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# Comparative assessment of compositional constituents and antioxidant effects in ginseng sprouts (*Panax ginseng*) through aging and fermentation processes

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ABSTRACT

The main objectives of this research was to demonstrate fluctuations of compositional constituents and antioxidant effects in aging (AGS) and fermentation (FAGS) processes regarding beneficial qualities of food industry from dried ginseng sprouts (DGS). Moreover, our wok is the first to compare the influential factors on antioxidant and physicochemical properties. Total amino acids (4537 → 2129 → 2450 mg/100g) and volatiles (3538 → 1015 → 325 ng/g) decreased considerably during DGS → AGS → FAGS processes, specifically, arginine (2490 → 1034 → 1351 mg/100g) and β-farnesene (1940 → 202 → 19 ng/g) showed the predominant decrease rates. Total ginsenoside contents also decreased with 37.39 → 33.83 → 34.52 mg/g, however, the deglycosilated ginsenoside F2 (2.15 → 3.56 → 4.59 mg/g, 2.1-fold) and compound K (CK) (0.75 → 2.98 → 4.07 mg/g, 5.4-fold) increased with high variations. Interestingly, ginsenoside Re decreased with the highest variation rate (6.47 → 2.45 → 1.53 mg/g, 4.2-fold). The antioxidant capacities increased remarkably with approximately 2 times in DGS → AGS → FAGS steps as follows: ABTS assay > DPPH assay > hydroxyl radical scavenge > FRAP at 1000 μg/mL. In particular, processed ginseng sprouts were observed high values of total phenolic content (TPC) (2.4 → 4.9 → 5.5 GAE/g), total flavonoid content (TFC) (0.5 → 0.9 → 1.3 RE/g), and of maillard reaction products (MRP) (2.0 → 2.8 → 2.9 $OD_{420nm}$) than DGS. Our results suggest that AGS and FAGS may be utilized as potential candidates on beneficial compositions and natural antioxidants for functional foods.

## 1. Introduction

For several decades, many researchers have evaluated that processed foods using crops, fruits, vegetables, and other natural sources as well as their products play an important role in human health aspects owing to the compositional qualities (Ahmed Bekhit et al., 2015; Akpabli-Tsigbe et al., 2021; Cho et al., 2017; Eom et al., 2018). Moreover, the use of the effective processing skills, such as fermentation, germination, heat treatment, aging, enzymatic hydrolysis, and microorganism has increased tremendously in medical and food industries because of the increase of health-promoting metabolites and functional properties (Akpabli-Tsigbe et al., 2021; Chu et al., 2020; Hwang et al., 2021; Jung et al., 2017). These above processes are also increasingly of interest in several fields using diverse natural sources, based on the beneficial aspects of non-toxicity and safety (Lee et al., 2015, 2018; Szambelan et al., 2020; Wang et al., 2022). The earlier researches have demonstrated that the inexpensive process techniques were observed high compositional qualities and strong biological activities in crops and edible plants (Ayestarán et al., 2019; Dewir et al., 2010; Eom et al., 2018; Jung et al., 2017). These observations have reported that the metabolite profiles and concentrations are associated with biotransformation and biochemical modification by process methods (Eom et al., 2018; Hsu et al., 2013; Xu et al., 2018). Numerous researchers are currently searching for effective process methods related to metabolites and biological abilities from natural materials for the manufacture of supplements through nutraceutical agents. In our continued screening of the

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processing sources, we found that the 50% ethanol extracts of ginseng sprouts through cultivated method showed considerable variations of compositional components and antioxidant abilities.

Ginseng (*Panax ginseng*, Araliaceae family) has been one of the most excellent natural crop for healthy and medicinal foods in East Asia and worldwide (Dewir et al., 2010; Dong et al., 2017; Eom et al., 2018; Jung et al., 2017). This species plays significant role in the manufacture of nutraceutical agents and functional foods for human health benefits concern to the compositional qualities (Guo et al., 2021; Jung et al., 2019; Kim et al., 2007; Liu et al., 2020). Several studies have reported that the ginseng metabolites are associated with the biological abilities such as antiaging, antidiabetes, anticancer, and anti-inflammatory (Eom et al., 2018; Kim et al., 2007; Renchinkhand et al., 2015; Saidi et al., 2020). In particular, the ginsenoside derivatives of tetracyclic triterpene saponines have been mainly attributed to be quantified for evaluating ginseng derived food products owing to their abundant concentration (Cheng et al., 2008; Chi & Ji, 2005; Lee et al., 2015; Xu et al., 2018). Furthermore, it is well established that fluctuations of ginsenosides differed remarkably depending on cultivation region and age (Dewir et al., 2010; Guo et al., 2021), however, there have been no comprehensive information through other environmental conditions in ginseng sprouts. Specifically, little information is available on the comparison and demonstration of other compositional components including ginsenosides. Although many literatures have established that the various ginseng types, such as mountain-cultivated ginseng, mountain wild ginseng, and cultivated ginseng possessed high metabolite contents and excellent biological capacities according to the environmental factors (Dewir et al., 2010; Dong et al., 2017; Lee et al., 2021; Xu et al., 2018), there are only few reports concern to compositional compositions and antioxidant properties in effective processing techniques using microorganism from ginseng sprouts.

Herein, our research was the first to elucidate variations of compositional constituents (ginsenosides, volatile compounds, amino acids, and fatty acids) and antioxidant properties (radical and FRAP) in ginseng sprouts (GS) of cultivated type from the food manufacturing methods by aging and fermentation processes. In addition, the potential antioxidant patterns were investigated with the fluctuations of total phenolic content (TPC), total flavonoid content (TFC), and mallard reaction product (MRP) in processed ginseng sprouts (AGS and FAGS). We also examined for the first time the comparisons of physicochemical properties of pH, acidity, and reducing sugar.

## 2. Materials and methods

### 2.1. Plant and strains

Ginseng (6-year-old) was collected (date: August 15, 2019) in the experimental field of Simmani Wild Farm Association Co., Hamyang-gun, Gyeongnam in South Korea. After collecting, ginseng samples were grown at 25 °C for 30 days with artificial soil under a growth chamber to gain the ginseng sprouts. The harvested samples were carefully washed with deionized water to remove the soil and dust residual, and then were air-dried for 3 days at 45 °C under chamber. Two probiotic strains including *Lactobacillus brevis* BMK184 and *L. plantarum* P1201 were acquired in fermented beverage plants and *mulkimchi* by previously described methods (Lee et al., 2018).

### 2.2. Chemicals and instruments

Folin-Ciocalteu phenol reagent, sodium carbonate ($Na_2CO_3$), gallic acid, sodium nitrite ($NaNO_2$), aluminum chloride ($AlCl_3$), sodium hydroxide (NaOH), rutin, and (+)-glucose, and (+)-xylose were supplied from Sigma-Aldrich (St. Louis, MO, USA). 2,2-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), iron (III) chloride ($FeCl_3$), acetate buffer, 2,4,6-tripyridyl-s-triazine (TPTZ), 2-deoxyribose, ferric chloride, sodium acetate, potassium persulfate, ascorbic acid, Trolox, and butylated hydroxytoluene (BHT) were also obtained from Sigma-Aldrich. Ginsenoside standards were approved by KOC Biotech Co., Ltd. (Daejeon, Korea), and the amino acid and fatty acid standards were provided by Sigma-Aldrich. Moreover, the used other chemicals and solutions were performed by analytical grade. All measurements including compositional components and antioxidant properties were conducted by various instruments in supplementary data (section 2.2).

### 2.3. Preparation of aging and fermentation processes from ginseng sprouts

Ginseng sprouts were washed with clean sterile water, and then air-dried for 1 day at 25 °C. The collected ginseng sprouts were dried with an incubator 55 °C during 3 days to gain dried ginseng sprouts (DGS). To obtain aged ginseng sprouts (AGS), the DGS sample was steamed at 100 °C for 1 h, and then were aged at 75 °C for 3 days in aging container (SI–70S2, Sinil, Daegu, Korea). This process was conducted using the method described previously (Kamizake et al., 2016), with some modifications. AGS was fermented at 30 °C for 3 days using *L. plantarum* P1201 strain to make fermented aged ginseng sprouts (FAGS) sample with modification by Lee et al. (2018). Three ginseng samples (DGS, AGS, and FAGS) were dried for 5 days at 50 °C to remove moisture by the methods of Chen et al. (2018) and Zhang et al. (2021) with slight modifications. The manufacturing methods exhibited Fig. S1, and their samples were kept dry state at 30 °C prior to analysis.

### 2.4. Measurement of physicochemical properties

The pH value was measured using a pH meter (MP 200, UK) and the acidity value was evaluated by titration of a 0.1 mol/L NaOH solution with lactic acid (%). The reducing sugar was confirmed using a dinitrosalicylic (DNS) acid method, as the reported in earlier study (Piao et al., 2019). The reducing sugar content was expressed as mg/g using a standard curve (glucose) (Miller, 1959).

