



Effect of vibration on the bacterial growth on strawberry fruits

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Abstract

The effects of vibration on the growth of aerobic mesophilic bacteria and coliform bacteria, and the survival of inoculated *Escherichia coli* O157:H7 or *Listeria monocytogenes* were studied on the strawberry fruits. Post-vibrated strawberries were stored at 10°C and 25°C and the periodic microbial count was observed for 4 days. It was found that vibrated strawberries caused increase of aerobic mesophilic and/or coliform bacteria on post storage incubation at 25°C (P<0.01), while vibration treatment did not affect the growth of mesophilic bacteria, if stored at 10°C. *L. monocytogenes* levels on vibrated strawberries stored at 10°C and 25°C were slightly lower than that of non-vibrated strawberries, but the difference was not significant. *E. coli* O157:H7 level for vibrated fruits stored at 10°C showed slightly but significantly lower value than that for non-vibrated fruits (P<0.01). On the other hand, *E. coli* O157:H7 levels on vibrated and non-vibrated strawberries stored at 25°C increased during storage (P<0.05). The results showed that vibration caused a significant increase of bacteria on strawberry fruits stored at 25°C, but non-significant when stored at 10°C.

Key words: Strawberry, vibration, damage, storage, temperature, aerobic mesophilic bacteria, coliform bacteria, *Escherichia coli* O157:H7, *Listeria monocytogenes*.

Introduction

Strawberry is very popular fruit in Japan for unique shape, color, flavor and many health advantages. The wholesale quantity of strawberries in 2004 is 140,752 tons/year, and the wholesale price is 982 Yen per kg ¹, that is the highest in major fruits and vegetables in Japan ¹. However, strawberries are extremely susceptible to damage by shock and vibration. Therefore, there is a need to develop a novel method to maintain the quality of strawberry fruits during transportation and/or distribution. Several studies revealed the physical damage, changes of chemical components and physiological reactions of strawberries after harvest including transportation ²⁻¹³.

Several foodborne outbreaks implicated strawberries as the possible vehicle of hepatitis A virus, norovirus and parasite *Cyclospora cayatanensis*, etc. ¹⁴. In addition, *Salmonella* was isolated from strawberries imported into the United States ¹⁵ and *E. coli* O157:H7 grew in wounds on apples ¹⁶⁻¹⁸. Kärenlampi and Hänninen showed the survival of *Campylobacter jejuni* on several fresh produces during storage ¹⁹. Han and Linton reported the affection of temperature and pH on the population change of *Escherichia coli* O157:H7 and *Listeria monocytogenes* in strawberry juice and/or acidified media ²⁰. Moreover, there are many reports on the effectiveness of the sanitizer to control microorganisms on strawberry fruit ²¹⁻²⁵. On the other hand, the survival of microorganisms on the fresh cut fruits and/or whole fruit were reported ²⁶⁻²⁷, however, there are few reports available for the bacterial growth on strawberry fruits. Therefore, this study was undertaken to evaluate the periodic growth of aerobic mesophilic bacteria, coliform bacteria in non-inoculated strawberry

fruits and the survival of inoculated *Listeria monocytogenes* and *Escherichia coli* O157:H7 on strawberry fruits after vibration operation.

Materials and Methods

Sample collection: Strawberries (*Fragaria x ananassa*, cv. Tochiotome) were harvested from a farm and transported to the laboratory with careful handling on the day of experiment. Fully ripe fruits were harvested with short stem left to avoid the bruise and to allow inocula operation. Only unblemished strawberries were screened and packed into sterilized container. Strawberries were not washed before experiments because usually washing is not a common practice during shipment.

