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Production of benzoic acid as a natural compound in fermented skim milk using commercial cheese starter

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ABSTRACT

In this study, we investigated the production of natural benzoic acid (BA) in skim milk fermentation by 5 kinds of commercial cheese starters. Five kinds of starter were inoculated into 10% reconstituted skim milk, and then the culture was incubated at 2-h intervals for 10 h at 30, 35, and 40°C. In fermentation by MW 046 N+LH 13, the starter for making raclette, BA was highly detected after 8 h at 30 and 35°C. In fermentation by LH 13, the starter for making berg, BA steadily increased and was highly detected at 40°C. In fermentation by TCC-3+TCC-4, the starter for making Caciocavallo and mozzarella, BA was detected after 2 h at 40°C. Also, BA was detected after 4 and 8 h at 35 and 30°C, respectively. In fermentation by Flora-Danica, the starter for making Gouda, BA was increased until 6 h and decreased after 6 h at all temperatures. Among the 5 kinds of fermentation, the level of BA was the highest in fermentation by Flora-Danica at 6 h at 35°C, at 14.55 mg/kg.
Key words: benzoic acid, cheese starter, skim milk, fermentation

INTRODUCTION

Benzoic acid is widely used in the food industry as a food preservative. Characterized by effective antibacterial properties (Daeschel, 1997), it has antimicrobial activity against an extensive range of bacteria, yeasts, and molds involved in food intoxication and food spoilage (Chipley, 1993). In several studies, it has been demonstrated that benzoic acid effectively inhibits pathogens such as *Listeria monocytogenes* (El-Shenawy and Marth, 1988), *Aspergillus* (Rusul and Marth, 1988), *Vibrio* sp. (Beuchat, 1980), *Escherichia coli* (Splittstoesser et al., 1996), and *Penicillium* sp. (Thompson, 1997).

According to the US Food and Drug Administration, benzoic acid and its potassium and sodium salts are regarded as generally recognized as safe (Boer and Nielsen, 1995). Although benzoic acid is extensively used in food and it is generally recognized as safe, several adverse effects, such as metabolic acidosis, asthma, urticarial, hyperpnoea, and convulsions, were observed in experimental animals and humans given very high dose of benzoic acid. For these reasons, the use of benzoic acid as food additives is limited by specific regulations in different countries (Tfouni and Toledo, 2002). The Joint FAO/WHO Expert Committee on Food Additives (WHO, 2000) has evaluated and established an acceptable daily intake for benzoic acid, benzoate salt, benzaldehyde, benzyl acetate, and benzyl alcohol of 0 to 5 mg/kg of BW.

However, even if not added as a food preservative, benzoic acid also occurs naturally in several foods and commodities, such as fruits, vegetables, spices, and nuts, and also in dairy products at low concentrations (Nagayama et al., 1983; Nagayama et al., 1986; Heimhuber and Hermann, 1990). Sieber et al. (1995) analyzed and surveyed natural occurrence of benzoic acid in various types of cultured dairy products and cheeses. Those authors found that benzoic acid appears to generate as a by-product of the microbial degradation of either hippuric acid or phenylalanine in these products. Also, oxidation of benzaldehyde produced by certain strains of lactic acid bacteria may contribute to benzoic acid generation. The production of several organic acids, including benzoic acid, could be different depending on the fermentation starters and the fermented condition (González de Llano et al., 1996; Mroueh et al., 2008).

The objective of our study was to investigative the change of natural benzoic acid produced from fermentation using 5 kinds of commercial cheese starter cultures (TCC-3, TCC-4, LH 13, MW 043 N, and Flora-Danica) at different temperature for establishment of the allowable level of naturally produced benzoic acid in cheese.

