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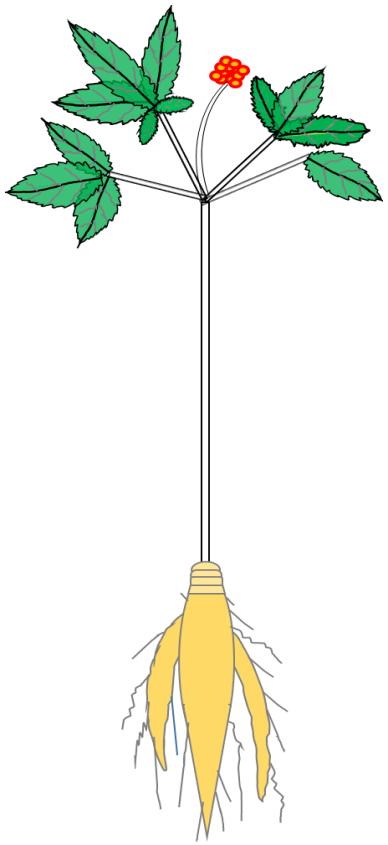
Characterizing New Zealand forest grown ginseng

(*Panax ginseng* C.A. Meyer and *Panax quinquefolius* L.)



Wei Chen

2021

Characterizing New Zealand forest grown ginseng (*Panax ginseng* C.A. Meyer and *Panax quinquefolius* L.)

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Wei Chen

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Abstract

Ginseng is a slow-growing perennial herbaceous plant of the genus *Panax* (Araliaceae family). Ginseng root has been a significant source for natural medicines and has been used for thousands of years in East Asia. Ginsenosides are the unique and bioactive components in ginseng. They are affected by the growing environment and conditions. With the increasing demand for ginseng, wild ginseng is becoming rare, and most of the world's supply of ginseng is from farmed ginseng. In New Zealand (NZ), ginseng has been grown as a secondary crop under a pine tree canopy with an open wild environment for more than 15 years. However, there is neither research on the chemical composition of NZ grown ginseng, nor reports on its biological activity. In this thesis, an LC-QTOF-MS/MS method was developed for characterizing and quantifying ginsenoside components of NZ-grown ginseng. A total of 102 ginsenosides were detected and identified from NZ-grown *P. ginseng*. The total content of ginsenosides in various parts of ginseng varied and was not dependent on age. In the underground parts, ginsenosides Rb1, mRb1, and Re were the main components. Furthermore, the average content of total ginsenosides in NZ-grown ginseng was 40.1 ± 3.2 mg/g (n = 14), which showed significantly ($p < 0.05$) higher concentration than that of China-grown ginseng (16.5 ± 1.2 mg/g, n = 113) and Korea-grown ginseng (21.1 ± 1.6 mg/g, n = 106). The individual ginsenosides Rb1, Re, Rf, and Rg1 from NZ-grown ginseng were 2.2, 2.9, 1.7, and 1.3 times higher than that of ginseng grown in China, respectively. In addition, fifty and forty-three ginsenosides were identified from various parts of NZ-grown *P. ginseng* and NZ-grown *P. quinquefolius* L., respectively, and 29 ginsenosides were found in both ginseng species. In both plants, concentration of Rb1 was highest in the underground parts (fine root, rhizome, and main root), and ginsenoside Re was highest in the aboveground parts (stem and leaf). In terms of improving the use of ginseng resources, ginseng leaves

can be made into black ginseng leaves by steaming for ginsenoside transformation, and the less-polar ginsenoside (Rg3) can be greatly enriched through converting major ginsenosides in the post-harvest process, such as high temperature treatment at low pH. THP-1 cell line was used to evaluate the effects of different NZ-grown ginseng fractions on the productions of inflammatory cytokines. Less-polar ginsenoside fraction extracts (LPG) showed stronger anti-inflammatory effects than high-polar ginsenoside fraction extracts (HPG) on inhibiting pro-inflammatory cytokine (TNF-α, IL-1β, and IL-6) production. Particularly, 100 μg/mL LPG not only significantly inhibited the production of pro-inflammatory cytokines TNF-α, IL-1β, and IL-6, but also remarkably enhanced the production of anti-inflammatory cytokine IL-10. This thesis fills a gap in the chemical composition and biological activity of NZ-grown ginseng and provides methods for improving active components through the post-harvest process.

Keywords: *Panax ginseng*, *Panax quinquefolius* L., Asian ginseng, American ginseng, New Zealand grown ginseng, ginsenosides, LC-MS, HPLC, anti-inflammatory

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List of abbreviations

8-OHdG: 8-hydroxy-2′-deoxyguanosine

11β-HSD1: 11β-hydroxysteroid dehydrogenase type I

Ac: acetyl

Ach: acetylcholine

AChE: acetylcholinesterase

AG: American ginseng

AGEs: advanced glycation end-products

AKT: protein kinase B

ALT: alanine aminotransferase

AMPK: adenosine 5′-monophosphate (AMP)-activated protein kinase

ap2: activating protein

Araf: α-L-arabinofuranosyl

Arap: α-L-arabinopyranosyl

AST: aspartate aminotransferase

AUC: area under curves

Bcl-xL: B-cell lymphoma-extra-large

BG: black ginseng

BPC: base peak chromatogram

Bu: butenoyl

BUN: blood urea nitrogen

BW: body weight

CASP3: caspase-3

CAT: catalase

CCN2: connective tissue growth factor

CEBPα: CCAAT/enhancer binding protein alpha

CHOP: C/EBP homologous protein

CK-MB: creatinine kinase MB

CoA: coenzyme A

CTGF: connective tissue growth factor

cTnI: cardiac troponin I

EIC: extracted ion chromatogram

eNOS: endothelial nitric oxide synthase

ET-1: endothelin-1

FBG: fasting blood glucose

FI: fasting insulin

FOXO1: forkhead box protein O1

FRG: fermented red ginseng

G6Pase: glucose-6-phosphotase

GAP: glyceraldehyde 3-phosphate

Glc: β-D-glucopyranosyl

GlcA: glucuronic acid

GLP-1: glucagon-like peptide-1

GLUT: glucose transporter protein;

GLUT1: glucose transporter protein 1

GLUT2: glucose transporter protein 2

GLUT4: glucose transporter protein 4

GOT: glutamic-oxaloacetic transaminase

GPT: glutamic-pyruvic transaminase

GRP78: glucose-regulated protein 78

GSH (-PX): glutathione peroxidase

HbA1c: hemoglobin A1c

HDL: high- density lipoprotein

HDL-C: high-density lipoprotein cholesterol

HGF: hepatocyte growth factor

HOMA-IR: homeostasis model assessment-insulin resistance

HPG: high-polar ginsenoside fraction

HPLC: high performance liquid chromatogram

ig: intragastric injection

IL: interleukin

ip: intraperitoneal injection

IPP: isopentenyl diphosphate

IR: insulin receptor

IRS: insulin receptor substrate

iv: intravenous injection

JNK: c-Jun N-terminal protein kinase

KRG: Korean red ginseng

LC-MS: Liquid chromatography coupled with mass spectrometry

LDL: low-density lipoprotein

LDL-C: low-density lipoprotein cholesterol

LOD: limit of detection

LOQ: limit of quantification

LPG: less-polar ginsenoside fraction

LPL: lipoprotein lipase

LPS: lipopolysaccharide

Mal: malonyl

MAPK: mitogen-activated protein kinase

MDA: malondialdehyde

MEP: 2-methyl-D-erythritol 4-phosphate

MS: mass spectrometry

MVA: mevalonic acid

NGE: non-ginsenoside fraction extract

NF-κB: nuclear factor–κB

NO: nitric oxide

NZ: New Zealand

NZPG: New Zealand-grown Asian ginseng

NZPQ: New Zealand-grown American ginseng

OA: oleanolic acid

OT: octillol

PAE: polyacetylene

PEPCK: phosphoenolpyruvate carboxykinase

PG: *Panax ginseng*

PI: postprandial insulin

PI3K: phosphoinositide 3 kinase

PPAR: peroxisome proliferators-activated receptors

PPAR-γ2: peroxisome proliferator-activated receptor gamma 2

PPARδ: peroxisome proliferator-activated receptors δ

PPD: protopanaxadiol

PPDS: protopanaxadiol synthase

PPT: Protopanaxatriol

PPTS: protopanaxatriol synthase

PQ: *Panax quinquefolius*

PTP-1B: protein tyrosine phosphatases-1B

QTOF: quadrupole time of flight

RCT: randomized controlled trial

RG: red ginseng

Rha: α-L-rhamnopranosyl

RSD: relative standard deviation

SCr: serum creatinine

SGL: steamed ginseng leaves

Sicam-1: soluble intercellular adhesion molecule 1

SOD: superoxide dismutase

STAT3: signal transducer and activator of transcription 3

STZ: streptozotocin

T2D: type 2 diabetes

T-AOC: total anti-oxidative capacity

TC: total cholesterol

TG: triglycerides

TGE: total ginseng extract

TGF-β: transforming growth factor beta

TLC; Thin layer chromatogram

TNF: tumor necrosis factor

UCP-2: uncoupling protein 2

UCr: urinary creatinine

VEGF: vascular endothelial growth factor

WG: white ginseng

Xyl: β-D-xylopyranosyl

β2-MG: β2-microglobulin

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1. **Chen W**, Balan P, and Popovich DG (2020) Ginsenosides analysis of New Zealand–grown forest *Panax ginseng* by LC-QTOF-MS/MS. J Ginseng Res, 44(4): 552-562. doi: 10.1016/j.jgr.2019.04.007

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12. **Chen W.**, Balan P., Popovich D. G. Ginsenosides profile of New Zealand wild grown *Panax ginseng* using LC-QTOF-MS/MS. The 12th International Symposium on Ginseng, 23-26th Oct 2018, Seoul, South Korea. (**Riddet Student Travel Award**)

13. **Chen W.**, Balan P., Popovich D. G. Ginsenosides analysis for New Zealand wild grown *Panax ginseng*. Annual Meeting of the Nutrition Society of New Zealand, 29-30th Nov 2018, Auckland, New Zealand.

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Chapter One

Background, objectives, and thesis structure

1.1 Background

Ginseng is a slow-growing perennial herbaceous plant belonging to the genus *Panax* (family Araliaceae). Ginseng root has been used as an important source of traditional herbal medicine in East Asia for thousands of years [1]. At least twelve ginseng species have been discovered [2]. Among them, *Panax ginseng* (*P. ginseng*, known as Asian ginseng) and *Panax quinquefolius* (*P. quinquefolius*, known as American ginseng) are the two most important ginseng species due to their medicinal properties. Ginseng has multiple biological activities including anti-ageing, anti-inflammatory, anti-diabetes, and anti-cancer [3-5] and has beneficial effects on the central nervous system, immune system, and cardiovascular system [6, 7]. Since the Song Dynasty (about 1078 A.D.), ginseng has been used to treat diabetes, which was known as Xiaoke disease [8]. Nowadays, ginseng is widely used to develop health-related products and has become one of the best-selling herbs around the world [9].

Phytochemical studies have found that the active ingredient of ginseng is ginsenosides [6, 8]. Ginsenosides are a type of triterpenoid saponins, also called ginseng saponins, which are the unique components in ginseng. According to the chemical structure of the sapogenins, ginsenosides are usually classified into three

main categories: the protopanaxadiol (PPD) type, protopanaxatriol (PPT) type, and oleanolic acid (OA) type (Figure 1.1) [2]. PPD type and PPT type ginsenosides belong to dammarane type saponins, consisting of a four-ring steroid-like aglycone with sugars attached at the C20 and C3 or C6 positions. OA type ginsenosides contain a six-ring oleanolic acid aglycone and sugars link to the C3 and C23 positions of aglycone. Because the composition of ginsenosides can be varied by attaching different kinds and numbers of sugars on the skeleton structure of aglycones, there are various ginsenosides in ginseng.

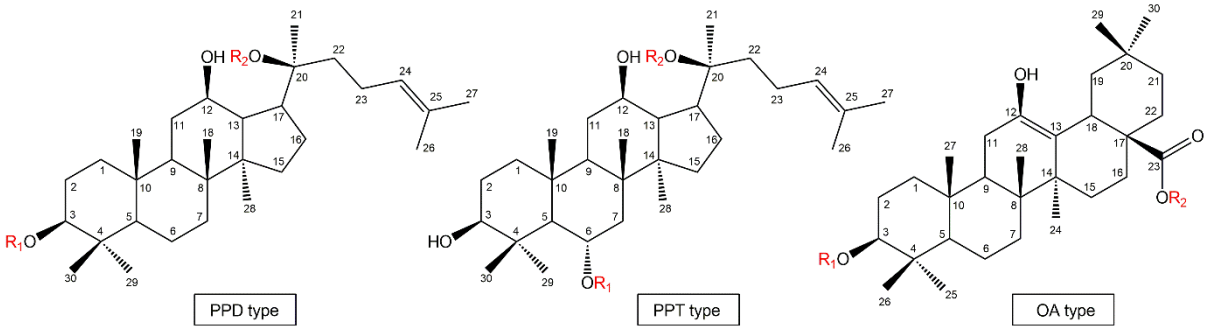

Figure 1.1 The chemical structures of PPD type, PPT type, and OA type ginsenosides and the numbering of skeleton structures

Additionally, the concentration of ginsenosides can also be affected by the growing environment, including light, soil, humidity, temperature, local climate, and seasonal changes [10]. Even within the plant, the type and concentration of ginsenosides are not all the same and are thought to be dependent on the ginseng's age [11], harvest time [12], storage [13], and processing methods [14]. Therefore, the characterization of ginsenosides is very important for ginseng research and practical application, since the content of ginsenosides is regarded as a significant index of the medicinal quality of ginseng [15].

With the increasing demand for ginseng, wild ginseng is becoming very rare, so many ginseng plants have been cultivated and produced commercially in the Northern Hemisphere, such as China, Korea, Japan and other places [16]. In New Zealand (NZ),

P. ginseng (Asian ginseng) has been grown around the Rotorua and Pureora regions in the North Island for about 15 years, and *P. quinquefolius* (American ginseng) has been grown around Nelson in the South Island for many years. Unlike farmed ginseng in other countries, ginseng in NZ has become an established secondary crop grown under a natural pine forest canopy in an open-field simulation of a wild environment (Figure 1.2). Specifically, no pesticides or chemical fertilizers are used, so the ginseng grown naturally.

Figure 1.2 The growing environment of ginseng in New Zealand

Except for several articles about ginseng cultivation two decades ago, there has been no thorough analysis for NZ-grown ginseng; the ginsenoside composition of NZ-grown ginseng, and how it differs compared to ginseng grown in the Northern Hemisphere, is unknown. Thus, the purpose of this thesis is to find the answers to these questions and provide theoretical support for the application of NZ-grown ginseng through conducting this PhD research.

1.2 Objectives

The broad objectives of this project were to add more knowledge about NZ-grown ginseng, provide evidence for the quality control of NZ-grown ginseng, and to promote the research of NZ-ginseng and the development of the NZ-ginseng industry.

Two ginseng species, Asian ginseng (*P. ginseng*) and American ginseng (*P. quinquefolius*), will be used in this thesis. If it is not specified, NZ grown ginseng refers to NZ forest grown Asian ginseng (*P. ginseng*).

The specific objectives of this thesis were:

- To modify and apply a method for quantifying ginsenosides from ginseng extract by high-performance liquid chromatography (HPLC).
- To modify and apply a method for profiling the ginsenoside components of NZ-grown ginseng using liquid chromatography coupled with mass spectrometry (LC-MS).
- To analyze the ginsenoside components of NZ-grown ginsengs and explore the accumulation law of ginsenosides in ginseng of different ages.
- To compare the ginsenoside components of various tissues of NZ-grown Asian ginseng (*P. ginseng*) and American ginseng (*P. quinquefolius*).
- To investigate the differences in ginsenoside content between ginseng grown in NZ and ginseng grown in the Northern Hemisphere (China and Korea).
- To investigate the ginsenoside transformation during the creation of black ginseng leaf.
- To manipulate the ginsenoside profiles and enrich the active ingredients (ginsenoside Rg3) through post-harvest processes.
- To evaluate the effect of NZ-grown ginseng fractions on cytokine production in THP-1 cell model.

1.3 Thesis structure

This thesis contains ten chapters, which can be briefly introduced based on the following six sections.

Section one is Chapter 1, which introduces the background of ginseng, ginsenosides, and NZ-grown ginseng, why this research was undertaken, objectives of this thesis, and thesis structure.

Section two is a literature review, containing Chapters 2 and 3. In Chapter 2, more than 200 ginsenosides isolated and identified from Asian ginseng and American ginseng were described and classified into seven categories according to chemical structures. Also, the ginsenoside transformation pathways of biosynthesis and degradation were summarized and the connections between ginsenosides were determined. This review has been published as a book chapter in Studies in Natural Products Chemistry [17]. Chapter 3 reviews the biological activities of ginseng extracts and ginsenosides, and mainly focuses on the anti-diabetic effect of ginseng in cell studies, animal trials, and human trials, and summarizes the possible mechanisms of ginseng's anti-diabetic effect. This review has been published in Molecules [18].

Section three contains Chapters 4 to 6 and focuses on ginsenoside analysis. Specifically, Chapter 4 applied an LC-QTOF-MS method to characterize ginsenosides and quantitatively analyze ginsenoside components of NZ-grown *P. ginseng* in different parts (main root, fine root, rhizome, stem, and leaf) with different ages. This study has been published in the Journal of Ginseng Research [19]. In Chapter 5, the contents of total ginsenosides and individual ginsenosides were obtained by quantifying ten batches of NZ-grown ginseng, which was compared with that of Northern Hemisphere-grown ginseng (data were extracted from publications). This study has been published in Molecules [20]. Chapter 6 compared the ginsenoside profiles and components of various tissues between NZ-grown Asian ginseng (*P. ginseng*) and NZ-

grown American ginseng (*P. quinquefolium*). This study has been published in Biomolecules [21].

Section four includes Chapters 7 and 8, which focus on manipulating ginsenoside components to improve concentrations of rare active less-polar ginsenosides. In Chapter 7, the change of ginsenosides was monitored when raw ginseng leaf was steamed nine times to create black ginseng leaf, simulating the production process of black ginseng root. This study has been published in Molecules [22]. In Chapter 8, the ginsenoside profile was manipulated through acid treatment, alkali treatment, different pH treatment, and high temperature treatment, respectively. The less polar ginsenosides (Rg3) were greatly enriched from major ginsenosides. This study has been accepted to be published by Traditional Medicine Research.

Section five is Chapter 9, which presents a bioactivity study of NZ-grown ginseng, and mainly focuses on evaluating the effect of different ginseng fractions on pro-inflammatory and anti-inflammatory cytokines production using a THP-1 cell model. This work has been published in Molecules [23].

Section six is Chapter 10 and summarizes the highlights of this PhD research and provides directions for future work.

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Chapter Two

Comparison of the ginsenoside composition of Asian ginseng (*Panax ginseng*) and American ginseng (*Panax quinquefolius* L.) and their transformation pathways ——literature review

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Ginseng is a slow-growing perennial herbaceous plant, belonging to the genus *Panax* of the family Araliaceae. As the two most popular species, Asian ginseng (*Panax ginseng*) and American ginseng (*Panax quinquefolius* L.) have been commonly used for their medicinal properties. Ginseng saponins, known as ginsenosides, the major and unique components of ginseng, have attracted lots of interest. This comprehensive literature review aims at exploring differences in ginsenoside composition between Asian ginseng and American ginseng. From the years between 1963 to the middle of 2017, at least 196 ginsenosides were identified from the roots, leaves, stems, flower buds and fruits of Asian ginseng, and 99 ginsenosides were isolated from American ginseng. Among these saponins, 51 ginsenosides were found in both ginseng varieties. The chemical structures of ginsenosides were described and classified into seven subtypes based on the different skeleton structures. Some ginsenosides are artefacts of the extraction process. The possible biotransformation pathways of ginsenosides, including biosynthesis pathways, degradation pathways, and thermal conversion pathways, were reviewed, and some other interesting saponins were predicted to be potentially present in ginseng based on these biosynthesis pathways. This work will provide a comprehensive reference for the chemical compositions and transformations of ginseng.

Key words: Asian ginseng, American ginseng, *Panax ginseng*, *Panax quinquefolius* L., ginsenosides.

2.1 Introduction

Ginseng is a slow-growing perennial herbaceous plant, belonging to the genus *Panax* of the family Araliaceae. Ginseng root has been a significant source of natural medicines and has been used for thousands of years in East Asia; it is one of the best-selling medicinal plants in the world [1]. Currently, at least twelve species of ginseng have been characterized in the genus *Panax*; some of the better known are *Panax ginseng* (Asian ginseng or Korean ginseng), *Panax quinquefolius* (American ginseng), *Panax notoginseng* (Chinese ginseng, sanqi), *Panax japonicus* (Japanese ginseng), *Panax vietnamensis* (Vietnamese ginseng) among others [2]. Asian ginseng and American ginseng are the two most popular species and are used for their purported medicinal properties.

Ginseng contains multiple active classes of compounds, which include saponins, polysaccharides, alkaloids, polyacetylenes, and phenolic acids. Ginseng saponins, known as ginsenosides are the major bioactive constituents of ginseng. Ginseng plays a significant role in traditional Chinese medicine and healthcare products, Asian ginseng and American ginseng have similar composition of ginsenosides and have similar effects on anti-diabetic activity [3, 4], cancer prevention [5, 6], and immunoregulatory activity [7, 8], and neuro regulation activity [9, 10]. To some extent, the different growing techniques can lead to differences in the content and variety of ginsenosides, which can further influence clinical applications. From a Traditional Chinese Medicine standpoint, American ginseng is viewed as having a cooling effect in nature for increasing 'Yin' energy. It is best used during humid weather conditions, on the contrary, Asian ginseng, especially Korean red ginseng, has a warming effect on promoting 'Yang' energy, which is great in the cooler climates as a tonic to improve overall health and restore the body to balance [11]. Ginseng research is increasing. Since the year 2000, over 3400 articles, including 242 reviews, were published on Asian ginseng, and the research is expected to exponentially increase in the future [12].

There were 425 American ginseng publications sourced from google scholar as of 15 November 2017.

Although there are several reviews available regarding ginsenosides saponins [2, 13-16], there is no comprehensive review focusing on the compositional differences in ginsenosides between Asian and American ginseng. Herein, this literature review summarizes saponins derived from Asian and American ginseng between 1963 to the middle of 2017. The objective of this review was to find the structural relationship between ginsenosides and characterize the possible transformation pathways of ginsenosides. Specifically, I hope to outline how one ginsenoside can relate to another.

2.2 Chemical diversity of saponins derived from Asian ginseng and American ginseng

Ginsenosides are the major bioactive class of compounds from ginseng. Ginsenosides share a four-ring steroid-like skeleton with a glycosidic link to the sugar moiety (Figure 2.1). Variation in types, amount and structural positions of sugar residues leads to the diverse ginsenoside structures [17]. The types of monosaccharides attached to ginsenosides aglycone are pyran arabinose (Arap), furan arabinose (Araf), rhamnose (Rha), xylose (Xyl), glucose (Glc) and glucuronic acid (GlcA). Among these sugar residues, glucose is prone to the further attachment of small molecules such as acetyl, malonyl, and butenyl groups. The sugar residues usually form one or two glycosyl units or chains linked to the C3, C6 or C20 position of the ginsenoside structure. Apart from the diversity of attached sugar residues, modifications of the side-chain at chemical structure positions C17 or C20, dehydrogenization at positions C3, C5 or C12, and stereoisomerism further enrich the structural diversity of ginsenosides [18].

Over the past half-century, at least 198 ginsenosides were identified from the roots, leaves, stems, flower buds and fruits of Asian ginseng, and 104 ginsenosides were

isolated from American ginseng. Among these saponins, 49 ginsenosides were found in both ginseng varieties. Ginsenosides were usually classified into four categories known as protopanaxadiol type (PPD), protopanaxatriol type (PPT), oleanolic acid type (OA) and octillol type (OT) according to the structure of the sapogenins [15]. However, given recent discoveries and the increasing amount of compounds, 253 saponins derived from Asian ginseng and American ginseng were further classified into seven subtypes based on common chemical structural characteristics.

2.2.1 Basic PPD- and PPT-type ginsenosides

PPD-type ginsenosides have sugar residues attached to the β-OH at position C3 and/or C20, and in the PPT group, the attachment of sugar unit(s) occurs at the C6 of α-OH and/or C20 of β-OH. Both belong to the dammarane-type saponins (Figure 2.1) and are abundant in Asian ginseng and American ginseng varieties. Up to now a total of 109 saponins (see Table 2.1, **1**-**109**) of the basic PPD-type (**1**-**65**) and PPT-type (**66**-**109**) ginsenosides have been isolated from Asian ginseng and American ginseng. Among the 109 ginsenosides, 60 of them were only observed in the Asian ginseng, 17 ginsenosides were only found in the American ginseng. The remaining 32 ginsenosides, including 20 typical PPD ginsenosides, such as ginsenoside Rb1, Rb2, Rb3, Rc, Rd, Rg3, Rh2, *etc*. and 12 typical PPT ginsenosides, like ginsenoside Re, Rg1, Rg2, Rh1, *etc*. were jointly found in both ginsengs (Table 2.1). There are several chemical differences between Asian ginseng and American ginseng. For example, the ratios of PPD-group/PPT-group, Rb1/Rg1, and the presence or absence of Rf have been widely used to differentiate Asian ginseng and American ginseng [15]. The presence of ginsenoside Rf usually identifies Asian ginseng, and for Asian ginseng, the PPD/PPT ratio of less than 2 and Rb1/Rg1 of less than five have also been used for identification.

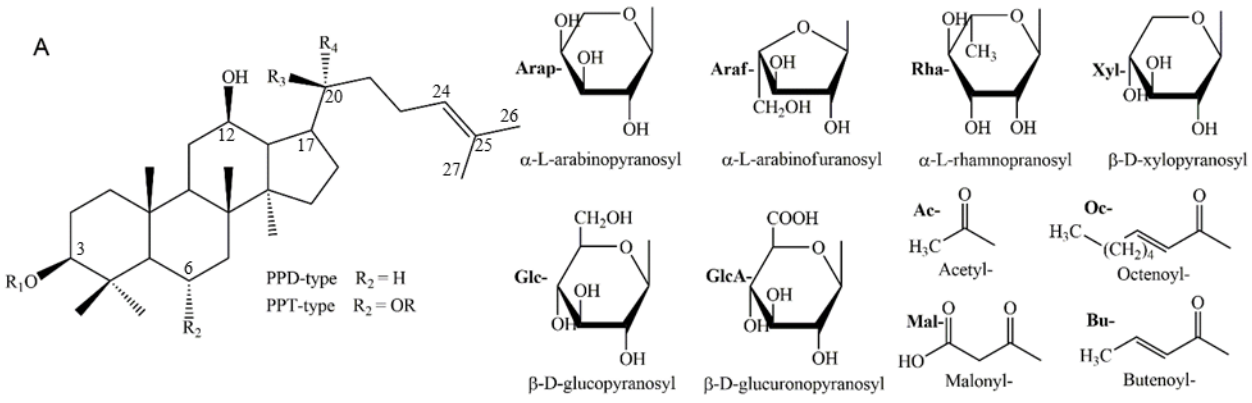


Figure 2.1 Chemical structures of basic PPD and PPT aglycone and monosaccharide residues.

Table 2.1 lists the structural information of 109 typical PPD and PPT ginsenosides derived from Asian and American ginseng. To my knowledge, recent newly discovered ginsenosides, described after 2015 are reviewed here for the first time. The new ginsenosides Rb4 and Rb5 have at the C3 position of the ring a sugar chain with three glucose (Rb4) and four glucose (Rb5), respectively, and these were isolated and identified from biotransformation products of *P. ginseng* with a complex enzyme system [19]. To investigate the less polar ginsenosides in the flower buds of *P. ginseng*, resulted in the isolation of 20(*S*)/20(*R*)-Me-G-Rg3, less polar ginsenosides, with an attachment of methyl to the OH at C20 instead of sugar residues [20]. In addition, abundant malonyl substituted triterpenoid saponins, including 16 PPD types of m-floral-G-Rb1, -Rb2, -Rc1, -Rc2, -Rc3, -Rc4, -Rd1, -Rd2, -Rd3, -Rd4, -Rd5, -Rd6 and m-Rb1, -Rb2, -Rc, -Rd and 3 PPT types of m-floral-G-Re1, -Re2, -Re3, were reported in the flower buds of *P. ginseng* [21].

Table 2.1 Structural information of PPD and PPT ginsenosides derived from *P. ginseng* (PG) and *P. quinquefolius* (PQ)

No.	Name	M.F.	R1 / R2	R3	R4	Source	References
PPD-type (A)							
1	PPD	$C_{30}H_{52}O_3$	H / H	OH	CH_3	Both (roots)	[22, 23]
2	G-Ra1	$C_{58}H_{98}O_{26}$	Glc(2-1)Glc / H	OGlc(6-1)Arap(4-1)Xyl	CH_3	PG (roots)	[24]
3	G-Ra2	$C_{58}H_{98}O_{26}$	Glc(2-1)Glc / H	OGlc(6-1)Araf(4-1)Xyl	CH_3	PG (roots)	[24]
4	G-Ra3	$C_{59}H_{100}O_{27}$	Glc(2-1)Glc / H	OGlc(6-1)Glc(3-1)Xyl	CH_3	PG (roots)	[25]
5	G-Ra4	$C_{62}H_{102}O_{27}$	Glc(2-1)Glc(6)Bu / H	OGlc(6-1)Arap(4-1)Xyl	CH_3	PG (roots)	[26]
6	G-Ra5	$C_{60}H_{99}O_{27}$	Glc(2-1)Glc(6)Ac / H	OGlc(6-1)Arap(4-1)Xyl	CH_3	PG (roots)	[26]
7	G-Ra6	$C_{58}H_{96}O_{24}$	Glc(2-1)Glc(6)Bu / H	OGlc(6-1)Glc	CH_3	PG (roots)	[26]
8	G-Ra7	$C_{57}H_{93}O_{23}$	Glc(2-1)Glc(6)Bu / H	OGlc(6-1)Arap	CH_3	PG (roots)	[26]
9	G-Ra8	$C_{57}H_{94}O_{23}$	Glc(2-1)Glc(4)Bu / H	OGlc(6-1)Araf	CH_3	PG (roots)	[26]
10	G-Ra9	$C_{57}H_{94}O_{23}$	Glc(2-1)Glc(6)Bu / H	OGlc(6-1)Araf	CH_3	PG (roots)	[26]
11	G-Rb1	$C_{54}H_{92}O_{23}$	Glc(2-1)Glc / H	OGlc(6-1)Glc	CH_3	Both (roots)	[27, 28]
12	Ac-G-Rb1	$C_{56}H_{94}O_{24}$	Glc(2-1)Glc(6)Ac / H	OGlc(6-1)Glc	CH_3	PQ (roots)	[29]
13	G-Rb2	$C_{53}H_{90}O_{22}$	Glc(2-1)Glc / H	OGlc(6-1)Arap	CH_3	Both (roots)	[27, 30]
14	G-Rb3	$C_{53}H_{90}O_{22}$	Glc(2-1)Glc / H	OGlc(6-1)Xyl	CH_3	Both (roots)	[31, 32]
15	G-Rb4	$C_{54}H_{92}O_{23}$	Glc(2-1)Glc(4-1)Glc / H	OGlc	CH_3	PG (fungal transformed)	[19]
16	G-Rb5	$C_{60}H_{102}O_{28}$	Glc(2-1)Glc(4-1)Glc(4-1)Glc / H	OGlc	CH_3	PG (fungal transformed)	[19]
17	G-Rc	$C_{53}H_{90}O_{22}$	Glc(2-1)Glc / H	OGlc(6-1)Araf	CH_3	Both (roots)	[27, 28]
18	G-Rd	$C_{48}H_{82}O_{18}$	Glc(2-1)Glc / H	OGlc	CH_3	Both (roots)	[27, 32]
19	G-Rg3	$C_{42}H_{72}O_{13}$	Glc(2-1)Glc / H	OH	CH_3	Both (roots)	[32, 33]
20	20R-G-Rg3	$C_{42}H_{72}O_{13}$	Glc(2-1)Glc / H	CH_3	OH	Both (roots)	[33, 34]
21	20S-Me-G-Rg3	$C_{43}H_{74}O_{13}$	Glc(2-1)Glc / H	OCH_3	CH_3	PG (flower buds)	[20]
22	20R-Me-G-Rg3	$C_{43}H_{74}O_{13}$	Glc(2-1)Glc / H	CH_3	OCH_3	PG (flower buds)	[20]
23	G-Rh2	$C_{36}H_{62}O_9$	Glc / H	OH	CH_3	Both (roots)	[32, 33]

24	20R-G-Rh2	$C_{36}H_{62}O_9$	Glc / H	CH_3	OH	Both (leaves)	[35, 36]
25	G-Rs1	$C_{55}H_{92}O_{23}$	Glc(2-1)Glc(6)Ac / H	OGlc(6-1)Arap	CH_3	Both (red ginseng)	[18, 37]
26	G-Rs2 (Rs11)	$C_{55}H_{92}O_{23}$	Glc(2-1)Glc(6)Ac / H	OGlc(6-1)Araf	CH_3	PG (red ginseng, roots)	[37, 38]
27	G-Rs3	$C_{44}H_{74}O_{14}$	Glc(2-1)Glc(6)Ac / H	OH	CH_3	Both (red ginseng)	[23, 39]
28	20R-G-Rs3	$C_{44}H_{74}O_{14}$	Glc(2-1)Glc(6)Ac / H	CH_3	OH	PQ (red ginseng)	[23]
29	G-F2	$C_{42}H_{72}O_{13}$	Glc / H	OGlc	CH_3	Both (leaves)	[28, 40]
30	G IV (Ra6)	$C_{58}H_{96}O_{24}$	Glc(2-1)Glc(6)Bu / H	OGlc(6-1)Glc	CH_3	PG (roots)	[26, 41]
31	m-G-Ra3	$C_{62}H_{102}O_{30}$	Glc(2-1)Glc(6)Mal / H	OGlc(6-1)Glc(3-1)Xyl	CH_3	PG (roots)	[42]
32	m-G-Rb1	$C_{57}H_{94}O_{26}$	Glc(2-1)Glc(6)Mal / H	OGlc(6-1)Glc	CH_3	Both (flower buds; roots)	[43, 44]
33	m-G-Rb2	$C_{56}H_{92}O_{25}$	Glc(2-1)Glc(6)Mal / H	OGlc(6-1)Arap	CH_3	Both (flower buds; roots)	[23, 43]
34	m-G-Rb3	$C_{56}H_{92}O_{25}$	Glc(2-1)Glc(6)Mal / H	OGlc(6-1)Xyl	CH_3	PG (roots)	[45]
35	m-G-Rc	$C_{56}H_{92}O_{25}$	Glc(2-1)Glc(6)Mal / H	OGlc(6-1)Araf	CH_3	Both (flower buds; roots)	[43, 44]
36	m-G-Rd	$C_{51}H_{84}O_{21}$	Glc(2-1)Glc(6)Mal / H	OGlc	CH_3	Both (flower buds, roots)	[43, 44]
37	m-floral-G Rb1	$C_{57}H_{94}O_{26}$	Glc(2-1)Glc / H	O(4-Mal)Glc(6-1)Glc	CH_3	PG (flower buds)	[21]
38	m-floral-G Rb2	$C_{57}H_{94}O_{26}$	(3-Mal)Glc(2-1)Glc / H	OGlc(6-1)Glc	CH_3	PG (flower buds)	[21]
39	m-floral-G Rc1	$C_{56}H_{92}O_{25}$	(6-Mal)Glc(2-1)Glc / H	OGlc(6-1)Xyl	CH_3	PG (flower buds)	[21]
40	m-floral-G Rc2	$C_{56}H_{92}O_{25}$	(4-Mal)Glc(2-1)Glc / H	OGlc(6-1)Arap	CH_3	PG (flower buds)	[21]
41	m-floral-G Rc3	$C_{56}H_{92}O_{25}$	(3-Mal)Glc(2-1)Glc / H	OGlc(6-1)Arap	CH_3	PG (flower buds)	[21]
42	m-floral-G Rc4	$C_{56}H_{92}O_{25}$	(3-Mal)Glc(2-1)Glc / H	OGlc(6-1)Araf	CH_3	PG (flower buds)	[21]
43	m-floral-G Rd1	$C_{51}H_{84}O_{21}$	(2-Mal)Glc(2-1)Glc / H	OGlc	CH_3	PG (flower buds)	[21]
44	m-floral-G Rd2	$C_{51}H_{84}O_{21}$	(3-Mal)Glc(2-1)Glc / H	OGlc	CH_3	PG (flower buds)	[21]
45	m-floral-G Rd3	$C_{51}H_{84}O_{21}$	(4-Mal)Glc(2-1)Glc / H	OGlc	CH_3	PG (flower buds)	[21]
46	m-floral-G Rd4	$C_{51}H_{84}O_{21}$	Glc(2-1)Glc / H	OGlc(3)Mal	CH_3	PG (flower buds)	[21]
47	m-floral-G Rd5	$C_{51}H_{84}O_{21}$	Glc(2-1)Glc / H	OGlc(6)Mal	CH_3	PG (flower buds)	[21]
48	m-floral-G Rd6	$C_{54}H_{86}O_{24}$	(6-Mal)Glc(2-1)Glc / H	OGlc(6)Mal	CH_3	PG (flower buds)	[21]
49	m-noto-G-R4	$C_{62}H_{102}O_{30}$	Glc(2-1)Glc(6)Mal / H	OGlc(6-1)Glc(6-1)Xyl	CH_3	PG (roots)	[46]
50	Gypenoside XVII	$C_{48}H_{82}O_{19}$	Glc / H	OGlc(6-1)Glc	CH_3	Both (leaves)	[47, 48]

51	Gypenoside IX	$C_{47}H_{80}O_{18}$	Glc / H	OGlc(6-1)Xyl	CH_3	Both (leaves)	[36, 49]
52	G-RAo	$C_{60}H_{100}O_{28}$	Glc(2-1)Glc(2-1)Glc / H	OGlc(6-1)Glc	CH_3	PQ (roots)	[50]
53	P-g-RC1	$C_{50}H_{84}O_{20}$	Glc(2-1)Glc(6)Ac / H	OGlc	CH_3	Both (rhizomes)	[18, 47]
54	P-g-F8	$C_{55}H_{92}O_{23}$	(6-Ac)Glc(2-1)Glc / H	OGlc(6-1)Arap	CH_3	PQ (flower buds)	[18]
55	Q I	$C_{52}H_{86}O_{19}$	Glc(2-1)Glc(6)Bu / H	OGlc	CH_3	PQ (roots)	[28]
56	Q II	$C_{62}H_{104}O_{24}$	Glc(2-1)Glc(6)Oc / H	OGlc(6-1) Glc	CH_3	PQ (roots)	[28]
57	Q III	$C_{50}H_{84}O_{19}$	(6-Ac)Glc(2-1)Glc / H	OGlc	CH_3	PQ (roots)	[28]
58	Q V	$C_{60}H_{102}O_{28}$	Glc(2-1)Glc / H	OGlc(6-1) Glc(4-1)[α-D]Glc	CH_3	PQ (roots)	[28]
59	Q-L10	$C_{47}H_{80}O_{17}$	Glc / H	OGlc(6-1)Arap	CH_3	PQ (leaves and stems)	[51]
60	Q-L14	$C_{47}H_{80}O_{17}$	Glc(2-1)Glc / H	OArap	CH_3	PQ (leaves and stems)	[51]
61	Q-Jb	$C_{59}H_{100}O_{27}$	Glc(2-1)Glc / H	OGlc(6-1)Glc(6-1)Araf	CH_3	PQ (roots)	[52]
62	Q-R1	$C_{56}H_{94}O_{25}$	Glc(2-1)Glc(6)Ac / H	OGlc(6-1)Glc	CH_3	Both (rhizoems)	[37]
63	Noto-G R4	$C_{60}H_{102}O_{29}$	Glc(2-1)Glc / H	OGlc(6-1)Glc(6-1)Glc	CH_3	PG (roots)	[25]
64	Noto-G Fe	$C_{47}H_{80}O_{17}$	Glc / H	OGlc(6-1)Araf	CH_3	PG (roots and rhizomes)	[53]
65	Vina-G-R16	$C_{47}H_{80}O_{17}$	Glc(2-1)Xyl / H	OGlc	CH_3	PG (roots)	[26]
PPT-type (A)							
66	PPT	$C_{30}H_{52}O_4$	H / OH	OH	CH_3	Both (leaves)	[23, 54]
67	G-Re	$C_{48}H_{82}O_{18}$	H / OGlc(2-1)Rha	OGlc	CH_3	Both (roots)	[32, 55]
68	G-Re1	$C_{48}H_{82}O_{19}$	H / OGlc	OGlc(3-1)Glc	CH_3	PG (roots)	[56]
69	G-Re2	$C_{48}H_{82}O_{19}$	H / OGlc(3-1)Glc	OGlc	CH_3	PG (roots)	[56]
70	G-Re3	$C_{48}H_{82}O_{19}$	H / OGlc	OGlc(4-1)Glc	CH_3	PG (roots)	[56]
71	G-Re4	$C_{47}H_{80}O_{18}$	H / OGlc	OGlc(6-1)Araf	CH_3	PG (roots)	[56]
72	G-Re6	$C_{46}H_{76}O_{15}$	H / OGlc	OGlc(6)Bu	CH_3	PG (roots)	[56]
73	m-G-Re	$C_{51}H_{84}O_{21}$	H / O(6-Mal)Glc(2-1)Rha	OGlc	CH_3	PG (roots)	[45]
74	m-floral-G Re1	$C_{51}H_{84}O_{21}$	H / O(6-Mal)Glc(2-1)Rha	OGlc	CH_3	PG (flower buds)	[21]
75	m-floral-G Re2	$C_{51}H_{84}O_{21}$	H / OGlc(2-1)Rha	OGlc(2)Mal	CH_3	PG (flower buds)	[21]
76	m-floral-G Re3	$C_{51}H_{84}O_{21}$	H / OGlc(2-1)Rha	OGlc(4)Mal	CH_3	PG (flower buds)	[21]

77	G-Rf	$C_{42}H_{72}O_{14}$	H / OGlc(2-1)Glc	OH	CH_3	PG (roots)	[55]
78	20-OGlc Rf	$C_{48}H_{82}O_{19}$	H / OGlc(2-1)Glc	OGlc	CH_3	PG (roots)	[31]
79	G-Rf-1a	$C_{42}H_{72}O_{14}$	H / OGlc(4-1)Glc	OH	CH_3	PG (red ginseng)	[57]
80	G-Rg1	$C_{42}H_{72}O_{14}$	H / OGlc	OGlc	CH_3	Both (roots)	[32, 58]
81	Ac-G-Rg1	$C_{44}H_{74}O_{15}$	H / OGlc(6)Ac	OGlc	CH_3	Both (roots)	[28, 45]
82	m-G-Rg1	$C_{45}H_{74}O_{17}$	H / OGlc(6)Mal	OGlc	CH_3	PG (roots)	[45]
83	G-Rg2	$C_{42}H_{72}O_{13}$	H / OGlc(2-1)Rha	OH	CH_3	Both (flower buds; roots)	[32, 55]
84	Ac-G-Rg2	$C_{44}H_{74}O_{14}$	H / O(6-Ac)Glc(2-1)Rha	OH	CH_3	Both (roots)	[45, 59]
85	20R-G-Rg2	$C_{42}H_{72}O_{13}$	H / OGlc(2-1)Rha	CH_3	OH	Both (berries)	[32, 33]
86	20R-Ac-G-Rg2	$C_{44}H_{74}O_{14}$	H / O(6-Ac)Glc(2-1)Rha	CH_3	OH	Both (roots)	[45, 59]
87	Ac-G-Rg3	$C_{50}H_{84}O_{20}$	H / OGlc(4-1)Glc	OGlc(6)Ac	CH_3	PG (red ginseng)	[38]
88	G-Rg18	$C_{48}H_{82}O_{18}$	H / OGlc	OGlc(2-1)Rha	CH_3	PG (red ginseng)	[38]
89	G-Rh1	$C_{36}H_{62}O_9$	H / OGlc	OH	CH_3	Both (roots)	[33, 60]
90	20R-G-Rh1	$C_{36}H_{62}O_9$	H / OGlc	CH_3	OH	Both (roots)	[33, 60]
91	G-Rh19	$C_{36}H_{62}O_9$	Glc / OH	OH	CH_3	PG (stems and leaves)	[61]
92	G-F1	$C_{36}H_{62}O_9$	H / OH	OGlc	CH_3	Both (flower buds; roots)	[40, 60]
93	G-F3	$C_{41}H_{70}O_{13}$	H / OH	OGlc(6-1)Arap	CH_3	Both (leaves; flower buds)	[18, 40]
94	G-F5	$C_{41}H_{70}O_{13}$	H / OH	OGlc(6-1)Araf	CH_3	PG (flower buds)	[62]
95	G-Ia	$C_{42}H_{72}O_{14}$	Glc / OH	OGlc	CH_3	Both (leaves)	[18, 63]
96	Floral-Q E	$C_{53}H_{90}O_{22}$	H / OGlc(2-1)Rha	OGlc(6-1)Xyl	CH_3	PQ (flower buds)	[18]
97	Floral-G-M	$C_{53}H_{90}O_{22}$	H / OGlc(2-1)Rha	OGlc(6-1)Araf	CH_3	PG (flower buds)	[64]
98	Floral-G-N	$C_{53}H_{90}O_{22}$	H / OGlc(2-1)Rha	OGlc(6-1)Arap	CH_3	PG (flower buds)	[64]
99	Floral-G-P	$C_{53}H_{90}O_{23}$	Glc(2-1)Glc / OH	OGlc(6-1)Arap	CH_3	PG (flower buds)	[64]
100	Koryo-G-R1	$C_{46}H_{76}O_{15}$	H / OGlc(6)Bu	OGlc	CH_3	PG (roots)	[65]
101	Q F6	$C_{47}H_{80}O_{18}$	H / OGlc	OGlc(6-1)Araf	CH_3	PQ (fruits)	[66]
102	Q-L17	$C_{47}H_{80}O_{18}$	H / OGlc	OGlc(6-1)Xyl	CH_3	PQ (leaves and stems)	[67]
103	Q-Ja	$C_{54}H_{92}O_{23}$	H / OGlc(2-1)Rha	OGlc(4-1)Glc	CH_3	PQ (roots)	[52]

104	Noto-G R1	$C_{47}H_{80}O_{18}$	H / OGlc(2-1)Xyl	OGlc	CH_3	PG (roots)	[56]
105	Noto-G R2	$C_{41}H_{70}O_{13}$	H / OGlc(2-1)Xyl	OH	CH_3	PG (roots)	[56]
106	Noto-G N	$C_{48}H_{82}O_{19}$	H / OGlc(4-1)Glc	OGlc	CH_3	PG (roots)	[56]
107	Vina-G R4	$C_{48}H_{82}O_{19}$	Glc(2-1)Glc / OH	OGlc	CH_3	PG (leaves)	[68]
108	Ye- D	$C_{44}H_{74}O_{15}$	H / OGlc(6)Ac	OGlc	CH_3	PG (roots)	[56]
109	Saponin I [a]	$C_{37}H_{62}O_{10}$	CHO / OH	Glc	CH_3	PG (leaves)	[69]

[a]: 3-formyloxy-20-*O*-*β*-D-glucopyranosyl-20(*S*)-protopanaxatriol

In the literature, there are some discrepancies in the ginsenoside naming, for example, G-Ra6, one of six new PPD-type ginsenosides isolated from the root of *Panax ginseng*, was identified as (20*S*)-3-*O*-[β-D-6-*O*-[(*E*)-but-2-enoyl] glucopyranosyl-(1→2)-β-D-glucopyranosyl]-20-*O*-[β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl] protopanaxadiol [26]. However, the same compound was named ginsenoside IV by another research group [41]. The same situation happened between ginsenosides Rs2 [37] and Rs11 [38], which have the same structure identified from *Panax ginseng* roots.

