The Sterilization Effects of the Low Temperature Steam-Heating Process on Vegetables

Shouhei Yaghi* Chikao Otoguro** Takeshi Sumino*** Kentaro Kaneko*  

Fresh-cut vegetables are generally contaminated with various bacteria. Staphylococcus aureus and E. coli have been isolated from some samples of vegetables. In this study, we estimated the sterilization effect of the low temperature steam-heating process on fresh-cut vegetables, cabbage, lettuce, cucumber, Chinese chive, welsh onion, carrot, pumpkin and potato. We measured aerobic plate count (APC), coliform count and the E. coli O-157 : H 7 count in the fresh-cut vegetables that are washed with tap water, treated with sodium hypochlorite acid solution (150 ppm available chlorine), or processed by low temperature steam-heating process. As the result, the low temperature steam-heating method greatly decreased the APC and the coliform count in the vegetables and suppressed the growth of the aerobic or facultative anaerobic bacteria and the coliform bacteria in the vegetables stored at 10°C. In conclusion, the low temperature steam-heating process for fresh-cut vegetables is an excellent new method for greatly decreasing the aerobic and coliform bacteria, and improving the microbial storage stability while holding the similar quality.

Key word : vegetables, sterilization, Steam-Heating, sodium hypochlorite acid, aerobic plate count, coliform count

Introduction

Kaneko et al. reported that the γ-Amino butyric acid and alanine in the cabbages and Chinese cabbage increase when the vegetables are steam-heated at low temperature (below 100°C) while glutamic acid decrease, the leafstalk of steam-heated Chinese cabbages show a harder texture than that of the fresh, and E. coli is not detected in the broccolis inoculated with E. coli and then steam-heated at 60°C for 5 min). Kaneko et al. proposed the low temperature steam-heating process in which low temperature drying at atmosphere pressure or lower has been joined to low temperature steam-heating). This process is a new technology in which substance transformation process by steam-heating is joined to physical transformation process by drying. The Gifu Aguri Foods Co. Ltd. built a new vegetable-processing plant in November, 2004 and has been producing vegetable products using the low temperature steam-heating process.

Fresh-cut vegetables are generally contaminated with bacteria at 5 to 6 log CFU / g3). Staphylococcus aureus and E. coli have been isolated from some samples of vegetables4). The levels of microbial contamination are very different in various vegetables5). The most extensively-used sterilization method for vegetables is the treatment with chlorine (sodium hypochlorite acid) solution. However, chlorine solution results in only a 0.5 to 1.7 log reduction of bacteria of vegetables6). Gamma rays are effective for sterilization of foods, without residual activity, or do not make food harmful to human health2). Food irradiation, except for potato sprout inhibition, is not legally authorized in Japan.

In this study, we estimated the sterilization effect of the low temperature steam-heating process on fresh-cut vegetables ; cabbage, lettuce, cucumber, Chinese chive, welsh onion, carrot, pumpkin, and potato.

We measured the aerobic plate count, the coliform count, and the E. coli O-157 : H 7 count in the fresh-cut vegetables that are washed with tap water, treated with sodium hypochlorite acid solution (150 ppm available chlorine), or processed by low temperature steam-heating process.

Material and Methods

1. Sodium hypochlorite acid solution treatment and low temperature steam-heating process for fresh-cut vegetables

1) Materials

Vegetables used for this experiment were cabbage (Brassica oleracea), lettuce (Lactuca sativa), cucumber (Cucumis sativus), Chinese chive (Allium tuberosum), welsh onion (Allium fistulosum), which were purchased
at some food markets in Gifu city in 2004 and 2005. We cut the cabbage into thin strips (1-2 mm), the lettuce into rectangles (3 x 1 cm), the cucumber into thick slices (1.0 cm), the Chinese chive and the welsh onion into strips (3 cm). The fresh-cut vegetables were washed in running water for 5 min.

2) Sodium hypochlorite acid solution treatment

The fresh-cut vegetables washed with water were treated with 20-fold volume of sodium hypochlorite acid (NaClO) solution for 5 min. The solution contained 150 ppm available chlorine and the pH was adjusted to 6.0 with acetic acid. After rinsing with tap water, the sterilized samples were drained of water using a drainer (Pearl Kinzoku Co. Ltd., Tokyo).

