Research article

Changes in the ginsenoside content during the fermentation process using microbial strains

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ABSTRACT

Background: Red ginseng (RG) is processed from Panax ginseng via several methods including heat treatment, mild acid hydrolysis, and microbial conversion to transform the major ginsenosides into minor ginsenosides, which have greater pharmaceutical activities. During the fermentation process using microbial strains in a machine for making red ginseng, a change of composition occurs after heating. Therefore, we confirmed that fermentation had occurred using only microbial strains and evaluated the changes in the ginsenosides and their chemical composition.

Methods: To confirm the fermentation by microbial strains, the fermented red ginseng was made with microbial strains (w-FRG) or without microbial strains (n-FRG), and the fermentation process was performed to tertiary fermentation. The changes in the ginsenoside composition of the self-manufactured FRG using the machine were evaluated using HPLC, and the 20 ginsenosides were analyzed. Additionally, we investigated changes of the reducing sugar and polyphenol contents during fermentation process.

Results: In the fermentation process, ginsenosides Re, Rg1, and Rb1 decreased but ginsenosides Rh1, F2, Rg3, and Compound Y (C.Y) increased in primary FRG more than in the raw ginseng and RG. The content of phenolic compounds was high in FRG and the highest in the tertiary w-FRG. Moreover, the reducing sugar content was approximately three times higher in the tertiary w-FRG than in the other n-FRG.

Conclusion: As the results indicate, we confirmed the changes in the ginsenoside content and the role of microbial strains in the fermentation process.

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1. Introduction

Korean ginseng, the root of Panax ginseng that has been a widely used traditional medicine in Asian countries for thousands of years, is composed of saponins, phenols, amino acids, and polysaccharides. Many studies have demonstrated that ginseng compounds have pharmacological effects, including anticancer, antioxidant, anti-inflammatory, antihypertensive, antidiabetic, anti-stress, and neuroprotective effects [1,2].

Ginseng saponins, called ginsenosides, play a key role in the pharmacological and biological activities, and thus they are regarded to be principal components. Ginseng saponins contain steroids and triterpenoids depending on an aglycone structure, and triterpenoids include oleanane and dammarane. Dammarane, the main component of ginseng, can be classified as protopanaxadiol (PPD) and protopanaxatriol (PPT) [3,4]. Among these, the PPD and PPT-type ginsenosides are the major ginsenosides in ginseng, and these major ginsenosides can be transformed into deglycosylated ginsenosides and minor ginsenosides. The converted minor ginsenosides have more effective pharmacological properties than the major ginsenosides [5,6].

Ginsengs are classified as fresh, white, and red ginseng (RG), which is made by steaming and drying fresh ginseng [5,7]. Ginseng is usually administered orally, and there are individual differences in its bioavailability depending on the ability of the human intestinal bacteria to transform it. Therefore, these processes may increase the absorption and efficacy of ginseng compounds [8]. Fermented red ginseng (FRG) is processed using several methods including heat

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treatment, mild acid hydrolysis, microbial conversion, and enzyme conversion, to transform the major ginsenosides into minor ginsenosides. Using the microbial strains, side reactions such as epimerization and hydration can be avoided [3,9]. In addition, ginsenoside conversion can occur via heat over the course of fermentation using the microbial strains in a fermenting machine for making RG.

In previous studies, the conversion of ginsenoside was investigated in FRG made under diverse fermentation conditions, including using different strains of microorganisms [10-12]. According to earlier studies, Rb1 and Rg1 were the two main ginsenosides contained in RG that decreased, whereas the Rh2 and Rc content increased, leading to improved antioxidant activity by the transformation of ginsenoside [13,14]. Additionally, it has been reported that the concentrations of pharmaceutically active Rh1, Rg2, and compound K (C.K) were undetectable in RG but detectable in FRG. Additionally, the pharmaceutical action of FRG effectively increased by fermentation because it is associated with several substances such as sugar and phenolic hydroxyl group, and reducing these components minimizes oxidative stress and free radicals [13,15].

