Microorganism Control in Packed Tofu Manufacture with Electrolyzed Water

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Abstract

Electrolyzed water was applied to control microorganisms during the soybean soaking process of packed tofu processing. Acidic electrolyzed water (pH 2.1; oxidation reduction potential, 1185; 100 ppm of available chlorine) and alkaline electrolyzed water (pH 11.7; oxidation reduction potential, -120) were prepared by electrolysis of 0.075% sodium chloride solution. Mixed electrolyzed water (pH 6.5; oxidation reduction potential, 891; 50 ppm of available chlorine) was prepared by mixing acidic and alkaline electrolyzed water to adjust pH to 6.5. Sterilization effects during soybean soaking and quality of soymilk and tofu produced from soybeans soaked in the three types of electrolyzed water were analyzed. Results show that microorganisms in soybeans can be effectively sterilized by using acidic electrolyzed water or mixed electrolyzed water as soybean soaking water, although the available chlorine concentration, which can be considered to reflect the electrolyzed water condition, decreased time dependently. We conclude that mixed electrolyzed water is the best disinfectant among the three types of electrolyzed water, due to weakly acidic pH, stability, and lack of damage to soymilk and tofu.

Key words: anti-microbial activity, heat-resistant bacteria, hypochlorous acid, mixed electrolyzed water, sterilization

Introduction

Tofu is important in Asian diets as a traditional food. Nowadays, it has become a popular food world-wide because of its nutritional value, including proteins containing essential amino acids and isoflavone[1]. However, since the relatively high water activities (Aw, the amount of water that is available for bacterial growth), nutrient, and pH values of tofu products are suitable for microorganism growth, tofu products decay easily and have a short shelf life. Therefore, control of microorganisms in tofu manufacture is a critical need. For this purpose, tofu factories rinse soybean thoroughly, which decreases the number of microorganism in the material, and protect washed soybean from contamination by microorganisms during processing[2]. In particular, some soybean microorganisms, such as *Bacillus* species, have heat-resistant spores. Rinsing the seeds, even thoroughly, with tap water cannot eliminate these bacteria[3].

Electrolyzed water (EW), which is prepared by the electrolysis of aqueous sodium chloride solution to strong acidic and alkaline forms, has unique properties in functional water. Generally, acidic EW is used as a strong bactericide and alkaline EW is used as a "detergent". Acidic EW is very effective in killing harmful microorganisms[4] in food industries, including organisms on cutting boards[5] and vegetables[6, 7]. Acidic EW has high acidity (< pH 2.7), positive oxidation-reduction potential (ORP), and high available chlorine concentration (ACC). Available chlorine in EW is a mixture of hypochlorous ion, hypochlorous acid, and chlorine, whose ratio depends on pH value[8, 9]. When acidic EW was exposed to light and/or elevated temperature, chlorine, whose concentration increases in response to acidity[8], is gradually released from acidic EW by evaporation[10]. Although chlorine is highly effective in killing microorganisms in simple aqueous systems, its activity is decreased by the presence of organic materials in foods[4]. Oomori et al. showed that the free available chlorine of acidic EW decreased rapidly during incubation with organic materials and was transformed into combined available chlorine forms of N-chloro derivatives, which had lower anti-microbial activities[11]. As a result, acidic EW loses its sterilizing capability under conditions of practical use[10]. Hypochlorous acid around available chlorine primarily acts as a bactericide[7]. Hypochlorous ion, on the other hand, has about 20 times less anti-microbial activity than hypochlorous acid since a high activation energy is required to penetrate the cell membrane because of its negative charge[9].

We focused on the distribution pattern of available chlorine in response to pH. The concentration of chlorine, which is a major component of available chlorine at strongly acidic pH values, is decreased when pH values are more than 4[7]. Hypochlorous ion is increased at alkaline pH[7, 8]. Under weakly acidic conditions around pH 6.5, concentrations of chlorine and hypochlorous ion become minimal[8], which makes EW more stable and more active as a bactericide. Thus, we prepared "mixed EW" at pH 6.5 by mixing acidic EW and alkaline EW. The anti-microbial effects of acidic EW, alkaline EW and mixed EW used as soaking water of soybean materials in tofu processing were assessed. The effects of EW soaking on tofu processing were estimated by soymilk yield and tofu gel strength.

