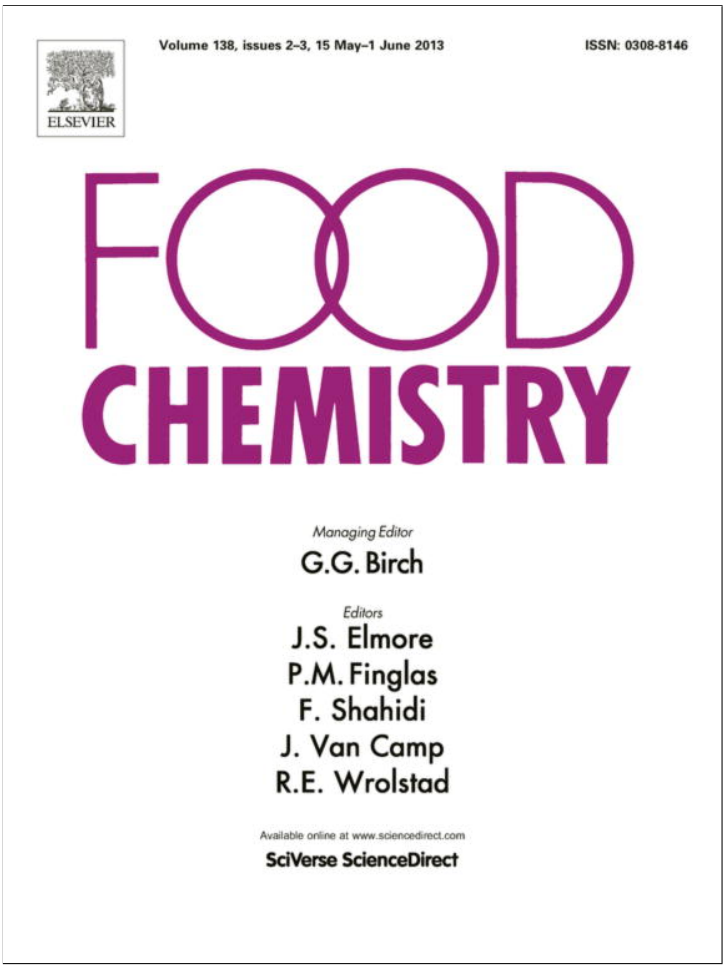

Food Chemistry 138 (2013) 876–883

Contents lists available at SciVerse ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Increase in antioxidant and anticancer effects of ginsenoside Re–lysine mixture by Maillard reaction ☆

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ARTICLE INFO

Article history:
Received 14 September 2012
Received in revised form 30 November 2012
Accepted 2 December 2012
Available online 8 December 2012

Keywords:
Panax ginseng
Maillard reaction
Ginsenoside
Lysine
Antioxidant
Anticancer effect

ABSTRACT

Ginsenosides are the main active components of *Panax ginseng*. Structural changes in diol type ginsenosides along with generation of Maillard reaction products (MRPs) are strongly associated with increased free radical-scavenging activities. Ginsenoside Re, one of the major triol type ginsenosides of *P. ginseng*, possesses a hydrophobic four-ring steroid-like structure with hydrophilic sugar moieties at carbons-3 and -20. The aim of the present study was to identify changes in the structure, antioxidant and anticancer effects of ginsenoside Re upon Maillard reaction. Ginsenoside Re was transformed into less-polar ginsenosides, namely Rg_2, Rg_6 and F_4 by heat-processing. Free radical-scavenging activity of the ginsenoside Re–lysine mixture increased upon heat processing. This improved free radical-scavenging activity mediated by antioxidant MRPs, which were generated through Maillard reaction of a glucosyl moiety separated from carbon-20 of ginsenoside Re and lysine. The increased anticancer effect of ginsenoside Re–lysine mixture upon heat processing was mainly derived from the generation of less-polar ginsenosides through the regulation of Bcl-2 and Bax, as well as caspase-dependent apoptotic pathway. These results reported here have shed significant new lights on the mechanism of increased antioxidant and anticancer effects of *P. ginseng* upon heat processing.

1. Introduction

The use of bioactive dietary agents is considered as attractive approach for the prevention and treatment of several types of cancers. Epidemiological as well as experimental studies have shown that diets rich in vegetables and fruits are beneficial for the chemotherapeutic, exerting the activity to inhibit proliferation and inducing apoptosis against malignancies including gastric cancer (Kim et al., 2005; Shih, Yeh, & Yen, 2005; Zou & Chang, 2011). Especially, antioxidant compounds, such as γ-linoleic acid, curcumin, or resveratrol, are well known to act as anticancer agents, including inhibition of cell cycle, enhancement of apoptosis, and antiangiogenicity (Gagliano et al., 2010; Karmakar, Banik, Patel, & Ray, 2006; Miyake, Banadiba, & Colquhoun, 2009).

