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ARTICLE

Fermentation of hot pepper juice by *Bacillus licheniformis* to reduce pungency

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Abstract Capsaicin, an active component of hot pepper, has considerable health benefits; however, the pungency of hot pepper has limited its usage. This study aimed to reduce the pungency of hot pepper by fermentation to enhance its application in the food industry. Hot pepper juice was fermented by *Bacillus licheniformis* SK1230, which was previously isolated as a capsaicin-degrading bacterium. *B. licheniformis* SK1230 was inoculated into the juice of red or green pepper, and the mixture was then fermented for 19 days to determine the degradation level of capsaicinoids. It was observed that with a gradual increase in pH, the growth of *B. licheniformis* SK1230 increased to 1.0×10^9 CFU/mL after day 1, and its viability persisted until the end of fermentation. The sugar content of green pepper drastically reduced at day 1 and that of red pepper reduced at day 5. The total polyphenol content of the medium containing red pepper was about 2-fold higher than that of the medium containing green pepper. The antioxidant activity of the medium containing red pepper was also higher than that of the medium containing green pepper; however, the activity gradually decreased during the course of the fermentation period. The capsaicinoid content of both types of peppers radically decreased at day 5. Thus, we suggest that a fermentation period of 5 days would be optimal for the degradation of capsaicin with minimal loss of antioxidant activity and would provide higher polyphenol content.

Keywords *Bacillus licheniformis* 1230 · Capsaicin · Dihydrocapsaicin · Fermentation · Green pepper · Pungency · Red pepper

Introduction

Peppers (*Capsicum* spp.) are cultivated worldwide and have wide applications in food and medicine industries (Hill et al. 2013). The principal phytochemical compounds of peppers, namely, capsaicinoids, flavonoids, anthocyanins, vitamins C and E, phenolics, carotenoids, and dietary fibers, are active components that impart considerable health benefits (Gonzalez-Aguilar et al. 2008; Park et al. 2010; Zimmer et al. 2012). Peppers have also been known to be effective for the treatment of arthritis, rheumatism, stomach aches, skin rashes, and flesh wounds (Meghvansi et al. 2010). Moreover, the ability of peppers to modify fat and energy metabolism in humans has been reported. By acting on the central nervous system, capsaicin confers anti-obesity effects by controlling appetite, reducing fat concentration in the blood, and restricting the generation of white fat cells (Hsu and Yen 2007). Furthermore it was reported that capsaicin decreased the total lipid level in the liver of mice fed a high-fat diet supplemented with fermented

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pepper powder (Yeon et al. 2013). Therefore, peppers have popularly been used as a main ingredient in diet recipes intended for weight loss and as an active component for anti-obesity treatment.

Despite their various health benefits, the strong pungent taste of peppers, which is attributed to capsaicinoids, considerably limits their use. Therefore, an effective reduction in the pungency of peppers is important and beneficial for its wider industrial application. Several researchers have attempted to reduce or eliminate the pungent taste of peppers; these attempts can be broadly categorized into two approaches. The first approach is the development of non-pungent cultivars of peppers. Capsiate is a capsaicin-like compound without pungency, which is found in a non-pungent cultivar of red pepper (Shintaku et al. 2012). It shows biological activities similar to those shown by capsaicin, particularly with regard to fat and energy metabolism in humans. The second approach is microbial fermentation. It has been reported that some bacteria can degrade capsaicin and capsaicin degradation product by microbial fermentation have a similar bioactivities of capsaicin on the swimming capacity of mice (Lee et al. 2010). Therefore, it should be noted that capsaicin fermentation can extend the availability of peppers with decreasing pungency. In our previous study, we have proven the capsaicin degradation ability of *Bacillus licheniformis* SK1230 [JQ864313] isolated from Korean traditional pickled pepper (Cho et al. 2014).