### 2.5. Determination of amino acid and fatty acid contents

The amino acid contents (23 non-essential and 8 essential compositions) in ginseng sprouts performed as the previous method (Hwang et al., 2021). In short, sample (1 g) was added to 4 mL distilled water, and then heated on heating block. After cooling at 25 °C, the crude solution was mixed with 10 g/100 mL sulfosaliylic acid (5 mL) at 60 °C for 2 h to drive hydrolysis. The mixture solution was centrifuged for 3 min at 3000×*g* and the supernatants were filtered with 0.42 μm syringe filter. Their contents were evaluated in mg/100 g by an automatic amino acid analyzer (L-8900, Hitachi, Tokyo, Japan). Fatty acid compositions were demonstrated by the boron trifluoride-catalyzed methylation technique (Lee et al., 2018). Each sample extract (2 mL) was added to 3 mL of 0.5 mol/L NaOH in methanol, and then heated in a heating block at 100 °C for 10 min. The reaction mixture was cooled to 25 °C and was mixed to 14 g/100 mL borontrifluride in methanol. After heating for 30 min at 100 °C to fatty acid methylation, the crude solution was saturated with 28 g/100 mL NaCl (6 mL) and isooctane (2 mL). And then, the mixture solution was analyzed by GC and their concentrations were measured in mg/100 g (dry weight). The fatty acid compositions were determined by a GC 7980 with SP-2560 capillary column (0.25 mm × 100 m i.d., 0.25 μm) and flame ion detector. Other GC conditions were as follows: temperature profile: 150 °C for 1 min, increased from 150 to 230 °C at 2.5 °C/min; carrier gas: nitrogen, 1 mL/min; inlet and detector temperature: 250 °C.

### 2.6. Preparation of samples and calibration curves for ginsenoside contents

The pretreatment procedure of samples and calibration curves for ginsenoside evaluation was prepared by following the modification

methods described by Kim et al., 2010 and Lee et al., 2018. The GA samples were pulverized for 3 min using a coffee grinder (HR2860, Philips, Netherlands). The powdered source (1.0 g) was extracted using 20 mL of 50% ethanol in a shaking incubator for 12 h at 25 °C. The supernatant was filtered through a syringe filter (0.45 μm, Whatman Inc., Maidstone, UK) before HPLC analysis. For quantification, the calibration curves were prepared by comparing the peak areas using eight concentrations (0.5, 1, 2, 5, 10, 50, 100, 200 μg/mL) of each standard material (stock solution 1000 μg/mL using DMSO) from HPLC chromatograms. The correlation coefficients regarding all ginsenoside curves were obtained higher than 0.998.

### 2.7. HPLC conditions for ginsenoside analysis

The quantification of ginsenosides in samples was carried out using HPLC analysis by the method described by Xu et al. (2018) with a slight modification. A 20 μL sample of the 50% ethanol extract was injected onto an analytical reversed phase C18 column (TSK-ODS100Z, 4.6 × 250 nm, 5 μm, Tosoh Corp., Tokyo, Japan). The mobile phase was performed with water (elution A) and acetonitrile(elution B) by the following gradient program: 0–10 min 19% B, 15 min 20% B, 30 min 23% B, 42 min 30% B, 75 min 35% B, 80 min 60% B, 100 min 90% B, and reconditioned with 100% B for 3 min. Other HPLC conditions were as follows: detection, 203 nm; flow rate, 1.0 mL/min; column temperature, 25 °C.

### 2.8. Quantification methods and GC/MS conditions for volatile compounds

Each sample (1 g; DGS, AGS, FAGS) was mixed with saturated sodium chloride solution (10 mL) in glass headspace vial, and then the internal standard (octanal-$d_{16}$, 10 ng/g) was added as described previously with some modifications (Bryant & McClung, 2011; Lee et al., 2014). Volatiles were extracted using a 2 cm solid-phase micro--extraction (SPME) fiber coated with divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS, Supelco, Bellefonte, PA) for 30 min at 40 °C and the SPME fiber was injected into the GC inlet and remained for 30 s. The chromatographic separations and calculated methods of volatiles are presented in supplementary data (section 2.8). The individual volatile content was calculated as follows:

Concentration (ng/g) = (extracted ion peak area / extracted ion peak area of internal standard) × (10 ng/g)

### 2.9. TPC, TFC, and MRP analyses

TPC and TFC were determined by the earlier reported procedure by Lee et al. (2021) with some modifications. MRP was confirmed non-enzymatic browning method (Lertsiri et al., 2001). Further explanation of this section is displayed in supplementary data (section 2.9).

### 2.10. Measurement of antioxidant properties

The antioxidant properties on radical scavenging abilities including DPPH, ABTS, and hydroxyl as well as FRAP in the 50% ethanol extracts of GS samples were demonstrated by the previous techniques (Dravie et al., 2020; Herraiz & Galisteo, 2015; Lee & Cho, 2021). The detailed experimental methods were expressed in supplementary data (section 2.10).

### 2.11. Statistical analysis

All measurements of compositional constituents (ginsenoside, amino acid, fatty acid, and volatile compound) and antioxidant characteristics (radical, FRAP, TPC, TFC, and MRP) were expressed as the mean ± SD (standard derivation) values of three independent experiments with triplicate samples ($n = 3$). The significant differences among samples were determined by Tukey's multiple test ($p < 0.05$) through ANOVA procedure using the Statistical Analysis System (SAS) software (ver. 9.4; SAS institute, Cary, NC, USA).

## 3. Results and discussion

### 3.1. Physicochemical properties in processed ginseng sprouts by aging and fermentation methods

It is well-known that the pH value was decreased and the acidity value was increased in the processing skills (Lee et al., 2018; Renchinkhand et al., 2015). Furthermore, the reducing sugar (glucose, ribose, etc.) content has been recognized as an excellent factor owing to the formation of amino acids, volatile compounds, and sensory traits for human health effects (Fu et al., 2021). However, little information is available on the physicochemical properties of the GS quality through food processing types for the development of human functional foods. The pH and acidity values showed mild variations and the reducing sugar was detected remarkable differences (Table S1). In more details, the pH data were slightly decreased in the range of 5.9 (DGS) → 5.1 (AGS) → 4.9 (FAGS) according to the processing steps, whereas the acidity values were found to be increase with 2.8 (DGS) → 3.8 (AGS) → 4.5 (FAGS). Moreover, change in the reducing sugar exhibited remarkable differences as 3.5 (DGS) → 21.1 (AGS) → 30.9 (FAGS). The current results showed similarity by comparison with the earlier literatures that reported ginseng or red ginseng was observed with a decrease of pH and the increase of acidity after fermentation (Jung et al., 2019; Renchinkhand et al., 2015). These phenomenon may be responsible for the environmental conditions of the action glycoside hydrolases in processing techniques (Hwang et al., 2021; Jung et al., 2019; Lee et al., 2021; Liu et al., 2021), and our data provides important information on the physicochemical properties of processed ginseng sprouts.

### 3.2. Comparisons of amino acid and fatty acid compositions in processed ginseng sprouts by aging and fermentation methods

Total amino acid (AA) contents including 23 non-essential amino acids (NEAAs) and 8 essential amino acids (EAAs) were analyzed according to the aging and fermentation processes of ginseng sprouts. As shown in Table 1, total AAs considerably decreased with 4537.7 → 2129.9 → 2450.6 mg/100 g, especially, the AGS source was observed the greatest decrease (approximately more 2 times) compared with DGS. In DGS sample, the NEAA contents exhibited 8 times great contents with 4015.9 mg/100 g than essential compositions (521.8 mg/100 g). The most predominant component showed arginine (55% of total AAs), with 2490.0 mg/100 g, followed by glutamic acid (271.2 mg/100 g), aspartic acid (252.1 mg/100 g), GABA (242.9 mg/100 g), aspartic acid-$NH_2$ (186.6 mg/100 g), and the remaining amino acids displayed low contents (each composition <150 mg/100 g). After aging process, total amino acids were remarkably decreased approximately 2.1 times with 4537.7 → 2129.9 mg/100 g (NEAAs: 4015.9 → 1967.4 mg/100 g, 2 times; EAAs: 521.8 → 162.4 mg/100 g, 3.2 times) compared to DGS. Specifically, arginine exhibited significant difference as 2490.0 → 1034.9 mg/100 g with decreasing rate of 2.4 times. Other AAs in NEAAs exhibited the following order with mildly variations: aspartic acid (252.1 → 150.8 mg/100 g) > GABA (242.9 → 107.9 mg/100 g) > alanine (112.5 → 26.4 mg/100 g) > proline (82.4 → 17.6 mg/100 g) > tyrosine (78.5 → 37.2 mg/100 g). The EAA contents of AGS also slightly decreased as follows: leucine (101.3 → 26.0 mg/100 g) > lysine (87.4 → 18.8 mg/100 g) > phenylalanine (72.8 → 23.9 mg/100 g) > threonine (72.0 → 19.5 mg/100 g). Although many AAs were detected lower contents after the aging process, four AAs, namely glutamic acid, GABA, cystathionine, and ornithine exhibited increase rates with 271.2 →

