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Fermentation Characteristics for Extruded Hair of Tissue Cultured Mountain Ginseng

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Abstract

Effects of extrusion conditions (barrel temperature and moisture content) and fermentation time on the antioxidant properties of root hair of tissue cultured raw mountain ginseng (MG) were investigated. The barrel temperature/moisture combinations were: 110°C/25% (MG1), 140°C/25% (MG2), 110°C/35% (MG3) and 140°C/35% (MG4). Red ginseng (RG) was also investigated. The contents of 2,2-diphenyl-1-picrylhydrazyl (DPPH) and polyphenolic increased after fermentation in RG and even more in MG, while extruded ginseng samples exhibited little change. The increases noted with MG and RG occurred during the first 4 days of fermentation. DPPH radical scavenging activity decreased after extrusion and was significantly higher in MG (20.93%) than RG (1.63%) on the first day of fermentation. DPPH radical scavenging activity in the barrel temperature/moisture combinations were 19.01% (MG1), 14.45% (MG2), 20.37% (MG3) and 15.78% (MG4). The content of polyphenolic compounds in ginseng samples displayed a similar trend. Acidic polysaccharide in RG and MG1~MG4 were higher than MG, but decreased during fermentation. Crude saponin in RG and MG1~MG4 decreased after 15 days of fermentation, while increasing in MG.

Key words: tissue cultured mountain ginseng, extrusion, antioxidant activity, crude saponin

INTRODUCTION

Ginseng is one of the most highly regarded herbal medicines in the Orient, where it has gained an almost magical reputation for promoting health and general body vigor, while also prolonging life (1,2). Ginsenosides are the most important active components in ginseng roots and are attributed with cardio-protective, immunomodulatory, anti-fatigue, and hepatoprotective physiological and pharmacological effects (3). Hairy roots, which have been induced in ginseng, can grow more rapidly and produce higher levels of ginsenosides than suspended cells and adventitious roots (4,5). With the aims of reducing expense and time, and to gain more control of experimental conditions than field grown plants, bioreactor root suspension cultures of *P. ginseng* are a primary alternative method for the large-scale production of raw mountain ginseng (MG) (6). Therefore, root suspension bioreactor cultures of ginseng were used in our research.

Fermentation is widely used in the food industry not only to improve the sensory characteristics of a product, but also to eliminate certain undesirable constituents and make nutrients more accessible, while preserving and even improving the nutritional properties (7). Ginseng root is added to traditional rice wine during fermentation (8). The use of enzyme-digested (saccharified) ginseng meal as a substrate for alcohol and yeast fermentation has been studied (9), as has the use of ginseng root hair, white and red ginseng (RG) in the preparation of ginseng wine (10).

Extrusion is a multi-step process involving high pressure, high shear, heating, mixing, cutting, crushing, and pressing. Extrusion technology has been explored for starch pretreatment and as ginseng pretreatment prior to the release of ginseng active components and red ginseng production in Korea since 2003 (11-15).

The objective of this research was to compare the fermentation characteristics of extruded root hair of tissue cultured MG and RG.

MATERIALS AND METHODS

Materials

RG root hair was purchased from a local market in Chungcheongnam-do, Korea. Root hair of tissue cultured

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MG was purchased from CBN BIOTECH (Chungcheongbuk-do, Korea). High grade chemical reagents including 2,2-diphenyl-1-picryhydrazyl radical (DPPH), gallic acid, Folin-Ciocalteu's phenol and galacturonic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA). Distiller's yeast containing *Aspergillus usamii* and *Rhizopus japonicas*, and yeast (*Saccharomyces cerevisiae*) were purchased from Koreaenzyme Company (Hwaseng, Korea).