In this study, we evaluated the effect of fermentation using only microbial strains according to ginsenosides and chemical composition. The fermentation process was performed with or without microbial strains to tertiary fermentation. The changes in ginsenoside composition of the self-manufactured FRG using a machine were evaluated by HPLC, and the amount of reducing sugar and polyphenol content were measured.

2. Materials and methods

2.1. Materials

Six-y-old ginseng root with a disheveled-hair shape cultivated from Geumsan (Korea) was used. Standard ginsenoside Rg1, Re, Rb1, Re, Rb2, Rh1(s), Rh1(r), Rd, F1, F2, Rg3(s), Rg3(r), Rh2(s), Rh2(r), compound O (C.O), compound Y (C.Y), compound K (C.K), PPT(s), PPD(s), and PPD(r) were purchased from the Ambo Institute (Seoul, Korea). All reagents in the experiment were of HPLC grade and purchased from Fisher Scientific (Fair Lawn, NJ, USA).

2.2. Reflux extraction

To examine the ginsenoside content of raw ginseng and RG, a reflux extraction was conducted according to the Health Functional Food Code of Korea [16]. Ginseng and RG were freeze-dried completely for 3 d and were prepared into a powder form. One gram of both ginseng powder and RG powder was put into a flask, and 50 mL of 50% methanol was added and reflux extracted at 80°C for 1 h using a heating mantle as the extraction apparatus (EAM9402-03, MTOPS, Yangju, Korea). Then, the extracted samples were cooled and centrifuged at 1,811g for 10 min, and the supernatant liquid was transferred to a conical tube. Samples were concentrated using a centrifugal vacuum concentrator (EZ-2 plus, Genevac, Ipswich, England), and then the concentrate was dissolved in an acetonitrile solution (water:acetonitrile = 80:20).

2.3. Fermentation conditions

RG was prepared using a machine for making red ginseng (HK9600, Jinhongtech, Korea) by steaming raw ginseng for 18 h, after which time 6 L of water was added to 300 g of RG and the RG extract was incubated for 48 h. The prepared RG extract was fermented for 75 h with a mix of 10 microbial strains, including *Saccharomyces cerevisiae*, *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus brevis*, and *Bacillus subtilis*. During this fermentation process, the temperature was monitored using a temperature data logger (17516, Testo, Germany; Fig. 1). In the same way, the fermentation procedure was followed without microbial strains to the tertiary fermentation. Additionally, the same amount of manufactured RG and commercial 6-y-old RG from Geumsan (Korea) were fermented to compare the manufactured RG and the commercial RG.

2.4. Reducing sugar content

Reducing sugar was measured using the dinitrosalicylic acid (DNS) method. DNS reagent was prepared by mixing 3,5-dinitrosalicylic acid (Sigma-Aldrich, St. Louis, MO, USA) and Rochelle salt (Sigma). Five hundred microliters of samples were added to 0.5 mL of DNS reagent, reacted in a boiling water bath for 5 min, and then cooled immediately for 10 min on ice. The absorbance was measured at 550 nm using a microplate reader (E-Max, Molecular Device Co., Sunnyvale, CA, USA), and the analyses were performed in triplicate. To determine the amount of reducing sugar, the values were averaged and a standard calibration curve was created using dextrose (Sigma-Aldrich, St. Louis, MO, USA) as a reference [6,17].
2.5. Analysis of polyphenol

Total phenolic compound contents of the samples were determined using the Folin-Denis method. Five hundred microliters of sample were mixed with 0.5 mL Folin-Denis reagent (Sigma-Aldrich, St. Louis, MO, USA) and equilibrated for 3 min. Five hundred microliters of 10% sodium carbonate (Na2CO3) was added and allowed to stand for 1 h. The absorbance was measured at 750 nm using a microplate reader. The total polyphenol concentration was calculated from a standard calibration curve using gallic acid (Sigma-Aldrich, St. Louis, MO, USA) and (4.6 250 mm, 5 mm; Waters, Milford, MA, USA) equipped with a liquid handler, UV detector, and binary pump. A Waters Symmetry C18 column (4.6 × 250 mm, 5 μm; Waters) was used. The gradient elution was employed using solvent A (water) and solvent B (acetonitrile) as follows: 0–5 min, 80% A and 20% B; 5–15 min, 80% A and 20% B; 15–19 min, 72% A and 28% B; 19–23 min, 67% A and 33% B; 23–50 min, 59% A and 41% B; 50–78 min, 20% A and 80% B; 78–85 min, 20% A and 80% B; and 85–90 min, 80% A and 20% B. The flow rate was 1.0 mL/min. UV absorption was measured at a wavelength of 203 nm. The content of ginsenosides was analyzed by HPLC, and the HPLC chromatograms of the 20 standard ginsenosides are shown in Fig. 2. All samples were filtered through a syringe filter (0.45 μm), and 10 μL of sample was injected into the HPLC.