2.2.2 C20 side-chain varied PPD- and PPT- type ginsenosides

As shown in Figure 2.2, the side chain attached to C20 can also vary. PPD- and PPT- type aglycones have a four-ring dammarane structure with multiple side chains varied between C22 and C27. The variations in the side chain are mostly involved in peroxidization (B-i, B-ii, B-xvi), hydroxylation (B-iii, B-v, B-vi, B-vii, B-viii, B-xii, B-xv), methoxylation (B-iv, B-xiv), degradation (B-iv, B-ix, B-x), carbonylation (B-ix, B-x, B-xi), dehydrogenation (B-xi, B-xiii), and most of these variations are also accompanied by rearrangement or integration reactions.

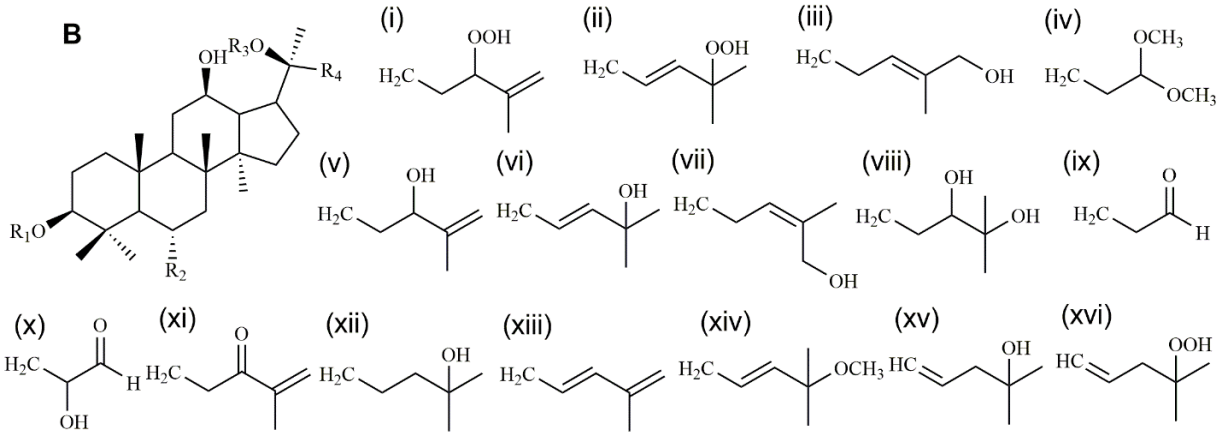


Figure 2.2 Chemical structures of C20 side-chain variations of PPD and PPT ginsenosides

Table 2.2 Structural information of C20 side-chain variations of PPD and PPT ginsenosides derived from *P. ginseng* (PG) and *P. quinquefolius* (PQ)

No.	Name	M.F.	R1	R2	R3	R4	Source	References
C20 side-chain varied PPD-type (B)								
110	25-OH-PPD	$C_{30}H_{54}O_4$	H	H	H	B(xii)	PG (fruits)	[22]
111	G I	$C_{48}H_{82}O_{20}$	Glc(2-1)Glc	H	Glc (24S)	B(i)	Both (flower buds)	[18, 70]
112	G II	$C_{48}H_{82}O_{20}$	Glc(2-1)Glc	H	Glc (24R)	B(i)	Both (flower buds)	[70]
113	G III	$C_{48}H_{80}O_{19}$	Glc(2-1)Glc	H	Glc	B(xi)	Both(flower buds)	[71]
114	G V	$C_{54}H_{92}O_{24}$	Glc(2-1)Glc	H	Glc(6-1)Glc	B(v)	PG (roots)	[41]
115	G-Rg7	$C_{36}H_{60}O_9$	Glc	H	Glc	B(v)	PG (leaves)	[72]
116	G-Rg12	$C_{42}H_{72}O_{15}$	Glc(2-1)Glc	H	H	B(xvi)	PG (roots)	[73]
117	G-Rh12	$C_{36}H_{64}O_{11}$	H	H	Glc	B(viii)	PG (leaves)	[49]
118	G-Rh13	$C_{36}H_{62}O_9$	H	H	Glc	B(vi)	PG (leaves)	[49]
119	Floral-G-E	$C_{42}H_{72}O_{15}$	Glc(2-1)Glc	H	H	B(ii)	PG (flower buds)	[74]
120	Floral-G-F	$C_{42}H_{72}O_{15}$	Glc	H	Glc	B(ii)	PG (flower buds)	[74]
121	Floral-G-G	$C_{50}H_{84}O_{21}$	Glc(2-1)Glc(6)Ac	H	Glc	B(ii)	PG (flower buds)	[75]
122	Floral-G-Kb	$C_{45}H_{76}O_{19}$	Glc(2-1)Glc	H	Glc	B(ix)	PG (flower buds)	[76]
123	Floral-G-Kc	$C_{45}H_{76}O_{20}$	Glc(2-1)Glc	H	Glc	B(x)	PG (flower buds)	[76]
124	Floral-G-O	$C_{53}H_{90}O_{24}$	Glc(2-1)Glc	H	Glc(6-1)Araf	B(ii)	PG (flower buds)	[64]
125	Floral-G-Tc	$C_{53}H_{90}O_{24}$	Glc(2-1)Glc	H	Glc(6-1)Arap (24S)	B(i)	PG (flower buds)	[77]
126	Floral-G-Td	$C_{53}H_{90}O_{24}$	Glc(2-1)Glc	H	Glc(6-1)Arap (24R)	B(i)	PG (flower buds)	[77]
127	Floral-Q D	$C_{42}H_{72}O_{15}$	Glc	H	Glc	B(i)	PQ (flower buds)	[18]
128	Koryo-G-R2	$C_{54}H_{92}O_{24}$	Glc(2-1)Glc	H	Glc(6-1)Glc	B(xv)	PG (roots)	[65]
129	Vina-G-R8	$C_{48}H_{82}O_{19}$	Glc(2-1)Glc	H	Glc	B(vi)	PQ (leaves)	[78]
130	Vina-G-R13	$C_{48}H_{84}O_{20}$	Glc(2-1)Glc	H	Glc	B(viii)	PG (leaves)	[68]
131	Q-L1	$C_{48}H_{80}O_{18}$	Glc(2-1)Glc	H	Glc	B(xiii)	PQ (leaves and stems)	[79]
132	Q-L2	$C_{48}H_{82}O_{19}$	Glc(2-1)Glc	H	Glc	B(vii)	PQ (leaves and stems)	[79]

133	Q-L3	$C_{47}H_{80}O_{18}$	Glc	H	Glc(6-1)Xyl	B(vi)	PQ (leaves and stems)	[80]
134	Q-L16	$C_{54}H_{94}O_{25}$	Glc(2-1)Glc	H	OGlc(6-1)Glc	B(viii)	PQ (leaves and stems)	[51]
135	Q-La	$C_{54}H_{92}O_{23}$	Glc(2-1)Glc	H	Glc(6-1)Arap	B(xiv)	PQ (leaves)	[81]
136	Q-Lc	$C_{54}H_{92}O_{23}$	Glc(2-1)Glc	H	Glc(6-1)Xyl	B(xiv)	PQ (leaves)	[78]
137	majoroside-F1	$C_{48}H_{82}O_{19}$	Glc(2-1)Glc	H	Glc	B(v)	PQ (leaves)	[78]
138	Gypenoside LXIX	$C_{53}H_{90}O_{23}$	Glc(2-1)Glc	H	Glc(6-1)Xyl	B(vi)	PQ (leaves)	[78]
139	Gypenoside LXXI	$C_{53}H_{90}O_{23}$	Glc(2-1)Glc	H	Glc(6-1)Glc	B(v)	PQ (leaves)	[78]
140	Noto-G A	$C_{54}H_{92}O_{24}$	Glc(2-1)Glc	H	Glc(6-1)Glc	B(vi)	PQ (roots)	[28]
141	Noto-G C	$C_{54}H_{92}O_{25}$	Glc(2-1)Glc	H	Glc(6-1)Glc	B(i)	PQ (roots)	[28]
142	Noto-G E	$C_{48}H_{82}O_{20}$	Glc(2-1)Glc	H	Glc	B(ii)	PQ (flower buds)	[18]
143	Noto-G K	$C_{48}H_{82}O_{18}$	Glc(2-1)Glc	H	Glc(6-1)Glc	B(ii)	PQ (roots)	[28]
C20 side-chain varied PPT-type (B)								
144	25-OH-PPT	$C_{30}H_{54}O_5$	H	OH	H	B(xii)	PG (fruits)	[22]
145	G-Re5	$C_{42}H_{72}O_{15}$	H	OGlc(2-1)Glc	H	B(iii)	PG (roots)	[56]
146	G-Re7	$C_{48}H_{82}O_{19}$	H	OGlc(2-1)Rha	Glc	B(xv)	PG (red ginseng)	[38]
147	20*S*-G-Rf2	$C_{42}H_{74}O_{14}$	H	OGlc(2-1)Rha	OH(*S*)	B(xii)	PG (roots)	[45]
148	20*R*-G-Rf2	$C_{42}H_{74}O_{14}$	H	OGlc(2-1)Rha	OH(*R*)	B(xii)	PG (red ginseng)	[82]
149	G-Rh6	$C_{36}H_{62}O_{11}$	H	OH	Glc	B(ii)	PG (leaves)	[72]
150	G-Rh11	$C_{36}H_{60}O_{10}$	H	OH	Glc	B(xi)	PG (leaves)	[49]
151	G-Rh20	$C_{42}H_{72}O_{14}$	H	OGlc(2-1)Rha	H	B(xv)	PG (stems and leaves)	[61]
152	G-Ki	$C_{36}H_{62}O_{10}$	H	OH	Glc	B(vii)	PG (leaves)	[63]
153	G-Km	$C_{36}H_{62}O_{10}$	H	OH	Glc	B(iii)	PG (leaves)	[63]
154	G-M7ed	$C_{36}H_{62}O_{10}$	H	OH	Glc	B(v)	PG (leaves, flower buds)	[83]
155	G-SL1	$C_{36}H_{62}O_{11}$	H	OGlc	H	B(i)	PG (steamed leaves)	[84]
156	G-ST2	$C_{36}H_{62}O_{10}$	H	OGlc	H	B(vi)	PG (steamed leaves)	[85]
157	Floral-G-A	$C_{42}H_{72}O_{16}$	H	OGlc	Glc	B(i)	PG (flower buds)	[74]
158	Floral-G-B	$C_{42}H_{72}O_{16}$	H	OGlc	Glc	B(ii)	PG (flower buds)	[74]
159	Floral-G-C	$C_{41}H_{70}O_{15}$	H	OH	Glc(6-1)Glc	B(i)	PG (flower buds)	[74]

160	Floral-G-D	$C_{41}H_{70}O_{15}$	H	OH	Glc(6-1)Araf		B(ii)	PG (flower buds)	[74]
161	Floral-G-H	$C_{50}H_{84}O_{21}$	Glc(2-1)Glc(6)Ac	OH	Glc		B(i)	PG (flower buds)	[75]
162	Floral-G-I	$C_{48}H_{82}O_{20}$	H	OGlc(2-1)Rha	Glc		B(ii)	PG (flower buds)	[75]
163	Floral-G-J	$C_{48}H_{82}O_{20}$	H	OGlc(2-1)Rha	Glc		B(i)	PG (flower buds)	[75]
164	Floral-G-K	$C_{48}H_{82}O_{21}$	Glc(2-1)Glc	OH	Glc		B(ii)	PG (flower buds)	[75]
165	Floral-G-Ka	$C_{36}H_{62}O_{11}$	H	OH	Glc		B(i)	PG (flower buds)	[76]
166	Floral-G-La	$C_{48}H_{82}O_{19}$	H	OGlc(2-1)Rha	Glc	(24α)	B(v)	PG (flower buds)	[75]
167	Floral-G-Lb	$C_{48}H_{82}O_{19}$	H	OGlc(2-1)Rha	Glc	(24β)	B(v)	PG (flower buds)	[75]
168	Floral-G-Ta	$C_{36}H_{60}O_{10}$	H	OH	Glc		B(xi)	PG (flower buds)	[77]
169	Floral-G-Tb	$C_{35}H_{62}O_{11}$	H	OH	Glc		B(iv)	PG (flower buds)	[77]
170	Floral-Q A	$C_{36}H_{62}O_{11}$	H	OGlc	H		B(ii)	PQ (flower buds)	[18]
171	Floral-Q B	$C_{42}H_{72}O_{15}$	H	OGlc(2-1)Rha	H		B(i)	PQ (flower buds)	[18]
172	Floral-Q C	$C_{42}H_{72}O_{15}$	H	OGlc(2-1)Rha	H		B(ii)	PQ (flower buds)	[18]
173	Q-L9	$C_{42}H_{74}O_{15}$	H	OGlc(2-1)Rha	H		B(viii)	PQ (leaves and stems)	[86]
174	Ginsenjilinol	$C_{42}H_{72}O_{15}$	H	OGlc(2-1)Glc	H		B(iii)	PG (roots and rhizomes)	[53]
175	Saponin II [b]	$C_{30}H_{52}O_5$	H	OH	H		B(iii)	PG (leaves)	[69]

[b]: 26-hydroxyl-24(*E*)-20(*S*)-protopanaxatriol

Table 2.2 shows the structural information about C20 side-chain variations for PPD- and PPT- type ginsenosides. Thirty-four C20 side-chain variations in PPD-type ginsenosides were found (**110-143**) and thirty-two C20 side-chain variations were found for PPT-type ginsenosides (**144-175**). Among these variations, 47 and 22 ginsenosides were isolated from Asian and American ginseng, respectively, and three of them, ginsenoside I, II and III (**111**-**113**) were common in both ginsengs. Interestingly, most of these saponins exist in the flower buds of ginseng.

2.2.3 C17 side-chain variations of PPD and PPT ginsenosides

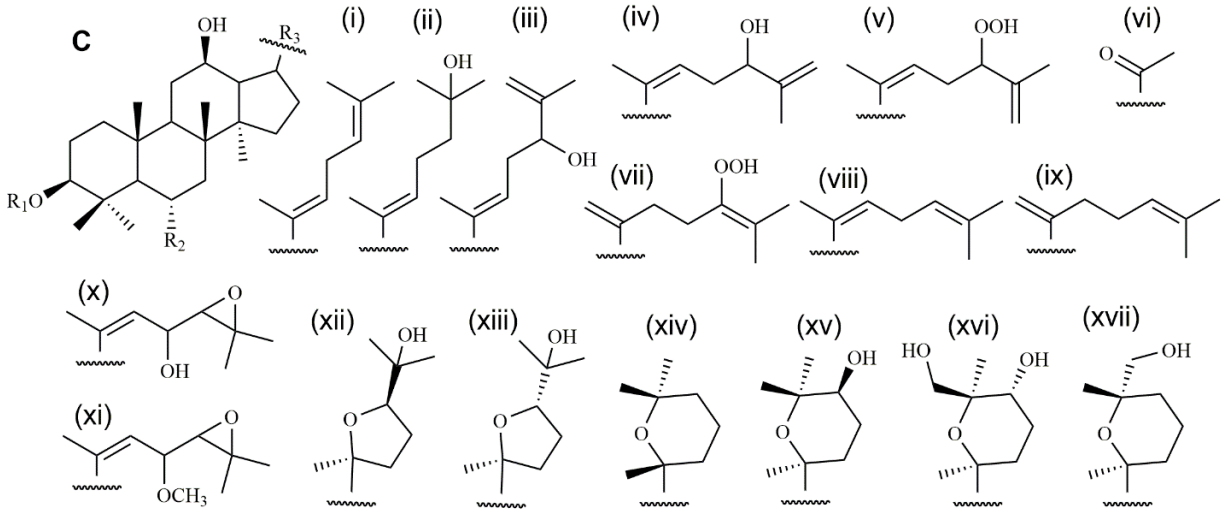


Figure 2.3 Chemical structures of C17 side-chain variation of PPD and PPT aglycones

C17 side-chain varied for both PPD- and PPT- type ginsenosides. PPD and PPT share a four-ring dammarane structure with modification at C20 either dehydration to form a double bond or cyclization to form an oxygen-containing ring. Thus, one of the unique features for these ginsenosides is that there is no substitute group or sugar residue at C20 (Figure 2.3).

Table 2.3 Structural details of C17 side-chain variations of PPD and PPT ginsenosides derived from *P. ginseng* (PG) and *P. quinquefolius* (PQ)

No.	Name	M.F.	R1	R2	R3	Sources	References
C17 side-chain varied PPD-type (C)							
176	PD	$C_{30}H_{52}O_3$	H	H	C(xiv)	PG (hydrolysis)	[54]
177	24,26(OH)$_2$-PD	$C_{30}H_{52}O_5$	H	H	C(xvi)	PG (red ginseng)	[87]
178	24-OH-PD	$C_{30}H_{52}O_4$	H	H	C(xv)	PG (red ginseng)	[87]
179	DH PPD I	$C_{30}H_{50}O_2$	H	H	C(i)	PG (red ginseng)	[2]
180	DH PPD II	$_{30}H_{50}O_2$	H	H	C(ix)	PG (red ginseng)	[2]
181	Saponin III [C]	$C_{30}H_{52}O_4$	H	H	C(xvii)	PQ (stems and leaves)	[88]
182	G-Rg5	$C_{42}H_{70}O_{12}$	Glc(2-1)Glc	H	C(i)	Both (heat-treated)	[34, 89]
183	G-Rg11	$C_{42}H_{70}O_{14}$	Glc(2-1)Glc	H	C(x)	PG (heated roots)	[90]
184	23-MeO-G-Rg11	$C_{43}H_{72}O_{14}$	Glc(2-1)Glc	H	C(xi)	PG (red ginseng)	[57]
185	G-Rh3	$C_{36}H_{60}O_7$	Glc	H	C(viii)	Both (leaves)	[23, 35]
186	G-Rh10	$C_{36}H_{62}O_8$	Glc	H	C(ii)	PG (heated roots)	[90]
187	G-Rh15	$C_{42}H_{70}O_{13}$	Glc(2-1)Glc	H	C(iv)	PG (stems and leaves)	[91]
188	G-Rk1	$C_{42}H_{70}O_{12}$	Glc(2-1)Glc	H	C(ix)	Both (heat-treated)	[23, 92]
189	G-Rk2	$C_{36}H_{60}O_7$	Glc	H	C(ix)	Both (heat-treated)	[23, 92]
190	G-Rs4	$C_{44}H_{72}O_{13}$	Glc(2-1)Glc(6)Ac	H	C(i)	Both (sun ginseng)	[23, 93]
191	20Z- G-Rs4	$C_{44}H_{72}O_{13}$	Glc(2-1)Glc(6)Ac	H	C(viii)	PG (red ginseng)	[57]
192	G-Rs5	$C_{44}H_{72}O_{13}$	Glc(2-1)Glc(6)Ac	H	C(ix)	PG (sun ginseng)	[23, 93]
193	G-Rz1	$C_{42}H_{70}O_{12}$	Glc(2-1)Glc	H	C(viii)	PG (heat-treated)	[94]
C17 side-chain varied PPT-type (C)							
194	PT	$C_{30}H_{52}O_4$	H	OH	C(xiv)	PG (hydrolysis)	[95]
195	DH PPT I	$C_{30}H_{50}O_3$	H	OH	C(i)	PG (red ginseng)	[2]
196	DH PPT II	$C_{30}H_{50}O_3$	H	OH	C(ix)	PG (red ginseng)	[2]
197	Ocotillol	$C_{30}H_{52}O_5$	H (β-OH)	OH	C(xii)	PQ (stems and leaves)	[96]

198	3α- Ocotillol	$C_{30}H_{52}O_5$	H (α-OH)	OH	C(xii)	PQ (stems and leaves)	[96]
199	G-Rg4	$C_{42}H_{70}O_{12}$	H	OGlc(2-1)Rha	C(viii)	PG (leaves)	[97]
200	G-Rg6	$C_{42}H_{70}O_{12}$	H	OGlc(2-1)Rha	C(ix)	Both (red ginseng)	[23, 98]
201	G-Rg8	$C_{42}H_{70}O_{12}$	H	OGlc(2-1)Rha	C(x)	PQ (roots)	[60]
202	20E-G-Rg9	$C_{42}H_{70}O_{13}$	H	OGlc(2-1)Glc	C(i)	PG (red ginseng)	[99]
203	20Z- G-Rg9	$C_{42}H_{70}O_{13}$	H	OGlc(2-1)Glc	C(viii)	PG (red gnseng)	[99]
204	G-Rg10	$C_{42}H_{70}O_{13}$	H	OGlc(2-1)Glc	C(ix)	PG (red ginseng)	[99]
205	G-Rh4	$C_{36}H_{60}O_8$	H	OGlc	C(ix)	Both (red ginseng)	[23, 100]
206	12-OGlc- G-Rh4	$C_{42}H_{70}O_{13}$	H	OGlc	C(i) 12-OGlc	PG (heated roots)	[90]
207	G-Rh5	$C_{36}H_{60}O_9$	H	OGlc	C(iii)	PG (leaves)	[72]
208	G-Rh14	$C_{42}H_{70}O_{13}$	H	OGlc(2-1)Rha	C(iv)	PG (stems and leaves)	[91]
209	G-Rh16	$C_{36}H_{60}O_8$	Glc	OH	C(viii)	PG (stems and leaves)	[91]
210	G-Rk3	$C_{36}H_{60}O_8$	H	OGlc	C(ix)	Both (heat-treated)	[23, 92]
211	G-Rs6	$C_{38}H_{62}O_9$	H	OGlc(6)Ac	C(i)	PG (sun ginseg)	[93]
212	G-Rs7	$C_{38}H_{62}O_9$	H	OGlc(6)Ac	C(ix)	PG (sun ginseng)	[93]
213	G-F4	$C_{42}H_{70}O_{12}$	H	OGlc(2-1)Rha	C(viii)	Both (red ginseng)	[23, 101]
214	20*E*-G-F4	$C_{42}H_{70}O_{12}$	H	OGlc(2-1)Rha	C(i)	Both (roots, red ginseng)	[60, 101]
215	G-SL2	$C_{42}H_{70}O_{14}$	H	OGlc(2-1)Rha	C(v)	PG (steamed leaves)	[84]
216	G-SL3	$C_{42}H_{70}O_{14}$	H	OGlc(2-1)Rha	C(vii)	PG (steamed leaves)	[84]
217	G-ST1	$C_{36}H_{60}O_{10}$	H	OGlc	C(v)	PG (steamed leaves)	[85]
218	Noto-G R10	$C_{30}H_{50}O_9$	H	OGlc	C(vi)	PG (steamed leaves)	[85]
219	P-g RT2	$C_{41}H_{70}O_{14}$	H	OGlc(2-1)Xyl	C(xii)	PQ (metabolites)	[36]
220	P-g RT5	$C_{36}H_{62}O_{10}$	H	OGlc	C(xiii)	PQ (flower buds)	[18]
221	P-g F11	$C_{42}H_{72}O_{14}$	H	OGlc(2-1)Rha	C(xiii)	PQ (roots, flower buds)	[18]
222	24*S*-P-g-F11	$C_{42}H_{72}O_{14}$	H	OGlc(2-1)Rha	C(xii)	Both (rhizomes)	[18, 45]
223	Vina-G R1	$C_{44}H_{74}O_{15}$	H	O(6-Ac)Glc(2-1)Rha	C(xiii)	PQ (flower buds)	[18]
224	Saponin IV [d]	$C_{24}H_{40}O_4$	H	OH	C(vi)	PG (leaves)	[102]

[c]: dammar-20S,25S-epoxy-3β,12β, 26-triol; [d]: 3β,6α,12β-triol-22,23,24,25,26,27-hexanordammaran-20-one

Variations may also be accompanied by H_2O-addition (C-ii), hydroxylation (C- iii, C-iv, C-xv, C-xvi, C-xvii), peroxidization (C-v, C-vii), degradation (C-vi), epoxidation (C-x, C-xi) and so on. The PPD- or PPT- type ginsenoside with a hydroxyl at C20 can lose an H_2O between C20 and C21 to form terminal olefin (C-ix), or between C20 and C22 to form an isomer double structure (C-i, C-viii) depending on the configuration of α/β-OH at C20 when the extraction involves steam or heat-process [2]. That may be the reason why most of these ginsenosides in this subtype were identified from steamed ginseng, known as Sun ginseng or Red ginseng. Structural information for eighteen C17 side-chain varied PPD (**176**-**193**) and thirty-one C17 side-chain varied PPT (**194**-**224**) are shown in Table 2.3. Forty-one ginsenosides were identified from Asian ginseng and 11 of them are found among the 19 ginsenosides derived from American ginseng.

2.2.4 Oleanolic acid types

The oleanolic acid type of saponins share a five-ring oleanane structure, which can be varied at ring position C3 of hydroxyl and C28 of carboxyl (Figure 2.4 and Table 2.4), thus, this type of saponin is not highly diversified. Eight oleanolic acid type saponins (**225**-**232**) were isolated from Asian and American ginseng. Most of these compounds have gluconic acid attached to C3. Ginsenoside Ro (**226**), a representative saponin of oleanolic acid type, was reported abundant in the roots of Asian ginseng and American ginseng [41].

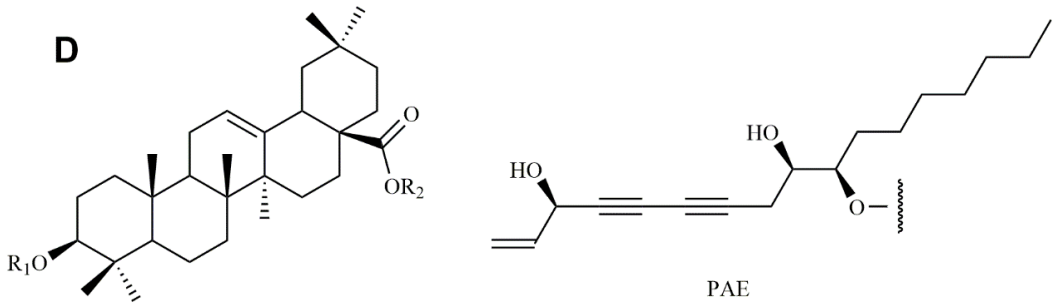


Figure 2.4 Chemical structures of oleanolic acid type, PAE refers to polyacetylene

Table 2.4 Structural variations of oleanolic acid saponins derived from *P. ginseng* (PG) and *P. quinquefolius* (PQ)

No.	Name	M.F.	R1	R2	Sources	References
225	G-Ri	$C_{35}H_{56}O_7$	H	Araf	PG (roots)	[103]
226	G-Ro	$C_{48}H_{76}O_{19}$	GlcA(2-1)Glc	Glc	Both (roots)	[27, 44]
227	G-RoMe	$C_{49}H_{78}O_{19}$	(6-Me)GlcA(2-1)Glc	Glc	PG (rhizomes)	[104]
228	G-RoBu	$C_{52}H_{84}O_{19}$	(6-Bu)GlcA(2-1)Glc	Glc	PG (red ginseng)	[57]
229	Poly Ro	$C_{65}H_{100}O_{21}$	(6-PAE)GlcA(2-1)Glc	Glc	PG (roots)	[105]
230	chikusetsusaponin IVa	$C_{42}H_{66}O_{14}$	GlcA	Glc	Both (metabolites)	[36, 106]
231	zingibroside R1	$C_{42}H_{66}O_{14}$	GlcA(2-1)Glc	H	Both (metabolites)	[36, 106]
232	Calenduloside E	$C_{36}H_{56}O_9$	GlcA	H	PQ (metabolites)	[36]

2.2.5 C3/C12 dehydrogenized types

The most obvious feature of this type of compounds is that the hydroxyl at position C3 and/or C12 changes to carbonyl by dehydrogenization, along with C20 variations. As shown in Figure 2.5 and Table 2.5, eleven saponins (**233**-**243**) were described, in which six of them were isolated from Asian ginseng and the other five were from American ginseng.

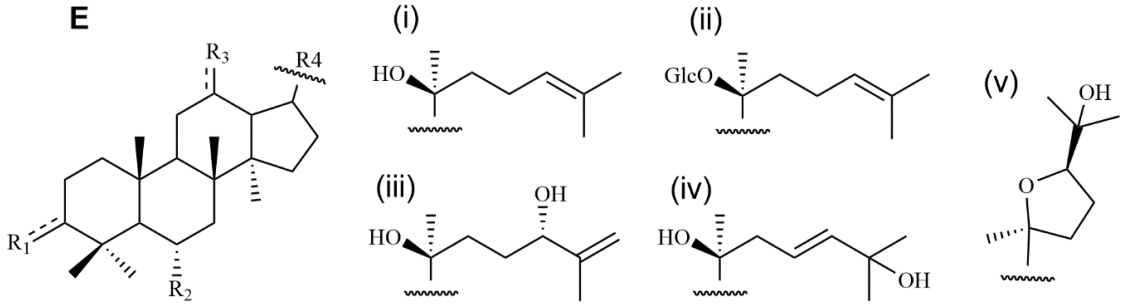


Figure 2.5 Chemical structures of C3/C12 dehydrogenized types

Table 2.5 Structural details of C3/C12 dehydrogenized type of ginsenosides derived from *P. ginseng* (PG) and *P. quinquefolius* (PQ)

No.	Name	M.F.	R1	R2	R3	R4	Sources	References
233	P-g-G1	$C_{42}H_{70}O_{14}$	OGlc(2-1)Glc	H	O	E(v, 24R)	PQ (heat treated)	[107]
234	P-g-G2	$C_{42}H_{70}O_{14}$	OGlc(2-1)Glc	H	O	E(v, 24S)	PQ (heat treated)	[107]
235	Vina-G R3	$C_{48}H_{82}O_{17}$	OGlc(2-1)Glc	H	H	E(ii)	PQ (leaves and stems)	[80]
236	G-Rh8	$C_{36}H_{60}O_{9}$	OH	OH	O	E(ii)	PG (leaves)	[72]
237	G-Rh17	$C_{42}H_{70}O_{13}$	O	OGlc(2-1)Rha	OH	E(i)	PG (stems and leaves)	[91]
238	P-g R1	$C_{30}H_{50}O_{5}$	O	OH	OH	E(v)	PQ (leaves and stems)	[108]
239	P-g RT6	$C_{36}H_{60}O_{10}$	O	OGlc	OH	E(v)	PQ (leaves and stems)	[108]
240	Saponin V [e]	$C_{30}H_{50}O_{4}$	O	OH	OH	E(i)	PG (leaves)	[109]
241	Saponin VI [f]	$C_{30}H_{48}O_{4}$	O	OH	O	E(i)	PG (leaves)	[110]
242	Saponin VII [g]	$C_{30}H_{48}O_{5}$	O	OH	O	E(iii)	PG (leaves)	[110]
243	Saponin VIII [h]	$C_{30}H_{48}O_{5}$	O	OH	O	E(iv)	PG (leaves)	[110]

[e]: 3-keto-20(*S*)-protopanaxatriol

[f]: 6*α*,20(*S*)-dihydroxydammar-3,12-dione-24-ene

[g]: 6*α*,20(*S*),24(*S*)-tri-hydroxydammar-3,12-dione-25-ene

[h]: 6*α*,20(*S*),25-trihydroxydammar-3,12-dione-23-ene

2.2.6 12, 23-oxepane-dammar type

Saponins of 12,23-oxepane-dammar type may be derived from basic PPD or PPT types by forming an ether bond between C12 and C23 to become the fifth ring with seven-

member heterocycle. A total of seven 12,23-oxepane-dammar type ginsenosides (**244**-**250**) were found in Asian ginseng and American ginseng, and their structures were displayed in Figure 2.6 and Table 2.6.

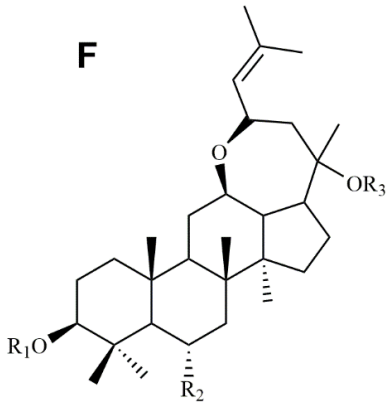


Figure 2.6 Chemical structures of 12,23-oxepane-dammar type

Table 2.6 Structural information of 12,23-oxepane-dammar type ginsenosides derived from *P. ginseng* (PG) and *P. quinquefolius* (PQ)

No.	Name	M.F.	R1	R2	R3	Sources	References
244	G-Rh9	$C_{36}H_{60}O_9$	H	OH	Glc	PG (leaves)	[72]
245	G-Rh18	$C_{48}H_{80}O_{18}$	H	OGlc(2-1)Rha	Glc	PG (stems and leaves)	[61]
246	G-La	$C_{42}H_{72}O_{13}$	Glc	H	Glc	PG (leaves)	[111]
247	Q-Lb	$C_{53}H_{88}O_{22}$	Glc(2-1)Glc	H	Glc(6-1)Xyl	PQ (leaves)	[81]
248	Q-Ld	$C_{48}H_{80}O_{18}$	H	OGlc(2-1)Rha	Glc	PQ (leaves)	[112]
249	Q-Le	$C_{48}H_{80}O_{18}$	Glc(2-1)Glc	H	Glc	PQ (leaves)	[112]
250	Saponin IX[i]	$C_{42}H_{70}O_{14}$	H	OGlc	Glc	PG (leaves)	[113]

[i]: 3,6,20(*S*)-trihydroxy-12,23-epoxydammar-24-ene,6,20-di-*O*-*β*-D-glucopyranoside

2.2.7 C5 dehydrogenized type

C5 dehydrogenized type is a minor saponin in ginseng, which may be derived from basic PPD and PPT types by dehydrogenization reaction between C5 and C6 to form a double bond in the second ring, shifting the hydroxyl from C6 to C7. Three ginsenosides (**251**-**253**) have been found in Asian ginseng and American ginseng (Figure 2.7 and Table 2.7).

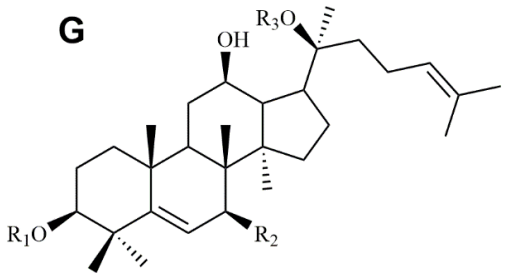


Figure 2.7 Chemical structures of C5 dehydrogenized type

Table 2.7 Structural information of C5 dehydrogenized type ginsenosides derived from *P. ginseng* (PG) and *P. quinquefolius* (PQ)

No.	Name	M.F.	R1	R2	R3	Sources	References
251	G-Rh7	$C_{36}H_{60}O_9$	H	OH	Glc	PG (leaves)	[72]
252	Q IV	$C_{54}H_{90}O_{24}$	Glc(2-1)Glc	OH	OGlc(6-1) Glc	PQ (roots)	[28]
253	Noto-G G	$C_{48}H_{80}O_{19}$	Glc(2-1)Glc	H	Glc	PQ (flower buds)	[18]

Overall, 253 ginsenosides were described in this chapter. On the aspect of the source, 149 ginsenosides were only found in Asian ginseng, 55 saponins were only observed in American ginseng, and 49 ginsenosides coexisted in both plant species. On the aspect of structure, the majority of ginseng saponins are PPD-type and PPT-type ginsenosides,

only 29 ginsenosides have different skeleton structures. Also, there is a small difference between the amount of PPD-type and PPT-type ginsenosides. Details about the distribution of ginseng saponins exhibit in Figure 2.8.

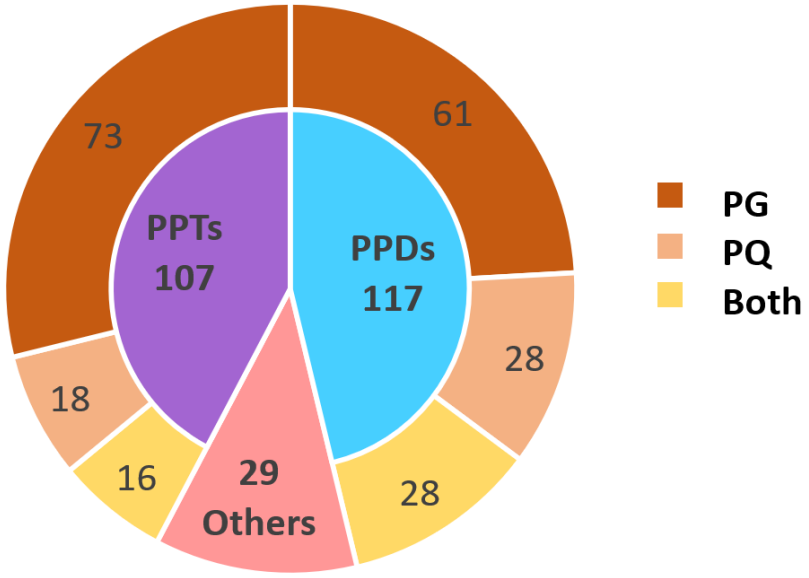


Figure 2.*8* Distributions of 253 saponins reviewed in this chapter.

2.3 Possible transformation pathway

2.3.1 Biosynthesis pathways of ginsenosides

As described above, hundreds of ginseng saponins have been found in Asian and American ginseng, and all of them belong to the triterpenoid group of compounds. In plants, triterpenes are synthesized from isopentenyl diphosphate (IPP) which is produced via MVA (mevalonic acid) pathway in the cytosol and MEP (2-methyl-D-erythritol 4-phosphate) pathway in the chloroplast [114]. In *P. ginseng*, biosynthesis of triterpenes and sterols have the same precursor 2,3-oxidosqualene produced by a series of synthesis reactions initiated from acetyl CoA through the MVA pathway [115]. 2,3-

oxidosqualene is cyclized and converted to β-amyrin by β-amyrin synthase and dammarenediol by dammarenediol-II synthase, respectively. Then β-amyrin is converted to oleanane-type ginsenosides, like G-Ro, through several steps, such as hydroxylation and glycosylation [116]. And dammarenediol is added a hydroxyl at C12 by protopanaxadiol synthase (PPDS) to form protopanaxadiol, which is further hydroxylated at C6 to yield protopanaxatriol under the catalysis of protopanaxatriol synthase (PPTS) [117, 118]. The possible biosynthetic route of ginsenosides is shown in Figure 2.9. Under the processes of glycosylation and/or acetylation, malonylation, butenoylation, PPD and PPT are converted to basic PPD-type (Rh2, Rg3, Rd, Rc, Rb1, Rb2, etc.) and PPT-type (Rh1, Rg1, Rg2, Rf, Re, etc.) ginsenosides (Figure 2.1). These can be further modified (carbonylation, dehydration, epoxidation, hydroxylation, etc.) to produce the different variations in PPD-type and PPT-type ginsenoside structure as shown in Figures 2.2 and 2.3.

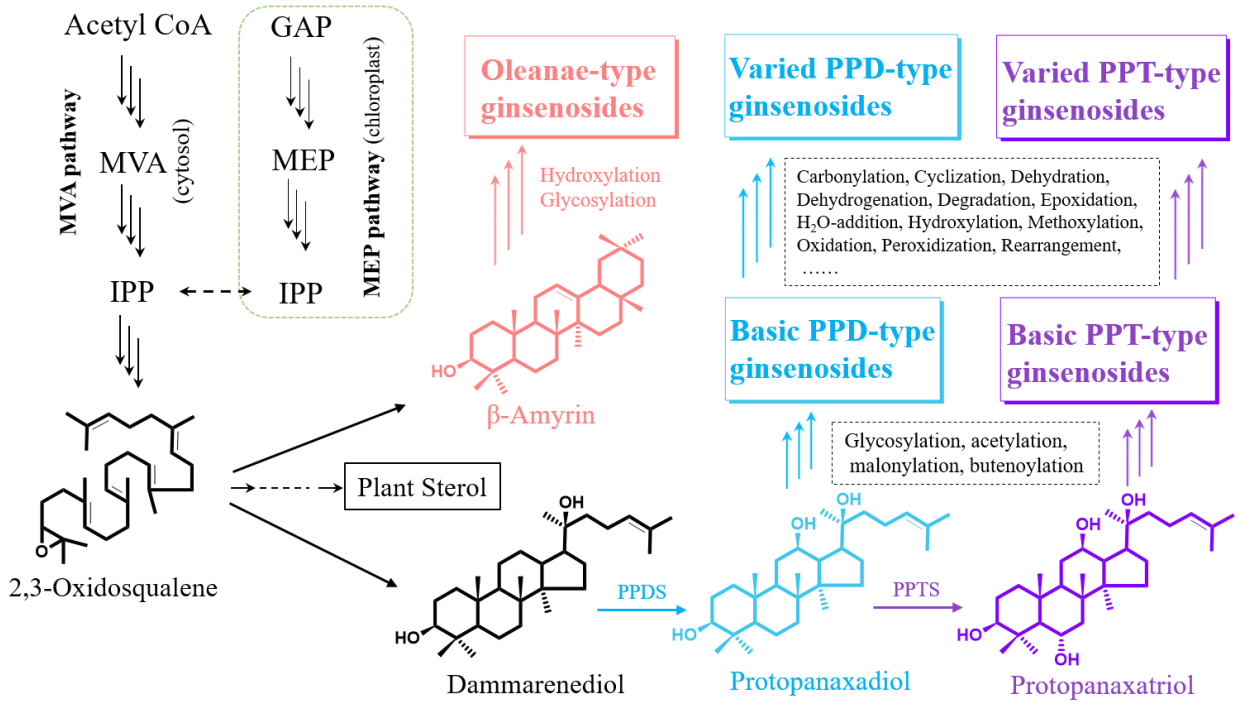


Figure 2.9 Possible biosynthetic pathways of ginsenosides. (CoA, coenzyme A, GAP, glyceraldehyde 3-phosphate; IPP, isopentenyl diphosphate; MEP, 2-methyl-D-erythritol 4-phosphate; MVA, mevalonic acid; PPDS, protopanaxadiol synthase; PPTS, protopanaxatriol synthase)

2.3.2 Degradation pathways of the major ginsenosides

Ginsenosides are the major and unique components in ginseng and share a similar structure. In *Panax ginseng*, ginsenosides account for at least 4% of the dry weight of roots [119]. PPD-type and PPT-type ginsenosides are the main saponins, among these compounds, ginsenoside Rb1, Rb2, Rc, Rd, Re, Rf, and Rg1 make up the seven major ginsenoside constituents [120, 121]. While studies [122-124] have demonstrated that these major ginsenosides have limited absorption from the gastrointestinal tract, they have low

concentration in the blood, and cannot be efficiently utilized by human body. On the contrary, some minor ginsenosides such as Rg2, Rg3, Rh1, and Rh2 which are deglycosylated from major ginsenosides, are reported to be more dynamically absorbed into the bloodstream and likely act as the bioactive component of ginseng [124]. Some major ginsenosides have a high concentration in ginseng roots, but they have a large molecule weight, and they are not the active forms in human body. The active forms of the ginsenosides usually have a small molecule weight, this conversation can occur in the stomach and intestine when people consume the ginseng products. Some microbial communities, especially intestinal microflora, are able to transform major ginsenosides into smaller and more active forms [125, 126].

Figure 2.10 shows the detailed degradation pathways of the major ginsenoside Rb1, Rb2, and Rc based on the hypothesis that only one monosaccharide can be removed from ginsenosides of either the C3 position or the C20 position at one time. There are two glucosides (-Glc-Glc) attached to both the C3 and C20 position of ginsenoside Rb1, which initially lose a sugar to either form ginsenoside Rd with a Glc removal from C20 or produce the compound M9 by removing a Glc from C3. In the same way, ginsenoside Rd can again lose a sugar from C3 position to generate compound F2 or lose one from C20 position to produce ginsenoside Rg3, then compound F2 and ginsenoside Rg3 continue to lose a Glc at C3 position to produce compound M1 (C-K) and ginsenoside Rh2, respectively. Similarly, compound M9 can also be de-glycosylated to form compound M1 and ginsenoside Rh2 via compound F2 or M13, respectively. Compound M1 and ginsenoside Rh2 can further lose a single glycosyl to yield the protopanaxadiol aglycone. Except for the same side sugar units (-Glc-Glc) at C3 position compared to ginsenoside Rb1, ginsenoside Rb2 and Rc have a terminal arabinopyranose (-Glc-Arap) and an arabinofuranose (-Glc-Araf) residue attached to a C20 position of the aglycone, respectively. This leads to the Rb2 → M6 → M2 → M1 pathway for ginsenoside Rb2 and

Rc → M7 → M3 → M1 pathway for ginsenoside Rc, except for the common pathway Rd → F2/Rg3 → M1/Rh2. These pathways were supported by the detection of some metabolic products (M1, M2, M3, M6) in the microbial transformation of ginsenoside Rb1, Rb2 and Rc [127, 128]. The metabolites, ginsenoside Rh2 and compound M1, may further lose their single glucose to obtain the protopanaxadiol aglycone.

