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Effects of pre-fermentation enzyme treatments of leaves, stems, and roots of ginseng on *Lactobacillus* fermentation characteristics and ginsenoside metabolite formation

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Abstract−The extraction and enzymatic treatment conditions of ginseng leaves, stems and roots for the production of fermented ginseng were optimized in order to enhance the extraction of oligosaccharide, which is a *Lactobacillus* growth-activating factor. Additionally, the effects of enzymatic hydrolysis on *Lactobacillus* fermentation characteristics and metabolites of ginsenoside were investigated. The ginseng leaves were found to be more suitable for the raw material of fermented ginseng products because ginseng leaves have higher carbohydrate and crude saponin content than ginseng roots. The optimized conditions were found as particle size of ginseng raw material below 0.15 mm, pH 5.0-5.5, reaction temperature of 55-60 °C, Ceremix concentration of 1%, and reaction time of 2 h. It was shown that the polysaccharides of ginseng were hydrolysed to oligosaccharide by the enzymatic hydrolysis of ginseng leaves, stems and roots. The total oligosaccharide content increased by the enzyme treatment up to 2.2-fold, 5.3-fold and 2.3-fold in ginseng leaves, stems and roots, respectively, compared to control (no treatment). It was found that the enzymatic treatment promoted the *Lactobacillus* growth, resulting in more significant change in total oligosaccharide consumption and total acidity. The content of several metabolites of ginsenoside, such as Compound K, Rg_1, Rh_1 and Rg_3, was selectively increased by combining the enzymatic treatment and *Lactobacillus* fermentation. Especially, in the case of enzyme treatment using ginseng leaves, Compound K formation was enhanced up to three-fold compared to control (no treatment). Moreover, in case of combined treatment of enzyme and fermentation, Compound K formation was significantly promoted up to ten-fold.

Key words: Fermented Ginseng, Enzyme Treatment, Oligosaccharide, *Lactobacillus* Fermentation

INTRODUCTION

Ginseng, a perennial herb that belongs to the Araliaceae Panax family, is indigenous to Korea, China and eastern regions of Siberia. Known for having great nutritional value, an ability to boost immunity and its ability to revitalize the body, it has long been used as a medication since ancient times. Ginseng is known to have the beneficial properties of being an anti-diabetic [1], an anti-inflammatory [2] and an anti-allergetic [3,4]. It is also known for its ability to improve the central nervous system [5], reinforce overall immunity [6], and lower blood pressure [7]. The primary medicinal components of ginseng originate from saponins that are made up of at least 40 kinds of ginsenosides. These benefits also originate from biologically active non-saponins which include polyacetylene, phenolic compounds, acid polysaccharides, peptides, and alkaloids [8-10].

Focus has been shifting to the development of new techniques that can maximize the beneficial properties of ginseng. Research is also being conducted to develop a new, multi-beneficial ginseng product [11-13]. There are research reports on the augmentation of the beneficial effects of ginseng saponin via conversion by heat treatment [14,15], acid treatment [16,17], alkali treatment [18,19].

Microbially, much attention is also being paid to methods in changing the structure of ginsenosides via fermentation. The conversion of ginseng saponin by enzymes originating from microorganisms has been studied, the conversion from ginsenoside Rb_1 to Rd by the enzymes produced by *Rhizopus* sp. [20] and the conversion of Rb_1, Rb_2 by â-glucosidase originating from the bacteria found in human intestines. Park et al. [21] have also reported on the conversion of ginsenoside Rb1 via β-glucosidase originating from bacteria found in human intestines. Yu et al. [22] has presented results on the hydrolysis of ginsenosides by ginsenoside α-L-ramnosisase and Ko et al. [23] have reported that ginsenoside Rg_2, Rh_1, F_1 and other metabolites from a protopanaxatriol-type saponin mixture (Re and Rg1) were obtained by the lactase isolated from *Penicillium sp.*

Ginseng saponins are formed by a combination of one or many saccharides of the triterpenoid series, dammarane framework (glucose, arabinose, xylose, rhamnose, etc). When taken orally, the active ingredient of ginseng is degraded and shows pharmacological action in the gastrointestinal system. However, plant saponins are not metabolized by human digestive enzymes, and are rather known to be metabolically absorbed by *Lactobacillus* and other enterobacteria [24]. It was confirmed that the active ingredient of ginseng is not the ginseng saponin itself, but the metabolites that are transformed from the ginseng saponins by the microorganisms in the intestinal tract [24]. It was found that the metabolic pathway and metabolic rates varied based on the condition of the host that the enterobacteria inhabit [25].