2.6. HPLC analysis

Ginsenosides were identified by comparing the retention time of the samples to those of standard samples using an analytical reversed-phase HPLC system (GX-281 HPLC, Gilson, Inc., Middleton, WI, USA) and (4.6 250 mm, 5 mm; Waters) was equipped with a liquid handler, UV detector, and binary pump. A Waters Symmetry C18 column (4.6 × 250 mm, 5 μm; Waters) was used. The gradient elution was employed using solvent A (water) and solvent B (acetonitrile) as follows: 0–5 min, 80% A and 20% B; 5–15 min, 80% A and 20% B; 15–19 min, 72% A and 28% B; 19–23 min, 67% A and 33% B; 23–50 min, 59% A and 41% B; 50–78 min, 20% A and 80% B; 78–85 min, 20% A and 80% B; and 85–90 min, 80% A and 20% B. The flow rate was 1.0 mL/min. UV absorption was measured at a wavelength of 203 nm. The content of ginsenosides was analyzed by HPLC, and the HPLC chromatograms of the 20 standard ginsenosides are shown in Fig. 2. All samples were filtered through a syringe filter (0.45 μm), and 10 μL of sample was injected into the HPLC.

3. Results and discussion

3.1. Polyphenol and reducing sugar contents in FRG

Table 1 shows the chemical compositions of RG and FRGs under various conditions. The polyphenol and reducing sugar are active antioxidants and thus prevent lipid oxidation [5,6]. The polyphenol activities in FRGs were measured and compared to their activities in RG. Although significant differences were not identified in phenolic compound and reducing sugar contents between RG and FRGs, both contents were slightly increased as a result of the fermentation process. The chemical composition of FRG with microbial strains (w-FRG) or without microbial strains (n-FRG) was also analyzed. The polyphenol content ranged from 27.023 μg/mL to 32.852 μg/mL in the w-FRGs and from 24.922 μg/mL to 29.765 μg/mL in the n-FRGs. The total reducing sugar contents ranged from 2.006 μg/mL to 3.433 μg/mL in the w-FRGs and from 1.178 μg/mL to 1.376 μg/mL in the n-FRGs. Compared to the n-FRGs, the w-FRGs had higher levels of polyphenol and reducing sugar, which could be because the microbial strains have a positive effect on the fermentation of RG.

3.2. Changes of ginsenoside composition in FRG

Fig. 3 shows the HPLC chromatogram of reflux-extracted raw ginseng, extracted RG, primary FRG with microbial strains (w-FRG1), and primary FRG without microbial strains (n-FRG1). The secondary FRG and tertiary FRG with microbial strains (w-FRG2 and w-FRG3) or without microbial strains (n-FRG2 and n-FRG3) are not shown. The total ginsenoside contents of the w-FRGs and n-FRGs yielded by the fermentation process were 191.2162 μg/mL (FRG1), 105.3750 μg/mL (FRG2), 92.7093 μg/mL (FRG3), 133.6902 μg/mL (n-FRG1), 92.7093 μg/mL (n-FRG2), and 82.4604 μg/mL (n-FRG3) in Table 2. As fermentation progressed, the total ginsenoside content was reduced and when the fermentation was performed with microbial strains, ginsenoside concentration was higher than performed without microbial strains.

