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Antimicrobial effectiveness of ultra-fine ozone-rich bubble mixtures for fresh vegetables using an alternating flow

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ABSTRACT

The consumption of fresh vegetables has been increasing in part owing to an increase in awareness of their health benefits. Chemicals are used for washing fresh vegetables in washing fresh vegetables to remove surface micro-organisms. However, the effect of these chemicals on human health must be considered, and the chemical concentrations used should be minimal. Previous studies have examined washing fresh vegetables with electrolyzed water, fine bubbles, and ultra-fine bubbles with strong mechanical action. A technique using fine and ultra-fine bubbles containing ozone and chlorine has also been developed. Although the efficiency of these techniques for washing fresh vegetable was high, the observations of fine and ultra-fine bubbles were limited. In the present study, the effect of fine and ultrafine bubbles on the washing rate of fresh vegetables in an alternating flow, which has a stronger mechanical action for washing, was investigated. Although all results in no mechanical action were the almost same (\equiv 6.1 log cfu/g), the difference between the washing result (5.9 log cfu/g) for deionized water and those (5.3 log cfu/g) for ultra-fine bubble was obtained in an alternating flow. Moreover, the resultant viable bacterial count (3.7 log cfu/g) for ultra-fine ozone-rich bubble was less than that (4.6 log cfu/g) for sodium hypochlorite alone. There were substantial differences between washing with deionized water alone and with ultra-fine (ozone-rich) bubble mixtures. Stronger washing effects were obtained when ultra-fine (ozone-rich) bubble mixtures were combined with an alternating flow. Furthermore, surface tension, free radicals, mechanical action, and limitation of the effect was discussed for understanding the experimental results.

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1. Introduction

The consumption of fresh vegetables and processed vegetables, such as salads and pickles, has been gradually increasing owing to better awareness of their health benefits. However, there is also increasing concern about safety and hygiene over the presence of micro-organisms such as bacteria, fungi and viruses. There are still outbreaks of food poisoning originating from vegetables despite advances in safety regulations. Therefore, chemicals including sodium hypochlorite (SH), electrolytic water, and ozone water have

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been used to remove micro-organisms on vegetables. In particular, SH has been widely studied and used [\(Zhang and Farber, 1996;](#page--1-0) [Niemira, 2007](#page--1-0)). However, the complete removal of microorganisms is not achieved, even with these chemicals. Furthermore, when SH is exposed to air, light, metals, and organic substances, its effects are lost. SH also causes inflammation in skin ([Abadias et al., 2008](#page--1-0)). Fine bubbles (FBs; microbubbles) and ultrafine bubbles (UFBs; nanobubbles) have been attracting attention in various fields. These types of bubbles have a larger specific surface area and slower velocity in water than normal-sized bubbles, and their properties become more unusual as the bubble size decreases. The previous studies for FBs and UFBs were summarized in [Table 1.](#page-1-0) Moreover, the details were as follows: [Takahashi \(2005\)](#page--1-0) reported that the surfaces of FBs and UFBs are negatively charged. * Corresponding author.

FBs have been used to remove oil adhering to walls ([Akuzawa et al.,](#page--1-0) [2010\)](#page--1-0), to clean laundry [\(Ushida et al., 2013](#page--1-0)), as an angiographic agent ([Iijima and Moriyasu, 2007\)](#page--1-0), to reduce frictional resistance ([Sanders et al., 2006](#page--1-0)), for long-term storage of fish [\(Wang et al.,](#page--1-0) [2008\)](#page--1-0), and to inactivate norovirus [\(Kozima et al., 2006\)](#page--1-0). The reported applications for UFBs are limited compared with those for FBs. The reduction of frictional resistance on channel walls ([Maali](#page--1-0) [and Bhushan, 2013\)](#page--1-0) and washing effects [\(Chen et al., 2009](#page--1-0)) of UFBs have been reported. The washing effects in fresh vegetables of chemicals, FBs, and UFBs have been examined. [Koseki and Itoh](#page--1-0) [\(2000a\)](#page--1-0) investigated washing and sterilization with electrolyzed water, and they studied the synergetic effect of the combination with physical effects (agitation, ventilation, and ultrasonication). They reported that the combination of strong acidic electrolyzed water with agitation produced a much stronger washing (bactericidal) effect than electrolyzed water without agitation. The viable bacterial count (VBC) of cucumbers after washing was up to 1% of the initial count. [Abadias et al. \(2008\)](#page--1-0) reported that neutral electrolyzed water had a similar effect to SH on lettuce cultured with micro-organisms. [Forghani and Oh \(2013\)](#page--1-0) washed Chinese cabbage, lettuce, sesame leaf, and spinach with weakly acidic electrolyzed water and reported that the total number of bacteria was decreased by 1.5 log cfu/g. They observed a synergetic effect between weak acidic electrolyzed water and ultrasonication. [Zhang et al. \(2013\)](#page--1-0) investigated the inactivation of Bacillus subtilis with FBs containing ozone. The average particle diameter of the FBs decreased as the ozone concentration increased. The maximum bacterial eradicable rate was obtained at an average particle diameter of 49.7 μ m. [Ushida et al. \(2012\)](#page--1-0) conducted a laundry cleaning test with UFBs. They reported that the mechanical action was important in washing. Although the use of chemicals for washing fresh vegetables is essential in modern microbial removal processes, the health effects of chemicals must be considered, and a minimal concentration of chemicals should be used. These studies suggest that electrolyzed water, FBs, and UFBs have a strong mechanical action suitable for washing fresh vegetables. The technique for generating FBs and UFBs containing ozone and chlorine may also be appropriate. However, observations of the FBs and UFBs have been limited. In the present study, we investigate the effect of FBs and UFBs on the bacterial eradication rate in fresh vegetables in an alternating flow, which provides stronger mechanical action in washing. The effectiveness of the method is discussed.

