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Inhibitory effects of garlic and other spices on biogenic amine production in *Myeolchi-jeot*, Korean salted and fermented anchovy product

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ABSTRACT

This study was carried out to reduce biogenic amine contents in *Myeolchi-jeot*, Korean salted and fermented anchovy (*Engraulis japonicus*). The effects of a variety of spices including ginger, garlic, green onion, red pepper, clove and cinnamon, on biogenic amine production were determined by HPLC. The greatest inhibitory effect on biogenic amine production was observed in the culture treated by garlic extract. In the culture, the contents of putrescine, cadaverine, histamine, tyramine and spermidine were reduced by up to 11.2%, 18.4%, 11.7%, 30.9% and 17.4%, respectively, compared to control. The other spice extracts tested showed less or no effect in reducing biogenic amine contents. In addition, the extract of garlic showed the highest antimicrobial activity against the amine producers tested. The extract of garlic at a concentration of 5% (weight basis) was finally applied to the ripening of *Myeolchi-jeot in situ*, and then overall production of biogenic amines in *Myeolchi-jeot* was found to be reduced by up to 8.7%, compared to control. Consequently, it is expected that the findings of this study might be helpful for enhancing the safety of *Myeolchi-jeot*.

1. Introduction

The presence of biogenic amines such as histamine and tyramine in fermented foods can pose a health risk to consumers if the amine-metabolizing capacity of the human body is saturated (Joosten & Nuñez, 1996), although ordinary administration of the amines does not provoke adverse reactions (Askar & Treptow, 1986). Histamine intoxications are enhanced by other biogenic amines which inhibit activities of intestinal histamine-metabolizing enzymes such as diamine oxidase and histamine-*N*-methyltransferase (Stratton, Hutkins, & Taylor, 1991). Histamine poisoning (scombroid poisoning) causes allergy-like symptoms such as dizziness, sneezing, flush, headache, diarrhea, itching, tingling, nausea, and hypotension in sensitive individuals (Joosten, 1988; Stratton et al., 1991; Askar & Treptow, 1993). Tyramine has been identified as the major mutagen precursor (Ochiai, Wakabayashi, Nagao, & Sugimura, 1984) and causes nausea, respiratory distress, hot flushes, sweating, heart palpitation, headache, a bright red rash, oral burning, and a rise in blood pressure (Rice, Eitenmiller, & Koehler, 1976; Joosten, 1988). In addition, the simultaneous occurrence of biogenic polyamines, including putrescine, cadaverine, spermidine, spermine and agmatine, can cause additional negative physiological effect such as carcinogenicity when these amines react with nitroso compounds (Smith, 1980; Scanlan, 1983). A hazardous level of histamine for human health has been suggested as 500 mg/kg (Askar & Treptow, 1993). Also, 100 ~ 800 mg/kg of tyramine in foods are known to be toxic (Brink, Damirik, Joosten, & Huis in't Veld, 1990).

Jeotkal (sometimes, *Jeotgal*) is a traditional Korean salted and fermented seafood, and is popularly taken not only as side dishes, but also as an ingredient in kimchi. *Jeotkal* contains large amounts of precursor amino acids of biogenic amines because it is made by fermenting a mixture of the muscles and visceras of seafoods and salts with digestive enzymes and microbes causing breakdown of proteins into amino acids. *Myeolchi-jeot* is made of anchovies (*Engraulis japonicus*), which is the most frequently consumed *Jeotkal* in Korea. Since *Myeolchi-jeot* is salted and fermented for a long period (few years) to develop taste, it contains relatively large amounts of biogenic amines (Mah, Han, Oh, Kim, & Hwang, 2002) that may be related to its microbial flora (Mah, Ahn, Park, Sung, & Hwang, 2003). The presence of histidine decarboxylase activity has been described in various microbial groups, such as pseudomonads (López-Sabater, Rodrıguez-Jerez, Hernández-Herrero, & Mora-Ventura, 1994), sporulated micro organisms (Taylor, Guthertz, Leatherwood, Tillman, & Lieber, 1978; Rodrıguez-Jerez, Colavita, Giaccone, & Parisi, 1994) and lactic acid bacteria (Maijala, Eerola, Aho, & Hirn, 1993; Joosten & Nuñez, 1996). Meanwhile, it has been known that the microflora in *Jeotkals* include the bacteria *Achromobacter*, *Bacillus*, *Brevibacterium*, *Flavobacterium*, *Halobacterium*,

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Leuconostoc, *Micrococcus*, *Pediococcus*, *Pseudomonas*, *Staphylococcus*, *Sarcina*, and the yeasts *Saccharomyces* and *Torulopsis* (Mheen, 1993; Um & Lee, 1996). In *Myeolchi-jeot*, *Bacillus licheniformis* brings an increase of the amine contents during the storage at an ambient temperature (Mah et al., 2003). Furthermore, *B. licheniformis* has been known to produce histamine in retail canned anchovies (Lee et al., 2005). Therefore, *B. licheniformis* can be suggested as an excellent microorganism for the investigation on biogenic amine production in anchovy products.

The inhibitory effects of clove and cinnamon on amine-producing bacteria have been reported by Wendakoon and Sakaguchi (1993a, 1993b) and Shakila et al. (1996). However, no information is available on the effects of spices used popularly in East Asia on biogenic amine formation either *in vitro* or *in situ*. In this study, the inhibitory effects of spices on strong biogenic amine-producing strains of *B. licheniformis* isolated from *Myeolchi-jeot* were determined to control biogenic amine formation in Korean salted and fermented fish products, particularly *Myeolchi-jeot*. It was also performed to evaluate the effect of material selected on the biogenic amine production during the ripening of *Myeolchi-jeot in situ*.