3) Low temperature steam-heating process

The fresh-cut vegetables (300-500 g) sterilized with the sodium hypochlorite acid solution treatment were laid in 2-3 cm thick in the plastic basket (33 x 25 x 7.5 cm) and were heated using a steam creating apparatus (SCR-03-0003, Unimac Co. Ltd., Kagawa) at 98% RH and 55°C for 5 min, and at 60°C for 3 and 5 min. The time it took to increase the temperature inside the apparatus to the preset temperature was 15 min. The samples were immediately chilled in a refrigerator (5°C), dried using a cold air drying apparatus (Miikan 21, Unimak Co., Ltd., Kagawa) at 35°C and 50% RH for 20 min, and cooled to 5°C.

2. The shelf life of vegetables processed in a vegetable-processing plant

1) Materials and sodium hypochlorite acid solution treatment

Fresh-cut vegetables used for the experiment were cabbage, cucumber, carrot (Daucus carota), pumpkin (Cucurbita moschata) and potato (Solanum tuberosum) which were manufactured in the vegetable-processing plant of Gifu Aguri Foods Co. Ltd. The vegetables were cut as follows: cabbage into thin strips (1-2 mm wide), cucumber into thick slices (1.0 cm thick), carrot into long sticks (1 x 1.5 cm and 3-4 cm long), pumpkin into rectangles (2 x 4 cm), and potato into wedges (2-3 cm radius). The fresh-cut vegetables were treated using the above sodium hypochlorite acid solution treatment.

2) Low temperature steam-heating process

Steam-heating of the vegetables sterilized with the sodium hypochlorite acid solution treatment were processed using a large (2.5 m x 6 m x 2 m) steam creating apparatus (Takinoukiki, Food machinery Co., Ltd., Nagoya) at 98% RH. The large apparatus designed from the above SCR-03-0003 has 6 removable shelves with 16 baskets (88 x 55 x 5.5 cm) placed 3.5 cm apart. Fresh-cut cabbage, cucumber, and carrot were steam-heated at 60°C for 5 min. pumpkin was steam-heated at 80°C for 30 min, and potato was steam-heated at 85°C for 30 min. The time it took to increase the temperature inside the apparatus to the preset temperature was 15-20 min. The samples (150 g each) were selected from the vegetables (50-100 kg each) that had been heated with low-temperature steam at a vegetable-processing plant, kept in plastics containers, and stored at 10°C for 5 days and at 25°C for 48 hrs.

3. Sterilization effect of the low temperature steam—heating process on E. coli O-157 : H 7

This sterilization experiment used broccoli with complicated florets that were difficult to clean thoroughly. We entrusted the experiment of E. coli O-157 : H 7 to Kotou Microbe Laboratories Co. Ltd. (Kouriyama, Japan). The broccolis were prepared by inoculation with 0.4 ml E. coli culture-broth grown overnight in Heart infusion broth (Eiken, Japan). The contaminated broccolis steam-heated at 98% RH, 50 and 55°C for 5 min and at 60°C for 3 and 5 min.

4. Microbiological analyses

1) Aerobic plate count and coliform count

Aerobic plate count (APC) and coliform count were counted on Standard Method Agar (Eiken, Japan) and Desoxycholate Agar (Eiken, Japan) respectively. The vegetables (10 g) were transferred into 90 ml sterile normal saline and pummeled with a stomacher (Oregano, Tokyo) for 2 min. Serially diluted samples were poured onto appropriate media and the plate incubated at 37°C for 48 hr. The number of bacterial colonies in each plate was manually counted and converted to log CFU (Colony forming units) per gram. Total number of E. coli O-157 was counted using the method established by the Ministry of Health, Labor and Welfare. When the bacteria count is under 300 CFU/g, the data expresses <2.5 log CFU/g.

2) Coliform group

All the strains developing red colony on Desoxycholate Agar do not belong to the coliform group defined as being gram-negative bacillus and fermenting lactose into acid and gas. Thus, we ascertain the validity of using Desoxycholate Agar for the Chinese chive, of which the coliform count showed the largest count. The isolated strains developing red colonies were tested by biochemical confirmation tests, indole production, Methyl red test.
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Table 1. Effects of low temperature steam-heating on total aerobic plate count (APC) and coliform count of various vegetables