Materials and Methods

Materials

Soybeans (cv. Enrei) harvested in October 1999 were purchased from Kaneko Seeds Co., Ltd. (Gunma, Japan) and stored at 5℃ in plastic bags. Soybean-casein digest agar (Daigo) was obtained from Nichiiko Pharmaceutical Co., Ltd (Toyama, Japan). Glucono-d-lactone (GDL) as a coagulant was purchased from Wako Pure Chemical Industries (Osaka, Japan). All water used in this study was prepared by Milli-Q SP UF (Milipore, MA, USA), and other chemicals were reagent grade.

Preparation of EW

Acidic EW and alkaline EW were produced by electrolysis of 0.075% NaCl solution with an electrolyzed water unit (Daikin Industries Ltd., Osaka, Japan). Mixed EW was prepared by adjusting pH value to 6.5 and ACC to 50 ppm with acidic EW, alkaline EW and water. Freshly prepared EW was used in all experiments. pH and ORP were measured by a pH meter (METTLER TOLEDO MP 320), and available chlorine concentration (ACC) of EW was determined by the iodometric titration method[12].

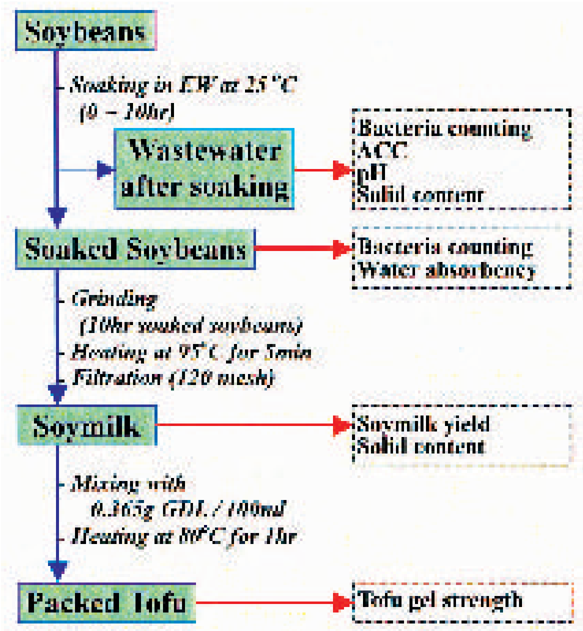

Fig.1 Process followed in microorganism control experiment using electrolyzed water.

Experimental conditions and methods for soybean soaking and tofu processing

Fig. 1 shows the process followed in carrying out our experiment. Four treatments were compared: acidic EW, alkaline EW, mixed EW as soaking water, and sterilized water as a control. 20 g samples of soybeans were soaked in 60 ml EW at 25℃ for 0.1, 0.5, 1, 2, 4, 6, 8, and 10 hr individually. Soaked soybeans and wastewater were separated after soybean soaking in EW. The number of bacteria, residual ACC, pH and solids content in the wastewater were determined. The surviving population of bacteria and water absorbency in soaked soybeans were measured. To estimate the effects of EW soaking, soymilk and tofu were prepared from 10 hr-soaked soybeans. To control differences in water absorbency after EW soaking, the amount of water for soybean grinding was calculated as 6 times the original soybean weight (as dried matter) minus the quantity of water absorbed into the soybeans. Soaked soybeans and water were ground into a slurry using a testing mixer. The slurry was heated at a rate of increase of 15℃/min until temperature reached 95℃, using a Model 55KW ohmic heating system (FRONTIER ENGINEERING Co. Ltd., Tokyo, Japan), and held at 95℃ for 5 min. Soymilk was separated by filtration of the slurry with a 120-mesh nylon filter and immediately cooled on an ice bath. Packed tofu was processed by coagulation of soymilk with GDL. Cooled soymilk (100 ml) was gently stirred for 2 min at 4℃ after addition of GDL (0.356 g) as a tofu coagulant, sealed with aluminum foil, and then heated at 80℃ for 1 hr in a water bath. Prepared tofu was left at 4℃ overnight before gelling property analysis.

