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Decrease in the number of microbial cells on chinese cabbage by rapid hygrothermal pasteurization using saturated water vapor

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Abstract

Decontamination effect of rapid hygrothermal pasteurization using saturated water vapor (RHP) treatment on Chinese cabbage was compared with that of sodium hypochlorite (NaClO) solution. RHP treatment was performed by passing Chinese cabbage sample through a processing chamber saturated with water vapor. NaClO treatment followed by RHP treatment was also performed. RHP treatment showed greater or equal decontamination effect compared to NaClO solution on mesophilic bacteria, coliform bacteria, lactic acid bacteria and yeast. NaClO followed by RHP treatment showed higher decontamination effect compared to RHP treatment or NaClO solution only. No significant decontamination effect on bacterial spores was observed after the all treatments tested. The cutting of Chinese cabbage to smaller size resulted in increasing the effect of RHP treatment. On the other hand, wetting the surface of Chinese cabbage by water reduced the effect of RHP treatment.

Introduction

Normally, leafy vegetables such as Chinese cabbage carry 2 to 7 log colony-forming units (CFU)/g of mesophilic bacteria including coliform or spoilage bacteria such as *Pseudomonas*, as their normal microflora (Daeschel *et al*., 1987; Nguyen-The and Carlin, 1994; Inatsu *et al*., 2005). Furthermore, some foodborne outbreaks in lightly fermented (salted) vegetables have been also reported (Ozaki *et al*., 2003; Inatsu *et al*., 2004). Most of the contaminated microorganisms might be transported via fresh vegetables.

In recent years, many studies have demonstrated on surface decontamination techniques to reduce microbial risk involved in fresh fruits and vegetables. Most of these researches have focused on the use of various chemicals such as chlorine, chlorinated water, erythrobate, potassium chloride, including some of the newest sanitizing agents, chlorine dioxide, ozone and organic acids (Abreu *et al*., 2003; Allende *et al*., 2006; Liu *et al*., 2007). Although some of these agents were found to be effective, consumers are demanding a reduction in the overall use of chemicals on fresh products due to their harmfulness and consumers health concerns. Especially chlorine using, the formation of carcinogenic chlorinated by-products such as chloramines and trihalomethanes, called attention to several safety concerns regarding chemical hazard to both human and environment (Abreu *et al*., 2003).

We constructed new rapid hygrothermal pasteurization (RHP) by using saturated water vapor at the dew point of 100˚C for the decontamination of microbes on fresh fruits and vegetables (Tirawat *et al*., 2010). This treatment is performed by passing with free-falling fresh fruits and vegetables through a processing chamber saturated with water vapor at

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100˚C. The saturated water vapor at 100˚C leads to an immediate intensive condensation on the surfaces of fruit and vegetable. While the high energy of latent heat of vaporization (40.8 kJ/mol) releases to the surface of fruit and vegetable for changing from vapor to water drop, this latent energy was thought to be sufficient and effective for inactivating microorganisms on fruit and vegetables surface. However, not only the air-present steam results in a lower temperature than the air-absent steam at the selected pressure (Scruton, 1989) but also during pasteurization under the air-present steam condition, the vapor condensation or heat transfer is interfered by air barriers around treating samples. Therefore, surface pasteurization by "Rapid Hygrothermal Pasteurization" is effective in the air-absent steam condition than in the air-present steam condition. We already reported that RHP using saturated water vapor at the dew point of 100˚C can reduce mesophilic microorganism load and preserve quality attributes in many kinds of fresh-cut fruits and vegetables (Tirawat *et al*., 2010). However, little is known about the decontamination effect of RHP on the other microorganisms.

In this study, besides mesophilic bacteria, we investigated the decontamination effect of RHP treatment on coliform bacteria, lactic acid bacteria and yeast using Chinese cabbage as a sample. In addition, the decontamination effect of RHP was compared to conventional method, sodium hypochlorite solution. Inactivation effect of sodium hypochlorite treatment followed by RHP treatment was also determined. Additionally, effects of sample size and the presence of water droplets of sample surface on bactericidal efficacy were also evaluated.

Materials and Methods

Material

Chinese cabbage *Brassica rapa* var. *Pekinensis* was purchased from local market and stored at 4˚C for a maximum of 1 day before used in the experiments.