Name	C3	C20
Rb1	OGlc-Glc	OGlc-Glc
Rb2	OGlc-Glc	OGlc-Arap
Rc	OGlc-Glc	OGlc-Araf
Rd	OGlc-Glc	OGlc
Rg3	OGlc-Glc	OH
F2	OGlc	OGlc
Rh2	OGlc	OH
M1(C-K)	OH	OGlc
M2(C-Y)	OH	OGlc-Arap
M3(Mc)	OH	OGlc-Araf
M6(C-O)	OGlc	OGlc-Arap
M7(Mb)	OGlc	OGlc-Araf
M9(Gyp-XVII)	OGlc	OGlc-Glc
M13(Gyp-LXXV)	OH	OGlc-Glc
PPD	OH	OH

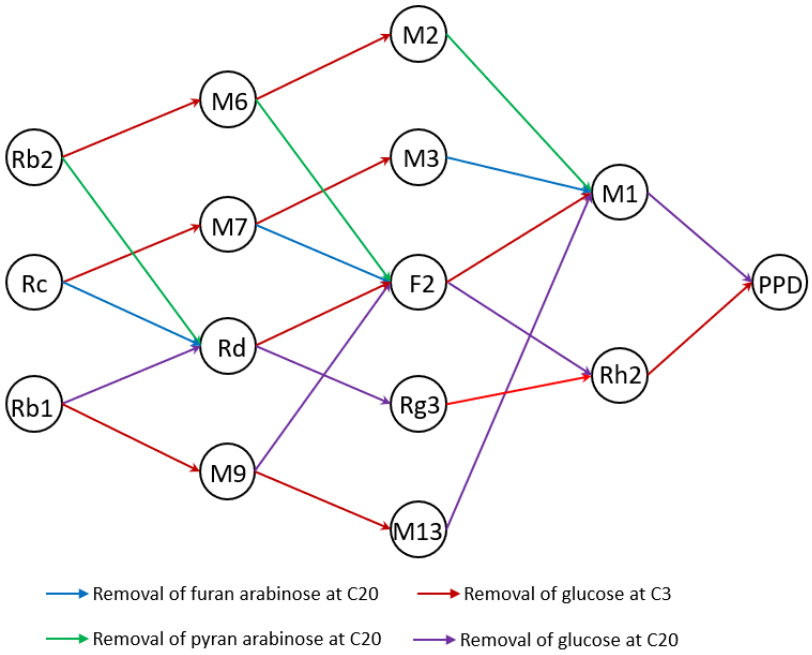


Figure 2.10 Possible biotransformation pathways of ginsenoside Rb1, Rb2 and Rc.

PPT-type ginsenosides are another important variety of saponins in ginseng species. They have a similar degradation pathway to that of PPT-type ginsenosides, compared to the degradation of major protopanaxadiol ginsenosides Rb1, Rb2 and Rc, ginsenosides Re and Rg1, the major protopanaxatriol, follow a comparatively simpler route. As shown in Figure 2.11, ginsenoside Re can lose the terminal rhamnose from –Glu-Rha sugar linkage at C6 to form ginsenoside Rg1 or lose glucose at C20 to produce ginsenoside Rg2. Then, ginsenoside Rg1 can again lose a sugar from C6 position to generate compound F1

or from C20 position to produce ginsenoside Rh1 which can also form by losing the terminal rhamnose from the C6 position of ginsenoside Rg2. On the other hand, 20-glucose-ginsenoside Rf may lose glucose from C20 position to yield ginsenoside Rf and further lose glucose from C6 position to gain ginsenoside Rh1, too. Compound F1 and ginsenoside Rh1, the main metabolites detected from the degradation of the major PPT-type ginsenoside [127, 129], may again lose the single glucose to yield the aglycone protopanaxatriol.

Name	C6	C20
Re	OGlc-Rha	OGlc
20-Glc-Rf	OGlc-Glc	OGlc
Rf	OGlc-Glc	OH
Rg1	OGlc	OGlc
Rg2	OGlc-Rha	OH
Rh1	OGlc	OH
F1	OH	OGlc
PPT	OH	OH

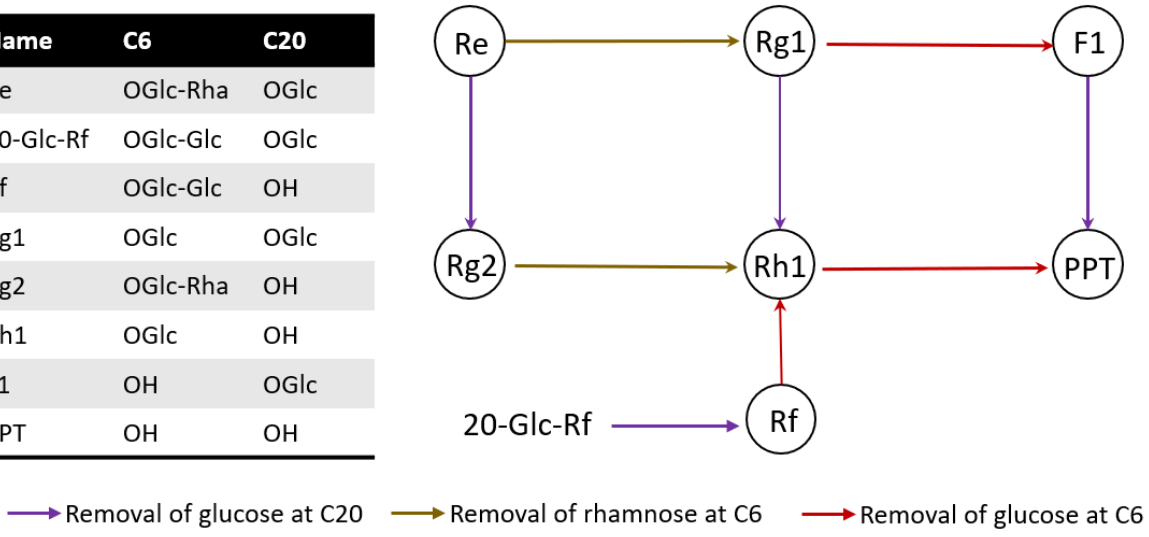


Figure 2.11 Possible biotransformation pathways of ginsenoside Re

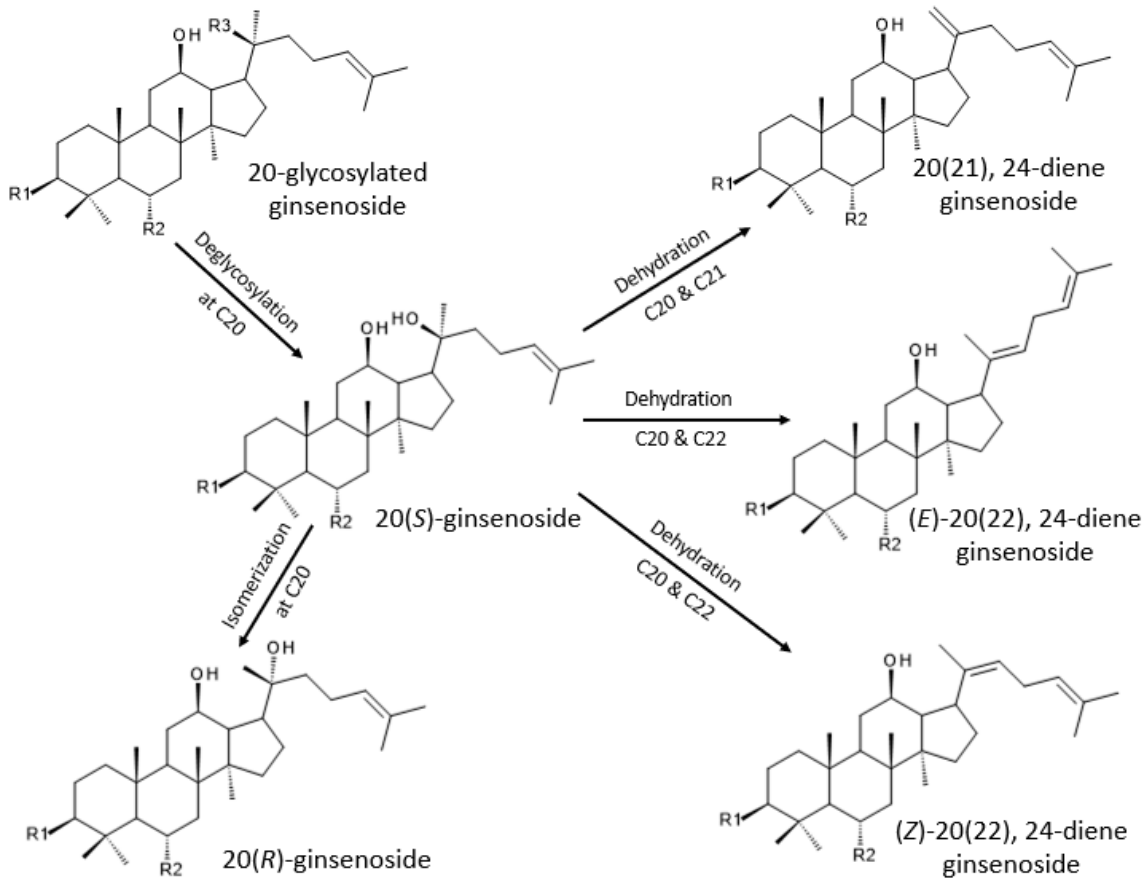
2.3.3 Thermal conversion pathways of ginsenosides

Various commercial ginseng products are available such as fresh ginseng root, white ginseng, and red ginseng. Most harvested ginseng roots (fresh ginseng) are air-dried and dehydrated to produce white ginseng. Meanwhile, some others are steamed at 95-100 °C for several hours before drying, which leads to Maillard reaction of sugars to produce red ginseng [130]. Red ginseng is known for containing unique less polar ginsenoside profile

which is different from that of white ginseng [131]. During the heat treatment, malonyl ginsenosides, such as m-Rb1, m-Rb2, m-Rc, and m-Rd which are thermally unstable ginsenosides, can be transformed. The loss of the malonyl group produces neutral ginsenosides-Rb1, -Rb2, -Rc and -Rd, which can further degrade into ginsenoside-Rg3 through deglycosylation [132]. Moreover, apart from the demalonylation and deglycosylation, some ginsenosides, with a hydroxyl at C-20 position tend to lose an H_2O to form a double bond either between C-20 and C-21 or between C-20 and C-22 to yield less polar ginsenosides during the steaming process [2, 133].

For instance, ginsenosides PPD, Rs3, Rh2 and Rg3 are the PPD-type ginsenosides, which have a hydroxyl at C-20, so these ginsenosides can lose an H_2O to form a double bond when they are steamed or heated. Actually, the corresponding modified PPD-type ginsenosides (dPPD I, dPPD II, Rs4, Rs5, Rh3, Rk2, Rg5, Rk1, and Rz1) were found in the steamed roots of ginseng. Similarly, the PPT-type ginsenosides, which have a hydroxyl at C-20, like ginsenosides PPT, Rh1, Rg2 and Rf, can also lose an H_2O to form a double bond when they are steamed or heated. Accordingly, dPPT I and dPPT II, Rh4 and Rk3, F4 and Rg6, Rg9 and Rg10, which are modified from ginsenosides PPT, Rh1, Rg2 and Rf by dehydration, respectively, were found in the steamed roots of ginseng except for F4 from leaves [134].

Another interesting finding is that Rs6 and Rs7, two PPT-type modified ginsenosides with a double bond between C-20 & C-22 and between C-20 & C-21, respectively, were detected in the steamed roots of ginseng. Rs6 and Rs7 may be mostly modified from ginsenoside X (the structure is shown in Figure 2.12) by losing an H_2O to form their double bonds. There is no record about ginsenoside X in current literature, thus I speculate that compound X exists but has not been detected in ginsenoside so far. There may be another pathway to form Rs6 and Rs7 by adding acetyl (Ac) to C6 of glucose of Rh4 and Rk3, respectively.



NO.	Lose a H_2O between		Substituent group
	C20-C21	C20-C22	
G-PPD	dPPD II	dPPD I	R1=OH; R2=H
G-Rh2	Rk2	Rh3	R1=OGlc; R2=H
G-Rg3	Rk1	Rg5 (Rz1)	R1=OGlc(2-1)Glc; R2=H
G-Rs3	Rs5	Rs4	R1=OGlc(2-1)Glc(6)Ac; R2=H
G-PPT	dPPT II	dPPT I	R1=OH; R2=OH
G-Rh1	Rk3	Rh4 (20(E)Rh4)	R1=OH; R2=OGlc
G-Rg2	Rg6	F4	R1=OH; R2=OGlc(2-1)Rha
G-Rf	Rg10	20(Z)Rg9 (Rg9)	R1=OH; R2=OGlc(2-1)Glc
G-X	Rs7	Rs6	R1=OH; R2=OGlc(6)Ac

Figure 2.12 Dehydration pathway for some ginsenosides under steamed or heated conditions.

Multiple ginsenosides are biosynthesized based on the skeleton of ginsenosides PPD and PPT, so ginsenosides especially basic PPD- and PPT-types can be degraded gradually to yield the aglycones PPD and PPT. The possible transformation pathways of basic PPD and PPT -type ginsenosides were summarized based on degradation pathways and thermal conversion pathways and are shown in Figures 2.13 and 2.14.

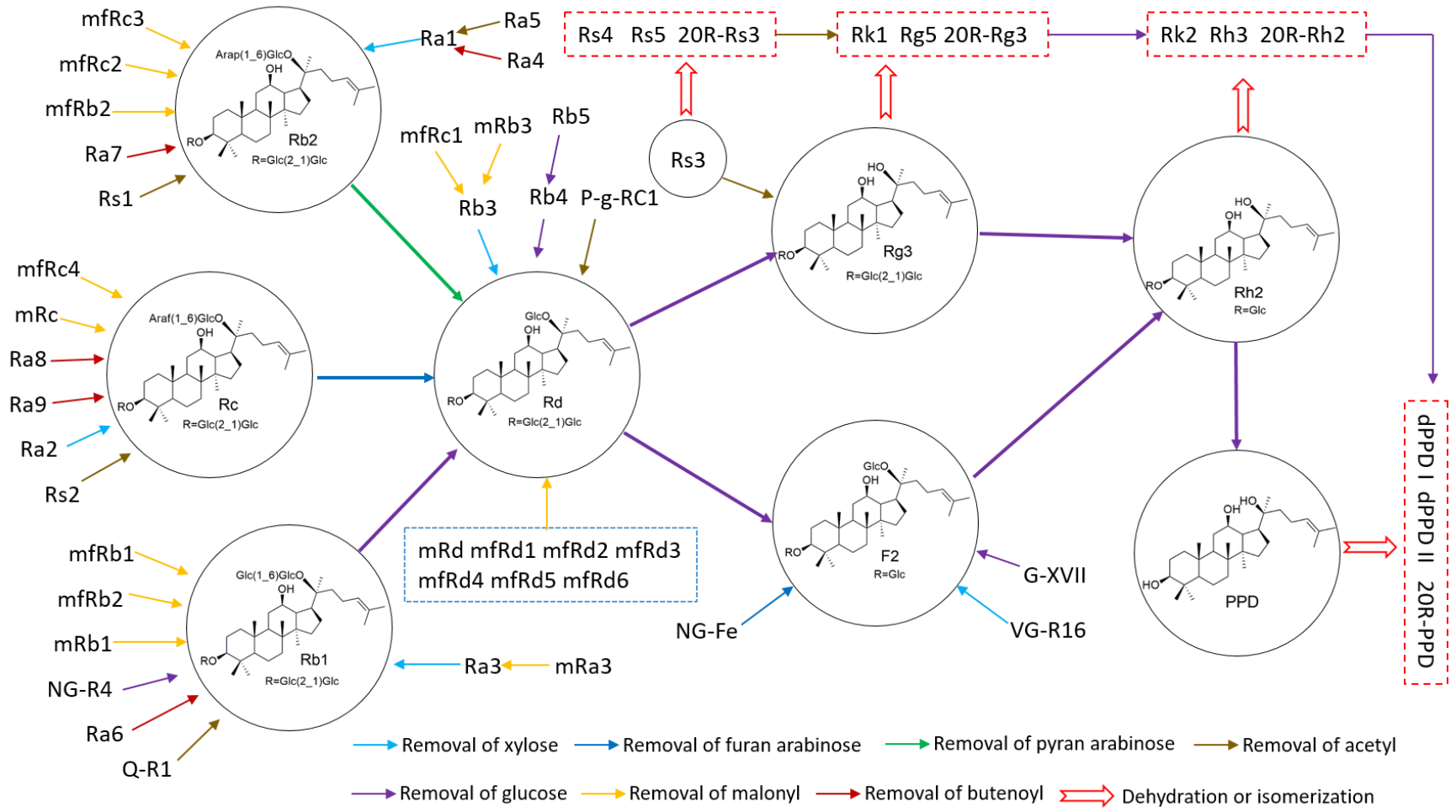


Figure 2.13 Possible transformation pathways of PPD-type ginsenosides

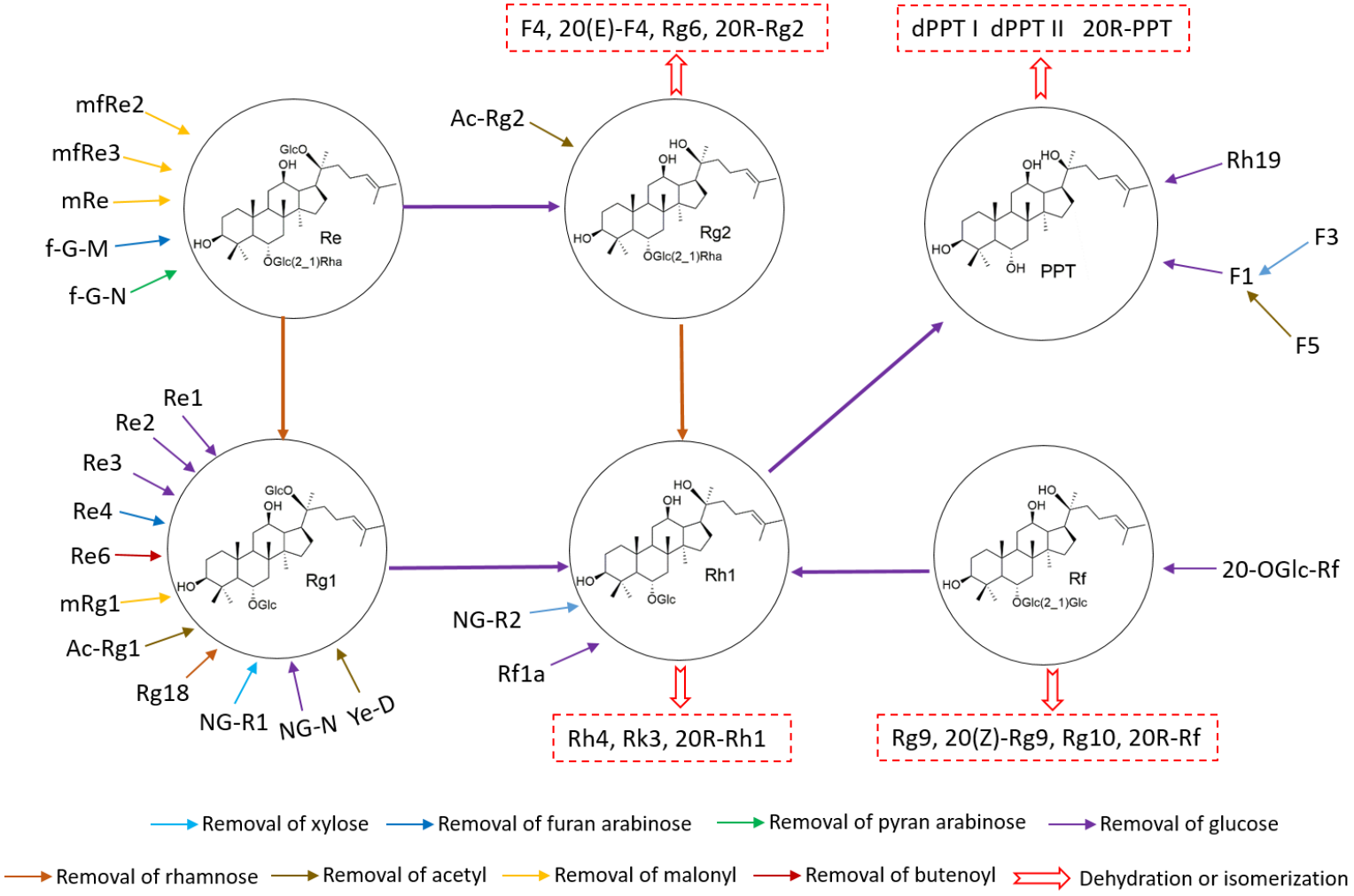


Figure 2.14 Possible transformation pathways of PPT-type ginsenosides

2.4 Conclusion

Ginseng has been used for thousands of years as an important medicinal plant resource in Asia. Ginsenosides are the unique active component of ginseng and hundreds of ginsenosides have been identified from the Asian and American ginseng. This review provides a comprehensive guide to the structural information of ginsenosides isolated from the two most popular ginseng varieties and compared differences in ginsenoside composition of the Asian and American ginseng. Based on structural characteristics and transformation pathways, the relationship of ginsenosides was also outlined. With the development of separation and identification technology, increasing new ginsenosides, as well as biotransformation metabolites will be discovered in the future.

2.5 References

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Chapter Three

Anti-diabetic effect of ginseng extracts and ginsenosides ——literature review

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CHAPTER THREE ABSTRACT

Ginseng is one of the most valuable and commonly used Chinese medicines not only in ancient China but also worldwide. Ginsenosides, also known as saponins or triterpenoids, are thought to be responsible for the beneficial effects of ginseng. This chapter summarizes recent publications on anti-diabetic studies of ginseng extracts and ginsenosides in cells, animals, and humans. It seems that the anti-diabetic effect of ginseng is positive for type 2 diabetic patients but has no significant impact on prediabetes or healthy adults. Regulation of insulin secretion, glucose uptake, anti-oxidative stress, and anti-inflammatory pathways may be the mechanisms involved with ginseng's anti-diabetic effects. Taken together, this summary provides evidence for the anti-diabetes effects of ginseng extracts and ginsenosides as well as the underlying mechanisms of their impact on diabetes.

3.1 Introduction

The main type of diabetes is type 1 diabetes, which is caused by insulin deficiency, and type 2 diabetes, which is characterized by insulin resistance [1]. The majority of diabetes is type 2 diabetes, and it is a formidable challenge for public health [2]. According to the International Diabetes Federation statistics, 463 million people aged 20–79 years worldwide had type 2 diabetes in 2019, and this number is estimated to reach 700 million by 2045 [3]. There is also a huge population with prediabetes, which is very likely to develop into type 2 diabetes [4]. Without proper management (blood glucose control, including drug intervention and diet management), diabetes can cause serious health problems, especially diabetic complications. For example, it can damage eyes, kidneys, and nerves, and it also causes heart disease, stroke, and can even necessitate limb removal [5]. Thus, the proper treatment of diabetes is important. Although many researchers are looking at ways to reduce insulin resistance to treat type 2 diabetes, type 2 diabetes is still a severe disease and cannot be cured [6]. Currently, management of diabetes mainly focuses on insulin or its peptide derivatives, anti-diabetic oral drugs, and diet control [7]. Insulin and its peptide derivative treatments need to be injected via vein every day, leading to inconvenience and burdens for patients [8]. Long-term oral administration of chemical drugs can be harmful to many diabetic patients because of drug toxicities [8]. Researchers are exploring the possibilities offered by complementary and alternative medicine, such as traditional herbal medicine.

There has been increasing popularity in Chinese medicine, especially since the 2015 Nobel Prize was awarded to Tu Youyou, who discovered that artemisinin from traditional Chinese medicine can treat malaria [9]. This popularity indicates that even in modern times, ancient Chinese herbal remedies can be valuable as an alternative therapy for relieving serious diseases. One of the Chinese medicinal herbs with promising anti-diabetic effects is ginseng [10]. In fact, ginseng has long been used to

treat diabetes in China. In the Song Dynasty (1078 A.D.), official documentation (Formularies of the Bureau of People's Welfare Pharmacies) recorded that ginseng was employed to cure Xiaoke disease, which is nowadays known as diabetes [10]. As medicinal intervention management to diabetes, the Asian (*P. ginseng*) and American (*P. quinquefolius* L) ginsengs are the two most widely used varieties and have recently attracted a lot of attention from those trying to understand the molecular mechanisms of ginseng's anti-diabetic effects.

Ginseng contains diversified components, including saponins, polysaccharides, polyacetylenes, phenols, and alkaloids. Ginseng saponins, known as ginsenosides, are an important class of natural triterpene saponins, which are thought to be responsible for the anti-diabetic effect in ginseng. So far, nearly two hundred ginsenosides have been detected from ginseng plants and heat-processed ginseng products [11]. Most known ginsenosides are classified as members of the dammarane family, and consist of a four-ring, steroid-like structure, such as ginsenosides Rb1, Rg3, Re, and Rg1 (Structures are shown in Figure 3.1). Increasing number of studies suggest that ginseng extracts, processed ginseng extracts, and ginsenosides show anti-diabetic effects, although the mechanism of action is still not clear. A systematic summary of anti-diabetic studies of ginseng will be helpful to explore its anti- diabetic mechanism.

Until 2011, there were eleven publications (twelve human trials) about ginseng extracts being used for diabetes intervention. Four of them studied American ginseng root extracts, five of them studied Asian ginseng root extracts, two used ginsenosides (Rb1 and Rg1, Re), and one tested eight types of ginseng (American, American-wild, Asian, Asian-red, Vietnamese-wild, Siberian, Japanese- rhizome, and Sanchi ginseng) on acute postprandial glycemic indices in healthy humans. Through summarizing these twelve human trials, along with *in vitro* cell studies and *in vivo* animal studies, the authors deduced that ginseng may modulate insulin production/secretion, glucose metabolism and uptake, or inflammatory pathway to exert anti-diabetic effects [12]. Recently, many significant researches including human trials, *in vitro* and *in vivo*

studies have been conducted on diabetes intervention with ginseng extracts or ginsenoside. In order to better know the progress and basis for the evaluation of the anti-diabetic effects of ginseng, this current review summarizes the research into type 2 diabetes intervention with ginseng extracts or ginsenosides conducted in human trials, *in vivo* animal studies, and *in vitro* cell studies since 2012.

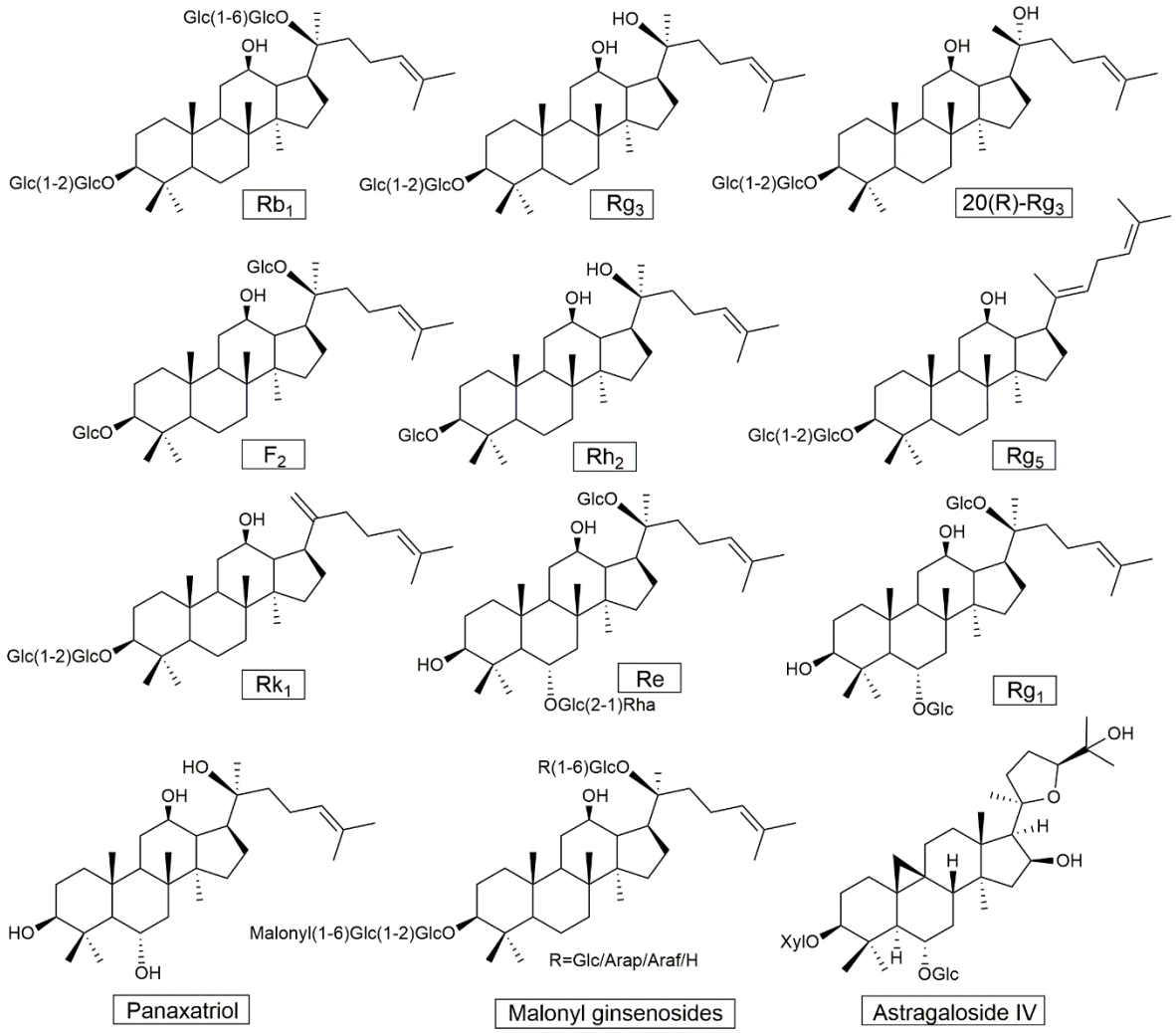

Figure 3.1 Chemical structures of triterpenoid saponins mentioned in this chapter. Glc, Rha, Arap, Araf, and Xyl refer to β-D-glucopyranosyl, α-L-rhamnopranosyl, α-L-arabinopyranosyl, α-L-arabinofuranosyl, and β-D-xylopyranosyl, respectively.

3.2 Anti-diabetic effects of ginseng in human trials

Although ginseng has been used as a natural herbal medicine for a long time in East Asia, interest in the anti-diabetic efficacy of ginseng has drawn increased attention from modern medicine. An increasing number of human trials are exploring the efficacy of ginseng intervention in diabetes. Shishtar et al. found ginseng significantly reduced fasting blood glucose compared to the control (−0.31 mmol/L [95% CI: −0.59 to −0.03], $p = 0.03$) based on a meta-analysis of sixteen randomized controlled clinical trials; there was no significant effect on fasting plasma insulin and glycated haemoglobin [13]. Another meta-analysis included eight trials that suggested ginseng supplementation showed significant differences on fasting glucose (−0.306 mmol/L [95% CI: −0.539 to −0.074], $p = 0.01$), postprandial insulin (−2.132 mmol/L [95% CI: −3.706 to −0.558], $p = 0.008$), and HOMA-IR (−0.397 mmol/L [95% CI: −0.679 to −0.115], $p = 0.006$) compared to the control group; there was no significant difference on postprandial glucose and fasting insulin between ginseng treatment and control groups [14]. Since 2012, there have been 13 human trials published that have focused on the anti-diabetic effect of ginseng (Asian ginseng (*P. ginseng*) and American ginseng (*P. quinquefolius* L.)). Different ginseng extracts or differently processed ginseng products exhibited varied impact on diabetic patients. The information of ginseng extracts mentioned in this chapter is shown in Table 3.1.

Table 3.1 Ginseng materials mentioned in this chapter.

Types	Samples	Processing	Main Components (mg/g Dry Weight)	Ref.
White ginseng	WKGE	Shade dried, Soxhlet extracted by water	1.9 Re, 1.9 Rc, 1.8 Rb2, 1.6 Rb1, 1.2 Rg1, 0.6 Rf, ($\times 10^{-3}$)	[15]
	KWG	Air dried	9.1 Re, 3.0 Rg1, 2.4 Rb1, 1.3 Rc, 0.7 Rb2	[16]
	TCMGAR	Air dried	11.2 Rg3, 4.2 Rd, 4.1 Rb1, 3.8 Rh2, 2.3 Rb2, 2.0 Rc	[17]
	AG	Air dried	96.7 total ginsenosides (PPD:PPT = 3.03:1)	[18]
Red ginseng	KRG	Steamed and air dried	1.9 Rb1, 1.3 Rg3, 1.0 Rd, 0.9 Rb2	[19]
	KRG	Steamed at 90–100 °C for 3 h and air dried	51.6 Rb1, 28.9 Rg1, 22.2 Rc, 21.6 Re, 18.2 Rb2	[20]
	KRG	Steamed and air dried	2.4 Rb1, 1.6 Rg1, 1.0 Rc, 0.9 Rb1, 0.6 Re	[21]
	KRG	Steamed and air dried	4.6 Rb1, 2.8 Rc, 2.3 Rb2, 1.4 Rg2, 1.2 Rg3, 1.2 Re	[22]
	KRG	Steamed and air dried	8.0 Rb1, 3.3 Rc, 2.8 Rb2, 2.5 Rg3, 1.5 Rf, 1.3 Re, 1.2 Rg1, 1.0 Rd	[23]
	KRG	Steamed and air dried	16.6 total ginsenosides (PPD:PPT = 1.65:1)	[24]
	ARG	Steamed in autoclave and dried	Related fatty acids, 58.1 cinnamic acid, 50.1 ferulic acid	[25]
Black ginseng	BGE	Steamed and dried, repeat several cycles, extracted by 70% ethanol at 70 °C for 12 h	5.6 C-K, 4.7 Rg5, 1.7 Rg3, 1.5 Rb1, 0.8 Rg2, 0.7 Rc	[26]
	GBG05-FF	Repeated steaming at 95 °C for 6 h and drying at 60 °C, extracted by 70% ethanol at 80 °C for 8 h	11.7 Rg5, 6.9 Rk1, 5.2 Rg3, 1.9 Rh4	[27]
Fermented ginseng	FRG	RGE incubated with yeast at 40 °C for 12 h	4.9 Rg3, 4.8 Rb1, 3.4 Rb2, 2.9 Rg2, 1.8 Re, 1.4 C-K, 0.8 Rg1	[28]
	FRG	RGE incubated with *L. plantarum* at 35–40 °C for 15 d	4.9 C-K, 3.5 Re, 3.3 Rb1, 3 Rb2, 2.4 Rd, 2 Rc	[29]
	FSGB	SGB incubated with *L. plantarum* at 30 °C for 72 h	Quinic acid, linoleic acid, palmitic acid	[30]

	FGE	GE incubated with microorganism	61.0 C-K, 27.7 Rg3, 12.1 Rh1, 9.5 Rd, 8.2 Rg2, 3.1 Rh2, 2.3 Rb2	[31]
	GS-E3D	RGE incubated with 10% pectin lyase at 50 ℃ for 5 d	30.2 Rb1, 17.6 Rb2, 14.0 Rc, 12.6 Re, 5.9 Rg1, 4.7 Rf, 2.7 Rg3, 1.5 Rg5	[32]
	VEG	GE incubated with vinegar (pH2.3) at 90 ℃ for 6 h	40.5 Rg3, 4.1 Rc, 4.0 Rd, 1.9 Rb2, 1.1 Rf	[33]
	HGE	GE incubated with an enzyme solution	7.5 Rg1, 6.3 C-K, 5.4 Rb1, 1.9 Re, 0.7 Rd	[34]
Fractioned ginseng	Rg3-RGE	RG multiple extracted by 55% ethanol	51.7 Rg3, 3.9 Rb1, 3.7 Rh1, 3.6 Rg2, 1.6 Rd, 1.5 Rb2	[35]
	GB	Removed seeds and air dried, refluxed with 70% ethanol for 10 h	110.6 Re, 21.1 Rc, 19.0 Rb2, 16.6 Rg1, 16.5 Rd, 8.4 Rg2, 7.7 Rb1	[36]

WKGE: wild Korean ginseng extract; KWG: Korean white ginseng; TCMGAR: tissue culture raised mountain ginseng adventitious root; AG: American ginseng; KRG: Korean red ginseng; ARG: American red ginseng; BGE: black ginseng extract; GBG50-FF: black ginseng ethanol extract; FRG: fermented red ginseng; FSGB: fermented steam-dried ginseng berry; FGE: fermented ginseng extract; GS-E3D: pectin modified red ginseng extract; VEG: vinegar extract of ginseng; HGE: hydrolyzed ginseng extract; Rg3-RGE: Rg3-enriched red ginseng extract; GB: ginseng berry extract.

Ten clinical trials on Asian ginseng have been reported in recent years. In one trial, thirty-six diabetic patients were randomized to receive 1.5 g/day of ginsam, which is enriched ginsenoside Rg3, though an acetic acid extraction from *P. ginseng*, or a daily placebo for eight weeks. HbA1c levels and fasting blood glucose in the ginsam-treated group significantly reduced by 0.6% and 21.4 mg/dL compared to the placebo group [33]. Park *et al.* reported that 23 impaired fasting glucose participants were randomly administrated with 960 mg/day of hydrolyzed Asian ginseng extract or a placebo for eight weeks. The hydrolyzed ginseng extract, containing 7.5 mg/g of Rg1, 6.3 mg/g of compound K, 5.4 mg/g of Rb1, 1.9 mg/g of Re, 0.7 mg/g of Rd, 0.4 mg/g of Rb2, and 0.3 mg/g of Rc, significantly decreased fasting plasma glucose ($p = 0.017$) and postprandial glucose ($p = 0.01$) compared to the placebo but there were no significant differences in fasting plasma insulin and postprandial insulin between the groups [34]. These results showed that hydrolyzed ginseng extract cut the absorption of glucose in the intestinal lumen. Further large-scale and long-term studies are needed to fully evaluate whether it can decrease insulin secretion.

The ginseng berry has a different ginsenoside composition and contains more plentiful ginsenosides than its root [37]. An *in vivo* study using *ob*/*ob* diabetic mice reported that after being treated with 150 mg/kg ginseng berry extract, fasting blood glucose significantly decreased to 150 mg/dL on day 5 and 129 mg/dL on day 12. The same experiment was carried out with ginseng root extract. Although the same dose of ginseng root extract significantly reduced the fasting blood glucose to 143 mg/dL on day 5, the fasting blood glucose had not decreased by day 12 (155 mg/dL) [38]. This study showed that ginseng berry extract exhibited more potent anti-hyperglycemic effects compared to ginseng root extract administered at the same concentrations using *ob*/*ob* diabetic mice. Recently, a 12-week, randomized, double-blind, placebo-controlled clinical trial was completed with 72 participants with a fasting glucose level ranging from 100–140 mg/dL. The study showed that ginseng berry extract significantly reduced fasting blood glucose and postprandial glucose at 60 min in an

oral glucose tolerance test after a 12-week treatment, but it did not regulate serum glucose to normal levels [36], indicating that the ginseng berry extract failed to show anti-hyperglycemic effects in this human study. It should be noted that the participants in this study were prediabetes rather than type 2 diabetic patients. The anti-hyperglycemic effect was observed in the *ob*/*ob* diabetic mice model [38]. Maybe the anti-hyperglycemic effect of ginseng berry extract is positive for type 2 diabetes rather than prediabetes. Further investigation of people with type 2 diabetes is needed to test this hypothesis.

Participants with impaired fasting glucose, impaired glucose tolerance, or type 2 diabetes consuming 5 g/day Korean red ginseng (KRG) extract were found to have significantly reduced blood glucose. Specifically, −1.2 ± 0.6 mmol/L of serum glucose and –1.0 ± 0.3 mmol/L of whole blood glucose were decreased at a 30 min oral glucose tolerance test after a 12-week intervention [24]. The plasma insulin sensitivity index increased by 33% compared to the placebo group in this 12-week randomized, double-blind and placebo-controlled trial [24].

In a randomized, double-blind, placebo-controlled trial involving forty-two subjects, 2.7 g/day of fermented red ginseng given for four weeks significantly increased postprandial insulin of subjects with impaired fasting glucose or type 2 diabetes (35.5 μU/mL in the placebo group vs. 56.3 μU/mL in the ginseng group, $p = 0.040$). Moreover, in the ginseng treatment group, their postprandial glucose and glucose area under curves (AUC) significantly reduced by 17.2% ($p = 0.0001$) and 27.4% ($p = 0.002$) compared to the baseline values. However, the ginseng treated group did not show a remarkable difference in fasting blood glucose and insulin relative to the placebo [29]. It is known that fermentation can increase the bioavailability (absorption and bioactivity) of ginseng due to small ginsenosides (ginsenoside Rg3, compound K, etc.) produced through the fermentation process. As reported by Bang et al. in the clinical trial [24], 5 g/day of Korean red ginseng can reduce fast blood glucose and glucose AUC. However, in Oh et al study [29], the fermented red ginseng only modulated

postprandial glucose and insulin, not fasting glucose and insulin. The short period of time (four weeks) may be responsible for the lack of significant differences in fasting glucose and insulin because the improvement of fasting glucose and insulin is usually expected over a long-term treatment, while postprandial glucose and insulin are more sensitive to slight interventions in glycemic control. Long-term clinical trials for fermented red ginseng are needed.

These studies suggest that different Asian ginseng extracts consistently reduce fasting blood glucose or postprandial glucose in type 2 diabetic patients and could be considered as an optional therapy for managing type 2 diabetes. However, other results from human studies cast doubts about this view.

Sixty-eight obese subjects without diabetes were administrated 6 g/day of KRG (orally) or a placebo for 12 weeks in a randomized, double-blind, and placebo-controlled trial. This study showed that there was no significant effect on the insulin level and the insulin sensitivity index between the ginseng treatment group and placebo group [20]. Another randomized, double-blind, placebo-controlled 8-week trial conducted among fifty obese women found that there were significant improvements in the obesity index between before and after treatment with 6 g/day KRG, but no significant difference between the KRG treatment group and the placebo group [39]. Both studies indicate that KRG does not enhance insulin sensitivity in obese people without diabetes. In both trials, the regulation of glucose homeostasis was not observed in the prediabetes situation for subjects who were obese without diabetes, impaired glucose tolerance or mild type 2 diabetes. Recently, a multicenter, double-blind, randomized, and placebo-controlled trial was conducted on 1000 healthy adults. The test group consumed 2 g/day of KRG for 24 weeks, which was found to be safe and well-tolerated in healthy adults, and there were no significant abnormal changes from the anthropometric, laboratory, and vital sign measurements between the KRG group and the placebo group [23]. Therefore, it seems that the anti-diabetic effect of Asian

ginseng is positive for type 2 diabetic patients, but there has no significant effect on prediabetes or healthy adults.

Besides the human trials using Asian ginseng, there have been three trials using American ginseng on type 2 diabetic patients. In one study, thirty type-2 patients were involved in a randomized, placebo-controlled crossover trial. HbA1c levels were 0.31% lower (p = 0.011) after treatment with 6 g konjac-glucomannan-based fiber blended together with 3 g American ginseng per day for 12 weeks. Plasma lipids of the ginseng intervention group also significantly decreased compared to the placebo control group [40]. This study did not consider the effect of konjac- glucomannan-based fiber on diabetes. The study did not report whether the konjac- glucomannan-based fiber blend or the American ginseng was responsible for reducing the glucose levels in the trial subjects. The study could be improved by planning another group administrated with konjac-glucomannan-based fiber or adding it to the placebo. Vuksan et al. [18] conducted a randomized, double-blind, and placebo-controlled crossover clinical study to assess the anti-diabetic efficacy and safety of American ginseng. Twenty-four individuals with well-controlled type 2 diabetes completed the study. Using a double-blind, crossover design, the participants were randomized to receive either 3 g/day of American ginseng extract or a placebo for eight weeks together with their original treatment. The efficacy was assessed by HbA1c, and safety was assessed by liver and kidney function testing. The researchers found that American ginseng significantly reduced HbA1c levels (−0.3%, p = 0.041), fasting blood glucose (−0.7 mmol/L, p = 0.008), and systolic blood pressure (−5.6 mmHg, p <0.001) compared to the placebo. Furthermore, the beneficial changes did not affect the parameters of safety, including livers function and kidney function, specifically, the urea, creatinine, and alanine amino transferase. Another human study specifically tested the safety of American ginseng as an adjunct to conventional therapy in type 2 diabetes [41]. Seventy-four type 2 diabetic patients were given 3 g/day American ginseng extract or a placebo for 12 weeks. The investigators found that there was no significant difference between the

ginseng treatment and the placebo in the safety parameters, such as kidney function (urates and creatinine), liver function (AST and ALT), and hemostatic function (PT and INR). These studies suggest that American ginseng extract is effective and safe as an additional treatment in the management of type 2 diabetes.

Together, these human studies show that both Asian ginseng and American ginseng do indeed decrease blood glucose in type 2 diabetics. At the same time, it should be noted that this efficacy was observed in several limited ginsenosides (6–7 ginsenosides analyzed), small sample sizes (23–94 subjects), and short-term studies (4–12 weeks). Larger scale clinical trials are needed to completely illuminate the long-term benefits of this herbal supplement in the management of type 2 diabetes. The studies are summarized in Table 3.2.

Table 3.2 Effects of ginseng on diabetes-related parameters in human studies.