Analytical methods

The number of bacteria in soaked soybeans and wastewater for each soaking time was evaluated as total viable aerobic bacteria. Soaked soybeans were homogenized with 9 times (w/w) sterilized 0.9% NaCl dosage. An aliquot (1mL) of the mixture or wastewater was inoculated into soybean-casein digest agar and cultured at 35℃ for 48 hr and then forming units (CFU) were counted.

The moisture content of soybean samples and the solids content of soymilk and soaking water were measured by A.O.A.C. methods[13]. The gel strength of prepared tofu (60 mm in diameter and 25 mm in height) was determined using a Creep Meter (Rheoner II RE2-330055S, Yamaden Company Ltd.), with a plunger diameter of 5 mm and a press speed of 1 mm/s.

Results

Sterilizing effects of EW

The moisture content of the original soybean sample was 10.6 ± 0.3%, and approximately 300 CFU/g of microorganisms were detected in the original soybeans.

Table 1. Physicochemical properties of three EW solutions and sterilized water

Solution	pH	ACC (ppm)	ORP (mV)	CFU /mL
Acidic EW (AC)	2.1	100 ±3	1185	<10 (0)
Alkaline EW (AL)	11.7	0	-120	<10 (0)
Mixed EW (M)	6.5	50 ± 2	891	<10 (0)
Sterilized water (Q)	8.2	0	N. D.	<10 (0)

ACC, available chlorine concentration; ORP, oxidation-reduction potential; CFU, colony forming unit; EW, electrolyzed water.

Table 1 shows the physicochemical properties of EW used in the experiment. The ACC of acidic EW and mixed EW were 100 ppm and 50 ppm, respectively. No microorganisms were found in the water before soaking. The sterilizing effects of EW under the different soaking times were evaluated by microorganism analyses of soaked soybeans (Fig. 2a) and wastewater after soybean soaking (Fig. 2b). Acidic EW acted as a strong bactericide and decreased bacteria counts by about 2 log CFU/g within 6 min of soaking. Similar anti-microbial activity was observed in mixed EW (Fig. 2a). No detectable bacteria was found in wastewater from acidic EW or mixed EW after soybean soaking (Fig. 2b). The number of bacteria in soybeans soaked in sterilized water decreased initially but then increased after 1 hr soaking. Alkaline EW soaking showed results intermediate between acidic EW, mixed EW, and sterilized water (Fig. 2a).

ACC and pH changes of EW during soybean soaking

Fig. 3 shows that the ACC of wastewater after acidic EW and mixed EW soaking decreased rapidly at the first 2 hr of soaking, and then decreased gradually until the termination of the experiment. After 10 hr of soaking, the residual ACC of acidic EW was about 5ppm, but no detectable ACC remained in the mixed EW wastewater.

The acidity of acidic EW gradually decreased as the time of soybean soaking progressed (Fig. 4). The pH values of mixed EW dropped after soaking, and then increased over time. Sterilized water after soaking showed a decrease in pH values over time. Although alkaline EW maintained high pH values during the initial 2 hr of soaking, alkalinity was lost after 4 hr of soaking. The final pH values for all wastewaters including that of alkaline EW were weakly acidic (Fig. 4).