RHP treatment

An apparatus for rapid hygrothermal pasteurization method was designed and constructed (Figure 1). The cuboid processing chamber (50 cm×30 cm with 100 cm height) was jointed with two steam spreaders, connected with a steam boiler (Miura Co., Ltd., Aichi, Japan). The flow of the steam into the chamber was controlled by a steam regulator. The top of the chamber has 13.5×33.2 cm square hole opened to the atmosphere for dropping samples into. Steam release from this top hole was prevented by an air curtain with push-pull type blowers. The bottom was also opened to release air from the chamber in order to saturate

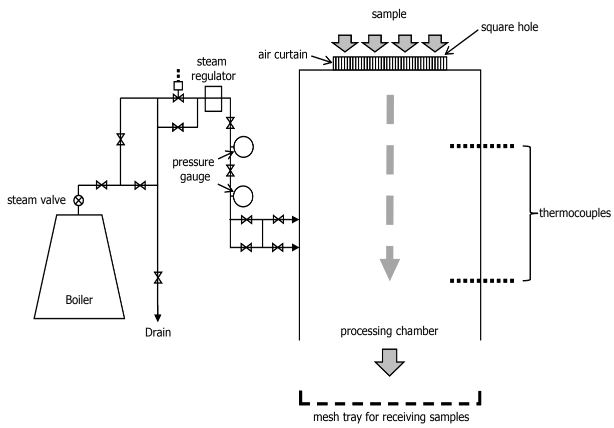

Figure 1. Schematic diagram of the rapid hygrothermal pasteurization apparatus

Table 1. The used agar plates and incubation conditions for viable counts of naturally inoculated microorganisms

microorganism	agar plate	conditions for incubation	
		temperature (°C)	period (h)
mesophilic bacteria	Pearl Core standard agar (Eiken Chemical, Co. Ltd, Tokyo, Japan)	32	48
coliform bacteria	XM-G agar (Nissui Pharmaceutical, Co., Ltd, Tokyo, Japan)	32	24
lactic acid bacteria	Difco Lactobacilli MRS Broth containing 1.2% agar (Becton, Dickinson and Company, NJ, USA)	30	48
heat tolerant spore	Pearl Core standard agar (Eiken Chemical)	32	48
yeast	polydextrose agar (Eiken Chemical) containing 2% NaCl, 0.2% sodium propionate and 0.1% chloramphenicol	28	72

water vapor. Two thermocouples were equipped for monitoring temperature within the chamber during the whole procedure. When the chamber reached saturation state, confirmed by reaching temperature of processing chamber to 100˚C at atmospheric pressure, the samples were loaded into the square hole and dropped it by free-falling through the chamber. The time needed to pass Chinese cabbage through the processing chamber was about 0.5 s. The samples that fell through the steam chamber were received by mesh tray for avoiding bruises.

Sodium hypochlorite treatment

Sodium hypochlorite (NaClO) solution was prepared by diluting sodium hypochlorite (NaClO) solution to obtain 100 mg/L chlorine solution. The pH of NaClO solution was adjusted to 7.0 ± 0.5 using hydrochloric acid (HCl) (Martín-Diana *et al*., 2007; López-Gálvez *et al*., 2010). NaClO treatment was performed by washing Chinese cabbage (3 × 3 cm) in a glass container with approximately 100 g/L of the NaClO solution for 1 min with agitation. After that the NaClO solution remained on the sample surface was removed by a manually operated spinner for 1 min. The samples were then subjected to microbial analysis. As another set of experiment, combined treatment of NaClO-RHP was also performed. In detail, the samples after removing NaClO solution on their surfaces were then treated with RHP immediately.

The effect of sample size on decontamination effect of RHP

To evaluate the effect of sample size, pieces of uncut Chinese cabbage leafs with average size of about 25 cm × 15 cm and pieces of cut samples (about 3 cm × 3 cm) of Chinese cabbage were subjected to RHP treatment. Then mesophilic bacteria count was determined.

The effect of the presence of minuscule water droplets on decontamination effect of RHP

The presence of water droplets may affect the decontamination effect of RHP, because RHP treatment uses latent heat of water vapor. Chinese cabbage pieces (3 cm × 3 cm) were sprayed with tap water and then treated by RHP. In contrast, the cut cabbage without water spraying was used as control. Decontamination effect was estimated by determining mesophilic bacterial count.

