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RESEARCH ARTICLE

Application of Slightly Acidic Electrolyzed Water and Ultrasound for Microbial Decontamination of Kashk

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Abstract Application of slightly acidic electrolyzed water (SAEW) in combination with ultrasound for decontamination of kashk was investigated. SAEW had a pH of 5.3-5.5, an oxidation reduction potential of 545-600 mV, and an available chlorine concentration of 20-22 mg/L. Kashk is a dairy product with a unique aroma and a high nutritive value produced in Iran. A 2/1 SAEW/kashk ratio showed 1.42, 1.13, 1.24, and 1.37 log CFU/mL microbial reductions in *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, and *Aspergillus fumigatus*, respectively, at room temperature. A combination of SAEW treatment with ultrasound (SAEW+ultrasound) resulted in 1.87, 1.67, 1.71, and 1.91 log CFU/mL reductions in *S. aureus*, *B. cereus*, *E. coli,* and *A. fumigatus*, respectively. The developed hurdle approach can be a useful tool for sanitization of kashk and similar products. Application of SAEW+ultrasound in dairy microbial decontamination is first reported herein.

Keywords: slightly acidic electrolyzed water, kashk, ultrasound, microbial decontamination, hurdle

Introduction

Humans have used dairies for thousands of years but consumption of acid-fermented milk products has increased tremendously only recently as consumer preferences have changed due to changes in lifestyle (1). Acid-fermented milk products are now widely used around the world (2). Dried fermented milk products are produced in the region between the eastern Mediterranean and the Indian subcontinent (1). Depending on additives, ingredients, and region, dried fermented milk products can have different names, such as tarhana, kishk, chura, kadhi, or kashk (3).

Kashk is a low-fat dried yogurt lacking cereal additives produced in Iran that is used in or with many Iranian foods (4). It is produced both traditionally and industrially in both liquid and dried forms (Fig. 1). Dried forms are intended for long term storage and are usually produced traditionally. Traditional production of kashk is carried out by villagers in rural areas while industrial production takes place in factories using conversion of dried kashk to liquid kashk. Kashk has nutritional properties of high calcium, phosphorous, and protein contents, with some fat and carbohydrates, and a pleasing taste and aroma that make it a product of consumer preference. There have been reports on kashk, especially, traditional kashk, as a source of microbial contamination (1,4). Therefore, improvement in kashk hygiene for the production process is of great importance for public health.

Slightly acidic electrolyzed water (SAEW) with a pH value of 5.0-6.5 contains a high concentration of hypochlorous acid (HOCl). The antimicrobial effect of SAEW has been proved and its application is widely accepted as an environmentally friendly sanitization method (5,6). SAEW is generated based on electrolysis of a dilute hydrochloric acid (HCl) and/or NaCl solution in an electrolytic chamber without a membrane (7). Antimicrobial activity and a low available chlorine content make SAEW an important agent for food sanitization that reduces corrosion of surfaces and damage to human health (8,9). Therefore, during the last decade there has been growing interest in new applications of SAEW in the food industry.