Material	Design (Sample Size and Subjects)	Drug Treatment and Duration	Results								Ref.
			BW	HbA1c	FBG	FI	PG	PI	HOMA-IR	Safety	
Vinegar extract of ginseng (VEG)	RCT (72 type 2 diabetic patients)	Four group (*n* = 18/group): 1500, 2000, 3000 mg of VEG, or placebo daily; 8 weeks	#	+	+	+	+	#	#	*	[33]
Korean red ginseng (KRG)	RCT (50 obese women)	6 g/d of KRG *n* = 24, or placebo *n* = 26; 8 weeks	+	#	-	#	#	#	#	#	[39]
Korean red ginseng (KRG)	Multicenter, RCT (1000 healthy adults)	2 g/d of KRG *n* = 495, or placebo *n* = 505; 24 weeks.	-	#	#	#	#	#	#	*	[23]
Korean red ginseng (KRG)	RCT (68 obese participants without diabetes)	6 g/d of KRG *n* = 34, or placebo *n* = 34; 12 weeks	-	#	-	-	#	#	-	#	[20]
Korean red ginseng (KRG)	RCT (41 type 2 diabetic patients)	5 g/d of KRG *n* = 21, placebo *n* = 20; 12 weeks	-	-	+	+	+	+	+	#	[24]
Fermented red ginseng (FRG)	RCT (42 impaired fasting glucose or type 2 diabetic patients)	2.7 g/d of FRG *n* = 21, placebo *n* = 21; 4 weeks	#	#	-	-	+	+	#	*	[29]
Hydrolyzed ginseng extract (HGE)	RCT (23 impaired fasting glucose participants)	960 mg/d of HGE *n* = 12, placebo *n* = 11; 8 weeks	-	#	+	-	+	-	-	*	[34]
Korean white ginseng (KWG)	RCT crossover trial (25 type 2 diabetic patients)	1 g, 3 g, 6 g KWG, or 3 g placebo together with 50g glucose-load, acute test	#	#	-	#	-	#	#	*	[16]

Ginseng berry extract (GBE)	RCT (72 participants)	1 g/d GBE n = 34, placebo n = 38; 12 weeks	-	-	+	-	+	-	-	*	[36]
American ginseng (AG)	RCT parallel trial (74 type 2 diabetic patients)	3 g/d of AG n = 35, placebo n = 39; 12 weeks	#	#	#	#	#	#	#	*	[41]
American ginseng (AG)	RCT crossover trial (39 type 2 diabetic patients)	6 g/d of fiber from KGB together with 3 g AG; 12 weeks	-	+	-	-	#	#	#	*	[40]
American ginseng (AG)	RCT crossover trial (24 type 2 diabetic patients)	3 g/d AG or placebo with original treatment; 8 weeks	-	+	+	-	#	#	#	*	[18]

BW: body weight; FBG: fasting blood glucose; FI: fasting insulin; HbA1c: hemoglobin A1c; HOMA-IR: homeostasis model assessment-insulin resistance; PG: postprandial glucose; PI: postprandial insulin; RCT: randomized controlled trial. +: indicates the positive outcome with significant difference compared to the control (p <0.05); -: indicates no significant differences compared to the control; #: indicates not tested in the literature; *: indicates the safety profiles were unaffected.

3.3 Potential mechanisms of the anti-diabetic effect of ginseng

Apart from the clinical evidence for the anti-diabetic effect of ginseng, there have also been animal studies conducted to explore the underlying mechanisms of the anti-diabetic effect of ginseng, along with some cell experiments *in vitro*. Different processed ginseng extracts and some individual ginsenosides (Structures are shown in Figure 3.1) have been reported to exhibit an anti-diabetic effect in cell and animal experiments (Tables 3.3 and 3.4). Although the anti-diabetic mechanism of ginseng has not been entirely clarified, the available data indicate that the regulation of blood glucose by ginseng is possibly related to the following four aspects.

Table 3.3 Effects of ginseng on diabetes-related parameters in animal studies.

Material	Dose [Route of administration]	Duration	Animal	Molecular mechanism	Ref.
Ginseng extracts					
Fermented steam-dried ginseng berry (FSGB)	0.5 g/kg [ig]	7 wk	*db*/*db* mice	Decreased the blood glucose and body weight; increased the immune cell population and GLUT1 expression.	[30]
Ginseng berry (GB)	100, 200 mg/kg [ig]	10 wk	STZ-induced mice	Enhanced beta-cell proliferation and glucose tolerance, decreased blood glucose.	[42]
Tissue culture raised mountain ginseng adventitious root (TCMGARs)	125, 250, 500 mg/kg [diet]	4 wk	STZ-induced rats	Significantly reduced the blood glucose, TC, and TG levels.	[17]
Ginseng berry (GB)	0.05% [diet]	6 m	C57BL/6 mice (15 months old mice)	Increased the parameters of insulin sensitivity, IRS, AKT, and FOXO1; decreased PPAR-γ.	[43]
Wild Korean ginseng extract (WKGE)	100, 200, 300 mg/kg [ig]	8 wk	STZ-induced rats	Significantly reduced blood glucose, ALT and alkaline phosphatase levels.	[15]
Black ginseng extract (BGE)	50, 100, 200 mg/kg [ig]	5 wk	STZ-induced mice	Reduced hyperglycemia and NF-κB; increased the insulin/glucose ratio and β-cell function.	[26]
Black ginseng ethanol extract (GBG05-FF)	300, 900 mg/kg [diet]	4 wk	*db*/*db* mice	Reduced the parameters of fasting blood glucose, glucose tolerance, HbA1c, TG, TC levels, and lipid accumulation; enhanced the phosphorylation of the AMPK, and up-regulated the expressions of GLUT2 and GLUT4.	[27]
Red ginseng (RG)	100, 200 mg/kg [ig]	8 wk	ICR mice for type 1 (STZ induced)	Improved the threshold shift of hearing, delayed latencies, and signal intensity decrease in type 2 diabetic mice; changes with no significance in type 1 diabetic mice.	[22]

			db/*db* mice for type 2		
Korean red ginseng (KRG)	200 mg/kg [ig]	10 wk	STZ-induced rats	39 genes were upregulated more than two-fold; 84 genes were down-regulated.	[44]
Pectin lyase-modified red ginseng extract (GS-E3D)	25, 50, 100 mg/kg [ig]	6 wk	STZ-induced rats	Decreased the urinary levels of albumin, 8-OHdG, and AGEs; suppressed oxidative stress.	[32]
Rg3-enriched red ginseng extract (Rg3-RGE)	2.5, 5 mg/kg [ig]	12 wk	$LDL^{-/-}$ mice	Reduced the levels of glucose, TG, LDL, AST, and AST; inhibited atheroma formation.	[35]
Korean red ginseng (KRG)	200 mg/kg [diet]	12 wk	Goto Kakizaki rats	Reduced blood glucose, PTP-1B, UCP-2, and PARP; enhanced the production of GLUT-4 and insulin.	[21]
Fermented red ginseng (FRG)	0.5%, 1% [diet]	16 wk	*ob*/*ob* mice	Decreased body weight, blood glucose, and hyperlipidemia; increased the expressions of IR, LPL, GLUT1, GLUT4, PPAR-γ, and PEPCK.	[28]
Red ginseng extract (RGE)	2 g/kg [ig]	5 wk	STZ-induced rats	Metformin or RGE administered alone can reduce the FBG, but co-administration recovered the FBG to the control level.	[19]
Fermented ginseng extract (FGE)	0.1% *w*/*w* [diet]	8 wk	*db*/*db* mice	Decreased blood glucose, HbA1c, TNF-α, and lymphocytes; increased adiponectin, serum insulin, PPAR-γ2, and GLUT-2.	[31]
Ginseng polysaccharides	0, 12.5, 25, 50, 100, 200 mg/kg [ig]	10 d	STZ-induced ICR mice	Increased the concentration of insulin, SOD, and glycogen; decreased the content of MDA.	[45]
Ginseng powder	150 mg/kg [ig]	7 d	STZ-induced rats	Enhanced the expression of PPAR-δ and the phosphorylation of troponin1.	[46]
American red ginseng (ARG)	150 mg/kg [ip]	30 d	*db*/*db* mice	Reduced blood glucose, plasma cholesterol and LDL; increased glycogen and HDL.	[25]

American ginseng	200 mg/kg [ig]	2 m (type 1), 4 m (type2)	C57BL/6 mice for type 1 (STZ-induced), *db*/*db* mice for type 2	Increased stroke volume, ejection fraction, cardiac output, and left ventricle pressure; reduced oxidative stress.	[47]
American ginseng saponin (PQS) of stem and leaf	30, 60 mg/kg [ig]	8 wk	STZ-induced rats	Up-regulated the expressions of HGF, NO, ET-1, TNF-α, and slCAM-1.	[48]
Ginsenosides					
Panaxatriol	11 mg/d + 45 min/d (15 m/min) exercise [diet]	6 wk	KK-Ay/Ta Jcl (KKAy) mice	Significantly lowered blood glucose; no significant differences in body weight; significantly improved insulin resistance.	[49]
Rb1	10 mg/kg [ip]	1 wk	C57BL/6 mice (high fat)	Decreased fasting blood glucose, glucose tolerance, and 11β-HSD1; increased insulin sensitivity.	[50]
Re	5, 10, 20 mg/kg [ig]	4 wk	C57BL/6 mice (high fat)	Decreased the parameters of TG, TC, LDL-C, GOT, and GPT; increased HDLC, regulating ACh, AChE, MDA, SOD, and GSH via JNK pathway.	[51]
Rg1	10 mg/kg [ip]	4 wk	STZ-induced rats	Improved angiogenesis, increased eNOS activation, up-regulated VEGF expression, and inhibited apoptosis.	[52]
Rg1	10,15, 20 mg/kg [ip]	12 wk	STZ-induced rats	Decreased the parameters of cTnI, CK-MB, MDA, apoptosis, and CASP3; increased expressions of SOD, CAT, GSH, and Bcl-xL.	[53]
Rg1	10,15, 20 mg/kg [iv]	12 wk	STZ-induced rats	Reduced the expressions of cTnI, GRP78, and CHOP; inhibited endoplasmic reticulum stress-induced apoptosis.	[54]
Rg1	56.25 μM/kg [iv]	10 d	STZ-induced rats	Reduced the cerebral infarction volume; promoted neuronal recovery.	[55]

Rg1	25, 50 mg/kg [ig]	8 wk	STZ-induced rats	Reduced blood glucose levels and insulin resistance index; increased the parameters of TC, TG, LDL-C, AST, and ALT.	[56]
Rg3	0.5 mg/kg [ig]	Once	*db*/*db* mice	Increased the production of GLP-1 and insulin; decreased blood glucose.	[57]
Rh2	5 mg/kg [iv]	4 wk	STZ-induced rats	Decreased fasting blood glucose, ratio of heart weight/body weight, and PPAR-δ; increased STAT3, CCN2, and fibronectin.	[58]
Malonyl ginsenosides (MGR)	50, 100 mg/kg [ip]	3 wk	STZ-induced rats (high fat)	Significantly lowered FBG levels; reduced TG and TC; increased glucose disposal; no effect on body weight.	[59]
Ginsenoside Rg1 + Astragaloside IV	(50 Rg1 + 16 IV) mg/kg [ig]	8 wk	STZ-induced rats	Lowered MDA level and increased levels of CAT, GSH-PX, and T-AOC. Significantly reduced the levels of BUN, SCr, β_2-MG, and UCr. Diminished the mRNA overexpression of TGF-β1 and CTGF; increased Smad7 expression.	[60]
Rg3	10 mg/kg/2 days [ip]	4 wk	STZ-induced ApoE$^{-/-}$ mice	20(*S*)-Rg3 showed stronger anti-proliferative and anti-migratory effects than 20(*R*)-Rg3; (*S*)-isomer significantly reduced the plaque size of diabetic atherosclerosis.	[61]
Rg3	0.5 mg/kg [ig]	3 m	STZ-induced rats (high fat)	Inhibited inflammatory response (inhibited the expression of TGF-β_1, IL-1, IL-6, IL-12) and promoted the activation of PI3K and MAPK signaling pathways to prevent lung tissue damage induced by hyperglycemia.	[62]

11β-HSD1: 11β-hydroxysteroid dehydrogenase type I; 8-OHdG: 8-hydroxy-2′-deoxyguanosine; Ach: acetylcholine; AChE: acetylcholinesterase; AGEs: advanced glycation end-products; AKT: protein kinase B; ALT: alanine aminotransferase; AMPK: 5′ adenosine monophosphate-activated protein kinase; AST: aspartate aminotransferase; Bcl-xL: B-cell lymphoma-extra-large; BUN: blood urea nitrogen; CASP3: caspase-3; CAT: catalase; CCN2: connective tissue growth factor; CHOP: C/EBP homologous protein; CK-MB: creatinine kinase MB; CTGF: connective tissue growth factor; cTnI: cardiac troponin I; eNOS: endothelial

nitric oxide synthase; ET-1: endothelin-1; FBG: fasting blood glucose; FOXO1: forkhead box protein O1; GLP-1: glucagon-like peptide-1; GLUT1: glucose transporter 1; GLUT-2: glucose transporter protein 2; GLUT-4: glucose transporter protein 4; GOT: glutamic-oxaloacetic transaminase; GPT: glutamic-pyruvic transaminase; GRP78: glucose-regulated protein 78; GSH (-PX): glutathione peroxidase; HbA1c: hemoglobin A1c; HDL: high- density lipoprotein; HDL-C: high-density lipoprotein cholesterol; HGF: hepatocyte growth factor; IL: interleukin; IR: insulin receptor; IRS: insulin receptor substrate; JNK: c-Jun N-terminal protein kinase; LDL: low-density lipoprotein; LDL-C: low-density lipoprotein cholesterol; LPL: lipoprotein lipase; β2-MG: β2-microglobulin; MAPK: mitogen-activated protein kinase; MDA: malondialdehyde; NF-κB: nuclear factor–κB; NO: nitric oxide; PEPCK: phosphoenolpyruvate carboxykinase; PI3K: phosphoinositide 3 kinase; PPAR-γ2: peroxisome proliferator-activated receptor gamma 2; PPARδ: peroxisome proliferator-activated receptors δ; PTP-1B: protein tyrosine phosphatases-1B; SCr: serum creatinine; Sicam-1: soluble intercellular adhesion molecule 1; SOD: superoxide dismutase; STAT3: signal transducer and activator of transcription 3; STZ: streptozotocin; T-AOC: total anti-oxidative capacity; TC: total cholesterol; TG: triglycerides; TGF-β: transforming growth factor beta; TNF-α: tumor necrosis factor alpha; UCP-2: uncoupling protein 2; UCr: urinary creatinine; VEGF: vascular endothelial growth factor. ip: intraperitoneal injection; iv: intravenous injection; ig: intragastric injection.

Table 3.4 Effects of ginseng on diabetes-related molecular targets in cell line studies.

Material	Cell line	Drug dose	Molecular mechanism	Ref.
Ginseng total saponin	Mouse podocytes	1 μg/mL for 6, 24, and 48 h	Ginseng total saponins improved p130Cas protein of podocytes.	[63]
Ginsenoside Rg3	Human NCI-H716	10 μM	Enhanced secreting of GLP-1.	[57]
Ginsenoside Rg3	Mouse islet cell	4 μM	Increased glucose-induced insulin secretion (2.3-fold higher); enhanced islet function and attenuated apoptosis.	[64]
Ginsenoside Rg3	Human pulmonary cell BEAS-2B	25, 50, 75, 100 μg/mL	No effect on the viability of BEAS-2B cells; high concentration could induce apoptosis compared with the control group.	[62]
Ginsenoside Rg1	C2C12 muscle cell	10, 20, 40 μM	Enhanced glucose uptake and GLUT4 by AMPK pathways.	[65]

Ginsenoside Re	3T3-L1 cell	3, 10, 30, 60 μM	Increased glucose uptake and the expressions of PPAR-γ2, IRS-1, ap2, and adiponectin genes expressions; helped the translocation of GLUT4 to the membranes; inhibited the expression and production of TNF-α.	[66]
Ginsenoside F2	3T3-L1 cell	10, 50, 100 μM	Reduced the content of lipid accumulation; down-regulated expression of PPAR-γ and perilipin.	[67]
Ginsenoside Rg5:Rk1 mixture	3T3-L1 cell	10, 100 μg/mL	Inhibited lipid accumulation and reduced TG level. Decreased the mRNA level of STAT3, PPAR-γ, CEBPα, and ap2; reduced protein expression of PPAR-γ and CEBPα.	[68]

AMPK: adenosine 5'-monophosphate (AMP)-activated protein kinase; ap2: activating protein; CEBPα: CCAAT/enhancer binding protein alpha; GLP-1: glucagon-like peptide-1; GLUT4: glucose transporter protein 4; IRS-1: insulin receptor substrate 1; PPAR: peroxisome proliferators-activated receptors; STAT3: signal transducer and activator of transcription 3; TNF: tumor necrosis factor.

First, ginseng modulates blood glucose levels by improving β-cell function and enhancing insulin sensitivity. *P. ginseng* berry extract increased β-cell proliferation and insulin secretion to improve glycemic control in streptozotocin (STZ) -induced diabetic mice [42]. Ginseng berry extract could also improve insulin sensitivity in C57BL/6 mice over 15 months old, likely by increasing the activation of IRS-1 and AKT [43]. Kim et al. found that 200 mg/kg of black ginseng extract inhibited β-cell apoptosis and improved islet architecture, which led to the enhancement of β-cell function and reduction of hyperglycemia in STZ-treated mice [26]. While exploring the anti-diabetic component of ginseng extracts, both PPD-type ginsenosides and PPT-type ginsenosides were investigated in animal studies. Ginsenoside Rb1, one of the main PPD-type ginsenosides, was found to reduce symptoms of decreased insulin sensitivity and elevated blood glucose caused by the high-fat diet induction of type 2 diabetic mice [50]. Using the STZ-induced diabetic rat model, researchers found that a main PPT-type ginsenoside, Rg1, can lower insulin resistance and blood glucose, and also improve the blood lipid profile and liver function [56], suggesting that Rg1 may be a potential adjuvant therapy for type 2 diabetic patients with fatty liver disease. As well as the main components in fresh ginseng extracts, heat-treated ginsenoside Rg3 has also been tested. Ginsenoside Rg3 is the main metabolite degraded from other abundant ginsenosides during heat-processing fresh ginseng to manufacture red ginseng and black ginseng. Kim et al. screened the GLP-1 release ability of 15 ginsenosides and found that Rg3 showed the strongest GLP-1 secretion (about 27 pM/mg) effect in NCI-H716 cells. In this *in vivo* trial, 10 μM Rg3 significantly raised the production of GLP-1 and insulin to reduce blood glucose in *db*/*db* mice [57]. In mouse islet cells, insulin secretion was significant: 2.3 times higher in the 4 μM Rg3 treatment group compared to control group [64]. Taken together, Rg3 is presumably the main active anti-diabetic ingredient of ginseng, although administration with other ginsenosides has also shown hypoglycemic effects; these ginsenosides can be hydrolyzed to Rg3 in the gastrointestinal tract [69] to further exert pharmacological effects.

Second, ginseng can enhance glucose uptake by up-regulating the expression of glucose transporters (GLUT). One percent Fermented red ginseng (FRG) significantly decreased the body weight of *ob*/*ob* mice compared with the controls after 4 weeks of administration during the 16-week treatment period; blood glucose was also significantly reduced after 16-weeks treatment with FRG [28]. Furthermore, the study found that the expressions of GLUT1 and GLUT4 were significantly up-regulated by FRG in liver and muscle. This suggests that FRG may decrease blood glucose by enhancing glucose uptake in skeletal muscle. Similar results were also observed in *db*/*db* mice treated with ginseng extracts. Fermented steam-dried ginseng berry (FSGB) at the dose of 0.5 g/kg [30] significantly decreased body weight and blood glucose in*db*/*db* mice during a 35-day treatment; furthermore, the plasma insulin in the FSGB treated group (17.1 ± 0.5 ng/mL) significantly increased approximately twice as much compared to the untreated *db*/*db* group (8.2 ± 0.6 ng/mL). GLUT1 mRNA expression was up-regulated by FSGB in the L6 skeletal muscle cells. During an 8-week treatment with 0.1% fermented ginseng extract (FGE) in *db*/*db* mice, the body weight, fasting blood glucose, and HbA1c level significantly (p <0.05) decreased compared to control groups [31]. FGE intervention significantly enhanced the mRNA expression of GLUT2 and lowered the glucose-6-phosphatase (G6Pase) expression in liver tissues [31]. G6Pase plays an important role in glucose homeostasis because it is the main enzyme of gluconeogenesis and glycogenolysis in the liver. The reduction of G6Pase and increase of GLUT2 expressions mean FGE may improve the transport of blood glucose into the liver, which leads to decreasing the blood glucose level in *db*/*db* mice. Moreover, the FGE group exhibited a higher level of insulin secretion than the control group in the islet primary cell culture; the FGE treated group had weaker damage and inflammation than the control group *in vivo* hematoxylin-eosin staining assay [31]. These suggest that FGE has a protective effect on the pancreas and improves the production/secretion of insulin. Kang et al. found that 900 mg/kg black ginseng ethanol extract (GBG05-FF) significantly reduced fasting blood glucose, glucose tolerance, and plasma HbA1c after four weeks of treatment. Furthermore, GBG05-FF

up-regulated the expressions of GLUT2 in the liver and GLUT4 in the muscles [27], indicating that black ginseng can enhance glucose uptake in surrounding tissues to regulate blood glucose levels. Other beneficial physiological changes, such as a reduction of total cholesterol and triglycerides, and increased PPAR-γ and PEPCK were also observed after administration with fermented ginseng berry [31] or black ginseng [27]. Ginsenosides Rg1 [65] and Re [66] increased expressions of GLUT4 through AMPK pathways in C2CI2 muscle cells and via increasing PPAR-γ activity in 3T3-L1 cells, respectively. Furthermore, KRG at a dose of 0.2 g/kg/d for a 12-week treatment period enhanced insulin action and secretion in diabetic Goto-Kakizaki rats by up-regulating the GLUT4 expression in adipose tissue and down-regulating the expressions of UCP2 and PARP in the pancreas, and PTP-1B in adipose tissue and skeletal muscle [21].

Third, suppression of oxidative stress through increasing SOD activity and decreasing MDA production was reported to occur in treatment with either ginseng polysaccharides in STZ-induced ICR diabetic mice [45], or ginsenoside Rg1 in STZ-induced diabetic rats [53]. Administration of GS-E3D (pectin lyase-modified red ginseng extract) for six weeks significantly decreased urinary levels of albumin, 8-hydroxy-2′-deoxyguanosine (8-OHdG), and advanced glycation end-products (AGEs) in STZ-induced diabetic rats. Moreover, all symptoms of diabetic nephropathy were improved by GS-E3D treatment via suppressing the renal accumulation of AGEs and oxidative stress [32]. Tissue culture raised mountain ginseng adventitious root (TCMGARs) extracts at dosage levels of 250, and 500 mg/kg significantly reduced the blood glucose, total cholesterol, and triglyceride content in STZ-induced diabetic rats [17]. In an $LDL^{-/-}$ mouse model, Rg3-enriched red ginseng extract (44.91 mg/g, 66.6% occupy for total ginsenosides) significantly decreased the levels of glucose, triglyceride, low-density lipoprotein, alanine aminotransferase and aspartate aminotransferase compared to the control [35]. In a high-fat induced C57BL/6 mouse model, ginsenoside Re significantly reduced fasting blood glucose levels and related

biochemical parameters, including cholesterol, low-density lipoprotein cholesterol, total triglyceride, glutamic-pyruvic transaminase, and glutamic-oxaloacetic transaminase. Re also regulated the level of ACh, AChE, and oxidative stress-related parameters (MDA, SOD, GSH) via the JNK pathway [51].

In addition to the above three potential anti-diabetic mechanisms, ginseng can also show anti-diabetic activity through other pathways. Several animal studies have found that ginseng processed products or individual ginsenosides regulate blood glucose in diabetic mouse or rat models, accompanied by regulating the expression of TNF-α and eNOS [31, 48, 52], suggesting that diabetes is associated with inflammation, and effective modulation by ginseng on inflammation may be able to prevent the development of insulin resistance. Recently, Shen et al. reported that Rg1 released in brain tissue using nano-drug delivery systems reduced the volume of cerebral infarction and improved neural recovery in diabetic rats with cerebral infarction [55], indicating that the hypoglycemic effect of Rg1 may be regulated through the central nervous system. Additionally, in an STZ-induced type 1 diabetic rat model, ginsenoside Rh2 reduced fasting blood glucose and improved cardiac function via enhancing PPAR-δ signalling in diabetic rats with cardiac fibrosis [58]. In the 3T3-L1 cells, researchers that found ginsenoside F2 [67], and a ginsenosides Rg5 + Rk1 mixture [68] could reduce lipid accumulation and down-regulate expression of PPAR-γ.

Based on studies on diabetes intervention with ginseng *in vivo* and *in vitro*, the potential mechanism of ginseng extracts (ginsenosides) on type 2 diabetes is summarized in Figure 3.2.

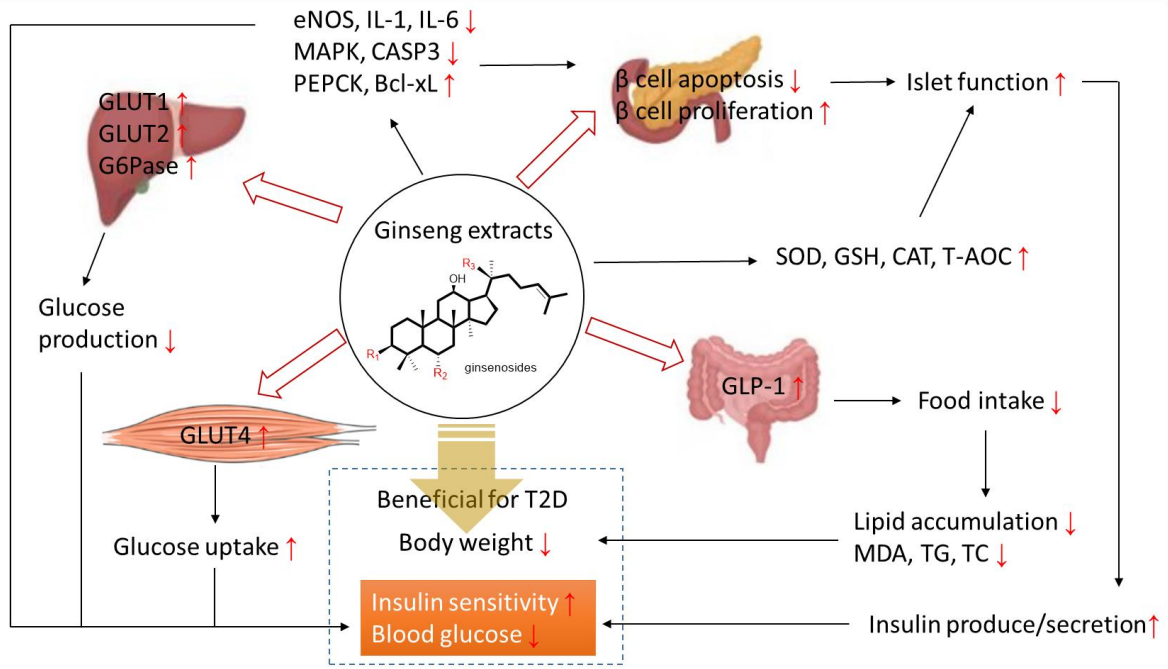

Figure 3.2 The potential mechanism of ginseng extracts (ginsenosides) on type 2 diabetes. Bcl-xL: B-cell lymphoma-extra-large; CASP3: caspase-3; CAT: catalase; eNOS: endothelial nitric oxide synthase; G6Pase: glucose-6-phosphotase; GLP-1: glucagon-like peptide-1; GLUT: glucose transporter protein; GSH (-PX): glutathione peroxidase; IL: interleukin; MAPK: mitogen-activated protein kinase; MDA: malondialdehyde; PEPCK: phosphoenolpyruvate carboxykinase; SOD: superoxide dismutase; T-AOC: total anti-oxidative capacity; TC: total cholesterol; TG: triglycerides.

3.4 Conclusion

Human, animal, and cell studies have shown that different processed ginseng extracts and specific ginsenosides possess beneficial effects on diabetes, especially type 2 diabetes. Most studies of individual ginsenosides have focused on Rb1, Re, or Rg1, which are the main components of ginseng and are easily obtained. However, these ginsenosides have a large molecule structure, resulting in poor systemic

bioavailability. Reeds et al. reported on overweight and obese participants with impaired glucose tolerance or newly diagnosed type 2 diabetes who consumed ginseng root extract (8 g/day), ginsenoside Re (250–500 mg/day), or placebo for 30 days; ginsenosides Rb1, Rb2, and Re were not detected in plasma after treatment with ginseng root extract or ginsenoside Re [70]. However, another study conducted in healthy volunteers showed that ginsenoside Re and its potential metabolites (including Rg2, F1, Rh1, and PPT) were detected in plasma after oral administration of ginsenoside Re [71]. It seems that the large molecule ginsenosides (Rb1, Rc, Re) may be a form of storage for saponins in ginseng plants rather than the active form *in vivo*. The related but smaller molecule ginsenosides (Rg3, Rh1) may be the components that exert therapeutic effects. This is also supported by the evidence found in experiments with red ginseng, fermented ginseng, and black ginseng. Therefore, the metabolic ginsenosides (Rg3, Rh1) need to be investigated to determine the active form of ginsenosides *in vivo*.

Another concern is that the results from clinical data for different processed ginseng extracts are inconsistent; some studies showed that the ginseng extracts possessed anti-hyperglycemic or diabetes-related effects, while others did not. These results could be caused by subject factors and/or drug reasons. The physical activity, body weight, diabetic degree, and sample size of volunteers may affect the outcomes of clinical trials. Another influencing factor is the variability of ginseng extracts. Different sources, species, or extraction processes lead to different ginseng ingredients. Even when using the same species and the same extraction process, different batches of ginseng can have various components, which influence the curative effect. Thus, characteristic detection and quality control are needed for ginseng products in clinical applications.

3.5 References

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Chapter Four

Ginsenosides analysis of New Zealand grown forest *Panax ginseng* using liquid chromatography coupled with quadrupole time-of-flight tandem mass spectrometry

This chapter has been published in the following peer-reviewed open access publication.

Chen, W.; Balan, P.; Popovich, D. G. Ginsenosides analysis of New Zealand grown forest *Panax ginseng* using LC-QTOF-MS/MS. **Journal of Ginseng Research**, 2020, 44(4): 552-562. https://doi.org/10.1016/j.jgr.2019.04.007

Ginsenosides are the unique and bioactive components in ginseng. Ginsenosides are affected by the growing environment and conditions. In New Zealand (NZ), *Panax ginseng* Meyer (*P. ginseng*) is grown as a secondary crop under a pine tree canopy with an open-field forest environment. There is no thorough analysis reported about NZ grown ginseng. Ginsenosides from NZ-grown *P. ginseng* in different parts (main root, fine root, rhizome, stem, and leaf) with different ages (6-year, 12-year, 13-year, and 14-year old) were extracted using ultrasonic extraction and characterized by liquid chromatography coupled with quadrupole time-of-flight tandem mass spectrometry (LC-QTOF-MS/MS). Twenty-one ginsenosides in these samples were accurately quantified and relatively quantified with 13 ginsenoside standards. All compounds were separated by 40 min, and a total of 102 ginsenosides were identified by matching MS spectra data with 23 standard references or published known ginsenosides from *P. ginseng*. The quantitative results showed that the total content of ginsenosides in various parts of *P. ginseng* varied, which was not obviously dependent on age. In the underground parts, the 13-year old ginseng root contained more abundant ginsenosides among tested ginseng samples; while in the aboveground parts, the greatest amount of ginsenosides was from the 14-year sample. Additionally, the amount of ginsenosides is higher in the leaf and fine root, and much lower in stem compared to other parts of *P. ginseng*. This study provides the first ever comprehensive report on NZ grown wild simulated *P. ginseng*.

4.1 Introduction

Panax ginseng Meyer, known as Korean ginseng or Asian ginseng, is a slow-growing perennial plant, belonging to the family Araliaceae. The ginseng root was used as an important Chinese medicine for more than two thousand years. Ginseng has been reported to have anti-ageing [1], anti-fatigue [2], anti-inflammatory [3], anti-cancer [4], anti-diabetic [5] properties. It has been approved as a new food resource by the Chinese government in 2012 [6] and it can be directly added to food products. In recent years, ginseng has been widely used as a tonic food and healthcare product, in a variety of commercial health products, such as ginseng tea, ginseng honey, ginseng milk, ginseng capsules, ginseng candy, and cosmetics have been developed [7].

Ginseng saponins, known as ginsenosides, are the unique and active components in ginseng. Up to now, more than 100 ginsenosides have been isolated from the roots, rhizomes, stems, leaves, flower buds, fruits, and processes ginseng products [8]. According to the chemical structure of the sapogenins, ginsenosides can be mainly divided into three categories: the protopanaxadiol (PPD) type, including Rb1, Rb2, Rb3, Rd, Rg3, Rh2 and others; the protopanaxatriol (PPT) type, including Re, Rf, Rg1, Rg2, Rh1 and others; and the oleanolic acid (OA) type, including Ro and others [9]. The amount of ginsenosides can be influenced by the surrounding environment, including the local geo-climate, seasonal changes, external conditions such as light, temperature, humidity and soil fertility [10]. Even within the plant, the type and amount of ginsenosides vary and are dependent on ginseng age [11], harvest time [12], storage [13] and processing methods [14]. The variability in the amount and composition of ginsenosides is largely dependent on the place of origin, that is why the Chinese medicine emphasizes the concept of 'daodi' referring to an area, which can produce the best quality medicinal herbs. The wild Asian ginseng grows in cool and shady forests ranging from Korea and north-eastern China to the Russian Far East [6]. With the increasing demand for ginseng, the wild ginseng is becoming rare,

leading to the cultivation of ginseng in China, Korea, and Japan. Most of the world supply of ginseng is from farmed ginseng [15]. Studies on ginsenosides from ginseng grown in different regions showed that the content and composition of ginsenosides are diverse [9, 16].

In the north island of New Zealand (NZ), *P. ginseng* has been grown in Turangi around Lake Taupo for about 15 years. It has become established as a secondary crop grown under a natural pine forest canopy in an open-field simulation of a wild environment. It is hypothesized that ginseng grown in NZ under pine may have a unique ginsenoside profile based on how it is grown, such as the unique pumice soil around Lake Taupo, the cool winters in the high altitude of Mountain Ruapehu, the high intensity of ultraviolet rays, and seasonal changes opposite to the northern hemisphere.

Many analytical methods have been established to quantify ginsenosides, including TLC [17], HPLC equipped with a UV detector [18], a diode array detector (DAD) [19], an evaporative light scattering detector (ELSD) [20] or a mass spectrometric detector (MS) [21]. Liquid chromatography coupled with quadrupole time-of-flight tandem mass spectrometry (LC-QTOF-MS/MS) can provide advanced structural information with high sensitivity, specificity, and versatility in characterizing complex natural product samples. It has been successfully employed as a powerful tool for ginsenosides analysis [6, 7].

My objective of this Chapter was to measure ginsenosides from different parts of *P. ginseng* grown in NZ. The ginsenosides were extracted using ultrasonic waves and characterized by LC-QTOF-MS/MS. Currently, there is no validated method for NZ grown ginseng, and the program of liquid chromatograph including the flow rate, injection volume, temperature, time of program, and the ratio of mobile phase were modified based on the literature [22]. The stability (intra-day stability and inter-day stability), accuracy and precision, recovery rate, limit of detection, and limit of

quantification were validated in this study. The profiles and contents of ginsenosides from *P. ginseng* main root, fine root, rhizome, stem, and leaf with different ages were investigated. To my knowledge, this is the first-ever comprehensive report about the ginsenoside profile of NZ wild-simulated grown ginseng.

4.2 Materials and methods

4.2.1 Ginseng samples

Fresh *P. ginseng* plants (6-, 12-, 13-, 14-year old) were harvested in November 2017 from pine forest around Taupo and Rotorua, NZ. The ages of ginseng plants were identified by Mr. Glen Chen who planted the ginseng in the forest (there are different ginseng blocks marked the date of plantation in the forest). Five parts including main root (root body), fine root (root hair), rhizome (neck between root and stem), stem, and leaf of *P. ginseng* (Figure 4.1) were detached, rinsed with water and lyophilized at -68 °C. The dried *P. ginseng* was powdered using a CG2B coffee grinder (Breville, Sydney, Australia) and then passed through a 40-mesh sieve before extraction.

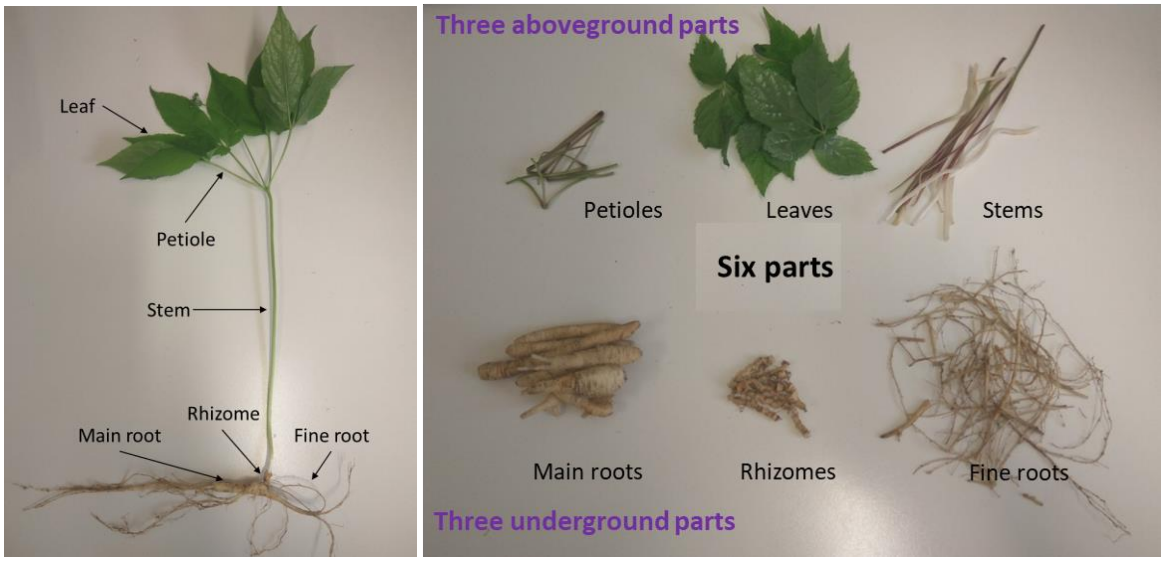


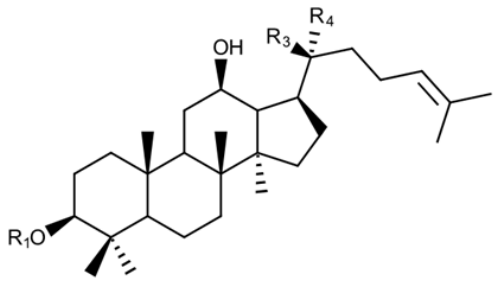
Figure 4.1 The whole plant of NZ-grown *P. ginseng* and various parts of ginseng materials analyzed in this study (petioles are too little and combined into stems for analysis)

4.2.2 Standard samples, chemicals, and reagents

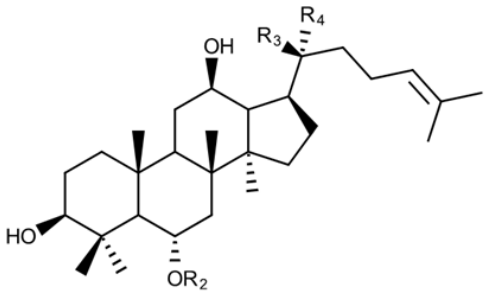
Twenty-three reference standards of ginsenosides Rg1, Re, Rf, Rb1, 20S-Rg2, 20R-Rg2, 20S-Rh1, 20R-Rh1, Rc, Rb2, Rb3, Rd, 20S-Rg3, 20R-Rg3, 20S-Rh2, 20R-Rh2, Rh3, Rh4, Rk2, Rk3, 20S-PPT, 20S-PPD, and F2 were purchased from Star Ocean Ginseng Ltd (Suzhou, China). The purities of all reference standards were above 98.0%. LC-MS grade acetonitrile (MeCN) and water were purchased from Merck (Phillipsburg, NJ, USA). LC-grade methanol (MeOH) and formic acid (HCOOH) was obtained from Fisher Chemical (Pittsburgh, PA, USA). Water (for extraction) was obtained from a Milli-Q Ultra-pure water system (Millipore, Billerica, MA, USA). Other reagents used in this study were of analytical grade.

4.2.3 Preparation of samples and reference standards

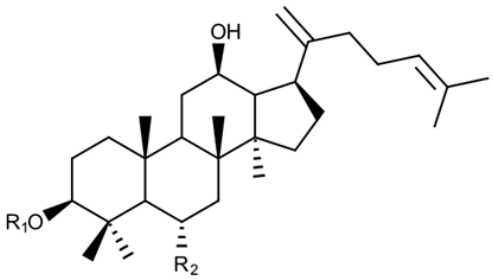
The ginsenosides in the *P. ginseng* material were ultrasonically extracted three times using a Q700 Sonicator (Melville, NY, USA). Approximately 1 g of the main root, stem, leaf, rhizome, and fine roots were separately extracted in 10 mL 70% (v/v) aqueous MeOH 20 kHz for 10 min at no more than 40 ℃ (the extraction was programmed to sonicate at 15% amplitude for 2 min. It was shut off for 1 min to cool between extraction and then re-started for 5 cycles) The second extraction repeated the procedure with a Q700 Sonicator, and the third extraction was continuously extracted for 1 h in an ultrasonic water bath (60 Hz). The three extracts were mixed and filtered through a 0.22 μm syringe filter before LC-MS analysis.



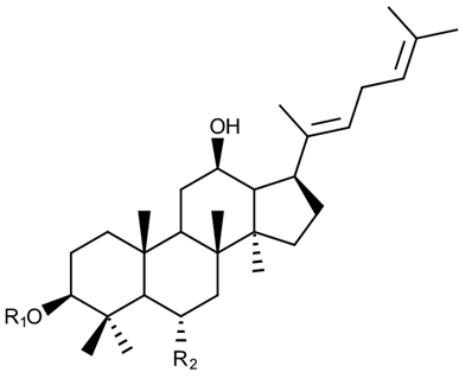
Name	Formula	R1	R3	R4
20S-PPD	$C_{30}H_{52}O_3$	H	OH	CH3
20R-PPD	$C_{30}H_{52}O_3$	H	CH3	OH
G-Rb1	$C_{54}H_{92}O_{23}$	Glc(2-1)Glc	OGlc(6-1)Glc	CH3
G-Rb2	$C_{53}H_{90}O_{22}$	Glc(2-1)Glc	OGlc(6-1)Arap	CH3
G-Rb3	$C_{53}H_{90}O_{22}$	Glc(2-1)Glc	OGlc(6-1)Xyl	CH3
G-Rc	$C_{53}H_{90}O_{22}$	Glc(2-1)Glc	OGlc(6-1)Araf	CH3
G-Rd	$C_{48}H_{82}O_{18}$	Glc(2-1)Glc	OGlc	CH3
20S-Rg3	$C_{42}H_{72}O_{13}$	Glc(2-1)Glc	OH	CH3
20R-Rg3	$C_{42}H_{72}O_{13}$	Glc(2-1)Glc	CH3	OH
20S-Rh2	$C_{36}H_{62}O_9$	Glc	OH	CH3
20R-Rh2	$C_{36}H_{62}O_9$	Glc	CH3	OH
G-F2	$C_{42}H_{72}O_{13}$	Glc	OGlc	CH3



Name	Formula	R2	R3	R4
20S-PPT	$C_{30}H_{52}O_4$	H	OH	CH3
20R-PPT	$C_{30}H_{52}O_4$	H	CH3	OH
G-Re	$C_{48}H_{82}O_{18}$	Glc(2-1)Rha	OGlc	CH3
G-Rf	$C_{42}H_{72}O_{14}$	Glc(2-1)Glc	OH	CH3
G-Rg1	$C_{42}H_{72}O_{14}$	Glc	OGlc	CH3
20S-Rg2	$C_{42}H_{72}O_{13}$	Glc(2-1)Rha	OH	CH3
20R-Rg2	$C_{42}H_{72}O_{13}$	Glc(2-1)Rha	CH3	OH
20S-Rh1	$C_{36}H_{62}O_9$	Glc	OH	CH3
20R-Rh1	$C_{36}H_{62}O_9$	Glc	CH3	OH



Name	Formula	R1	R2
Rk2	$C_{36}H_{60}O_7$	Glc	H
Rk3	$C_{36}H_{60}O_8$	H	OGlc



Name	Formula	R1	R2
Rh3	$C_{36}H_{60}O_7$	Glc	H
Rh4	$C_{36}H_{60}O_8$	H	OGlc

Figure 4.2 The chemical structures and molecular formulas of reference standards in this chapter.

Thirteen reference standards (structures are shown in Figure 4.2) of ginsenosides Rg1 (1.154 mg/mL), Re (0.923 mg/mL), Rf (1.462 mg/mL), Rb1 (0.769 mg/mL), Rg2 (0.615 mg/mL), Rh1 (1.000 mg/mL), Rc (1.077 mg/mL), Rb2 (0.846 mg/mL), Rb3 (0.629 mg/mL), Rd (0.692 mg/mL), Rg3 (1.154 mg/mL), Rh2 (1.077 mg/mL), and F2 (0.692 mg/mL) were dissolved in 70% MeOH, then mixed and diluted with 70% MeOH to obtain a series of mixture standard solutions of different concentrations. The solutions were filtered through a 0.22 μm syringe filter prior to LC-MS analysis.

4.2.4 HPLC-QTOF-MS

An Agilent 1290 liquid chromatograph (Agilent, MA, USA) equipped with a quaternary pump, an online degasser, an auto-sampler, a heated column compartment, and a UV detector was used. The ginsenosides were separated by a Zorbax Extend-C18 (2.1 x 100 mm, 3.5 μm) column (Agilent, USA) at a temperature of 33 °C. The binary gradient elution solvent consisted of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B). The gradient elution program was as follows: 0-4 min, 20% B; 4-10 min, 20-30% B; 10-25 min, 30-32.5% B; 25-27 min, 32.5-60% B; 27-39 min, 60-95% B; 39-40 min, 95% B; 40-40.5 min, 95-20% B; 40.5-45 min, 20% B. The flow rate was changed with the gradient: 0-27 min, 0.2 mL/min; 27-45 min, 0.25 mL/min. The wavelength was set at 203 nm and the injected volume was 1 μL.

An Agilent 6530 Quadrupole Time of Flight (QTOF) Mass Spectrometer (Agilent, USA) equipped with an electrospray ionization source was used for ESI-MS analysis [6]. Nitrogen (>99.998%) was used for nebulizer gas and curtain gas. The ion polarity was set to negative mode. The optimized parameters were set as follows: gas temperature: 350 °C; drying gas: 10.0 L/min; nebulizer: 37 psi; capillary voltage: 3500 V; fragmentor: 220 V; skimmer: 65 V. The reference masses in negative ion mode were at *m*/*z* 121.0509 and 922.0098. The mass spectrometer was in full scan ranges of *m*/*z* 100-2200 for MS and MS/MS. The acquisition rate was 4 spectra/s for MS and 1 spectrum/s for MS/MS. Data acquisition was controlled by the Agilent MassHunter Workstation Software (Version B.06.00, Agilent Technologies, Santa Clara, CA, USA).

