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# Fermented ginseng offers new skin care efficacy

Ginseng has a long history of use by humans for its health-giving properties. In fact its Genus name, Panax, means 'all heal', and it has a reputation across the world for its ability to induce energy, vitality and boost the immune system. However in the centuries of its use, it has largely been taken as an oral medicine and not topically.

Korean company, GFC, now offers the cosmetics industry an opportunity to tap into the power and history of this famous medicinal root with its new fermented ginseng ingredient which harnesses for the first time the potency of ginseng for use in skin care products.

The efficacy of fermented ginseng fruit was analysed in a series of both *in vitro* and *in vivo* experiments to determine its viability for use in anti-ageing products. The results reveal how the fermented ginseng ingredient performs in comparison with normal ginseng.

## Materials and method

### Production of fermented ginseng fruit

The *Lactobacillus brevis* strain GFC01 was cultivated by placing it in an MRS medium at 37˚C. After the extract was sterilised from the fruit of the ginseng in a medium, the activated *Lactobacillus* ($1\times10^9$ CFU/mL) was innoculated into the extract at a 10% density. Over the course of 5 days, the inoculated extract samples were kept at 37˚C and removed for use in analysis.

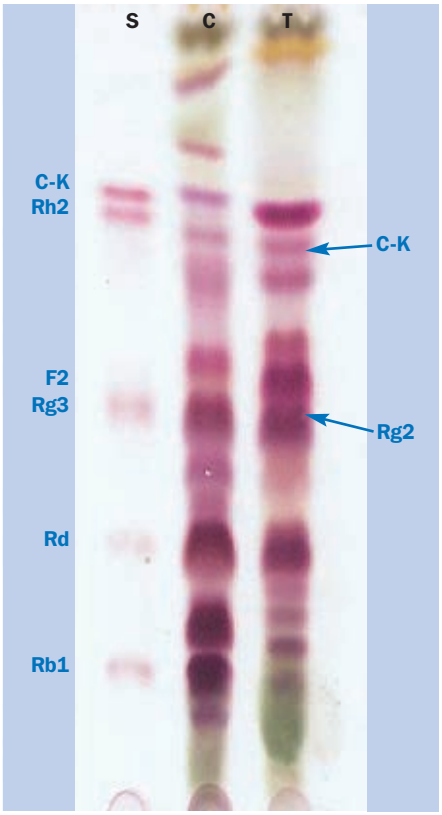



**Figure 1:** *Thin-layer chromatography (TLC) analysis of metabolites of ginsenoside. Major ginsenoside was used as the substrate to yield ginsenoside Rg2 (1) and compound K (2). Developing solvent: $CHCl_3$/MeOH/$H_2O$ (65:35:10, by vol., lower phase).* **S:** *Saponin standards;* **C:** *Normal ginseng;* **T:** *Fermented ginseng.*

### Analysis of ginsenosides by thin-layer chromatography

TLC was performed with silica gel plates (60F254, Merck, Darmstadt, Germany), and $CHCl_3$-$CH_3OH$-$H_2O$ (65:35:10, V/V, lower phase) was used as its developing solvent. The spots on the TLC plates were detected through spraying with 10% $H_2SO_4$, followed by heating at 110˚C for 10 min.

### Analysis of ginsenosides by high-performance liquid chromatography

The HPLC-grade acetonitrile and water were purchased from SK Chemicals (Ulsan, Korea). The reaction mixture was extracted in n-butanol saturated with $H_2O$ and evaporated in vacuum. Additionally, the residue was dissolved in $CH_3OH$ and injected for HPLC analysis. This experiment also employed a C18 (250 × 4.6 mm, particle size 5 μm) column using acetonitrile (solvent A) and distilled water (solvent B) mobile phases at A/B ratios of 15:85, 21:79, 58:42, 90:10, and 15:85; with run times of 0-5, 5-25, 25-80, 70-82, and 82-100 min, respectively. The flow rate was 1.6 mL/min and the sample was detected at UV 203 nm.