Recently, much interest has been brought to the subject of research on the validity and medicinal effectiveness of ginseng leaves and stems, and on the application of such components [26]. It was found that the saponin content of ginseng leaves was approximately 4 to 5

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times higher than that of the roots, and at least 9 times higher than that of the stems [27-30]. The composition of ginsenosides in different parts of ginseng is not so different; the application value of ginseng leaves and stems as a new natural resource is being reevaluated.

Until now, although there have been many reports on the production of fermented ginseng though the use of *Lactobacillus*, a pre-fermentation enzyme treatment process to increase the efficiency of the fermented ginseng manufacturing process has not been studied. This research aims to optimize the extraction conditions for the enzyme treatment process of ginseng leaves, stems and roots for the extraction of oligosaccharide, a growth factor for *Lactobacilli*. Also, the promotion of *Lactobacillus* propagation by enzyme treatment, as well as the formation of fermentation metabolites, was investigated.

MATERIALS AND METHODS

1. Materials and Reagents

Ginseng leaves, stems and roots aged 6 years were obtained from the Gimpo Agricultural Technology Center (Gimpo, Korea). The polysaccharide breakdown enzyme Ceremix was purchased from Nordisk A/S (Copenhagen, Denmark). For thin layer chomatography (TLC), silca gel 60 F_{254} plate (Merck Co., Germany) was used, and a standard reagent mixture of five kinds of maltooligosaccharides (maltose monohydrate, maltotetraose, maltotriose, maltopentaose, maltoheptaose, maltohexaose) were purchased from Sigma Chemical Corp. (MO, USA). The microorganisms used in the production of fermented ginseng were *Lactobacillus platarum* MIN 107, a microorganism isolated from human excrement, and was obtained from the New Pharm Corporation (Gimpo, Korea). The medium used was MRS broth (Difco Co., MD, USA) and all other reagents were first grade reagents.

2. General Component and Crude Saponin Content Analysis

General components, which include analysis of moisture content, crude ash, crude lipid, crude protein, crude fiber and carbohydrates, were analyzed. The general component analysis followed the Food Code [31]: The crude saponin content was quantified using Red Ginseng Component Analysis Guidelines contained within the Food Code [31]. Ginseng leaves, stems and roots were each divided into 5 g samples and suspended in 50 ml of an aqueous butanol solution. Extraction was performed using a water bath (SWB-10, Jeio Tech, Korea) for 1 h at 85 ºC, and the extraction was repeated three times. Afterwards, the mixture of the fluid extract was separated in a centrifuge (Union 55R, Hanil Co., Korea) for 10 min at 3,200 rpm (1,969 ×g). After the supernatant was poured into separatory funnel, 50 ml of distilled water was added, and the mixture was left alone for 6 h. The layer of water was removed and the aqueous butanol solution layer was enriched at 80 ºC. 50 ml of ether was added, and after 30 min of heating at 46 ºC, the sample was dried at 105 ºC for 20 min in a drying oven. After cooling, the crude saponin content was calculated from the remaining mass.

3. Enzyme Treatement

The method of enzyme treatment was already established in the previous report [26]. Ginseng leaves, stems and roots were pulverized with a KT-34 machine (Korea Medi Co., Ltd., Korea), and separated by particle size (0.15 mm). Distilled water containing 6% (w/v) pulverized ginseng was mixed with 1% (w/w) Ceremix, and incubated at 55-60 ºC for 2 h in a water bath (SWB-10). After enzymatic hydrolysis, the mixture was centrifuged at 3,200 rpm (1,969 ×g) for 15 min by using a Union 55R centrifuge.

4. Thin Layer Chomatography

For thin layer chomatography analysis, 8 ml of isopropanol was added to 1 ml of the enzyme treated ginseng leaves, stems and roots extracts, respectively. These mixtures were then run in a centifuge for 15 min at 3,200 rpm. After centrifugal separation, the supernatant was separated and enriched with nitrogen gas. The enriched sample was then freeze dried. It was dissolved in 1 ml of distilled water and used as the TLC analysis sample. Maltoologosaccharide mix (a mixture of maltose monohydrate, maltotetraose, maltotriose, maltopentaose, maltoheptaose and maltohexaose) was used as the standard for TLC analysis.

5 μl of the standard reagents of 0.5% (w/v) glucose, sucrose and maltooligosaccharide mix, as well as the sample (5 μl), were added in drops to the TLC plate. N-propanol, nitromethane and water were mixed into a 9 : 6 : 5 ratio and developed over 3 h. 3 g of phenol was dissolved into 100 ml of a 5% sulfuric solution prepared with methanol and used as a chomotropic reagent. After the sample was removed, chromotropic reagent was sprayed on the plate. After drying, the plate was placed in a drying oven for 5 min at 100 ºC.