Effect of EW as soaking water on soymilk and tofu quality

Soybeans soaked in acidic EW or mixed EW absorbed water at the same rate as soybeans soaked in sterilized water. However, the absorbency effect of alkaline EW showed higher values (Fig. 5 and Table 2). The solids content of wastewater after soybean soaking in mixed EW was almost the same as the solids content after soaking in sterilized water, whereas acidic EW and

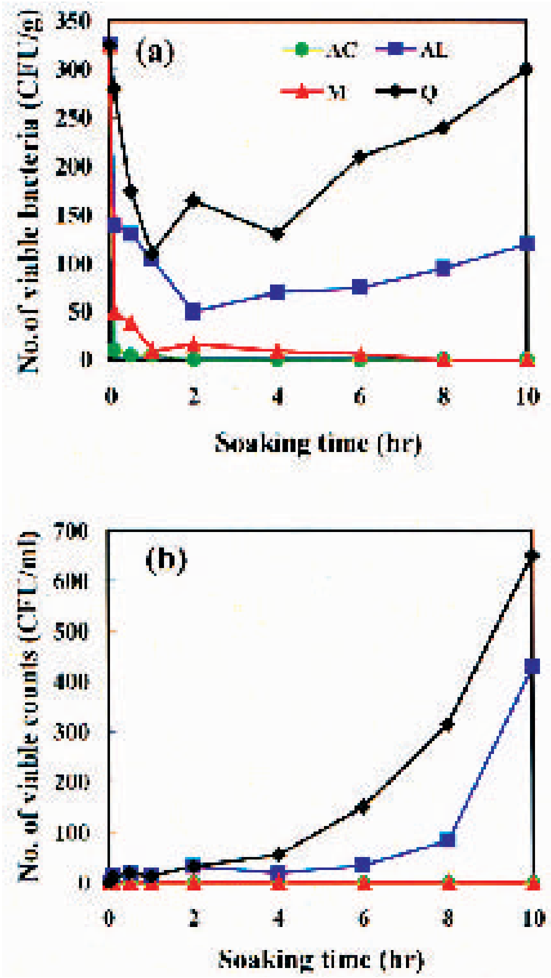

Fig. 2 Changes over time of viable bacteria counts in soaked soybeans (Fig. 2a) and wastewater (Fig. 2b) after soaking in electrolyzed water.
Soybeans were soaked in the four types of water (AL, alkaline electrolyzed water; AC, acidic electrolyzed water; M, mixture of AC and AL; Q, sterilized water) described in Table 1. The surviving bacterial population was determined and expressed as colony forming units (CFU) as described in the text.

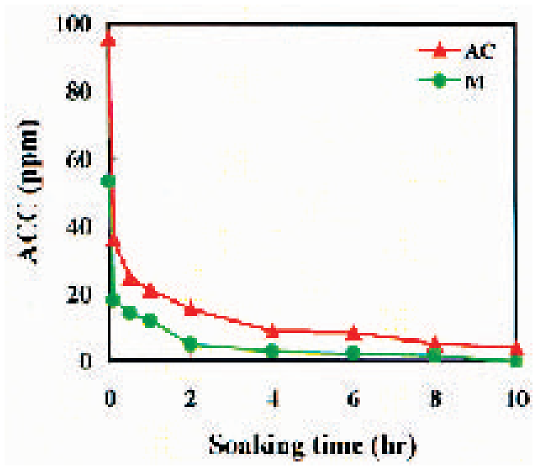

Fig. 3 Changes in available chlorine concentration in wastewater after soybean soaking in two types of electrolyzed water.
Available chlorine concentration of wastewater after soybean soaking in acidic electrolyzed water (AC) and mixed electrolyzed water (M) was determined.

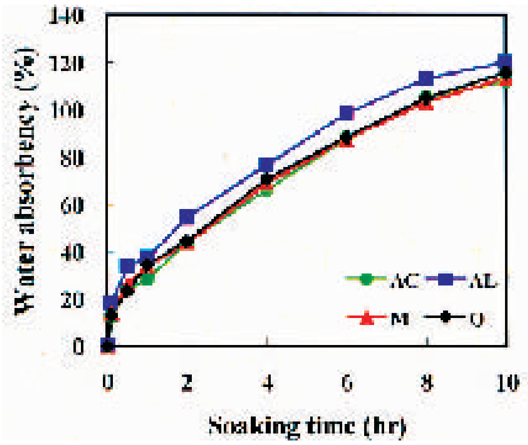

Fig. 4 pH of wastewater of four types of solutions after soybean soaking.
The pH values of wastewater were analyzed after soybean soaking in electrolyzed water (AL, alkaline electrolyzed water; AC, acidic electrolyzed water; M, mixture of AC and AL; Q, sterilized water).