<table>
<thead>
<tr>
<th>Sample treatment</th>
<th>Cabbage</th>
<th>Lettuce</th>
<th>Cucumber</th>
<th>Chinese chive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total bacteria</td>
<td>Coliforms</td>
<td>Total bacteria</td>
<td>Coliforms</td>
</tr>
<tr>
<td>Control</td>
<td>5.9 ±0.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.7±0.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.2±0.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.7±0.5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>NaClO</td>
<td>150 ppm, 5 min</td>
<td>4.8±0.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;2.5±0.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.9±0.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Steam-heating</td>
<td>55°C, 10 min</td>
<td>&lt;2.5±0.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;2.5±0.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;2.5±0.0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>heating</td>
<td>60°C, 3 min</td>
<td>&lt;2.5±0.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;2.5±0.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;2.5±0.0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>60°C, 5 min</td>
<td>&lt;2.5±0.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;2.5±0.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;2.5±0.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;2.5±0.0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>
| Control : Sample was tested immediately after washing with water without sterilization with NaClO. Data (log CFU/g) expressed as mean ± SD (n=6).

Means in the same column with different letters are significantly different (p<0.05).

Results and Discussion

1. Sterilization of cut vegetables performed with sodium hypochlorite acid solution and low temperature steam-heating treatment

Table 1 shows the aerobic plate count (APC) and coliforms count in the fresh-cut cabbages, lettuces, cucumbers and chinese chives treated with sodium hypochlorite acid (NaClO) solution (150 ppm available chlorine, pH 6.0) or processed with steam-heating at 50 and 60°C for 3, 5 and 10 min.

1) Sterilization in cabbages and lettuces

The APC of the unprocessed cabbages was 5.9 log CFU/g and the coliform count was 4.7 log CFU/g. Treatment with the NaClO solution reduced the APC to 4.8 log CFU/g, the coliform count to <2.5 log CFU/g. However, process with steam-heating at 55°C for 10 min and 60°C for 3 and 5 min greatly reduced the APC and coliform count to <2.5 log CFU/g. The APC of the unprocessed lettuces was 6.2 log CFU/g, the coliform count was 4.7 log CFU/g. Treatment with the NaClO solution reduced the APC to 4.9 log CFU/g, the coliform count to <2.5 log CFU/g. However, process with steam-heating at 55°C for 10 min and 60°C for 3 and 5 min greatly reduced the APC and coliform count to <2.5 log CFU/g.

As a result, the APC and the coliform count of cabbages and lettuces steam-heated at 55°C for 10 min and 60°C for 3 min or longer were reduced to <2.5 log CFU/g. In addition, there were no significant differences in the quality, color, taste, and texture, between the processed and fresh cabbage as previously reported<sup>1</sup>. However, there was a slight change of only texture in lettuce.

2) Sterilization in cucumber

The APC of the unprocessed cucumber was 6.0 log CFU/g, the coliform count was 5.3 log CFU/g. The treatment with the NaClO solution reduced the APC to 5.1 log CFU/g, the coliform count to 3.9 log CFU/g. The sterilization effect of NaClO solution for the cucumbers was low compared with that for the cabbages and the lettuces. The process with steam-heating at 55°C for 10 min limitedly reduced the APC to 4.5 log CFU/g. However, the steam-heating at 60°C for 3 and 5 min greatly reduced the APC and coliform count to <2.5 log CFU/g.

As a result, the steam-heating for cucumber required higher heating temperature than that for cabbage and lettuce. This is mainly because the sterilization of cucumber is difficult because a surface of cucumber is covered with thin wax repelling water, pustules, biofilm and stomata<sup>6,7,8</sup>.

3) Sterilization in Chinese chive

Of the unprocessed Chinese chives the APC was 7.0 log CFU/g and the coliform count was 6.4 log CFU/g. The treatment with the NaClO solution reduced the APC to 6.1 log CFU/g and the coliform count to 4.8 log CFU/g. The process with steam-heating at 55°C for 10 min further reduced the APC to 4.8 log CFU/g. However, steam-heating at 60°C for 3 and 5 min greatly reduced the APC and coliform count to <2.5 log CFU/g. Chinese chive sometimes decay due to a disease caused by Pseudomonas Marginalis. The Chinese chives used in this experiment were heavily contaminated in comparison to the cabbages, lettuces, and cucumbers. Furthermore, the chlorine sterilization for the Chinese chives showed a
weaker effectiveness comparing to the cabbages and lettuces. The effective sterilization using steam-heating for the Chinese chives required the higher heating temperature than that for the cabbages and lettuces. This result was similar to that of the cucumbers. From the results, the process of steam-heating at 60°C for 3 min or longer is highly effective in sterilization for cabbage, lettuce, cucumber, and Chinese chive.