Root of ginseng, *Panax ginseng* C.A. Meyer (Araliaceae), is heat-processed to improve its medicinal efficacy in Korea. Specifically, the free radical-scavenging activities of *P. ginseng* increase upon heat processing (Kang, Kim, Pyo, & Yokozawa, 2006; Keum et al., 2000; Kim et al., 2000). Ginsenosides are regarded as the main active components of *P. ginseng*, and we previously identified that structural changes in ginsenosides in response to heat processing are strongly associated with increased free radical-scavenging activities (Kang, Yokozawa, Yamabe, Kim, & Park, 2007; Yamabe et al., 2011). Ginsenosides are 30-carbon glycosides derived from the triterpenoid dammarane, as shown in Fig. 1. In our previous research, we found that diol type ginsenosides gradually transform into 20(*S*)-Rg_3, 20(*R*)-Rg_3, Rk_1, and Rg_5 upon heat processing, and the sugar moieties at carbon-20 separate.

Maillard reaction products (MRPs) are generated whenever reducing sugars are heated in the presence of amino acids, peptides, or proteins, which mainly occurs during the heat processing of foods such as coffee, bread, and meat (Chen & Kitts, 2012). Interestingly, MRPs are known as a major class of compounds with enhanced antioxidant activity generated by thermal treatment of various crude drugs or foods (Cai, Luo, Sun, & Corke, 2004; Morales & Jiménez-Pérez, 2001; Yanagimoto, Ochi, Lee, & Shibamoto, 2004).

☆ This paper was studied with the support of the MEST (Ministry of Education Science and Technology) and KOFST (The Korean Federation of Science and Technology Societies). This work was also supported by the Korea Institute of Science and Technology institutional program.

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http://dx.doi.org/10.1016/j.foodchem.2012.12.004

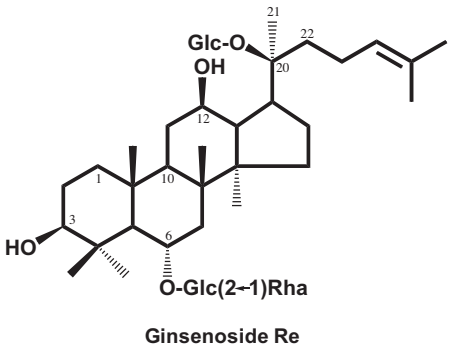

Fig. 1. Structure of ginsenoside Re. -Glc, D-glucopyranosyl; -Rha, L-rhamnopyranosyl.

The generation of MRPs, although limited to the reaction between glucosyl moieties separated from diol type ginsenosides and glycine, was shown to be positively correlated with free radical-scavenging activity (Lee et al., 2008). However, little is known about the structural and biological changes in triol type ginsenosides upon Maillard reaction.

Ginsenoside Re, one of the major triol type ginsenosides in *P. ginseng*, possesses a hydrophobic four-ring steroid-like structure with hydrophilic sugar moieties at carbons-3 and -20 (Fig. 1). The aim of the present study was to identify changes in the structure as well as antioxidant and anticancer effects of ginsenoside Re upon Maillard reaction.

2. Materials and methods

2.1. Chemicals and reagents

1,1-Diphenyl-2-picrylhydrazyl (DPPH) and Folin–Ciocalteu's phenol reagent were purchased from Sigma Aldrich (Seoul, South Korea). Ginsenoside Re (Fig. 1(A)) was isolated and identified from *P. ginseng* by the reported method (Oura, Hiai, Odaka, & Yokozawa, 1975). Ginsenosides 20(*S*)-Rg_2, 20(*R*)-Rg_2, Rg_6/F_4 were purchased from Ambo Institute (Seoul, South Korea). Monoclonal antibodies against cleaved caspase-8 and GAPDH and polyclonal antibodies against cleaved caspase-3, cleaved caspase-9, Bcl-2, Bax and poly-(ADP-ribose) polymerase (PARP) were purchased from Cell Signaling Technology, Inc. (Cell Signaling). Other chemicals and reagents were of high quality and obtained from commercial sources.

2.2. Maillard reaction model experiment using ginsenoside Re and lysine

The same amounts (w/w) of ginsenoside Re and lysine, a frequently used amino acid in Maillard reaction model experiments, were steamed together at 120 °C for 3 h as reported previously (Kang et al., 2006). The reaction was conducted in aqueous solution to contain approximately 5 mM of ginsenoside Re and 34 mM of lysine. After drying at 50 °C for 3 days, un-treated and heat-processed ginsenoside Re–lysine mixtures at 120 °C were prepared. The glucose–lysine mixture heat-processed at 120 °C was also prepared to examine the effects of MRPs (Lee et al., 2008; Yamabe et al., 2011).

2.3. Analysis and structural confirmation of ginsenosides

Analytical reversed-phase HPLC system was composed of a solvent degasser (Agilent, G1322A), binary pump (Agilent, G1312C), an autosampler (Agilent, G1329B) and model ZAM 3000 Evaporative Light Scattering Detector (ELSD) (Young Lin, Korea). ELSD conditions were optimised in order to achieve maximum sensitivity: temperature of the nebulizer was set for 50 °C, and N_2 was used as the nebulizing gas at a pressure of 2.0 bar. The Phenomenex Luna C18 column (150 × 4.6 mM, 5 μm) was used, and the mobile phase consisted of a binary gradient of solvent A (acetonitrile:water:5% acetic acid in water = 15:80:5) and solvent B (acetonitrile:water = 80:20) at a flow rate of 1.0 mL/min. The gradient flow program was as follows: initial; 0% B, 6 min; 30% B, 18 min; 50% B, 30 min; 100% B, 37 min; 100% B, 42 min; 0% B.