4.3 Results and discussion

4.3.1 Identification of the detected ginsenosides in different parts of NZ-grown ginseng.

To investigate the chemical constitutes of NZ-grown *P. ginseng*, Zorbax Extend-C18 column and LC-QTOF-MS/MS was employed to separate and detect the ginsenosides extracted from the main roots, fine roots, rhizomes, stems and leaves of ginseng. The ginsenosides were well separated by gradient elution system of 0.1% formic acid aqueous solution (A) and 0.1% formic acid aqueous solution-acetonitrile (B). The base peak chromatogram (BPC) profiles are displayed in Figure 4.3. The negative ion mode was chosen to analyze the ginsenosides, for it provided more peaks and much clearer fragment information than that of the positive ion mode (Appendix1 Figure S4.1). Twenty-three ginsenoside reference standards were used to optimize the chromatographic conditions and to gain the fragmentation information of ginsenosides. In the negative mode of the full-scan MS, ginsenosides exhibited the deprotonated ion $[M-H]^-$ and adduct ion $[M+HCOO]^-$ due to the formic acid in the mobile phase (Appendix1 Figure S4.2). Different types of ginsenosides were well-differentiated and characterized in the negative MS/MS spectrum. In the other words, the PPD-type, PPT-type, and OA-type ginsenosides produced [(20S)-protopanaxadiol-H]$^-$ at *m*/*z* 459 ($C_{30}H_{51}O_3$), [(20S)-protopanaxatriol-H]$^-$ at *m*/*z* 475 ($C_{30}H_{51}O_4$), and [oleanolic acid -H]$^-$ at *m*/*z* 455 ($C_{30}H_{47}O_3$), respectively (Appendix1 Figure S4.3). Thus, aglycones could be easily identified by finding its [aglycon-H]$^-$ ion. Glycosidic units could be recognized by calculating the neutral loss molecular weight, for example, the mass differences of 176, 162, 146, and 132 Da mostly correspond to the glucuronyl (GluA), glucosyl (Glc), rhamnosyl (Rha), and pentosyl [arabinopyranosyl (Arap) or arabinofuranosyl (Araf) or xylosyl (Xyl)] group, respectively. Among these sugar residues, glucose is prone to further attachment of

small molecules such as the acetyl, malonyl, and butenoyl groups, which would also observe the mass differences of 43, 87, and 69 Da.

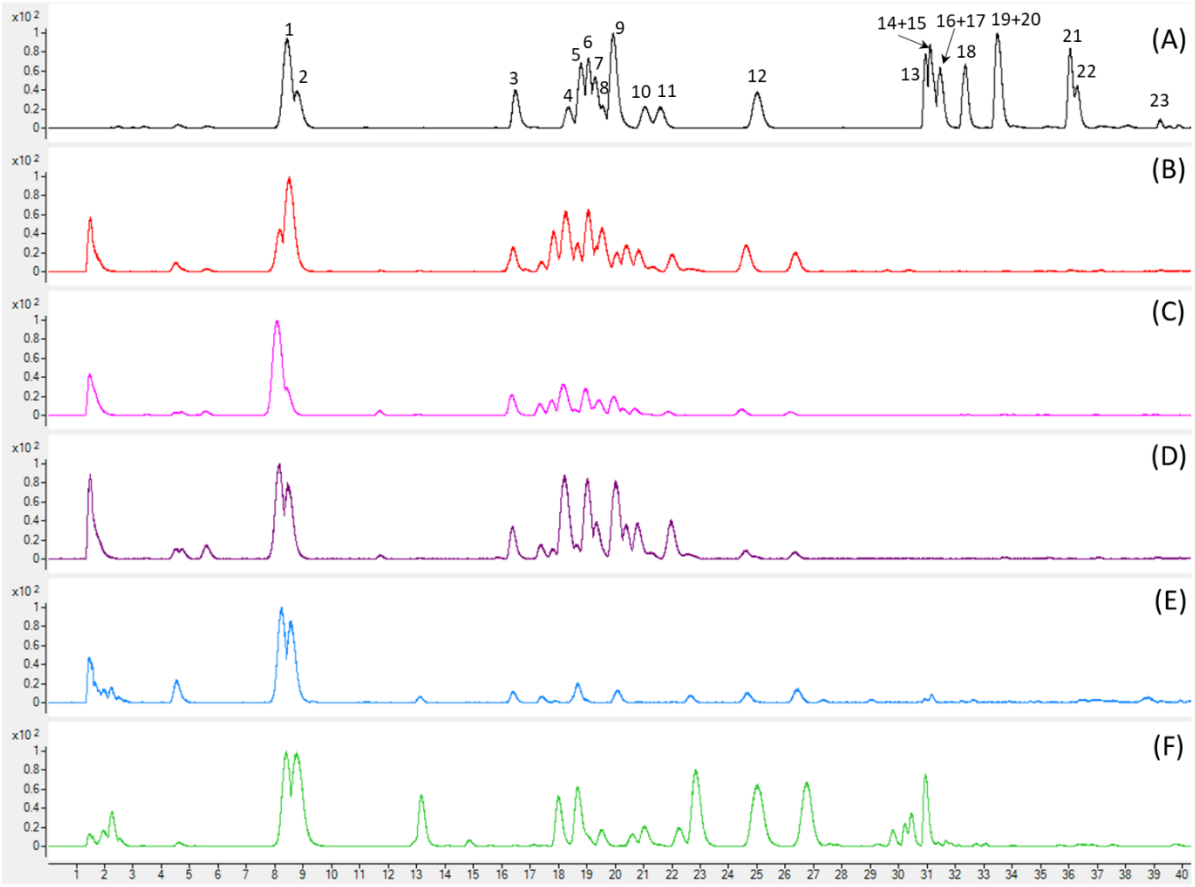


Figure 4.3 The base peak chromatogram (BPC) profiles of reference standards and different parts of ginseng. (A) reference standards: 1, Rg1; 2, Re; 3, Rf; 4, Rb1; 5, 20S-Rg2; 6, 20S-Rh1; 7, 20R-Rg2; 8, Rc; 9, 20R-Rh1; 10, Rb2; 11, Rb3; 12, Rd; 13, F2; 14, Rk3; 15, Rh4; 16, 20S-Rg3; 17, 20R-Rg3; 18, 20S-PPT; 19, 20S-Rh2; 20, 20R-Rh2; 21, Rk2; 22, Rh3; 23, PPD. (B) ginseng fine roots; (C) ginseng main roots; (D) ginseng rhizomes; (E) ginseng stems; (F) ginseng leaves.

Table 4.1 Compounds identified from different parts of NZ-grown *P. ginseng*

Peak No.	R.t	Measured value [ion form]	Molecular formula	Identity	References
1 [m,f,r,l]	3.60	815.4775 [M-H]; 861.4825 [M+HCOO]	$C_{42}H_{72}O_{15}$	Rg12/Re5/Ginsenjilinol/f-G-E/f-G-F	[23-26]
2 [m,f,r,l]	4.53	931.5308 [M-H]; 977.5340 [M+HCOO]	$C_{47}H_{80}O_{18}$	Re4/NG-R1/Q F6	[6, 27]
3 [m,f,r,s,l]	4.91	961.5376 [M-H]; 1007.5434 [M+HCOO]	$C_{48}H_{82}O_{19}$	Re1/Re2/Re3/NG-N/VG-R4/20glc-Rf	[6, 28]
4 [m,s,l]	5.26	931.5291 [M-H]; 977.5329 [M+HCOO]	$C_{47}H_{80}O_{18}$	Re4/NG-R1/Q F6	[6, 27]
5 [m]	5.49	699.4341 [M+HCOO]	$C_{36}H_{62}O_{10}$	G-Ki/G-Km/G-ST2	[29, 30]
6 [m,f,r,s,l]	5.82	931.5315 [M-H]; 977.5319 [M+HCOO]	$C_{47}H_{80}O_{18}$	Re4/NG-R1/Q F6	[6, 27]
7 [m,f,r,l]	6.09	799.4956 [M-H]; 845.4905 [M+HCOO]	$C_{42}H_{72}O_{14}$	Rf-1a	[31]
8 [m,f,r,s,l]	8.54	799.4824 [M-H]; 845.4934 [M+HCOO]	$C_{42}H_{72}O_{14}$	Rg1	[6]
9 [m,f,r,s,l]	8.93	945.5430 [M-H]; 991.5506 [M+HCOO]	$C_{48}H_{82}O_{18}$	Re	[6]
10 [f]	9.78	447.2190 [M-H]; 493.2236 [M+HCOO]	$C_{29}H_{52}O_{3}$	Unknown	-
11 [f]	10.25	445.1943 [M-H]; 491.2083 [M+HCOO]	$C_{29}H_{50}O_{3}$	Unknown	-
12 [l]	11.74	699.4281 [M+HCOO]	$C_{36}H_{62}O_{10}$	G-Ki/G-Km/G-ST2	[29, 30]
13 [m,f,r,l]	11.93	841.4988 [M-H]; 877.4923 [M+HCOO]	$C_{44}H_{74}O_{15}$	Ac-Rg1	[32]
14 [m,f,r,l]	12.94	885.4872 [M-H]	$C_{45}H_{74}O_{17}$	m-Rg1	[32]
15 [m,f,r,s,l]	13.21	1031.5461 [M-H]	$C_{51}H_{84}O_{21}$	m-Re	[32]
16 [m,f,r,s,l]	13.23	987.5541 [M-H]	$C_{50}H_{84}O_{19}$	Ac-Re	[32]
17 [m,f,r,s,l]	13.31	841.4970 [M-H]	$C_{44}H_{74}O_{15}$	Yesanchinoside D / Ac-panajaponol A	[6]
18 [m,l]	13.70	815.4794 [M-H]	$C_{42}H_{72}O_{15}$	Rg12/Re5/Ginsenjilinol/f-G-E/f-G-F	[23-26]

19 [m,l]	14.13	815.4814 [M-H]	$C_{42}H_{72}O_{15}$	Rg12/Re5/Ginsenjilinol/f-G-E/f-G-F	[23-26]
20 [m,f,r,s,l]	14.92	961.5363 [M-H]; 1007.5475 [M+HCOO]	$C_{48}H_{82}O_{19}$	Re1/Re2/Re3/NG-N/VG-R4/20glc-Rf	[6, 28]
21 [l]	15.56	977.5317 [M+HCOO]	$C_{47}H_{80}O_{18}$	Re4/NG-R1/Q F6	[6, 27]
22 [r]	15.62	973.5369 [M-H]	$C_{49}H_{82}O_{19}$	Unknown (Ac-Re4 / Ac-NG-R1)[&]	-
23 [l]	15.66	829.4933 [M+HCOO]	$C_{42}H_{72}O_{13}$	G-La/Rg2 isomer	[6, 33]
24 [r]	15.95	1117.5375 [M-H]	$C_{54}H_{86}O_{24}$	mf-Rd6/isomer	[5]
25 [r]	16.03	1185.5369 [M-H]	$C_{58}H_{90}O_{25}$	Unknown (Bu-mf-Rd6)[&]	-
26 [l]	16.06	1047.5379 [M-H]	$C_{51}H_{84}O_{22}$	Unknown (m-Re1 / isomer)[&]	-
27 [l]	16.07	1003.5479 [M-H]	$C_{50}H_{84}O_{20}$	Unknown (Ac-Re1/isomer)[&]	-
28 [f,r]	16.17	1371.6822 [M+HCOO]	$C_{62}H_{102}O_{30}$	m-Ra3/m-NG-R4	[6, 34]
29 [f,r]	16.42	1239.6375 [M-H]	$C_{59}H_{100}O_{27}$	Ra3/NG-R4	[6]
30 [s]	16.46	915.5235 [M-H]	$C_{47}H_{80}O_{17}$	NG-Fe/VG-R16	[6]
31 [m,f,r,l]	16.52	845.4944 [M+HCOO]	$C_{42}H_{72}O_{14}$	Rf	[6]
32 [m,f,r]	16.98	1325.6428 [M-H]	$C_{62}H_{102}O_{30}$	m-Ra3/m-NG-R4	[6, 34]
33 [l]	17.14	845.4905 [M+HCOO]	$C_{42}H_{72}O_{14}$	Rf isomer	[6]
34 [l]	17.46	977.5333 [M+HCOO]	$C_{47}H_{80}O_{18}$	Re4/NG-R1/Q F6	[6, 27]
35 [m,f,r,s]	17.53	769.4774 [M-H]; 815.4818 [M+HCOO]	$C_{41}H_{70}O_{13}$	NG-R2/G-F3/G-F5	[6]
36 [m,r]	17.74	841.4952 [M-H]	$C_{44}H_{74}O_{15}$	Yesanchinoside D / Ac-panajaponol A	[6]
37 [m,f,r]	17.75	1239.6355 [M-H]	$C_{59}H_{100}O_{27}$	Ra3/NG-R4	[6]
38 [f,r]	17.81	1277.6038 [M-H]	$C_{62}H_{102}O_{27}$	Ra4	[35]
39 [m,r,s,l]	18.04	815.4797 [M-H]	$C_{42}H_{72}O_{15}$	Rg12/Re5/Ginsenjilinol/f-G-E/f-G-F	[23-26]
40 [m,f,r]	18.04	1209.6314 [M-H]	$C_{58}H_{98}O_{26}$	Ra1/Ra2	[6]

41 [m,f]	18.28	1239.6407 [M-H]	$C_{59}H_{100}O_{27}$	Ra3/NG-R4	[6]
42 [m,f,r,s,l]	18.55	1107.5998 [M-H]	$C_{54}H_{92}O_{23}$	Rb1	[6]
43 [l]	18.72	815.4778 [M-H]	$C_{42}H_{72}O_{15}$	Rg12/Re5/Ginsenjilinol/f-G-E/f-G-F	[23-26]
44 [m,f,r,s,l]	18.84	829.4976 [M+HCOO]	$C_{42}H_{72}O_{13}$	Rg2	[6]
45 [m,f]	18.85	1295.6288 [M-H]	$C_{61}H_{100}O_{29}$	Unknown (m-Ra1/m-Ra2)[&]	-
46 [m,f,r,s,l]	19.08	683.4389 [M+HCOO]	$C_{36}H_{62}O_{9}$	Rh1	[6]
47 [m,f,r]	19.11	1325.6348 [M-H]	$C_{62}H_{102}O_{30}$	m-Ra3/m-NG-R4	[6, 34]
48 [m,f,r,s,l]	19.29	1149.6098 [M-H]	$C_{56}H_{94}O_{24}$	Ac-Rb1	[6]
49 [m,f,r,s,l]	19.32	1193.6004 [M-H]	$C_{57}H_{94}O_{26}$	m-Rb1	[6]
50 [m,f,r,s,l]	19.61	1077.5894 [M-H]	$C_{53}H_{90}O_{22}$	Rc	[6]
51 [m,f,r]	19.81	1209.6327 [M-H]	$C_{58}H_{98}O_{26}$	Ra1/Ra2	[6]
52 [f,r]	19.84	1277.6039 [M-H]	$C_{62}H_{102}O_{27}$	Ra4	[35]
53 [m]	20.03	683.4399 [M+HCOO]	$C_{36}H_{62}O_{9}$	F1/ Rh19	[36, 37]
54 [m,f,r,s,l]	20.32	955.4945 [M-H]	$C_{48}H_{76}O_{19}$	Ro	[6]
55 [m]	20.46	1209.6316 [M-H]	$C_{58}H_{98}O_{26}$	Ra1/Ra2	[6]
56 [m,f,r]	20.71	1295.6322 [M-H]	$C_{61}H_{100}O_{29}$	Unknown (m-Ra1/m-Ra2)[&]	-
57 [m,f,r,s,l]	20.72	1119.6013 [M-H]	$C_{55}H_{92}O_{23}$	Ac-Rc	[6]
58 [m,f,r,s,l]	20.72	1163.5926 [M-H]	$C_{56}H_{92}O_{25}$	m-Rc	[38]
59 [m,f,r]	20.78	1251.6374 [M-H]	$C_{60}H_{100}O_{27}$	Ra5/isomer	[6]
60 [r]	20.89	1209.6296 [M-H]	$C_{58}H_{98}O_{26}$	Ra1/Ra2	[6]
61 [m,f,r,s,l]	21.17	1077.5897 [M-H]	$C_{53}H_{90}O_{22}$	Rb2	[6]
62 [m,f,r]	21.42	1149.6114 [M-H]	$C_{56}H_{94}O_{24}$	PQ-R1 / Ac-Rb1isomer	[6]
63 [m,f,r]	21.42	1193.5997 [M-H]	$C_{57}H_{94}O_{26}$	mf-Rb1 / mf-Rb2/ m-Rb1isomer	[5, 6]

64 [m,f,r,s,l]	21.63	1077.5896 [M-H]	$C_{53}H_{90}O_{22}$	Rb3	[6]
65 [f,r]	22.21	1209.6274 [M-H]	$C_{58}H_{98}O_{26}$	Ra1/Ra2	[6]
66 [m,f,r,s,l]	22.37	1163.6592 [M-H]	$C_{56}H_{92}O_{25}$	m-Rb3	[32]
67 [m,f,r,s,l]	22.42	1119.6017 [M-H]	$C_{55}H_{92}O_{23}$	Ac-Rb3	[32]
68 [m,f,r,s,l]	22.93	683.4299 [M+HCOO]	$C_{36}H_{62}O_{9}$	F1/Rh19	[36, 37]
69 [m,f,r,s,l]	22.99	1163.5864 [M-H]	$C_{56}H_{92}O_{25}$	m-Rb2/m-Rb3/m-Rc isomer	[32]
70 [m,f,r,s]	23.13	1119.5989 [M-H]	$C_{55}H_{92}O_{23}$	Ac-Rc/Ac-Rb2/Ac-Rb3/Rs1/Rs2	[6, 39]
71 [m,f,r]	23.26	1251.6388 [M-H]	$C_{60}H_{100}O_{27}$	Ra5/isomer	[6]
72 [m,f,r]	23.37	1295.6295 [M-H]	$C_{61}H_{100}O_{29}$	Unknown (m-Ra1/m-Ra2)[&]	-
73 [m,f,r,s,l]	25.11	945.5363 [M-H]; 991.5445 [M+HCOO]	$C_{48}H_{82}O_{18}$	Rd	[6]
74 [m,r,s]			$C_{42}H_{66}O_{14}$	Chikusetsusaponin IVa/Zingibroside R1	[6]
	25.34	793.4404 [M-H]			
75 [m,f,r,s]	25.41	1163.5861 [M-H]	$C_{56}H_{92}O_{25}$	m-Rb2/m-Rb3/m-Rc isomer	[32]
76 [m,f,r,s]	25.44	1119.5963 [M-H]	$C_{55}H_{92}O_{23}$	Ac-Rc/Ac-Rb2/Ac-Rb3/Rs1/Rs2	[6, 39]
77 [m]	26.11	1163.5858 [M-H]	$C_{56}H_{92}O_{25}$	m-Rb2/m-Rb3/m-Rc isomer	[32]
78 [m]	26.11	1119.5969 [M-H]	$C_{55}H_{92}O_{23}$	Ac-Rc/Ac-Rb2/Ac-Rb3/Rs1/Rs2	[6, 39]
79 [l]	26.59	679.4421 [M-H]; 725.4446 [M+HCOO]	$C_{38}H_{64}O_{10}$	Unknown (Ac-Rh1)[&]	-
80 [m,f,r,s]	26.86	1031.5488 [M-H]	$C_{51}H_{84}O_{21}$	m-Rd	[6]
81 [m,f,r,s]	26.88	987.5577 [M-H]	$C_{50}H_{84}O_{19}$	Ac-Rd	[6]
82 [m,f,r,s]	27.69	1031.5374 [M-H]	$C_{51}H_{84}O_{21}$	m-Rd isomer	[6]
83 [m,f,r,s]	27.70	987.5575 [M-H]	$C_{50}H_{84}O_{19}$	Ac-Rd isomer	[6]
84 [m,r]	28.85	991.5488 [M+HCOO]	$C_{48}H_{82}O_{18}$	Rd isomer/Re isomer	[6]
85 [m,s,l]	29.40	1117.5453 [M-H]	$C_{54}H_{86}O_{24}$	mf-Rd6/isomer	[5]

86 [m,f,r,s,l]	29.85	987.5550 [M-H]	$C_{50}H_{84}O_{19}$	Ac-Rd isomer /Ac-Re isomer	[6]
87 [m,f,r,s,l]	29.87	1031.5467 [M-H]	$C_{51}H_{84}O_{21}$	m-Rd isomer /m-Re isomer	[6]
88 [m,r,l]	30.39	915.5314 [M-H]	$C_{47}H_{80}O_{17}$	NG-Fe/VG-R16	[6]
89 [m,f,r,s,l]	30.40	961.5425 [M-H]	$C_{48}H_{82}O_{19}$	Re1/Re2/Re3/NG-N/VG-R4/20glc-Rf	[6, 28]
90 [m,f,r]	30.55	1001.5308 [M+HCOO]	$C_{48}H_{76}O_{19}$	Ro isomer	[6]
91 [m,f,r]	30.55	957.5381 [M-H]	$C_{49}H_{82}O_{18}$	Unknown (Ac-NG-Fe)[&]	-
92 [m,f,r]	30.95	1015.5485 [M+HCOO]	$C_{49}H_{78}O_{19}$	Me-Ro	[40]
93 [m,f,r,l]	30.98	829.4936 [M+HCOO]	$C_{42}H_{72}O_{13}$	F2	[32]
94 [m,f,r,s,l]			$C_{42}H_{66}O_{14}$	Chikusetsusaponin IVa/Zingibroside	[6]
	31.17	793.4434 [M-H]		R1	
95 [s]	31.21	825.5006 [M-H]	$C_{44}H_{74}O_{14}$	Ac-Rg3	[6]
96 [m,f,r,s,l]	31.47	829.4934 [M+HCOO]	$C_{42}H_{72}O_{13}$	Rg3	[6]
97 [l]	31.67	675.3591 [M-H]; 721.3636 [M+HCOO]	$C_{40}H_{52}O_{8}$	Unknown	-
98 [l]	31.88	675.3556 [M-H]; 721.3614 [M+HCOO]	$C_{40}H_{52}O_{9}$	Unknown	-
99 [l]	32.71	653.3698 [M-H]; 699.3791 [M+HCOO]	$C_{36}H_{62}O_{10}$	G-Ki/G-Km/G-ST2	[29, 30]
100 [l]	32.85	513.3057 [M-H]; 559.3106 [M+HCOO]	$C_{27}H_{46}O_{9}$	Unknown	-
101 [m,f,r,l]	33.09	667.4455 [M+HCOO]	$C_{36}H_{62}O_{8}$	Rh2 isomer	[6]
102 [m,f,r,s,l]	33.48	667.4376 [M+HCOO]	$C_{36}H_{62}O_{8}$	Rh2	[6]

[m], main root; [f], fine root; [r], rhizome; [s], stem; [l], leaf. [&] the possible ginsenosides which has not been reported.

A total of 102 compounds were detected from the main root, fine root, rhizome, stem, and the leaf of the 12-year old *P. ginseng*. Thirteen ginsenosides (peaks 8, 9, 31, 42, 44, 46, 50, 61, 64, 73, 93, 96, 102) were unambiguously identified by comparison with the reference standards. Since there are many isomeric forms of ginsenosides, the others were assigned by comparing the empirical molecular formulas and fragmentation information with those of the literature. In some cases shown below, the mass spectrometry analysis had identical *m*/*z* and through MS/MS analysis it was possible to group them into possible groups based on the probable aglycone. The information of compounds was shown in Table 4.1.

The above peaks were assigned based on comparing with the data of published known ginsenosides. Interestingly, some potential new compounds were also found based on my data. For example, peaks 45, 56, and 72 were observed $[M-H]^-$ ions at *m*/*z* 1295.6288, indicating the molecular formulas were $C_{61}H_{100}O_{29}$. In the MS/MS spectra, the mass difference between *m*/*z* 1295.6288 and *m*/*z* 1209.6236 suggested that malonyl was lost from the $[M-H]^-$ ion. After losing the malonyl group, the fragmentation pathway was similar to that of Ra1 and Ra2. Thus, peak 45, 56, and 72 were deduced as malonyl Ra1 (m-Ra1), malonyl Ra2 (m-Ra2), and isomers, respectively. The $[M-H]^-$ ion of peak 91 was at *m*/*z* 957.5381, indicating the molecular formula was $C_{49}H_{82}O_{18}$. The mass difference between *m*/*z* 957.5381 and *m*/*z* 915.5355 suggested that acetyl was removed from the $[M-H]^-$ ion. After losing the acetyl group, the fragmentation pathway was similar to that of NG-Fe. Thus, peak 91 was deduced as acetyl NG-Fe (Ac-NG-Fe) or its isomer. In the same way, the other five potential new compounds of peaks 22, 25, 26, 27, and 79 gave their $[M-H]^-$ ions at *m*/*z* 973.5369, 1185.5369, 1047.5379, 1003.5479, and 679.4421 were tentatively deduced as acetyl Re4 (Ac-Re4) or isomer, butenoyl mf-Rd6, malonyl Re1 (m-Re1) or isomer, acetyl Re1 (Ac-Re1) or isomer, and acetyl Rh1 (Ac-Rh1) or isomer, respectively. Further studies need to be conducted to confirm their structures, for there is no report about these ginsenosides. While there are another five unknown compounds (peaks 10, 11, 97, 98, and 100) with small

molecular weight, their $[M-H]^-$ ions were at *m*/*z* 447.2190, 445.1943, 675.3591, 675.3556, and 513.3057, respectively. Their adduct $[M+HCOO]^-$ ions were also observed in the MS spectra.

Based on the above results of the qualitative analysis, a total of 102 compounds were identified from the NZ-grown *P. ginseng*. Among them, 76, 69, 74, 44, and 57 ginsenosides presented in the main root, fine root, rhizome, stem, and leaf, respectively. The underground parts (main root, fine root, and rhizome) had more diversified ginsenosides than the aboveground parts (stem and leaf). From the Table 4.1, we can see that the main common ginsenosides, such as Rb1, Rb2, Rb3, Rc, Rd, Re, Rg1, Rg2, Rg3, Rh1, Rh2, etc., exist in various parts of the ginseng plant. However, there are also special ingredients in each part which were not detected in other parts. Specifically, five (peaks 5, 53, 55, 77, and 78), two (peaks 10 and 11), four (peaks 22, 24, 25, and 60), two (peaks 30 and 95), and thirteen (peaks 12, 21, 23, 26, 27, 33, 34, 43, 79, and 97-100) compounds were detected only in the main root, fine root, rhizome, stem, and leaf, respectively.

4.3.2 Valuation of quantitative analytical method

Thirteen ginsenoside references were used to conduct quantitative analysis. The calibration curves were plotted based on linear regression of the integrated peak areas (y) of extracted ion chromatograms (EICs) to concentrations (x, ng) of 13 ginsenoside references in the standard solution at five different concentrations. The regression equations of calibration curves, correlation coefficient, and linear ranges for the ginsenoside standards are shown in Table 4.2. The correlation coefficients are no less than 0.9986, which show good linearity. Under the present chromatographic conditions, the LOD and LOQ, which are in the range from 0.3 to 2.9 mg/L and from

1.0 to 9.5 mg/L, were determined at a signal-to-noise ratio (S/N) of about 3 and 10, respectively.

Table 4.2 The regression equations, linear ranges, limits of detection and limits of quantification of 13 ginsenosides

Ginsenosides	Calibration curve	r^2	Concentration range (mg/L)	LOD (mg/L)	LOQ (mg/L)
Rg1	y=117288x+46652	0.9994	3.0-192.3	2.8	9.5
Re	y=74382x-12033	0.9999	2.4-153.8	0.9	3.0
Rf	y=68030x+39879	0.9996	2.5-162.4	2.0	6.8
Rb1	y=40414x+29985	0.9986	2.0-128.2	2.1	6.9
Rg2	y=142443x+52824	0.9997	1.6-102.5	1.0	3.4
Rh1	y=161372x+41690	0.9994	1.7-27.8	0.9	2.9
Rc	y=36973x-30003	0.9995	1.9-119.7	1.6	5.5
Rb2	y=42370x-36168	0.9994	2.2-141.0	2.1	6.9
Rb3	y=41463x-24949	0.9998	1.8-57.7	1.1	3.7
Rd	y=76766x+18313	0.9988	2.1-67.3	2.9	9.5
F2	y=114456x+3865.7	0.9995	2.4-38.4	1.2	3.9
Rg3	y=100975x+2937.7	0.9998	2.0-32.1	0.6	2.0
Rh2	y=194885x-27449	0.9999	2.3-18.7	0.3	1.0

y is the integrated peak area and x is the amount of analyte (ng). LOD, limit of detection; LOQ: limit of quantification.

Intra-day and inter-day variations were employed to evaluate the precision of this LC-MS method for determining ginsenoside. Thirteen ginsenoside standards with the known concentrations were mixed and tested. In the intra-day variability experiment, the test was conducted within one day and three parallel experiments were carried out. While for the inter-day precision, the mixed standard solutions were examined in

three different days. The relative standard deviation (RSD) was used to describe variations. As shown in Table 4.3, the validations of intra-day and inter-day precisions are from 0.1% to 4.9% and from 3.4% to 6.5%, respectively.

Table 4.3 Inter-day variation and intra-day variation (RSD) of the standards

Name	m/z	Inter-day		Intra-day	
		Con.(μg/ml)	RSD	Con.(μg/ml)	RSD
Rg1	845.5	138.3	5.5%	139.6	2.0%
Re	991.5	94.3	6.5%	94.2	2.4%
Rf	845.5	76.2	6.4%	75.9	1.2%
Rb1	1107.5	79.3	3.4%	77.7	0.7%
Rg2	829.5	65.3	4.5%	66.0	4.0%
Rh1	683.5	55.7	4.9%	54.4	1.3%
Rc	1077.5	98.4	3.9%	97.1	0.5%
Rb2	1077.5	84.1	4.8%	82.4	2.1%
Rb3	1077.5	87.7	5.0%	87.0	0.1%
Rd	991.5	90.7	6.1%	91.6	2.3%
F2	829.5	57.0	4.9%	57.1	3.8%
Rg3	829.5	73.8	3.6%	74.4	4.9%
Rh2	667.5	68.7	4.7%	69.0	1.1%

The recovery experiment was carried out to evaluate the accuracy of the method. The recoveries were expressed according to the following formula: recovery (%) = (found amount - original amount) / added amount×100 [41]. Thirteen ginsenoside standards with known amount were added into a certain amount (0.5 g) of the sample. The mixture was extracted and analyzed according to Section 4.2.3 and 4.2.4. The recoveries of 13 ginsenosides are listed in Table 4.4. It indicates that the proposed

method in this chapter has good accuracy with the recoveries of ginsenosides between 88.2 and 108.0 %, and the RSD ranging from 1.1% to 13.4%.

Table 4.4 The recovery of 13 ginsenosides

Ginsenosides	Original (μg)	Added (μg)	Found (μg)	Recovery (%)	RSD (%)
Rg1	222.6	11.0	232.9 ± 1.4	94.3	13.0
Re	169.6	10.6	178.9 ± 1.2	88.2	10.9
Rf	69.3	4.4	73.8 ± 0.3	102.5	6.9
Rb1	211.1	16.5	225.1 ± 1.6	94.5	9.4
Rg2	15.3	1.5	16.9 ± 0.1	106.0	9.0
Rh1	2.7	1.0	3.8 ± 0.0	105.1	1.9
Rc	75.0	5.4	80.2 ± 0.2	96.3	3.8
Rb2	72.1	6.4	78.3 ± 0.2	97.8	3.3
Rb3	19.8	1.7	21.6 ± 0.2	106.8	13.4
Rd	41.1	2.6	43.7 ± 0.3	100.3	10.3
F2	0.1	1.4	1.6 ± 0.0	106.2	1.8
Rg3	0.7	1.2	2.0 ± 0.0	108.0	3.0
Rh2	0.1	1.4	1.4 ± 0.0	92.0	1.1

4.3.3 Ginsenoside content of NZ-grown *P. ginseng*

To comprehensively evaluate the content of ginsenosides in NZ-grown *P. ginseng*, accurate quantification and relative quantification were used to analyze the different parts of ginseng samples of different ages. Specifically, thirteen ginsenosides were accurately quantified by their linear regression equations of standard curves, and some ginsenosides without reference standards, including ginsenosides m-Rg1, m-Re, m-Rd, m-Rb1, m-Rb2, m-Rb3, and m-Rc, were relatively quantified by the regression equations of their corresponding neutral ginsenosides. The EICs of the quantified ginsenosides are shown in the supplementary material (Appendix 1 Figure S4.4).

Ginsenoside contents were analyzed in five ginseng parts (main root, fine root, rhizome, stem, and leaf) with different ages (6-, 12-, 13-, 14-year old). Apart from the individual ginsenoside content, the total ginsenoside amount, the ratio of PPD-type to PPT-type (PPD/PPT), and the ratio of neutral ginsenoside to malonyl ginsenoside (G/m-G) were also described in this chapter. The total ginsenoside amount is the sum of all the quantified ginsenosides, similarly, the PPD-type amount and PPT-type amount are the sum of all the quantified PPD-type ginsenosides and PPT-type ginsenosides, respectively; the malonyl ginsenoside amount is the sum of seven quantified malonyl ginsenosides (m-Re, m-Rg1, m-Rb1, m-Rb2, m-Rb3, m-Rc, and m-Rd), and the neutral ginsenoside amount is the sum of corresponding neutral ginsenosides (Re, Rg1, Rb1, Rb2, Rb3, Rc, and Rd).

4.3.3.1 Ginsenoside content in different ages of *P. ginseng* main root

The content of ginsenosides from *P. ginseng* main root with different ages is shown in Table 4.5. Compounds Rg1, Re, Rb1, and m-Rb1 are the four main ginsenosides in the main root of NZ-grown *P. ginseng*, and the content of ginsenoside Rg1 is the highest in different ages. Different from the literature [11] that the content of ginsenosides in root increases with increase in age of *P. ginseng* from one to five years, I found that the content of ginsenosides is not directly related to age. In the main root, the number of neutral ginsenosides are more than that of malonyl ginsenosides, their ratio ranged from 1.6 to 2.2. The ratio of PPD-type to PPT-type decreased with age before 12-year old and then increased with age. At 12th year, the contents of the two type ginsenosides were almost equal.

Table 4.5 Contents (mg/g) of ginsenosides in different ages of *P. ginseng* main root (mean ± S.D.)

	ginsenosides	6-yr old	12-yr old	13-yr old	14-yr old
Accurate Quantification	Rg1	6.2 ± 0.1	7.7 ± 0.1	9.3 ± 0.2	7.1 ± 0.3
	Rf	1.9 ± 0.1	2.4 ± 0.1	2.7 ± 0.1	2.1 ± 0.1
	Re	5.7 ± 0.1	6.1 ± 0.1	6.3 ± 0.3	4.2 ± 0.2
	Rg2	0.5 ± 0.0	0.4 ± 0.0	0.6 ± 0.0	0.4 ± 0.0
	Rh1	#	#	#	#
	Rb1	6.3 ± 0.6	5.3 ± 0.5	5.8 ± 0.5	5.2 ± 0.6
	Rc	1.5 ± 0.3	1.4 ± 0.1	2.2 ± 0.2	1.5 ± 0.2
	Rb2	1.2 ± 0.4	0.9 ± 0.1	1.9 ± 0.2	1.2 ± 0.2
	Rb3	0.7 ± 0.0	0.3 ± 0.0	0.4 ± 0.1	0.3 ± 0.0
	Rd	1.4 ± 0.2	0.4 ± 0.0	0.5 ± 0.1	0.3 ± 0.1
	Rh2	#	#	#	#
	F2	#	#	#	#
	Rg3	#	#	#	#
Relative Quantification	Ro	2.1 ± 0.1	2.0 ± 0.0	2.6 ± 0.0	2.1 ± 0.1
	mRg1	0.6 ± 0.1	0.9 ± 0.1	1.0 ± 0.1	0.8 ± 0.1
	mRe	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
	mRd	1.5 ± 0.0	0.5 ± 0.0	0.5 ± 0.0	0.4 ± 0.0
	mRb1	6.1 ± 0.4	6.0 ± 0.6	7.2 ± 0.5	6.8 ± 0.4
	mRc	1.6 ± 0.3	1.4 ± 0.1	2.6 ± 0.2	1.9 ± 0.1
	mRb2	2.8 ± 0.3	1.0 ± 0.1	2.8 ± 0.2	1.8 ± 0.1
	mRb3	0.9 ± 0.1	0.2 ± 0.0	0.5 ± 0.0	0.4 ± 0.0
Total amount		40.9 ± 0.5	36.7 ± 0.1	47.0 ± 0.7	36.7 ± 0.9
PPD-type/PPT-type		1.6 ± 0.0	1.0 ± 0.0	1.2 ± 0.0	1.4 ± 0.0
Ginsenosides/m-Ginsenosides		1.9 ± 0.2	2.2 ± 0.3	1.8 ± 0.2	1.6 ± 0.2

not quantified. Each sample was extracted and tested three times.

4.3.3.2 Ginsenoside content in different ages of *P. ginseng* fine root

Similar to the main root, the ginsenoside content of fine root in 13-year old is the highest, reaching at 131.3 ± 2.9 mg/g (Table 4.6). Different from the main root, the content of ginsenoside Re is the highest in the fine root, and it is about 3 to 6 times higher than that of Rg1 in different ages, which is consistent with the results observed

by Shi et al. [11]. Ginsenosides Rb1 and m-Rb1 are the two most abundant in the remaining quantified saponins. The content of malonyl PPD-type ginsenosides is higher than that of the corresponding neutral ginsenosides. However, the amount of total malonyl ginsenosides is lower than that of neutral ginsenosides apart from 13-year old sample, the PPD-type ginsenosides are more 1.8 times higher than PPT-type ginsenosides content.

Table 4.6 Contents (mg/g) of ginsenosides in different ages of *P. ginseng* fine root (mean ± S.D.)

	ginsenosides	6-yr old	12-yr old	13-yr old	14-yr old
Accurate Quantification	Rg1	5.3 ± 0.1	3.3 ± 0.2	4.8 ± 0.2	3.7 ± 0.2
	Rf	2.6 ± 0.0	2.9 ± 0.1	3.6 ± 0.3	3.2 ± 0.2
	Re	16.2 ± 0.1	20.0 ± 0.3	22.3 ± 0.7	19.4 ± 0.8
	Rg2	1.8 ± 0.1	2.2 ± 0.1	2.5 ± 0.3	2.2 ± 0.1
	Rh1	#	#	#	#
	Rb1	11.7 ± 0.4	7.6 ± 0.8	11.9 ± 0.9	12.3 ± 1.3
	Rc	7.3 ± 0.6	3.9 ± 0.4	6.5 ± 0.7	5.3 ± 0.8
	Rb2	6.7 ± 0.7	2.6 ± 0.4	6.0 ± 1.0	4.2 ± 0.7
	Rb3	1.3 ± 0.1	0.7 ± 0.1	1.4 ± 0.2	1.1 ± 0.2
	Rd	5.6 ± 0.5	5.2 ± 0.7	5.5 ± 0.9	3.8 ± 0.6
	Rh2	#	#	#	#
	F2	0.2 ± 0.0	#	#	#
	Rg3	#	#	#	#
Relative Quantification	Ro	0.9 ± 0.0	0.7 ± 0.0	2.0 ± 0.0	1.5 ± 0.1
	mRg1	0.4 ± 0.0	0.5 ± 0.0	0.7 ± 0.1	0.4 ± 0.0
	mRe	0.4 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.5 ± 0.0
	mRd	7.2 ± 0.5	7.3 ± 0.3	8.8 ± 0.2	6.0 ± 0.2
	mRb1	16.7 ± 1.6	13.8 ± 1.1	21.3 ± 0.4	18.6 ± 0.6
	mRc	11.6 ± 1.2	7.4 ± 0.6	14.2 ± 0.7	9.5 ± 0.5
	mRb2	13.8 ± 1.3	5.3 ± 0.4	16.1 ± 0.8	9.0 ± 0.5
	mRb3	2.5 ± 0.3	1.2 ± 0.1	3.2 ± 0.1	1.9 ± 0.0
Total amount		112.2 ± 2.5	84.8 ± 0.4	131.3 ± 2.9	102.5 ± 3.2
PPD-type/PPT-type		3.2 ± 0.1	1.9 ± 0.0	2.7 ± 0.1	2.4 ± 0.0
Ginsenoside/m-Ginsenoside		1.0 ± 0.1	1.2 ± 0.2	0.9 ± 0.1	1.1 ± 0.1

not quantified. Each sample was extracted and tested three times.

4.3.3.3 Ginsenoside content in different ages of *P. ginseng* rhizome

The rhizome is an important part of ginseng, linking the root and stem. It contains diversified ginsenosides according to above qualitative analysis. From Table 4.7, we can see that rhizome also have abundant ginsenosides. The contents of ginsenosides Rb1 and m-Rb1 are the two highest in the rhizome, which is a large difference from the main root and fine root. Interestingly, the increase in the total amount of ginsenosides from a 6-year old to a 14-year old was low (92.2 mg/g to 115.2 mg/g). Moreover, both the ratio of PPD-type to PPT-type and the ratio of neutral ginsenoside to malonyl ginsenoside remained at a stable value of about 2.30 and 1.35, respectively.

Table 4.7 Contents (mg/g) of ginsenosides in different ages of *P. ginseng* rhizome (mean ± S.D.)

	ginsenosides	6-yr old	12-yr old	13-yr old	14-yr old
Accurate Quantification	Rg1	8.1 ± 0.6	10.7 ± 0.8	13.3 ± 0.9	9.6 ± 0.8
	Rf	2.6 ± 0.2	3.2 ± 0.3	4.4 ± 0.4	3.6 ± 0.3
	Re	11.8 ± 0.7	11.4 ± 0.8	11.3 ± 0.8	14.4 ± 1.1
	Rg2	0.8 ± 0.0	0.9 ± 0.1	0.9 ± 0.1	1.3 ± 0.1
	Rh1	#	#	#	#
	Rb1	15.2 ± 1.9	15.5 ± 1.8	16.2 ± 1.9	15.0 ± 1.9
	Rc	5.0 ± 0.8	5.2 ± 1.0	6.1 ± 1.1	7.2 ± 0.9
	Rb2	3.5 ± 0.6	4.2 ± 0.7	4.8 ± 0.9	5.0 ± 0.8
	Rb3	0.9 ± 0.1	1.0 ± 0.1	1.0 ± 0.1	1.3 ± 0.2
	Rd	1.0 ± 0.2	1.1 ± 0.2	1.5 ± 0.3	1.0 ± 0.2
	Rh2	#	#	#	#
	F2	#	#	#	#
	Rg3	#	#	#	#
Relative Quantification	Ro	10.6 ± 0.2	9.4 ± 0.8	9.1 ± 0.8	14.1 ± 1.6
	mRg1	0.6 ± 0.0	0.8 ± 0.0	1.0 ± 0.0	0.6 ± 0.0
	mRe	0.2 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0
	mRd	1.3 ± 0.0	1.5 ± 0.0	2.0 ± 0.0	1.5 ± 0.1
	mRb1	17.9 ± 0.2	18.4 ± 0.1	18.8 ± 0.1	19.1 ± 0.9
	mRc	6.3 ± 0.1	6.8 ± 0.0	8.5 ± 0.1	9.9 ± 0.2
	mRb2	5.4 ± 0.0	7.5 ± 0.0	8.6 ± 0.1	9.1 ± 0.2
	mRb3	1.1 ± 0.0	1.39±0.06	1.62±0.08	2.20±0.05
Total amount		92.2 ± 4.9	99.2 ± 6.9	109.3 ± 6.8	115.2 ± 9.3
PPD-type/PPT-type		2.4 ± 0.0	2.3 ± 0.0	2.2 ± 0.0	2.4 ± 0.0
Ginsenoside/m-Ginsenoside		1.4 ± 0.2	1.4 ± 0.1	1.3 ± 0.2	1.3 ± 0.1

not quantified. Each sample was extracted and tested three times.

4.3.3.4 Ginsenoside content in different ages of *P. ginseng* stem

Consistent with other published results [11, 42], the content of ginsenosides in the stem is also the lowest in this study (Table 4.8). Compared to the underground parts (main root, fine root, and rhizome), ginsenosides distributed in the stem have some differences. For example, ginsenosides Rb1 and m-Rb1, which are the main ingredients in underground parts, were not quantified due to the low amount. The highest total amount of ginsenosides is the 14-year old sample (12.3 ± 1.0 mg/g). Different from other parts that the amount of PPD-type ginsenosides is higher than that of PPT-type, in the stem, the amount of PPT-type ginsenosides is much higher, about four folds that of PPD-type ginsenosides. The ratio of neutral ginsenoside to malonyl ginsenoside is also high, ranges from 2.4 to 2.7.

Table 4.8 Contents (mg/g) of ginsenosides in different ages of *P. ginseng* stem (mean ± S.D.)

	ginsenosides	6-yr old	12-yr old	13-yr old	14-yr old
Accurate Quantification	Rg1	1.8 ± 0.2	2.6 ± 0.2	2.0 ± 0.2	2.7 ± 0.2
	Rf	0.2 ± 0.0	0.4 ± 0.1	0.2 ± 0.0	0.4 ± 0.0
	Re	2.7 ± 0.4	3.3 ± 0.5	1.9 ± 0.3	3.6 ± 0.5
	Rg2	0.2 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	0.4 ± 0.0
	Rh1	#	#	#	#
	Rb1	#	#	#	#
	Rc	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
	Rb2	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
	Rb3	#	#	#	#
	Rd	0.3 ± 0.2	0.3 ± 0.2	0.2 ± 0.1	0.3 ± 0.2
	Rh2	#	#	#	#
	F2	0.1 ± 0.0	#	#	#
	Rg3	#	#	#	#
Relative Quantification	Ro	0.8 ± 0.0	1.6 ± 0.1	0.5 ± 0.0	2.2 ± 0.1
	mRg1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1

	mRe	0.8 ± 0.1	1.0 ± 0.2	0.7 ± 0.1	1.2 ± 0.2
	mRd	1.1 ± 0.1	1.2 ± 0.1	0.7 ± 0.1	1.2 ± 0.0
	mRb1	#	#	#	#
	mRc	#	0.1 ± 0.0	#	#
	mRb2	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.2 ± 0.0
	mRb3	#	#	#	#
Total amount		8.2 ± 0.9	11.1 ± 1.1	6.5 ± 0.7	12.3 ± 1.0
PPD-type/PPT-type		0.3 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
Ginsenosides/m-Ginsenosides		2.4 ± 0.3	2.5 ± 0.4	2.7 ± 0.4	2.6 ± 0.5

not quantified. Each sample was extracted and tested three times.