A previous study has shown that the total ginsenoside content decreased as the extraction time increased [19]. Similarly, in this study, the ginsenoside content of FRG1 showed particularly high levels of total ginsenosides compared to that of FRG3 and thus, it is possible that transformation to unmeasured ginsenosides occurred. Additionally, it was determined that microbial strains have a positive effect on the fermentation of RG based on the difference in the total ginsenoside levels among FRGs with or without microbial strains.

To investigate changes of ginsenoside composition, a fermentation process was conducted three times. Tables 3 and 4 show the amount and ratio of PPD and PPT-type ginsenoside, respectively. Rb1, one of the main ginsenosides contained in RG, was identified at high levels in RG and was decreased significantly with repeated fermentation. Thus, it was considered that Rb1 changed into low-molecular and was transformed. In particular, w-FRG1 had a higher ratio (%) of 20(S)-Rg3 and 20(R)-Rg3 (22.3% and 10.0%, respectively) compared to the ratios for raw ginseng, RG and RG fermented under other conditions. Further, CY showed a lower ratio (%) in w-FRGs with microbial strains than n-FRGs. Re and Rg1 were extremely rare.

**Table 1**
The phenolic compound and reducing sugar contents of fermented red ginseng with or without microbial strains

| Contents   | RG | w-FRG1 | w-FRG2 | w-FRG3 | n-FRG1 | n-FRG2 | n-FRG3 |
|------------|----|--------|--------|--------|--------|--------|--------|
| Polyphenol | 24.565 | 27.023 | 29.687 | 32.852 | 24.922 | 27.791 | 29.765 |
| (mg/mL)    |     |        |        |        |        |        |        |
| Reducing   | 1.089 | 2.006  | 2.801  | 3.433  | 1.178  | 1.257  | 1.376  |
| sugar      |     |        |        |        |        |        |        |
| (mg/mL)    |     |        |        |        |        |        |        |

n-FRG, fermented red ginseng with no microbial strains; RG, red ginseng; w-FRG, fermented red ginseng with microbial strains
in FRGs yielded by the various fermentation conditions. 20(S)-Rh1 and 20(R)-Rh1 had a relatively high ratio (%) in FRGs compared to the ratios in raw ginseng and RG.

Moreover, the same amount of manufactured RG by the machine and commercial RG were fermented, and then the total ginsenoside concentration was analyzed to examine the useful component of manufactured FRGs. As a result, when primary fermentation was conducted, the total ginsenoside contents of the manufactured FRGs were higher than those of the commercial FRGs with or without microbial strains (Fig. 4). Although the manufactured FRG2 and FRG3 had lower ginsenoside levels than the commercial FRGs, the primary fermentation was considered a stable and suitable condition based on results of repeated fermentation and chemical composition in view of a result obtained both manufactured RG and commercial RG.

4. Conclusion

The minor ginsenosides of Korean ginseng are known to be produced by the hydrolysis of sugar moieties of the major

| Table 2 |
|------------------|------------------|------------------|------------------|------------------|------------------|
| Total ginsenoside concentration of fermented red ginseng (Primary FRG, secondary fermented red ginseng, and tertiary fermented red ginseng) with or without microbial strains |
| w-FRG1 | w-FRG2 | w-FRG3 | n-FRG1 | n-FRG2 | n-FRG3 |
| Total ginsenoside concentration (μg/mL) | 191.2162 | 105.3750 | 92.7093 | 133.6902 | 92.7093 | 82.4604 |

FRG1, primary red ginseng; FRG2, secondary red ginseng; FRG3, tertiary red ginseng; n-FRG, fermented red ginseng with no microbial strains; w-FRG, fermented red ginseng with microbial strains
ginsenosides. For the transformation of the major ginsenosides to the minor ginsenosides, FRG is processed using several methods, including by fermentation using microbial strains. Because ginsenoside conversion may occur by heating during fermentation with microbial strains, we intended to confirm the fermentation process with or without microbial strains.

Similar to the results for the measurement of polyphenol and reducing sugar contents, the total ginsenoside levels of the w-FRGs was higher than those of the n-FRGs, indicating the importance of microbial strains. The changes in ginsenoside composition were identified, and indicated that deglycosylated ginsenosides including Rg3 and Rh2 increased and that Rb1, a major ginsenoside, decreased.