For effective fermentation, it is necessary to obtain detailed information regarding fermentation profiles such as the growth rates of starter culture, the alterations that can occur in biologically active compounds, and the biological activities associated with the degradation of capsaicinoids. In the present study, we aimed to elucidate and compare the characteristics of red and green pepper juices fermented by *B. licheniformis* SK1230. We also evaluated the alterations in the total polyphenol content and antioxidant activity as well as in the degradation of capsaicinoids during the fermentation period.

Materials and methods

Preparation of hot pepper juice and solid content measurement

Green and red peppers (*C. annuum* cv. Chungyang) were purchased from a Korean local farm located at Korea. The peppers were washed several times under tap water and were wiped well by using a paper towel. Juice was extracted from each pepper by using a mechanical juicer (DA5000, Korea) and stored at −70 °C until further use. Prepared juice was dried at 105 °C for overnight to determine solid content.

Fermentation medium and culture conditions

Bacillus minimal medium (BMM) was used for the fermentation of pepper juice (Brans et al. 2004). It was composed of 33.5 mM $Na_2HPO_4{\cdot}7H_2O$, 22 mM KH_2PO_4, 8.6 mM NaCl, 18.7 mM NH_4Cl, 1 mM $MgSO_4$, 0.1 mM $CaCl_2$, and 10 mM glucose. *B. licheniformis* SK1230 grown in Luria–Bertani (LB) broth was inoculated into BMM (1 % v/v) and incubated for 16 h to prepare the seed culture. The fermentation medium consisted of 50 % (v/v) pepper juice in BMM without glucose. The initial pH of the medium was adjusted to 6.5 with 10 N NaOH, and the medium was autoclaved at 121 °C for 15 min before use. Finally, the seed culture of *B. licheniformis* was inoculated (5 % v/v) into the fermentation medium for pepper juice. Briefly, 50 mL of seed culture was inoculated into 1 L of pepper juice medium and mixed vigorously. Subsequently, 30 mL of fermentation media broth was aseptically dispensed into a sterilized Erlenmeyer flask. In total, 54 flasks containing red or green pepper juice fermentation broth were prepared, three of which were used for triplicate analysis at each sampling point during fermentation. Fermentation parameters were investigated at nine fermentation time points: 0, 1, 3, 5, 7, 9, 11, 15, and 19 days.

Viable cell count and pH

The growth of *B. licheniformis* during the fermentation of pepper juice was determined by viable cell count. Briefly, a sample from each fermentation time point was serially diluted with sterile 0.8 % NaCl solution, and the diluted samples were then spread on LB medium. After overnight incubation, the colonies visible on the medium were counted, and the result was calculated as $\log_{10}$ (CFU/mL). pH was measured using a pH meter (Model 735P; ISTEK, Korea).

Sample preparation

To determine the soluble sugar content, total polyphenol content, and antioxidant activity, the supernatant was prepared by centrifuging the fermentation broth at 10,000 rpm for 5 min at room temperature and filtered using a 0.45-μm syringe filter (PVDF Membrane Filter; PALL, USA). The filtrate was used as a sample for analysis.

Reduction of sugar content

To evaluate the substrate utilization, the soluble sugar content in the fermentation broth was determined. The

soluble sugar concentration was analyzed by the DNS (3,5-dinitrosalicylic acid) reaction, according to Miller's (1959) method, with slight modification. Briefly, 1.5 mL of culture supernatant was mixed with 1.5 mL of DNS solution, and the mixture was incubated at 90 °C for 10 min. After cooling to room temperature, 0.5 mL of 40 % potassium sodium tartrate solution was added to the reaction mixture, and the optical density was measured at 575 nm by using a spectrophotometer (UV-1601; SHIMADZU, Japan). The sugar concentration was calculated by regression analysis, with a standard curve of glucose.

Total polyphenol content

The total polyphenol content was determined by reaction with phenol reagent (Folin–Ciocalteu reagent; Sigma, USA), according to Juan and Chou's (2010) method, with slight modification. Briefly, 0.1 mL of the culture supernatant was mixed with 0.1 mL of 1 N phenol reagent, and the mixture was maintained for 3 min at room temperature. Subsequently, 0.3 mL of 1 N Na_2CO_3 was added to the mixture and maintained for 90 min at room temperature. Aliquots of 2 mL distilled water were added to the mixture, and the optical density was measured at 725 nm. Gallic acid was used as a standard compound, and the concentration was represented as gallic acid equivalents.