Test strains: Four rifampicin-resistant strains of *Escherichia coli* O157:H7 (MN28, MY29, CR3 and DT66) and five rifampicin-resistant strains of *Listeria monocytogenes* (JCM7671, JCM7672, JCM7676, ATCC43256 and ATCC49594) were used for this study. The details were as follows: Enterohemorrhagic *E. coli* O157:H7 strains CR-3, MN-28, MY-29 and DT-66 were isolated from bovine feces, which were provided by the Laboratory of Zoonosis, National Institute of Animal Health, Tsukuba, Japan. Strains of *L. monocytogenes* were American Type Culture Collection (ATCC, Manassas VA) 43256 (Mexican-style cheese), ATCC 49594 (derived from *L. monocytogenes* strain Scott A) and Japan Collection of Microorganisms (JCM) 7676 (roast beef), 7672 (salami sausage) and 7671 (lax ham). The rifampicin-resistant derivatives of these strains were used in this study to facilitate the recovery of test strains from strawberry during storage.

Inoculation procedure: Each strain of *Escherichia coli* O157:H7 and *Listeria monocytogenes* was cultured in Tryptic soy broth (10 ml) supplemented with 50 µg of rifampicin (TSB/Rif) per ml at 37°C. Cultures were transferred to TSB/Rif by loop at three successive 24-h intervals immediately before use as inocula. An equal volume of cell suspensions of four or five strains of each pathogen was combined to give approximately equal populations of each strain. These cocktails were diluted with sterile phosphate buffer saline (PBS; 10 mM, pH 7.2) to prepare final inoculums (approximately 5.0×10^6 CFU/ml). The inoculum was maintained at $20 \pm 1^\circ\text{C}$ and applied to the strawberry fruits. Strawberry fruits were immersed in the inoculum and eased slowly for a few seconds tweezing with stem. Excessive water was drained off and strawberries were kept in a clean bench for 2 h to allow drying the surface. The strawberry fruits were then packed into sterilized container and vibrated.

Vibration operation: A three-dimensional vibrator (VTVH-5, Saginomiya Seisakusho, Inc., Japan) was used. This equipment consists of three vertical actuators (Z-axis), two horizontal actuators (Y-axis) and another horizontal actuator (X-axis) that is right-angled to Y-axis. These actuators were driven by hydraulic system connecting through personal computer (PC). An actuator for X-axis was operated to generate the horizontal vibration in the current study. The vibration direction, acceleration, frequency and time were horizontal, $9.8 \text{ m}\cdot\text{s}^{-2}$, 7 Hz, 60 s, respectively. The vibration acceleration and time were decided by pre-test to make sufficient injury on strawberry fruits. The sterilized container containing strawberries was fixed on the vibration table with a rope, and the vibration treatment was done under the conditions described above.

Storage conditions: Strawberry fruits were stored at 10°C and 25°C after vibration. Periodic viable counts of aerobic mesophilic bacteria and coliform bacteria in non-inoculated strawberry fruits were observed for up to 4 days. The survival of inoculated *L. monocytogenes* and *E. coli* O157:H7 in strawberry fruits was also observed for up to 4 days.

Microbiological analyses: Twenty-five grams of vibrated, non-vibrated or post-vibrated samples were aseptically transferred to stomacher bags, and 225 ml of phosphate buffered saline (PBS, pH 7.2) was added to each bag. The bag contents were pummeled for 60 s in a stomacher (ILU Instrument, model CE-97, Barcelona, Spain) at medium speed. Serial decimal dilutions were prepared with PBS and were surface-plated (0.1 ml, in duplicate) on Standard Plate Agar (SPA) for aerobic mesophilic bacterial counts and Desoxycholate agar (Nissui) for coliform bacteria, sorbitol MacConkey agar (Nissui) supplemented with 50 µg/ml of rifampicin for *E. coli* O157:H7 and *Listeria* selective Oxford agar (Oxoid) supplemented with 50 µg/ml of rifampicin for *L. monocytogenes*. All the plates were then incubated at 37°C for 48 h.

Sensory evaluation: Eighteen panelists were volunteered from the staff, Food Company, researchers and students at National Food Research Institute. The criteria for participation in the analysis were a willingness to evaluate the appearance and aroma of treated and untreated strawberry samples and availability during scheduled testing. No panelists or data were eliminated.

The panelists, all volunteers, were informed about the nature of the study. The test area was free of extraneous odors and sound, and panelists were instructed not to talk during testing.

