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Comparing the effects of high hydrostatic pressure and thermal pasteurization combined with nisin on the quality of cucumber juice drinks

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ABSTRACT

The aim of this study was to evaluate the effects of high hydrostatic pressure (HHP) at 400 MPa/4 min and 500 MPa/2 min and thermal pasteurization at 85 °C/15 s with 100 IU/mL nisin on natural microbial flora, chlorophyll *a* and *b*, color, lipoxygenase activity and four C_9 key odorants of (E,Z)-2,6-nonadienal, (E,Z)-3, 6-nonadien-1-ol, (E)-2-nonenal and (Z)-6-nonenal in cucumber juice drinks over 50 days of storage at 4 °C. Yeast and molds (Y&M) were completely inactivated by all treatments, and their levels were below the detection limit during storage. Nisin with HHP or thermal pasteurization had a synergistic effect on the inactivation of total aerobic bacteria (TAB). For all the quality attributes studied in this article, their retention was significantly better in the HHP-treated samples than in the thermally pasteurized samples during storage. The samples treated by 500 MPa/2 min with 100 IU/mL nisin exhibited a longer shelf life as compared with other treated samples.

Industrial relevance: This research paper presents a fair comparison of cucumber juice drinks treated by HHP and thermal pasteurization which is quite scarce. The available data are provided for the evaluation and application of HHP and thermal pasteurization in the beverage industry and could be used to establish safety criteria for the commercial production of high quality cucumber juice beverages.

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1. Introduction

Cucumber is an important vegetable that offers a good flavor and a low caloric content. Freshly squeezed cucumber juice drinks are becoming increasingly popular in China, particularly in restaurants. Because of cucumber juice's sensitivity to thermal pasteurization, which can be detrimental to the color and flavor of cucumber juice drinks, there is considerable demand for a minimal-processing treatment that will retain the juice's quality. High hydrostatic pressure (HHP) is a nonthermal pasteurization technology that could meet the consumers' demand for minimally processed (Liao & Hu, 1998), fresh tasting and microbiologically safe food (Patterson, 2005; San Martín, Barbosa-Cánovas, & Swanson, 2002).

The most important factor that can affect the shelf life and microbiological safety of cucumber juice drinks is the presence and growth of spoilage microorganisms and pathogens. In general, pathogens, yeast and molds (Y&M) are very sensitive to HHP (Linton, McClements, & Patterson, 1999; Ogawa, Fukuhisa, Kubo, & Fukumoto, 1990; Parnell, 2003; Patterson, 2005; Ramaswamy, Riahi, & Idziak, 2003; Raso, Calderón, Góngora, Barbosa-Cánovas, & Swanson, 1998; Vega-Gálvez et al., 2012; Yen & Lin, 1996) and Gram-negative bacteria tend to be more susceptible to pressure than Gram-positive (Patterson, 2005), while nisin exhibits antimicrobial activity toward a wide range of Gram-positive bacteria (Arauz, Jozala, Mazzola, & Vessoni Penna, 2009; Corbo et al., 2009; Delves-Broughton, Blackburn, Evans, & Hugenholtz, 1996; Kim, Choi, Bajpai, & Kang, 2008) and shows little or no activity against Gram-negative bacteria (Boziaris & Adams, 1999; Helander & Mattila-Sandholm, 2000) and Y&M (Arauz et al., 2009; Boziaris & Adams, 1999; Delves-Broughton et al., 1996). Previous studies have shown that nisin has a synergistic effect on the inactivation of bacteria when combined with HHP (Hauben, Wuytack, Soontjens, & Michiels, 1996; Kalchayanand, Sikes, Dunne, & Ray, 1994; Mertens & Knorr, 1992), while the study of combination effects on the quality of fruit and vegetable products is very limited, hence this combination has been evaluated in this study.

Color and flavor are very important indicators of cucumber products and are the two attributes that are most obviously damaged by thermal pasteurization. Because of the breakdown of chlorophyll *a* and *b* to yellow-olive colored pheophytins as a result of thermal pasteurization (Clydesdale & Francis, 1976), it is useful to study the concentration of chlorophyll in cucumber juice drinks.