Antioxidant activity

The antioxidant activity of the culture broth was determined by measuring its free-radical scavenging activity by using α,α-diphenyl-β-picrylhydrazyl (DPPH; Sigma). Briefly, 4 mL of diluted supernatant was mixed with 1 mL of DPPH solution (0.15 mM in 99 % ethanol), and the mixture was incubated for 30 min at room temperature. After adding 2 mL of distilled water, the optical density of the mixture at 517 nm was measured using a spectrophotometer. Ascorbic acid was used as the standard compound, and its activity was represented as ascorbic acid equivalents (ACE).

Capsaicinoid content

At each fermentation time point, all samples were dried by lyophilization and were finely ground before analysis. The sample powder was mixed with the solvent (acetic acid: methanol, 1:9, v/v) in a ratio of 1:25, and extraction was performed for 3 h at room temperature, with agitation (100 rpm). The solvent mixture was filtered through a filter paper (Whatman No. 42, UK). The filtrate was concentrated by evaporation (EYERA, Japan) and redissolved in methanol. Finally, the concentrated sample was filtered through a 0.45-μm syringe filter before analysis. The capsaicinoid content in the sample was determined by high-performance liquid chromatography (HPLC) (Agilent 1100 series; Agilent Technologies, USA) at room temperature on a SUPELCOSIL™ LC-ABZ HPLC column (250 mm × 4.6 mm × 5 μm) (Supelco, Inc., USA). The mobile phase consisted of 70 % methanol and 1 % acetic acid, and its flow rate was set at 0.7 mL/min. Peaks were detected at 280 nm.

Results

Fermentation parameters

The solid content of red and green pepper juice was 0.16 and 0.29 g/mL, respectively. The pH, viable cell count, and sugar content of the fermented pepper juice were determined (Fig. 1). The pH of the fermented juices of red as well as green peppers gradually increased from an initial pH of 6.5 to a pH of 9.0 at day 19 (Fig. 1A). During the first 10 days of fermentation, green pepper juice showed greater increment in pH as compared to that shown by red pepper juice.

Rapid bacterial growth was detected on day 1, with a slight increment in the cell count of both red and green pepper juices (Fig. 1B). The bacterial cell count in the juices of both types of pepper exceeded 1.0×10^9 CFU/mL from day 7 to day 19, which indicated normal bacterial growth. This result suggested that *B. licheniformis* SK1230 is able to ferment or metabolize some ingredients of hot peppers as a sole carbon source.

The alterations in sugar content are shown in Fig. 1C. Interestingly, sugar utilization was delayed in the first 3 days of fermentation of red pepper juice, and suddenly increased from day 3 to day 5. In contrast, approximately 80 % of the sugar in the green pepper juice was utilized on the first day of fermentation.

Total polyphenol content and antioxidant activity

At the initial time points in fermentation, the total polyphenol content of the medium containing red pepper juice was about 2-fold higher than that of the medium containing green pepper juice (Fig. 2A). The total polyphenol content of the medium containing red pepper juice increased from 216 mg/L at the start of fermentation to 263 mg/L at day 5. Thereafter, the polyphenol content decreased until day 13 and was subsequently restored to its initial level. In case of green pepper juice, the total polyphenol content was almost unchanged compared to red pepper juice.