Panelists evaluated samples and marked on unstructured hedonic score sheets shown a line of very disagreeable to very agreeable in individual booths. Five pieces of treated and non-treated strawberry samples at room temperature from 8 different sample groups were given to each panel member for evaluation, and the effects of temperature, storage and vibration on appearance and aroma were scored.

Results

Growth of aerobic mesophilic bacteria and coliform bacteria: Changes in population of aerobic mesophilic and coliform bacteria in non-inoculated strawberries are shown in Figs. 1 and 2, respectively. Population of aerobic mesophilic bacterial counts on vibrated and non-vibrated fruits at 10°C were found $3.5\text{--}3.7 \log_{10}$ CFU/g at day 0, and increased little at day 2 and remained constant throughout the 4 days incubation period. However, the population of aerobic mesophilic bacteria on vibrated fruits stored at 25°C for 4 days showed significant ($P < 0.01$) increase ($0.2\text{--}1.3 \log_{10}$ CFU/g) compared to non-vibrated fruits during the same storage period. Coliform bacteria showed a trend unlike aerobic mesophilic bacteria. The population of coliform bacteria was found below detection level on vibrated or non-vibrated samples at 10°C. However, the populations of coliform bacteria of vibrated fruits were increased ($> 2.0 \log_{10}$ CFU/g) significantly ($P < 0.01$) at Day 2 compared to non-vibrated fruits at 25°C, and/or remained constant throughout the storage period.

Survival of *L. monocytogenes* and *E. coli* O157:H7: The survival of *L. monocytogenes* and *E. coli* O157:H7 on strawberries is shown in Figs 3 and 4, respectively. The population of *L. monocytogenes* at 10°C and 25°C was on the increase by $0.3\text{--}0.7 \log_{10}$ CFU/g in the first day, respectively, and remained constant and/or on the decrease during storage. The vibrated strawberries showed slightly lower population than non-vibrated strawberries ($0.1\text{--}1.0 \log_{10}$ CFU/g). The difference between vibrated and non-vibrated fruits was significant ($P < 0.01$) on Day 2 storage sample at 10°C and 3 days at 25°C. The population of *E. coli* O157:H7 at 10°C in non-vibrated strawberries was constant up to 2 days of storage, and then decreased slightly. However, vibrated strawberry fruits showed significantly lower population ($0.3\text{--}0.8 \log_{10}$ CFU/g) than non-vibrated strawberries ($P < 0.01$). The population of *E. coli* O157:H7 at 25°C in both non-vibrated and vibrated strawberries was increased by maximum of $1.4 \log_{10}$ CFU/g and $1.7 \log_{10}$ CFU/g within 1 day and were constant throughout the incubation period for both vibrated and non-vibrated strawberry fruits.

Sensory evaluation: The sensory characteristics of appearance and aroma of non-inoculated vibrated and non-vibrated strawberry samples stored at 25°C and 10°C were evaluated and the results are shown in Fig. 5. The sensory characteristics of appearance evaluated by the panelists decreased significantly at 25°C ($P < 0.01$) compared to that at 10°C immediately after the vibration treatment. Non-vibrated strawberry fruits did not show any change ($P > 0.05$) after 3-day storage at 10°C. However, discoloration was observed around the damaged spot on vibrated strawberries, but the sensory score was not changed significantly

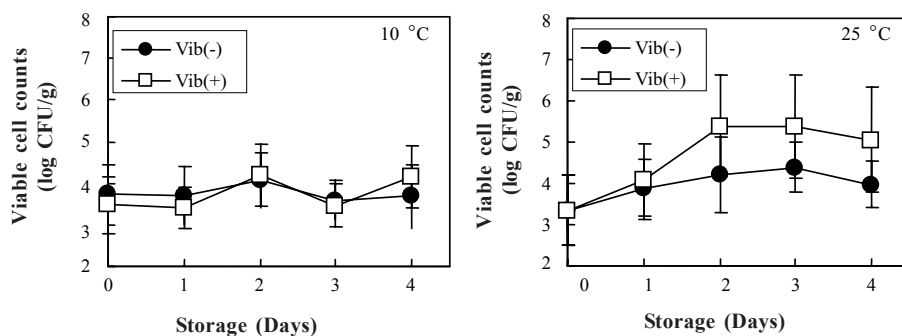


Figure 1. Growth of aerobic mesophilic bacteria on the vibrated (square open symbols) and non-vibrated (circle closed symbols) strawberries stored at 10°C and 25°C. Values are the average of quintuple samples from each of 5 experiments (n=25). Error bars represent the standard deviation.