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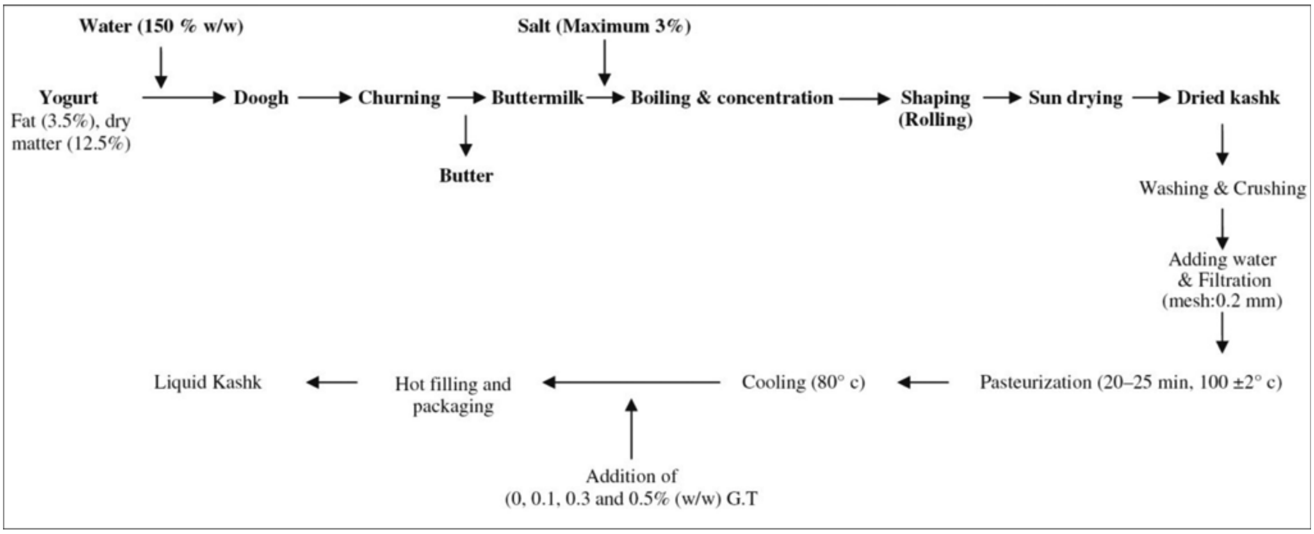

Fig. 1. Schematic production process for dried and liquid kashk (1).

Ultrasound is a form of energy generated as high frequency sound waves (>16 kHz) that can not be detected by the human ear (10). Application of ultrasound as an antimicrobial agent has a long history, although its application for food decontamination is more recent (11,12). Microbial inactivation by ultrasound is mainly attributed to cavitation, a phenomenon that disrupts cellular structures and functions (13). Ultrasound alone may not effectively inactivate bacteria on food (14); however, combined with other treatments, ultrasound may result in higher bacterial lethality (15,16). It has also been reported that ultrasound is more efficient in a saline solution or in water (14). Hence, the aim of this study was (A) to evaluate the efficiency of SAEW for microbial decontamination of the liquid dairy product kashk, (B) to investigate the sanitization effect of ultrasound alone in kashk, and (C) to develop a simple, safe, and production friendly microbial decontamination method for sanitization of kashk as part of a production process.

Materials and Methods

Sample preparation and microbial inoculation Kashk samples were bought from rural markets in Shahsavar, Iran in June-July 2014 and transported to a laboratory in Shahsavar, Iran. For destruction of background microflora, 25 mL kashk samples were autoclaved insterile flasks prior to experimentation. Three different bacterial strains and one fungal strain were used for this study due to their previously reported presence in kashk (4,17). *Escherichia coli* O157: H7 (B0273), *Bacillus cereus* (ATCC 12480), *Staphylococcus aureus* (ATCC 25923), and *Aspergillus fumigatus* (ATCC 28216) were obtained from the Department of Food Science and Biotechnology, Kangwon National University, Korea. Bacterial stock cultures (100 μL) were transferred to 10 mL of tryptic soy broth (TSB) (BD Diagnostics, Sparks, MD, USA) and incubated at 35ºC for 24 h. *A. fumigatus* (100 μL) was transferred into 10 mL of yeast and mold broth (YM broth) (BD Diagnostics) and incubated at 25ºC for 5 days. Following incubation, the microbial culture was sedimented by centrifugation (3,000×*g* for 10 min at 4ºC), and resuspended in 10 mL of 0.1% buffered peptone water (BPW), pH 7.3 (BD Diagnostics) to obtain a final concentration of approximately 7 log CFU/mL. Actual starting concentrations were confirmed based on plating serial dilutions of bacteria and *Aspergillus* on TSA and YB agar (BD Diagnostics), respectively. For inoculation, 250 μL of a microbial suspension was inoculated into 25 mL of kashk in a 500 mL sterile glass beaker resulting in initial pathogen inocula levels of approximately 5 log CFU/mL. After inoculation, the beaker was shaken in a shaking incubator at 4ºC for 5 min at 150 rpm to obtain a homogenous inoculum level in kashk.

