Analysis of Volatile Constituents in Fermented Brown Rice and Rice Bran by *Aspergillus oryzae* (FBRA)

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In recent years, consumers' interest in health foods has increased significantly. Among these health foods, fermented foods are used traditionally in Japanese food culture and have contributed to the maintenance of people's health. Recently, the biological effects of fermented brown rice and rice bran by *Aspergillus oryzae* (FBRA) have been comprehensively studied, and inhibitory effects on carcinogenesis have been reported. Regarding the bioactive chemical constituents in FBRA, the involvement of ferulic acid on the biological activity has been reported. In this study, we quantitatively investigated the dependence on fermentation time of the production of ferulic acid and related compounds in FBRA. In addition, we analyzed the generation of aroma-active compounds by fermentation.

Key Words: fermented brown rice by *Aspergillus oryzae*, ferulic acid, 4-vinylguaicol, GC-MS, SPME

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Introduction

In recent years, the awareness of consumers has changed, and their interest in health foods has increased significantly. The increasing demand for such health foods can be explained by the steady increase in life expectancy, and the desire of older people for improved quality of life [1]. Among these health foods, fermented foods, such as black rice vinegar, soy sauce (shoyu), fermented soy beans (miso, natto and tempeh) are used traditionally in Japanese food culture and have contributed to the maintenance of people's health [2, 3]. The effects of the fermentation of soy beans by Aspergillus oryzae have been analyzed by Hong et al., who have reported that fermentation increases the amount of small-size peptides (<20 kDa) compared with raw soybeans, while significantly decreasing large-size peptides (>60 kDa) [4]. In addition, Supriyati indicated that fermentation of rice bran by Bacillus amyloliquefaciens reduces the crude fiber content significantly [5]. Rajalakshmi et al. reported on changes in the chemical compositions of the traditional Indian fermented foods, Idli and Khaman, and that large and significant increases in thiamine and riboflavin were observed with fermentation [6]. These results show that fermentation changes the ingredients in food into forms that can be easily absorbed as nutrition.

Fermented brown rice by Aspergillus oryzae and abbreviated to FBRA is a processed food prepared by fermenting brown rice and rice bran with Aspergillus oryzae. The effects of FBRA have been extensively studied. Kuno et al. reported that FBRA has inhibitory effects on carcinogenesis of the colon, liver and esophagus in rodents [7]. In addition, Kataoka et al. reported that a diet containing FBRA suppresses acute colitis induced by dextran sulfate sodium [8].

Antioxidative effects are suggested to be involved in the potential chemopreventive effects of FBRA. Sakurai et al. investigated the influence of the fermentation of brown rice and rice bran by A. oryzae on the bioactive chemical constituents by comparing them before and after fermentation and detected slight increases in the concentration of ferulic acid, one of the antioxidants in brown rice [9]. In this study, we investigated the dependence on fermentation time of the amounts of ferulic acid and compounds related to this in FBRA together with the generation of aroma-active compounds by fermentation.

Experimental

Materials and sample preparation

FBRA was produced by the fermentation of 10% brown rice and 90% rice bran by A. oryzae at 40 °C for 44 hr. Every 4 hr., 25 g of the sample was collected from the fermentation batch.

Standard samples and reagents

Ferulic acid, p-coumaric acid and caffeic acid were purchased from Nacalai tesque Co., Ltd. (Kyoto, Japan). 4-Vinylguaiacol and guaiacol were purchased from Wako Pure Chemical Ind. Co. Ltd. (Tokyo, Japan) and Tokyo Kasei Co. Ltd. (Tokyo, Japan), respectively. Trifluoromethyl cinnamic acid was purchased from Sigma-Aldrich Co. Llc. (St Louis, MO). All chemicals were of analytical grade, and the chromatographic solvents were of LC-MS grade (Wako Pure Chemical Ind. Co. Ltd., Tokyo, Japan).

Analytical instruments

GC-MS analyses were carried out using a Shimazu 2010 GC system (Shimazu, Tokyo, Japan) equipped with an AOC-20i autosampler and DB-5MS capillary column (0.25 mm × 30 m, 0.1 μm film thickness, Agilent Technology, California, USA). The analytical conditions were as follows: injector and transfer line temperature, 270°C; oven temperature programmed from 50 to 300°C at a ramp rate of 10°C/min; carrier gas, helium (1.2 ml/min); split less mode; ionization energy, 70 eV; ionization current, 300 μA.

Quantitative analysis of ferulic acid and its related compounds was performed by LC–MS using a Thermo Scientific TSQ-7000 mass spectrometer equipped with an ESI interface. The ESI parameters were as follows: source voltage, -4.0 kV, capillary temperature, 200°C, nebulizer gas, 1.5 L/min. The mass spectrometer was operated in the selected reaction monitoring (SRM) mode: caffeic acid; m/z 359.00 → m/z 178.65-179.25, coumaric acid; m/z 208.89 → m/z 118.76-119.36, ferulic acid; m/z 238.92 → m/z 133.74-134.34, vinyl guaiacol; m/z 150.91 → m/z 90.68-91.28, trifluoromethyl cinnamic acid (internal standard); m/z 260.88 → m/z 170.67-171.27.
A Waters Atlantis T3 column (2.1 mm i.d.× 150 mm) was used and the column temperature was maintained at 40°C. The mobile phase was a binary eluent of (A) 0.1 % HCOONH_4 solution and (B) CH_3CN under the following gradient conditions: 0–30 min linear gradient from 10% to 100% B, 30–40 min isocratic at 100% B. The flow rate was 0.15 mL/min. A manual Solid Phase Micro Extraction (SPME) holder and 100 µm polydimethylsiloxane (PDMS) fiber (Supelco, Bellefonte, Pa) were used for the extraction procedure. The fiber was conditioned following the supplier’s instructions prior to use.