Structures of ginsenosides were confirmed by preparative isolation, nuclear magnetic resonance (NMR) spectroscopy and mass spectrometer. Ginsenosides mixture was separated by reversed-phase preparative HPLC, eluted with solvent A (acetonitrile:water: 5% acetic acid in water = 15:80:5) and solvent B (acetonitrile: water = 80:20) [(100:0) → (70:30) → (50:50) → (0:100) → (0:100) → (100:0)] to furnish 4 ginsenosides (20(*S*)-Rg_2, 20(*R*)-Rg_2, F4 and Rg_6). NMR spectra were recorded in pyridine-d_5 on a Varian UNITY Plus 500 MHz spectrometer (Palo Alto, CA, USA). NMR chemical shifts were referenced to the residual solvent peaks (δ_H 7.22, 7.56, 8.74 and δ_C 123.5, 135.5, 149.9 for pyridine-d_5). Low-resolution ESI-MS data were measured with an Agilent Technologies VS/Agilent 1100 system (Santa Clara, CA, USA).

2.4. DPPH radical-scavenging activity test

In microwells, 100 μL of an aqueous solution of the sample (control: 100 μL of DW) was added to an ethanolic solution of DPPH (100 μL, 60 μm) according to the method of Hatano et al. (1989). After mixing gently and letting stand for 30 min at room temperature, the absorbance at 540 nm was determined using a microplate reader (Tecan SPECTRAFluor; Tecan UK, Goring-on-Thames, UK). Trolox was used as a positive control compound.

2.5. Measurement of browning compound levels

The extent of browning was measured by the reported method with minor modifications (Kitts, Chen, & Jing, 2012; Yamabe et al., 2011). The samples (4 mg) were dissolved in 1 mL of distilled water (DW)–ethanol (1:1, v/v), and the absorbance at 420 nm was measured in a 1 cm glass cuvette using a UV–Vis spectrophotometer (UV-1200; Shimadzu, Kyoto, Japan). The fluorescent intensity was measured with a microplate reader (Tecan SPECTRAFluor; Tecan UK, Goring-on-Thames, UK) at excitation/emission wavelengths of 330/420 nm for advanced MRPs. Measurement was repeated three times for each sample.

2.6. Measurement of total phenolic contents

The total phenolic contents of samples were determined by the Folin–Ciocalteu method (Singleton, Orthofer, & Lamuela-Raventós, 1999). Contents were expressed as milligramms of gallic acid equivalents (GAE) per gram of each sample. Measurement was repeated three times for each sample.

2.7. Antiproliferative effect on gastric cancer cells

The human gastric cancer AGS cell line was purchased from the American Type Culture Collection (ATCC, VA, USA). Cells were grown in RPMI1640 medium (Cellgro, VA, USA) supplemented with 10% foetal bovine serum (Gibco BRL, MD, USA), 100 units/ml penicillin and 100 μg/ml streptomycin and incubated at 37 °C in a humidified atmosphere with 5% CO_2. AGS cells were treated with different concentrations of compounds for 24 h, and cell proliferation was measured using the Cell Counting Kit-8 (CCK-8, Dojindo Laboratories, Japan) according to the manufacturer's recommendations.

2.8. Western blotting analysis

AGS cells were grown in 6-well plates and treated with the indicated concentration of compounds for 24 h. Whole-cell extracts were then prepared according to the manufacturer's instructions using RIPA buffer (Cell Signaling, MA, USA) supplemented with 1× protease inhibitor cocktail, 1 mM phenylmethylsulfonyl fluoride (PMSF) and 1 mM sodium orthovanadate (Na_3VO_4). Proteins (whole-cell extracts, 30 μg/lane) were separated by electrophoresis in a precast 4–15% Mini-PROTEAN TGX gel (Bio-Rad, CA, USA) blotted onto PVDF transfer membranes and analysed with epitope-specific primary and secondary antibodies. Bound antibodies were visualised using ECL Advance Western Blotting Detection Reagents (GE Healthcare, UK) and a LAS 4000 imaging system (Fujifilm, Japan).

2.9. Statistical analysis

Statistical significance was determined through analysis of variance (ANOVA) followed by a multiple comparison test with a Bonferroni adjustment. P values of less than 0.05 were considered statistically significant.