4) Microbiological aspects of the red colonies on Desoxycholate Agar

The Chinese chives had a problem with high count of coliform, forming a red colony on Desoxycholate Agar, in comparison to the other vegetables. Thus, we examined the microbiological aspects of the strains isolated from the Chinese chives. Among the 80 isolate strains which developed red colonies, 60 (75.0%) belonged to the coliform group which is defined as acid and gas production form lactose and Gram-negative bacillus (Table 2). We performed the species identification for 14 strains using the Micro Station system. These strains were identified as Raoultella terrigena, Enterobacter aerogenes, Pantoea agglomerans and Raoultella aquatilis species (Table 3).

Table 2. Gas and acid production from lactose by strains, forming red colonies on the Desoxycholate Agar, isolated from Chinese chive.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Coliforms (log CFU/g)</th>
<th>Red colonies No. (R)</th>
<th>Gas and acid No. (P)</th>
<th>Ratio (P/R×100) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.4</td>
<td>11</td>
<td>9</td>
<td>81.8</td>
</tr>
<tr>
<td>2</td>
<td>6.4</td>
<td>12</td>
<td>10</td>
<td>83.3</td>
</tr>
<tr>
<td>3</td>
<td>6.4</td>
<td>12</td>
<td>5</td>
<td>41.7</td>
</tr>
<tr>
<td>4</td>
<td>5.9</td>
<td>12</td>
<td>9</td>
<td>75.0</td>
</tr>
<tr>
<td>5</td>
<td>5.9</td>
<td>11</td>
<td>6</td>
<td>54.5</td>
</tr>
<tr>
<td>6</td>
<td>5.2</td>
<td>12</td>
<td>11</td>
<td>91.7</td>
</tr>
<tr>
<td>7</td>
<td>7.4</td>
<td>10</td>
<td>10</td>
<td>100.0</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>80</td>
<td>60</td>
<td>75.0 ± 19.1</td>
</tr>
</tbody>
</table>

Mean ± SD 6.0 ± 0.7

These species are frequently isolated from soil\(^9\). Therefore, we consider that the Chinese chives were heavily contaminated with soil bacteria.

5) Sterilization of broccoli contaminated with E. coli O-157 : H7

Table 4 shows the changes of E. coli count in the broccolis, contaminated with E. coli O-157 : H7, steam-heated at 50 and 60°C for 3 and 5 min. The E. coli count of the unprocessed broccolis was 7.3 log CFU/g, but the process with steam-heating at 60°C for 3 and 5 min greatly reduced the E. coli count to <2.5 log CFU/g. This result is consistent with the report of broccoli contaminated with fecal E. coli\(^1\). Thus, we consider that process of steam-heating at 60°C for 3 min or longer is effective for sterilization of E. coli O-157 : H7.

The most extensively-used sterilization method for fresh-cut vegetables is the method using chlorine disinfectant. However, chlorine or other disinfectants have only a limited effect\(^2\). When shredded turnip root was heated with hot water at 55°C for 10 min, there was an approximately 0.9 log reduction in the population of Gram-positive bacteria and a significant 5.0 log reduction in the population of Gram-negative bacteria\(^9\).

The method using hot water has problems, e. g. loss of water-soluble useful component, wastewater treatment, and hot-water contamination. The heating method using superheated steam (over 100°C) is an effective technique\(^12\). However, effective enzyme reactions, e. g. hardening appearance by pectin esterase, hardly occur because of very short process time (10–30 sec).

2. Microbial storage stability of fresh-cut vegetables manufactured in a vegetable-processing plant

Table 5 shows the changes of the APC and the coliform count in the samples stored at 10°C for 3 and 5...
days. The APC in the processed samples; cabbages, cucumbers, carrots, pumpkins, and potatoes, were <2.5 log CFU/g. After the three-day-storage, the APC in these samples increased to 2.8 (cucumber) to 4.0 (carrot) log CFU/g, and after the five-days-storage, the APC in these samples increased to 3.5 (potato) to 5.6 (carrot) log CFU/g. However, the increases of these coliform counts were not detected in any of the samples. The APC and the coliform count in all the vegetables stored for 5 days passed the standard (under 5.0 log CFU/g) in Vegetables Cut Business Roundtable Corp.