The antioxidant activity was found to gradually decrease as fermentation proceeded in red or green pepper juice containing medium (Fig. 2B). Antioxidant activity during the fermentation of red pepper juice was observed to be higher than that observed during the fermentation of green pepper juice when evaluated throughout the fermentation

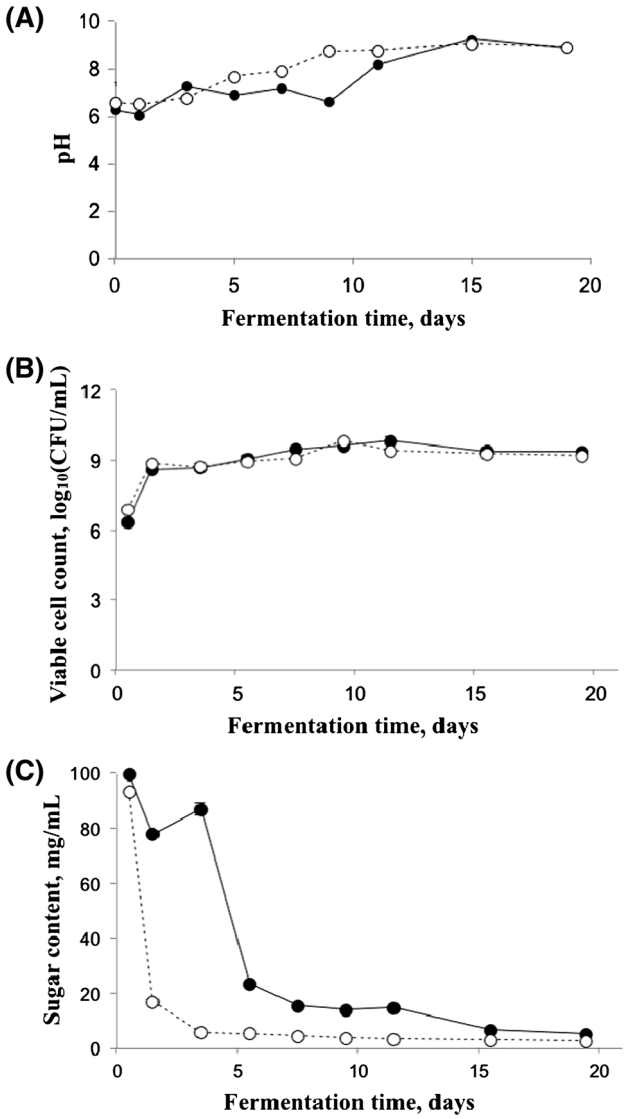

Fig. 1 Parameters for the fermentation of pepper juice by *B. licheniformis* SK1230. The *filled* and *empty circles* depict red and green peppers, respectively. (**A**) pH profiles; (**B**) viable cell counts; and (**C**) sugar content

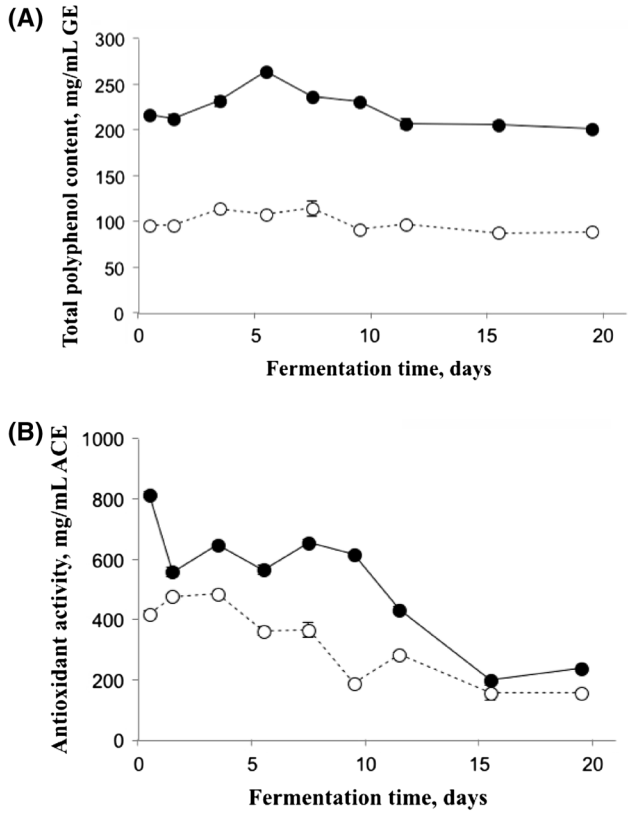

Fig. 2 Changes in the (**A**) total polyphenol content and (**B**) antioxidant activity during the fermentation of pepper juice by *B. licheniformis* SK1230. The *filled* and *empty circles* depict red and green peppers, respectively

period. A steady increase in antioxidant activity was observed during the fermentation of red pepper juice from day 1 to day 9, which decreased thereafter until day 15. In contrast, the antioxidant activity during the fermentation of green pepper juice gradually decreased as fermentation progressed.