Lipoxygenase (LOX) is an enzyme found in many plants and animals. It catalyzes the oxygenation of polyunsaturated fatty acids to form fatty acid hydroperoxides, and linoleic and linolenic acid are the major polyunsaturated fatty acids in plant tissues (Baysal & Demirdöven, 2007).

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LOX plays a role in the production of off flavors in soybeans (Sheu & Chen, 1991) and many vegetables, such as tomatoes (Shook, Shellhammer, & Schwartz, 2001). However, LOX-produced odorant compounds are desirable in cucumber products (Avdiushko, Ye, Kuc, & Hildebrand, 1994; Baysal & Demirdöven, 2007; Li, Wu, Li, Hang, & Zhu, 2008; Robinson, Wu, Domoney, & Casey, 1995; Wardale & Lambert, 1980). C_9 odorant compounds, the key aroma compounds of cucumber, are the result of LOX degradation of linoleic and linolenic acid that occurs rapidly after the tissue is disrupted (Grosch & Schwarz, 1971; Palma-Harris, McFeeters, & Fleming, 2002). (E,Z)-2,6-nonadienal with the threshold of 0.01 ng/mL (Boehlens & van Gemert, 1986) is the most important C_9 odorant (Palma-Harris, McFeeters, & Fleming, 2001) and is primarily responsible for the fresh cucumber odor (Fleming, Cobb, Etchells, & Bell, 1968; Fross, Dunstone, Ramshaw, & Stark, 1962; Schieberle, Ofner, & Grosch, 1990) in fresh cucumbers. (E,Z)-3,6-nonadien-1-ol is the stable form of (E,E)-3,6-nonadien-1-ol, which is the intermediate product of (E,Z)-2,6-nonadienal (Phillips & Galliard, 1978). (E)-2-nonenal, with the threshold of 0.08 ng/mL (Fazzalari, 1978), was found to be the second most important odor compound, with approximately 2% the odor impact of (E,Z)-2,6-nonadienal (Palma-Harris et al., 2002). (Z)-6-nonenal is known to have a typical melon aroma (Schieberle et al., 1990) and is the assistant aroma and flavor-producing substance in cucumbers (Kemp, Knavel, & Stolz, 1972; Rymal & Nakayama, 1974). Although several studies on cucumber odorants have been conducted (Avdiushko et al., 1994; Fleming et al., 1968; Fross et al., 1962; Grosch & Schwarz, 1971; Liu & He, 2004; Liu, He, & Liu, 2002; Palma-Harris et al., 2001, 2002; Phillips & Galliard, 1978; Rymal & Nakayama, 1974; Schieberle et al., 1990; Wardale & Lambert, 1980), most of these were concerned with the form or detection of cucumber flavor. However, information about the effect of HHP on the key odorants of cucumber juice is not available.

The aim of this study was to compare the effect of HHP (400 MPa/4 min and 500 MPa/2 min) and thermal pasteurization (85 °C/15 s) with 100 IU/mL nisin on natural microbial flora, chlorophyll *a* and *b*, color, LOX activity and four C_9 key odorants of (E,Z)-2,6-nonadienal, (E,Z)-3,6-nonadien-1-ol, (E)-2-nonenal and (Z)-6-nonenal in cucumber juice drinks during 50 days of storage at 4 °C.

2. Materials and methods

2.1. Preparation of cucumber juice drink

Healthy, fresh cucumber (pH 6.60 and total soluble solids of 1.9 °Brix) was purchased at the local market (in Beijing, China) and then cleaned with tap water and cut into pieces. Approximately 10 kg cucumber pieces were juiced with a mechanical juice extractor (Joyong Electric Appliance Co., Shandong, China) and diluted 2.5-fold with distilled water. Food-grade sucrose was added to the juice to achieve total soluble solids of 3.0 °Brix to improve the taste. The final pH of the drink was 6.60.