### Cell culture

Normal human dermal fibroblasts (MCTT, Seoul, Korea) were plated in 100 mm tissue culture dishes and maintained in

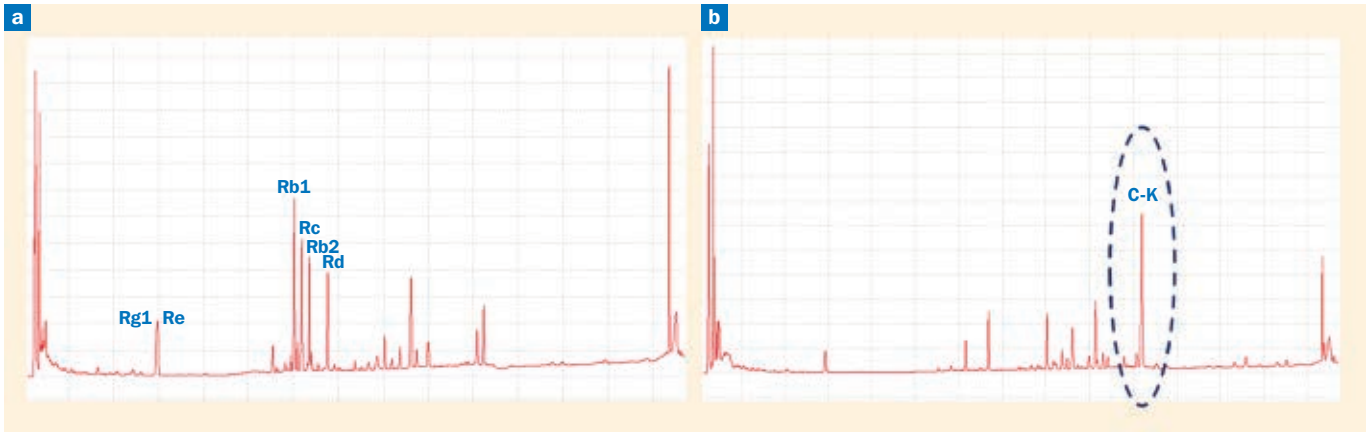



**Figure 2:** *High-performance liquid chromatography (HPLC) analysis of the bioconversions of ginsenosides.* **a)** *Normal ginseng;* **b)** *Fermented ginseng.*

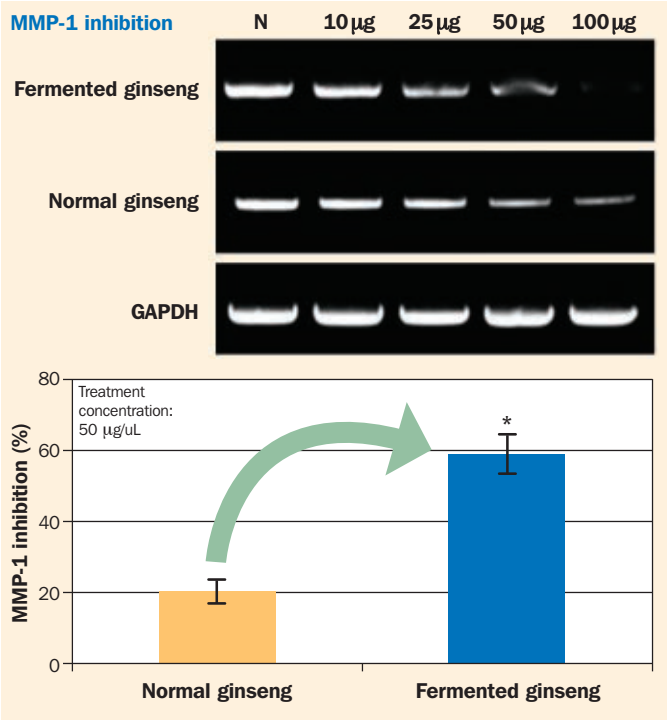



**Figure 3:** *Effects of fermented ginseng on tissue inhibitors of MMP-1 production in dermal fibroblasts.*

DMEM supplemented with 10% heat-inactivated FBS and 1% penicillin-streptomycin (Gibco-BRL, Gaithersburg, MD, US) at 37˚C in an atmosphere containing 5% $CO_2$. All experiments were performed using only those cells between passages 6 and 10.

**UV irradiation and sample treatment**

UVB irradiation and sample treatment were performed according to the method previously reported by Hwang *et al*. 25 NHDFs were irradiated with UVB for 40 seconds. The concentrations of the fermented ginseneg fruit extract treatment were 0.1, 1, and 10 mg mL. Control cells were kept under the same culture conditions without UVB exposure. For RT-PCR, cells were harvested 24 hours after UVB irradiation. MMP-1 secretion was assessed in the supernatants harvested 72 hours after UVB irradiation.