**Table 1**
Variation of amino acid contents through aging and fermentation processes from ginseng sprouts.

| Amino acid | Content (mg/100 g) [a] through processing steps[b] | | |
|---|---|---|---|
| | DGS | AGS | FAGS |
| Non-essential amino acids (NEAAs) | | | |
| Proline | 82.4 ± 4.1[a] | 17.6 ± 0.9[b] | 13.4 ± 0.7[c] |
| Hydroxyproline | 1.2 ± 0.1[a] | ND[c] | 1.0 ± 0.1[a] |
| Aspartic acid | 252.1 ± 12.6[a] | 150.8 ± 7.5[b] | 252.7 ± 12.6[a] |
| Serine | 83.6 ± 4.2[a] | 21.7 ± 1.1[b] | 21.5 ± 1.1[b] |
| Aspartic acid - $NH_2$ | 186.6 ± 9.3[a] | 13.6 ± 0.7[c] | 21.8 ± 1.1[b] |
| Glutamic acid | 271.2 ± 13.6[b] | 334.2 ± 16.7[a] | 354.2 ± 17.7[a] |
| Sarcosine | 21.6 ± 1.1[a] | 8.7 ± 0.4[b] | 2.1 ± 0.1[c] |
| Aminoadipic acid | 5.7 ± 0.3[a] | 4.4 ± 0.2[b] | 5.6 ± 0.3[a] |
| Glycine | 12.8 ± 0.6[a] | 8.6 ± 0.4[b] | 12.8 ± 0.6[a] |
| Alanine | 112.5 ± 5.6[a] | 26.4 ± 1.3[b] | 30.7 ± 1.5[b] |
| Citrulline | 1.9 ± 0.1[b] | ND | 7.1 ± 0.4[a] |
| α-Aminobutyric acid | 12.5 ± 0.6[b] | 52.0 ± 2.6[a] | 12.0 ± 0.6[b] |
| Cystine | 44.5 ± 2.2[a] | 11.2 ± 0.6[b] | 9.6 ± 0.5[bc] |
| Cystathionine | ND | 39.9 ± 2.0[a] | 34.0 ± 1.7[a] |
| Tyrosine | 78.5 ± 3.9[a] | 37.2 ± 1.9[b] | 22.9 ± 1.1[c] |
| β-Alanine | 27.6 ± 1.4[a] | 26.3 ± 1.3[a] | 24.6 ± 1.2[a] |
| β-Aminoisobutyric acid | 21.6 ± 1.1[a] | 28.5 ± 1.4[a] | 23.2 ± 1.2[a] |
| γ-Aminobutyric acid (GABA) | 242.9 ± 12.2[a] | 107.9 ± 5.4[b] | 59.3 ± 3.0[c] |
| Aminoethanol | 37.3 ± 1.9[a] | 9.9 ± 0.5[b] | 10.5 ± 0.5[b] |
| Ornithine | 24.5 ± 1.2[b] | 32.0 ± 1.6[a] | 35.4 ± 1.8[a] |
| 3-Methylhistidine | 1.1 ± 0.16[b] | 1.8 ± 0.1[a] | 0.6 ± 0.0[c] |
| Anserine | 4.0 ± 0.2[b] | ND | 20.3 ± 1.0[a] |
| Arginine | 2490.0 ± 124.5[a] | 1034.9 ± 51.8[bc] | 1351.8 ± 67.6[b] |
| Total | 4015.9 | 1967.4 | 2327.1 |
| Essential amino acids (EAAs) | | | |
| Threonine | 72.0 ± 3.6[a] | 19.5 ± 1.0[b] | 15.4 ± 0.8[bc] |
| Valine | 64.3 ± 3.2[a] | 39.5 ± 2.0[b] | 15.0 ± 0.8[c] |
| Methionine | 13.3 ± 0.7[a] | ND | ND |
| Isoleucine | 54.2 ± 2.7[a] | 25.5 ± 1.3[b] | 13.3 ± 0.7[c] |
| Leucine | 101.3 ± 50.5[a] | 26.0 ± 1.3[b] | 13.3 ± 0.7[c] |
| Phenylalanine | 72.8 ± 3.6[a] | 23.9 ± 1.2[b] | 25.2 ± 1.3[b] |
| Lysine | 87.4 ± 4.4[a] | 18.8 ± 0.9[c] | 27.8 ± 1.4[b] |
| Histidine | 56.5 ± 2.8[a] | 9.3 ± 0.5[c] | 13.6 ± 0.7[b] |
| Total | 521.8 | 162.4 | 123.6 |
| Total amino acids (NEAAs + EAAs) | 4537.7 | 2129.9 | 2450.6 |

[a] All values are presented as the mean ± SD of triplicate determination. Different letters correspond to the significant differences relating to the processing steps using Tukey's multiple test ($p < 0.05$).

[b] Processing steps: DGS, dried ginseng sprouts; AGS, aged ginseng sprouts; and FAGS, fermented and aged ginseng sprouts.

[c] ND: not detected.

334.2, 12.5 → 52.0, ND→39.3, and 24.5 → 32.0 mg/100 g by comparing other amino acids of DGS (Table 1). During fermentation of AGS, the total AA contents were slightly increased (2129.9 → 2450.6 mg/100 g) with 1967.4 → 2327.1 (NEAAs) and 162.4 → 123.6 (EAAs) mg/100 g, specifically, aspartic acid (150.8 → 252.7 mg/100 g) and arginine (1034.9 → 1351.8 mg/100 g) showed high increase rates when compared to the other compositions. Also, this processing step exhibited mildly variations in amino acids compared to aging process. Among various compositions, the arginine production can be correlated to the ornithine amount as previously reported data (Diana et al., 2014), however, their inter-relation may be not affected by the aging and fermentation processes. The most abundant arginine content may be an important component in determining the functional and quality factor in processed products of ginseng sprouts due to multiple biological functions (Zou et al., 2019). Based on the above considerations, the variations of individual and total amino acids in ginseng sprouts may be associated with the environmental parameters concern to microorganism, temperature, time, and metabolite biotransformation in the process steps, as compared to the previous results of other crops (Liu et al., 2020; Subrota et al., 2013; Wang et al., 2022). Overall, our findings exhibited that the AA contents of processed ginseng sprouts were detected in remarkably lower amounts (AGS: 2129.9 and FAGS: 2450.6 mg/100 g) than raw sample (DGS: 4537.7 mg/100 g). The decrease patterns of AAs in the current work were similar to the earlier data regarding the total AA contents in processing methods (steam, aging, and fermentation) (Eom et al., 2018), and the AAs in diverse ginseng products (fresh > frozen > white > stoved > red > black ginseng) (Wang et al., 2015)

For the quantitative analysis regarding changes in fatty acids, we analyzed fatty acid compositions (6 saturated and 9 unsaturated fatty acids) using GC. As revealed in Table S2 (supplementary material), palmitic acid (C16:0) and linoleic acid (C18:2c) exhibited high contents with 121 and 280 mg/100 g in DGS sample. The stearic acid (C18:0) (23 mg/100 g) and α-linolenic acid (C18:3n3) (62 mg/100 g) contents were also detected high rates by comparison with other compositions (<20 mg/100 g). During the aging and fermentation processes, the total fatty acids exhibited mildly differences with 562 → 695 → 660 mg/100 g, and the main components, palmitic acid and linoleic acid showed as follows: 280 → 345 → 309; 121 → 146 → 144 mg/100 g. Also, the stearic acid and α-linolenic acid contents were detected weak variations with 23 → 28 → 29 and 62 → 99 → 85 mg/100 g (Table S2). In other words, the fatty acid contents were not detected remarkable variations in aging and fermentation processes, as comparing the previous data concern to thermal processing and storage skills as well as fermentation method (Cho et al., 2017; Rather et al., 2021). Although the increase rates of the linoleic and linolenic acids were reduced phytochemicals (Dewir et al., 2010; Wu et al., 2009), their patterns may be not related to the accumulation or reduction of phytochemicals (ginsenosides) in the current study. Overall, the individual and total fatty acids displayed mildly differences in these above processing methods, and the total SFAs and USFAs also exhibited slightly variations with 179.5 → 214.6 → 221.4 and 382.5 → 481.0 → 439.3 mg/100 g. The distribution patterns of fatty acids through aging and fermentation processes of ginseng sprouts were consistent with the previous data that the fatty acid contents in ginseng seeds exhibited slight changes by the fermentation technique (Lee et al., 2017). Therefore, the fatty acid contents may be not a key factor and an important ingredient in demonstrating the quality of processed ginseng products.