4.3.3.5 Ginsenoside content in different ages of *P. ginseng* leaf

Similar with stem, the leaf from 14-year old ginseng contained the highest amount of ginsenosides, reaching 152.1 ± 6.7 mg/g (Table 4.9), and ginsenosides Rb1 and m-Rb1 were still at a low level. Interestingly, as age increases, there is an increasing trend in the content of Re and m-Re from 15.3 ± 2.4 mg/g to 27.4 ± 2.7 mg/g and from 8.4 ± 0.1 mg/g to 15.1 ± 1.0 mg/g, respectively. Compare this to the content of Rg1, which has an opposite trend, decreasing from 15.7 ± 2.7 mg/g to 7.5 ± 1.5 mg/g. In leaf, ginsenosides Rd and m-Rd become another two main ingredients because of their high contents up to 19.7 ± 1.2 mg/g and 26.6 ± 4.2 mg/g in the 14-year old samples, respectively. In addition, ginsenoside Rc, m-Rc, Rb2, and m-Rb2 also have relatively moderate content. What's more, ginsenoside F2, which is almost undetectable in other parts, is found at around 5 mg/g in the leaf. Ginsenoside Rf has a relatively modest content in other parts, but only a bit (about 0.2 mg/g) in the leaf. In line with underground parts, the amount of PPD-type ginsenoside is higher than that of PPT-type ginsenoside, and the content of neutral ginsenoside is also higher than that of malonyl ginsenoside.

Table 4.9 Contents (mg/g) of ginsenosides in different ages of *P. ginseng* leaf (mean ± S.D.)

	ginsenosides	6-yr old	12-yr old	13-yr old	14-yr old
Accurate Quantification	Rg1	15.7 ± 2.7	10.3 ± 1.7	8.0 ± 1.8	7.5 ± 1.5
	Rf	0.3 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1
	Re	15.3 ± 2.4	20.7 ± 2.9	21.0 ± 2.1	27.4 ± 2.7
	Rg2	0.3 ± 0.1	0.6 ± 0.2	0.8 ± 0.2	1.1 ± 0.2
	Rh1	0.3 ± 0.1	0.2 ± 0.1	0.2 ± 0.0	0.2 ± 0.1
	Rb1	0.5 ± 0.1	0.7 ± 0.2	0.2 ± 0.0	1.1 ± 0.2
	Rc	6.9 ± 1.7	7.3 ± 2.0	4.0 ± 0.9	9.3 ± 1.9
	Rb2	7.9 ± 2.1	8.5 ± 2.2	4.5 ± 0.8	10.8 ± 2.1
	Rb3	0.9 ± 0.2	0.9 ± 0.2	0.5 ± 0.1	1.3 ± 0.2
	Rd	16.4 ± 2.2	15.9 ± 2.1	11.8 ± 1.4	19.7 ± 1.2
	Rh2	#	#	#	#
	F2	5.4 ± 0.6	2.2 ± 0.6	5.1 ± 0.5	4.7 ± 0.6
	Rg3	0.3 ± 0.1	0.2 ± 0.0	0.2 ± 0.1	0.4 ± 0.1
Relative Quantification	Ro	0.8 ± 0.1	1.5 ± 0.1	0.4 ± 0.0	0.6 ± 0.0
	mRg1	0.5 ± 0.4	0.5 ± 0.2	0.3 ± 0.1	0.2 ± 0.1
	mRe	8.4 ± 0.1	11.3 ± 0.6	14.2 ± 1.5	15.1 ± 1.0
	mRd	20.2 ± 2.6	19.9 ± 1.9	19.5 ± 2.9	26.6 ± 4.2
	mRb1	1.2 ± 0.1	1.5 ± 0.2	0.7 ± 0.1	2.2 ± 0.2
	mRc	5.4 ± 0.4	5.0 ± 0.6	5.1 ± 0.3	8.4 ± 0.4
	mRb2	9.2 ± 0.5	8.9 ± 0.5	8.6 ± 0.1	13.3 ± 0.2
	mRb3	1.3 ± 0.2	1.1 ± 0.3	1.2 ± 0.2	2.0 ± 0.3
Total amount		117.1 ± 10.3	117.5 ± 12.6	106.5 ± 4.2	152.1 ± 6.7
PPD-type/PPT-type		1.9 ± 0.1	1.7 ± 0.1	1.4 ± 0.1	1.9 ± 0.1
Ginsenoside/m-Ginsenosides		1.4 ± 0.3	1.3 ± 0.2	1.0 ± 0.2	1.2 ± 0.2

not quantified. Each sample was extracted and tested three times.

Many previous studies about the chemical analyses of ginseng focused on the main neutral ginsenosides, such as Rg1, Re, Rf, Rb1, Rb2, Rc and Rd [43-47], and the less studied malonyl ginsenosides (mRb1, mRb2, mRb3, mRc and mRd), although malonyl

ginsenosides are abundant in *P. ginseng* [12]. It is more difficult to analyze malonyl ginsenosides by HPLC using a mobile phase without phosphate buffer than that of neutral ginsenosides [48]. Therefore, many previous studies on ginseng extracts pay more attention to neutral ginsenosides and ignoring malonyl ginsenosides. Compared to the traditional HPLC, LC-QTOF-MS/MS is effective in characterizing various ginsenosides in a mixture. QTOF-MS/MS can not only provide the exact mass values of the compound, but also give the fragment ions which provide structural information for the elucidation of ginsenosides in the complex mixture.

In the present study, we employed LC-QTOF-MS/MS technology to profile the various parts of NZ-grown *P. ginseng*. Not only were neutral ginsenosides analyzed, but malonyl ginsenosides were analyzed as well. Specifically, thirteen neutral ginsenosides were accurately quantified by matching the standard references, and seven malonyl ginsenosides (m-Rg1, m-Re, m-Rb1, m-Rb2, m-Rb3, m-Rc, and m-Rd) were relatively quantified using their corresponding neutral ginsenosides' curves after extracting their EIC peaks. This will provide a new thought to analyze adduct linked neutral ginsenosides.

Many publications have reported variations in the content of total and individual ginsenosides in *P. ginseng* are depending on cultivation age, harvest time, proceeding method, geographical origin, and various environmental conditions. Chung's study reported that the total amount of ginsenoside in fresh ginseng can be significantly affected by cultivation age, and not affected by the cultivation region [49]. For the individual ginsenoside, one published result showed that only the contents of the main PPT-type ginsenosides, like Rg1 and Re, increased along with cultivation age of *P. ginseng*; the contents of the main PPD-type ginsenosides, including Rb1, Rc, and Rd, did not show a direct relation to cultivation ages, and it varied widely from different cultivation locations [50]. Another report held the opinion that the content of ginsenoside Re was affected by genetic factors; the amounts of Rb1, Rc, and Rb2 were

affected by cultivation environment; and Rg1 and Rd were affected by both genetic factors and cultivation environment [51].

In this study, the total content of ginsenosides in various parts of *P. ginseng* varied, which did not increase with ages. The content of individual ginsenosides fluctuated with age and did not increase strictly with age. To some extent, the results also suggested that the contents of ginsenosides are affected by other factors in addition to age, especially in the natural environment of the wild, they may be affected more by other uncontrollable factors such as soil, moisture, and light. Consistent with results in the literature [11, 42], this study also indicated that the content of ginsenosides is higher in the leaf and fine root, and much lower in the stem than in other parts of *P. ginseng*. In line with the view that ginsenoside Rg1, Re, and Rb1 are the main components in *P. ginseng* [52], some other ginsenosides, such as mRb1 in underground parts, Ro in the rhizome, Rd and m-Rd in the leaf, also have a high concentration in *P. ginseng*. It is also said that the ratio of Rb1 to Rg1 is less than 5.0, and the ratio of PPD-group to PPT-group is less than 2.0 in *P. ginseng* [52]. In this study, it is easy to find Rb1:Rg1 < 5.0 in all tested samples, but the value of PPD-group to PPT-group didn't keep < 2.0 all the time. As shown in Figure 4.4, in addition to maintaining a much lower level in the stem, the ratio of PPD type to PPT type is less than 2.0 in main root and leaf, but greater than 2.0 in fine root and rhizome. The main reason for this phenomenon is caused by the different definitions of PPD-group and PPT-group.

As stated above, many previous studies were limited to neutral ginsenosides, so the PPD-group and PPT-group were defined as the sum of neutral PPD ginsenosides (Rb1, Rb2, Rb3, Rc, and Rd) and neutral PPT ginsenosides (Rg1, Re, and Rf), respectively [20, 45, 53]. While the amounts of PPD-type and PPT-type ginsenosides were defined as the sum of all quantified PPD-type and PPT-type ginsenosides in this study, containing malonyl ginsenosides, as well. Because *P. ginseng* have abundant malonyl ginseng and most of malonyl ginsenosides are PPD-type, resulting in an increased ratio of PPD-group to PPT-group. The ratio is less than 1.82 if the figures of malonyl

ginsenosides were moved out from the PPD-type and PPT-type amounts. Although *P. ginseng* are rich in malonyl ginsenosides , their total amounts are less than that of the neutral ginsenosides (Figure 4.3), the ratio of neutral ginsenosides to malonyl ginsenosides showed an obvious difference in various parts, the highest in the stem, followed by main root, leaf, and fine root.

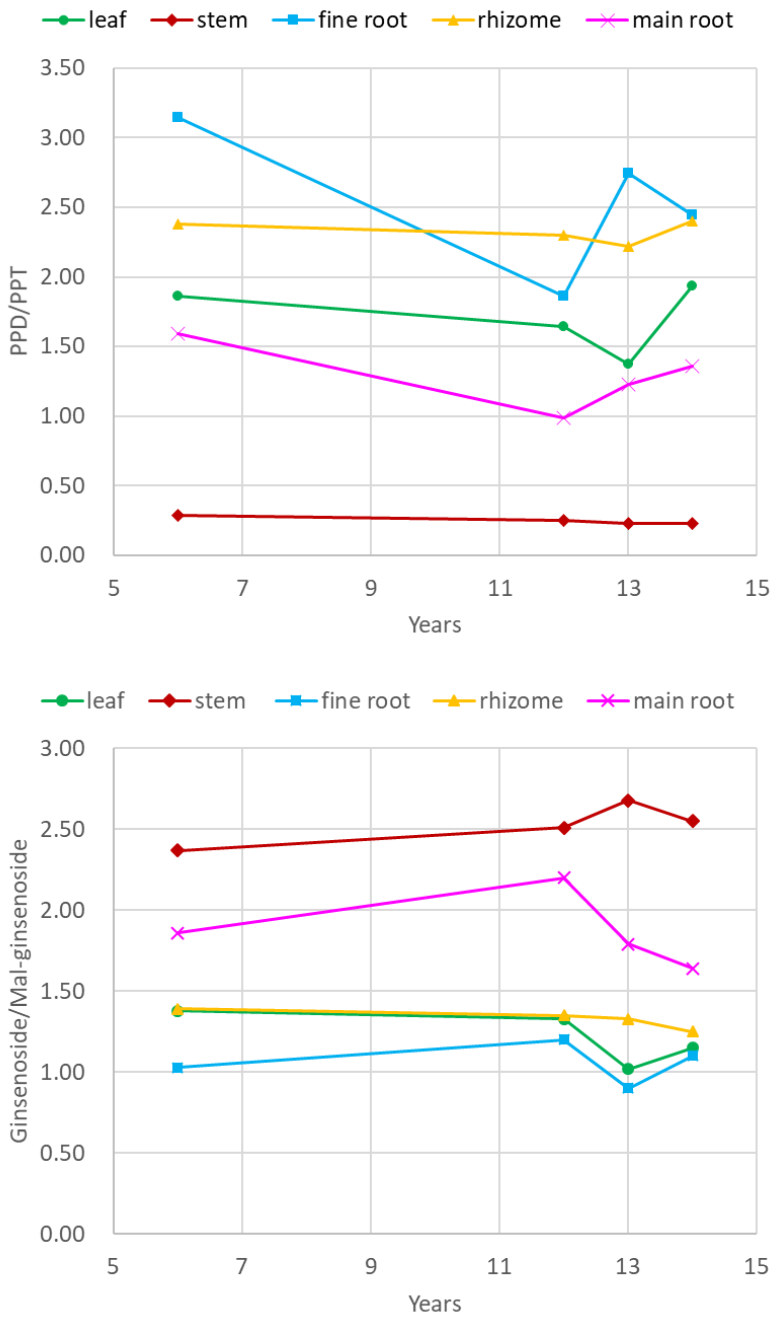


Figure 4.4 The changes of PPD-type/PPT-type and neutral-ginsenoside/malonyl-ginsenoside in different parts of *P. ginseng*.

4.4 Conclusion

In this chapter, an efficient LC-QTOF-MS/MS method was established to profile the main root, fine root, rhizome, stem, and leaf of NZ-grown *P. ginseng*. A total of 102 ginsenosides were identified by matching retention time with reference standards and comparing MS or MS/MS features with published known ginsenosides. Among them, 76, 69, 74, 44, and 57 ginsenosides were identified in the main root, fine root, rhizome, stem, and leaf, respectively. To systematically analyze the ginsenosides in different parts of NZ-grown *P. ginseng* with different ages, thirteen neutral ginsenosides were accurately quantified using their standard references, and seven malonyl ginsenosides were relatively quantified by integrating EIC peaks and calibrating with their corresponding neutral ginsenoside standards. The total content of ginsenosides in various parts of *P. ginseng* varied and did not increase strictly with age. In the underground parts (main root and fine root), the 13-year old sample contained the highest level of ginsenoside content; while in the aboveground parts (stem and leaf), the most amount of ginsenosides was from the 14-year sample. Additionally, the content of ginsenosides is higher in the leaf and fine root, and much lower in the stem than in other parts of *P. ginseng*. The total content of ginsenosides is in the order of leaf > fine root >> main root >> stem. These results provide the first systematic data about the ginsenosides in NZ wild simulated grown ginseng. Traditionally, the whole ginseng root was used for medical purpose or food purpose. From this study, the underground parts of ginseng contain abundant ginsenosides, which can provide the material base for using NZ-grown *P.* ginseng.

4.5 References

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Chapter Five

Analysis of ginsenoside content (*Panax ginseng*) from different regions (New Zealand, China, and Korea)

This chapter has been published in the following peer-reviewed open access publication.

Chen, W.; Balan, P.; Popovich, D. G. Analysis of ginsenoside content (*Panax ginseng*) from different regions. **Molecules**, 2019, 24, 3491; doi:10.3390/molecules24193491

Recently *Panax ginseng* has been grown as a secondary crop under a pine tree canopy in New Zealand (NZ). Aim of the study is to compare the mean content of ginsenosides from NZ grown ginseng and its original native locations (China and Korea) grown ginseng. Ten batches of NZ-grown ginseng were extracted using 70% methanol and analyzed using LC-MS/MS. The mean content of ginsenosides from China- and Korea- grown ginseng were obtained by collecting data from 30 and 17 publications featuring China- and Korea- grown ginseng, respectively. The mean content of total ginsenosides in NZ-grown ginseng was 40.1 ± 3.2 mg/g (n = 14), which showed significantly ($p < 0.05$) higher concentration than that of China-grown ginseng (16.5 ± 1.2 mg/g, n = 113) and Korea-grown ginseng (21.1 ± 1.6 mg/g, n = 106). For the individual ginsenosides, except for the ginsenosides Rb2, Rc, and Rd, ginsenosides Rb1, Re, Rf, and Rg1 from NZ-grown ginseng were 2.2, 2.9, 1.7, and 1.3 times higher than that of ginseng grown in China, respectively. Ginsenosides Re and Rg1 in NZ-grown ginseng were also 2.1 and 1.6 times higher than ginseng grown in Korea. From the accumulation of ginsenosides, NZ volcanic pumice soil may be more suitable for ginseng growth than its place of origin.

5.1 Introduction

Panax ginseng (P. ginseng), as one of the most important medicinal plant, root was used for first aid, health care, and the treatment of coma, gastrointestinal disease and cardiovascular disease in ancient China [1]. Pharmacological studies found ginseng possesses diverse bioactive effects, such as anti-aging, anti-stress, anti-tumor, anti-inflammatory, and anti-diabetes [2]. It has a beneficial impact on brain function, liver function, immune function, and sexual function [3]. Since the use of traditional Chinese herbs for medicinal and dietary purposes is becoming increasingly popular in Western countries, ginseng is becoming one of the best-selling herbs in the world [4].

Ginsenosides are known as the key active ingredient of ginseng [2]. The diversity of ginseng efficacy is related to the structural variability of ginsenosides, also known as triterpenoid saponins [5]. More than a hundred ginsenosides have been reported in *P. ginseng* (Chapter two) [6]. Among them, ginsenosides Rb1, Rb2, Rc, Rd, Re, Rg1 (chemical structures are shown in Figure 5.1) are the most abundant and are usually regarded as the main ginsenosides. These six ginsenosides are normally used to evaluate ginsenoside abundance and for quality control. The accumulation of ginsenosides in ginseng is variable and can be influenced by the surrounding environment, including soil fertility, temperature, light, and humidity [7]. Even in the same area, the type and amount of ginsenosides are dependent on ginseng age [8], harvest time [9], and storage process [10]. Since ginseng's bioactive properties depend largely on the contents and types of ginsenosides, accurately knowing the amount of ginsenosides is not only important for the pharmacological evaluation of ginseng products, but also for assessing the quality of ginseng from different regions.

With the increasing popularity of ginseng around the world, ginseng is also cultivated outside its place of origin (Northeast China, and Korea). Recently ginseng has been grown in New Zealand (NZ). NZ has a unique geographical environment, such as the

volcanic pumice soil, high-intensity UV rays, and open-wild environment. NZ-grown ginseng showed abundant ginsenosides in the underground parts (roots, rootlets) and above ground parts (stem and leaf) based on our previous study [11]. It is hypothesized that NZ-grown ginseng may contain different content of ginsenosides compared to China-grown ginseng and Korea-grown ginseng based on the environment where it grows. To compare the average amount of ginsenosides between NZ-grown ginseng and ginseng grown in China and Korea, the amount of ginsenosides from NZ forest-grown *P. ginseng* was analyzed using HPLC-QTOF-MS/MS technology and compared to published data of China-grown *P. ginseng* and Korea-grown *P. ginseng*. Thus, this study not only provides the information about the differences of ginsenoside content in ginseng from different places of origin, but it also provides a reference data for the saponin content from ginseng produced in different areas. To my knowledge, this is the first article focusing on ginsenoside content of *P. ginseng* in different regions (Northern and Southern Hemisphere).

5.2 Materials and methods

5.2.1 Analysis of ginsenosides content of NZ-grown ginseng

5.2.1.1. Ginseng samples

All the NZ-grown *P. ginseng* samples were collected from different areas at different seasons in the pine forest around Taupo and Rotorua (Table 5.1). The sizes of ginseng roots were categorized according to their weight and the hardness of ginseng roots was determined by hand. The samples were rinsed with water, lyophilized at – 68 °C and powdered using a CG2B coffee grinder (Breville, Sydney, Australia), and then passed through a 40-mesh sieve before extraction.

Table 5.1 Information of ten batches of ginseng samples.

NO	Sample	age	NO	Sample	age
1	Root, large and hard	13	6	Root, regular	12
2	Root, small and hard	13	7	Root, regular	12
3	Root, small and soft	13	8	Root, regular	12
4	Root, normal size and hard	8	9	Root, regular	12
5	Root, small and hard	8	10	Root, regular	12

5.2.1.2 Standard samples, chemicals, and reagents

Thirteen reference standards of ginsenosides Rb1, Rb2, Rb3, Rc, Rd, Re, Rf, Rg1, Rg2, Rg3, Rh1, Rh2, and F2 were purchased from Star Ocean Ginseng Ltd (Suzhou, China). The purities of all reference standards were above 98.0%. HPLC-grade methanol (MeOH) and formic acid (HCOOH) was purchased from Fisher Chemical (Pittsburg, PA, USA). LC-MS-grade acetonitrile (MeCN) and water were obtained from Merck (Phillipsburg, NJ, USA). Water (for extraction) was obtained from a Milli-Q Ultra-pure water system (Millipore, Billerica, MA, USA). Other reagents used in this study were of analytical grade.

5.2.1.3 Sample preparation and HPLC-QTOF-MS/MS analysis

Ginsenosides were extracted three times from ginseng samples using a Q700 sonicator (Qsonica, Melville, NY, USA) and analyzed by an Agilent 1290 liquid chromatograph coupled with quadrupole time-of-flight tandem mass spectrometry (Agilent, MA, USA) according to previous methods (Chapter four) [11]. Briefly, 0.7 g dried ginseng root powder was mixed with 10 mL 70% (v/v) aqueous MeOH and extracted at 20 kHz for 10 min at no more than 40 ℃. (The extraction was carried out for 5 cycles, each cycle contained 2 min ultrasonically extraction at 15% amplitude and 1 min for cooling between extraction). The supernatant was collected after centrifugation at 1788 xg for 10 min and the sediment was extracted twice more. The three extracts were mixed and filtered through a 0.22-μm filter before the LC/MS analysis.

Thirteen reference standards of ginsenosides Rb1 (0.769 mg/mL), Rb2 (0.846 mg/mL), Rb3 (0.629 mg/mL), Rc (1.077 mg/mL), Rd (0.692 mg/mL), Re (0.923 mg/mL), Rf (1.462 mg/mL), F2 (0.692 mg/mL), Rg1 (1.154 mg/mL), Rg2 (0.615 mg/mL), Rg3 (1.154 mg/mL), Rh1 (1.000 mg/mL), and Rh2 (1.077 mg/mL) were dissolved in 70% MeOH and then mixed and diluted with 70% MeOH to obtain a series of mixture standard solutions of different concentrations. The solutions were filtered through a 0.22- μm syringe filter before LC/MS analysis.

An Agilent 1290 liquid chromatograph (Agilent, MA, USA) equipped with an online degasser, an auto-sampler, a quaternary pump, and a heated column compartment, and an Agilent 6530 Quadrupole Time of Flight Mass Spectrometer (Agilent, MA, USA) equipped with an electrospray ionization source were used for LC/MS analysis [11]. The separation was based on a Zorbax Extend-C18 (2.1 X 100 mm, 3.5 μm) column (Agilent, USA) at a temperature of 33 ℃. The binary gradient elution solvent consisted of water (A) and acetonitrile (B) (Both A and B containing 0.1% formic acid). The gradient elution program was as follows: 0-4 min, 80% A; 4-10 min, 80-70% A; 10-25 min, 70-67.5% A; 25-27 min, 67.5-40% A; 27-39 min, 40-5% A; 39-40 min, 5% A; 40-40.5 min, 5-80% A. The flow rate was changed with the gradient: 0-27 min, 0.2 mL/min; 27-40.5 min, 0.25 mL/min. The injected volume was 1 μL. The mass spectrometer data were collected from *m*/*z* 100- 2200 in negative ion model and nitrogen (>99.998%) was used for nebulizer gas and curtain gas. The gas temperature and flow rates were 350 ℃ and 10.0 L/min, respectively. The pressure of nebulizer was 37 psi. The voltages of capillary, fragmentor, and skimmer were 3500 V, 220 V, and 65 V, respectively. The reference masses in negative ion mode were at *m*/*z* 121.0509 and 922.0098. The acquisition rates were 4 spectra/s for MS and 1 spectrum/s for MS/MS. Mass data were analyzed with Agilent MassHunter Workstation software (version B.06.00; Agilent Technologies, Santa Clara, CA, USA).

5.2.2 Analysis of ginsenosides content from China and Korea grown ginseng

Ginsenosides content of China and Korea grown ginseng obtained from publications based on the following search criteria.

5.2.2.1 Search strategy

The following search terms were applied to search the following electronic databases from their inception to April 2019.

The following search terms were used in Web of Science: TITLE: (Content* OR level* OR amount* OR quantif* OR determinat* OR investigat* OR analy*) AND TOPIC: (Ginsenoside*) AND TITLE: ("*Panax ginseng*" OR "Korean ginseng"). Refined by: LANGUAGES: (ENGLISH OR CHINESE) AND DOCUMENT TYPES: (ARTICLE). Timespan: All years. Databases: WOS, BIOABS, CABI, CCC, FSTA, KJD, MEDLINE, RSCI, SCIELO. Search language=Auto.

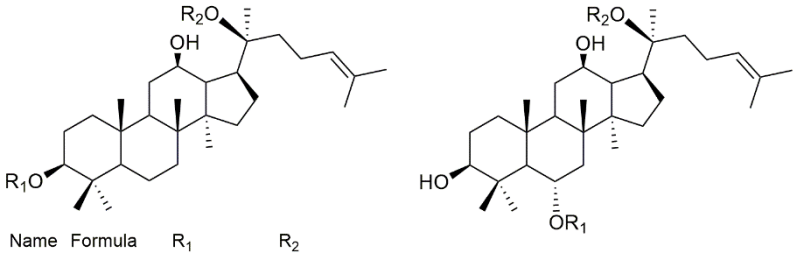
The following search terms were used in PubMed database: (ginsenoside [Title] AND content [Title]) OR (ginsenosides [Title] AND content [Title]) OR (ginsenoside [Title] AND contents [Title]) OR (ginsenosides [Title] AND contents [Title]) OR (ginsenosides [Title] AND investigation [Title]) OR (ginsenosides [Title] AND quantification [Title]) OR (ginsenosides [Title] AND determination [Title]) NOT rat [Title]).

The following search terms were used in Science Direct: Find articles with these terms "content of ginsenosides"; Title, abstract, keywords: "ginsenoside, *Panax ginseng*"; Article type: research articles.

The following search terms were used in Scopus: TITLE-ABS-KEY (ginsenosides) AND (LIMIT-TO (ACCESSTYPE (OA))) AND (LIMIT-TO (SUBJAREA, "PHAR") OR LIMIT-TO (SUBJAREA, "CHEM") OR LIMIT-TO (SUBJAREA, "AGRI")) AND (LIMIT-TO (EXACTKEY- WORD, "Article") OR LIMIT-TO (EXACTKEYWORD, "Ginsenoside") OR LIMIT-TO (EXACTKEYWORD, "Ginsenosides") OR LIMIT-TO (EXACTKEYWORD, "Ginseng")) AND (LIMIT-TO (LANGUAGE, "English") OR LIMIT-TO (LANGUAGE, "Chinese")) AND (LIMIT-TO (SRCTYPE, "j")) AND (EXCLUDE (EXACTKEYWORD, "Animals") OR EXCLUDE (EXACTKEYWORD, "Animal Experiment ")) AND (EXCLUDE (EXACTKEYWORD, "Human")).

5.2.2.2 Inclusion criteria

The following inclusion criteria were defined: (a) the literature are experimental articles; (b) the study measured six common ginsenosides (ginsenosides Rb1, Rb2, Rc, Rd, Re, and Rg1) or seven common ginsenosides (structures are shown in Figure 5.1) that includes Rf; (c) the above common ginsenosides are extracted from ginseng root (*P. ginseng*); (d) the articles contain the number of common ginsenosides.



Name	Formula	R_1	R_2
Rb1	$C_{54}H_{92}O_{23}$	Glc(2-1)Glc	Glc(6-1)Glc
Rb2	$C_{53}H_{90}O_{22}$	Glc(2-1)Glc	Glc(6-1)Arap
Rc	$C_{53}H_{90}O_{22}$	Glc(2-1)Glc	Glc(6-1)Araf
Rd	$C_{48}H_{82}O_{18}$	Glc(2-1)Glc	Glc

Name	Formula	R_1	R_2
Re	$C_{48}H_{82}O_{18}$	Glc(2-1)Rha	Glc
Rf	$C_{42}H_{72}O_{14}$	Glc(2-1)Glc	H
Rg1	$C_{42}H_{72}O_{14}$	Glc	Glc

Figure 5.1 The Chemical structures of seven main ginsenosides. Glc, Arap, Araf, and Rha refer to β-D-glucopyranosyl, α-L-arabinopyranosyl, α-L-arabinofuranosyl, and α-L-rhamnopyranosyl, respectively.

5.2.2.3 Exclusion criteria

The following exclusion criteria were defined: (a) excludes articles about ginsenoside contents in the animal or human tissue samples, such as plasma, urine, and feces, etc.; (b) excludes articles that the materials contain other ingredients, such as Chinese herbs formula, commercial ginseng products (c) excludes articles that ginseng materials did not grow in the forest or farmland, such as cultured laboratory samples.

5.2.2.4 Data extraction

Investigators reviewed the titles, abstracts, and full text of the resulting articles for inclusion. Data were extracted from each publication meeting the inclusion criteria. The values of ginsenosides (Rb1, Rb2, Rc, Rd, Re, Rf, and Rg1) content (mean) were collected based on grown locations (China, Korea, and NZ). When ginsenosides content were reported using bar charts in the literature, the estimated values were collected using ruler assistance.

5.2.3 Data analysis

The level of ginsenosides of NZ-grown ginseng was expressed as mean ± SD, and the mean contents of ginsenosides of different regions from the literature were calculated and expressed as mean ± SE. One-way ANOVA and Sidak's multiple comparisons test were used to analyze the difference among groups. A p-value < 0.05 was considered to be statistically significant.

5.3 Results and discussion

5.3.1 The ginsenoside contents of NZ-grown ginseng

In this chapter, the amount of 21 ginsenosides in 10 batches of ginseng samples were analyzed using LC-MS/MS described in previous methods (Chapter four) [11]. Briefly, thirteen ginsenosides (Rb1, Rb2, Rb3, Rc, Rd, Re, Rf, Rg1, Rg2, Rg3, Rh1, Rh2, and F2)

were accurately quantified by their linear regression equations of standard curves, and some ginsenosides without reference standards, such as ginsenosides m-Rb1 (m = malonyl), m-Rb2, m-Rb3, m-Rc, m-Rd, m-Re, and m-Rg1, were relatively quantified by the regression equations of their corresponding neutral ginsenosides. As shown in Table 5.2, apart from the individual ginsenoside content, the total ginsenoside amount, the ratio of protopanaxadiol (PPD)-type to protopanaxatriol (PPT)-type (PPD/PPT) and the ratio of neutral ginsenoside to malonyl ginsenoside (G/m-G) are also described in this section.

Table 5.2 The content (mg/g) of ginsenosides in ten batches of NZ-grown ginseng samples (mean ± SD).

Compounds	Ginseng samples				
	1	**2**	**3**	**4**	**5**
Rg1	9.3 ± 0.2	8.5 ± 0.0	14.6 ± 0.2	6.9 ± 0.0	3.2 ± 0.0
Rf	2.4 ± 0.1	2.8 ± 0.1	4.0 ± 0.1	2.0 ± 0.0	1.1 ± 0.0
Re	4.4 ± 0.0	12.3 ± 0.1	11.7 ± 0.2	5.4 ± 0.0	5.4 ± 0.0
Rg2	0.4 ± 0.0	0.9 ± 0.0	0.9 ± 0.0	0.1 ± 0.0	0.4 ± 0.0
Rh1	#	#	0.1 ± 0.0	#	#
Rb1	8.3 ± 0.1	14.9 ± 0.0	20.0 ± 0.2	6.0 ± 0.0	2.9 ± 0.0
Rc	4.1 ± 0.1	5.7 ± 0.1	8.6 ± 0.0	3.1 ± 0.0	2.2 ± 0.0
Rb2	3.5 ± 0.0	4.6 ± 0.0	8.2 ± 0.0	2.9 ± 0.0	1.8 ± 0.0
Rb3	0.8 ± 0.0	1.1 ± 0.0	1.7 ± 0.0	0.5 ± 0.0	0.4 ± 0.0
Rd	0.7 ± 0.0	0.8 ± 0.0	1.7 ± 0.0	1.3 ± 0.0	0.9 ± 0.0
Rh2	#	#	0.1 ± 0.0	#	#
F2	#	#	#	#	#
Rg3	#	#	#	#	#
Ro	3.2 ± 0.0	2.1 ± 0.0	2.8 ± 0.0	3.4 ± 0.0	0.6 ± 0.0
m-Rg1	0.7 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	1.5 ± 0.0	0.4 ± 0.0
m-Re	0.1 ± 0.0	0.3 ± 0.0	0.1 ± 0.0	0.4 ± 0.0	0.3 ± 0.0
m-Rd	1.1 ± 0.0	1.3 ± 0.0	1.5 ± 0.0	3.8 ± 0.1	1.9 ± 0.0
m-Rb1	11.5 ± 0.1	13.9 ± 0.1	13.2 ± 0.1	16.6 ± 0.0	6.1 ± 0.1
m-Rc	5.4 ± 0.0	5.2 ± 0.0	5.1 ± 0.1	9.9 ± 0.3	4.7 ± 0.1
m-Rb2	6.9 ± 0.1	5.8 ± 0.1	7.0 ± 0.1	12.9 ± 0.1	5.7 ± 0.0

m-Rb3	1.3 ± 0.0	1.1 ± 0.0	1.1 ± 0.1	2.3 ± 0.0	0.9 ± 0.1
Total	64.0 ± 0.2	81.6 ± 0.2	102.7 ± 0.9	78.9 ± 0.3	38.7 ± 0.2
PPD/PPT	2.5 ± 0.0	2.2 ± 0.0	2.1 ± 0.0	3.6 ± 0.0	2.6 ± 0.0
G/m-G	1.1 ± 0.0	1.71±0.00	2.34±0.01	0.6 ± 0.0	0.8 ± 0.0

Compounds	Ginseng samples				
	6	**7**	**8**	**9**	**10**
Rg1	14.0 ± 0.1	15.5 ± 0.0	8.2 ± 0.3	12.8 ± 0.0	9.2 ± 0.1
Rf	3.1 ± 0.0	4.1 ± 0.1	2.8 ± 0.0	3.1 ± 0.1	3.3 ± 0.0
Re	4.5 ± 0.0	8.4 ± 0.1	8.0 ± 0.1	6.4 ± 0.1	10.2 ± 0.4
Rg2	0.3 ± 0.0	0.7 ± 0.0	0.7 ± 0.0	0.5 ± 0.0	0.9 ± 0.0
Rh1	0.1 ± 0.0	0.1 ± 0.0	#	0.1 ± 0.0	0.1 ± 0.0
Rb1	9.1 ± 0.1	12.6 ± 0.0	9.9 ± 0.1	12.0 ± 0.1	11.6 ± 0.1
Rc	2.5 ± 0.1	4.0 ± 0.0	4.0 ± 0.0	4.2 ± 0.0	5.6 ± 0.2
Rb2	2.2 ± 0.1	3.6 ± 0.1	3.6 ± 0.0	3.8 ± 0.1	5.2 ± 0.1
Rb3	0.5 ± 0.0	0.8 ± 0.0	0.8 ± 0.1	0.8 ± 0.0	1.1 ± 0.0
Rd	0.8 ± 0.0	1.9 ± 0.0	0.8 ± 0.0	0.8 ± 0.0	1.1 ± 0.0
Rh2	#	#	#	#	#
F2	#	#	#	#	#
Rg3	#	#	#	#	#
Ro	2.8 ± 0.0	2.6 ± 0.0	1.1 ± 0.0	3.0 ± 0.0	1.1 ± 0.0
m-Rg1	0.9 ± 0.0	0.7 ± 0.0	0.3 ± 0.0	0.5 ± 0.0	0.3 ± 0.0
m-Re	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
m-Rd	1.0 ± 0.0	2.0 ± 0.0	0.9 ± 0.0	0.9 ± 0.0	1.2 ± 0.0
m-Rb1	9.7 ± 0.1	11.3 ± 0.1	8.8 ± 0.3	9.9 ± 0.1	9.1 ± 0.0
m-Rc	2.4 ± 0.0	3.4 ± 0.1	3.5 ± 0.1	3.3 ± 0.0	4.4 ± 0.1
m-Rb2	2.7 ± 0.0	3.8 ± 0.0	4.1 ± 0.1	4.0 ± 0.0	5.7 ± 0.0
m-Rb3	0.5 ± 0.0	0.7 ± 0.0	0.8 ± 0.0	0.7 ± 0.1	1.0 ± 0.0
Total	57.1 ± 0.2	76.5 ± 0.1	58.3 ± 0.7	66.6 ± 0.3	71.0 ± 1.0
PPD/PPT	1.4 ± 0.0	1.5 ± 0.0	1.8 ± 0.0	1.7 ± 0.0	1.9 ± 0.0
G/m-G	2.0 ± 0.0	2.1 ± 0.0	1.9 ± 0.0	2.1 ± 0.0	2.0 ± 0.0

not quantified. The total ginsenoside amount is the sum of all the quantified ginsenosides. The PPD-type amount and PPT-type amount are the sum of all the quantified PPD-type ginsenosides and PPT-type ginsenosides, respectively. G/m-G is the ratio of neutral ginsenoside amount to malonyl ginsenoside amount; the malonyl ginsenoside amount is the sum of seven quantified malonyl

ginsenosides (m-Re, m-Rg1, m-Rb1, m-Rb2, m-Rb3, m-Rc, and m-Rd), and the neutral ginsenoside amount is the sum of corresponding neutral ginsenosides (Re, Rg1, Rb1, Rb2, Rb3, Rc, and Rd).

Among those samples, the ginsenoside concentrations were varied. The highest amount (102.7 ± 0.9 mg/g) and the lowest amount (38.7 ± 0.2 mg/g) were from the third batch and fifth batch of samples, respectively. Ginsenosides Rg1, Rb1, m-Rb1, and Re are the four major ginsenosides in most samples except the fourth and fifth batches of samples, while in the fourth and fifth batches of samples, the major ginsenosides give way to the ginsenosides m-Rb1, m-Rb2, m-Rc, and Rg1 based on their concentrations. The high amount of m-Rb1, m-Rb2, and m-Rb3 lead to the higher ratios of PPD/PPT and lower ratios of G/m-G in sample four and five. Both ratios are approximately 2 from other samples.

Usually, ginseng refers to the whole ginseng root including the main root body, root hair, and rhizome. In Chapter 4, ginsenosides from different parts including main root, fine root, rhizome, stem, and leaf were analyzed [11], it was found that the underground parts, including main root, fine root, and rhizome, have very similar ginsenoside composition. In this chapter, ginsenosides were determined from the whole underground part. There is another study reporting ginsenosides analysis for NZ-grown ginseng [12], which only detected seven main ginsenosides in four batches of ginseng samples. In order to compare with China and Korea-grown ginseng, the data from Table 1 and Follett's publication [12] are used to calculate the mean content of ginsenosides of NZ grown ginseng.

5.3.2 The mean content of ginsenosides in different regions

To obtain the mean content of ginsenosides from China-grown ginseng and Korea-grown ginseng, I searched the scientific literature for ginsenosides analysis from

ginseng grown in China and Korea. A total of 524 articles were located, and 151 potentially relevant studies were selected to full-text review after removing the duplicates (N = 230), abstract only (N = 13) and screening based on title and abstract according to the inclusion criteria. After exclusion of 102 articles for the reasons given in Figure 5.2, 49 articles were included (article lists are supplied in Appendix 2). Among these articles, 30 articles were about ginsenosides analysis for China-grown ginseng, 17 for Korea-grown ginseng, and 2 for NZ-grown ginseng.

After extracting the data from the inclusion articles, the amount of ginsenosides from 113 batches of China-grown ginseng roots aged from 3 to 30-year olds was used to calculate the mean content of ginsenosides of China-grown ginseng. A total of 106 batches of Korea-grown ginseng roots with different ages (1 to 10-year olds) were analyzed from 17 publications. For NZ-grown ginseng, there were 14 batches of samples tests, including ten batches of ginseng roots in this chapter and four from the literature.

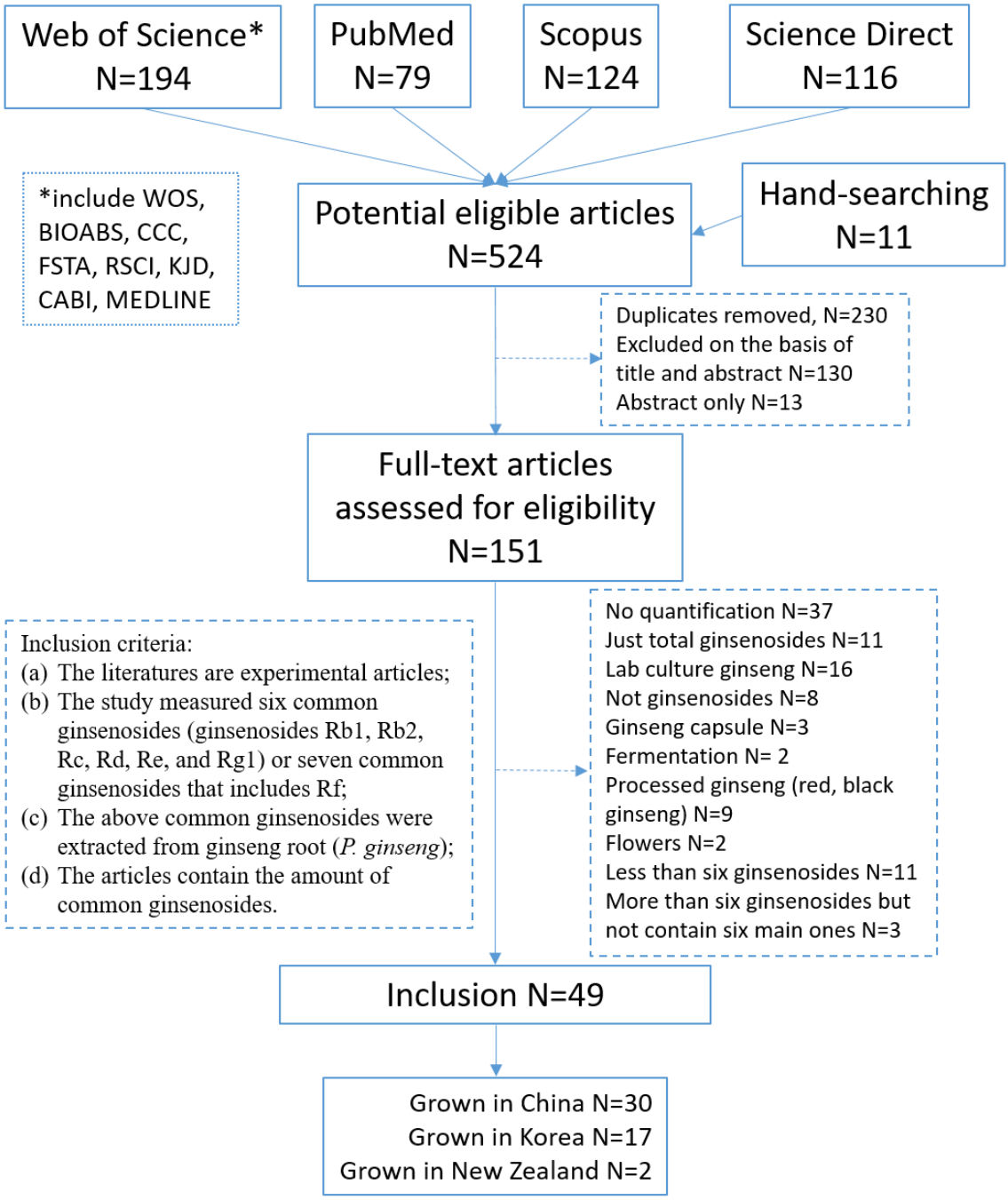


Figure 5.2 Flow diagram of study search and inclusion criteria.

As shown in Figure 5.3, the mean content of total ginsenosides from NZ-grown ginseng (40.1 ± 3.2 mg/g, n = 14) is significantly ($p < 0.05$) 1.4 times higher than that of China-grown ginseng (16.5 ± 1.2 mg/g, n = 113) and 90% higher compared with that of

Korean-grown ginseng (21.1 ± 1.6 mg/g, n = 106). There is no significant difference in the mean content of total ginsenosides for ginseng grown in China and Korea.

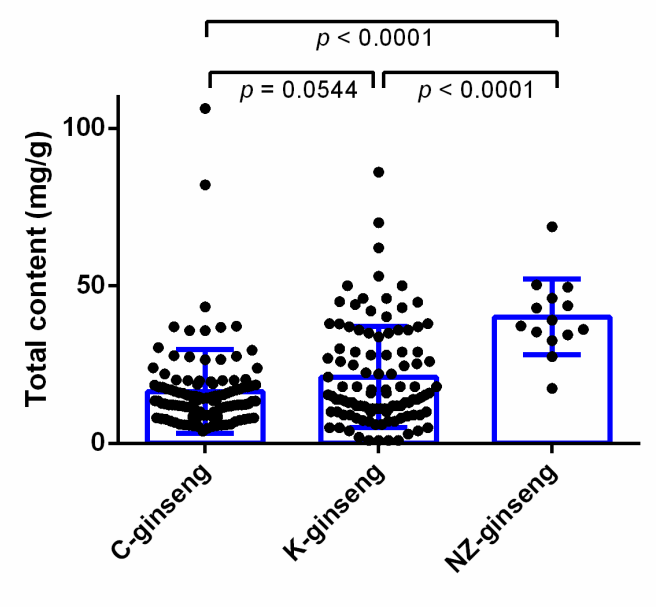


Figure 5.3 The mean content of total ginsenosides in the ginseng roots grown in three different countries. Data were analyzed by one-way ANOVA and Sidak's multiple comparisons test using Graph pad prism 6 software and expressed as mean ± SE. Differences were considered significant if $p < 0.05$. Each dot represents one batch of ginseng sample test datum. C, K, and NZ-ginseng refers to China (n = 113), Korea (n = 106), and New Zealand (n = 14) grown ginseng, respectively.

The mean contents of seven individual ginsenosides are shown in Figure 5.4, the mean concentrations of ginsenoside Rb1, Re, Rf, and Rg1 from NZ-grown ginseng are significantly ($p < 0.05$) 2.2, 2.9, 1.7, and 1.3 times higher than that of China-grown ginseng, respectively. The ginsenosides Re, Rf, and Rg1 of NZ ginseng are 3.1, 1.6, and 2.6 times that of Korean ginseng, respectively. The mean content of PPD type ginsenosides Rb2, Rc, and Rd are not significantly different between the three growing regions of ginseng.