Degradation of capsaicin and dihydrocapsaicin

The alterations in capsaicin and dihydrocapsaicin contents of red and green pepper juices during fermentation are shown in Fig. 3. Capsaicin in both red and green pepper juices was completely degraded by day 7, whereas dihydrocapsaicin was completely degraded by day 5. The initial content of capsaicin and dihydrocapsaicin in red pepper juice was 1753 and 490 ppm, respectively, which decreased to 71 ppm at day 7 and to 24 ppm at day 5, respectively (Fig. 3A). The degradation of capsaicin and dihydrocapsaicin in green pepper juice showed a pattern similar to that observed in the degradation of capsaicin and dihydrocapsaicin in red pepper juice. The initial content of capsaicin and dihydrocapsaicin in green pepper juice was 2380 and 504 ppm, respectively, which decreased to 309 ppm and 36 ppm, respectively, at day 5 (Fig. 3B). The capsaicin content of green pepper juice was 1.36-fold higher than that of red pepper juice, whereas the dihydrocapsaicin content did not show much difference before and after fermentation between red and green pepper juices.

Discussion

Peppers (*Capsicum annuum* L. cv Chungyang) are popular in Korea for their strong taste and have considerable health benefits owing to their various constituents such as vitamins, capsaicin, and phenolic compounds. Peppers are

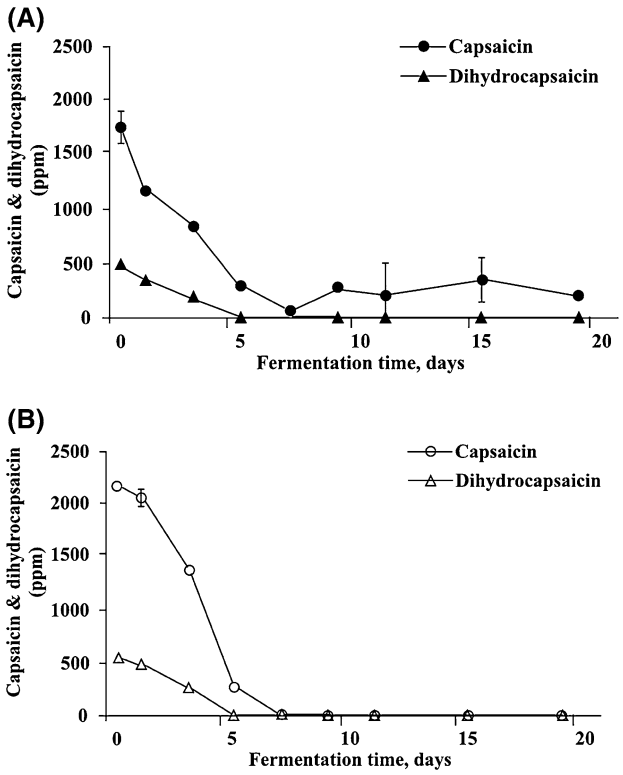

Fig. 3 Changes in the contents of capsaicin and dihydrocapsaicin in the fermentation of (**A**) red and (**B**) green pepper juices by *B. licheniformis* SK1230

fermented for two different purposes: one is to reduce the pungency of peppers, which strongly restricts their use (Lee et al. 2010) and the other is to produce natural vanillin flavors (van den Heuvel et al. 2001). These biological processes are mediated by microorganisms. Therefore, nutrient supplementation of culture medium is crucial to ensure appropriate microbial growth. It has been reported that peppers contain about 10–15 % sugar, with fructose (~10 % in dry matter) and glucose (~7 % in dry matter) being the major constituents (Choi et al. 2000). The crude protein and crude fat contents of peppers were reported to be 10 and 11 % (including the seed), respectively (Hwang et al. 2001). These nutrient concentrations are sufficient for microbial growth.