5. Production of Fermented Ginseng

The *Lactobacillus platarum* MIN 107 was cultivated by placing it in an MRS medium at 37 ºC. After the extract was sterilized from the respective parts of the ginseng in a medium, the activated *Lactobacillus* (1×10^9 CFU/ml) was innoculated into the extract at a 1% density. Over the course of 120 h, the innoculated extract samples were kept at 37 ºC and removed for use in analysis.

6. Assesment of Fermentation Characteristies

6-1. Acidity

For each part of the ginseng (leaves, stems and roots), the acidity of the enzyme treated fermentation extract was measured. The pH was adjusted until the 10 ml sample was up to a pH of 8.3 and the consumed volume of 0.05 N-NaOH was converted to lactic acid content (%) [32].

6-2. Viable Cell Count

1 ml of each part of the ginseng (leaves, stems and roots), the acidity of the enzyme treated fermentation extract was added to sterile water to reach a dilution of $10^{7\text{-}9}$ times. The viable cell count was measured as follows. 1 ml of the diluted solution was divided in a petri dish, and the middle layer was cultivated by using an MRS agar (Difco, MD, USA) medium. After the bacteria were cultivated in the medium for 36 to 48 h at a temperature of 37 ºC, a number of colonies formed were counted. The number of colonies was used as the logarithmic value of a colony forming unit (CFU) per 1 ml of each part (leaves, stems and roots) of the enzyme-treated fermentation ginseng extract. The resulting values were calculated by repeating the procedure 3 times, and averaged.

6-3. Analysis of Total Oligosaccharide Content

The reducing sugar content and crude polysaccharide content were subtracted from the total oligosaccharide content, and the remaining value was assumed to be the total oligosaccharide content. The reducing sugar content was analyzed by using the DNS method [33], with glucose as the standard. 1 ml of each part (leaves, stems and roots) of the enzyme treated fermentation ginseng extract was mixed with 3 ml of DNS reagent and was reacted at 85 ºC for

5 min. The resulting reaction was added to 25 ml of distilled water. A microplate reader (VERSAmax, Molecular Device, CA, USA) was used to measure the absorbance of 200 μl of the sample that was reacted on a 96 well-plate.

7. Ginseng Metabolites Analysis

Ginseng metabolite analysis was done by using the HPLC systems (Alliance 2695, Waters, MA, USA). The metabolites were separated Xterra MS C_{18} (3.5 μm×150 mm, Waters, MA, USA) columns. HPLC operation on was done at a speed of 0.25 ml/min, using acetonitrile and water as mobile phases [34,35]. The mobile phase was A (acetonitrile) and B (water): 0-20 min, A 20%; 20-31 min, A from 20 to 32%; 31-40 min, A from 32 to 43%; 40-70 min, A from 45 to 100%; and 70-80 min, the column eluted by 100% A.

RESULTS AND DISCUSSION

1. Analysis of General Components and Crude Saponin Content

Using ginseng leaves, stems and roots aged 6 years from Gimpo were used as raw material for the examination of general components and crude saponin content. The carbohydrate content of ginseng leaves, stems and roots was found to be 69.0%, 82.6% and 80.2%, respectively, as shown in Table 1. These results were compartively higher than a reported case where Kim et al. [36] had found the carbohydrate content of the 6 year aged ginseng leaves, stems and roots to be 55.4%, 36.1% and 65.6%, respectively. The crude saponin content of the ginseng leaves, stems and roots was 24.8%, 4.6% and 5.3%, respectively, and therefore correspond with the research done by Kim et al. [36], in that the crude saponin content of the ginseng leaves was higher, compared to the stems and/or roots. Research done by Chang et al. [27] also presented that in the production of tea using ginseng leaves, as opposed to roots, the saponin content was at least 6.86% (dry basis) and up to 7.5%. As both the carbohydrate and crude saponin content of the ginseng leaves was found to be higher than the ginseng roots, it was suggested that the ginseng leaves would be a better raw material to use in the production of fermented ginseng by enzyme treatment and *Lactobacillus* fermentation.