2.2. Preparation of the nisin solution

One gram of commercial nisin preparation containing 1×10^6 IU/g (Zhejiang Silver-Elephant Bio-engineering Co., Zhejiang, China) was dissolved in 100 mL 0.02 M sterile citric acid solution with a pH of approximately 2.5 and a nisin concentration of 1×10^4 IU/mL. The resulting solution was filtered through a 0.2-μm Millipore filter (Lee, Heinz, & Knorr, 2003) and stored at 4 °C for a maximum of 1 week before use.

2.3. Thermal and HHP processing of cucumber juice drinks

The cucumber juice drink prepared in Section 2.1 was divided into 4 portions. The first portion (without nisin) and the second portion (with a nisin concentration of 100 IU/mL) were poured into 100-mL polyethylene terephthalate bottles for processing. A nisin concentration of 100 IU/mL was achieved by adding 10 mL of the nisin solution prepared in Section 2.2 to every liter of cucumber juice drink. HHP treatments were conducted using a hydrostatic pressurization unit (HHP-650, Baotou Kefa Co., Ltd., Inner Mongolia, China) with a capacity of 7.0 L at ambient temperature (approximately 25 °C). This unit pressurized at 2 MPa/s, and the decompression time was 7 to 8 s. Distilled water was used as the pressure transmitting fluid. The pressure-holding treatment time in this study did not include the pressure-increase and decompression times.

The third portion (without nisin) and the fourth portion (with a nisin concentration of 100 IU/mL) were pasteurized in a pilot scale pasteurizer with a tubular heat exchanger (Armfield FT74, HTST/UHT processing unit, Hampshire, England) and then cooled to 20 °C. The pasteurized samples were aseptically transferred into aseptic 100-mL polyethylene terephthalate bottles identical to the ones used for the HHP-treated samples.

According to our preliminary research, HHP (400 MPa/4 min and 500 MPa/2 min) and thermal pasteurization (85 °C/15 s) can achieve almost the same inactivation of total aerobic bacteria (TAB) and Y&M. Therefore, the cucumber juice drink samples in this study were processed using these treatments.

2.4. Storage conditions

The treated samples were subsequently stored at 4 ± 2 °C in darkness. Physico-chemical and microbiological analyses were carried out after 0, 5, 10, 15, 25, 35 and 50 days of storage. For all kinds of samples, three different batches ($n=3$) were considered and analyzed separately.

2.5. Microbial analysis

To count the viable cells of natural microorganisms in the cucumber juice drink samples, the total plate count (TPC) method was used. Each untreated or treated sample was serially diluted with sterile 0.85% NaCl solution, and 1.0 mL of each dilution was plated into duplicate plates with the appropriate agar. Nutrient agar (Beijing Land Bridge Technology Co. Ltd., Beijing, China) was used to detect the viable TAB after incubation at 37 °C for 48 ± 2 h. Rose Bengal agar (RBA, Beijing Land Bridge Technology Co. Ltd., Beijing, China) was used to detect the viable Y&M cells, and the plates were incubated at 27 °C for 72 to 120 h. After incubation, the colonies were counted. Log N/N_0 was calculated to determine the inactivation effect, where N_0 was the number of initial microorganisms in untreated sample, and N was the corresponding viable number of microorganisms after thermal pasteurization or HHP treatments. The initial TAB count in the cucumber juice drink samples was 1.04×10^5–3.50×10^5 CFU/mL, and the initial Y&M was 5.60×10^3–2.35×10^4 CFU/mL. The fluctuations in the initial counts of natural microorganisms in the cucumber juice drink samples for each experiment were ascribed to the juice preparation process.

2.6. Physicochemical characteristic analysis

The samples were equilibrated at 25 °C to measure pH, titrable acidity (TA) and total soluble solid (TSS), simultaneously. The pH value was measured at 25 °C using a Thermo Orion 868 pH meter (Thermo Fisher Scientific, Inc., MA, U.S.A). TA was measured using an automatic titrimeter (851 GPD titrino, Metrohm, Switzerland). The results were expressed as percentages of citric acid content. TSS was determined using a WAY-2S Digital Abbe Refractometer (Shanghai Precision & Scientific Instrument Co., Shanghai, China) at 25 °C, and the results were reported as °Brix.

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