison with the initial count, it may not be considered biologically significant for a processing antimicrobial intervention. By comparison, 3% citric acid application resulted in significant ($P<0.01$) 1.7 log reduction in *S.* Typhimurium and 1.52 log reduction in *E. coli* levels.

Table 1: Sensory evaluation scores of fresh cheese

Cheese treatments	Flavour (1-10 points)	Body and texture (1-5 points)	Appearance and colour (1-5 points)	Total (20 points)
Control	10 [a]	4.9 [a]	4.7 [a]	19.6 [a]
T_2	7 [b]	3 [b]	3 [b]	13 [b]
T_3	9.9 [a]	4.9 [a]	4.8 [a]	19.6 [a]
T_4	9.8 [a]	4.9 [a]	4.8 [a]	19.5 [a]

Means having different letters are significantly differ (P<0.01)



Means having different letters are significantly differ (p<0.05)

DISCUSSION

Yield of cheese: The increase in yield of cheese could be explained by the denaturation and precipitation of whey proteins and/or by higher retention of water in the soft curd formed (Zaki *et al.*, 1974; Abdel-Razig, 1996).

Sensory properties: Gobbetti *et al.* (1998) remind that the flavour intensity score of cheese was probably due to the combination of the higher concentration of lactic, citric and acetic acids and free amino acids and soluble peptides. Very few sensory differences between the control samples and the treated samples were detected by a consumer panel. Thus, organic acid treatments did not cause serious adverse sensory changes. Use of these antimicrobial treatments can be a promising intervention (Harris *et al.*, 2006).

Bacteriological analysis: Organic acids are considered weak acids meaning the antimicrobial effect of organic acids is mainly caused by its undissociated forms (Malicki *et al.*, 2004). They passively diffuse through the bacteria cell wall and internalizing into neutral pH dissociating into anions and protons. Release of the protons causes the internal pH to decrease which exert inhibitory effects on the bacteria (Ricke, 2003). The pH at which the acid is half dissociated (pKa) of citric and acetic acids varies between 3 and 4 (Dibner and Buttin, 2002) which is near to pH of cheese making (4.6).

Somewhat lower results were recorded by Harris *et al.* (2006) on beef trim treated with 2% acetic acid

as *E. coli* O157:H7 and *S.* Typhimurium were reduced by 1.5 to 2.0 log cycles. Acetic acid is widely used as preservative substances and pH-adjusting ingredients in various foods. As preservatives, acetic acid gain their antimicrobial effect by lowering the pH-value below the growth range, and their undissociated forms of acid inhibit the metabolic mechanisms of organisms (Jay, 1978).

Similarly Skrivanova *et al.* (2006) found that citric acid 0.4% didn't influence growth of *E. coli* strains and *Salmonella* sp. Also Seo *et al.* (2013) recorded 0.28–0.57 log reductions of *E. coli* O157:H7 and *S.* Typhimurium after treatment with citric acid 1 and 2%. The addition of citric acid and acetic acid each reduced the growth of Enterobacteriaceae (Bradley *et al.*, 2011) and inhibit the growth of pathogenic bacteria such as *E. coli* (Makino *et al.*, 2000). The bactericidal effect of acids increased with the increasing concentration. However, the inhibitory activity of organic acids during the storage varied with the kinds and concentrations of the acids. As for total plate counts, acetic acid was found to have the highest bactericidal activity, whereas citric acid was found to be the most inhibitory for coliform and *S.* Typhimurium (Seoknam *et al.*, 2003). Rapid acidification in the early stages of the process of raw milk cheeses manufacture is a key factor to effectively control the development of pathogens with low core contamination in raw-milk (Millet *et al.*, 2006).

Difference between the effect of acetic and citric acids may be referred to that, lethal effects of these weak acids depend on concentration, pH of the environment and the dissociation constant of each acid beside adapted or resistant strains due to sub-lethal conditions (Foster and Hall, 1990).

Results of this study indicate that adding of vinegar to milk in the rate of 1% or citric acid 3% are effective antimicrobial intervention for kariesh cheese making. Given the significant microbial reductions observed it is an attractive and effective alternative for traditional process.

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السيطرة على بكتريا التسمم الغذائي اثناء تصنيع الجبن الحمضى باستخدام بعض الاحماض العضوية

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الجبن القريش هو جبن حمضى طرى مصنوع من الحليب الفرز وأكثر انتاجه في المنازل بالتخمر الطبيعي ومع أنه مصدر ممتاز للمواد الغذائية إلا ان إنتاجه عادة يكون بطريقة غير صحية لذا فهو يشكل خطورة على الصحة العامة بسبب استخدام الحليب الخام الملوث بشكل كبير. أجريت هذه الدراسة لتقييم فعالية استخدام حمض الخليك والستريك من مصادر طبيعية (الخل وعصير الليمون) بتركيزات مختلفة، كطريقة بديلة لإنتاج الجبن القريش للحد من مخاطر ميكروبى التسمم الغذائي سالمونيلا تيفيميوريم وايشريشيا كولاى. وقد أظهرت النتائج ان العائد من الجبن لا يختلف كثيرا ($P> 0.05$) بين حمض الستريك (٢ % و٣%) والعينة الضابطة، بينما كانت المعاملة بحمض الخليك هي الأقل معنويا ($P <0.01$). وبالنسبة للخواص الحسية، كانت المعاملة بعصير الليمون (٠.٧٥% و١%) هي الأقل في التقييم معنويا ($P <0.01$) من باقى المعاملات، في حين أن المعاملات الأخرى لم تختلف فيما بينها أو بين المجموعة الضابطة بشكل ملحوظ ($P> 0.05$). حمض الخليك ١٪ كان الأكثر فاعلية ($P<0.01$) في اختزال اعداد الميكروبات حيث اختزل السالمونيلا تيفيميوريم وايشريشيا كولاى بقيمة ٣.٥ و١.٨٤ وحدة لوغاريتمية لكل منهما على التوالي مقارنة مع العد المبدئ، ثم حمض الخليك ٠.٧٥٪ حيث اختزل ($P<0.01$) ١.٩ و ٠.٩ وحدة لوغاريتمية لكلا الميكروبين على التوالي. وبالنسبة للمعاملة بحامض الستريك فبينما لم يحقق تركيزى (٠.٧٥٪ و١٪) اى اختزال معنوى ($P> 0.05$) لكلا الميكروبين وحقق التركيز ٢٪ اختزالا معنويا ولكنه يعتبر ضعيفا من الناحية التطبيقية (٠.٥٩ و٠.٥٨ وحدة لوغارتمية) لكلا الميكروبين على التوالي. فإن تركيز ٣٪ من حامض الستريك حقق اختزالا معنويا ($P <0.01$) بلغ ١.٧ و١.٥٢ وحدة لوغاريتمية لكلا الميكروبين على التوالي بالمقارنة مع العد المبدئى. وتشير هذه النتائج إلى أن استخدام أى من حمض الخليك (الخل) ١٪ أو حمض الستريك ٣٪ مع اللبن الفرز بديل جيد لإنتاج جبن قريش صحي.