**Cell viability**

For the various treatments, the medium was removed and 3-[4,5-dimethylthiazol-2-yl]-2,5-dipheny lte trazolium bromide (MTT, 0.1 µg/mL$^{-1}$) was added followed by incubation at 37˚C for 2 hours in a $CO_2$ incubator. Formazancrystals were completely dissolved by DMSO, absorbance was measured at 570 nm using a microplate reader (Molecular Devices, US).

**Measurement of MMP-1 and procollagen type 1**

The concentrations of MMP-1 and procollagen type 1 in the medium were determined using commercially available ELISA kits (Human Total MMP-1 kit, R&D Systems, Inc., Minneapolis, MN, US; Procollagen Type 1 C-Peptide EIA Kit, Takara, Shiga, Japan) according to the manufacturer's instructions. Each sample was analysed in triplicate.

**Clinical evalution of anti-ageing effect**

Fifty female subjects with moderate photoageing (dyschromic facial skin with fine lines and wrinkles) between the ages of 30 and 50 were enrolled in the

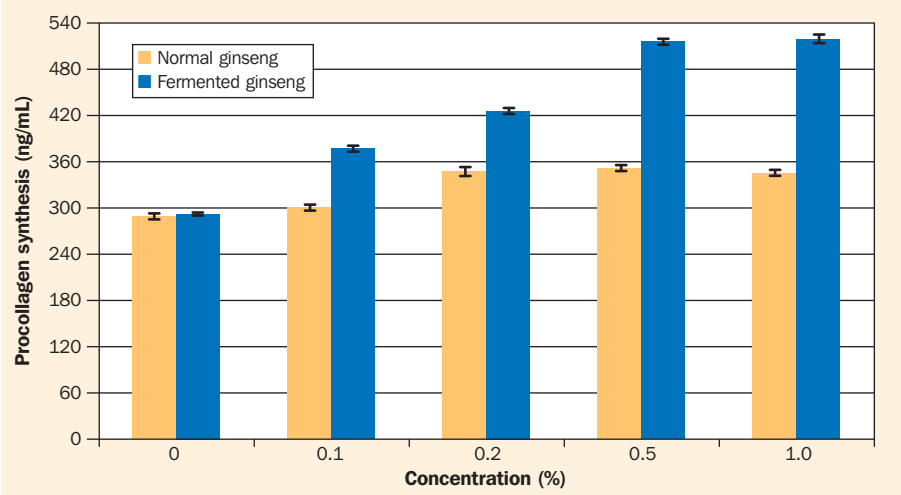
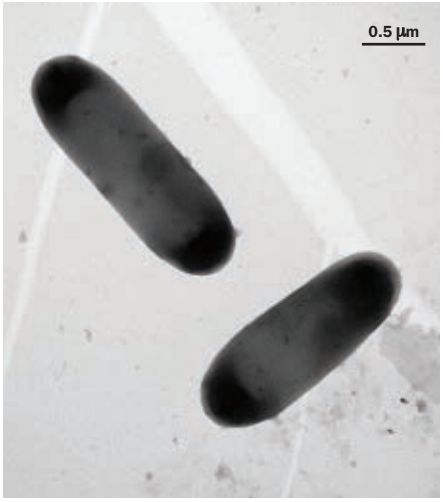



**Figure 4:** *Effects of fermented ginseng on procollagen type 1 peptide production in dermal fibroblasts.*




Lactobacillus brevis *strain GFC01 microorganism used during fermentation process.*

study and randomly divided into two equal groups of 25 subjects each. Subjects were given a commercial cream containing either 0% or 2% fermented ginseneg fruit extract w/w in blinded containers. Of the 25 subjects receiving the 0% formulation, 20 completed the study; of the 25 subjects receiving the 2% formulation, 20 completed the study. All study dropouts were for personal reasons and none were related to the efficacy, safety, or adverse events from the products. Clinical evaluations were performed at baseline, three weeks, and six weeks, including Antedra 3D (Miravex, Ireland) blinded expert grader assessments using a 0–4 scale (0 = no change, 1 = 25% improvement, 2 = 50% improvement, 3 = 75% improvement, 4 = 100% improvement) were made via analysis of high-resolution digital photographs in a blinded scenario for skin roughness/dryness, fine lines/wrinkles, and overall global improvement in photodamaged skin.