Extrusion process

For extrusion a THK31T co-rotating intermeshing twin-screw extruder (Incheon Machinery, Incheon, Korea) was used (Fig. 1). The screw was 768 mm in length and 32 mm in diameter, representing a length : diameter ratio of 24:1. The screw speed was 200 rpm and the feed rate was 100 g/min. The die dimension consisted of three 1 mm diameter holes arranged in a circle. The barrel temperature was adjusted to 110°C or 140°C. The moisture content of dried ginseng used for extrusion was adjusted to 25% and 35%. The barrel temperature/moisture combinations were: 110°C/25% (MG1), 140°C/25% (MG2), 110°C/35% (MG3), and 140°C/35% (MG4). RG was also investigated. The extruded material was directly dried in an oven at 50°C for 8 hr. The dried extrudate was ground, sieved through a 500 μm pore size filter and stored at 4°C.

Fermentation and chemical analysis

Ginseng (10 g), distiller's yeast (0.13 g) and yeast (0.05 g) were homogenized in 50 mL of distilled water. The homogenate was maintained in a constant temperature cabinet (27°C) for 0, 1, 2, 3, 4, 10 or 15 days. Periodically during each incubation period, a portion of each sample was collected and prepared for further analyses by centrifugation (7000 rpm, 4°C, 20 min) using a Mega 21R centrifuge (Hanil Science, Seoul, Korea).

DPPH radical scavenging assay

Fermentation samples were measured in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH as described previously (16). Briefly, 100 μL of the fermentation solution was added to 5 mL of a 0.1 mM methanol solution of DPPH. After 30 min incubation in the dark at room temperature, the absorbance was read against a blank at 517 nm. The DPPH radical-scavenging activity was calculated according to the following equation:

$$\text{Scavenging activity } (\%) = \frac{\text{Abs}_{\text{blank}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{blank}}} \times 100$$

where $\text{Abs}_{\text{blank}}$ is the absorbance of the control reaction (containing all reagents except the test compound), and $\text{Abs}_{\text{sample}}$ is the absorbance of the test compound.

Determination of polyphenolic compounds

The Folin-Denis method (17) was modified to quantify polyphenolic compound colorimetrically. An aliquot (0.5 mL) of the fermentation sample was dispersed in 6.5 mL of distilled water and 0.5 mL Folin-Ciocalteu's reagent. After 3 min at room temperature, 1 mL of saturated Na_2CO_3 and 1.5 mL of distilled water were added to the mixture, which was stored in the dark for 1 hr. The absorbency of the mixture was measured at 725 nm and converted to phenolic contents according to the calibration curve from various concentration of gallic acid (as gallic acid equivalents).

Determination of acidic polysaccharide

Acidic polysaccharide was analyzed by the Carbazole-sulfuric method (18), using galacturonic acid as a standard. Fermentation liquor (0.5 mL) was mixed with 0.25 mL of 0.1% carbazole ethanol and 3 mL of concentrated sulfuric acid prior to incubation at 80°C for 5 min. After allowing the solution to cool during 15 min at room temperature, the absorbance was recorded at 525 nm.

Determination of crude saponin

Crude saponin was extracted and analyzed as described previously (19,20). Briefly, 50 mL of methanol was added to 5 mL of fermentation sample. The mixture was stirred at 35°C for 1 hr and filtered through Whatman No. 41 paper. This step was repeated. The final filtrate was concentrated and 50 mL of distilled water

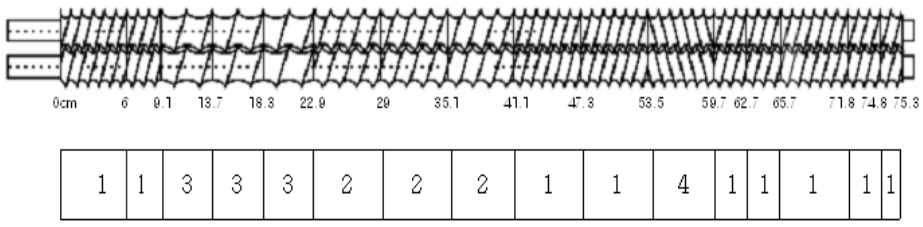

Fig. 1. Screw configuration of the THK31 co-rotating intermeshing twin-screw extruder.

was added to the residue. The distilled water layer was extracted by adding 50 mL of water-saturated butanol. The supernatant liquor was concentrated. After vacuum concentration, 50 mL of ethyl ether was added to the sample, which was then refluxed and extracted. After the ether was removed, the residue was dried at 105°C for 1 hr and weighed.

