Inactivation of *Alicyclobacillus acidoterrestris* in Orange Juice by High Electric Field Alternating Current

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*Alicyclobacillus acidoterrestris* spores inoculated in orange juice were inactivated by applying a high electric field alternating current (HEF-AC) to food for a short duration. This new method is effective for inactivating all microorganisms in liquid foods. HEF-AC was compared with a conventional heating method using two oil baths and a tubular heat exchanger for the inactivation of *A. acidoterrestris* spores in orange juice. Using an HEF-AC pilot plant system at a flow rate of 100L/h, *A. acidoterrestris* spores in orange juice were exposed to an electric field of 2.5 - 2.7 kV/cm at 20 kHz for 13.8 ms in an electrode unit, as the temperature of orange juice was increased from 35°C to above 110°C upon leaving the electrode. Holding times between the electrode and cooling unit varied from 0.3 - 0.9 s. Increasing the holding time of HEF-AC enhanced microbial inactivation, while changing the holding time after the electrode unit was replaced with a tubular heat exchanger had no effect. Comparative studies revealed that the HEF-AC inactivated *A. acidoterrestris* spores 30 times faster than conventional heating using two oil baths during the holding time.

Keywords: high electric field alternating current, *Alicyclobacillus acidoterrestris*, spore, inactivation

Introduction

Fruit products, such as juices, nectars, concentrates, and purees, are generally acidic (pH < 4.6), and therefore pasteurization in a temperature range of 85 - 95°C should be adequate to stabilize them at ambient temperature. Such a process inactivates all nonspore-forming microorganisms that cause spoilage. Any surviving bacterial spores usually do not germinate and grow under these acidic conditions (Splittstoesser et al., 1994). However, *Alicyclobacillus acidoterrestris*, a thermo-acidophilic, non-pathogenic, spore-forming microorganism, has been detected in several spoiled commercial pasteurized fruit juices. Cerny et al. (1984) reported that a new type of bacterium in aseptically packaged apple juice, which was later identified as *A. acidoterrestris*, causes a flat-sour spoilage and produces the offensive-smelling compound guaiacol, along with other taint chemicals. In addition, Baumgart (2003) reported 2,6-dibromophenol as an off-flavor compound associated with *A. acidoterrestris* spoilage. As *A. acidoterrestris* is a producer of off-flavors, it is considered to be an important target for quality control of acidic beverages.

The high electric field alternating current (HEF-AC) method was originally designed by Uemura and Isobe (2002) to inactivate *Escherichia coli* in liquid foods. *E. coli* inactivation by HEF-AC results from the combination of a high electric field with ohmic heating (Uemura and Isobe, 2002). Similarly, Geveke et al. (2004) applied a radio frequency electric field (18 kV/cm at 20 kHz) to *E. coli* in apple juice at a moderately low temperature (50°C), reducing the microorganisms to 3 log through the high electric field effect. The most popular nonthermal and electrical inactivation method uses a pulse electric field (PEF). Most reports have investigated inactivation of vegetative cells by PEF, but few have elucidated the limitations of PEF on inactivating spores. *Bacillus cereus* spores in apple juice were reduced by only 1 log with a PEF at 20 kV/cm for 10.4 pulses (Cserhalmi et al., 2002). On the other hand, Pagán et al. (1998) showed no inactivation of *Bacillus subtilis* spores with a PEF at 60 kV/cm for 75 pulses at room temperature, while HEF-AC inactivated *B. subtilis* spores in orange juice by 4 log (Uemura and Isobe, 2003). Thus, both the electric field effect and the sud-
den temperature rise of more than 100°C due to Joule heating may contribute to spore inactivation by HEF-AC. A major advantage of the HEF-AC method is its ability to retain most of the nutritional content, such as ascorbic acid, despite the thermal treatment, because the heating time is much shorter than for that of conventional heating.

In addition, the HEF-AC method can be easily scaled up, and an apparatus that processes liquid food at 100 L/h has already been developed (Inoue, 2007). This method is thought to achieve synergism from heating and electric treatment, but the mechanism of spore inactivation has not been sufficiently clarified. In this study, we compared the dynamic inactivation of *A. acidoterrestris* spores inoculated in orange juice by both the HEF-AC and conventional heating methods.

**Materials and Methods**

**Bacterial strain** The *A. acidoterrestris* strain (JCM21546) used in this study was obtained from the Japanese Collection of Microorganisms.

**Orange juice** Frozen concentrated orange juice from Louis Dreyfus Citrus (Brazil) was purchased from Nisshin Trading Co. and diluted five-fold with distilled water for use in the experiments. The juice had a Brix degree of 11.0 and pH 4.0.