### 3.3. Ginsenosides in processed ginseng sprouts by aging and fermentation methods

So far, to the best of our knowledge, there have been no systematic literatures to phytochemical conversion in ginseng sprouts from the processing skills such as aging and fermentation. Compared to ginsenosides, there is also limited information concerning their contents and patterns as a function of processing methods. For these above backgrounds, we evaluated 21 ginsenosides (11 PPD, 9 PPT, and 1 oleanane chemical structures) in ginseng sprouts of processed methods using HPLC analysis. The ginsenoside derivatives in processed ginseng sprouts were identified by comparing the retention times of ginsenoside standards (Fig. 1A and supplementary data section 3.3).

Ginsenosides were carried out from the peak areas by HPLC technique and their contents exhibited Table 2. In DGS sample, the most abundant ginsenoside was Re (6.47 mg/g), representing approximately 17.3% of the total content (37.39 mg/g), and ginsenoside Rd was the second main component (5.08 mg/g) with about 13.6%, followed by Rb1 (4.04 mg/g, 10.8%), Rc (3.65 mg/g, 9.8%), Rb2 (3.15 mg/g, 8.4%), and F2 (2.15 mg/g, 5.8%) (Table 2). The remaining compositions were observed low content (<2.00 mg/g). The individual ginsenoside content exhibited remarkable differences with ranges of 0.20–6.47 mg/g in DGS source, and their distributions showed as the following order: PPD (21.87 mg/g) > PPT (13.06 mg/g) > oleanane (2.46 mg/g). Our data were similar to the earlier studies that ginsenoside Rc, Rb2, Rd, Rb1, and Re were detected higher contents than other compositions in ginseng

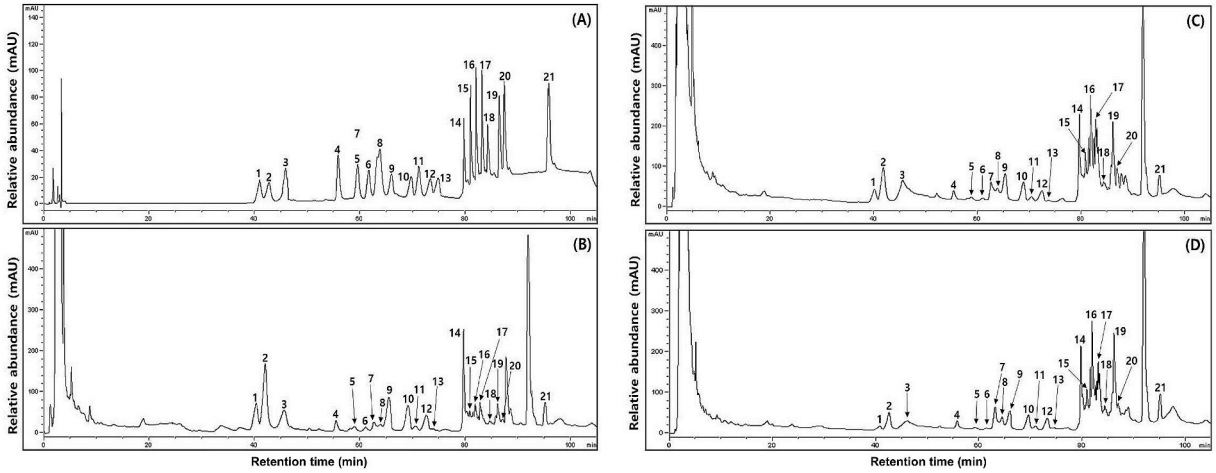


**Fig. 1.** Comparison of HPLC chromatograms concern to 21 ginsenoside derivatives in the 50% ethanol extracts of processed ginseng sprouts by aging and fermentation: (A) ginsenoside standards; (B) DGS; (C) AGS; (D) FAGS, 1: ginsenoside Rg1, 2: ginsenoside Re, 3: ginsenoside Ro, 4: ginsenoside Rf, 5: ginsenoside F5, 6: ginsenoside F3, 7: ginsenoside Rg2, 8: ginsenoside Rh1, 9: ginsenoside Rb1, 10: ginsenoside Rc, 11: ginsenoside F1, 12: ginsenoside Rb2, 13: ginsenoside Rb3, 14: ginsenoside Rd, 15: ginsenoside Rd2, 16: ginsenoside F2, 17: ginsenoside Rg3, 18: protopanaxtriol (PPT), 19: compound K (CK), 20: ginsenoside Rh2, 21: protopanaxdiol (PPD).

**Table 2**
Variation of ginsenoside contents through aging and fermentation processes from ginseng sprouts.

| Ginsenoside | Content (mg/g)[a] through processing steps[b] | | |
|---|---|---|---|
| | DGS | AGS | FAGS |
| Protopanaxdiol (PPD) types | | | |
| Ginsenoside Rb1 (**9**) | 4.04 ± 0.25[a] | 2.77 ± 0.19[b] | 2.12 ± 0.21[c] |
| Ginsenoside Rc (**10**) | 3.65 ± 0.18[a] | 2.60 ± 0.13[b] | 2.07 ± 0.11[c] |
| Ginsenoside Rb2 (**12**) | 3.15 ± 0.16[a] | 2.05 ± 0.10[b] | 1.71 ± 0.12[c] |
| Ginsenoside Rb3 (**13**) | 0.38 ± 0.02[a] | 0.21 ± 0.01[b] | 0.23 ± 0.01[b] |
| Ginsenoside Rd (**14**) | 5.08 ± 0.03[a] | 3.81 ± 0.19[c] | 4.42 ± 0.22[b] |
| Ginsenoside Rd2 (**15**) | 1.48 ± 0.07[c] | 2.79 ± 0.19[a] | 2.16 ± 0.21[b] |
| Ginsenoside F2 (**16**) | 2.15 ± 0.11[c] | 3.56 ± 0.18[b] | 4.59 ± 0.43[a] |
| Ginsenoside Rg3 (**17**) | 0.31 ± 0.02[c] | 1.17 ± 0.06[b] | 1.64 ± 0.08[a] |
| Compound K (CK) (**19**) | 0.75 ± 0.04[c] | 2.98 ± 0.15[b] | 4.07 ± 0.23[a] |
| Ginsenoside Rh2 (**20**) | 0.20 ± 0.01[c] | 1.04 ± 0.05[b] | 1.36 ± 0.07[a] |
| Protopanaxdiol (**21**) | 0.68 ± 0.03[b] | 0.75 ± 0.04[b] | 1.16 ± 0.06[a] |
| Total | 21.87 | 23.73 | 25.53 |
| Protopanaxtriol (PPT) types | | | |
| Ginsenoside Rg1 (**1**) | 1.85 ± 0.07[a] | 0.54 ± 0.03[b] | 0.39 ± 0.02[c] |
| Ginsenoside Re (**2**) | 6.47 ± 0.42[a] | 2.45 ± 0.17[b] | 1.53 ± 0.13[c] |
| Ginsenoside Rf (**4**) | 0.51 ± 0.03[a] | 0.50 ± 0.03[a] | 0.54 ± 0.03[a] |
| Ginsenoside F5 (**5**) | 0.32 ± 0.02[a] | 0.25 ± 0.01[b] | 0.15 ± 0.01[c] |
| Ginsenoside F3 (**6**) | 1.51 ± 0.05[a] | 0.63 ± 0.03[b] | 0.35 ± 0.02[c] |
| Ginsenoside Rg2 (**7**) | 0.82 ± 0.04[c] | 1.83 ± 0.09[b] | 2.07 ± 0.14[a] |
| Ginsenoside Rh1 (**8**) | 0.54 ± 0.03[c] | 0.78 ± 0.04[b] | 1.16 ± 0.06[a] |
| Ginsenoside F1 (**11**) | 0.48 ± 0.02[a] | 0.44 ± 0.02[a] | 0.36 ± 0.02[b] |
| Protopanaxtriol (**18**) | 0.56 ± 0.03[b] | 1.05 ± 0.09[ab] | 1.26 ± 0.13[a] |
| Total | 13.06 | 8.47 | 7.81 |
| Oleanane types | | | |
| Ginsenoside Ro (**3**) | 2.46 ± 0.12[a] | 1.63 ± 0.10[b] | 1.18 ± 0.11[c] |
| Total ginsenosides | 37.39 | 33.83 | 34.52 |

[a] All values are presented as the mean ± SD of triplicate determination. Different letters correspond to the significant differences relating to the processing steps using Tukey's multiple test ($p < 0.05$).

