Viable Bacterial Counts on Fresh-cut Salads and Bactericidal Effect of Electrolyzed Acidic Water

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The total viable bacterial count was determined with seven kinds of fresh-cut salad products for the duration of a year. Group A consisted of four different takeout salad products sold in sealed packages, while group B consisted of three different takeout salad products prepared and served at restaurants. Generally, the total viable bacterial counts of purchased salad products were strongly correlated with the ambient temperature and number of foodborne illnesses. When disinfected with electrolyzed acidic water, group A resulted in no significant bactericidal effect, while two samples in group B showed strong bactericidal effects. These results and the high initial bacterial counts in group B suggested possible cross-contamination during the serving process of the above two samples. The study indicated that successful prevention of microbial contamination requires thorough training of workers on sanitation control as well as low temperature distribution and storage.

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Consumption of salad products such as fresh-cut vegetables has been growing in recent years. This trend is related to the increase of nuclear families whose members eat meals at separate times. The growing number of foodborne illnesses¹⁾ and accidental mixtures of foreign matter suggest that salad products should require proper sanitation control. However, there are no legal regulations on bacterial counts, and some local governments establish their own target levels²⁾. Bacterial growth rate on fresh-cut salads depends on environmental conditions. Even if controlled in the production and processing stages, bacteria may grow on food products depending on their distribution, storage and display conditions. It is difficult for processors to ensure the quality and safety of their products until supplied to consumers.

Fresh-cut salads are mostly produced in bulk at vegetable processing plants, and then either

packaged and displayed at supermarkets and convenience stores, or transported in containers and served at fast food and family restaurants. Generally, the former type of salads remains sealed up until consumption and has little risk of cross-contamination during distribution. In contrast, the latter type of salads is prepared and served at each restaurant and has a possibility of cross-contamination. Other than cross-contamination, storage and display temperatures are also important factors for bacterial contamination. In addition, viable bacterial counts in raw vegetables vary with their origin and production season³⁾. It is interesting to study bacterial counts in fresh-cut salads at their consumption stage.

Electrolyzed acidic water is a new disinfectant that was listed as a food additive in 2002⁴⁾. It shows an equal bactericidal effect to the conventional sodium hypochlorite solution with a lower

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concentration for a shorter time and produces fewer carcinogens⁵⁾. Its applications are expected in food production sites as well as other various fields^{6)~8)}. ACHIWA *et al.*⁹⁾ reported that the storage time after inoculation affected the bacterial reduction of fresh-cut cabbage treated with electrolyzed acidic water. The results showed that the longer storage times after inoculation caused almost no effects of bacterial reduction, while the shorter storage times contributed to desirable effects. In this study, we determined the viable bacterial counts on packaged fresh-cut salad products and non-packaged takeout salads from retail outlets, and compared the bactericidal effects of electrolyzed acidic water for the two types of fresh-cut salads.

Materials and Methods

1. Materials and purchase dates

Four sealed packages of fresh-cut salads purchased from supermarkets and convenience stores were numbered from A-1 to A-4 (Group A), whereas three packages of takeout salads served by workers at fast food and family restaurants were numbered from B-1 to B-3 (Group B). The detailed ingredients of each sample were specified in Table 1.

A-1 was packed in a plastic bag mainly containing 1 mm wide fresh-cut cabbage with 3 cm square lettuce and other vegetables for decoration. It was displayed at a vegetable stand of the room temperature in January and in a refrigerated showcase in April. It was not seasoned with mayonnaise or dressing.

A-2 was packed in a plastic bag mainly containing 3 cm square lettuce. It was displayed in a refrigerated showcase and not seasoned with mayonnaise or dressing.

A-3 was packed in a plastic cup mainly containing 1 mm wide fresh-cut cabbage and seaweeds. It was displayed in a refrigerated

showcase and not seasoned with mayonnaise or dressing. The sampled part was not in contact with the seaweeds.

A-4 was packed in a plastic cup mainly containing 1 mm wide fresh-cut cabbage and red leaf lettuce. It was displayed in a refrigerated showcase and not seasoned with mayonnaise or dressing.