Viable count

Ten grams of Chinese cabbage leaves were mixed with 90 mL of sterile 0.85% sodium chloride solution in a stomacher bag (Eiken kizai Co., Ltd., Tokyo, Japan) and then homogenized with a stomacher (IUL Instrument, Barcelona, Spain) for 90 s. The resultant solution was serially diluted and its 0.1 mL was plated onto each type of agar plates in Table 1 for counting the number of mesophilic bacteria, coliform bacteria, lactic acid bacteria and yeast. Viable cells were enumerated as colony forming unit per gram of sample (CFU/g). For counting heat tolerant spores, the homogenized samples were heated at 80°C for 5 min before plating on Pearl core standard agar at 32°C for 48 h.

Results and Discussion

Comparison of decontamination efficacy among RHP treatment, NaClO solution treatment and combined treatment of NaClO-RHP on mesophilic bacteria, coliform bacteria, lactic acid bacteria and yeast

Mesophilic bacterial count of untreated sample was 6 log CFU/g (Figure 2A). RHP treatment reduced the mesophilic bacterial count to about 4.5 log CFU/g. The decontamination effect of RHP treatment on Chinese cabbage was similar to that on cucumber, cherry tomato and pineapple (Tirawat *et al*., 2010). On the other hand, the mesophilic bacterial count after NaClO treatment was about 5 log CFU/g. This decontamination effect was similar to the previous reports (Nicholi *et al*., 2004; Koide *et al*., 2009). These results indicated that decontamination effect of RHP treatment on mesophilic bacteria was prone to be higher than NaClO treatment. Mesophilic bacteria were also decreased to 4.1 log CFU/g when performed with combined treatment of NaClO-RHP. The combination treatment of NaClO with RHP treatment tended to increase the decontamination effect of RHP treatment.

Coliform bacterial count on untreated sample was about 3.2 log CFU/g (Figure 2B). Both NaClO and RHP treatments reduced the count to 2.7 log CFU/g. An increased decontamination effect on coliform bacteria was not observed when performed with combined treatment of NaClO-RHP. Residual minuscale droplets of NaClO solution may inhibit condensation of water vapor or heat transfer. Inadequate removal of NaClO solution can decrease the decontamination effect of RHP treatment. In addition, mesophilic bacteria survived after NaClO treatment might have high tolerance to RHP treatment.

The microbial populations of lactic acid bacteria and spore forming bacteria after treatment with

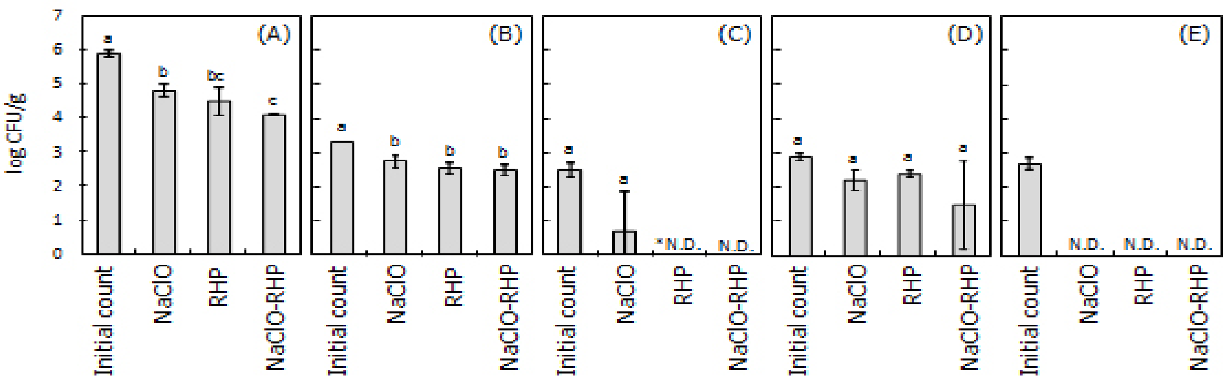

Figure 2. Decontamination effects of NaClO, RHP treatment and combined treatment of NaClO-RHP on mesophilic bacteria (A), coliform bacteria (B), lactic acid bacteria (C), spore forming bacteria (D) and yeast (E). *"N.D" means "not detected". Data represent mean ± SD of three replicated experiments. Different alphabet shows significant difference ($p<0.05$) in Tukey's multiple test (A, B and D) or Welch's t test (C).