**Sample preparation**

Ten milligrams of the sample in powder form was placed in a 10 ml screw cap vial (Sperco, Bellefonte, PA) with a PTFE septa-screw cap (Sperco, Bellefonte, PA). After piercing the septum with the SPME needle, the fiber was extended and exposed to the vapor above the sample at a temperature of 80°C. After 20 min the fiber was retracted and removed from the vial and was immediately thermally desorbed in the injection port of the GC–MS system at 270 °C for 5 min.

For the measurement, 5g of the powder was extracted with 14 ml of methanol under reflux conditions for 2 hr. After adding IS to the extract, the solvent was evaporated in vacuo.

**Results and discussion**

So far, there have been many reports on the biological activities of ferulic acid and its derivatives; for example, as an antioxidant defense mechanism to protect DNA and lipids from reactive oxygen species, and to prevent and treat disorders linked to oxidative stress, including Alzheimer’s disease, diabetes, cancers, hypertension and atherosclerosis [10]. Therefore, it is considered that ferulic acid and its derivatives play an important role in health foods. Ferulic acid is a ubiquitous plant constituent and it occurs in the seeds and leaves in its free form, its soluble-conjugate form, and covalently linked to lignin in the ratio 0.1:1:100 [11]. Yoshizawa et al. reported that ferulic acid and sinapic acid are transformed from bonded forms to free form during fermentation of rice grains by *A. oryzae* [12]. In addition, several reports have described the enzymatic or thermal degradation of ferulic acid by one-carbon cleavage of the side chain to form 4-vinylguaiacol (Figure 1) [13]. 4-Vinylguaiacol and guaiacol have significant phenolic, spicy and smoky flavors, which are key flavors in coffee, beer, wine and sake [14]. Recently, Jeong et al. reported that 4-vinylguaiacol arrests the growth of benzo(a)pyrene-treated NIH 3T3 cells by blocking the hyper-phosphorylation of retinoblastoma via regulating the expression of cell cycle-related proteins [15].

Selected ion monitoring (SRM) chromatograms of samples fermented for 28 hr and 44 hr are shown in Figure 2. No special purification was carried out, but ferulic acid and its derivatives were detected without interfering peaks. Figure 3 shows changes in the quantities of phenolic acid components and 4-vinylguaiacol with fermentation time, analyzed by LC–MS. The production of ferulic acid was evident 24 hr after the start of fermentation and reached to peak at 28 hr, while the formation of coumaric acid was not detected. Regarding 4-vinylguaiacol, a rapid increase was observed from 32 hr after the start of fermentation. The amount of 4-vinylguaiacol produced is much larger than the amount of ferulic acid produced, which would suggest 4-vinylguaiacol to be produced directly from the bound form of ferulic acid as well as from the free ferulic acid.
produced by decomposition.

To investigate the volatile compounds including 4-vinylguaiacol, generated in FBRA by fermentation, samples were taken every 4 hours after starting fermentation and analyzed by the SPME method. As already described, it is well known that ferulic acid is easily converted to 4-vinylguaiacol by a thermal treatment [13]. Therefore, the stability of ferulic acid during the extraction process in the SPME analysis depends on the temperature. The peak intensities of 4-vinylguaiacol in the GC-MS chromatograms at the respective extraction temperatures were examined and the results are shown in Figure 4. At 80 °C, no artificial 4-vinylguaiacol was detected.

![Figure 4](image_url)

**Fig. 4** The peak intensities of 4-vinylguaiacol in the GC-MS chromatograms at the respective extraction temperatures.

In Figure 5, the variations in volatile components in FBRA with fermentation time analyzed by the SPME method are shown. Yamabe et al. analyzed the variations in the amounts of lipid fractions during maturation of rice-koji Miso, and reported that triacylglycerol gradually decomposes into free fatty acids, with the distinct formation of fatty acid ethyl esters [16]. The present results on the production of volatile constituents during FBRA fermentation also indicate that the fatty acid esters were liberated prior to the generation of the free fatty acids, from 4 to 20 hours after the beginning of fermentation.

![Figure 5](image_url)

**Fig. 5** Variation in the amounts of volatile components in FBRA with fermentation time.

**Conclusion**

Recently, the biological activities of FBRA have been reported and the involvement of ferulic acid in these activities has been suggested. In this study, we examined the production of ferulic acid and its related compounds in FBRA, and analyzed the variations in the amounts of these with fermentation time. The results show that the generation of ferulic acid started 24 hr after the start of fermentation and reached to the maximum at 28 hr (2.9 mg/100 g of FBRA), while no significant increase in coumaric acid was observed. Also, a rapid increase in the amount of 4-vinylguaiacol was observed from 32 hr after the start of fermentation (9.6-15 mg/100 g of FBRA). Moreover, analysis of the volatile components in FBRA using SPME showed the formation of fatty acid esters came prior to the generation of free fatty acids.

**References and Notes**

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