2. Analysis of Saccharides in Enzyme-treated Ginseng

After the ginseng samples were treated with the complex enzyme Ceremix, thin layer chomatography (TLC) was performed to analyze the change in the saccharides of the ginseng leaf, stem and root extracts. 1 ml of the extracts of both the enzyme treated and the nonenzyme treated leaves, stems and roots were added to 8 ml of isopropanol, and separated in the centrifuge for 15 min at 3,200 rpm. After separation, the supernatant was removed and enriched with nitrogen gas. The enriched sample was freeze dried, dissolved in 1 ml of distilled water, and used as the analysis sample for thin layer chomatography. N-propanol, nitro methane and water were added in a 9 : 6 : 5 (v/v/v) ratio to 0.5% (w/v) glucose, sucrose and maltooligosaccharide mix, as well as 5 μl extracts of both the enzyme-treated leaves, stems and roots (1, 2, 3) and the non-enzyme treated leaves, stems and roots (4, 5, 6). This solvent mixture was developed for 3 h. It was atomized and dried in a drying oven for 5 min at 100 °C. As shown in Fig. 1, it seems that the gingseng roots had higher oligosaccharide content than ginseng leaves and stems. In addition, oligosaccharides of relatively higher molecular weight (such as maltotriose and maltotetraose: M2 and M3 in Fig. 1) were shown in TLC. It was also found that the test group for the ginseng roots also had maltopentaose. More importantly, it was confirmed that the degree of coloration of the oligosaccharide for the test group (enzyme treated ginseng leaves, stems and roots) was higher than for the control group (non-enzyme treated ginseng leaves, stems and roots). These results indicated that the enzymatic treatment enhanced the extraction of oligosaccharides from ginseng leaves, stems and roots.

For more quantitative verification of the oligosaccharde extraction from ginseng raw materials, the measurement of total oligosaccharide content was attempted.

Table 1. Proximate compositions of ginseng leaves, stems and roots
(dry basis, %)

Components	Leaves	Stems	Roots
Carbohydrate	69.0	82.6	80.2
Crude protein	15.5	8.1	12.5
Crude lipid	5.4	1.2	2.0
Crude ash	10.0	8.0	5.5
Crude saponin	24.8	4.6	5.3
Total oligosaccharide	0.9	2.0	3.2

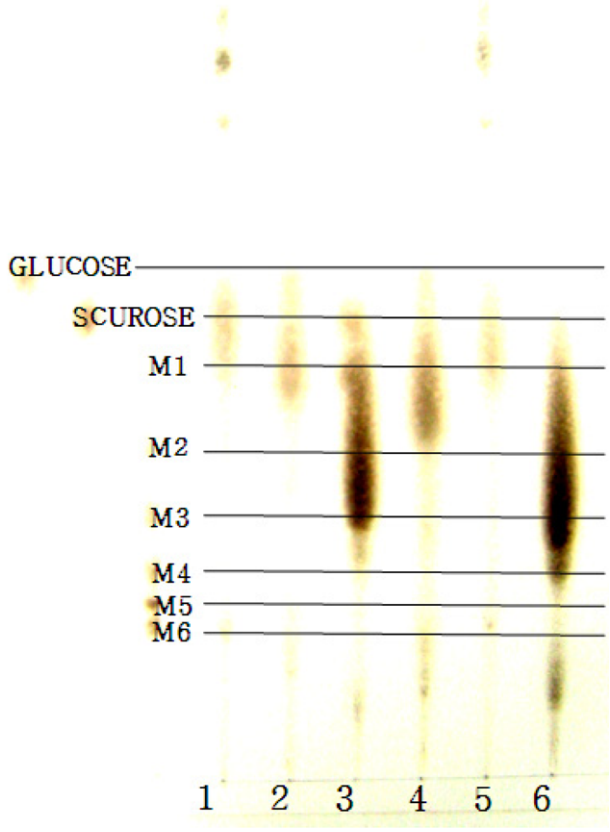

Fig. 1. Thin layer chomatography of enzyme treated ginseng leaves, stems and roots. No. 1: ginseng leaves, No. 2: ginseng stems, No. 3: ginseng roots, No. 4: enzyme treated ginseng leaves, No. 5: enzyme treated ginseng stems, No. 6: enzyme treated ginseng roots, M1: maltose monohydrate, M2: maltotriose, M3: maltotetraose, M4: maltopentaose, M5: maltohexaose, M6: maltoheptaose.

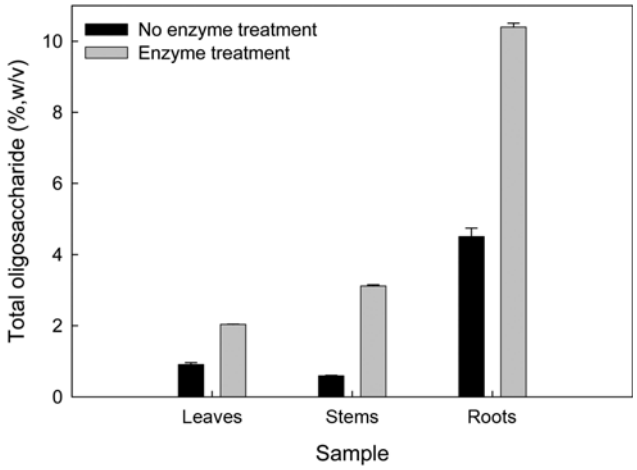

Fig. 2. The effects of enzyme treatment of ginseng on total oligosaccharide content.