B-1 and B-2 were packed in plastic cups mainly containing 3 cm square lettuce. They were displayed in a refrigerated showcase and not seasoned with mayonnaise or dressing.

B-3 was packed in a plastic tray mainly containing 3 cm square lettuce. It was purchased immediately after served. The part not seasoned with mayonnaise and dressing was sampled.

With both group A and group B, no visible contamination with dust and sand, discoloration or offensive odor was present. Each sample with different ingredients was purchased on the morning of experiment day and refrigerated for not more than three hours before examined in consideration of the previous results⁹⁾. Either cabbage or lettuce was used in the experiments, because both are leaf vegetables and either was included in all samples. Experiments were conducted four times in every three months on January 21, April 15, July 8 and October 28, 2003 with samples purchased from the same stores and restaurants.

2. Treatment water

Toyoake city water was used as tap water. Electrolyzed acidic water refers to water generated at the anode by electrolysis of dilute salt water through a membrane. The water electrolyzer ROX-20 TA by Hoshizaki Electric Co., Ltd. was used to produce electrolyzed acidic water. The pH of the generated water was determined with a pH meter (TOA, HM-10 P) and the available chlorine level by the iodine titration method¹⁰⁾.

The tap water had a pH of 7.0~7.4 and available chlorine of 0.1~0.2 mg/kg, while the electrolyzed acidic water had a pH of 2.5~2.7 and available chlorine of 20~40 mg/kg.

3. Bacterial counts

The purchased samples (10 g) and those treated with tap water or electrolyzed acidic water were mixed with sterile physiological saline solution (90 ml) and homogenized in a stomacher. Serial dilutions of this were poured in a standard agar (Nissui Pharmaceutical Co., Ltd.) plates and incubated at 37°C for 48 hours for total viable bacterial counts.

Table 1 Salad ingredients of samples

Sample	Ingredients
A-1	Cabbage, lettuce, carrot, red cabbage
A-2	Lettuce, paprika
A-3	Cabbage, onion, <i>wakame</i> seaweed, cucumber
A-4	Cabbage, red leaf lettuce, tomato, asparagus, corn
B-1	Lettuce, tomato, green pepper, onion
B-2	Lettuce, cabbage, paprika
B-3	Lettuce, tomato, potato, tuna

The counts of inherent bacteria in the purchased samples were considered as controls for each treatment. According to the previous report⁵⁾, the samples were treated for 30 seconds with agitation in the treatment waters of 20 times the sample weight and rinsed with tap water before assay for microbial counts. The results are means of triplicates.

Results and Discussion

1. Seasonal total viable bacterial counts of fresh-cut salad products

The seasonal and annual average bacterial counts were determined for each group of the salad products (Fig.1). No significant difference in the bacterial counts was observed between group A and group B. The average counts per group were correlated with the ambient temperatures and peaked in July.

The seasonal bacterial counts per sample showed marked increases up to $10^6 \sim 10^7$ CFU/g in July with every sample in group A (Fig.2). This is mainly attributed to the inherent high bacterial counts in the vegetable materials³⁾, maybe due to the ambient temperature in July. The average temperatures in this area (Nagoya, Japan) in 2003¹¹⁾ were 3.8°C in January, 14.8°C in April, 23.7°C in July, and 16.9°C in October. The annual total of patients with foodborne illness in 2002 amounted to 1931 in January, 1747 in April, 2729 in July, and 1875 in October¹⁾. These facts indicate a significant effect of temperatures on bacterial growth and foodborne illnesses. The increase in foodborne illnesses in July

Fig.1 Seasonal and annual average total viable bacterial counts per group

Vertical bars in graph show standard errors.

Fig.2 Seasonal total viable bacterial counts on each salad sample

Vertical bars in graph show standard errors (n = 3).

is also supported by the fact that most of the foodborne pathogenic bacteria rapidly grow around 37°C¹²⁾. The bacterial count of A-1 was relatively high in January, because it was displayed at a vegetable stand of the room temperature. In group B, the bacterial count of B-3 was significantly higher than the other samples in January and April. Considering the thorough practice of low temperature distribution, this may have been caused by cross-contamination in the serving process or bacterial growth due to insufficient cold storage after delivery to the restaurants. We will discuss this in the next section.