Preparation of SAEW SAEW was used in this study at a pH of 5.3-5.5, an oxidation reduction potential (ORP) of 545-600 mV, and an available chlorine concentration (ACC) of 20-22 mg/L. SAEW was produced using electrolysis of a dilute NaCl solution (0.9%) in a chamber without a membrane on an electrolysis device (model D-7; Dolki Co., Ltd., Wonju, Korea) at a setting of 1.75 A and 3.5 V. SAEW pH, ORP, and ACC values were measured immediately before sample treatment using a dual scale pH meter (Accumet model 15; Fisher Scientific Co., Fair Lawn, NJ, USA) bearing pH and ORP electrodes. A colorimetric method was used for the measurement of ACC using a digital chlorine test kit (RC-3F; Kasahara Chemical

Instruments Corp., Saitama, Japan) with a detection range of 0-300 mg/L (18).

Ultrasound treatment of kashk Ultrasound treatment was performed using a bench-top ultrasonic cleaner (JAC-4020; Kodo Technical Research Co., Ltd., Hwaseong, Korea) at a fixed frequency of 40 kHz and an acoustic energy density (AED) of 400 W/L at room temperature (22±2°C) or 1, 3, and 5 min. The ultrasound frequency and AED were selected based on previous study of optimum ultrasound treatment conditions (18,19). Controls (no treatment) were included for comparison of results. A rectangular tank (721×451×297 mm) was filled with 6 L of distilled water (DW) and a sterile glass beaker containing the sample was fixed at the center of the tank. The sample was kept completely under the DW surface level using a metal net. As a major representative bacteria, *E. coli* O157:H7 was used for the primary investigation of ultrasound treatment effect.

Treatment procedures Inoculated kashk samples of 25 mL were mixed with SAEW using different SAEW/kashk volume ratios (v/v), resulting in division of kashk samples into 5 treatment groups with 1/4, 1/2, 1/1, 2/1, and 3/1 SAEW/kashk (v/v) ratios, respectively. All treatments were performed at room temperature (22±2°C) for 1 min and controls were included (no treatment). Subsequently, the effect of treatment time on the SAEW decontamination efficacy was studied using 3 and 5 min treatment times with the optimum SAEW/kashk ratio. Optimal treatment was carried out at 40°C to investigate the effect of mild heat on the sanitization efficacy. Kashk samples were treated using an ultrasound/SAEW combination under previously optimized conditions. Following each SAEW treatment (with and without ultrasound), 225 mL of a neutralizing solution (0.85% NaCl containing 0.5% $Na_2S_2O_3$) was added to kashk samples (25 mL) and immediately mixed using 100 rpm agitation in shaking incubator for 1 min to stop the SAEW decontamination activity.

Microbiological analysis Following treatments, 10 mL aliquots of samples were aseptically and immediately transferred into a stomacher bag (Nasco-Fort Atkinson, Fort Atkinson, WI, USA) containing 90 mL of buffered peptone water (BPW) (BD Diagnostics) and homogenized for 1 min using a Seward stomacher (400 Circulator; Seward Ltd., Worthing, UK). After homogenization, 1 mL aliquots of samples were serially diluted in BPW and 0.1 mL diluents were spread plated on TSA and YMA (BD) for bacteria and *A. fumigatus*, respectively, for enumeration of surviving microorganisms. Subsequently, TSA plates were incubated at 35°C for 24 h and YMA plates at 25°C for 3-5 days. After incubation, surviving cells were enumerated and expressed as log CFU/mL. All analyses were conducted in duplicate with 3 replicates for each experiment (3 independent experiments including 2 replications for all treatments/conditions).

Statistical analysis Mean values of microbial populations (log CFU/mL) from each treatment were subjected to an analysis of variance (ANOVA) using IBM SPSS Statistics Version 19 (SPSS Inc., Chicago, IL, USA). Tukey's multiple range testing was used to determine significance differences between mean values at $p \le 0.05$. Standard deviations (SD) were also calculated.