3. Results and discussion

As shown in the HPLC chromatograms of the ginsenoside Re and ginsenoside Re–lysine mixture, ginsenoside Re and lysine were detected at about 2.0 and 8.5 min, respectively (Fig. 2(A) and (B)). However, upon heat processing at 120 °C, most of the ginsenoside Re disappeared, and the contents of less-polar ginsenosides were newly detected at about 13.0 and 19.0 min (Fig. 2(C) and (D)). Chemical structures of less-polar ginsenosides were determined basis on mass and NMR spectral data. The ESI-mass spectrum in negative-ion mode of peaks at about 13.0 min (Fig. 2(C) and (D)) showed $[M]^-$ peak at m/z 783.5 ($C_{42}H_{71}O_{13}$) corresponding to the structure of ginsenoside Rg_2 ($C_{42}H_{72}O_{13}$, MW = 784.5). The ESI-mass spectrum in negative-ion mode of peaks at about 19.0 min (Fig. 2(C) and (D)) showed $[M]^-$ peak at m/z 765.5 ($C_{42}H_{69}O_{12}$) corresponding to the structure of ginsenosides Rg_6 and F_4 ($C_{42}H_{70}O_{12}$, MW = 766.5). The ^1H NMR spectral data (Supplementary Figs. 1 and 2) of ginsenosides Rg_2 and Rg_6/F_4 were in accordance with those reported in the literatures (Ryu, Park, Eun, Jung, & Sohn, 1997; Ryu et al., 1996; Sanada, Kondo, Shoji, Tanaka, & Shibata, 1974). Therefore, the chemical structures of ginsenosides Rg_2, Rg_6 and F_4 were confirmed to be same as shown in Fig. 2(E). Ginsenoside Rg_2 was generated by elimination of the glycosyl residue at carbon-20 during the steaming process, and greater amounts of less-polar ginsenosides such as Rg_6 and F_4 were produced by the elimination of H_2O at carbon-20 of ginsenoside Rg_2 under the high pressure and temperature conditions (Lee et al., 2012). Therefore, ginsenoside Re gradually transformed into less-polar ginsenosides such as Rg_2, Rg_6 and F_4 upon heat processing with or without lysine (Fig. 2(E)). Considering the generated amounts of ginsenosides Rg_2, Rg_6 and F_4 from ginsenoside Re, the reaction rate was reduced when the ginsenoside Re was heat-processed with lysine.

The generation of colour is an obvious feature of caramelization and Maillard reaction, and brown-coloured antioxidant components are known to be formed by these reaction (Kitts, Chen, & Jing, in press; Samaras, Camburn, Chandra, Gordon, & Ames, 2005). The level of browning products (A_{420nm}) in ginsenoside Re and ginsenoside Re–lysine mixture increased from 0.042 and 0.041 to 0.068 and 0.090 after heat processing at 120 °C, respectively (Fig. 3(A)). In the case of the glucose–lysine mixture, the level of browning products increased to 0.550 after heat processing at 120 °C, which was 6.1 times higher than that of the ginsenoside Re–lysine mixture (Fig. 3(A)). The levels of advanced MRPs in ginsenoside Re, ginsenoside Re–lysine and glucose–lysine mixtures which measured by comparing fluorescence intensity were significantly increased after heat processing at 120 °C (Fig. 3(B)). The increased levels of advanced MRPs in glucose–lysine mixtures were 2.1 and 3.8 times higher than those of ginsenoside Re and ginsenoside Re–lysine mixture, respectively (Fig. 3(B)). Therefore, caramelization products and MRPs were generated after heat-processing of ginsenoside Re, ginsenoside Re–lysine and glucose–lysine mixtures.

Fig. 4 shows a comparison of the DPPH radical-scavenging activities of ginsenoside Re–lysine mixture and its MRPs. DPPH is used to test the ability of compounds or plant extracts to act as free radical scavengers or hydrogen donors (Hatano et al., 1989; Zhu, Hackman, Ensunsa, Holt, & Keen, 2002). No significant DPPH radical-scavenging activity of ginsenoside Re was confirmed and its activity was slightly increased after heat processing (Fig. 4(A)). Antioxidant activity of some caramelization products has been identified (Kitts et al., in press), and the slight increase in DPPH radical-scavenging activity of ginsenoside Re was supposed to be mediated by the caramelization products (Fig. 3). Ginsenoside Re–lysine mixture showed weak DPPH radical-scavenging activity, and its effect increased markedly after heat processing at 120 °C (Fig. 4(B)). The glucose–lysine mixture demonstrated no DPPH radical-scavenging activity, but its effect significantly increased after heat processing at 120 °C (Fig. 4(C)). It has been well discussed about the scavenging activity of various MRPs against reactive oxygen species such as DPPH, hydroxyl radical and superoxide anion, in cell free and cell culture systems (Chuyen, Ijichi, Umetsu, & Moteki, 1998; Jing & Kitts, 2004). Therefore, from the comparison of DPPH radical-scavenging activities of ginsenoside Re and its MRPs, the increased free radical-scavenging activity of the ginsenoside Re–lysine mixture upon heat processing was mainly mediated by the MRPs from glucose and lysine. These effects have been similarly observed in cases of ginsenoside Re–alanine and Re–serine mixtures upon heat processing (Lee et al., 2012a, 2012b).