As the fermentation process was repeated, polyphenol and reducing sugar content increased; however, the total ginsenoside contents were the highest when the RG was fermented only once. Therefore, these results provide a simple fermentation method and information about the proper fermentation conditions; further research is needed to determine other remarkable microbial strains.

Conflict of interest

The authors have no conflicts of interest to disclose.

References

[1] Lee HS, Lee HJ, Yu HJ, Ju DW, Kim Y, Kim CT, Kim CJ, Cho YJ, Kim N, Choi SY, et al. A comparison between high hydrostatic pressure extraction and heat extraction of ginsenosides from ginseng (Panax ginseng CA Meyer). J Sci Food Agric 2011;91:1466–73.

[2] Yi J-H, Kim M-Y, Kim Y-C, Jeong W-S, Bae D-W, Hur J-M, Jun M. Change of ginsenoside composition in red ginseng processed with citric acid. Food Sci Biotechnol 2010;19:647–53.

[3] Kim DH. Chemical diversity of panax ginseng, panax quinquifolium, and panax notoginseng. J Ginseng Res 2012;36:1–15.

[4] Kim SY, Lee H, Ra KS, Suh HJ, Kim SY, Shin K-S. Transformation of ginsenoside-rich fraction isolated from ginseng (panax ginseng) leaves in aqueous suspension. J Food Sci Biotechnol 2010;19:647–53.

[5] Bae SH, Lee HS, Kim MR, Kim SY, Kim JM, Suh HJ. Changes of ginsenoside content by mushroom mycelial fermentation in red ginseng extract. J Ginseng Res 2011;35:235–42.

Table 3

The protopanaxadiol-type ginsenoside amount of raw ginseng, red ginseng, and fermented red ginseng with or without microbial strains using 300 g of raw ginseng

| PPD-type ginsenoside (mg) | Raw Ginseng | RG | w-FRG1 | w-FRG2 | w-FRG3 | n-FRG1 | n-FRG2 | n-FRG3 |
|--------------------------|-------------|----|--------|--------|--------|--------|--------|--------|
| Rb1                      | 1391.3 (32.1) | 1915.3 (20.3) | 51.0 (4.4) | 25.5 (4.0) | 14.8 (2.7) | 52.9 (6.6) | 23.8 (4.3) | 13.7 (2.8) |
| Rd                       | 825.1 (1.9) | 352.3 (3.7) | ND     | ND     | ND     | ND     | ND     | ND     |
| C.O                      | 8.6 (0.2) | 13.8 (0.1) | ND     | ND     | ND     | ND     | ND     | ND     |
| F2                       | 2.8 (0.1) | 405.8 (4.2) | 63.3 (5.5) | 26.4 (4.2) | 18.4 (3.3) | 19.2 (2.4) | 17.1 (3.1) | 11.7 (2.4) |
| 20(R)-Rg3                | ND     | 673.9 (6.6) | 255.8 (22.3) | 70.4 (11.2) | 58.4 (10.5) | 62.6 (7.8) | 46.7 (8.4) | 50.3 (10.2) |
| 20(R)-Rg3                | ND     | 808.7 (8.4) | 115.1 (10.0) | 26.7 (4.2) | 33.4 (6.0) | 29.6 (3.7) | 38.0 (6.8) | 56.6 (11.5) |
| C.Y                      | 21.0 (0.5) | 476.7 (5.0) | 212.5 (18.5) | 226.6 (35.9) | 162.8 (29.4) | 316.8 (39.5) | 244.8 (44.1) | 223.6 (45.4) |
| C.K                      | ND     | ND     | ND     | ND     | ND     | ND     | ND     | ND     |
| 20(S)-Rh2                | ND     | ND     | ND     | ND     | ND     | ND     | ND     | ND     |
| 20(R)-Rh2                | ND     | ND     | ND     | ND     | ND     | ND     | ND     | ND     |
| Total                    | 1506.1 | 4644.6 | 697.5 | 375.6 | 128.7 | 481.1 | 370.4 | 356.0 |

Data are presented as n (%)

C.K, Compound K; C.Y, Compound Y; FRG1, primary red ginseng; FRG2, secondary red ginseng; FRG3, tertiary red ginseng; ND, not detected; n-FRG, fermented red ginseng with no microbial strains; PPD, protopanaxadiol; RG, red ginseng; w-FRG, fermented red ginseng with microbial strains.