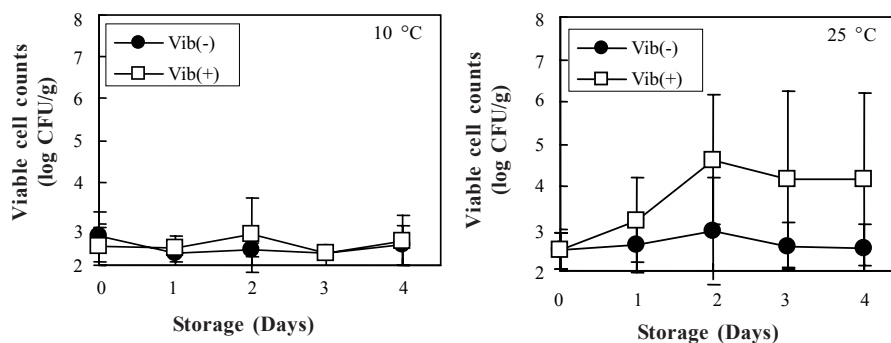


Figure 2. Growth of coliform bacteria on the vibrated (square open symbols) and non-vibrated (circle closed symbols) strawberries stored at 10°C and 25°C. Values are the average of quintuple samples from each of 5 experiments (n=25). Error bars represent the standard deviation.

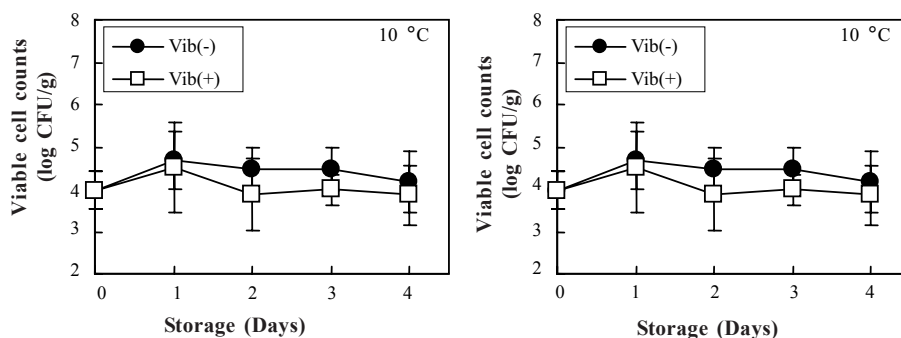


Figure 3. Survival of *L. monocytogenes* on the vibrated (square open symbols) and non-vibrated (circle closed symbols) strawberries stored at 10°C and 25°C. Values are the average of quintuple samples from each of 5 experiments (n=25). Error bars represent the standard deviation.

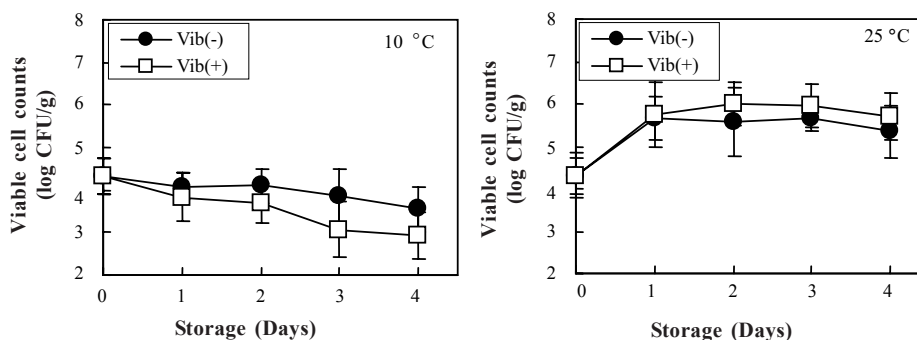


Figure 4. Survival of *E. coli* O157:H7 on the vibrated (square open symbols) and non-vibrated (circle closed symbols) strawberries stored at 10°C and 25°C. Values are the average of quintuple samples from each of 5 experiments (n=25). Error bars represent the standard deviation.