In this study, *B. licheniformis* SK1230 showed normal growth in culture media containing the juice of red or green peppers, with appropriate utilization of sugar as a substrate. The color of peppers is regarded as an important feature of their characteristics, and it differs among the different germ types of peppers (Castro et al. 2008; Hwang et al. 2011). In Korea, green and red peppers are harvested at different seasons, namely, in July and from August to October, respectively (Cho et al. 2004). Red pepper is a fully matured form of green pepper and is known to contain more sugar than the latter. The patterns of sugar utilization by the same starter cultures were found to be different in the two kinds of pepper, which could be attributed to the difference in the nutrient values between young and mature peppers.

The total polyphenol content during fermentation considerably differed between red and green peppers. The change in the color of pepper from green to red is typically attributed to the accumulation of pigments such as capsanthin and capsorubin (Hwang et al. 2012). Red pepper has potent antioxidant property, and the methanol extract of green pepper showed significant free-radical scavenging activity during lipid peroxidation (Conforti et al. 2007; Materska and Perucka 2005). In this study, the total polyphenol content in the medium with red pepper juice was 2-fold higher than that in the medium with green pepper juice. We observed that *B. licheniformis* SK1230 had no appreciable effect on the total phenol content of pepper juice during the fermentation.

We also observed that the antioxidant activity in the initial stages of fermentation of red pepper juice was almost 2-fold higher than that in the fermentation of green pepper juice and that this difference was proportional to the total polyphenol content. However, unlike the changes in the total phenol content that occurred throughout the fermentation period, the antioxidant activity continued to decrease in the media containing red or green pepper juice until day 19 and finally decreased to about 200 mg/mL ACE. In general, phenolic compounds and vitamins were considered relevant to antioxidant activity (Scalbert et al. 2005). In this study, fermentation did not decrease the total polyphenol content. Therefore, the decrease in antioxidant activity could be attributed to the destruction of vitamins during fermentation. A fresh Korean pepper contains approximately 46–243 mg/100 g of vitamin C (Cho et al. 2004). Antioxidant activity in the medium containing 50 % of red pepper juice was 800 mg/L ACE, which could majorly be attributed to the vitamin C content in pepper. Although the initial vitamin C content in pepper and its changes during the course of fermentation were not measured in this study, it could be assumed that the vitamin content is continuously degraded during fermentation.

Capsaicin and dihydrocapsaicin are the most important and representative components in peppers. All peppers used in this study that belonged to the same cultivars were reported to contain 1–2.6 g/kg of capsaicin and 0.5–0.8 g/kg of dihydrocapsaicin (Hwang et al. 2011). Surh (2002) reported that during the fermentation period, capsaicin content decreased from 24.7 to 15.5 g/mL and dihydrocapsaicin content decreased from 14.7 to 6.45 g/mL. Yeon et al. (2013) study reported that the dihydrocapsaicin and capsaicin contents of freeze-dried pepper powder considerably decreased during the fermentation period until day 20 and day 28, respectively.

However, in the present study, capsaicin and dihydrocapsaicin contents from pepper juice rapidly decreased and were almost degraded by day 5. This finding suggested that fermentation of red or green pepper juice by *B. licheniformis* SK1230 could lead to the degradation of capsaicin and dihydrocapsaicin while maintaining the polyphenol content, with gradual loss of antioxidant activity. The present study provides primary information on the fermentation profiles, particularly with regard to the physiochemical changes such as capsaicin degradation, polyphenol content, and antioxidant activity.

In conclusion, we postulate that fermentation of hot pepper juice by *B. licheniformis* SK1230 for 5 days leads to the near complete degradation of capsaicinoids while maintaining certain phenolic compounds and antioxidant activity.

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