Table 6 shows the changes of the APC and the coliform count in the samples stored at 25°C for 48 hrs. The APC and the coliform count in all the processed samples were <2.5 log CFU/g. After the 48-hours-storage, the rapid increases of the APC in all the samples were detected, while the increases of the coliform count were not detected.

Shimizu examined the APC of commercial fresh-cut vegetables (sell-by date of 3 days). In this report, the welsh onions (18 samples) had the highest 6.3 ± 0.9 (mean ± s. d.) log CFU/g, the lettuces (4 samples) had 5.5 ± 0.4 log CFU/g, the carrots (10 samples) had 5.2 ± 0.4 log CFU/g, the cabbages (20 samples) had 4.9 ± 0.6 log CFU/g. Furthermore, when fresh-cut cabbages were stored at 20°C, the APC exceeded 6 log CFU/g after 24 hrs storage, but when these were stored at 5°C, the APC did not increase. The Listeria monocytogenes inoculated into cabbage and lettuce hardly propagates at 10°C. The vegetables steam-heated at low temperature has a similar quality to the fresh-cut vegetables. For example, a steam-heated lettuce has a similar color to a fresh lettuce but a softer texture than a fresh lettuce. A steam-heated cabbage is somewhat harder than a fresh cabbage but has a similar color to a fresh cabbage. In conclusion, the low temperature steam-heating process for fresh-cut vegetables is an excellent new method for greatly decreasing the aerobic and coliform bacteria, and improving the microbial storage stability while holding the similar quality.

Since the main heat transfer of steam-heating is the condensation heat transfer of steam, the condensation heat transfer efficiency is different in the surface properties, e.g. hydrophilic or hydrophobic, in the steam-heated samples. Clarifying the relation between the surface properties of bacteria or vegetables and the sterilization effect of the steam-heating deserves further studies. Furthermore, the sterilization effect of the steam-heating on other food poisoning bacteria, e.g. Listeria monocytogenes or Yersinia enterocolitica, has been unidentified.

**Conclusion**

We measured the aerobic plate count (APC) and the coliform count in fresh-cut vegetables, cabbages, lettuces, cucumbers, chinese chives, welsh onions, carrots, pumpkins, and potatoes washed with tap water, treated with sodium hypochlorite acid (NaClO) solution (150 ppm).
available chlorine), and processed by low temperature steam-heating process. As the result, the low temperature steam-heating method greatly decreased the APC and the coliform count in the vegetables and suppressed the growth of the aerobic or facultative anaerobic bacteria and the coliform bacteria in the vegetables stored at 10°C. The results show:

1. When cabbages and lettuces were steam-heated at 55°C for 10 min and at 60°C for 5 min, the APC and the coliform count decreased to <2.5 log CFU/g.
2. When cucumbers and Chinese chives were steam-heated at 60°C for 3 min or longer, the APC and the coliform count decreased to <2.5 log CFU/g.
3. Among the 80 isolate strains which were isolated from Chinese chives and developed red colonies on Desoxycholate Agar, 60 (75.0%) belonged to the coliform group.
4. When broccolis inoculated with E. coli O-157 : H7 were steam-heated at 60°C for 3 min or longer, the E. coli count was decreased to <2.5 log CFU/g.
5. When fresh-cut vegetables processed by the low temperature steam-heating method in a vegetable-processing plant were stored at 10°C or lower, the microbial storage stability was gained for 5 days.

References

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低温蒸気加熱加工法による野菜類の殺菌

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和文抄録
一般にカット野菜は様々な微生物に汚染され、大腸菌やプドウ球菌が検出されることもある。この研究では、キャベツ、レタス、キュウリ、ニラ、長ネギ、ニンジン、カボチャ、ジャガイモなど野菜類を低温蒸気で加熱処理した場合の殺菌効果を検討した。洗浄、次亜塩素酸ナトリウム溶液（有効塩素150 ppm）、低温蒸気加熱処理した野菜類の一般生菌数及び大腸菌群数を測定した。低温蒸気加熱処理は野菜類の一般生菌数及び大腸菌群数を著しく減少させ、10℃で保存した野菜類の一般生菌及び大腸菌群の増殖を抑制した。低温蒸気加熱加工法は殺菌効果に優れ、処理した野菜が生鮮物と殆ど遜色のないことから、卓越した新殺菌技術といえる。

キーワード：野菜、殺菌、蒸気加熱、次亜塩素酸ナトリウム、一般生菌数、大腸菌群数

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