[b] Processing steps: DGS, Dried ginseng sprouts; AGS, aged ginseng sprouts; and FAGS, fermented and aged ginseng sprouts.

(Lee et al., 2021; Liu et al., 2020; Xu et al., 2018). After the aging process, the individual composition exhibited high changes, but the total ginsenoside contents showed mildly differences (37.39 → 33.83 mg/g). In other words, the AGS sample exhibited low contents in ginsenoside patterns by comparing those of DGS (Fig. 1B and C). The most abundant ingredient was ginsenoside Rd and its content exhibited 3.81 mg/g. And ginsenoside F2 was found to be the second major component with 3.56 mg/g, and the order of other ginsenosides were as follows: CK (2.98 mg/g) > Rd2 (2.79 mg/g) > Rb1 (2.77 mg/g) > Rc (2.60 mg/g) > Re (2.45 mg/g) > Rb2 (2.05 mg/g) (Table 2). Especially, ginsenoside Re was observed the highest variations with 6.47 → 2.45 mg/g and the remaining derivatives showed low contents (less than 2.00 mg/g) in the ranges of 0.21–1.83 mg/g. Five ginsenosides (Rb1, Rc, Rb2, Rd, and Re) also exhibited considerable decrease rates with high variations of 4.04 → 2.77, 3.65 → 2.60, 5.08 → 3.81, 3.15 → 2.05, and 6.47 → 2.45 mg/g after aging process (Fig. 1C and Table 2). Overall, the total PPD type displayed higher contents with 21.87 → 23.73 mg/g than DGS sample in the aging process, while the PPT type decreased with 13.06 → 8.47 mg/g. The ginsenoside Ro (oleanane type) was also observed low content (1.63 mg/g) when compared to the DGS sample (2.46 mg/g). This phenomenon confirms that the ginsenoside derivatives may be influenced by the environmental conditions including food processing skill as comparing the earlier data (Hsu et al., 2013; Jung et al., 2019; Xu et al., 2018). In other words, our data suggest that the increase rates of deglycosylated ginsenosides through the structure conversion of ginsenosides may be dependent to much degree on the sharp increase of β-glucosidase in ginseng sprouts during process step as supported by the earlier studies (Hsu et al., 2013; Kamizake et al., 2016; Kim et al., 2007; Lee et al., 2021). Moreover, the variations of ginsenoside contents through aging technique are consistent with the result of the previous literature (Saidi et al., 2020). Particularly, the secondary metabolites of aglycone structure have been considered important factor in the potential health properties owing to the rapid absorption and high bioavailability in the human body, compared with their glucoside types (Izumi et al., 2000; Lee et al., 2018). Thus, our understanding data regarding variations of ginsenosides in aging process using ginseng sprouts may be considered as important information for the development of human health functional foods and nutraceuticals.

The distribution profiles and concentrations of ginsenosides after fermentation displayed considerable differences in individual composition when compared to the aging sample (Fig. 1C and D). However, total ginsenoside contents of fermented sprouts exhibited slight differences with 33.83 → 34.52 mg/g (Table 2). Total PPD ginsenosides mildly increased with 23.73 → 25.53 mg/g, specifically, ginsenoside F2 and CK were observed the predominant contents with high variations of 3.56 → 4.59 and 2.98 → 4.07 mg/g. The individual PPD ginsenoside was also observed at slight higher content (1.16 mg/g) than aging process (0.75 mg/g) due to the bioconversion through fermentation (Table 2) (Cheng

et al., 2008; Chi & Ji, 2005; Lee et al., 2021). Therefore, the deglycosylated ginsenoside contents may be influenced by fermentation skill in diverse environment factors. These results were similar to the previously reported studies that soybean isoflavone glucosides were converted into aglycones after fermentation (Hwang et al., 2021; Lee et al., 2018). To summarize, the decrease rates of ginsenoside Rc (glucose moiety: 3, fructose moiety: 1), Rb1 (glucose moiety: 4), and Rb2 (glucose moiety: 4) may be transformed into the increase patterns of degradated ginsenosides Rd (glucose moiety: 3), Rg3 (glucose moiety: 2), F2 (glucose moiety: 2), and CK (glucose moiety: 1) because of various process conditions such as microorganism, time, and temperature concern to fermentation (Fig. 2).

In general, the fluctuation patterns of ginsenoside PPD derivatives in ginseng are associated with fermentation process with the rank order as follows: ginsenoside Rb1 (glucose moiety: 4) → Rd (glucose moiety: 3) → F2 (glucose moiety: 2) → Rh2 (glucose moiety: 1), CK (glucose moiety: 1) → PPD (glucose moiety: 0) or ginsenoside Rb1 (glucose moiety: 4) → Rg3 (glucose moiety: 2) → Rh2 (glucose moiety: 1) → PPD (glucose moiety: 0) (Fig. 2A) (Cheng et al., 2008; Chi & Ji, 2005; Renchinkhand et al., 2015). Moreover, the increase rates of ginsenoside Rh2 and CK can be transferred to the intermediate ginsenosides Rd, Rg3, and F2. Therefore, we assumed that the intermediate ginsenosides of Rd, Rg3, and F2 were converted to the initial ginsenosides (Rc, Rb1, and Rb2) in fermentation process. Our data may be primarily influenced that the variations of ginsenoside glucoside → deglycosylated ginsenoside type are attributed to the biotransformation under fermentation process as

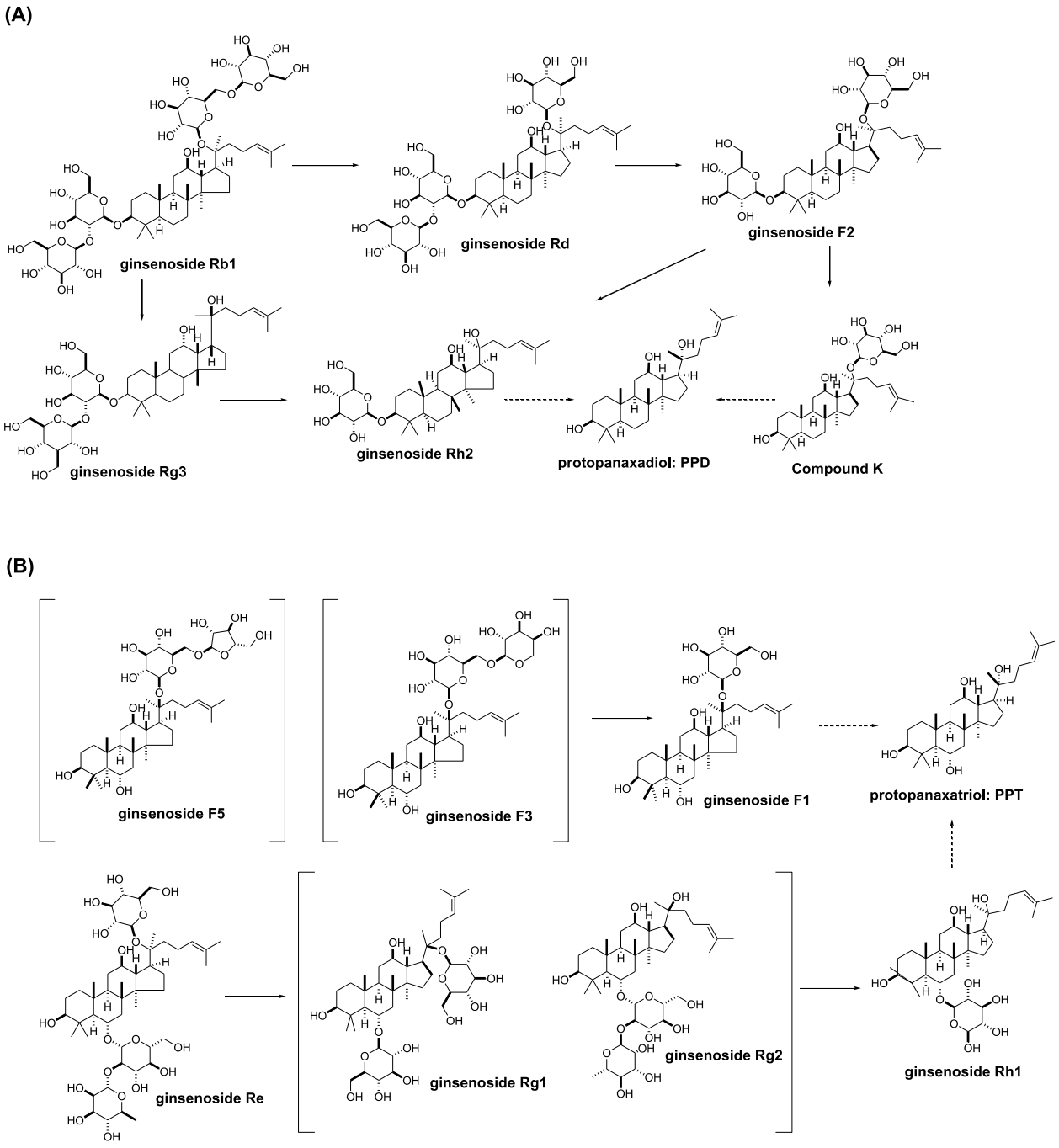


**Fig. 2.** Biotransformation processes of ginsenosides during aging and fermentation of ginseng sprouts: (A) Bioconversion of protopanaxdiol (PPD) type; (B) Bioconversion of protopanaxtriol (PPT) type.

