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

Tirawat, D., Noma, S., Kunimoto, H., Tameda, S., Nishibayashi, E. Igura, N. and Shimoda, M.

Laboratory of Food Process Engineering, Division of Food Biotechnology, Graduate School of Biosciences and Bioenvironmental Sciences, Kyushu University, 6-10-1 Hakozaki, Higashi-ku, Fukuoka City, 812-8581, Japan

R & D Laboratories, Tokai Pickling Co., Ltd., 78-1, Mukaigo, Mukoukusama, Toyohashi City, 441-8142, Japan

Laboratory of Food Process Engineering, Division of Food Biotechnology, School of Agriculture, Kyushu University, 6-10-1 Hakozaki, Higashi-ku, Fukuoka City, 812-8581, Japan

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 carcinogetic 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
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 atmosphere for dropping samples into. Steam release was controlled by a steam regulator. The top of the processing chamber was about 0.5 s. 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 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

![Figure 1. Schematic diagram of the rapid hygrothermal pasteurization apparatus](image-url)

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

<table>
<thead>
<tr>
<th>microorganism</th>
<th>agar plate</th>
<th>conditions for incubation</th>
<th>period (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>mesophilic bacteria</td>
<td>Pearl Core standard agar (Eiken Chemical, Co, Ltd, Tokyo, Japan)</td>
<td>32</td>
<td>48</td>
</tr>
<tr>
<td>coliform bacteria</td>
<td>DM agar (Nakal Pharmaceutical Co., Ltd, Tokyo, Japan)</td>
<td>22</td>
<td>24</td>
</tr>
<tr>
<td>lactic acid bacteria</td>
<td>(Eiken Chemical)</td>
<td>30</td>
<td>48</td>
</tr>
<tr>
<td>heat tolerant spore</td>
<td>Pearl Core standard agar (Eiken Chemical)</td>
<td>32</td>
<td>48</td>
</tr>
<tr>
<td>yeast</td>
<td>polydextrose agar (Eiken Chemical) containing 2% NaCl, 0.2% sodium propionate and 0.1% chloramphenicol</td>
<td>28</td>
<td>72</td>
</tr>
</tbody>
</table>
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 leaves 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 minuscule 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
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.

References