Generally, oligosaccharide analysis was carried out though HPLC using identified oligosaccharide standards. In this study, in order to verify the propagation effects by enzyme treatment, rather than analyzing the respective oligosaccharide content, the total enzyme oligosaccharide content was analyzed. However, analysis procedures for total oligosaccharide content have not been established in research reports, so the oligosaccharide content was estimated by subtracting the reducing sugar and crude polysaccharide content from the total saccharide content.

The reducing sugar content was analyzed via the DNS method [33]. Glucose was used for the standard calibration curve. As shown in Fig. 2, the total oligosaccharide content of the enzyme-treated ginseng leaves, stems and roots was 2.0%, 3.1% and 10.4%, respectively, which is 2.2 times, 5.3 times and 2.3 times increase over the content of the untreated ginseng leaves, stems and roots, respectively. As oligosaccharide is an important factor in *Lactobacillus* fermentation, and enzyme treatment is an important factor in the increase in oligosaccharide, it was assessed that enzyme treatment could increase the efficiency of the fermented ginseng production process.

3. Fermentation Characteristics of the Enzyme-treated Ginseng Leaves, Stems and Roots

Lactobacillus platarum MIN 107, isolated from human excrement, was used for the fermentation from respective parts of enzyme-treated ginseng leaves, stems and roots. During fermentation, the acidity, lactic acid bacterial count (LABC) and total oligosaccharide content were measured to verify the characteristics of the fermentation (Figs. 3, 4 and 5). The results indicated that the titratable acidity of the enzyme treatment solution of the respective parts of ginseng leaves, stems and roots before fermentation differed by 0.4%, 0.1% and 0.2%, respectively, regardless of whether it was enzyme treated. Although both the acidity of the non-enzyme-treated group and the enzyme-treated group had sharp increases during fermentation, it was found that 24 h after fermentation the respective acidities of the non-enzyme-treated ginseng leaves, stems and roots were 1.0%, 0.4% and 1.6%, and the respective acidities of the enzyme treated ginseng leaves, stems and roots were 1.1%, 0.6% and 2.3%, evidencing that the acidity of the group treated with the enzyme had

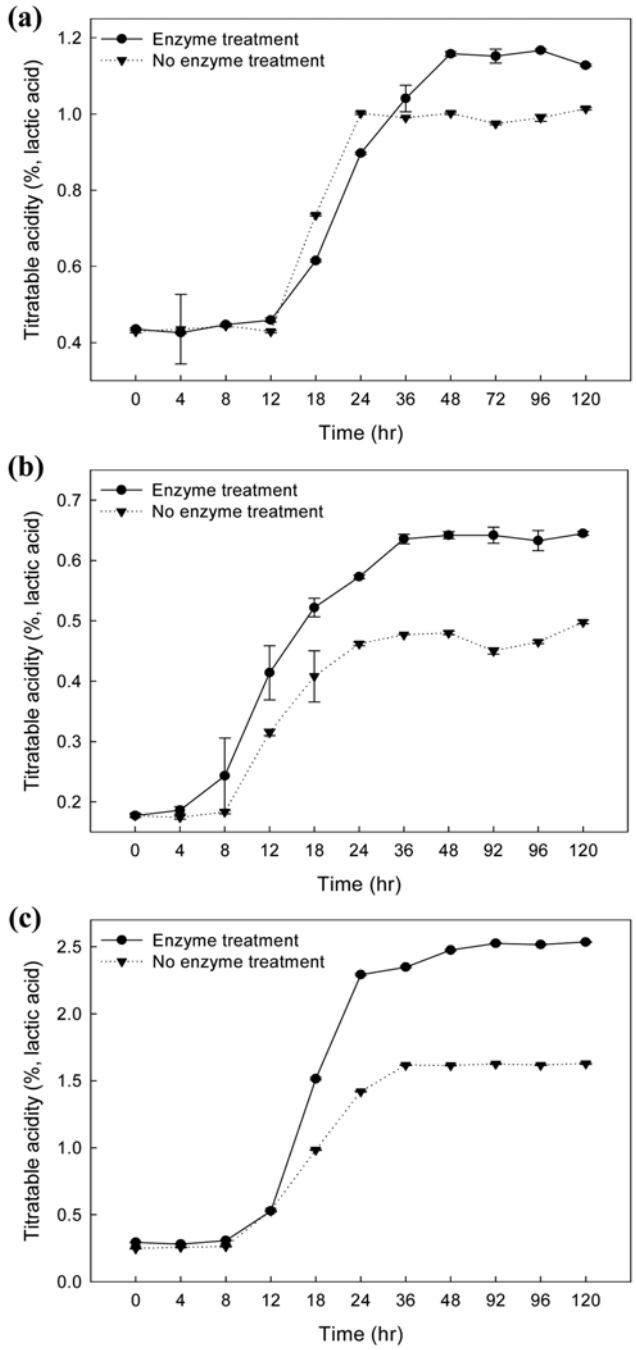

Fig. 3. The courses of titratable acidity in *Lactobacillus platarium* culture using ginseng (a) leaves, (b) stems and (c) roots.