Statistical analysis

Duplicate samples were used in each experiment and three measurements were taken for each sample. All experimental data are expressed as mean±SD.

RESULTS AND DISCUSSION

DPPH radical scavenging activity

DPPH activity is commonly used for the determination of primary antioxidant activity for pure antioxidant compounds, plant and fruit extracts and food materials (21). A near-linear correlation between DPPH radical scavenging activity and concentrations of polyphenolic compounds in various vegetables and fruits has been reported (22,23). Also, the amount of phenolics is related to the DPPH radical scavenging ability of ginseng (24).

In the present study, the DPPH radical scavenging activity of extruded ginseng samples did not change significantly during fermentation (Fig. 2). However, the DPPH radical scavenging activity of MG and RG increased during the first 4 days of fermentation and remained stable in the following period. The order of scavenging activity of fermentation samples was MG> extruded samples> RG (Fig. 2). These results indicated that DPPH radical scavenging activity decreased after extrusion, but was still greater than in RG, which can be attributed to the contribution of the root hair of MG in the extruded preparation. DPPH radical scavenging activity was higher in MG1 (19.01%) and MG3 (20.37%) than that in MG2 (14.45%) and MG4 (15.78%) on the first day of fermentation (Fig. 2). Examination of DPPH radical scavenging activity over the entire fermentation period revealed decreased activity with increasing die temperature. In contrast, moisture content decreased during extrusion. Die temperature influenced DPPH radical scavenging activity more than moisture content. As shown below, DPPH radical scavenging ability was similar to the pattern of polyphenols.

Polyphenolic compounds

Polyphenol contents showed no significant change with fermentation time in any ginseng sample (Fig. 3). During the first 2 days of fermentation, polyphenol contents increased slightly and then decreased slowly thereafter. The content of polyphenol compounds in tis-

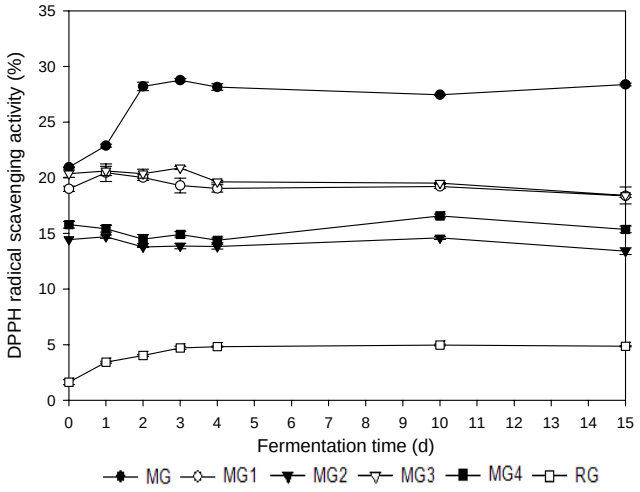

Fig. 2. DPPH radical scavenging activity during fermentation. RG: Root hair of red ginseng, MG: Root hair of tissue cultured mountain ginseng, MG1: Extruded by root hair of tissue cultured mountain ginseng (MG) (moisture content: 25%, barrel temperature: 110°C), MG2: Extruded by root hair of tissue cultured mountain ginseng (MG) (moisture content: 25%, barrel temperature: 140°C), MG3: Extruded by root hair of tissue cultured mountain ginseng (MG) (moisture content: 35%, barrel temperature: 110°C), MG4: Extruded by root hair of tissue cultured mountain ginseng (MG) (moisture content: 35%, barrel temperature: 140°C).