The phenolic contents of plants can influence their antioxidant activities, and MRPs are known as major contributors to the increased antioxidant activity in response to thermal treatment in various foods (Cai et al., 2004; Morales & Jiménez-Pérez, 2001; Yanagimoto et al., 2004). Fig. 5 shows the changes in total phenolic contents of the ginsenoside Re–lysine and glucose–lysine mixtures upon heat processing. The total phenolic content of the ginsenoside Re–lysine mixture increased after heat processing. Interestingly, the total phenolic content of the glucose–lysine mixture also significantly increased from 7.2 to 14.7 GAE upon heat processing, and its value was 2 times higher than that of the ginsenoside Re–lysine mixture. This increase in total phenolic contents upon heat processing was related to the increase in level of browning products (Figs. 3 and 5). On the other hand, Kitts et al. recently identified that 2 reductones, 5-hydroxymethyl-2-furfural (HMF) and 5-hydroxymethyl-2-furoic acid (HMFA), are antioxidant active components in glucose–lysine model system (Kitts et al., in press). Interference in the estimation of total phenolic contents may caused by the presence of reductones and reductone-like substances in our present study. Taken together, the free radical-scavenging activity of the ginsenoside Re–lysine mixture increased upon heat processing. Further, the increased free radical-scavenging activity of the ginsenoside Re–lysine mixture was mediated by the generation of caramelization products and MRPs obtained during the processing at high temperatures.

Numbers of researcher have discussed about the antioxidants possess anticancer activity (Gagliano et al., 2010; Karmakar et al., 2006; Miyake et al., 2009). To determine the antiproliferative activity of ginsenoside Re–lysine mixture, human gastric cancer AGS cell was used as the in vitro model. AGS cell line has been shown to grow in athymic mice and to have the same histochemical and

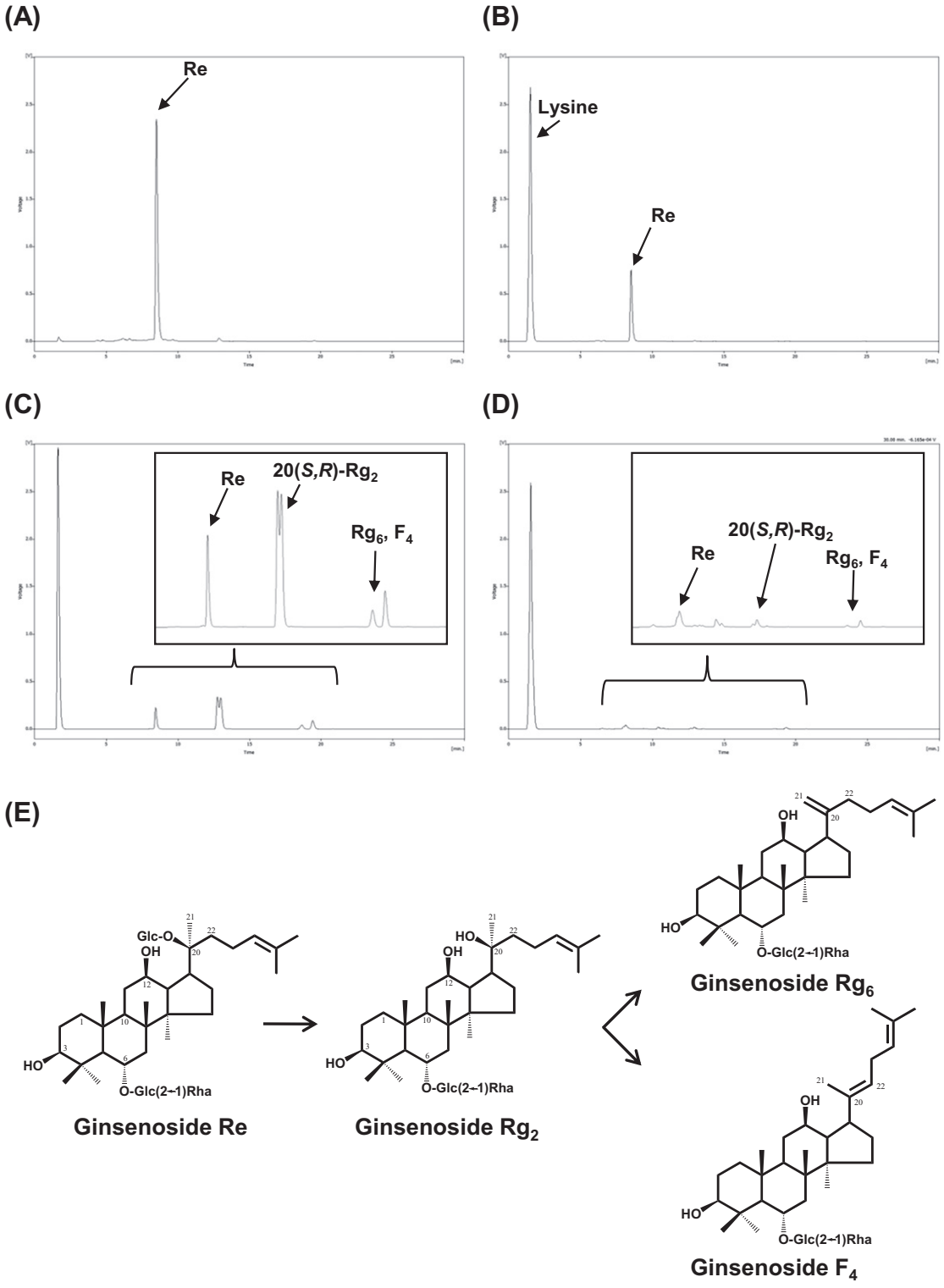

Fig. 2. Changes in HPLC chromatograms of ginsenoside Re and ginsenoside Re–lysine mixtures by heat processing. (A) HPLC chromatogram of ginsenoside Re before heat processing. (B) HPLC chromatogram of ginsenoside Re–lysine mixture before heat processing. (C) HPLC chromatogram of ginsenoside Re after heat processing. (D) HPLC chromatogram of ginsenoside Re–lysine mixture after heat processing. (E) Changes in chemical structure of ginsenoside Re during heat-processing.

cytological characteristics as the specimen taken from the patient (Shih et al., 2005), and recently this cell line has been widely used as a model system for evaluating cancer cell apoptosis (Barranco, Townsend, & Casartelli, 1983; Liu et al., 2003).