the obtained results by earlier studies (Dong et al., 2017; Liu et al., 2020; Xu et al., 2018). In the evaluation of PPT type (9 ginsenosides), the FAGS source possessed lower total ginsenoside contents (7.81 mg/g) than those of AGS sample (8.47 mg/g) (Fig. 1D and Table 2) and their distributions are present as follow, in decreasing order: Rg2 > Re > PPT > Rh1, with 2.07, 1.53, 1.26, and 1.16 mg/g, respectively, and the remaining ginsenosides exhibited low contents (<0.60 mg/g). Specifically, ginsenoside Re was detected remarkable differences with high variations (2.45 → 1.53 mg/g). The initial ginsenosides F5 (glucose moiety: 1 and fructose moiety: 1), F3 (glucose moiety: 2), and Re (glucose moiety: 3) may be transformed into deglycosidated ginsenosides F1, Rg1, and Rg2 during fermentation (Fig. 2B). We confirmed that the above three ginsenosides (F5, F3, and Re) may be converted into deglycosidated ginsenosides such as Rh1 (glucose moiety: 1) and PPT (glucose moiety: 0) according to the fermentation conditions (Fig. 2B). These phenomenon were coincident with the previous literatures that the increase rates of isoflavone aglycone were observed in fermented soybeans (Hwang et al., 2021; Lee et al., 2018). Although the PPT ginsenosides showed the decrease rates of sugar moieties after fermentation, the total PPT contents were not observed significant differences with 8.47 → 7.81 mg/g. Overall, ginsenosides of high molecular weight changed into low molecular owing to the decrease of their sugar chains under environmental factor of fermentation (Cheng et al., 2008; Chi & Ji, 2005; Jung et al., 2019). The fermentation technique using microbial transformation (*L. plantarum* P1201) has been the most employed for development of functional products due to the enhancement of the deglycosylated ginsenosides. It is well currently documented that the major ginsenoside Rb1 (4.04 → 2.12 mg/g), Rd (5.08 → 4.42 mg/g), and Re (6.47 → 1.53 mg/g) were transformed into Rg3 (0.31 → 1.64 mg/g), CK (0.75 → 4.07 mg/g), Rh2 (0.20 → 1.36 mg/g), Rg2 (0.82 → 2.07 mg/g), and Rh1 (0.54 → 1.16 mg/g) of high pharmaceutical effects after aging and fermentation processes (Fig. 2 and Table 2). Even though the AGS and FAGS samples had low total ginsenosides when compared to the DGS source, these materials may be recommended as human health agents for manufacturing of functional products in food industry related to the reduction of environmental hazards and costs, as well as valuable biological components and non-toxicity. Furthermore, these two processes may be considered an excellent food skills owing to the increase ratios of the deglycosylated ginsenosides including CK (4 times), PPD (2 times), and PPT (2 times) regarding high absorption and bioavailability in the human body (Izumi et al., 2000; Lee et al., 2018). Consequently, processed ginseng sprouts may be used an alternative sources for natural agents against various chronic diseases than those found in fresh ginseng sprouts (Chi & Ji, 2005; Hsu et al., 2013; Jung et al., 2019; Kim et al., 2010). The results of the present study may be contributed to enhance the values of ginseng sprouts regarding development of new processing foods.

### 3.4. Variations of volatile compounds in processed ginseng sprouts by aging and fermentation methods

Previous researchers have shown that processed techniques in food sources contributes to significant changes in the volatile profiles and their concentrations (Kelanne et al., 2022; Lee et al., 2014; Szambelan et al., 2020), however, little is known about the volatile compounds in ginseng sprouts under aging and fermentation methods. We investigated the fluctuations of the 21 volatile components in AGS and FAGS using processing skills compared to those of GS sample (Table 3). In DGS sample, the most abundant volatile component was observed β-farnesene, with 1940 ng/g more than approximately 55% of the total content (3538 ng/g). The second main composition exhibited β-elemene with 309 ng/g (8.7%) and the third volatile was detected with 295 ng/g in (−)-α-pinene (8.3%). The remaining volatiles showed low contents (<200 ng/g) with the ranges of ND–176 ng/g (Table 3). After the aging process, the total volatile contents significantly decreased approximately 3.5 times (3538 → 1015 ng/g) compared to DGS. In particular,

**Table 3**
Variation of volatile composition contents through aging and fermentation processes from ginseng sprouts.

| Volatile | Content (ng/g d.w.)[a] through processing steps[b] | | |
|---|---|---|---|
| | DGS | AGS | FAGS |
| (−)-α-Pinene | 295 ± 15[a] | 32 ± 3[b] | ND[c] |
| (+)-Camphene | 54 ± 2[a] | 3 ± 0[b] | ND |
| Hexanal | 44 ± 3[a] | 23 ± 1[b] | 23 ± 2[b] |
| (−)-β-Pinene | 176 ± 6[a] | 12 ± 1[b] | 2 ± 0[c] |
| β-Myrcene | 129 ± 14[a] | 15 ± 1[b] | 3 ± 0[c] |
| Heptanal | 8 ± 1[c] | 10 ± 0[b] | 18 ± 2[a] |
| Octanal | 14 ± 0[b] | 13 ± 2[b] | 18 ± 1[a] |
| (E)-2-Heptenal | 7 ± 1[c] | 49 ± 1[a] | 32 ± 1[b] |
| Sulcatone | 5 ± 0[a] | 7 ± 0[a] | 6 ± 0[a] |
| 2,3-Dimethylpyrazine | 1 ± 0[b] | 1 ± 0[b] | 4 ± 0[a] |
| Nonanal | 3 ± 0[b] | 10 ± 1[a] | 2 ± 0[b] |
| Trimethylpyrazine | 5 ± 0[b] | 4 ± 0[b] | 12 ± 1[a] |
| 2,3,5,6-Tetramethylpyrazine | 20 ± 1[a] | ND | 1 ± 0[b] |
| (+)-Longifolene | 57 ± 3[a] | 38 ± 1[b] | ND |
| Benzaldehyde | ND | 9 ± 1[a] | 8 ± 0[a] |
| β-Elemene | 309 ± 9[a] | 163 ± 3[b] | 43 ± 3 |
| Caryophyllene | 65 ± 4[a] | 14 ± 1[b] | 5 ± 0[c] |
| Aromadendrene | 7 ± 0[a] | 7 ± 0[a] | ND |
| β-Farnesene | 1940 ± 96[a] | 202 ± 2[b] | 19 ± 1[c] |
| β-(E)-Caryophyllene | 163 ± 7[b] | 225 ± 9[a] | 80 ± 4[c] |
| β-Selinene | 46 ± 2[a] | 8 ± 0[b] | 2 ± 0[c] |
| α-Selinene | 67 ± 1[a] | 19 ± 1[b] | 4 ± 0[c] |
| σ-Cadinene | 30 ± 2[a] | 5 ± 0[b] | 1 ± 0[c] |
| (Z,E)-α-Farnesene | 20 ± 2[a] | 1 ± 0[b] | ND |
| Calamenene | 19 ± 1[a] | 2 ± 0[b] | 1 ± 0[b] |
| Hexanoic acid | 23 ± 2[a] | 7 ± 0[b] | 9 ± 1[b] |
| β-Ionone | 7 ± 0[a] | 2 ± 0[b] | 1 ± 0[b] |
| 2-Acetylpyrrole | 3 ± 0[c] | 133 ± 11[a] | 31 ± 2[b] |
| (−)-Spathulenol | 21 ± 1[a] | 1 ± 0[b] | ND |

[a] All values are presented as the mean ± SD of triplicate determination. Different letters correspond to the significant differences relating to the processing steps using Tukey's multiple test ($p < 0.05$).
[b] Processing steps: DGS, dried ginseng sprouts; AGS, aged ginseng sprouts; and FAGS, fermented and aged ginseng sprouts.
[c] ND: not detected.