increased. During *Lactobacillus* fermentation, various organic acids and amino acids are produced as metabolites of the *Lactobacilli*. These acids have an influence on the titratable acidity, and are therefore being implied for use as a factor in the *Lactobacillus* growth index. According to the research of Lee et al. [11], in the case that mixed *Lactobacillus* are used with ginseng to produce ginseng-containing yogurt, the acidity in pre-fermentation is 0.29-0.36%. After 24 h of fermentation the titratable acidity level rises to 0.98-1.31%. Total oligosaccharide content, an important factor in *Lactobacillus* propagation, increases as a result of enzyme treatment. Therefore, it was determined that enzyme treatment is a factor in promoting

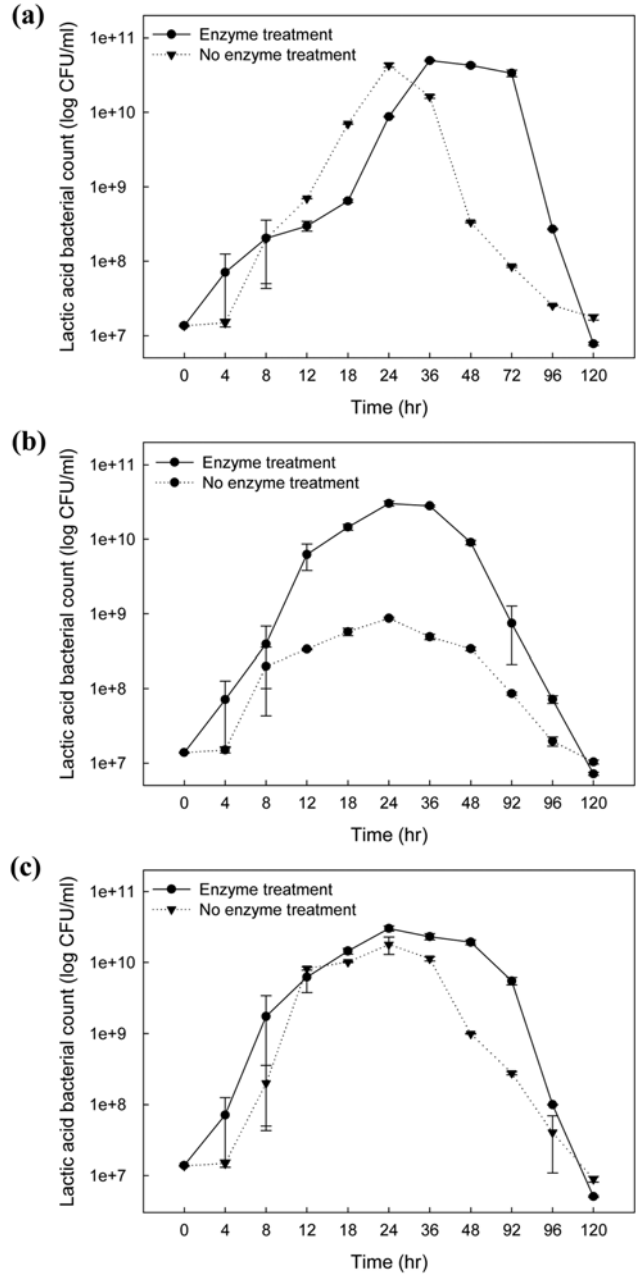

Fig. 4. The courses of lactic acid bacterial count in *Lactobacillus platarium* culture using ginseng (a) leaves, (b) stems and (c) roots.