Results and Discussion

Ultrasound wave effect Changes in the *E. coli* population after ultrasound treatment for 1, 3, and 5 min at a frequency of 40 kHz and an AED value of 400 W/L, compared to the control (no treatment) at room temperature (22±2°C), are shown in Fig. 2. The AED value used in this study was selected based on previous study (18,19) and on other reports (20) that ultrasound treatment at an AED value higher than 400-500 W/L was efficient. The 1 min ultrasound treatment of kashk resulted in a significant ($p \le 0.05$) reduction of 0.74 log CFU/mL in the initial population of *E. coli* O157:H7, compared with the control. This reduction was higher than reported in previous work (18) showing that ultrasound alone only resulted in a 0.35 log CFU/g reduction in bacterial counts, different from the work of Piyasena *et al.* (14) who reported that ultrasound does not effectively inactivate bacteria on food, but in agreement with the work of Ajlouni *et al.* (21) who reported a 0.98

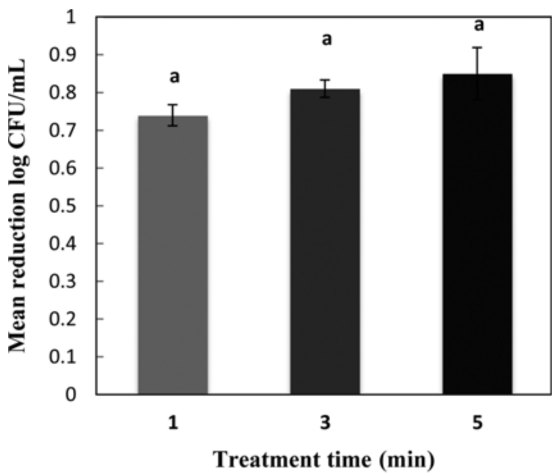

Fig. 2. Effect of treatment time on the sanitization efficacy of ultrasound for reduction of the *Escherichia coli* O157: H7 population at room temperature (22±2°C). Data are shown as a mean±SD of bacterial log reduction.

Table 1. Reductions in populations of inoculated microbes treated with SAEW for 1 min at room temperature (22±2°C) using different SAEW/kashk ratios (v/v)[1)]

Inocula	Log CFU/mL reductions after 1 min of SAEW treatment				
	SAEW/kashk Ratio 1/4	SAEW/kashk Ratio 1/2	SAEW/kashk Ratio 1/1	SAEW/kashk Ratio 2/1	SAEW/kashk Ratio 3/1
S. aureus	0.24±0.11[a]	0.28±0.06[a]	0.56±0.14[b]	1.42±0.28[c]	1.5±0.19[c]
B. cereus	0.28±0.09[a]	0.31±0.13[a]	0.42±0.05[ab]	1.13±0.31[c]	1.14±0.21[c]
E. coli	0.28±0.06[a]	0.36±0.15[a]	0.61±0.12[b]	1.24±0.18[c]	1.33±0.17[c]
A. fumigatus	0.34±0.12[a]	0.29±0.08[a]	0.38±0.14[ab]	1.37±0.23[c]	1.56±0.06[c]

[1)]Reductions (log CFU/mL) are reported as a mean±SD of triplicate determinations (each with 2 replications). Values with different lowercase letters in the same row differ significantly ($p \leq 0.05$). All reductions differed significantly ($p \leq 0.05$) from the control (no treatment).

log CFU/g reduction following ultrasound treatment at a frequency of 40 kHz. Other studies have also suggested that ultrasound alone is more efficient for microbial decontamination in a saline solution or liquid (14,16,22). Kashk is both liquid and saline, which makes it a good candidate for ultrasound treatment (23). Ultrasound creates local regions of high pressure and temperature in liquid environment (24) resulting in maximum cellular wall damage. Hence, a 1 min ultrasound treatment was used for further experimentation as it resulted in microbial reduction values similar to 3 and 5 min treatments, and a 1 min ultrasound treatment reduced the effect of the destructive nature of the treatment (22).