β-farnesene displayed remarkable difference with decrease rate (Table 3). Although the volatile contents decreased considerably, the β-(E)-caryophyllene and 2-acetylpyrrole mildly increased with 163 → 225 and 3 → 133 ng/g, respectively. During fermentation, the total volatile components also exhibited lower contents (3538; DGS → 1015; AGS → 325; FAGS ng/g) than those of the DGS and AGS samples, and the individual compound was detected with ND or minor amounts (<100 ng/g) (Table 3). These finding results suggest that the decrease rates of volatiles may be connected with the amino acid degradation and glucose conversion during processing periods (Chen et al., 2019). Our observations were coincident with the previous researches that the volatile compounds decreased during food processing skills (drying, fermentation, aging, and roasting methods) of currant, rice, soybean, and wine (Ayestarán et al., 2019; Choi et al., 2019; Kelanne et al., 2022). Overall, the volatile compounds of ginseng sprouts decreased considerably with processing techniques and their contents may be not excellent factor in quality of processed ginseng sprouts. This work was the first to demonstrate interrelation degrees between processing skills and volatile compositions from ginseng sprouts.

### 3.5. Comparisons of TPC, TFC, and MRP in processed ginseng sprouts by aging and fermentation methods

Although many studies have examined the possibility of increasing ingredients by processed ginseng, there are no previous reports concern to TPC and TFC in the processing methods of ginseng sprouts. Thus, we evaluate the changes in TPC and TFC throughout the process steps of aging and fermentation techniques from ginseng sprouts, and their values are summarized in Fig. 3A and B. Significant differences in TPC

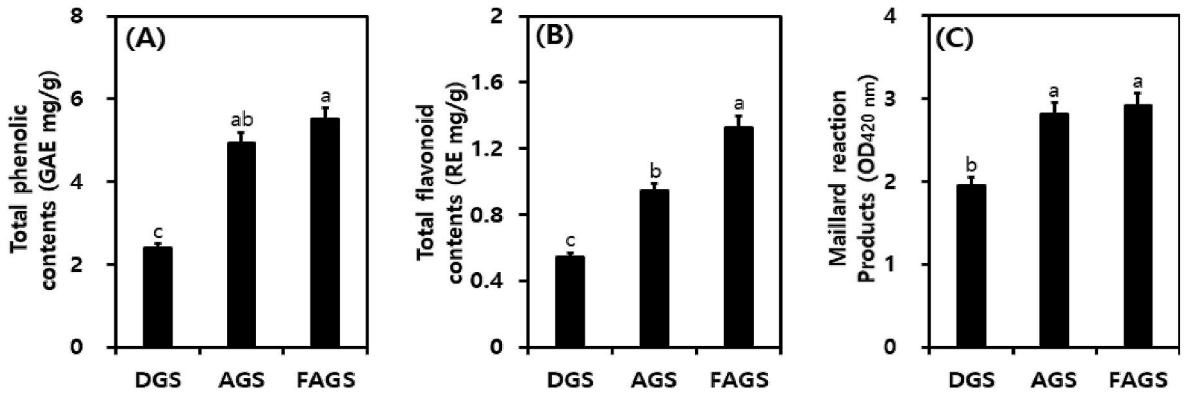


**Fig. 3.** Changes in TPC, TFC, and MRP in the 50% ethanol extracts through aging and fermentation processes of ginseng sprouts: (A) TPC; (B) TFC; (C) MRP, Different letters correspond to the significant differences relating to the processing steps using Tukey's multiple test ($p < 0.05$).

and TFC were observed among the three GS samples. In TPC analysis, the DGS sample was 2.35 GAE mg/g, and other sources (AGS and FAGS) showed high contents as follows: 5.52 (FAGS) > 4.94 GAE mg/g (AGS) (Fig. 3A). These results confirm that the phenolic metabolite (cinnamic acid, coumaric acid, caffeic acid, hydroxyl benzoic acid, etc.) contents and their variations in ginseng sprouts may be correlated with the sterilization, fermentation, and steaming methods of food processing steps (Eom et al., 2018; Jung et al., 2019; Kim et al., 2007; Lee et al., 2015). The TFC value also increased slightly with 0.54 (DGS) → 0.94 (AGS) → 1.33 RE/mg (FAGS), as presented in Fig. 2B, and our data are similar to the earlier work reporting that TFC of fermented black ginseng showed higher rates by comparing the raw ginseng (Jung et al., 2017). The findings of the current research indicate that the TPC and TFC distributions may be connected with the releasing of the hydroxyl moieties concern to phenolic structures and macromolecular decomposition in processed extracts of ginseng sprouts (Nooshkam et al., 2019; Zhang et al., 2020). After the aging and fermentation processes, the MRP rates also increased with remarkable variations as the results obtained in TPC and TFC, and their values increased as the following order:2.91 (FAGS) > 2.81 (AGS) > 1.95 (DGS) $OD_{420}$ nm (Fig. 3C). As support to these above results, the AGS and FAGS sources may be recommended as potential antioxidant agents in health benefits owing to the increase rates of MRPs as well as TPC and TFC (Fig. 3). The present results were consistent with previously data concern to changes in Maillard reaction products through the increase of food technology and heating temperature (Nooshkam et al., 2019; Xu et al., 2018; Zhang et al., 2020). We believe that the aging and fermentation skills may be provided many choices in the utilization and development of nutraceutical agents for the healthy life of human.

### 3.6. *Fluctuations of antioxidant properties against radicals in processed ginseng sprouts by aging and fermentation methods*

*In vitro* techniques, the radical scavenging assays (DPPH•, ABTS•+, •OH) and FRAP method were used to evaluate the potential antioxidant effects because of their stability, simplicity, and reproducibility (Hwang et al., 2021; Lee et al., 2021; Reddy et al., 2010). Additionally, it is commonly established that their effects are used to determine the antioxidant status of crops, fruits, vegetables, and other natural sources (Farhadi et al., 2020; Hazrati et al., 2019; Kim et al., 2007). The antioxidant abilities of DGS, AGS, and FAGS samples have confirmed by comparing the percentage scavenging of three radicals (DPPH, ABTS, and hydroxyl) and FRAP values with positive controls (BHT, Trolox, and ascorbic acid). In the preliminary experiments, we revealed that the DPPH radical scavenging activities of GS samples and BHT increased with increasing concentrations (500, 1000, 2000, 3000, and 5000 μg/mL). Also, we examined the antioxidant abilities against the radical scavenger of samples at 1000 μg/mL because of the dose-dependent variations in the inhibition rates (100% scavenging activities at 3000 and 5000 μg/mL). The antioxidant effects of DGS were observed significant differences according to their concentrations and test sources, as illustrated in Fig. 4, and the radical scavenging activities of AGS and FAGS increased by comparing the DGS sample. Firstly, the DPPH radical scavenging capacities showed considerable differences according to the GS sources and their concentrations. The inhibition percentages of DPPH radical in DGS at different concentrations were as follows: 46.6% at 1000 μg/mL, 30.4% at 500 μg/mL, and 13.8% at 250 μg/mL (Fig. 4A). Namely, the antioxidant properties on DPPH radical increased with dose-dependent variations in the increasing concentrations of DGS. After the aging and fermentation steps, the DPPH radical inhibitions exhibited higher scavenging activities than DGS sample. To be more specific, the effects of this radical displayed remarkable differences as 46.6 (DGS) → 85.8 (AGS) → 91.4% (FAGS) at 1000 μg/mL after process steps (Fig. 4A). The remaining concentrations also showed the increase rates as follows: 250 μg/mL: 13.8 (DGS) → 36.9 (AGS) → 47.9% (FAGS); 500 μg/mL: 30.4 (DGS) → 58.0 (AGS) → 68.7% (FAGS). Our results were consistent with the previous literatures reporting that processed ginseng products using thermal and fermentative skills had higher antioxidant capacities than fresh ginseng (Eom et al., 2018; Jung et al., 2019; Kim et al., 2007; Lee et al., 2015). Other crops and their products were also observed high antioxidant abilities in the processing methods (Akpabli-Tsigbe et al., 2021; Chu et al., 2020; Hwang et al., 2021; Wang et al., 2022). Therefore, processed GS extracts through aging and fermentation may be potent antioxidant agents due to the increase rates of approximately 20% DPPH radical scavenging activities (extract concentrations: 500 and 1000 μg/mL), even though their extracts showed low abilities by comparing the BHT (positive control; 95% at 1000 μg/mL).