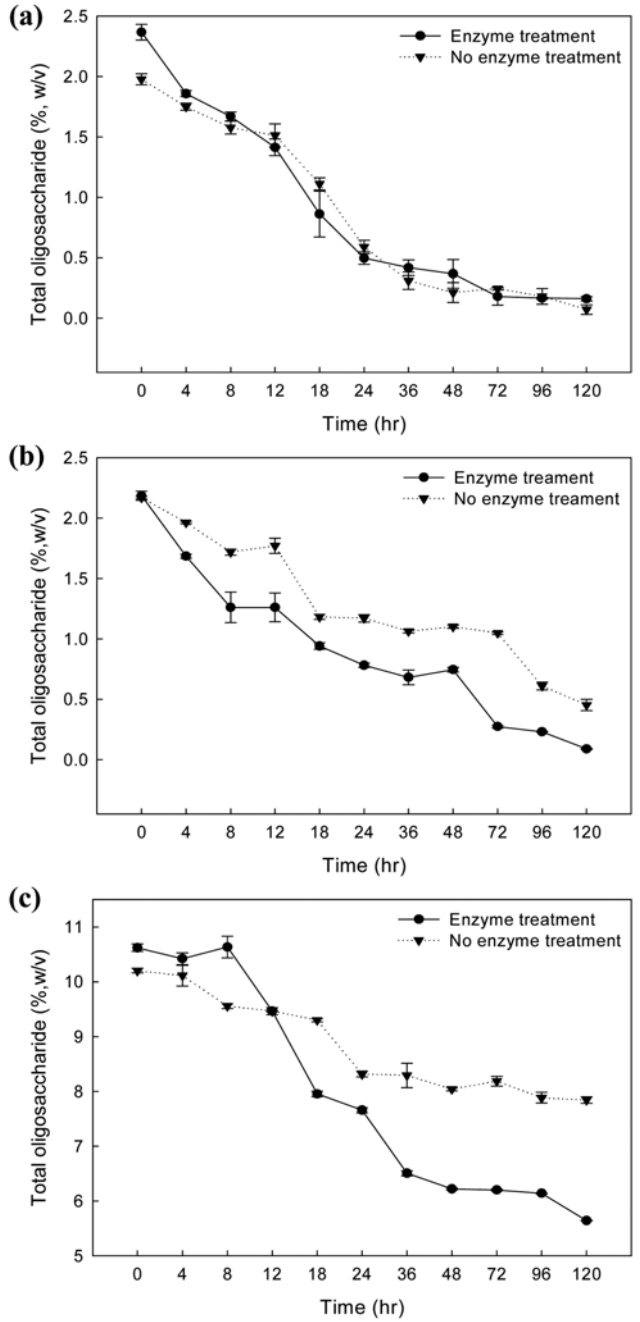

Fig. 5. The courses of total oligosaccharide in *Lactobacillus platarium* culture using ginseng (a) leaves, (b) stems and (c) roots.

the propagation of *Lactobacillus*.

When lactic acid bacterial counts were compared, from the time of initial innoculation to the time that fermentation progressed to 1×10^7 CFU/ml, the *Lactobacilli* in both the non-enzyme treatment group and the enzyme treatment groups had a steep increase in number. After 24-36 h of cultivation time, the *Lactobacillus* count for the non-enzyme treated leaves, stems and roots was 4.3×10^{10} CFU/ml, 8.6×10^8 CFU/ml and 1.8×10^{10} CFU/ml, respectively, and the count for the enzyme treated leaves, stems and roots was 4.9×10^{10} CFU/ml, 3.5×10^{10} CFU/ml and 3.5×10^{10} CFU/ml, respectively. To examine the effect of cultivation time, both the treated and non-treated groups were fermented with *Lactobacilli* for 120 h. The non-treated group showed noticeable decreases in the number of *Lactobacilli* after 36 h, but for 24-48 h, the treated leaves, stems and roots maintained a 2.1 times, 39 times and 2.4 times increase over the non-treated group.

As shown in Fig. 5, initially the total oligosaccharide content of the non-enzyme treated leaves, stems and roots was 1.9%, 2.5 and 10.0%, respectively, and oligosaccharide content of the enzyme-treated leaves, stems and roots was 2.4%, 2.2% and 10.6%, respectively. Although this shows that at the initial stage there was minimal difference, when *Lactobacilli* were added to the samples and fermented for 120 h, the consumption rate of the enzyme treated

samples was found to be higher. Therefore, it was concluded that the ginseng leaves, stems and roots of the enzyme-treated group showed higher levels of *Lactobacilli* propagation when compared to the non-enzyme-treated samples.

4. Analysis of Enzyme-treated and Fermented Ginseng

Ginsenosides are divided into approximately 40 groups [15]. With the exception of the ginsenoside R_o, all the remaining saponins in ginseng belong to the dammarane family. The dammarane family, depending on the non-saccharide section that the hydroxls (-OH) are bonded to, can be divided into the protopanaxadiol (PD) (locations C-3, C-12), or the protopanaxatriol (PT) systems (locations C-3, C-6, C-12). The PD ginsenosides consist of the ginsenoside Ra, Rb_1, Rb_2, Rc and Rd, and the PT ginsenosides consist of ginsenoside Re, Rf, Rg_1 and Rg_2. The six main ginsenosides are composed of the Rb_1, Rb_2, Rc, Rd, Re and Rg_1 saponins, and are reported to occupy at least 90% of the total saponins [15]. The possible transition path for the ginsenosides in the PD is reported to be $Rb_1 \rightarrow$ GypXVII, Rd→GypLXXV, F_2, $Rg_3 \rightarrow$C-K and $Rh_2 \rightarrow$aglycone-ppd, and the possible transition path for the ginsenoside in the PT is reported to be Re→Rg_2, $Rg_1 \rightarrow Rh_1$ and $F_1 \rightarrow$PPT [36].