**Preparation of *A. acidoterrestris* spore suspension** Spores were produced on plate nutrient agar (Eiken Chemical Co., Japan) adjusted at pH 4.0 with 13.3 µM tartaric acid (Wako, Japan) at 50°C for 10 days at which point at least 80% of the cells sporulated, as determined by microscopic examination. Spores were removed by stirring each tube using a vortex stirrer after adding 10 mL sterile distilled water. The spore crop was centrifuged at 4000 rpm for 10 min. The supernatant was decanted, and the pellet was resuspended. Spores were cleaned by repeated washing with centrifugation five times. The final pellets were resuspended in a sterile peptone solution. The suspension was heated and maintained at 80°C for 10 min and then stored at -80°C under sterile conditions until use.

**Conventional heating** Orange juice (10 mL) containing 10⁶ cfu/mL *A. acidoterrestris* spores was sealed in a test tube made of stainless steel (TV-100, Taiatsu Glass Co., Japan) (Fig. 1). A thermocouple sensor incorporated in the cap of the test tube measured the temperature at the center of the sample every second. Two oil baths were prepared; the temperature of oil bath 1 was set 15°C higher than the target temperature, and the temperature of oil bath 2 was set at the target temperature. A stainless steel test tube was heated in oil bath 1 until the sample temperature reached 3°C lower than the target temperature. The tube was then moved to oil bath 2. After the holding time, it was dipped in cold water. The sample temperature was increased from 40°C to 110°C over a 3-min period. Figure 4a shows the temperature profile of the sample with conventional heating using the oil baths. The stainless steel test tube was opened at a clean bench, and the content was decimally diluted in Ringer’s solution (DIGO, Wako, Japan). The diluted samples were surface-plated in triplicates onto nutrient agar with an adjusted pH 4.0. The plates were incubated at 50°C for 48 h before presumptive colonies were counted. All experiments were conducted in triplicate.

**Experiment design of HEF-AC** Figure 2 outlines the HEF-AC system. Raw orange juice containing 10⁴ cfu/mL

![Fig. 1. Schematic diagram of a conventional heating system using two oil baths. Tt: target temperature. Oil bath 1 is set at Tt + 15°C; oil bath 2 is set at Tt. The capped stainless steel test tube was moved from oil bath 1 to oil bath 2 when the temperature of the sample reaches Tt - 3°C.](image-url)
A. acidoterrestris spores was supplied from a tank at a constant flow rate of 100 L/h by a Nemo pump (NVL-TF, Heishin, Ltd., Japan). The internal pressure between the Nemo pump and the back-pressure valve at the exit was maintained at 0.5 MPa by adjusting the back-pressure valve. The power was a square-wave AC at a frequency of 20 kHz, with a maximum power voltage of 2000 V and an output power of 25 kW. The electrode unit comprised a Teflon insulator around a titanium electrode (6.0 mm in width, 2.0 mm from the electrode; electrode length 32 mm) (Figure 3). When the unit encountered the sample at a rate of 100 L/h, there was 13.8 ms of passage time between electrodes. A thermocouple inserted at the center of the pipe, 20 mm away from the electrode end, measured the sample temperature. Figure 4b plots the temperature history for HEF-AC.

**Tubular heat exchanger** A cylindrical heat exchanger (Kikkoman, Japan) with a 570-mL inner capacity was substituted for the electrode unit in the HEF-AC system. When
the unit encountered the sample at a rate of 100 L/h, there was a passage time of 20 s. Figure 4c plots the temperature history for HEF-AC using the cylindrical heat exchanger. *A. acidoterrestris* spores were inoculated into orange juice at a concentration of $10^4$ cfu/mL.

**Statistical Analysis** All experiments were performed in triplicate. The cfu counts after each treatment (n) were divided by the initial count ($N_0$) and were logarithmically converted to the survival rate. For analysis, values were averaged and the standard deviations were calculated using Excel (Microsoft Corp., Redmond, WA).

**Results and Discussion**

**Outlet temperature effect of HEF-AC** The influence of the outlet temperature (110°C to 125°C) on the *A. acidoterrestris* spore using HEF-AC is shown in Fig. 5. The survival rate is the spore reduction (inactivation) due to the temperature change at the outlet using HEF-AC. Spore inactivation increased with increasing temperature above 110°C.

**Holding time effect of HEF-AC** The influence of the holding times (0.3 - 0.9s) on *A. acidoterrestris* spores using HEF-AC at each outlet temperature is shown in Fig. 6a. A 0-s holding time indicates that the temperature at the outlet of the electrode reached the target temperature. Liquid flow through a fitting pipe of about 100 mm (shortest length) incorporating a thermocouple sensor, from the outlet of the electrode to the inlet of the cooling unit, takes 0.3 s. In the following experiments, extending the holding pipe 100 mm (200 mm) in the fitting pipe increases the liquid flow time to 0.6 s (0.9 s). Spore inactivation by HEF-AC increased as the holding time increased from 0.3 to 0.9 s. HEF-AC reduced *A. acidoterrestris* spores in orange juice by 3 logs at an outlet temperature of 125°C and a holding time of 0.9 s. When the approximation line of each temperature is extrapolated, all inactivation rates become less than 1-log reduction at a holding time of 0 s regardless of the outlet temperature. Thus, little inactivation occurred just after HEF-AC was applied at this holding time and inactivation mainly occurred during the holding time.