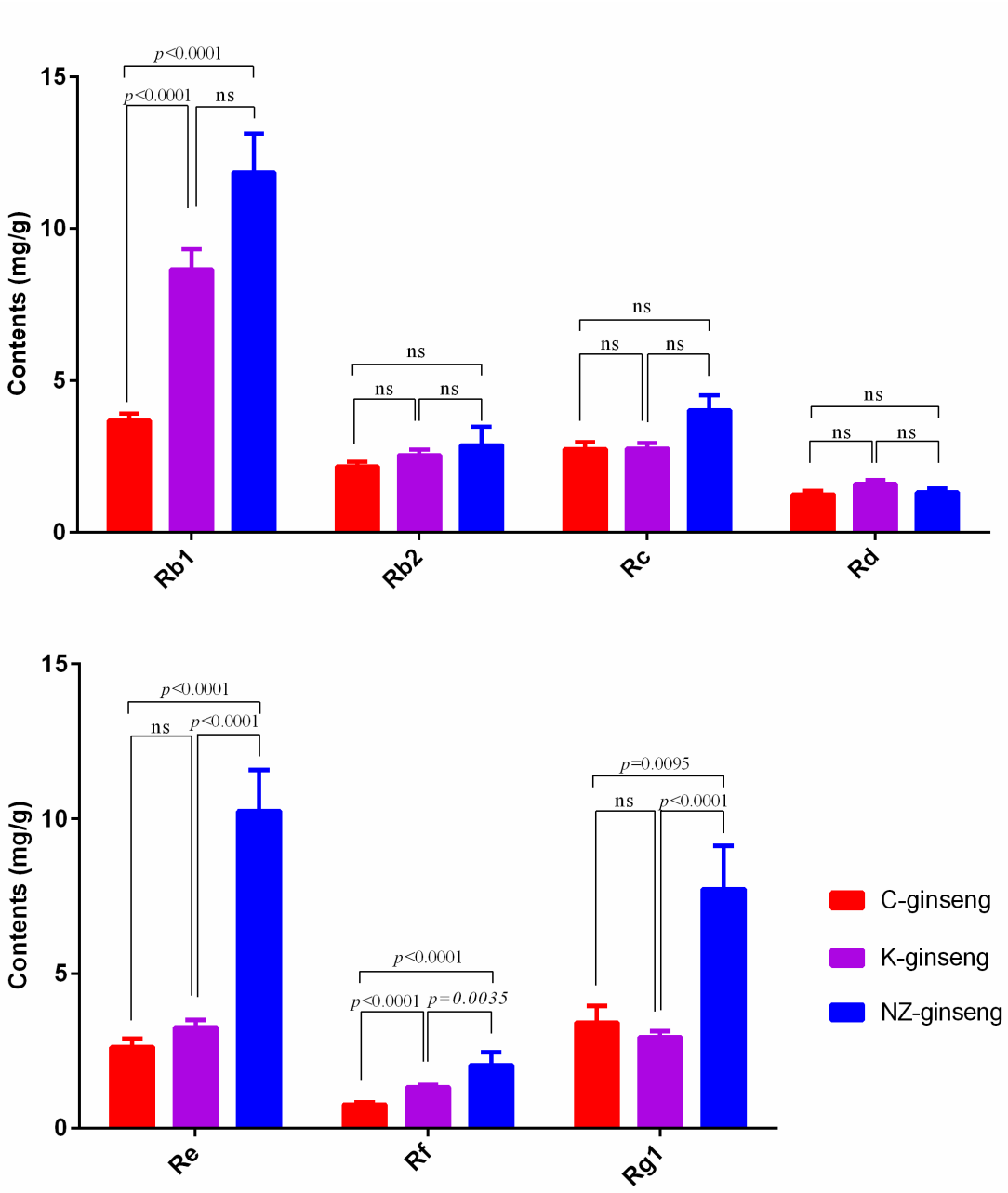
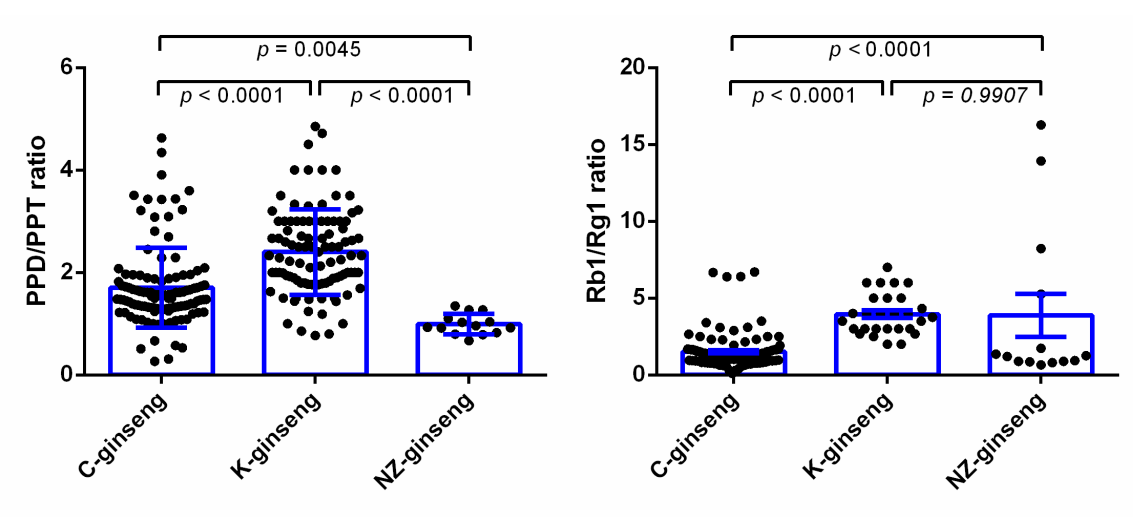


Figure 5.4 The mean content of individual ginsenosides in the ginseng roots grown in three different countries. Data were analyzed by one-way ANOVA and Sidak's multiple comparisons test using Graph pad prism 6 software and expressed as mean ± SE. Differences were considered significant if $p < 0.05$. C, K, and NZ-ginseng refers to China (n = 113), Korea (n = 106), and New Zealand (n = 14) grown ginseng, respectively.

It is reported that the ratios of PPD to PPT and Rb1 to Rg1 are less than 2.0 and less than 5.0 in *P. ginseng*, respectively [13]. Thus, I also calculated these ratios of ginsenosides from three countries (Figure 5.5). The PPD/PPT ratios of all the NZ-grown ginsengs are less than 2.0, although the mean ratio of PPD/PPT from China-grown ginseng is less than 2.0, many of the ratios are more than 2.0. While, most values of PPD/PPT from Korea-grown ginseng are more than 2.0, leading to the mean of PPD/PPT value > 2.0. Although the mean ratios of Rb1/Rg1 are less than 5.0 among three countries, some ginseng samples still have Rb1/Rg1 > 5.0. In addition, the ratios of Rg1 to Re is more than 1.0, and the ratio of Rb2 to Rc is more than 0.4 in *P. ginseng* [14]. From the mean level, the mean ratios of Rg1/Re and Rb2/Rc are more than 1.0 and 0.4, respectively, among the three regions' ginseng samples. However, neither the value of Rg1/Re keep > 1.0 all the time, nor the value of Rb2/Rc show > 0.4 in all samples.



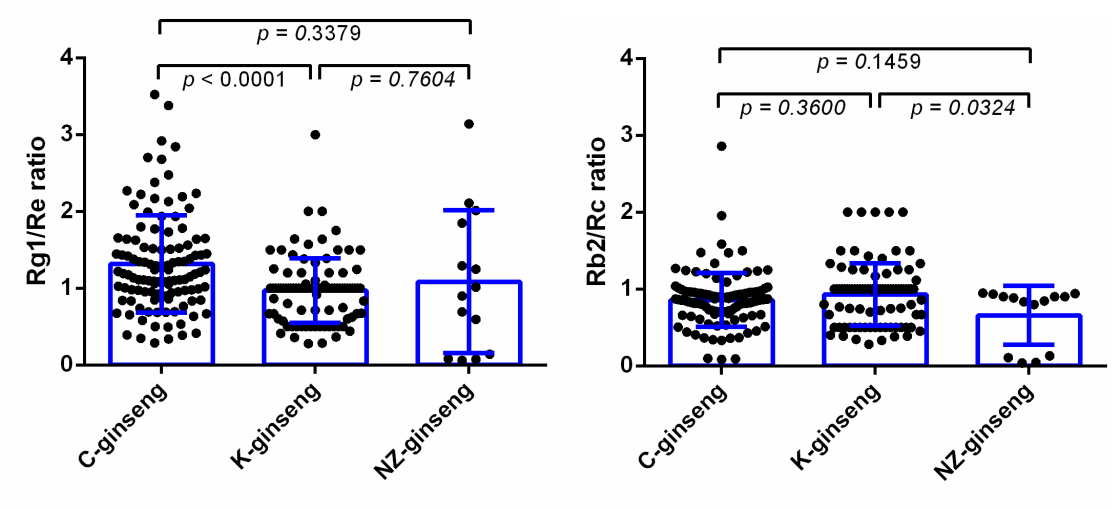


Figure 5.5 The PPD/PPT, Rb1/Rg1, Rg1/Re, and Rb1/Rc ratios of ginsenosides in the ginseng roots grown in three different countries. Data were analyzed by one-way ANOVA and Sidak's multiple comparisons test using Graph pad prism 6 software and expressed as mean ± SE. Differences were considered significant if $p < 0.05$. Each dot represents one batch of ginseng sample test datum. C, K, and NZ-ginseng refers to China (n = 113), Korea (n = 106), and New Zealand (n = 14) grown ginseng, respectively.

Since the contents of PPT type ginsenosides (Re, Rf, and Rg1) in NZ-grown ginseng are significantly higher than that of Chinese ginseng and Korean ginseng, besides Rb1, the concentrations of other PPD type ginsenosides (Rb1, Rc, and Rd) have no significant difference between three regions' ginseng, which lead to the lower PPD/PPT ratio in NZ-grown ginseng.

As the two most important sources of ginseng production, the total production by China and Korea is 72,229 tons, which was approximately 90.2% of the world ginseng production [15]. Many studies have reported the ginsenoside concentrations from China and Korea. However, they mainly focused on one batch or limited batches of sample analysis [16-18]. There are few studies concerning the comparison of ginsenosides between ginseng grown in different countries. It is unknown if they have

some differences in the ginsenosides content between China and Korea grown ginseng. In this chapter, the mean content of ginsenosides from hundreds of batches of ginseng samples represents the ginsenoside levels of ginseng grown in different regions. This study shows that there is neither a significant difference in the content of the total ginsenosides between China-grown ginseng and Korea-grown ginseng, nor remarkably differences in the individual ginsenoside contents of Rb2, Rc, Rd, Re, Rf, and Rg1 in both countries. This may be due to the geographical proximity of Northeast China and South Korea, and they have a similar growing environment for ginseng.

However, NZ-grown ginseng is grown in the Southern Hemisphere, apart from the similar growth conditions including the cold winter, temperate summer, and weakly acidic soil to that ginseng's natural habitat in Northeast of China and Korea [19], there are some different growing characteristics in NZ, including the volcanic pumice soil and high-intensity light radiation. A recent study showed that photosynthetically active radiation, soil and water potentially had a great impact on ginsenoside accumulation in ginseng roots [20]. The volcanic pumice soil can provide an excellent environment for root growth because of its unique properties such as dark soil color, unique consistency, low bulk density, and high water holding capacity [21]. Additionally, during the Southern Hemisphere summer, NZ receives, on average, 7% more radiation compared to a given latitude in the Northern Hemisphere summer [22]. It was reported that the total ginsenoside content increased significantly until light transmission rate increased by 20%, but the PPT type ginsenosides increased larger than PPD type ginsenoside, leading to the ratio of PPD/PPT eventually decreasing [23]. These evidences suggest that NZ's unique geographical environment encourages elevated ginsenosides (especially PPT type ginsenosides) content compared to ginseng grown in the Northern Hemisphere with similar latitudes. Notably, there are only 14 batches of NZ grown ginseng sample data, a bit smaller compared to hundreds of batches of sample data of Chinese ginseng and Korean ginseng.

5.4 Conclusion

This is the first article focusing on ginsenoside content of *P. ginseng* grown in different regions (China, Korea, and NZ). In this chapter, the mean content of ginsenosides from above three regions are described and compared. There is no significant difference in the mean content of total ginsenosides between China-grown ginseng and Korea-grown ginseng. NZ-grown ginseng has a significantly higher mean content of total ginsenosides than that of above two regions, especially higher concentrations of PPT type ginsenosides Re, Rf, and Rg1.

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Chapter Six

Comparison of ginsenoside components of various tissues of New Zealand forest-grown Asian ginseng (*Panax ginseng*) and American ginseng (*Panax quinquefolium* L.)

This chapter has been published in the following peer-reviewed open access publication.

Chen, W.; Balan, P.; Popovich, D. G. Comparison of ginsenoside components of various tissues of New Zealand forest-grown Asian ginseng (*Panax ginseng*) and American ginseng (*Panax quinquefolium* L.). **Biomolecules**, 2020, 10, 372; doi:10.3390/biom10030372

Asian ginseng (*Panax ginseng*) and American ginseng (*Panax quinquefolium* L.) are the two most important ginseng species for their medicinal properties. Ginseng is not only popular to consume, but it is also increasingly popular to cultivate. In the North Island of New Zealand (NZ), Asian ginseng and American ginseng have been grown in Taupo and Rotorua for more than 15 years. There are no publications comparing the chemical constituents between New Zealand-grown Asian ginseng (NZPG) and New Zealand-grown American ginseng (NZPQ). In this chapter, fourteen ginsenoside reference standards and LC–MS/MS technology were employed to analyze the ginsenoside components of various parts (fine root, rhizome, main root, stem, and leaf) from NZPG and NZPQ. Fifty and 43 ginsenosides were identified from various parts of NZPG and NZPQ, respectively, and 29 ginsenosides were found in both ginseng species. Ginsenoside concentrations in different parts of ginsengs were varied. Compared to other tissues, the fine roots contained the most abundant ginsenosides, not only in NZPG (142.5 ± 1.1 mg/g) but also in NZPQ (115.7 ± 3.5 mg/g). For the individual ginsenosides of both NZPG and NZPQ, concentration of Rb1 was highest in the underground parts (fine root, rhizome, and main root), and ginsenoside Re was highest in the aboveground parts (stem and leaf).

6.1 Introduction

Ginseng is a perennial herb and belongs to the genus *Panax* (Araliaceae family). There are more than twelve species of ginseng characterized in the genus *Panax*. Among them, Asian ginseng and American ginseng are the two most well-known species and are commonly used for their medicinal properties [1]. Asian ginseng (*Panax ginseng*) root has been used widely as a significant source of natural medicine for thousands of years in East Asia, particularly in China, Korea, and Japan. American ginseng (*Panax quinquefolium* L.), mainly grown in North America, is also well-known in Asian countries. As important Chinese medicine resources, Asian ginseng and American ginseng are considered to possess different properties in clinical applications. Asian ginseng, especially Korean red ginseng, has a warming effect and reinforces 'qi' to promote 'yang' energy, which benefits the spleen and lungs [2], and it was found to modulate the intestinal ecosystem and enhance the gut function [3]. American ginseng is viewed as having a cooling effect and can tonify 'qi' to nourish 'yin' energy, which means it can remove 'heat' and promote the production of body fluids [4]. These functional varieties may be due to differences in chemical composition, particularly in the bioactive triterpenoid saponins, popularly known as ginsenosides [5].

Ginsenosides, also known as triterpenoid saponins with a four-ring skeleton structure, are unique to ginseng species. So far, nearly 200 ginsenosides have been identified in Asian ginseng, and more than 100 in American ginseng; 49 ginsenosides coexist in both plant species (Chapter two) [6]. Most of them are classified as members of the dammarane family, including the protopanaxadiol (PPD) type and protopanaxatriol (PPT) type. In chemical analysis of ginseng, ginsenosides Rb1, Rb2, Rb3, Rc, Rd, Re, Rf, Rg1, and p-F11 are the most important compounds to be quantified due to their abundant content in ginseng plants. Among these compounds, ginsenoside Rf is considered to be only present in Asian ginseng, while ginsenoside p-F11 is considered to be only found in American ginseng [7]. Therefore, ginsenosides Rf and p-F11 are

used as marker compounds to differentiate Asian ginseng and American ginseng [5, 8]. There are some articles about the component analysis of Asian ginseng and American ginseng; they mainly focus on how to differentiate the two ginseng species [9] or the constituent analysis of ginseng roots [10, 11]. To my knowledge, there have been no comprehensive analyses of the ginsenoside components of ginseng tissues (fine root, rhizome, main root, stem, and leaf) between Asian ginseng and American ginseng. Furthermore, the vast majority of ginseng used in ginseng studies has been grown in the Northern Hemisphere; there are few reports about how ginseng grows in the Southern Hemisphere, such as those varieties in New Zealand (NZ).

NZ has a unique geographical environment, with features such as cold winters, temperate summers, adequate rainfall, unique volcanic pumice soil, and high-intensity UV rays. Summer solar radiation in NZ is on average 7% higher compared to an equivalent latitude in the Northern Hemisphere [12]. These factors may affect the accumulation and distribution of ginsenosides in ginseng plants. My previous study found that the average content of total ginsenosides from NZ grown *Panax ginseng* is significantly higher than that of ginseng grown in China or Korea (Chapter five) [13]. In this chapter, I used LC–MS/MS technology to analyze the ginsenoside components of various parts from New Zealand-grown Asian ginseng (NZPG) and New Zealand-grown American ginseng (NZPQ). To my knowledge, this is the first article focusing on ginsenoside component analysis of ginseng tissues between Asian ginseng and American ginseng, particularly for NZ-grown ginseng.

6.2 Materials and methods

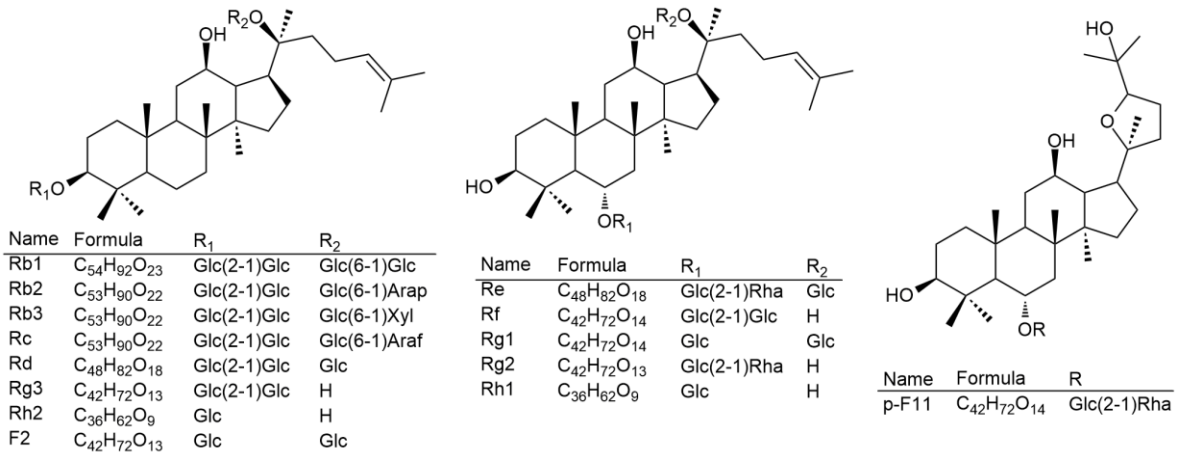
6.2.1 Ginseng samples

Eight-year-old Asian ginseng and American ginseng plants were harvested in November 2017 from a Taupo (NZ) pine forest. Five parts including fine root (root hair), rhizome (neck between root and stem), main root (root body), stem, and the leaf

of Asian ginseng and American ginseng were separated, rinsed with water, and lyophilized at –68 °C. The dried ginseng tissues were powdered using a CG2B coffee grinder (Breville, Sydney, Australia) and then passed through a 40-mesh sieve before extraction.

6.2.2 Chemicals and reagents

Fourteen reference standards of ginsenosides Rb1, Rb2, Rb3, Rc, Rd, Re, Rf, Rg1, Rg2, Rg3, Rh1, Rh2, F2, and 24(R)-pseudoginsenoside F11 (p-F11) were purchased from Star Ocean Ginseng Ltd. (Suzhou, China). The purities of the fourteen standards were no less than 98.0%. Their structures are shown in Figure 6.1. LC–MS-grade acetonitrile (MeCN) and water were supplied by Merck (Phillipsburg, NJ, USA). LC-grade methanol (MeOH) and formic acid (HCOOH) was purchased from Fisher Chemical (Pittsburgh, PA, USA). Water (for extraction) was obtained from a Milli-Q Ultra-pure water system (Millipore, Billerica, MA, USA). Other reagents used in this study were of analytical grade.



Name	Formula	R_1	R_2
Rb1	$C_{54}H_{92}O_{23}$	Glc(2-1)Glc	Glc(6-1)Glc
Rb2	$C_{53}H_{90}O_{22}$	Glc(2-1)Glc	Glc(6-1)Arap
Rb3	$C_{53}H_{90}O_{22}$	Glc(2-1)Glc	Glc(6-1)Xyl
Rc	$C_{53}H_{90}O_{22}$	Glc(2-1)Glc	Glc(6-1)Araf
Rd	$C_{48}H_{82}O_{18}$	Glc(2-1)Glc	Glc
Rg3	$C_{42}H_{72}O_{13}$	Glc(2-1)Glc	H
Rh2	$C_{36}H_{62}O_9$	Glc	H
F2	$C_{42}H_{72}O_{13}$	Glc	Glc

Name	Formula	R_1	R_2
Re	$C_{48}H_{82}O_{18}$	Glc(2-1)Rha	Glc
Rf	$C_{42}H_{72}O_{14}$	Glc(2-1)Glc	H
Rg1	$C_{42}H_{72}O_{14}$	Glc	Glc
Rg2	$C_{42}H_{72}O_{13}$	Glc(2-1)Rha	H
Rh1	$C_{36}H_{62}O_9$	Glc	H

Name	Formula	R
p-F11	$C_{42}H_{72}O_{14}$	Glc(2-1)Rha

Figure 6.1 The chemical structures of 14 ginsenoside reference standards.

6.2.3 Preparation of samples and reference standards

Ginsenosides were ultrasonically extracted three times from each part of the Asian ginseng and American ginseng using a Q700 sonicator (Qsonica, Melville, NY, USA) according to my previous methods (Chapters four and five) [13, 14]. Briefly, about 1 g of the fine root, rhizome, main root, stem, and leaf were separately extracted by 10 mL 70% (*v*/*v*) aqueous MeOH at 20 kHz for 10 min at no more than 40 °C. (The extraction was programmed for five cycles; each cycle contained ultrasonic extraction at 15% amplitude for 2 min and a cooling period of 1 min between extractions.) The supernatant was collected after centrifugation at 1788 xg (Thermo Scientific Multifuge 1S-R Centrifuge, Marshall Scientific, Hampton, NH, USA) for 10 min and the sediment was extracted twice more. The three extracts were mixed and filtered through a 0.22-micron syringe filter before the LC/MS analysis. Fourteen reference standards of ginsenosides Rb1 (0.769 mg/mL), Rb2 (0.846 mg/mL), Rb3 (0.629 mg/mL), Rc (1.077 mg/mL), Rd (0.692 mg/mL), Re (0.923 mg/mL), Rf (1.462 mg/mL), F2 (0.692 mg/mL), p-F11 (0.923 mg/mL), Rg1 (1.154 mg/mL), Rg2 (0.615 mg/mL), Rg3 (1.154 mg/mL), Rh1 (1.000 mg/mL), and Rh2 (1.077 mg/mL) were dissolved and diluted with 70% MeOH to obtain a series of standard solutions of different concentrations. The solutions were filtered through a 0.22-micron syringe filter before the LC-MS/MS analysis.

6.2.4 High-performance liquid chromatography coupled with quadrupole time-of-flight tandem mass spectrometry (HPLC-QTOF-MS)

An Agilent 1290 liquid chromatograph (Agilent, Lexington, MA, USA) equipped with an online degasser, a quaternary pump, an auto-sampler, a heated column compartment, a UV detector, and an Agilent 6530 Quadrupole Time-of-Flight Mass Spectrometer (Agilent, Lexington, MA, USA) equipped with an electrospray ionization source were used for LC-MS/MS analysis. The instrument setting was

consistent with my previous reports (Chapters four and five) [13, 14]. A double end-capped Zorbax Extend-C18 (2.1 × 100 mm, 3.5 μm) column (Agilent, Lexington, MA, USA) was used to separate compounds from the ginseng extract. The column temperature was controlled at 33 ℃. The binary gradient eluent consisted of mobile phase A (0.1% formic acid in water) and mobile phase B (0.1% formic acid in acetonitrile). The gradient elution program was as follows: 20% B at 0–4 min, 20–30% B at 6–10 min, 30–32.5% B at 10–25 min, 32.5–60% B at 25–27 min, 60–95% B at 27–39 min, 95% B at 39–40 min. The flow rate was changed with the gradient: 0–27 min, 0.2 mL/min; 27–40 min, 0.25 mL/min. The wavelength was set at 203 nm, and the injected volume was 1 μL. The mass spectrometer data were collected from *m*/*z* 100–2200 in negative ion mode, and nitrogen (>99.998%) was used for the nebulizer gas and curtain gas. The gas temperature and flow rates were 350 ℃ and 10.0 L/min, respectively. The pressure of the nebulizer was 37 psi. The voltages of capillary, fragmentor, and skimmer were 3500 V, 220 V, and 65 V, respectively. The reference masses in negative ion mode were at *m*/*z* 121.0509 and 922.0098. The acquisition rates were 4 spectra/s for MS and 1 spectrum/s for MS/MS. Mass data were analyzed with Agilent Mass Hunter Workstation software (version B.06.00; Agilent Technologies, Santa Clara, CA, USA).

6.3 Results and discussion

6.3.1 Identification of the detected ginsenosides in various parts of NZPG and NZPQ

To compare the ginsenoside components from various parts of the ginseng plants, Zorbax Extend-C18 column and LC-QTOF-MS/MS were used to separate and determine the ginsenosides extracted from the fine root, rhizome, main root, stem, and the leaf of NZPG and NZPQ. Fourteen ginsenosides reference standards (for

structures see Figure 6.1) were used to establish the chromatographic method. The base peak chromatograph (BPC) of the ginsenoside standards is shown in Figure 6.2.

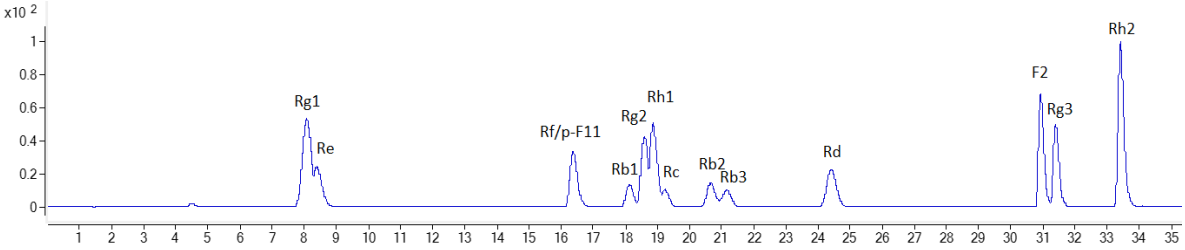


Figure 6.2 The base peak chromatogram (BPC) of 14 ginsenoside reference standards.

Although ginsenosides Rf and p-F11 are isomeric compounds and have the same retention time, I can differentiate them using mass spectrometry (MS). As shown in Figures 6.3A and 6.3C, ginsenosides Rf and p-F11 had similar $[M - H]^-$ ion at *m/z* 799.48 and $[M + HCOO]^-$ ion at *m/z* 845.49 in the negative ESI full scan MS. The abundance of *m/z* 799.48 $[M - H]^-$ and *m/z* 845.49 $[M + HCOO]^-$ are almost equal in ginsenoside Rf, while in ginsenoside p-F11, the abundance of *m/z* 845.59 $[M + HCOO]^-$ is about seven times that of *m/z* 799.48 $[M - H]^-$. In the MS^2, ginsenosides Rf and p-F11 have the same $[M - H]^-$ ion at *m/z* 799.48 and distinct product ions at *m/z* (637.42, 475.38) and (654.42, 491.37), respectively, which were obtained by the successive losses of the two sugar moieties (shown in Figure 6.3B and 6.3D). These distinct ions could be used as the characteristic ions to differentiate and quantify the two ginsenoside isomers.

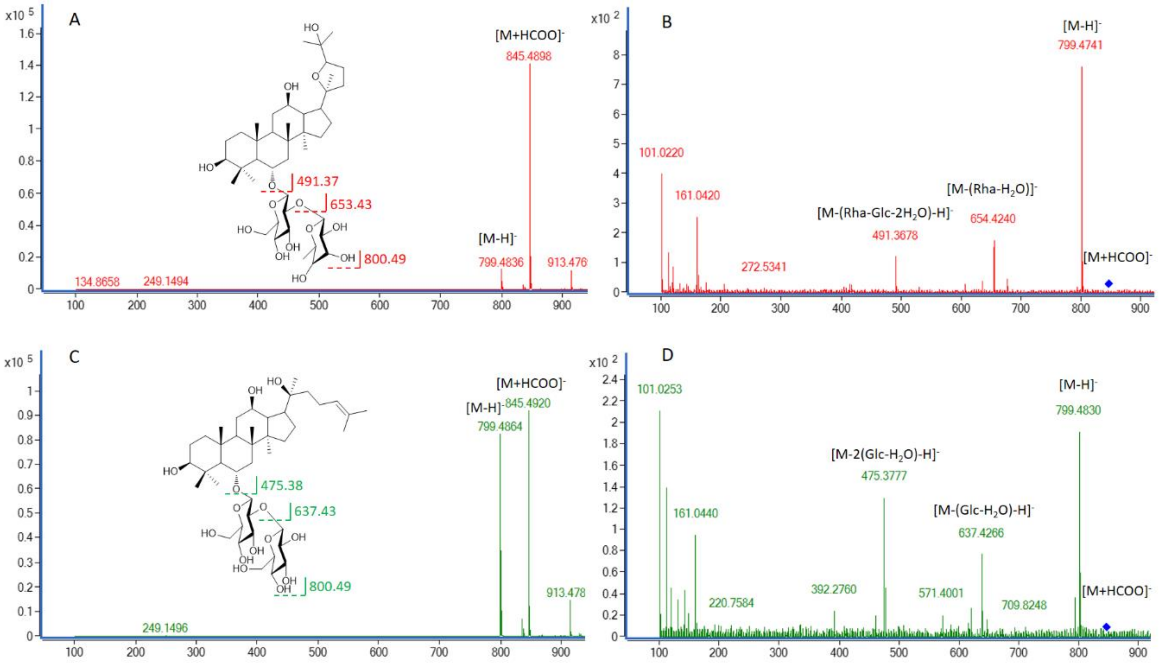
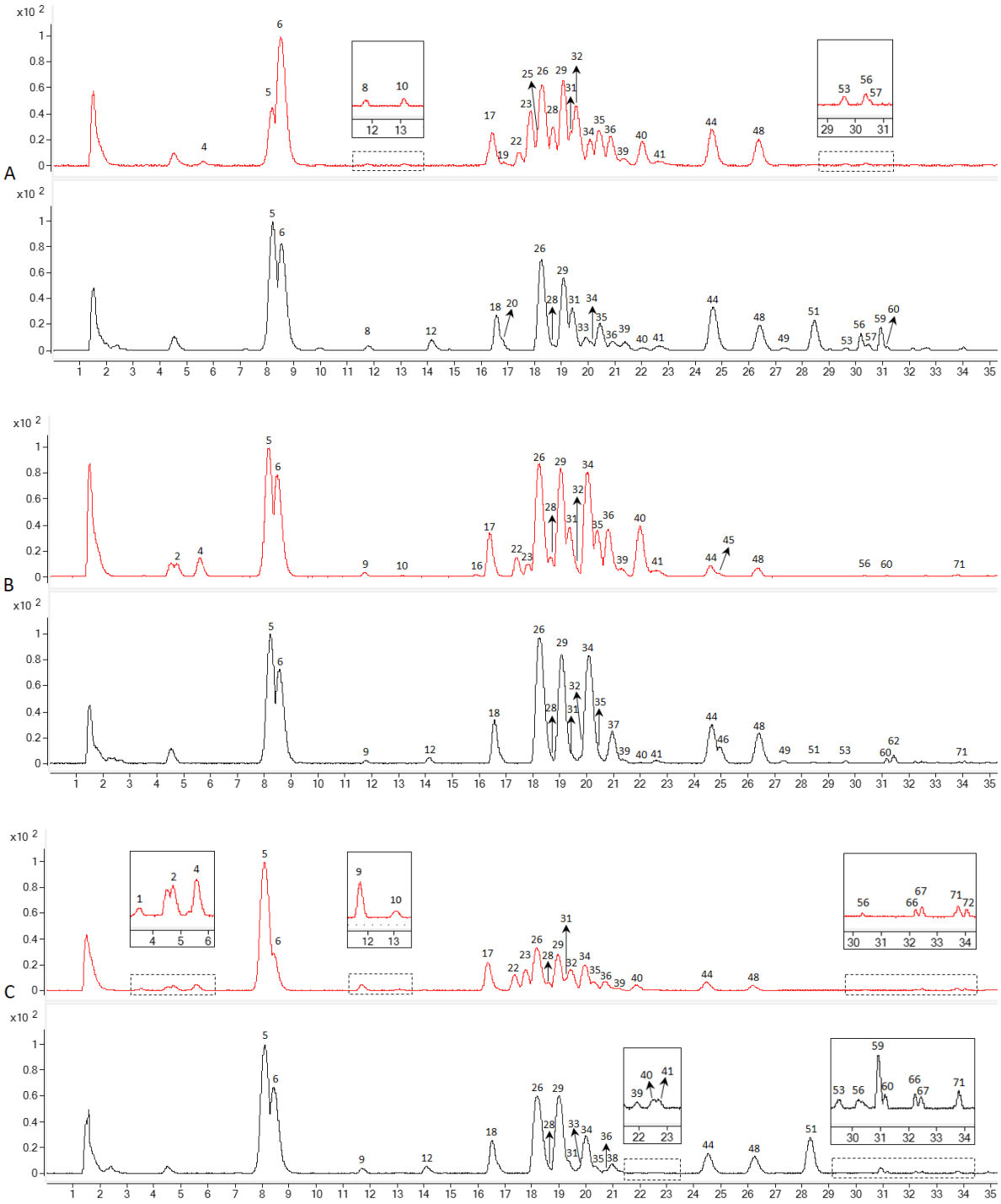


Figure 6.3 The MS (A, C) and MS/MS (B, D) of ginsenosides p-F11 (A, B) and Rf (C, D).

The BPC profiles of the various parts of NZPG and NZPQ are shown in Figure 6.4. A total of 72 potential ginsenosides were detected from the fine root, rhizome, main root, leaf, and stem of NZPG and NZPQ. The potential ginsenosides were identified using the same procedure as my previous report (Chapter four) [14]. Briefly, the PPD-type, PPT-type, and oleanolic acid-type ginsenosides could produce [(20S)-protopanaxadiol - H]$^-$ at *m*/*z* 459 ($C_{30}H_{51}O_3$), [(20S)-protopanaxatriol - H]$^-$ at *m*/*z* 475 ($C_{30}H_{51}O_4$), and [oleanolic acid - H]$^-$ at *m*/*z* 455 ($C_{30}H_{47}O_3$) in the negative MS^2 spectra, respectively. Thus, a different type of ginsenoside could be easily differentiated and characterized by finding its [aglycone - H]$^-$. Sugar residues could be identified by calculating the neutral loss molecular mass, for instance, the glucosyl (Glc), rhamnosyl (Rha), and pentosyl [arabinopyranosyl (Arap) or arabinofuranosyl (Araf) or xylosyl (Xyl)] groups correspond to the mass differences of 162, 146, and 132 amu in the MS/MS spectra, respectively. There are some small mass differences, such as 43 and 87 amu, which are mostly acetyl and malonyl groups. These groups are prone to attach with glucose.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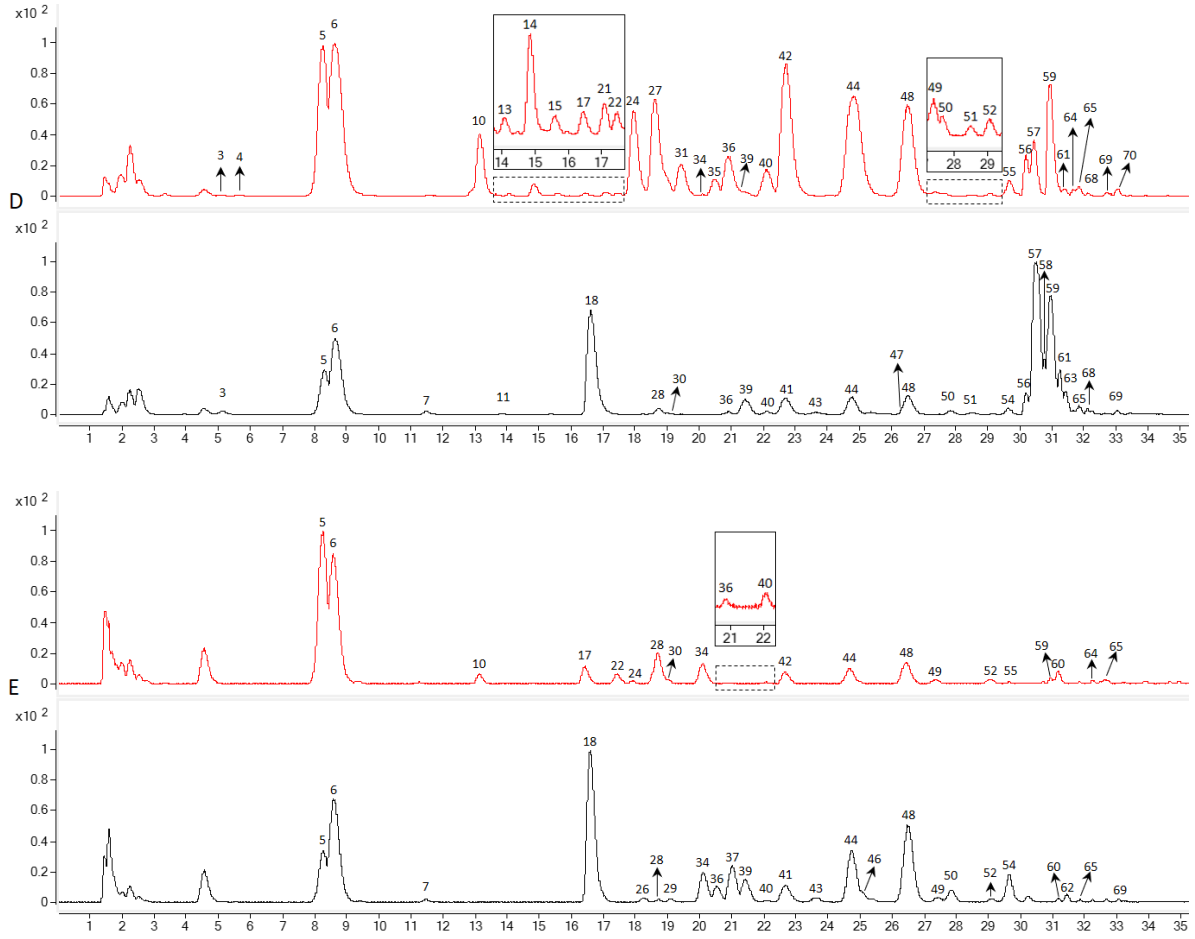


Figure 6.4 The base peak chromatogram (BPC) profiles of different parts of New Zealand-grown Asian ginseng (NZPG, red line) and New Zealand-grown American ginseng (NZPQ, black line). (A) Ginseng fine root, (B) ginseng rhizome, (C) ginseng main root, (D) ginseng leaf, (E) ginseng stem.

Fourteen ginsenosides (peaks 5, 6, 17, 18, 26, 28, 30, 31, 36, 39, 44, 59, 61, 70) were unambiguously identified by comparison with their reference standards. The other peaks were allocated by comparing the relative retention time, empirical molecular formulas, and fragmentation information with those in the literature. The information on 72 detected compounds is summarized in Table 6.1.

Table 6.1 The ginsenosides identified from various parts of New Zealand-grown *Panax ginseng* (PG) and *Panax quinquefolium* L. (PQ).

No.	R.t	Measured Value [ion form]	Formula	Source	Identification	Ref.
1	3.50	815.4775 [M - H]; 861.4822 [M + HCOO]	$C_{42}H_{72}O_{15}$	PG (m)	Ginsenjilinol	[15]
2	4.71	961.5291 [M - H]; 1007.5387 [M + HCOO]	$C_{48}H_{82}O_{19}$	PG (m, r)	20glc-Rf	[11]
3	5.09	931.5215 [M - H]; 977.5257 [M + HCOO]	$C_{47}H_{80}O_{18}$	PG (l); PQ (l)	NG-R1	[11]
4	5.62	931.5162 [M - H]; 977.5212 [M + HCOO]	$C_{47}H_{80}O_{18}$	PG (f, r, m, l)	Re4	[16]
5	8.19	799.4857 [M - H]; 845.4898 [M + HCOO]	$C_{42}H_{72}O_{14}$	PG (f, r, m, s, l); PQ (f, r, m, s, l)	Rg1	std
6	8.55	945.5370 [M - H]; 991.5439 [M + HCOO]	$C_{48}H_{82}O_{18}$	PG (f, r, m, s, l); PQ (f, r, m, s, l)	Re	std
7	11.49	799.4835 [M - H]; 845.4920 [M + HCOO]	$C_{42}H_{72}O_{14}$	PQ (s, l)	Rg1 isomer	[16]
8	11.53	841.4871 [M - H]	$C_{44}H_{74}O_{15}$	PG (f); PQ (f)	Ac-Rg1	[16]
9	11.70	885.4780 [M - H]	$C_{45}H_{74}O_{17}$	PG (m, r); PQ (m, r)	m-Rg1	[11]
10	13.17	1031.5386 [M - H]	$C_{51}H_{84}O_{21}$	PG (f, r, m, s, l)	m-Re	[11]
11	13.83	799.4821 [M - H]; 845.4770 [M + HCOO]	$C_{42}H_{72}O_{14}$	PQ (l)	Rg1 isomer	[16]
12	14.14	887.4954 [M + HCOO]	$C_{44}H_{74}O_{15}$	PQ (f, m, r)	Yesanchinoside D	[16]
13	14.12	815.4697 [M - H]	$C_{42}H_{72}O_{15}$	PG (l)	Re5	[15]
14	14.81	961.5322 [M - H]; 1007.5338 [M + HCOO]	$C_{48}H_{82}O_{19}$	PG (l)	VG-R4	[17]
15	15.55	829.4839 [M + HCOO]	$C_{42}H_{72}O_{13}$	PG (l)	G-La	[18]
16	15.88	1117.5382 [M - H]	$C_{54}H_{86}O_{24}$	PG (r)	mf-Rd6/isomer	[6]
17	16.40	799.4807 [M - H]; 845.4855 [M + HCOO]	$C_{42}H_{72}O_{14}$	PG (f, r, m, s, l)	Rf	std
18	16.55	799.4772 [M - H]; 845.4862 [M + HCOO]	$C_{42}H_{72}O_{14}$	PQ (f, r, m, s, l)	p-F11	std
19	16.84	1325.6255 [M - H]	$C_{62}H_{102}O_{30}$	PG (f)	m-Ra3/m-NG-R4	[6]
20	16.84	653.3698 [M - H]; 699.4271 [M + HCOO]	$C_{36}H_{62}O_{10}$	PQ (f)	G-Ki/G-Km/G-ST2	[6]

21	17.09	799.4497 [M - H]; 845.4817 [M + HCOO]	$C_{42}H_{72}O_{14}$	PG (l)	Rf isomer	[16]
22	17.40	769.4681 [M - H]; 815.4726 [M + HCOO]	$C_{41}H_{70}O_{13}$	PG (f, r, m, s, l)	NG-R2	[11]
23	17.82	1209.6224 [M - H]	$C_{58}H_{98}O_{26}$	PG (f, r, m)	Ra1	[11]
24	17.90	769.4739 [M - H]; 815.4754 [M + HCOO]	$C_{41}H_{70}O_{13}$	PG (s, l)	G-F5	[17]
25	18.02	1239.6273 [M - H]	$C_{59}H_{100}O_{27}$	PG (f)	Ra3/NG-R4	[17]
26	18.22	1107.5929 [M - H]	$C_{54}H_{92}O_{23}$	PG (f, r, m); PQ (f, r, m, s)	Rb1	std
27	18.61	769.4658 [M - H]; 815.4750 [M + HCOO]	$C_{41}H_{70}O_{13}$	PG (l)	G-F3	[17]
28	18.66	783.4858 [M - H]; 829.4930 [M + HCOO]	$C_{42}H_{72}O_{13}$	PG (f, r, m, s); PQ (f, m, s, l)	Rg2	std
29	19.04	1193.5932 [M - H]	$C_{57}H_{94}O_{26}$	PG (f, r, m); PQ (f, r, m, s)	m-Rb1	[11]
30	19.05	683.4329 [M + HCOO]	$C_{36}H_{62}O_{9}$	PG (s); PQ (l)	Rh1	std
31	19.31	1077.5818 [M - H]	$C_{53}H_{90}O_{22}$	PG (f, r, m, l); PQ (f, r, m)	Rc	std
32	19.49	1209.6254 [M - H]	$C_{58}H_{98}O_{26}$	PG (f, r, m)	Ra2	[11]
33	19.75	1193.5849 [M - H]	$C_{57}H_{94}O_{26}$	PQ (f, m)	m-Rb1 isomer	[16]
34	20.01	955.4865 [M - H]	$C_{48}H_{76}O_{19}$	PG (f, r, m, s, l); PQ (f, r, m, s)	Ro	[11]
35	20.38	1163.5798 [M - H]	$C_{56}H_{92}O_{25}$	PG (f, r, m, l); PQ (f, r, m)	m-Rc	[16]
36	20.80	1077.5813 [M - H]	$C_{53}H_{90}O_{22}$	PG (f, r, m, s, l); PQ (f, m, s, l)	Rb2	std
37	20.98	955.4853 [M - H]	$C_{48}H_{76}O_{19}$	PQ (r, s)	Ro isomer	[16]
38	20.95	1193.5892 [M - H]	$C_{57}H_{94}O_{26}$	PQ (m)	m-Rb1 isomer	[16]
39	21.35	1077.5782 [M - H]	$C_{53}H_{90}O_{22}$	PG (f, r, m, l); PQ (f, r, m, s, l)	Rb3	std
40	21.95	1163.5767 [M - H]	$C_{56}H_{92}O_{25}$	PG (f, r, m, s, l); PQ (f, r, m, s, l)	m-Rb2	[11]
41	22.59	1163.5782 [M - H]	$C_{56}H_{92}O_{25}$	PG (f, r, m); PQ (f, r, m, s, l)	m-Rb3	[11]
42	22.69	637.4292 [M - H]; 683.4310 [M + HCOO]	$C_{36}H_{62}O_{9}$	PG (s, l)	F1	[17]
43	23.56	1163.5822 [M - H]	$C_{56}H_{92}O_{25}$	PQ (s, l)	m-Rb3 isomer	[16]
44	24.65	945.5364 [M - H]; 991.5469 [M + HCOO]	$C_{48}H_{82}O_{18}$	PG (f, r, m, s, l); PQ (f, r, m, s, l)	Rd	std

45	24.59	1119.5871 [M - H]	$C_{55}H_{92}O_{23}$	PG (r)	Rs1	[19]
46	24.99	793.4306 [M - H]	$C_{42}H_{66}O_{14}$	PQ (r, s)	Zingibroside R1	[11]
47	25.84	1119.5844 [M - H]	$C_{55}H_{92}O_{23}$	PQ (l)	Rs2	[19]
48	26.35	1031.5348 [M - H]	$C_{51}H_{84}O_{21}$	PG (f, r, m, s, l); PQ (f, r, m, s, l)	m-Rd	[11]
49	27.36	987.5413 [M - H]	$C_{50}H_{84}O_{19}$	PG (s, l); PQ (f, r, s)	Ac-Rd	[18]
50	27.76	1031.5348 [M - H]	$C_{51}H_{84}O_{21}$	PG (l); PQ (s, l)	m-Rd isomer	[16]
51	28.41	945.5354 [M - H]; 991.5435 [M + HCOO]	$C_{48}H_{82}O_{18}$	PG (l); PQ (f, r, m, l)	GyXVII	[17]
52	29.12	1117.5369 [M - H]	$C_{54}H_{86}O_{24}$	PG (s, l); PQ (s)	mf-Rd6 isomer	[6]
53	29.56	987.5449 [M - H]	$C_{50}H_{84}O_{19}$	PG (f); PQ (f, r, m)	Ac-Rd isomer	[16]
54	29.63	1117.5314 [M - H]	$C_{54}H_{86}O_{24}$	PQ (s, l)	mf-Rd6 isomer	[6]
55	29.67	987.5436 [M - H]	$C_{50}H_{84}O_{19}$	PG (s, l)	Ac-Rd isomer	[16]
56	30.10	915.5285 [M - H]; 961.5338 [M + HCOO]	$C_{47}H_{80}O_{17}$	PG (f, r, m, l); PQ (f, m, l)	NG-Fe	[17]
57	30.42	915.5226 [M - H]; 961.5279 [M + HCOO]	$C_{47}H_{80}O_{17}$	PG (f, l); PQ (f, l)	VG-R16	[16]
58	30.75	1001.5258 [M + HCOO]	$C_{48}H_{76}O_{19}$	PQ (l)	Ro isomer	[16]
59	30.91	829.4906 [M + HCOO]	$C_{42}H_{72}O_{13}$	PG (s, l); PQ (f, m, l)	F2	std
60	31.15	793.4334 [M - H]	$C_{42}H_{66}O_{14}$	PG (r, s); PQ (f, m, r, s)	Chikusetsusaponin IVa	[11]
61	31.4	783.4806 [M - H]; 829.4874 [M + HCOO]	$C_{42}H_{72}O_{13}$	PG (l); PQ (l)	Rg3	std
62	31.43	793.4301 [M - H]	$C_{42}H_{66}O_{14}$	PQ (r, s)	Chikusetsusaponin IVa isomer	[16]
63	31.43	825.4879 [M - H]; 871.4974 [M + HCOO]	$C_{52}H_{72}O_{11}$	PQ (l)	Unknown	-
64	31.65	675.3529 [M - H]; 721.3579 [M + HCOO]	$C_{40}H_{54}O_{9}$	PG (s, l)	Unknown	-
65	31.83	675.3505 [M - H]; 721.3589 [M + HCOO]	$C_{40}H_{54}O_{9}$	PG (s, l); PQ (s, l)	Unknown	-
66	32.23	677.3731 [M - H]; 723.3730 [M + HCOO]	$C_{40}H_{54}O_{9}$	PG (m); PQ (m)	Unknown	-
67	32.46	677.3743 [M - H]; 723.3746 [M + HCOO]	$C_{40}H_{54}O_{9}$	PG (m); PQ (m)	Unknown	-
68	32.69	653.3673 [M - H]; 699.3744 [M + HCOO]	$C_{36}H_{62}O_{10}$	PG (l); PQ (l)	G-Ki/G-Km/G-ST2	[6]

69	33.05	513.2942 [M - H]; 559.3044 [M + HCOO]	$C_{27}H_{46}O_9$	PG (l); PQ (s, l)	Unknown	-
70	33.42	667.4373 [M + HCOO]	$C_{36}H_{62}O_8$	PG (l)	Rh2	std
71	33.81	595.2873 [M + HCOO]	$C_{33}H_{42}O_7$	PG (r, m, s); PQ (r, m)	Unknown	-
72	34.05	515.9695 [M - H]; 561.3247 [M + HCOO]	$C_{27}H_{48}O_9$	PG (m)	Unknown	-

Note: f, r, m, s, and l refer to fine root, rhizome, main root, stem, and leaf of ginseng, respectively.