In this study, ginseng leaves, stems and roots were analyzed in six groups: no treatment (N), enzyme treatment (E), fermentation (F), enzyme treatment and fermentation (E+F), as well as ginsenoside Rb_1, Rg_3, compound K (C-K), Rh_2 of the PD and ginsenoside Rg_1, Rh_1 of the PT as standard calibration curve. As shown in Table 2, when compared with the ginseng leaves, stems and roots of the no treatment (N) or enzyme treatment (E) groups, the fermentation (F) and the enzyme and fermentation (E+F) groups showed a selective increase in the specific metabolites C-K, Rg_1, Rh_1 and Rg_3. The research done states that as saccharides combined with ginseng saponins become degraded, the ginseng saponins are selectively converted [38]. In this research, in the ginseng leaves, the level of C-K in the enzyme-treatment (E) group increased to three-times the level of the no treatment (N) group, while the fermentation (F) and enzyme and fermentation group (E+F) showed at least a 10-fold selective increase.

Conclusively, it was found that the pre-fermentation enzymatic treatment of ginseng was helpful for the ginsenoside metabolite formation.

Table 2. Changes in ginsenoside metabolites content by different treatment of ginseng leaves, stems and roots

Material	method	Ginsenoside content (μg/g)					
		Rg_1	Rh_1	Rb_1	Rg_3	C-K	Rh_2
Leaves	N[a]	380.3	28.55	23.9	1.39	15.86	0.19
	E[b]	298.78	24.98	18.97	3.25	46.6	1.2
	F[c]	553.92	424.02	24.51	78.02	160.74	17.86
	E+F[d]	559.08	479.17	20.76	80.50	174.40	18.36
Stems	N[a]	56.04	9.81	1.56	0.38	3.84	0.02
	E[b]	58.25	9.96	1.08	0.19	2.07	0.01
	F[c]	237.46	40.53	0.31	1.01	0.97	0.42
	E+F[d]	266.76	37.54	0.13	1.62	2.82	0.34
Roots	N[a]	84.38	9.77	56.73	0.77	0.00	0.00
	E[b]	86.08	10.46	59.82	1.21	0.00	0.00
	F[c]	73.1	26.17	53.71	153.31	0.00	0.00
	E+F[d]	76.42	501.04	58.01	208.12	0.00	0.00

[a]N: no treatment

[b]E: enzyme treatment

[c]F: fermentation

[d]E+F: enzyme treatment and fermentation

CONCLUSIONS

To maximize the extraction of oligosaccharides, a factor in the propagation of *Lactobacilli* during fermented ginseng production, ginseng leaves, stems and roots was put though an enzyme treatment process to find the most optimal extraction conditions. It was not only the roots of ginseng that are high in carbohydrate and saponin content, but its leaves and stems are high in content as well. It was found that the ginseng leaves and stems are also good raw materials for the production of fermented ginseng by enzyme treatment and *Lactobacillus* fermentation. After ginseng leaves, stems and roots were crushed into 0.15 mm piece sizes, Ceremix was added at a level of 1% at pH 5.0-5.5 at pH 55-60 °C and allowed to undergo enzyme treatment for 2 h. The results of the thin layer chomatography on the enzyme-treated ginseng leaves, stems and roots revealed that olligosaccharides are released. It was also found that the total oligosaccharide content of the enzyme-treated group of ginseng leaves, stems and roots showed a respective 2.2 times, 5.3 times and 2.3 times greater increase than the non-treated group. As the *Lactobacilli* propagation in the enzyme-treated group of ginseng leaves, stems and roots was activated, there was a great change in both the total oligosaccharide consumption rate and the acidity level. The promotion effect of enzyme treatment on the formation of fermentation metabolites was investigated. When the "fermented" and "enzyme treatment and fermented" groups were compared to the "no treatment" and "enzyme-treated groups", the ginsenoside metabolites Compound K, Rg_1, Rh_1 and Rg_3 of the "fermented" and "enzyme-treated and fermented" groups selectively increased. In the case of the ginseng leaves, C-K increased three-fold in the "enzyme treatment" group as compared to the "no treatment" group, and the "fermentation" and "enzyme-treated and fermentation" group showed a selective increase of more than ten-fold.

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