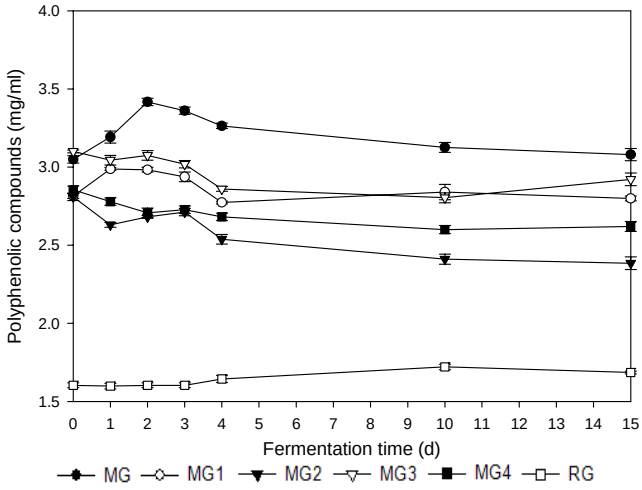

Fig. 3. The content of polyphenolic compounds during fermentation. Samples are the same as in Fig. 2.

cultured MG was higher than in RG (Fig. 3). Polyphenol contents decreased after extrusion and also decreased with increasing die temperature and the decreased moisture content that occurred during extrusion.

Antioxidant activities of individual phenolic compounds may depend on structural factors, such as the number of phenolic hydroxyl or methoxyl groups, flavone hydroxyls, keto groups, free carboxylic groups and other structural features. Dihydroxylation in both rings and in the 3-position in catechin, myricetin, quercein and

epicatechin is required for antioxidant activity as reported in various lipid systems (25). With this information, it is clear that there might be some chance of structural modification of components in ginseng during fermentation, which results in the change of polyphenol contents.

Previous studies have investigated the effect of fermentation length on the total phenolic content in some bean and tea substrates (25,26). In a 20-day solid fermentation of fava bean with *R. oligosporus*, the total phenolic level was reduced during the first 8 days of fermentation, but increased substantially thereafter (26). Total phenolic compounds in kombucha tea were reported to progressively increase with fermentation time (18 days) (25). The trends in the variation of total phenolic content in ginseng samples observed in the present study appear not to be consistent with these previous studies. Whether these discrepancies can be attributed to different materials remains unresolved.

Acidic polysaccharide

During the first day of fermentation, the content of acidic polysaccharides markedly decreased. The decrease continued over the next 3 days (Fig. 4). From day 4 onward, however, the acidic polysaccharides did not diversify further. The content of acidic polysaccharide in extruded MG and RG samples was higher than in raw MG. At the first day of fermentation, the contents of acidic polysaccharide in RG, MG and MG1～MG4 were 8.30, 4.59, 7.67, 6.75, 7.26 and 6.86 mg/mL, respectively. On the final day of fermentation the respective contents were 4.67, 4.14, 4.92, 4.05, 4.66 and 5.23 mg/mL. The content of acidic polysaccharide in the six samples differed on the first day of fermentation, but was similar by the final day (Fig. 4). Also, the content of acidic polysaccharide increased after extrusion but decreased after fermentation.

Previous studies have revealed that the acidic polysaccharide purified from the root of *Panax ginseng* inhibits adhesion of *Helicobacter pylori* to gastric epithelial cells, and the ability of *Porphyromonas gingivalis* to agglutinate erythrocytes (27-29). In addition, a polysaccharide obtained from *P. ginseng* has exerts a comparable inhibitory effect against *P. gingivalis*-mediated hemagglutination (30). In the present study, we conclude that fermentation is not an effective method for increasing the content of acidic polysaccharides, but extrusion is valid.

Crude saponin

As shown in Fig. 5, the content of crude saponin decreased after fermentation in all samples except MG. On the first day of fermentation, crude saponin in RG, MG and MG1～MG4 was 11.27, 10.64, 11.25, 11.14, 11.56 and 10.27 mg/mL, respectively. After extrusion, the content of crude saponin in the fermentation solution was increased in all samples except MG4. After 15 days of fermentation, the content of crude saponin in MG increased to the maximum. Crude saponin in extruded MG samples was higher than raw MG. At fermentation day 15, the content of crude saponin in RG, MG and MG1～MG4 was 9.90, 14.41, 9.77, 9.59, 10.28, and 9.75 mg/mL, respectively (Fig. 5). This indicated that the content of crude saponin in fermentation solution was not affected by the moisture content and barrel temperature during the extrusion process.