As indicated in Fig. 4B, the scavenging capacities against ABTS radical cation also exhibited considerable differences according to the process skills. The GS extracts of each concentration (250, 500, and 1000 μg/mL) increased with dose-dependent activation and the rank order was consistent with those of DPPH radical (FAGS > AGS > DGS). The highest capacity on this radical was found with 96.0% in FAGS sample at a concentration of 1000 μg/mL (79% at 500 μg/mL) and other sources were as follows: AGS (72.4%, 500 μg/mL) > DGS (18.8%, 500 μg/mL) (Fig. 4B). Interestingly, all GS extracts showed higher scavenging abilities in ABTS radical cation when compared to the DPPH radical. These differences may be considerably responsible for the reaction portions through chain breaking and hydrogen donating activities of ABTS radical cation as well as hydrogen donating capacities of DPPH radical concern to metabolites in GS extracts (Hwang et al., 2021; Lee et al., 2018). In particular, fermented GS extract at 1000 μg/mL was detected similar scavenging ability by comparing Trolox (positive control 92%). Our results were similar to previous literatures which showed that the antioxidant activities of crops increased remarkably owing to the biotransformation of secondary metabolites through food processing

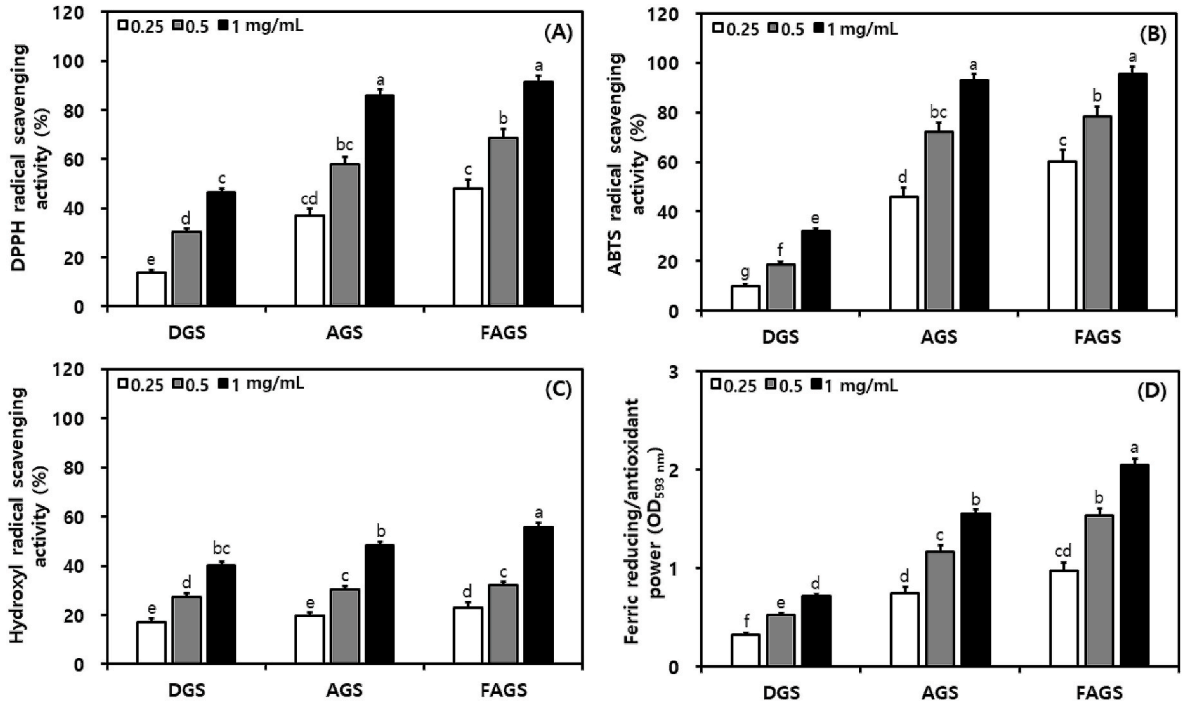


**Fig. 4.** Changes in antioxidant properties of the 50% ethanol extracts through aging and fermentation processes of ginseng sprouts: (A) DPPH radical scavenging activity; (B) ABTS radical scavenging activity; (C) hydroxyl radical scavenging activity; (D) FRAP, Different letters correspond to the significant differences relating to the processing steps using Tukey's multiple test ($p < 0.05$).

techniques (Akpabli-Tsigbe, 2021; Chu et al., 2020; Eom et al., 2018; Hwang et al., 2021). In the hydroxyl radical method, the AGS and FAGS materials exhibited lower scavenging abilities when compared to the DPPH radical and ABTS radical cation (Fig. 3C) and their effects increased with increasing concentrations (250 → 500 → 1000 μg/mL) of samples. Especially, the FAGS extract was observed higher scavenging activity than AGS source of aging process. To put it concretely, the hydroxyl radical showed scavenging capacities in the rank order of FAGS > AGS > DGS with 55.7 48.2, and 40.4% at 1000 μg/mL. Other concentrations exhibited as follows: FAGS > AGS > DGS (500 μg/mL: 32.0 > 30.4 > 27.3%; 250 μg/mL: 23.1 > 19.5 > 17.0%) (Fig. 4C), and their properties were lower than positive control (ascorbic acid; 62% at 500 μg/mL). As mentioned above results, the antioxidant effects on three radicals were as follows: ABTS > DPPH > hydroxyl. Our findings were demonstrated that the antioxidant activities in ginseng sprouts exhibited considerable differences with the increased patterns after aging and fermentation processes (Jung et al., 2019; Kim et al., 2007; Lee et al., 2015). To gain more information, we examined the FRAP assay regarding determine the extract activities of three ginseng sprouts to reduce $Fe^{3+}$ to $Fe^{2+}$ (Dravie et al., 2020) (Fig. 4D). This method would be an appropriate system for evaluating antioxidant because of its high reproducibility and sample procedure (de Torre et al., 2015; Dravie et al., 2020). The FRAP effects increased considerably with increasing concentrations of GS sources, as those of radical scavenging abilities. As indicated in Fig. 4D, the FRAP properties of AGS and FAGS exhibited higher capacities with remarkable differences than the DGS sample. Precisely, the FRAP assay exhibited high values in the rank order of FAGS > AGS > DGS with 2.05, 1.55, and 0.72 $OD_{420\ nm}$ at 1000 μg/mL. Other concentrations (DGS → AGS → FAGS processes; 500 μg/mL: 0.52 → 1.1 → 1.53 $OD_{420\ nm}$, 250 μg/mL: 0.32 → 0.75 → 0.98 $OD_{420\ nm}$) are also similar patterns as those of 1000 μg/mL extract (Fig. 4D). Our results were similar to the previously published data that the antioxidant properties exhibited high increase rates in germination and fermentation processes (Chu et al., 2020; Hwang et al., 2021; Sandoval-Sicairos et al., 2021). Consequently, processed ginseng sprouts may be utilized as an excellent sources of natural antioxidants for the development of functional foods. This work elucidated for the first time, the antioxidant capacities on radical and FRAP from ginseng sprouts by aging and fermentation processes.

## 4. Conclusions

The current research documented an excellent information regarding variations of physicochemical properties and compositional components in different ginseng sprouts using the aging and fermentation processes. We also observed that the fluctuations of antioxidant abilities and their potential factors. The reducing sugar and acidity were increased according to the order of DGS → AGS → FAGS, while the volatile and amino acid contents exhibited considerable decrease rates (3538 → 1015 → 325 ng/g; 4537.7 → 2129.9 → 2450.6 mg/100 g). The ginsenoside contents were also considerably decreased with 37.39 → 33.83 → 34.52 mg/g, however, ginsenoside F2 and CK of the deglycosidated transformation showed abundant contents with high variations of 2.15 → 4.59 and 0.75 → 4.07 mg/g. The antioxidant abilities of processed ginseng sprouts (FAGS > AGS) increased approximately 2 times as the rank order of ABTS > DPPH > hydroxyl > FARP. The influential factors on antioxidant including TPC, TFC, and MRP increased with value degrees of 2.4 → 5.5 GAE mg/g, 0.5 → 1.3 RE mg/g, and 1.9 → 2.9 $OD_{420\ nm}$ after processes. Consequently, the AGS and FAGS sources were observed high increase rates in deglycosylated ginsenosides and antioxidant activities. It is believed that processed ginseng sprouts may be utilized as commercial products concerning functional and nutraceutical sources in food industry. Future studies are needed to demonstrate the potential human health biological properties in processed ginseng sprouts for the development of diverse functional agents.

## Ethics statement

This manuscript did not include any human subjects and animal experiments.

## CRediT authorship contribution statement

**Kye Man Cho:** Conceptualization, Project administration, Investigation, Funding acquisition, Methodology, Supervision. **Hee Yul Lee:** Methodology, Data curation, Formal analysis. **Young Min Lee:** Formal analysis, Data curation. **Eun Young Seo:** Data curation, Visualization. **Du Hyun Kim:** Investigation, Resources. **Ki-Ho Son:** Software, Validation. **Jihyun Lee:** Formal analysis. **Du Yong Cho:** Formal analysis, Data curation. **Jin Hwan Lee:** Conceptualization, Data curation, Writing – original draft, Writing – review & editing.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2022.113644.

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