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Table 1. Commercial starters and their strains used in cheese manufacture

Starter[1]	Strains	Cheese
TCC-3 + TCC-4[2]	*Lactobacillus delbrueckii* ssp. *bulgaricus* + *Lactobacillus delbrueckii* ssp. *bulgaricus* *Streptococcus thermophilus*	Caciocavallo, mozzarella
LH 13	*Lactobacillus helveticus*	Berg
MW 046 N + LH 13[3]	*Lactococcus lactis* ssp. *cremoris* *Lactococcus lactis* ssp. *lactis* *Lactococcus lactis* ssp. *diacetylactis* *Leuconostoc mensenteriodes* ssp. *cremoris* + *Lactobacillus helveticus*	Raclette
Flora-Danica	*Lactococcus lactis* ssp. *cremoris* *Lactococcus lactis* ssp. *lactis* *Lactococcus lactis* ssp. *diacetylactis* *Leuconostoc mensenteriodes* ssp. *cremoris*	Gouda

[1]MW 046 N, LH 13, TCC-3, TCC-4 (Sacco, Cadorago, Italy), and Flora-Danica (Chr. Hansen, Hørshølm, Denmark).

[2]TCC-3 and TCC-4 were mixed in the ratio 1:1.

[3]MW 046 N and LH 13 were mixed in the ratio 7:3.

MATERIALS AND METHODS

Cheese Starters

Five kinds of commercial, freeze-dried direct vat set cheese starters, MW 046 N, LH 13, TCC-3, TCC-4 (Sacco, Cadorago, Italy), and Flora-Danica (Chr. Hansen, Hørshølm, Denmark), were used. Mixing proportions of starter for making cheese were used the ratio of commercial industries. Mixing proportion and strains were in Table 1.

Growth of Strain

The number of viable starter strain was determined by serial 10-fold dilution in 0.1% peptone water. About 10^5 cfu/mL of starter strain was inoculated into 10% reconstituted skim milk, and then the culture was incubated at 2-h intervals for 10 h at 30, 35, and 40°C. All pour plates were incubated aerobically at 37°C for 48 h using a bromo cresol-purple (BCP) plate count agar (Eiken, Tochigi, Japan)

pH and Acidity

The pH was measured using pH meter (Inolab 7110 pH BNC, WTW, Weilheim, Germany). For measuring acidity, 9 mL of sample was diluted with 18 mL of distilled water. Next, 0.5 mL of 1% phenolphthalein solution was added and the solution was titrated by 0.1 *N* NaOH solution until it became pink for 30 s. The acidity was determined using the following formula:

$$\text{Acidity (\%)} = [\text{titration amount of 0.1 NaOH (mL)} \times 0.009/\text{sample (g)}] \times 100.$$

HPLC-Photodiode Array Analysis of Benzoic Acid Contents

Standard and Chemicals. Commercial standard (Sigma Aldrich, St. Louis, MO) of benzoic acid was used. We purchased HPLC-grade solvents from J.T. Baker (Phillipsburg, NJ). Other reagents (analytical grade) were purchased from Wako (Tokyo, Japan).

Sample Preparation. Sample preparation was conducted in accordance with Korean Food Additives Codex (MFDS, 2014). Five grams of cheese sample was accurately weighed and added distilled water until the total volume was 50 mL. The sample was vortexed for 1 min, sonicated for 20 min, and then filtered. Five milliliters of filtrate was mixed with 1.5 mL of 0.1 *N* HCl and 0.5 mL of 0.005 *M* cetyltrimethylammonium chloride solution.

Sep-Pak C18 cartridges (Waters Associates, Milford, MA) were prepared before use by successively washing each cartridge with 10 mL of methanol and 10 mL of 0.005 *M* cetyltrimethylammonium chloride solution; at a flow rate of 2 mL/min, the mixed solution was applied to the Sep-Pak cartridge. After washing with 10 mL of water, the solution was eluted with 10 mL of methanol. The solution was filtered through a 0.45-μm filter paper.

HPLC-Photodiode Array Analysis. An HPLC with a photodiode array (Shiseido. Co. Ltd., Tokyo,