The degree of deformation applied to the plant cell tissue with shear stress by extrusion cooking is determined according to the screw dimension, and the shear stress affected by the changing shear rate (31-34).

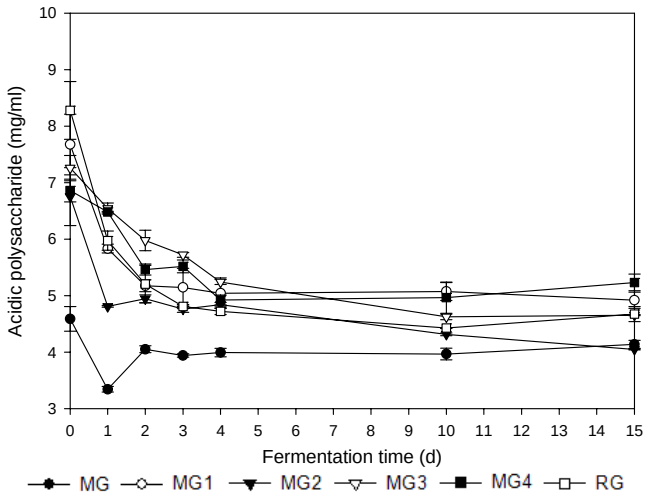

Fig. 4. The content of acidic polysaccharide during fermentation. Samples are the same as in Fig. 2.

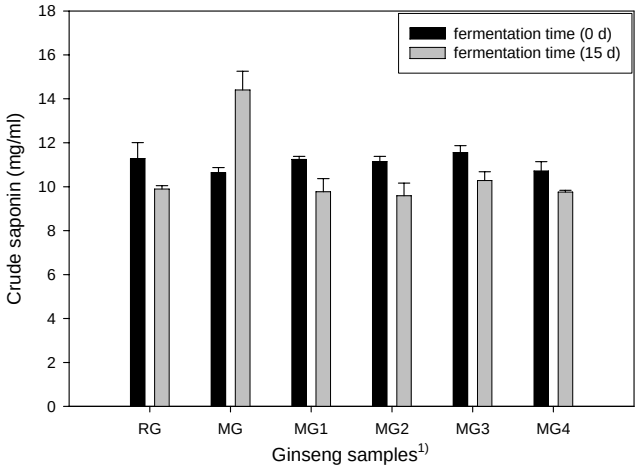

Fig. 5. The content of crude saponin in fermentation solution. [1)]Refers to Fig. 2.

As raw ginseng passes through the inside of the barrel, it experiences shear stress and high pressure due to the by input of heat and mechanical energy. As a result of this, the conversion of saponin occurs during the extrusion process. This is consistent with the observed increase in the content of crude saponin by the drying and extrusion processes (35).

CONCLUSION

DPPH radical scavenging activity and the content of polyphenolic compounds increased during fermentation in RG and MG, while extruded ginseng samples displayed little change. DPPH radical scavenging activity and polyphenolic compounds of MG and RG increased during the first 4 days of fermentation and remained stable thereafter. These results demonstrate that antioxidant activities increase during fermentation. But prolonged fermentation is not recommended because of accumulation of organic acids, which might reach harmful levels for direct consumption (36). The potential effectiveness or use of root hair of tissue cultured MG should be investigated further. The content of acidic polysaccharide decreased during fermentation. It continued to decrease during the following 3 days and halted thereafter. By the last day of fermentation, acidic polysaccharide in RG and tissue cultured mountain ginseng (MG and MG1～MG4) were comparable (Fig. 4). Furthermore, acidic polysaccharide was increased after extrusion, and was the highest in RG on the first day of fermentation. Crude saponin in RG and extruded ginseng samples decreased after 15 days of fermentation, while increasing in MG. After extrusion, the content of crude saponin in the fermentation solutions increased except for MG4. The content of crude saponin in fermentation solution was not affected by the moisture content and barrel temperature during the extrusion process.

In a conclusion, fermentation increases the antioxidant capability of ginseng. The potential effectiveness or use of root hair of tissue cultured MG during the extrusion process warrants further study.

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