Effect of the SAEW/kashk volume ratio on the microbicidal efficiency For investigation of the effect of SAEW/kashk volume ratios on microbicidal decontamination of SAEW in kashk, SAEW and kashk were mixed with different volume ratios (v/v) of 1/4, 1/2, 1/1, 2/1, and 3/1 and immediately incubated for 1 min at room temperature (22±2°C). Microbial reduction values resulting from different treatment ratios are shown in Table 1. Both the 1/4 and 1/2 SAEW/kashk ratios did not result in significant ($p>0.05$) changes in microbial counts, compared with the control (no treatment). However, the 1/1 ratio showed a significant ($p \leq 0.05$) difference, compared with the control, and the 2/1 and 3/1 ratios showed a great decontamination efficacy perhaps due to faster inactivation of SAEW at lower concentrations (1/1 SAEW/kashk ratio) due to organic materials present in kashk, compared with higher concentrations (2/1 and 3/1 SAEW/kashk ratio) (6). Therefore, the 2/1 SAEW/kashk ratio, which resulted in 1.42, 1.13, 1.24, and 1.37 log CFU/mL reductions in *S. aureus*, *B. cereus*, *E. coli,* and *A. fumigatus* was used for subsequent experimentation. Besides, consumption of less SAEW and faster drying of the final product (if necessary) would be advantages of a 2/1 ratio over use of a 3/1 SAEW/kashk (v/v) ratio.

Effect of the treatment time on the SAEW decontamination efficacy Effects of different SAEW treatment times (1, 3, and 5 min) on microbial reduction at room temperature (22±2°C) are shown in Fig. 3. Reductions resulting from SAEW treatment were always more than 1 log CFU/mL and significantly ($p<0.05$) differed from the control (no treatment), regardless of the treatment time, in agreement with previous reports that SAEW is an effective method for food decontamination (7,8,19,24), but different from previous studies suggesting that 3 min was the best treatment time for SAEW treatments (18,19,25,26). In this study, 1 min was used as the treatment time since 3 and 5 min treatment times resulted in similar degrees of microbial reduction, perhaps due to higher amounts of organic materials (protein/lipid) in kashk, compared with all the foods treated using SAEW in previous reports (1). This higher amount of organic material probably inactivated SAEW in treatment times longer than 1 min. Hypochlorous acid (HOCl), the main effective form of chlorine in electrolyzed water at a pH of 5.0 to 6.5, has different sensitivity levels to organic matter based on the environment (27,28). Therefore, after 1 min of treatment, the decontamination effect of SAEW was lost in kashk.

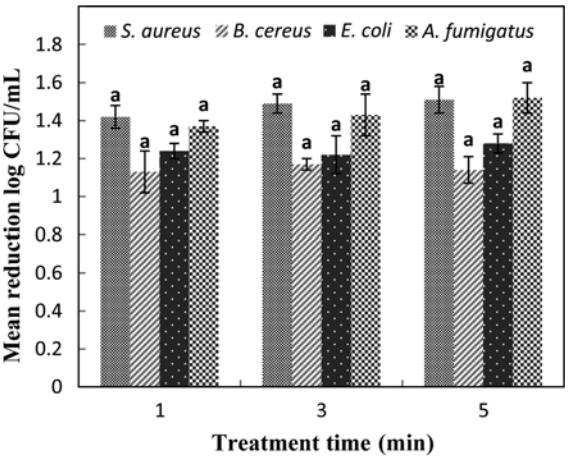

Fig. 3. Effect of SAEW treatment time on reduction of inoculated pathogen populations in kashk at room temperature (22±2°C). Vertical bars represent a mean±SD of triplicate independent determinations (each with 2 replications).

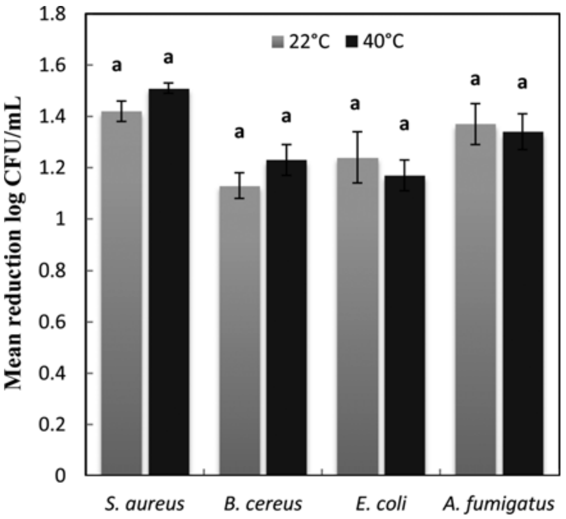

Fig. 4. Effect of mild heat on the microbial inactivation efficacy of SAEW in kashk. Vertical bars represent a mean±SD of triplicate independent determinations (each with 2 replications).