Ginsenoside Re–lysine mixture was found to suppress the AGS cell proliferation upon heat processing in a dose-dependent manner (Fig. 6(A) and (B)). The loss of cell proliferation following treatment of ginsenoside Re–lysine mixture without heat processing was not observed (Fig. 6(A) and (B)). Next, we sought to determine whether the antiproliferative effect of ginsenoside Re–lysine mixture is due to the ginsenoside Re or antioxidant MRPs. As a result, strong antiproliferative activity was found with the treatment of ginsenoside Re upon heat processing. However, the MRPs from glucose and lysine mixture showed significant suppression but less

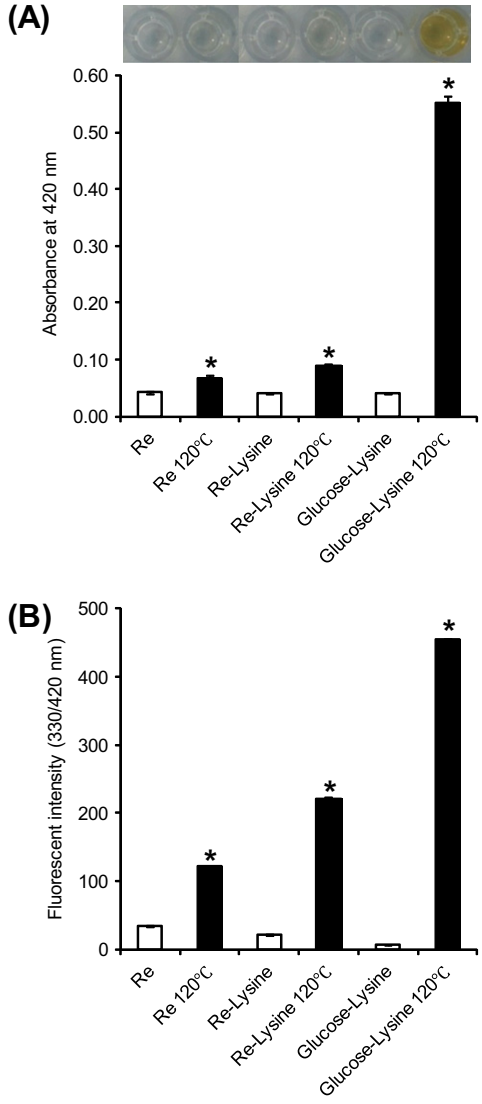

Fig. 3. Changes in browning compound levels of ginsenoside Re, ginsenoside Re–lysine and glucose–lysine mixtures by heat-processing. (A) The level of browning products. (B) The level of advanced MRPs. $^{*}p < 0.05$ compared to the not-treated value.

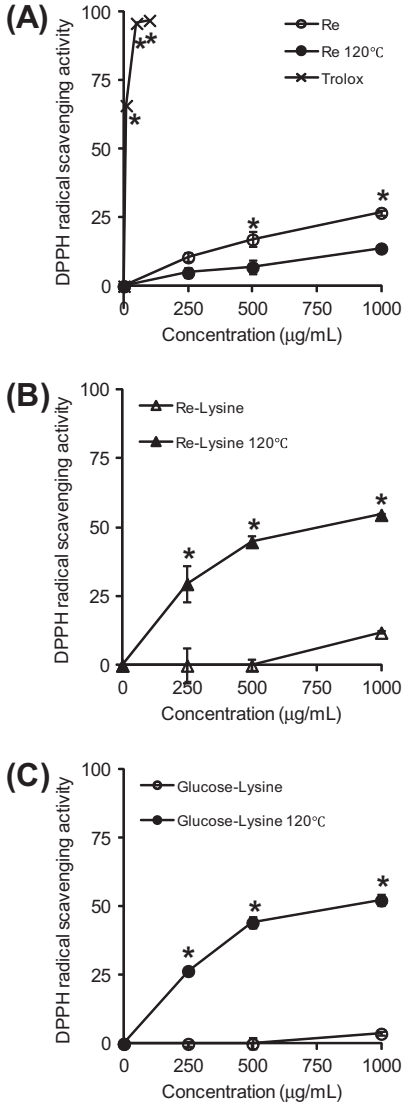

Fig. 4. Changes in DPPH radical-scavenging activities of ginsenoside Re, ginsenoside Re–lysine and glucose–lysine mixtures by heat-processing. (A) Comparison of DPPH radical-scavenging activity of ginsenoside Re upon heat processing. (B) Comparison of DPPH radical-scavenging activity of ginsenoside Re–lysine mixture upon heat processing. (C) Comparison of DPPH radical-scavenging activity of glucose–lysine mixture upon heat processing. $^{*}p < 0.05$ compared to the not-treated value.

activity compare to that of ginsenoside Re upon heat processing (Fig. 6(C)). Therefore, the antiproliferative efficacy of ginsenoside Re–lysine upon heat processing was mainly derived from less-polar ginsenosides such as Rg_2, Rg_6 and F_4 (Fig. 2), suggesting that this efficacy was not related to the antioxidant activity.