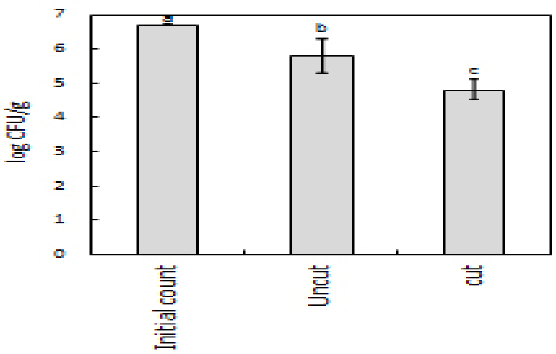

Figure 3. The effect of sample size (uncut or cut) on decontamination effect of RHP treatment against mesophilic bacteria. The sizes of uncut and cut samples were 25 cm × 15 cm and 3 cm × 3 cm, respectively. Data represent mean ± SD of three replicated experiments. Different alphabet shows significant difference ($p<0.05$) in Tukey's multiple test.

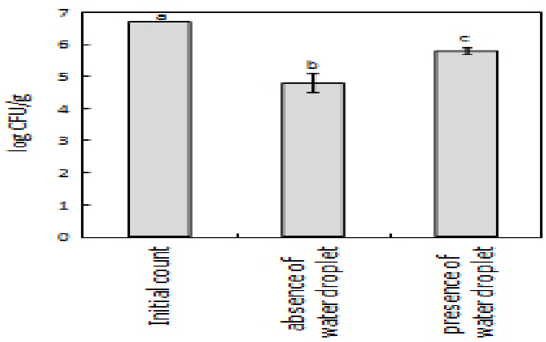

Figure 4. Effect of the presence of minuscule water droplets on decontamination effect of RHP treatment against mesophilic bacteria. Data represent mean ± SD of three replicated experiments. Different alphabet shows significant difference ($p<0.01$) in Tukey's multiple test.

100 mg/L of NaClO solution for 1 min, RHP, and combined treatment of NaClO-RHP are shown in Figures 2C and 2D, respectively. Initial count of lactic acid bacteria, 2.2 log CFU/g, was reduced to 0.5 log CFU/g after NaClO treatment. However, this decontamination effect was not significant because of the large deviation. Lactic acid bacteria were completely decontaminated by both RHP treatment and combined treatment of NaClO-RHP, suggesting that RHP has high decontamination effect against lactic acid bacteria.

The count of spore forming bacteria, tended to decrease after all of the treatments. Especially, combined treatment of NaClO-RHP reduced about 1.3 log CFU/ml. However, no significant difference was observed against initial spore count. The viable count of yeast, 2.5 log CFU/g, was not observed after all treatments including RHP treatment, NaClO treatment and combined treatment of NaClO-RHP (Figure 2E).

Foodborne illness outbreaks linked to fresh produce are becoming more frequent and widespread (Warriner *et al*., 2009). We previously showed that the inactivation effect of the RHP on Escherichia coli, used as a model of pathogenic bacteria, was 2.5 log CFU/ml (Tirawat, 2011). This result suggests that the RHP treatment also has a potential to inactivate pathogenic bacteria.

The effect of sample size on decontamination effect of RHP

The initial mesophilic bacteria count was 6.8 log CFU/g (Figure 3). For uncut sample (25 cm × 15 cm), RHP treatment reduced the count to 5.8 log CFU/ml. For cut sample (3 cm × 3 cm), decontamination effect of RHP treatment increased and mesophilic bacteria count was decreased to 5.2 log CFU/ml. Scruton (1989) mentioned that the presence of air can lowering temperature at the selected pressure because the vapor condensation or heat transfer can be interfered by air barriers around treating samples. It is considered that cutting of Chinese cabbage into smaller size reduced the volume of air and/or non-condensable gas during RHP treatment through free falling.

The effect of the presence of minuscule water droplets on decontamination effect of RHP

The mesophilic bacterial count of untreated sample was 6.8 log CFU/g (Figure 4). After RHP treatment, contaminated mesophiles was decreased by approximately 1.4 and 1.0 log CFU/g in the absence and presence of minuscule water droplets on Chinese cabbage, respectively. The lower decontamination effect of RHP in the presence of water droplets may be resulted from the interference of vapor condensation on the sample surface.

Conclusions

In this study, we investigated the decontamination effect of RHP treatment on various kind of microorganisms contaminated on Chinese cabbage. RHP treatment reduced the counts of mesophilic bacteria and coliform bacteria about 1.5 and 1.0 log CFU/g, respectively. RHP treatment completely inactivated lactic acid bacteria and yeast. These decontamination effects were greater than or equal to conventionally used NaClO treatment. In addition, the treatment time for NaClO (1 min) was reduced to about 0.5s in RHP treatment. These results suggested that RHP treatment can be used as a novel decontamination method for the vegetables including Chinese cabbage.

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