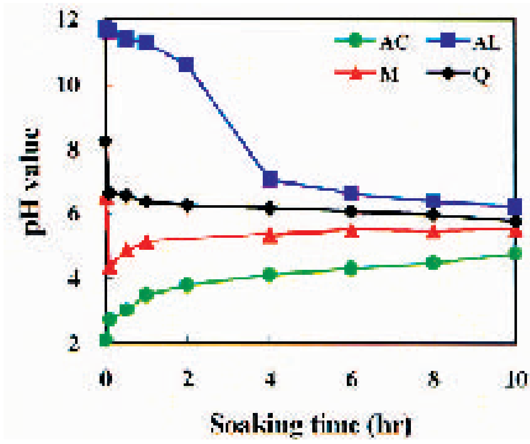

Fig. 5 Water absorption in soybeans soaked in 4 types of solutions.
The water absorbency of soybeans soaked in different soaking waters (AL, alkaline electrolyzed water; AC, acidic electrolyzed water; M, mixture of AC and AL; Q, sterilized water) was determined.

Table2. Effects of soybean soaking in four types solutions on soybeans, soymilk and tofu

	Soaking water			
	AL	AC	M	Q
Water absorbency of soybeans (%)	120.6	112.6	114.0	116.1
Solids content in wastewater (%)	0.51	0.47	0.37	0.32
Yield of soymilk (ml)	232.9	230.6	229.1	227.4
Solids content in soymilk (%)	10.85	11.04	10.60	10.64
Tofu gel strength (kPa)	15.14	15.90	17.68	17.78

AL, alkaline electrolyzed water; AC, acidic electrolyzed water; M, mixture of AC and AL; Q, sterilized water.

alkaline EW showed higher values. Although soymilk yields and solids content in soymilk were similar among the four types of soaking water, tofu consistencies produced from acidic EW and alkaline EW soaking, measured by tofu gel strength, were lower than the values from mixed EW and sterilized water. (Table 2)

Discussion

Packed tofu solidification is usually performed by heating soymilk for 40-60 min at 70-90℃[3,14], depending on the size of the containers[3], after mixing coagulant at a lower temperature. The heating process in packed tofu processing is necessary not only for solidification but also for sterilization[3]. However, some heat-resistant bacteria in soybean material may remain in the tofu and induce decay. LL (long-life) tofu is produced by ultra high temperature heating of soymilk in a clean room[3] but it has unfavorable brittleness and consistency due to overheating. Therefore, development of alternative methods to control microorganisms is a priority need. Acidic EW is widely used in many fields as a convenient and effective sterilizer[5-7,15]. In this paper, soybean soaking in acidic EW and mixed EW was applied as an alternative method in packed tofu processing. The results showed that acidic EW and mixed EW are very effective in killing all microorganisms in soybeans and keep the soaking water aseptic during soaking (Fig. 2).

The reason for the initial decrease in the number of microorganisms in soybeans after soaking in alkaline EW and sterilized water can be considered to be a simple elution effect from the original soybeans.

However, the decrease from alkaline EW was higher than from sterilized water. Alkaline EW, which is considered to be an aqueous sodium hydroxide solution, acts as a sanitizer and reduces the attachment of microorganisms on soybean surfaces[16]. Basic pH and negative ORP decrease the number of aerobic bacteria[4]. The sanitation effect, a basic pH value, and negative ORP result in alkaline EW having an anti-microbial activity. However, the negative ORP in alkaline EW is unstable and gradually changes to a positive value[10]. Alkalinity in EW is also transient, which might be due to a reaction of sodium hydroxide with carbon dioxide[10] from the atmosphere and/or from soybean seeds respiration. It is well known that some components in soybeans dissolve in soaking water[3]. Some materials dissolved from soybeans in this experiment might have brought the pH to weakly acidic values not only in alkaline EW soaking but also in the other tested solutions. These results suggest that alkaline EW is not suitable as a soaking water in tofu processing.