## Result and discussion

### Biotransformation by pathway

The process through which ginseng fruit major ginsenoside such as ginsenoside Rb1 and Re was decomposed by strain GFC01 was analysed using HPLC, as were any changes in reaction time. Figure 1 shows the concentrations of major ginsenosides and those of the decomposition products minor ginsenoside such as ginsenosides Rg2 and compound K (Figures 1 and 2).

After 120 hrs of reaction, ginsenoside Rb1 was simultaneously transformed into ginsenoside F2 and ginsenoside C-K (metabolite 1). And the majority of ginsenoside Re had been decomposed into ginsenoside Rg2 (metabolite 2) after 120 hrs of reaction. This result suggested that strain GFC01 exerts potent β-glucosidase activity, and that ginsenoside Rb1 and Re has converted in the following sequence: ginsenosides $Rb_1$ → ginsenoside Rd or F2 and ginsenosides C-K, ginsenoside Re ginsenoside Rg2 by the enzymes produced from strain GFC01, consecutively hydrolysing glucose of major ginsenoside.

### *In vitro* anti-ageing study

Normal ginseng and fermented ginseng treated cells showed no signs of cytotoxicity at any concentration (0.1–10 µg $mL^{-1}$). Irradiation of the fibroblasts with UVB decreased cell viability to approximately 21% of that of the non-irradiated cells.

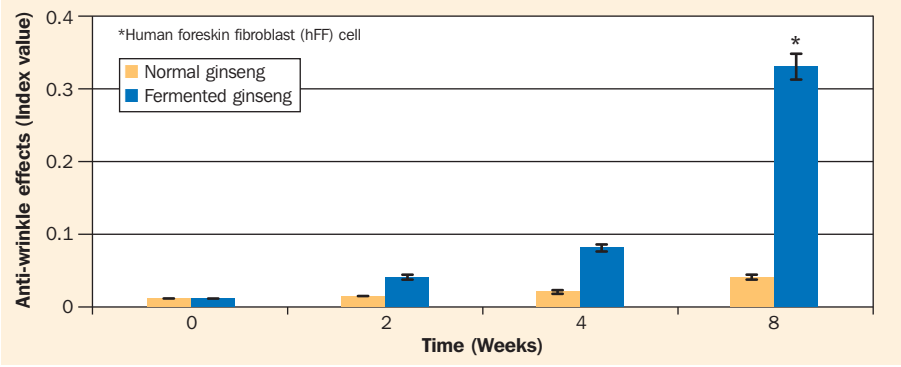



**Figure 5:** *Effect on wrinkle reduction* (in vivo).

Our present study is consistent with these results, as UVB-exposed cells had 520% higher MMP-1 expression than non-exposed cells. However, normal ginseng and fermented ginseng treatment strongly inhibited MMP-1 expression, decreasing it to 19% and 59% of the control in the 50 µg $mL^{-1}$ each treatment group.

We also studied the effects of fermented ginseng on the level of type 1 procollagen in UVA-irradiated cultured HDFs. The protein level was determined in the culture medium by ELISA. Type 1 procollagen expression level increased by 68% and 12% in the presence of 1% fermented ginseng, and 1% normal ginseng respectively (Fig. 4). Epigallocatechin gallate (EGCG), a component of green tea with known anti-ageing properties, was used as a control drug. EGCG decreased MMP-1 expression by a maximum of 62% and increased type 1 procollagen expression by 95% at 1.0 µM. These effects on protein expression were more pronounced in the fermented ginseng treated group than in the normal ginseng treated group.

## Conclusion

The studies show that the fermented ginseng extract from GFC is a highly effective addition to anti-ageing formulations and a much more viable cosmetic active than normal ginseng. The fermented ginseng extract ingredient showed particularly strong efficacy with its effect on procollagen type 1 peptide production in dermal fibroblasts, and also in the *in vivo* anti-wrinkle study.

Fermented ginseng extract therefore offers a great opportunity for cosmetic manufacturers to capitalise on ginseng's health-giving reputation and re-define it as an innovative and highly effective anti-ageing active ingredient. PC