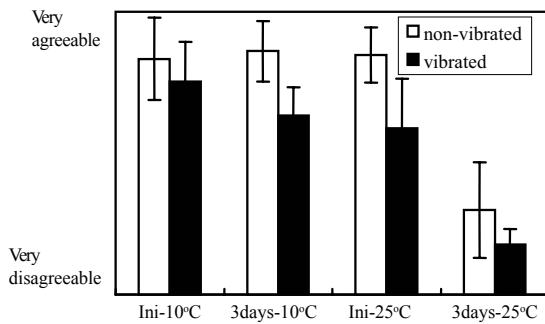


Figure 5. Effects of vibration subsequent storage and storage temperature on the sensory score. Values are the average of score standard deviation of quintuple samples from each of 2 experiments (n=18).

($P > 0.05$). Moreover, vibrated strawberry fruits showed more serious fungal growth with discoloration at 3-day storage at 25°C than non-vibrated strawberries (Fig 6). The appearance of both vibrated and non-vibrated samples declined significantly ($P < 0.01$) during storage, and after 3 days of storage at 25°C the appearance was found very disagreeable. The sensory characteristic of aroma of non-inoculated vibrated and non-vibrated strawberry samples stored at 25°C and 10°C was found similar to appearance and therefore was not shown in the figure.

Discussion

Strawberry is one of the most popular and expensive fruits in Japan because of appearance, flavor and food values, while the shortness of shelf life is a great problem²⁸. Damage of fruits caused by vibration or shock in transit or handling is considered one of the major factors of subsequent deterioration during distribution. However, the effect of physical damage on bacterial growth is not clear.

There are many factors affecting the bacterial growth in relation to the vibration damage. For example, the pericarp deterioration induces fungal growth on strawberry surface. Riordan *et al.*

suggest that the populations of *E. coli* O157:H7 increased due to the rise of pH caused by the presence of certain pathogenic fungus in wounds on apple surface at room temperature¹⁷. Yu *et al.* suggested that the decrease in bacterial population on the strawberry during storage was caused by drying of the bacteria, lack of nutrients or growth of competing microorganisms²⁵, although the leakage of juice is considered to cause bacterial growth. In addition, Han and Linton revealed that acids and lower pH contribute to an inhibitory effect on growth of *E. coli* O157:H7 and *L. monocytogenes*²⁰. Furthermore, Puupponen-Pimiä *et al.* reported that berry extracts inhibit the growth of Gram-negative bacteria but had no effect on Gram-positive bacteria^{29,30}.

In our study, the populations of aerobic mesophilic bacteria, coliform bacteria and *E. coli* O157:H7 at 25°C on the vibrated strawberry increased significantly ($P < 0.01$) compared to non-vibrated fruits. The liquid from damaged surface may be the main factor of bacterial growth on vibrated strawberry. In addition, fungal growth was observed on vibrated strawberries stored at 25°C and this fungal growth could subsequently increase the pH. Therefore, there is a possibility to increase of population of *E. coli* O157:H7 or aerobic mesophilic bacteria and coliform bacteria on strawberry.

As shown in Fig. 4, the population of *E. coli* O157:H7 at 10°C was significantly lower compared to non-vibrated strawberries ($P < 0.05$). It seems reasonable to suppose that the lowering of pH, caused by the leakage of citric or malic acid, which are the major organic acids in strawberry, reduced the population of *E. coli* O157:H7¹⁷ on bruised fruits.

The microbiological and sensory parameters in this study showed that vibration damage caused a significant increase of bacteria and fungal growth on strawberry stored at 25°C, but no significant changes occurred when stored at 10°C. Therefore, there is a need to maintain the cold temperature chain from harvest to distribution and through consumption.



Figure 6. Appearance of vibrated or non-vibrated strawberries stored 3 days at 10°C and 25°C after vibration treatment.

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