Since apoptosis is a mechanism by which cells undergo death to control cell proliferation and heat processed-ginsenoside Re possessed strong antiproliferative effect on AGS cell, we also confirmed the molecular mechanisms of apoptotic effects by Western blotting. The Bcl-2 family of proteins is important in regulating apoptosis, consisting of both pro-apoptotic (Bax, Bak, Bid, Bim, Bik, Blk, and Bcl-10) and anti-apoptotic (Bcl-2, Bcl-x, Bcl-XL, Bcl-XS, and Bcl-w) proteins, which share one or more of the four characteristic domains of homology entitled the Bcl-2 homology domains. The function of Bax is to promote cell apoptosis by translocation of the mitochondrial voltage-dependent anion channel membrane and disruption of the integrity of mitochondrial, resulting in the release of cytochrome *c* and other pro-apoptotic factors into the cytosol, leading to the activation of caspase. In contrast, anti-apoptotic protein Bcl-2 inhibits apoptosis by inhibiting the release of cytochrome *c* and apoptosis-inducing factor from mitochondria to the cytosol and by limiting the activation of caspase (Danial, 2007; Rosse, Olivier, & Monney, 1998). As shown in Fig. 7, examination of Bax and Bcl-2 expression during apoptosis indicated that treatments with heat processed-ginsenoside Re dose-dependently up-regulated the expression of pro-apoptotic protein Bax, whereas the expression of anti-apoptotic protein Bcl-2 was down-regulated with increasing concentration of heat processed-ginsenoside Re at 120 °C.

Caspases, which are cysteine proteases, play pivotal roles in cell apoptosis. Caspase-3 is an apoptosis executioner and activated by other activated caspases, such as caspase-8 and caspase-9, in response to pro-apoptotic signals. This enzyme has substrate specificity for the amino acid sequence Asp-Glu-Val-Asp (DEVD) and cleaves PARP into 83 and 24 kDa fragments, which respond to DNA fragmentation and eventually lead to apoptosis associating morphological changes (Ghobrial, Witzig, & Adjei, 2005;

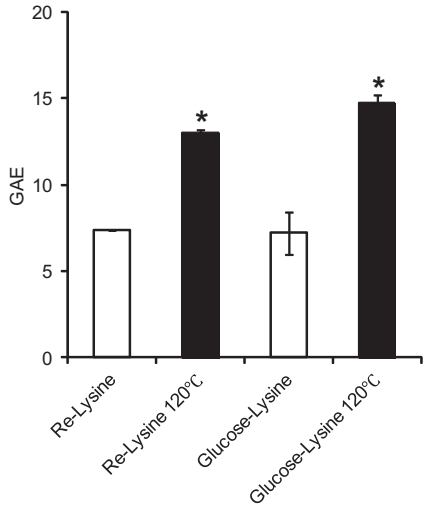

Fig. 5. Changes in total phenolic contents of ginsenoside Re–lysine and glucose–lysine mixtures upon heat processing. GAE: gallic acid equivalent. $^{*}p < 0.05$ compared to the not-treated value.

Thornberry & Lazebnik, 1998; Vaculova & Zhivotovsky, 2008). Here, we demonstrated that active forms (cleaved forms) of caspase-8, caspase-9, and caspase-3 were up-regulated in a dose-dependent manner after treatment of heat processed-ginsenoside Re at 120 °C (Fig. 7). Subsequently, PARP, a well-known substrate of caspase-3, was cleaved by the activated caspase-3, resulting in AGS cell apoptosis (Fig. 7).

Anticancer effects of several ginsenosides are identified and particularly diol type ginsenosides have higher anticancer activity against cancer cells than the triol type ginsenosides by triggering apoptotic cell death (Wang et al., 2007a). For example, Sin et al. have recently reported that ginsenoside Rg_3 induces senescence-like growth arrest in human glioma cancer through the Akt, which is associated with increased ROS levels, and p53/p21-dependent signaling pathways (Sin, Kim, & Kim, 2012). Ginsenoside Rg_3 and Rh_2 have also been known to induce apoptosis by direct activation of the mitochondrial pathway, that is, Rg_3 and Rh_2 stimulate the release of mitochondrial cytochrome c, activation of caspase-3 and Bax protein, inhibition of Bcl-2 protein and production of intracellular ROS in human hepatocellular carcinoma cells (Park, Kim, Kim, & Kang, 2012). Lee et al. have demonstrated that ginsenoside Rg_5 blocks the cell cycle of human hepatoma SK-HEP-1 cells via the down-regulation of cyclin E-dependent kinase activity (Lee, Lee, Kim, Park, & Lee, 1997). In addition, ginsenoside F_2 have been reported to induce apoptosis in breast cancer stem cells by activating the intrinsic apoptotic pathway and mitochondrial dysfunction (Mai et al., 2012). However, so far, there is no report or no positive evidence to show antiproliferative efficacy of ginsenoside Re, Rg_2, Rg_6 and/or F_4 except for our investigation (Wang et al., 2007b).