Mixed EW showed the same sterilizing activity as acidic EW despite weak acidity and undetectable available chlorine residue after 10 hr soaking. Acidic EW has high ORP and available chlorine[8, 9]. The anti-microbial activity of acidic EW has been attributed to relationships among pH and ORP and/or ACC[8, 9, 17, 18]. Several studies have shown that the main factor in the elimination of microorganisms is available chlorine and that hydroxyl free radical produced from hypochlorous acid in available chlorine acts on microorganisms[7-9,15, 18]. The preservability of acidic EW under strong acidic conditions is known to be relatively low due to the evaporation of chlorine gas[10] and the reactivity of chlorine with organic materials[4, 11]. Koseki *et al.* found a lower anti-microbial activity in a "Mixture" of EW in which pH had been adjusted to 7.1 by combining acidic EW and alkaline EW[18]. However, about 40% of the available chlorine in their EW "Mixture" was hypochlorous ion[9]. The lower activity of the "Mixture" in those studies corresponded to about 20 times less activity[9] of hypochlorous ion than hypochlorous acid. Thus, we designed our mixed EW to have weak acidity, so it would act as a more stable sterilizer than either acidic EW or the "Mixture" in Koseki's study. In our research, ACC in mixed EW remained nearly constant during storage at 5℃ for a week under shaded and sealed conditions, whereas ACC in acidic EW decreased to 5.5% at the end of the storage period (data not shown). Therefore, we concluded that the hypochlorous acid, which is the dominant form at weak acidic conditions, made mixed EW more stable than acidic EW.

The quality and yield of soymilk extracted from soaked soybean, which affects the quality and yield of tofu products, depends on the length and temperature of soaking due to differences in the water absorbency of soybean[3]. The water absorbency of soybeans after alkaline EW soaking was higher than after soaking in the other soaking waters (Fig. 5 and Table 2). EW soaking of soybeans might affect soymilk and tofu production. It is for this reason that we analyzed the effects of different soaking waters on the qualities of soymilk and tofu product. Soaking in acidic EW and alkaline EW both enabled the solids in soybeans to dissolve into the soaking water more easily than in sterilized water, giving microorganisms a chance to proliferate (see the data for "AL" in Fig. 2b). Tofu produced from mixed water, which had adequate anti-microbial activity, had the same quality as tofu made using sterilized water. Therefore, we concluded that using mixed EW, which is weakly acidic electrolyzed water prepared by a mixing of acidic EW and alkaline, as soaking water is more suitable than acidic EW for tofu processing. To apply mixed EW to other food processing, similar analyses of the efficiency, stability, and reactivity (safety) of mixed EW in the presence of other organic materials are needed.

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電解水を用いた充填豆腐製造の微生物制御

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概要

電解水を用いて充填豆腐製造工程における微生物制御を検証した。酸性電解水（pH2.1、酸化還元電位1185、有効塩素濃度100ppm）とアルカリ電解水（pH 11.7、酸化還元電位-120）は0.075%塩化ナトリウム水溶液を電気分解して調製した。混合電解水（pH 6.5、酸化還元電位 891、有効塩素濃度50ppm）は、酸性電解水・アルカリ電解水・蒸留水を混合して調整した。三種類の電解水を用い、大豆浸漬中の殺菌効果と浸漬された大豆から調製された豆乳と充填豆腐の品質を分析した。その結果、酸性電解水あるいは混合電解水を大豆浸漬水として用いた場合、電解水の状態を反映すると考えられている有効塩素濃度やpHは経時的に減少したが、これらの電解水が大豆中の微生物を効果的に殺菌することが明らかとなった。特に、pH値・安定性・豆乳や豆腐に影響を与えないことから、試験した三種類の電解水の中で混合電解水が最も良い殺菌剤であることが示された。

キーワード：殺菌活性、耐熱性菌、次亜塩素酸、混合電解水、消毒