2. Test fluids

The following test fluids (washing liquids) were used.

2.1. Water

The water used in this study was distilled and deionized by distillation apparatus (GSR-200, ADVANTEC Co. Ltd., Japan). In the study, it referred to as simply "water". The electric conductivity of the water was 0.055 μ S/cm. Before bubble generation and experiments, it was passed through 5.0 - μ m filters.

2.2. Sodium hypochlorite

For comparison, we conducted experiments with aqueous solutions of SH (NaClO, Wako Pure Chemical Industry Co. Ltd., Japan). The molecular weight of SH is 74, and the available chlorine content is approximately 12%. Before the experiments, it was stored in a commercial refrigerator (low temperature; $3-5$ °C). A mass concentration, C_m , of 200 ppm was used. The effective chlorine concentration (AQ-102P, Shibata Scientific Technology Co. Ltd., Japan) was 40 mg/L, and the pH was 9.5 ± 0.2 (pHep4; HANNA Instruments, Inc., USA) was measured.

2.3. Fine bubble mixture

FBs were prepared in water by using a FB generator (OM1-C200, Aura Tech Co. Ltd., Japan). The same generator was used as in our previous study ([Ushida et al., 2016](#page--1-0)), and the same particle diameter was observed. [Fig. 1](#page--1-0) (a) shows particle diameter D_b , which was measured with a microscope (VH-8000, Keyence Co. Ltd., Japan), plotted against elapsed time t_e . The FBs were observed at room temperature, $T = 20.0 \pm 2.0$ °C. At $t_e = 120$ s, D_h was approximately 80 μ m, and it gradually decreased. Finally, over $t_e = 420$ s, D_h was less than 20 μ m. The void fraction was set to 1.0 vol% on the generator.

2.4. Ultra-fine bubble mixture

UFBs were generated in water by using a UFB generator (Nano-Aqua MN-20, TECH Corporation Co. Ltd., Japan). [Fig. 1](#page--1-0)(b) shows the particle size distributions (number density, N_h , plotted against particle diameter, D_b) of the UFBs measured by a particle counter (NanoSight LM1-HS, Japan Quantum Design Co. Ltd., Japan). Peak diameter was 70 nm, and the average diameter was 110 nm. The volume fraction of air was set to 1.0 vol% on the generator. The UFBs were generated and mixed with SH (S-UFB; $C_m = 50$ ppm).

2.5. Electrolyzed water contained ultra-fine bubbles

Acidic electrolyzed water containing UFBs (EUB) was also used. EUB was produced from the gas generated by electrolysis of potassium chloride solutions (ozone and chlorine) in a high-speed swirling flow of electrolyzed water. The generator (EFB20AC-12TEST, TECH Corporation Co. Ltd. Japan) was similar to the UFB generator. The effective chlorine concentration (AQ-102P, Shibata Scientific Technology Co. Ltd., Japan) was 50 mg/L, and the pH was 4.0 ± 0.1 (pHep4; HANNA Instruments, Inc., USA).

2.6. Physical properties

The physical properties of the fluids were measured. Density, ρ , viscosity, μ , and static surface tension, σ_s , were measured by a Baumé scale, a capillary viscosity meter, and the ring method ([Ushida et al., 2012; Ushida et al., 2013](#page--1-0)), respectively. Good agreement was obtained, except for the static surface tension, between water and the other test fluids of $\rho = 1.0 \times 10^3$ kg/m³ and $\mu = 1.0 \times$ 10⁻³ Pa•s. However, the values of $\sigma_s = 65.4$ mN/m (FB), $\sigma_s = 63.0$ mN/m (UFB), $\sigma_s = 63.2$ mN/m (S-UFB), and $\sigma_s = 63.9$ mN/m (EUB) Download English Version:

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