Vegetable materials contain different types of bacteria^{13), 14)} which counts depend on the breed, production district, season³⁾, and cultivation method. The vegetable samples especially in group A may have been affected by the cultivation temperatures with extensive bacterial growth, indicating distinct relations between temperatures and bacterial counts.

2. Bactericidal effect of electrolyzed acidic water on fresh-cut salad products

The bacterial counts were determined with fresh-cut salads purchased seasonally and treated with tap water or electrolyzed acidic water (Fig.3-6). In January, the results of group A, except for A-1, showed that the disinfection treatment only reduced the bacterial counts by not more than 1 log CFU/g. In group B, the initial bacterial count of B-3 was markedly high, and the disinfection treatment reduced the bacterial counts of B-1 and

tap water. In the electrolyzed acidic water treatment, A-1-A-4 and B-2 showed no significant bactericidal effect, while B-1 and B-3 showed relatively strong effects throughout the year. ACHIWA *et al.*⁹⁾ reported that disinfectants became less effective on fresh-cut cabbage within at least three hours after contamination. In some seasons, B-1 and B-3 showed markedly high initial bacterial counts. Thus, the above results suggested the possibility of bacterial attachment to B-1 and B-3 samples during the serving process at the restaurants. The results of no significant bactericidal effect in group A indicated effective sanitation control at fresh-cut vegetable processing plants to prevent cross-contamination. However, A-1 in January and A-3 in July showed high initial bacterial counts, suggesting the effect of high temperatures during distribution and storage.

B-2 was probably distributed and stored in similar conditions to B-1 and B-3, but resulted in low initial bacterial counts and no significant bacterial reduction by the disinfection treatments. This result indicated thorough sanitation control of workers and workplaces all the year round.

Recently, food safety became major concerns for consumers. Food safety and quality control systems have been emphasized for the purpose of ISO, HACCP and traceability¹⁵⁾, and the widespread use of Internet also has enabled consumers to search for producers and cultivation methods of farm products¹⁶⁾. Legally, farm produce safety has been ensured by the Pesticides Control Act and Food Sanitation Law. Still, microbial safety of salads and other fresh-cut vegetables has not yet been legally secured. The Japanese Ministry of Health, Labor and Welfare suggested the maximum level of total viable bacterial count as 10^6 CFU/g in the "Sanitation Standard for Box Lunch and Delicatessen"¹⁷⁾ published by the name of a ministry manager in 1979. Some local governments established their own guidelines²⁾. For example, Miyagi and Kanagawa Prefectures specified the maximum level of 10^5 CFU/g in their guidelines. Some fresh-cut vegetable processors also set up their own shipping standards. Comparing the above results with these standards, most of the salad products purchased in July exceeded the specified levels of total viable bacterial count. This may be attributed to the inherent bacteria existing in the raw vegetables. Perhaps the population of microorganisms on the

samples in July became too great for electrolyzed acidic water containing 20~40 mg/kg available chlorine to remain effective. Other than microbial safety, taste, odor and appearance are also important elements of food products. It is necessary to ensure food safety without degrading these elements. In this regard, disinfection treatment with electrolyzed water will not affect the taste and odor of foods even when immediately followed by consumption⁵⁾. Therefore, it is an effective treatment to prevent foodborne illnesses on the verge of consumption of contaminated foods.

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市販サラダの生菌数ならびに 強酸性電解水による殺菌効果

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1年にわたり市販されているサラダ7種の一般生菌数を測定した。持ち帰り用密封状態で販売されているサラダ4種をグループAとし、各店舗で持ち帰り用に盛りつけられ販売されているサラダ3種をグループBとした。購入時のサラダの一般生菌数は、全体的には気温ならびに食中毒事故件数と関係が深かった。それらサラダを強酸性電解水により殺菌処理を行ったところ、Aグループでは減菌効果は低かったが、Bグループの2種では高かった。購入時のサラダの菌数が高かったことと殺菌処理による減菌効果から、Bグループの2種では盛りつけ時の二次汚染が示唆された。低温流通、低温保存に加え、作業員への衛生管理教育まで徹底させなければ微生物汚染は防止できないということが示された。

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