2. Materials and methods

2.1. Test micro organisms

Myeolchi-jeot isolate No. 1043, 1056, 1542 and 1553, identified as *B. licheniformis*, based on characteristics described in Bergey's Manual of Systematic Bacteriology and the pattern of fatty acid profile by the Microbial Identification System, and defined as strong amine producers in our previous study (Mah et al., 2003), were used for the determination of the inhibitory effects of spices on biogenic amine production. Stock cultures were maintained on nutrient agar (Difco, Becton–Dickinson Co., Sparks, MD, USA) slants at 4 °C.

2.2. Preparation of extracts of spices

Ethanol extracts of spices were prepared according to procedure described by Shakila, Vasundhara, and Rao (1996). Six types of spices, including fresh ginger, garlic, green onion, red pepper, dried clove and cinnamon, were purchased from a retail shop in Seoul, Korea. Ethanol extract of each spice was prepared by soaking 20 g of sliced spice in 100 ml of 95% ethanol at room temperature. The mixture was left 12 h and filtered through the Whatman paper No. 1 to obtain the spice extract.

2.3. Preparation of assay medium for HPLC analysis

To determine the inhibitory effects of spices on amino acid decarboxylase activity of biogenic amine-producing bacteria, an assay method described by Shakila et al. (1996) was employed with minor modifications, as follows: the ethanol extracts of different spices at varying levels (0.1, 0.5 and 1.0 ml, adjusted to 1 ml with ethanol) were aseptically added to 5 ml of the assay medium consisted of tryptic soy broth (TSB, Difco) with 0.5% L-histidine hydrochloride monohydrate, L-tyrosine, L-ornithine hydrochloride and L-lysine hydrochloride (pH 5.8) supplemented with 0.0005% pyridoxal-HCl (Sigma Chemical Co., St. Louis, MO, USA). The assay medium itself and the medium prepared by adding 1 ml of 95% ethanol served as control.

2.4. Biogenic amine production

A loop from the test organism was inoculated in 5 ml of the assay medium prepared without spice extract, and incubated at 30 °C for 24 h. One-milliliter of the culture was then transferred to 5 ml of the assay medium prepared with spice extract, which was incubated at 30 °C for 24 h. The culture contained about 10^8 CFU/ml of the test organism. Five milliliters of the broth culture were taken by a sterile syringe, filtered through a 0.2 μm membrane (Millipore Co., Bedford, MA, USA), and kept at −25 °C prior to HPLC analysis.

2.5. Determination of antimicrobial activity of spice

To determine the inhibitory effects of spices on the growth of strong amine producers, agar diffusion test (Mah et al., 2001) was carried out. Briefly, the disk containing 50 μl of the ethanol extract of spice was placed onto an agar medium inoculated with 0.1 ml of a 12-h culture of the amine producer. Then, the plate was incubated at 37 °C for 18 h, and the diameter of inhibition halo was measured.

2.6. Preparation of Myeolchi-jeot

Anchovies (*E. japonicus*, 9–12 cm long, 10–12 g/anchovy) were caught in the East Sea near the Geoje Island, Korea. Samples were salted and kept in ice onboard, and transported in ice pack to the Korea University, Seoul, Korea. Upon arrival, samples were immediately divided into three equal portions, each of 800 g, and then salt was added at the level of 15% to each lot (pre-salted raw anchovy). One lot of anchovy was treated with ethanol garlic extract and two lots were treated with ethanol and distilled water (solvent control). Anchovies were uniformly smeared with each solution at a concentration of 5% (weight basis), which is the level normally used for the manufacture of other types of *Jeotkal* products. All samples were held at 25 °C for 10 weeks. Periodically, samples were drawn for biogenic amine analysis.

2.7. Physico-chemical and microbial measurements

Several physico-chemical properties of *Myeolchi-jeot* samples were measured. pH was measured with a pH meter (pH meter 340, Corning Inc., NY, USA), the salinity by the AOAC (1990) method, and water activity by an electric hygrometer (Novasina, Talstrasse, Switzerland). Total plate count was made by plate count agar (PCA, Difco) with 3% NaCl added (Hernández-Herrero, Roig-Sagués, Rodríguez-Jerez, & Mora-Ventura, 1999). The number of colonies that appeared after 24 h at 30 °C and 37 °C were counted.

2.8. Determination of biogenic amines by HPLC

Analysis of biogenic amines in the filtered broth culture by HPLC was performed according to the procedure developed by Eerola, Hinkkanen, Lindfors, and Hirvi (1993) and modified by Ben-Gigirey, DE Sousa, Villa, and Barros-Velazquez (1998, 1999). Briefly, 1 ml of the filtered broth culture was added to 9 ml of 0.4 M perchloric acid (Merck KGaA, Darmstadt, Germany) and was mixed by a vortex mixer. The mixture was then centrifuged at 3000*g* for 10 min and the supernatant was filtered through Whatman paper No. 1. Derivatization of sample extract and amine standards was carried out as previously described (Mah et al., 2003). The equipment consisted of a Waters 2690 HPLC solvent delivery system, a Waters 996 photodiode array detector and Millennium 2010 software. A Nova-Pak C_{18}, 4 μm, 150 by 3.9 mm column (Waters, Milford, MA, USA) was used, with ammonium acetate (0.1 M; Merck; solvent A) and acetonitrile (Merck; solvent B) as the mobile phases at the flow rate of 1 ml/min. The program was set for a linear gradient starting from 50% of solvent B to reach 90% of the solvent at 19 min. The sample volume injected was 20 μl and the sample was monitored at 254 nm.

Analysis of biogenic amines in *Myeolchi-jeot* was carried out according to the procedure developed by Ben-Gigirey et al.