In summary, our investigation clearly demonstrated that the heat processing strengthened antioxidant and anticancer effects of ginsenoside Re, one of the major triol type ginsenosides, contained in *P. ginseng*. The free radical-scavenging activity of ginsenoside Re–lysine mixture increased upon heat processing due to the generation of antioxidant MRPs. However, the increased anticancer effect of ginsenoside Re–lysine mixture upon heat processing was

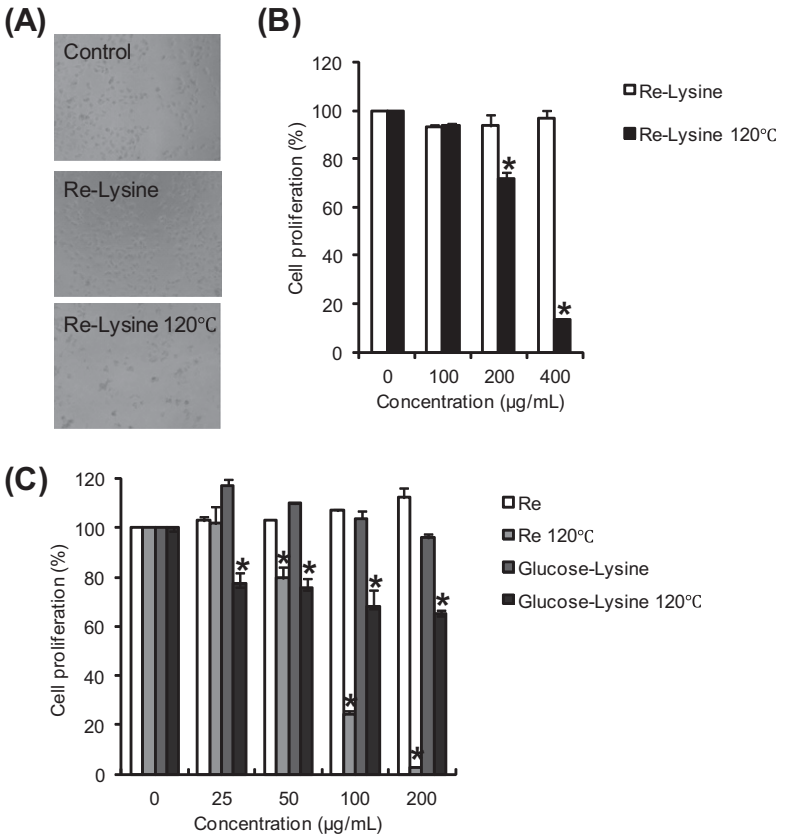

Fig. 6. Changes in effects of ginsenoside Re–lysine, ginsenoside Re and glucose–lysine mixtures upon heat processing on AGS cell proliferation. (A) Morphological changes were confirmed using phase-contrast microscopy. (B) Cells were treated with ginsenoside Re–lysine mixture with or without heat processing at different concentrations (100, 200, and 400 μg/mL) for 24 h. (C) Cells were treated with ginsenoside Re or glucose–lysine mixture with or without heat processing, respectively, at different concentrations (25, 50, 100, and 200 μg/mL) for 24 h. Relative cell proliferation was measured by the CCK-8 assay. $^{*}p < 0.05$ compared to the not-treated value.

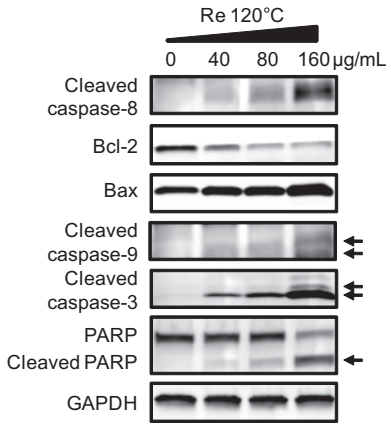

Fig. 7. Effect of heat processed-ginsenoside Re on pro- and anti-apoptosis proteins in AGS cells. Western blotting results showing the levels of cleaved caspase-8 (18 kDa), Bcl-2 (26 kDa), Bax (20.5 kDa), cleaved caspase-9 (35, 37 kDa), cleaved caspase-3 (17, 19 kDa), PARP (116 kDa), and cleaved PARP (85 kDa) in AGS cells treated with heat processed-ginsenoside Re at different concentrations (40, 80, and 160 μg/mL) for 24 h. Thirty micrograms of each protein were separated by SDS–PAGE. GAPDH (37 kDa) was used as an internal control.

mainly derived from less-polar ginsenosides and it was not related to the antioxidant MRPs. Molecular mechanisms involved in ginsenoside Re upon heat processing-induced apoptosis might attributed to the regulation of Bcl-2 and Bax, as well as caspase-dependent apoptotic pathway. A better understanding of the mechanism of the antiproliferative effect of ginsenoside Re upon heat processing will aid in the development of effective cancer chemotherapeutic strategies involving their use as potential anticancer adjuvants.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2012.12.004.

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