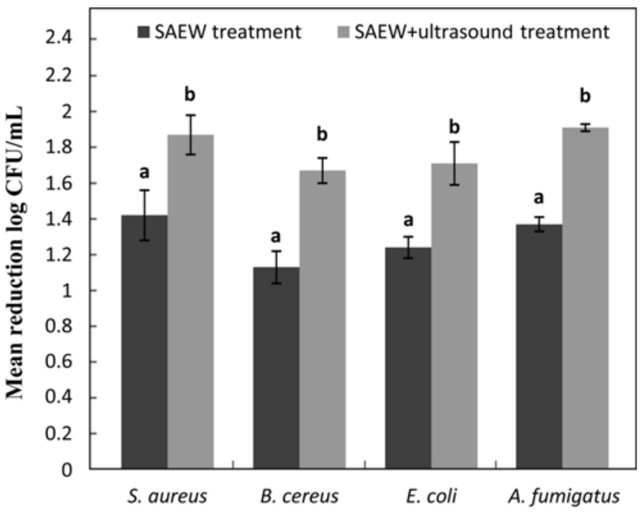

Fig. 5. Comparative microbial inactivation efficacy values of SAEW and SAEW+ultrasound treatments in kashk at room temperature (22±2°C). Vertical bars represent a mean±SD of triplicate independent determinations (each with 2 replications). Bars labeled with different letters for the same species indicate a significant difference ($p \leq 0.05$).

Effect of mild heat on the SAEW decontamination efficacy The effect of mild heat on the microbial inactivation efficacy of SAEW in kashk is shown in Fig. 4. Previous studies showed that mild heat can increase the microbicidal effect of SAEW (6,18,19,29). However, results obtained in this study revealed that mild heat did not improve the sanitization efficacy of SAEW in kashk, perhaps due to the different natures and properties of the foods treated in these studies. All of the above mentioned studies used SAEW for microbial decontamination of solid foods, such as vegetables. In this study, the SAEW-treated food was a liquid. Mild heat itself (40°C) is not sufficient to kill microbial cells and a synergistic effect with SAEW in solid samples might be due to enhancement of physical removal or detachment of microbial cells from the food surface, which does not exist in liquid kashk. Hence, mild heat does not improve the efficacy of SAEW microbial decontamination in kashk.

Combined treatment of kashk (SAEW+ultrasound) Comparative microbial inactivation efficacy values of SAEW and SAEW+ultrasound treatments in kashk are shown in Fig. 5. The SAEW+ultrasound treatment resulted in 1.87, 1.67, 1.71, and 1.91 log CFU/mL reductions in the populations of inoculated *S. aureus*, *B. cereus*, *E. coli*, and *A. fumigatus* in kashk, respectively. All values were significantly ($p \leq 0.05$) higher than for the SAEW treatment alone, in agreement with previous studies reporting the synergistic effect of electrolyzed water and ultrasound (18,19,22,30). This synergistic effect may have been due to cavitation, which disrupts cellular structures and functions, leading to faster intake of SAEW by microbial cells and more damage. Furthermore, the liquid environment of kashk with a semi-high concentration of NaCl enhances the ability of ultrasound to amplify the SAEW decontamination efficacy.

In conclusion, the newly developed hurdle sanitization method of SAEW+ultrasound, which was applied for the first time in dairy products, is an environmental friendly, cost effective, and easy to perform approach with a high degree of efficiency for decontamination of kashk. In addition, it is easy to use this method on site and application is production friendly and does not require any further steps, such as removal from a finished product. Therefore, SAEW+ultrasound is a useful approach for improvement of produce safety that can be widely used for microbial decontamination of kashk.

Disclosure The authors declare no conflict of interest.

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