Table 4

The protopanaxatriol-type ginsenoside amount of raw ginseng, red ginseng, and fermented red ginseng with or without microbial strains using 300 g of raw ginseng

| PPT-type ginsenoside (mg) | Raw Ginseng | RG | w-FRG1 | w-FRG2 | w-FRG3 | n-FRG1 | n-FRG2 | n-FRG3 |
|--------------------------|-------------|----|--------|--------|--------|--------|--------|--------|
| Rg1-Re                   | 1508.6 (34.8) | 372.4 (3.9) | 6.4 (0.6) | BQL    | BQL    | 5.4 (0.7) | BQL    | BQL    |
| 20(S)-Rh1                | 338.0 (0.8) | 612.6 (6.4) | 127.3 (11.1) | 81.1 (12.9) | 49.2 (8.9) | 111.6 (13.9) | 66.3 (11.9) | 43.7 (8.9) |
| 20(R)-Rh1                | 7.7 (0.2) | 355.5 (3.7) | 102.2 (8.9) | 91.6 (14.5) | 64.3 (11.6) | 106.5 (13.3) | 66.9 (12.0) | 55.4 (11.3) |
| F1                       | 9.3 (0.2) | 2.5 (0.0) | ND     | ND     | ND     | ND     | ND     | ND     |
| Total                    | 1559.5 | 1342.9 | 235.9 | 179.9 | 127.1 | 223.4 | 142.7 | 114.2 |

Data are presented as n (%)

BQL, below quantifiable limit; FRG1, primary red ginseng; FRG2, secondary red ginseng; FRG3, tertiary red ginseng; ND, not detected; n-FRG, fermented red ginseng with no microbial strains; PPT, protopanaxatriol; RG, red ginseng; w-FRG, fermented red ginseng with microbial strains.

Fig. 4. The comparison of total ginsenoside content in fermented red ginseng (FRG) using manufactured red ginseng (RG) using a machine for making RG with FRG or using commercial RG.
Lee HS, Kim MR, Park Y, Park HJ, Chang UJ, Kim SY, Suh HJ. Fermenting red ginseng enhances its safety and efficacy as a novel skin care anti-aging ingredient: in vitro and animal study. J Med Food 2012;15:1015–23.

Jang M, Min J-W, Yang D-U, Jung S-K, Kim S-Y, Yang D-C. Ethanol fermentation from red ginseng extract using Saccharomyces cerevisiae and Saccharomyces carlsbergensis. Food Sci Biotechnol 2011;20:131–5.

Quan JH, Kim YJ, Li GH, Choi KT, Yang DC. Microbial transformation of ginsenoside rb1 to compound K by Lactobacillus paralimentarius. World J Microbiol Biotechnol 2013;29:1001–7.

Lee EJ, Song MJ, Kwon GE, Ji GE, Sung MK. Oral administration of fermented red ginseng suppressed ovalbumin-induced allergic responses in female BALB/c mice. Phytomedicine 2012;19:896–903.

Kang B-H, Lee K-J, Hur S-S, Lee D-S, Lee S-H, Shin K-S, Lee J-M. Ginsenoside derivatives and quality characteristics of fermented ginseng using lactic acid bacteria. Korean J Food Preserv 2013;20:573–82.

Hong SY, Oh JH, Lee I. Simultaneous enrichment of deglycosylated ginsenosides and monacolin K in red ginseng by fermentation with Monascus pilosus. Biosci Biotechnol Biochem 2011;75:1490–5.

Quan L-H, Piao J-Y, Min J-W, Yang D-U, Lee HN, Yang DC. Bioconversion of ginsenoside rb1 into compound K by Leuconostoc citreum LH1 isolated from kimchi. Braz J Microbiol 2011;42:1227–37.