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Short communication

Continuous ohmic heating of commercially processed apple juice using five sequential electric fields results in rapid inactivation of *Alicyclobacillus acidoterrestris* spores

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ABSTRACT

Spores of *Alicyclobacillus acidoterrestris*, a spoilage bacterium, cause problems for the apple juice industry because they are resistant to thermal treatment. Here, we examined the sporicidal effect of an ohmic heating (OH) system with five sequential electric fields and compared it with that of conventional heating. Apple juice product (50 kg) inoculated with *A. acidoterrestris* spores were subjected to OH (electric field strength = 26.7 V/cm; frequency = 25 kHz) at 85–100 °C for 30–90 s. The effect of conventional heating was also examined under these conditions. OH treatment at 100 °C for 30 s resulted in total inactivation of the inoculum, with no recovery of viable cells (initial population = 4.8–4.9 log CFU/ml), whereas 3.6–4.9 log CFU/ml of the spores survived conventional heating. OH did not alter the quality (°Brix, color, and pH) of commercial apple juice ($p > 0.05$). These results suggest that the OH system is superior to conventional heating for rapid sterilization (30 s) of apple juice to assure microbiological quality in the absence of chemical additives.

1. Introduction

Alicyclobacillus acidoterrestris is frequently isolated from fruit juices and is responsible for spoilage of commercially processed apple juice products (Huang et al., 2015; Silva and Gibbs, 2001). Cerny et al. (1984) first documented apple juice spoilage in Germany in 1982; since then, several incidents have been reported in various countries (Gouws et al., 2005; Walker and Phillips, 2008a; Wisse and Parish, 1998; Yamazaki et al., 1996). Juice processors did not recognize spoilage by this bacterium; they were informed after consumer complaints. This is because there was no apparent sign (e.g., increased turbidity, swelling of the container due to gas production, or sediment) that led to rejection of the spoiled product prior to shipping (Chang and Kang, 2004; Pettipher et al., 1997). Thus, it is of importance to inactivate *A. acidoterrestris* before the final product is released.

A. acidoterrestris is a typical spore-forming bacterium with thermophilic and acidophilic characteristics that enable growth under a wide range of conditions (temperature = 20–70 °C; pH = 2.0–6.0) (Huang et al., 2015; Silva and Gibbs, 2001). In particular, spores formed by this bacterium have a high level of thermal resistance, which means that they can survive after the conventional hot-fill and hold pasteurization process (88–96 °C, 2 min) (Huang et al., 2015); thus it is generally considered an important target for sterilization in the juice industry (Ceviz et al., 2009; Murakami et al., 1998). According to a survey carried out by the National Food Processor Association in 1998, about 35% of commercial fruit juice manufacturers have experienced large-scale spoilage of fruit juice due to failed pasteurization; in other words, growth of acidophilic spore-formers in commercial juice products (Chang and Kang, 2004; Walls and Chuyate, 1998). The European Fruit Juice Association surveyed fruit processing industries in 2005 and found that 45% of respondents experienced problems related to *Alicyclobacillus* contamination (Steyn et al., 2011).

A number of researchers have examined a variety of techniques and methods designed to prevent and control contamination during juice manufacture. Traditional chemical antimicrobials such as oxidizing agents and organic acids or salts are used to inactivate soil-borne *A. acidoterrestris* on the surface of fruits or on production equipment (Lee et al., 2006; Lee et al., 2004; Orr and Beuchat, 2000), or to inhibit the growth of the bacterium in apple juice (Bevilacqua et al., 2008; Walker and Phillips, 2008b). The efficacy of alternative natural compounds, including bacteriocins (Grande et al., 2005; Pei et al., 2014), chitosan (Falcone et al., 2005), or plant extracts (Alberice et al., 2012;

Abbreviations: OH, ohmic heating.

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Bevilacqua et al., 2010), has also been examined. However, because consumers prefer natural or artificial chemical-free fruit juice products, there is a need for a method that can destroy spores in the absence of chemical additives (Lee et al., 2015).

Ohmic heating (OH) is a thermal treatment based on the passage of an electric current through food that contains sufficient water and electrolytes to generate heat within the product (Knirsch et al., 2010). It is potentially a highly effective method for inactivating heat-resistant *A. acidoterrestris* spores in apple juice because 1) the electrical conductivity of apple juice (1.3–2.0 S/m) is within the known range of electric conductivity (0.01–10 S/m) appropriate for OH application (Piette et al., 2004); 2) OH heats the product more rapidly than more general heating methods; and 3) the innate acidity of apple juice (pH 3.0–4.0) might be detrimental to heat-resistant spores during OH treatment.

Here, we examined the efficacy of an OH system based on five sequential electric fields (Ryang et al., 2015; Ryang et al., 2016) against *A. acidoterrestris* spores in commercial apple juice and compared the results with those obtained by conventional heating. In addition, we monitored several factors indicative of apple juice quality to determine the practical utility of the OH system.