**Holding-time effect of tubular heater** Figure 6b plots the inactivation effect of changes in holding time using the cylindrical heat exchanger at 110°C and 115°C. Less than

![Fig. 4. Temperature profiles for heating processes of juice using two oil baths (a), a tubular heater (b), and HEF-AC (c). The heating time was from 40°C to 115°C for 180 s using the oil baths, from 40°C to 120°C for 38 s using the tubular heater and from 40°C to 120°C for 0.014 s using HEF-AC. The holding time at 115°C was 180 s using the oil baths, 0.6 s using the tubular heater and 0.6 s using HEF-AC.](image)

![Fig. 5. Spore reduction using HEF-AC at different outlet temperatures. $N_0$: the initial spore concentration (cfu/mL); n: the treated spore concentration (cfu/mL). Each plot is represented as the mean ± standard deviation from three experiments.](image)
10 cfu/mL spores remained in the treated orange juice when the tubular heater temperature was 120°C and 125°C for a holding time from 0.3 - 0.9 s. The tubular heater reduces the spores by 4 logs at an outlet temperature of higher than 120°C. The reduction was thus achieved by raising the temperature from 115°C to 120°C for 3 s (Fig. 4b). No significant change was observed with holding time from 0.3 to 0.9 s at 110°C or 115°C. The spores were thus inactivated by the heat exchanger at a holding time of 0 s and stayed at the same level for a holding time from 0.3 to 0.9 s.

**Holding time effect of conventional heating**

Figure 7 indicates the inactivation effect of changes in holding time using two different temperature-controlled oil baths. The inactivation effects increased with increasing holding time at 105°C, 110°C, and 115°C. The survival rate at a holding time of 0 s increased with increasing target temperature from 105°C to 115°C for 24 s. The survival rate also increased as the holding time increased. However, the holding time using the oil baths may also be influenced by the total heating time, as compared in Fig. 6a and b.

**Arrhenius plot**

An Arrhenius plot has been used to compare the inactivation effect at the holding time of HEF-AC and conventional heating (Figure 8). As the activation energies for HEF-AC (Ea=177 kJ/mol) and conventional heating (Ea=155 kJ/mol) were similar, inactivation using HEF-AC may be attributable to mainly thermal effects. The inactivation speed of HEF-AC was 1.5 logs faster than that of conventional heating at 115°C. Thus, HEF-AC was determined to inactivate *A. acidoterrestris* 30 times faster than conventional heating for a given holding time. A comparison of inactivation by HEF-AC and conventional heating indicated that *A. acidoterrestris* spores are killed immediately with a

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**Fig. 6.** Inactivation effect of holding time using HEF-AC (a) or coaxial tubular heater (b). ▲: 110°C, ■: 115°C, ◆: 120°C, and ●: 125°C. N₀: the initial spore concentration (cfu/mL); n: the treated spore concentration (cfu/mL). Each plot is represented as the mean ± standard deviation from three experiments.

**Fig. 7.** Inactivation effect of holding time using conventional heating. ▲: 105°C, ■: 110°C and ◆: 115°C. N₀: the initial spore concentration (cfu/mL); n: the treated spore concentration (cfu/mL). Each plot is represented as the mean ± standard deviation from three experiments.

**Fig. 8.** Arrhenius plot of inactivation speed using HEF-AC and conventional heating. ■: HEF-AC; ◆: conventional heating using two oil baths. N₀: the initial spore concentration (cfu/mL); n: the treated spore concentration (cfu/mL).
short holding time.

Inoue et al. (2008) made quality comparisons between HEF-AC treated orange juice and an ultra-high-temperature (UHT)-treated orange juice. HEF-AC, equivalent to that used in this study, at 120°C for a 0.6-s holding time and UHT at 110°C for a 10-s holding time produced equal inactivation of *B. subtilis* spores by 4 logs. After HEF-AC treatment, 24% more linalool, 15% more limonene, 25% more β-carotene, 18% more hesperidin and 8% more L-ascorbic acid were retained in the orange juice than after UHT treatment.

**Conclusions**

Comparison of the HEF-AC and conventional heating methods showed HEF-AC to be advantageous. First, HEF-AC reduced *A. acidoterrestris* spores in orange juice by 3 logs at an outlet temperature of 125°C and a holding time of 0.9 s. HEF-AC slightly inactivated *A. acidoterrestris* spores through the electrode and significantly inactivated them through the holding pipe. Moreover, the inactivation rate of HEF-AC during the holding time was 30 times greater than that of conventional heating, and the total heating time, comprising the rising time and holding time, was greatly reduced, which contributed to retaining the nutrient components in orange juice.

**References**


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