2. Materials and methods

2.1. Preparation of spore suspension

A. acidoterrestris ATCC 49025 was obtained from the Korea Culture Center of Microorganisms (Seoul, Korea), stored at −20 °C in tryptic soy broth (Difco, Becton Dickenson; Sparks, MD, USA) containing 20% glycerol, and sub-cultured monthly. Stocks were transferred to orange serum agar (pH 3.7) (OSA; Difco) and re-activated by culture at 43 °C for 48 h. Each culture was spread-inoculated onto potato dextrose agar (PDA; Difco), the pH of which was adjusted to 5.6 with 10% tartaric acid. After 7 days of incubation at 43 °C, cells on the surface of the PDA were harvested and used as target spores for the experiments after microscopic confirmation of >80% sporulation (BX41TF; Olympus, Tokyo, Japan). Target spores were suspended in phosphate buffered saline (pH 7.2) (PBS; Difco) to yield 7–8 log CFU/ml of spore suspension and washed 3–4 times with buffered peptone water (BPW) by centrifugation at 4000 ×*g* for 20 min under refrigeration (4 °C) (Biofuge; Kendro-Laboratory, London, Germany). The spore suspension was freshly prepared on the day of experiment and immediately used.

2.2. Inoculation of apple juice

Commercial apple juice products (pH 3.5–3.6) were obtained directly from the manufacturer (NONGSHIM Co., Ltd.; Seoul, Korea) immediately before pasteurization. The composition of the commercial apple juice product was as follows: water, apple juice 10% concentrate, citric acid, maltodextrin, natural flavorings, steviol glycoside, and Vitamin C (exact proportion of each ingredient is confidential). The presence of *A. acidoterrestris* was examined in triplicate according to the method of Pettipher et al. (1997) and Bae et al. (2009). Briefly, 25 ml of apple juice was randomly collected from each batch and incubated with 225 ml of orange serum broth (OSB) for 48 h at 43 °C in triplicate. The pre-enriched sample was then streaked onto OSA and examined after culture for 48 h at 43 °C. Only batches of apple juice that had been confirmed as "*A. acidoterrestris* negative" were examined in the experiment (limit of detection = 1 CFU/75 ml). Spore suspensions were inoculated into *A. acidoterrestris*-free apple juice product to attain an initial spore count of 4–5 log CFU/ml.

2.3. OH

The OH system used in our previous studies was also used here (Ryang et al., 2016). Briefly, the system comprises a separate heating mixer (NONGSHIM Engineering Co. Ltd.; Seoul, Korea), a sample input unit, an OH unit with seven electrodes connected in an elbow-type arrangement (15 cm in length; pipe internal diameter, 3.5 cm) which generated five sequential electric fields, a holding unit, a cooling unit, and an output unit. An apple juice product containing *A. acidoterrestris* spores was pre-heated to 70 °C in a separate heating mixer and allowed to flow into the input unit within 10 min from the start of pre-heating. The quantity per experimental bath was 50 l, and the sample flow rate of the system was 50 l/h. The electric field strength was set at 26.7 V/cm at a frequency of 25 kHz (voltage = 400 V). After OH treatment in a holding unit at 85, 90, 95, or 100 °C for 30, 60, or 90 s, the inner temperature of the treated apple juice was allowed to fall to 70 °C. Thirty milliliters of apple juice were then collected from both the input and output units (untreated and treated samples, respectively) and immediately subjected to microbiological analysis. Inner temperature values, which was automatically and timely displayed on the OH system, were recorded every 10 s.

2.4. Conventional heating

Several tests have been performed to obtain by conventional heating the same temperature profile as with OH, by changing type and material of the experimental container, volume of the apple juice product for each experiment, and heating procedures. The completed procedure for each experiment was as follows. The prepared commercial apple juice product (100 ml) was put into a sterilized Erlenmeyer flask in 250 ml of volume and pre-heated in an oil bath (OB-25; Han Yang Scientific Equipment Co., Ltd., Seoul, Korea) at 70 °C. The flask containing apple juice product was submerged below the surface of the oil in the bath. Spore suspension was inoculated into apple juice (final concentration, 4–5 log CFU/ml), and the mouth of the flask was tightly sealed using parafilm M (Bemis Company, Inc., Neenah, WI, USA). The inoculated apple juice product was stirred for >10 min to simulate the separate heating mixer in the OH system then transferred to the other oil bath (OB-25) set at a target temperature (85, 90, 95, or 100 °C). After holding the flask containing the apple juice and *A. acidoterrestris* spores for a set time (30, 60, or 90 s), the treated sample was transferred to a water bath set at 70 °C and maintained for a further 60–90 s to replicate the cooling pattern of the OH system. To check inner temperature of the heated apple juice, un-inoculated apple juice was separately prepared and heated in the same way. Changes in inner temperature of the un-inoculated apple juice were measured using a probe thermometer and recorded every 10 s, from right after transferring the flask to the oil bath set at a target temperature.

2.5. Microbiological analysis

One milliliter of sample was obtained before and after each treatment and serially diluted (10-fold) with 9 ml of 0.85% sterile saline solution. The diluent (0.1 ml) was then spread-plated onto two OSA plates and additional 1 ml of undiluted sample was split and spread onto five OSA plates to achieve lower detection limit (1 CFU/ml). The inoculated plates were then incubated at 43 °C for 48 h, and typical colonies were counted to calculate the surviving population of *A. acidoterrestris* spores. Recovery of heat-shocked spores was examined only for the OH treated groups that showed complete inactivation of the inoculated *A. acidoterrestris* spores. The apple juice product (25 ml) after the OH treatment at each condition was incubated in 225 ml of OSB at 43 °C for 48 h, followed by additional enrichment on OSA plates at 43 °C for 48 h. Inactivation (CFU/ml) of *A. acidoterrestris* spores by each treatment was calculated using following equation: Inactivation = $\mathrm{Log}_{10}(N_t/N_0)$. In this equation, N_t and N_0 represent colony count number of *A. acidoterrestris* spores per 1 ml of apple juice product before and after each treatment, respectively.