Apart from eight unknown compounds, 64 ginsenosides were identified from NZPG and NZPQ. In the ginseng fine root, NZPG and NZPQ contain the same number (26) of ginsenosides, and 18 of them are in both ginsengs (Figure 6.4A). In the ginseng rhizome, there were 16 ginsenosides found in both ginseng species, and there were 10 and eight ginsenosides found only in NZPG and NZPQ respectively (Figure 6.4B). As the main source of medicinal material, the chemical constituents of the main root are essential for the pharmacological activity of ginseng. From Figure 6.4C, we can see that in addition to the common main compounds 5 (Rg1), 6 (Re), 26 (Rb1), 29 (mRb1), 34 (Ro), 44 (Rd), and 48 (mRd) in both ginseng species, NZPG and NZPQ also contain some unique secondary metabolites, such as compounds 17 (Rf), 22 (NG-R2/G-F3/G-F5), and 23 (Ra1/Ra2) in NZPG, and compounds 18 (p-F11), and 51 (Rd isomer) in NZPQ. This may be the reason why Asian ginseng and American ginseng not only have similar bioactive effects, including enhanced physical and sexual functions, general vitality, anti-stress, and anti-aging [4], but also have different medicinal applications. Compared to the underground parts (fine root, rhizome, and main root), ginseng leaf contained more abundant ginsenosides, especially in the less polar areas (Figure 6.4D). Specifically, 33 and 25 ginsenosides were identified from the leaves of NZPG and NZPQ, respectively; 15 of these ginsenosides were found in both species. In the aboveground parts, there were fewer ginsenosides in stems than in the leaves. Interestingly, NZPQ had more peaks than NZPG had in ginseng stems (Figure 6.4E), whereas in leaves, NZPG had more peaks than NZPQ. In general, 50 and 43 ginsenosides were identified from various parts of NZPG and NZPQ, respectively, and 29 ginsenosides were found in both ginseng plants. Details about the distribution of ginsenosides are shown in Figure 6.5.

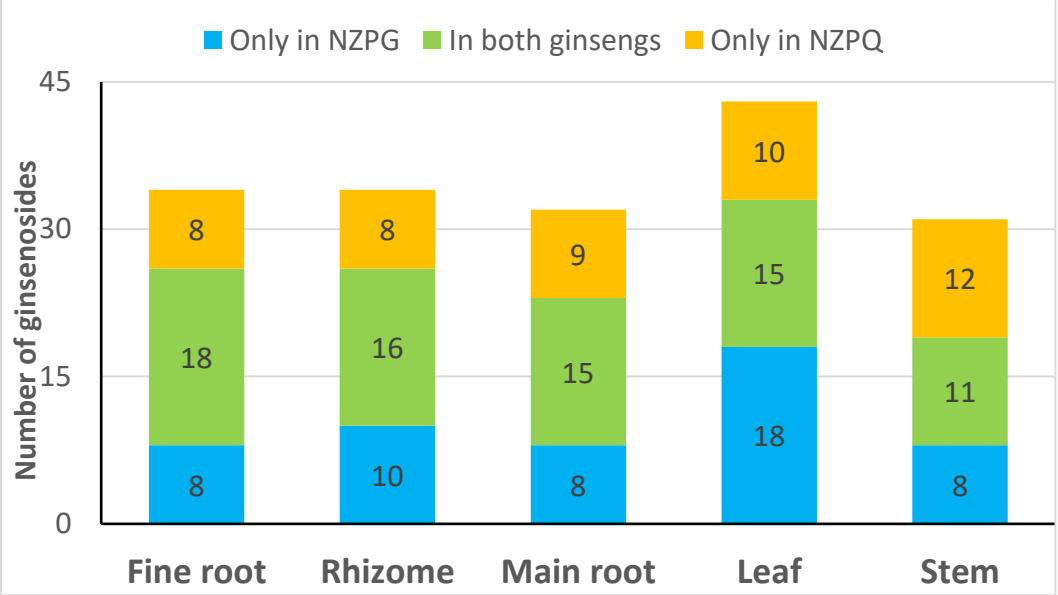


Figure 6.5 Distribution of ginsenosides in different parts of NZPG and NZPQ.

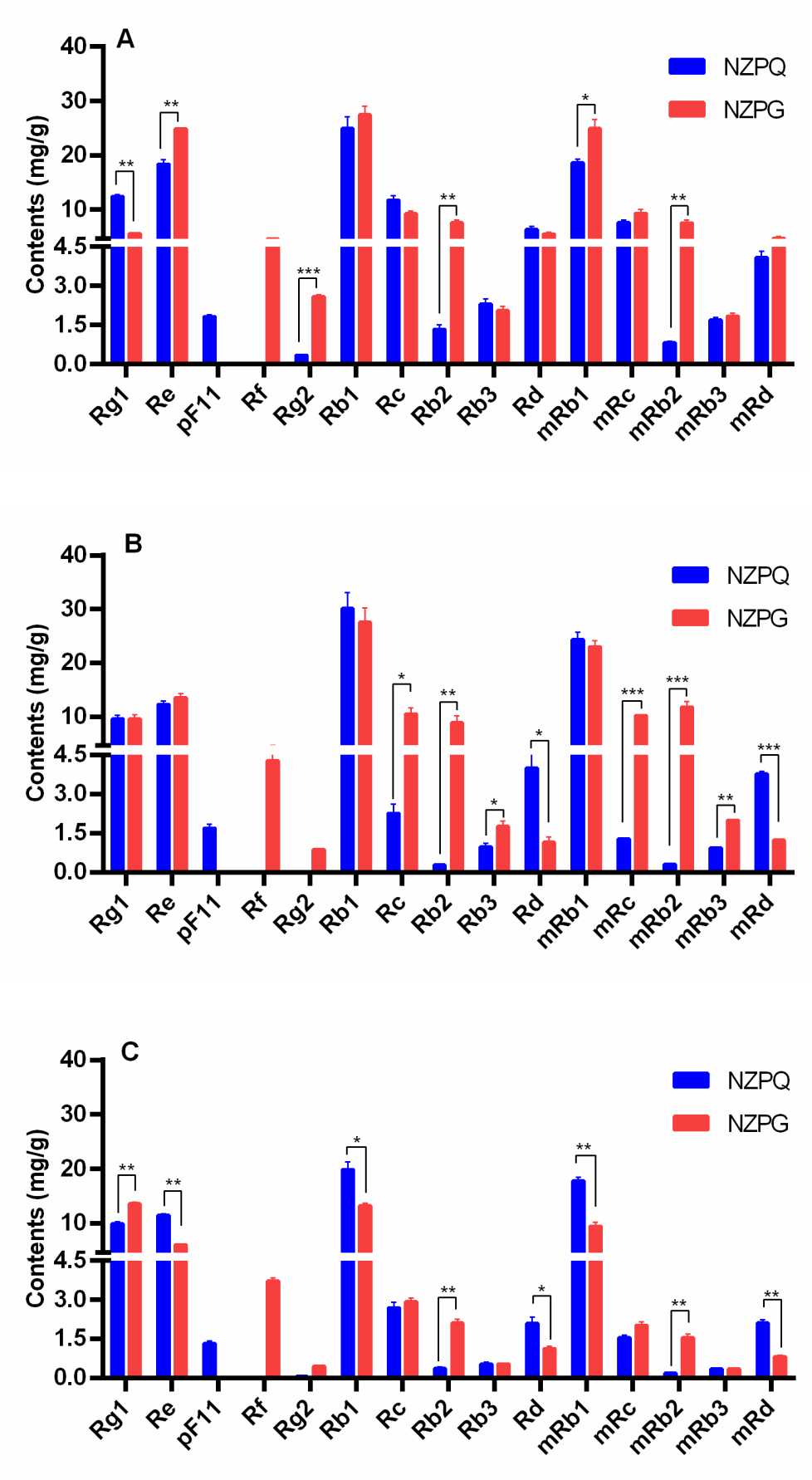
6.3.2. Quantification of the main ginsenosides in various parts of NZPG and NZPQ

To compare the differences in the content of ginsenosides between NZPG and NZPQ, the main ginsenosides in various parts of both ginseng plants were quantified according to my previous methods (Chapter four) [14]. Briefly, 14 ginsenosides (Rb1, Rb2, Rb3, Rc, Rd, Re, Rf, p-F11, F2, Rg1, Rg2, Rg3, Rh1, and Rh2) were accurately quantified by their linear regression equations of standard curves (the regression equations of calibration curves, correlation coefficient, and linear ranges for the ginsenoside standards are shown in Appendix 3); some ginsenosides without reference standards, such as ginsenosides mRb1 (m = malonyl), mRb2, mRb3, mRc, mRd, mRe, and mRg1, were relatively quantified by the regression equations of their corresponding neutral ginsenosides.

As shown in Figure 6.6, ginsenoside concentrations are varied in different ginseng tissues and different ginseng species. The four highest concentration saponins in the

underground parts (fine root, rhizome, and main root) are ginsenosides Rb1, mRb1, Re, and Rg1. The ginsenoside contents differ between the two ginseng species. In the fine root (Figure 6.6A), ginsenoside Rb1 has the highest concentration of saponins in both NZPQ (25.0 ± 2.1 mg/g) and NZPG (27.5 ± 1.6 mg/g). The contents of ginsenosides mRb1, Re, Rg2, Rb2, and mRb2 in NZPG are significantly higher than in NZPQ. The ginsenoside Rg1 content in NZPG is significantly lower than that in NZPQ. In the rhizome (Figure 6.6B), ginsenosides Rb1, mRb1, Re, and Rg1 have the four highest concentration saponins, and there are no significant differences in their concentrations between NZPQ and NZPG. However, the contents of PPD-type ginsenosides Rc, Rb2, Rb3, and their corresponding malonyl ginsenosides (mRc, mRb2, and mRb3) in NZPG are significantly higher than those in NZPQ. The contents of ginsenosides Rb1, mRb1, Re, and Rd in NZPG are significantly lower than those in NZPQ in the main root (Figure 6.6C), and the concentrations of ginsenosides Rg1, Rb2, and mRb2 in NZPG are more than those in NZPQ.

Compared to the underground parts, the distribution of ginsenosides in the aboveground parts (stem and leaf) is much different. Ginsenoside Re has the highest level of compound in the leaf and stem of both NZPG and NZPQ. Ginsenosides Rd and m-Rd became the second and third highest ingredients in NZPG leaves, while in NZPQ leaves, compound p-F11 became the major constituent, with the concentration ranking just after Re. Interestingly, ginsenosides Rb1 and mRb1, which are the main components in the underground parts, are very low and could not be quantified in some aboveground parts due to the low concentration (Figure 6.6D and 6.6E). As for the total ginsenoside content, the fine roots contain the most abundant ginsenosides not only in NZPG (142.5 ± 1.1 mg/g), but also in NZPQ (115.7 ± 3.5 mg/g); stems have the fewest ginsenosides. From the point of the total amount of ginsenoside, the main roots have similar concentrations between NZPG and NZPQ, while there is greater difference in the aboveground parts, especially in the ginseng leaves of both species (37.4 ± 3.4 mg/g in NZPQ vs. 91.5 ± 7.2 mg/g in NZPG).


A
B
C
NZPQ
NZPG
Contents (mg/g)
40
30
20
10
4.5
3.0
1.5
0.0
Rg1
Re
pF11
Rf
Rg2
Rb1
Rc
Rb2
Rb3
Rd
mRb1
mRc
mRb2
mRb3
mRd

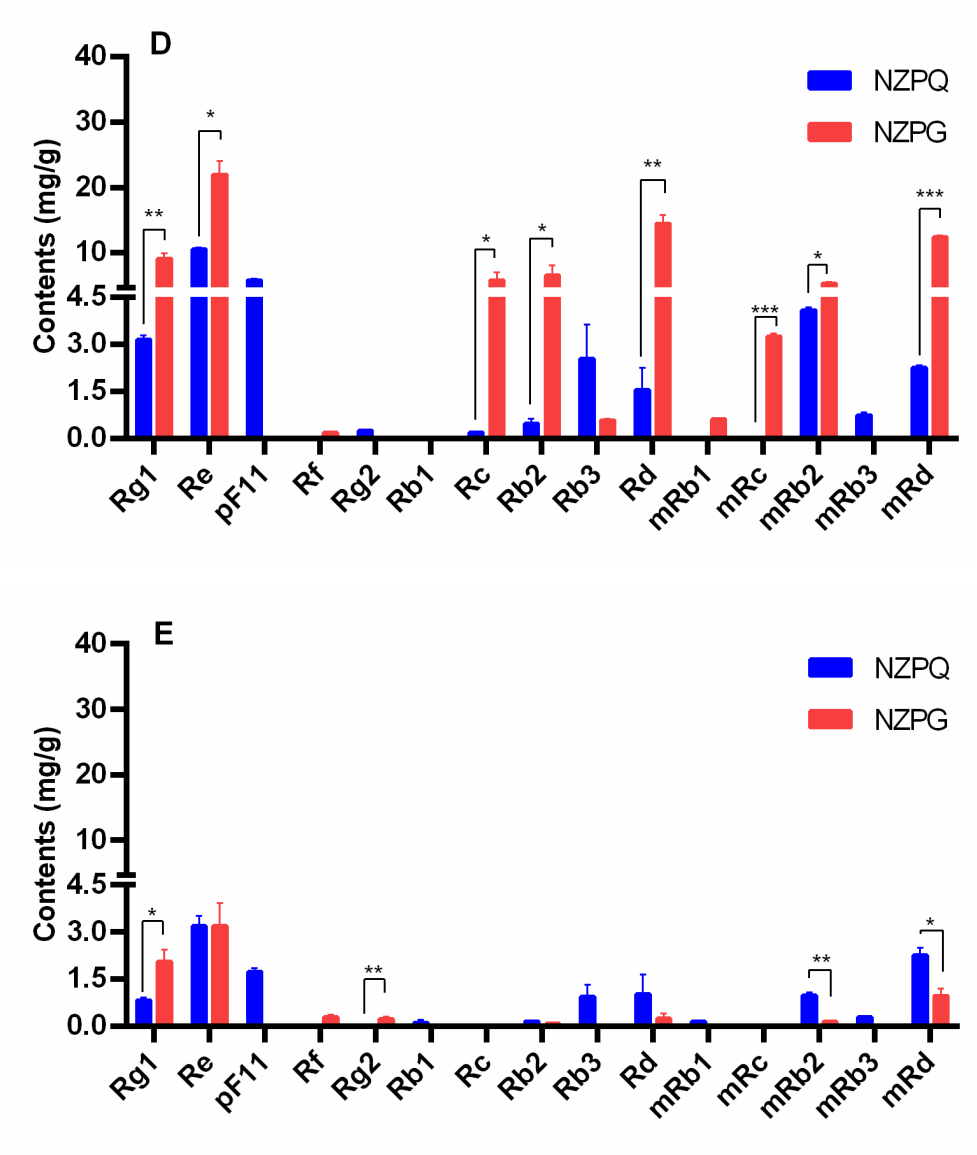
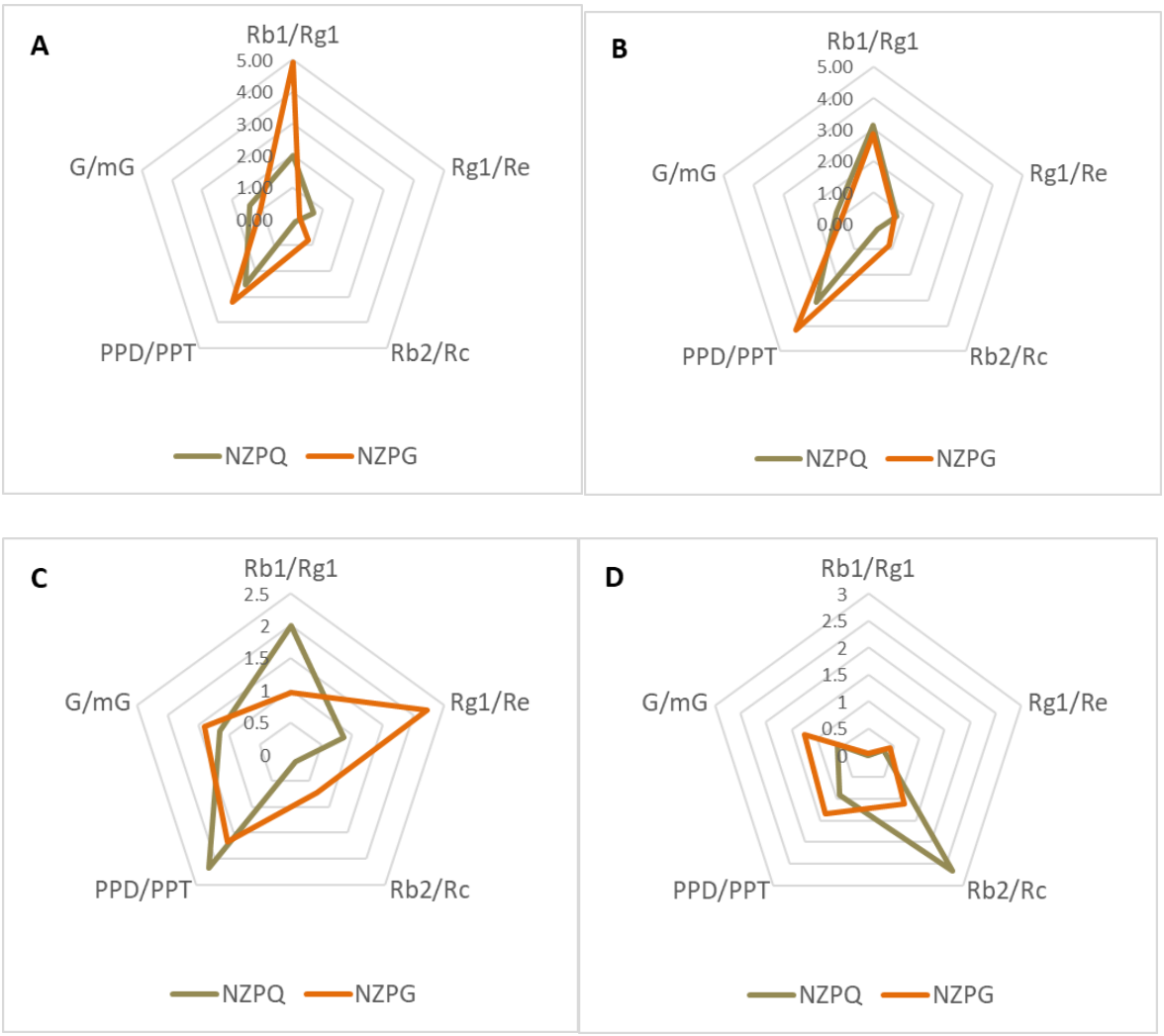


Figure 6.6 The ginsenoside contents from different parts of NZPQ and NZPG. (A) Fine root; (B) rhizome; (C) main root; (D) leaf; (E) stem. Data were expressed as mean ± SD and analyzed by t-test using Graph pad 8 software. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Differences were considered significant if p-value < 0.05.

Apart from the individual ginsenoside content and the total ginsenoside amount, the ratio of PPD-type to PPT-type (PPD/PPT), the ratio of neutral ginsenoside to malonyl ginsenoside (G/m-G), and the ratios of Rb1/Rg1, Rg1/Re, and Rb2/Rc are also described in this chapter. These ratios in the five ginseng parts vary in both ginseng species. The Rb1/Rg1 of NZPG (4.9 ± 0.3) is much higher ($p < 0.05$) than that of NZPQ (2.0 ± 0.1) in the fine roots (Figure 6.7A). There are similar ratios of ginsenosides in the

rhizome of both ginseng species (Figure 6.7B). In the main root, the Rb1/Rg1 in NZPG (1.0 ± 0.0) is significantly lower than in NZPQ (2.0 ± 0.1); the ratios of Rg1/Re and Rb2/Rc in NZPG are significantly higher than in NZPQ (Figure 6.7C). In the aboveground parts, NZPQ (2.7 ± 0.1) has a significantly higher ratio of Rb2/Rc compared to NZPG (1.1 ± 0.1) in the ginseng leaves (Figure 6.7D), and the ratio of PPD/PPT in NZPQ stems is remarkably higher than that in NZPG stems (Figure 6.7E).



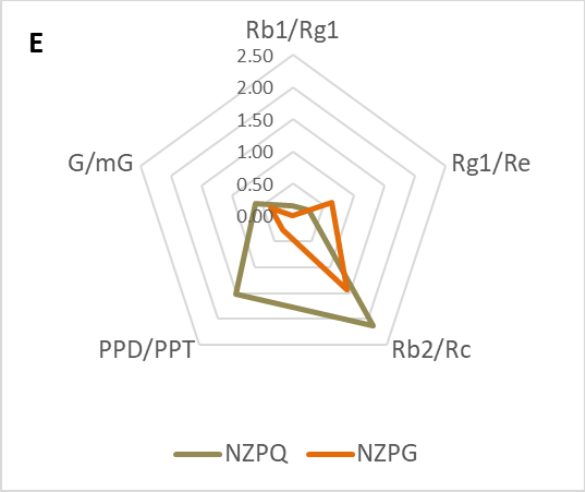


Figure 6.7 The ratios of Rb1/Rg1, Rg1/Re, Rb2/Rc, protopanaxadiol/protopanaxatriol (PPD/PPT), and G/mG in NZPQ and NZPG. (A) Fine root; (B) rhizome; (C) main root; (D) leaf; (E) stem. The PPD-type amount and PPT-type amounts are the sum of all the quantified PPD-type ginsenosides and PPT-type ginsenosides, respectively. G/mG is the ratio of neutral ginsenoside amount to malonyl ginsenoside amount; the malonyl ginsenoside amount is the sum of five quantified malonyl ginsenosides (mRb1, mRb2, mRb3, mRc, and mRd), and the neutral ginsenoside amount is the sum of corresponding neutral ginsenosides (Rb1, Rb2, Rb3, Rc, and Rd).

Consistent with other reports [7], I found ginsenoside Rf only in NZPG and p-F11 only in NZPQ. They can be used as a reference marker to differentiate between Asian ginseng and American ginseng. Notably, the two ginsenosides have very similar retention times in HPLC, and often co-elute. Thus, they are easily confused if identifications are made only according to the retention time of compounds. However, ginsenosides Rf and p-F11 can produce unique ions at *m/z* (637, 475) and (653, 491) in negative MS/MS spectrum, respectively, due to fragmentation differences in their sugar moieties. Therefore, LC-MS/MS can be an effective and sensitive method to identify ginsenoside Rf and p-F11 and further distinguish Asian ginseng and American ginseng, even without reference standards.

The literature reports that the contents of ginsenosides Rg1, Rb2, and Rc in Asian ginseng are higher than they are in American ginseng, and in Asian ginseng, the contents of Rb1, Re, and Rd are lower than they are in American ginseng [4]. In this chapter, I found that the contents of Rg1, Rb2, and Rc in NZPG are higher than they are in NZPQ only in the main root, and the contents of Rb1, Re, and Rd in NZPG are lower compared to the contents in NZPQ. However, in other parts, this rule has not been maintained. A few ginsenosides in the fine root (Rb2, Rd) and rhizome (Rg1, Re) do not comply with this rule, while, particularly in the leaf, the contents of these ginsenosides in NZPG are higher than that in NZPQ. A recent publication found the ratios of Rg1/Re < 0.15, Rb1/Rg1 > 2.15, and Rb2/Rc < 0.26 as the ginsenoside markers of American ginseng (as opposed to Asian ginseng) [9]. In the main root of New Zealand-grown ginseng, I found that Rb1/Rg1 > 2.15 and Rb2/Rc < 0.26 are in American ginseng and Rb1/Rg1 < 2.15, Rb2/Rc > 0.26, and Rg1/Re > 0.15 are in Asian ginseng, which is in line with the published report. In the rhizome, the ratios of these ginsenosides are similar between the two ginseng species. However, in other tissues, these ratios are waved and do not exhibit an obvious rule. This may be due to the uninterrupted interaction between the fine roots (root hair) and soil (microorganism), and the leaves and atmosphere (solar energy) leading to variation in ginsenosides in these parts. The transport of ginsenosides from various tissue parts needs to be explored in future experiments. Moreover, ginseng is typically grown in a farm setting under artificial shade conditions often involving pesticides and fertilizers [20, 21], and managed by unified practice. Whereas this ginseng is certified organic grown under the shade of a pine forest, accumulation of ginsenosides can be affected by some uncontrolled factors, including soil, moisture, and light [22].

6.4 Conclusion

This study systematically investigated the composition of ginsenosides in various tissues of NZPG and NZPQ through qualitative and quantitative analysis. The number of ginsenosides and the total contents of ginsenosides in different parts between two ginseng variations are summarized in Figure 6.8. The ginsenoside composition and content of the two kinds of ginseng are very different in the aboveground parts (leaf and stem), and in the underground parts (main root, fine root, and rhizome); Asian ginseng and American ginseng have relatively similar ginsenoside constituent in the number of ginsenosides as well as the total ginsenoside amounts.

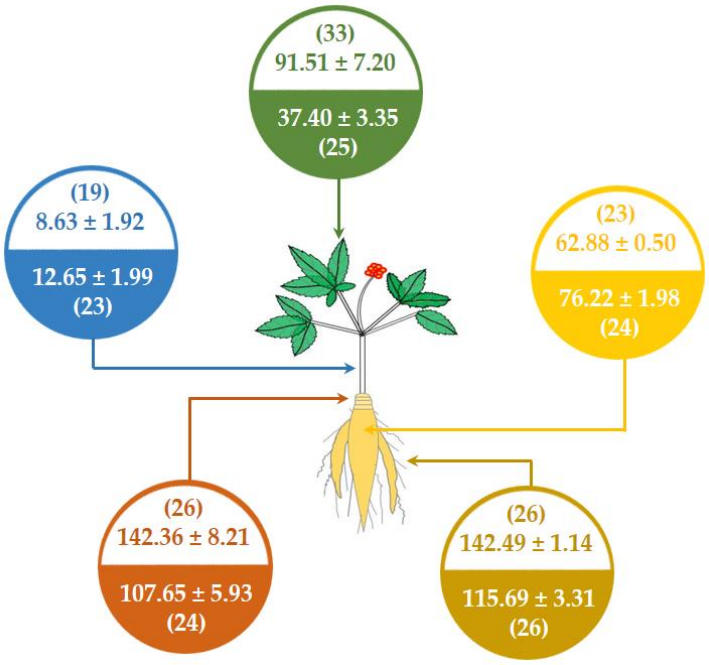


Figure 6.8 Comparison of ginsenoside components between New Zealand-grown Asian ginseng (colorless background) and New Zealand-grown American ginseng (colored background) in leaf (green), stem (blue), rhizome (red), fine root (brown), and main root (yellow). The numbers in parentheses refer to the number of ginsenosides identified by qualitative analysis, and the other numbers refer to the total content of ginsenosides (mg/g) quantified by quantitative analysis.

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Chapter Seven

Changes of ginsenoside composition in the creation of black ginseng leaf

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Ginseng is an increasingly popular ingredient in supplements for healthcare products and traditional medicine. Heat-processed ginsengs, such as red ginseng or black ginseng, are regarded as more valuable for medicinal use when compared to white ginseng due to some unique less polar ginsenosides produced during heat-treatment. Although ginseng leaf contains abundant ginsenosides, attention has mostly focused on ginseng root; relatively few publications have focused on ginseng leaf. Raw ginseng leaf was steamed nine times to make black ginseng leaf using a process similar to that used to produce black ginseng root. Sixteen ginsenosides were analyzed during each steaming while using high-performance liquid chromatography (HPLC). The contents of ginsenosides Rd and Re decreased and the less polar ginsenosides (F2, Rg3, Rk2, Rk3, Rh3, Rh4, and protopanaxatriol) enriched during steam treatment. After nine cycles of steaming, the contents of the less polar ginsenosides F2, Rg3, and Rk2 increased by 12.9-fold, 8.6-fold, and 2.6-fold, respectively. Furthermore, I found that the polar protopanaxadiol (PPD) -type ginsenosides are more likely to be converted from ginsenoside Rg3 to ginsenosides Rk1 and Rg5 via dehydration from Rg3, and from ginsenoside Rh2 to ginsenosides Rk2 and Rh3 through losing an H_2O molecule than to be completely degraded to the aglycones PPD during heat process. This study suggests that ginseng leaves can be used to produce less polar ginsenosides through heat processes, such as steaming.

7.1 Introduction

Panax ginseng (*P. ginseng*) root has been used as a natural medicinal herb for thousands of years in Asian countries, especially in China, Korea, and Japan. It has become one of the most popular and best-selling herbs in the world herb market [1, 2]. Ginseng saponins, known as ginsenosides, are thought to be the main bioactive components of ginseng and they have wide health-promoting effects, including anti-ageing [3], anti-stress [4], antioxidative [5], antifatigue [6], anti-diabetes [7], anticancer [8], enhanced liver function [9], improved immune system [10], and improved climacteric disorder and sexual function [11]. To date, nearly two hundred ginsenosides have been isolated and identified from various tissues of ginseng plants (Chapter two) [12]. Based on the chemical structures of aglycones moieties, ginsenosides are divided mainly into protopanaxadiol (PPD) type ginsenosides, such as Rb1, Rb2, Rc, Rd, Rg3, and so on, or protopanaxatriol (PPT) type ginsenosides, for example, Rg1, Re, Rg2, and Rh1, and so on (structures are shown in Figure 7.1).

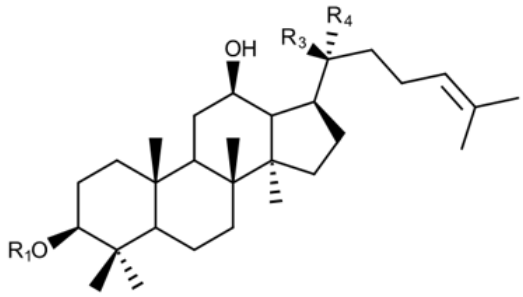
Traditionally, ginseng root has been regarded as the most used and valuable part for health care and medical applications. Thus, 'ginseng' in literature and media refers to ginseng root unless otherwise specified. Ginseng harvested from the wild or farm is fresh ginseng. Fresh ginseng easily decomposes because of the high water content (approximately 70-80%) and might coexist with soil microorganisms [13], thus it is rarely used. For long-term storage and improved pharmacological effects, fresh ginseng is usually processed before application and usage, mainly through heat-involved processes, such as steaming [14], toasting [15], baking [16], and fermentation [17]. The common commercially processed ginseng products are white ginseng (WG), red ginseng (RG), and black ginseng (BG). WG is produced by dehydrating fresh ginseng in sunlight; RG is produced by steaming fresh ginseng at 95-100 °C for 2-3 h; and BG is produced through nine cycles of steaming fresh ginseng at 95-98 °C for 3 h [18, 19]. WG mainly contains ginsenosides m-Rb1, m-Rb2, m-Rc, Re, and Rg1. The

main ginsenosides of RG are Rb1, Rb2, Rc, Rd, Re, and Rg1 [20], while BG contains some unique ginsenosides such as Rd, Rk1, Rg5, and Rg3, which are not present in WG, and exhibit more potent pharmacological effects than WG and RG [21-23]. The less polar ginsenosides in black ginseng can be deglycosylated from major polar ginsenosides during steaming fresh ginseng. Among the less polar ginsenosides, Rg3 is regarded as the bioactive component that exerts pharmacological effects, especially the anti-diabetic effect [24]. Increasing the content of ginsenoside Rg3 through post-harvest processing is an effective strategy to improve the pharmacological function of ginseng.

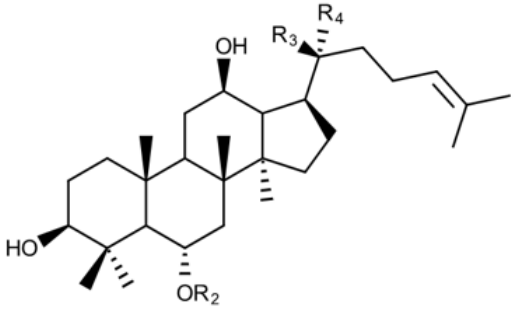
Phytochemical studies have found that the ginseng leaf contains abundant ginsenosides, and the total content of ginsenosides in the leaf is higher than in the root (Chapter four) [25-27]. Unlike ginseng root, ginsenosides Re and Rd are the major ginsenosides in the ginseng leaf [26, 27]. Moreover, ginseng leaf extract has many pharmacological activities that are similar to ginseng root extract [28]. Thus, ginseng leaves have advantages over ginseng root in terms of cost, source availability, and sustainability, for ginseng leaves can be harvested annually while ginseng root usually takes four to six years to be harvested. At a minimum, ginseng leaf can be used as a valuable source of ginsenosides Re and Rd [29, 30].

Steaming ginseng leaves can produce some new ginsenosides and enhance the leaves' potential. Five new dammarane ginsenosides (SL1-SL3, ST1, and ST2) were isolated from steamed ginseng leaves [31, 32]. Extracts from steamed ginseng leaves have been found to enhance cytotoxic effects on human leukemia HL-60 cells [32]. Another study found ginsenosides that were extracted from steamed ginseng leaves and flowers inhibited LPS-induced IL-12 production [33]. However, there are no publications concerning changes in ginsenoside profiles during the leaf steaming process. Ginsenoside Rd can be converted into Rg3 when ginseng is treated with heat-processing [12]. The hypothesis of this study is that the content of ginsenoside Rg3 may increase in black ginseng leaves by following the same process used to make

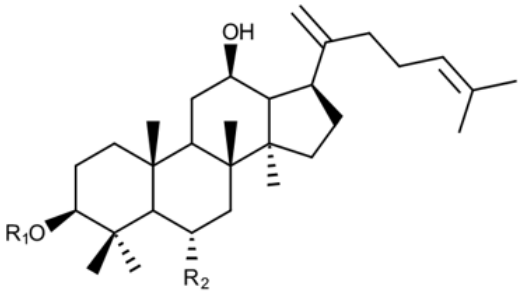
black ginseng root. This study investigated the changes of ginsenosides during steaming ginseng leaves to provide chemical information for the development and application of ginseng leaf.



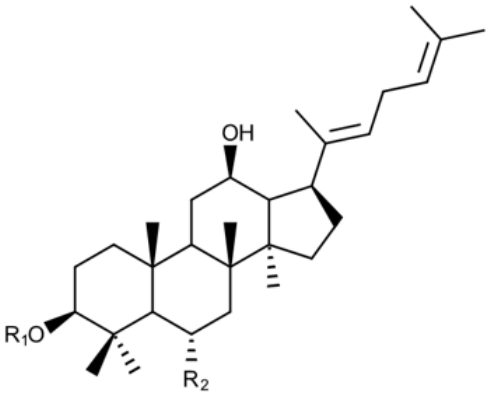
Name	Formula	R1	R3	R4
Rb1	$C_{54}H_{92}O_{23}$	glc(2-1)glc	Oglc(6-1)glc	CH_3
Rb2	$C_{53}H_{90}O_{22}$	glc(2-1)glc	Oglc(6-1)arap	CH_3
Rb3	$C_{53}H_{90}O_{22}$	glc(2-1)glc	Oglc(6-1)xyl	CH_3
Rc	$C_{53}H_{90}O_{22}$	glc(2-1)glc	Oglc(6-1)araf	CH_3
Rd	$C_{48}H_{82}O_{18}$	glc(2-1)glc	Oglc	CH_3
F2	$C_{42}H_{72}O_{13}$	glc	Oglc	CH_3
20S-Rg3	$C_{42}H_{72}O_{13}$	glc(2-1)glc	OH	CH_3
20R-Rg3	$C_{42}H_{72}O_{13}$	glc(2-1)glc	CH_3	OH
20S-Rh2	$C_{36}H_{62}O_9$	glc	OH	CH_3
20R-Rh2	$C_{36}H_{62}O_9$	glc	CH_3	OH
20S-PPD	$C_{30}H_{52}O_3$	H	OH	CH_3
20R-PPT	$C_{30}H_{52}O_3$	H	CH_3	OH



Name	Formula	R2	R3	R4
Re	$C_{48}H_{82}O_{18}$	Oglc(2-1)rha	Oglc	CH_3
Rf	$C_{42}H_{72}O_{14}$	Oglc(2-1)glc	OH	CH_3
Rg1	$C_{42}H_{72}O_{14}$	Oglc	Oglc	CH_3
20S-Rg2	$C_{42}H_{72}O_{13}$	Oglc(2-1)rha	OH	CH_3
20R-Rg2	$C_{42}H_{72}O_{13}$	Oglc(2-1)rha	CH_3	OH
20S-Rh1	$C_{36}H_{62}O_9$	Oglc	OH	CH_3
20R-Rh1	$C_{36}H_{62}O_9$	Oglc	CH_3	OH
20S-PPT	$C_{30}H_{52}O_4$	OH	OH	CH_3
20R-PPT	$C_{30}H_{52}O_4$	OH	CH_3	OH



Name	Formula	R1	R2
Rk2	$C_{36}H_{60}O_7$	glc	H
Rk3	$C_{36}H_{60}O_8$	H	Oglc



Name	Formula	R1	R2
Rh3	$C_{36}H_{60}O_7$	glc	H
Rh4	$C_{36}H_{60}O_8$	H	Oglc

Figure 7.1 The chemical structures and molecular formulas of reference standards in this chapter. The glc, arap, xyl, araf, and rha refer to β-D-glucopyranosyl, *α*-L-arabinopyranosyl, β-D-xylopyranosyl, *α*-L-arabinofuranosyl, and *α*-L-rhamnopyranosyl, respectively.

7.2 Materials and methods

7.2.1 Ginsenoside standards, chemicals, and reagents

Twenty-five reference standards of ginsenosides (structures are shown in Figure 7.1) (Rb1, Rb2, Rb3, Rc, Rd, Re, Rf, Rg1, 20S-Rg2, 20R-Rg2, 20S-Rg3, 20R-Rg3, 20S-Rh1, 20R-Rh1, 20S-Rh2, 20R-Rh2, Rh3, Rh4, Rk2, Rk3, F2, 20S-PPD, 20R-PPD, 20S-PPT, and 20R-PPT) were purchased from Star Ocean Ginseng Ltd (Suzhou, Jiangsu, China). The purities of all reference standards were no less than 98.0%. HPLC-grade methanol (MeOH) and acetonitrile (MeCN) were purchased from Fisher Chemical (Pittsburg, PA, USA). Water (deionized) was obtained from a Milli-Q Ultra-pure water system (Millipore, Billerica, MA, USA). Other reagents used in this study were of analytical grade.

7.2.2 Ginseng leaf preparation

Fresh ginseng leaves (*P. ginseng*) were collected in May 2019 from pine forests around Taupo, NZ. Two hundred grams of fresh ginseng leaves were steamed in a household steamer at 98-99 ℃ for 30 min. After steaming, the leaves were dried in a drying oven at 60 ℃ for 1.5 h. This process (steaming at 98-99 ℃ for 30 min and oven-drying at 60 ℃ for 1.5 h) was repeated nine times. The fresh leaves (SGL0) were dried in a drying oven at 60 ℃ for 1.5 h. The color parameters were measured with a CR-400 Chroma Meter (Konica Minolta, Ramsey, NJ, USA). Each sample (Figure 7.2) was ultrasonically extracted three times using a Q700 sonicator (Qsonica, Melville, NY, USA) as previous methods (Chapter four) [27, 34]. Briefly, 0.7 g dried steamed ginseng leaf pieces were incubated with 10 mL 70% (v/v) aqueous MeOH for 30 min and extracted at 20 kHz for 10 min at no more than 40 ℃ (10 min extraction contained five cycles, each cycle consisted of 2 min ultrasonic extraction at 15% amplitude and 1 min for cooling between extraction). The supernatant was collected after centrifugation (1788 xg for

10 min) and the sediment was extracted twice more. The three extracts were mixed and filtered through a 0.22-micron filter before HPLC injection.

Figure 7.2 Pictures of steamed ginseng leaves (SGL) at different steaming cycles.

7.2.3 Ginsenoside reference standards preparation

Twenty-five reference standards of ginsenosides (Rb1 (0.769 mg/mL), Rb2 (0.846 mg/mL), Rb3 (0.629 mg/mL), Rc (1.077 mg/mL), Rd (0.692mg/mL), Re (0.923 mg/mL), Rf (1.462 mg/mL), Rg1 (1.154 mg/mL), 20S-Rg2 (0.615 mg/mL), 20R-Rg2 (0.846 mg/mL), 20S-Rg3 (1.154 mg/mL), 20R-Rg3 (0.769 mg/mL), 20S-Rh1 (1.000 mg/mL), 20R-Rh1 (0.769 mg/mL), 20S-Rh2 (1.077 mg/mL), 20R-Rh2(1.231 mg/mL), Rh3 (0.769 mg/mL), Rh4 (0.923 mg/mL), Rk2 (0.692 mg/mL), Rk3 (0.923 mg/mL), F2 (0.692 mg/mL), 20S-PPD (0.769 mg/mL), 20R-PPD (1.077 mg/mL), 20S-PPT (1.231 mg/mL), and 20R-PPT (0.769 mg/mL)) were dissolved in 70% MeOH and then mixed and diluted with 70% MeOH to obtain a series of mixture standard solutions of different concentrations. The solutions were filtered through a 0.22-micron syringe filter before HPLC injection.

7.2.4 HPLC analysis

The HPLC instrument used in this chapter was a Shimadzu prominence LC-20A UFLC stack HPLC system (Shimadzu, Kyoto, Japan) equipped with a DGU-20A3 degasser, LC-20AD pump, CTO column oven, SPD-20A detector, and SIL-20A autosampler. A Waters Symmetry Shield RP18 column (4.6 mm × 250 mm, 5 μm, 100 Å) was used. The mobile phase consisted of deionized water (A) and acetonitrile (B). The gradient elution program was as follows: 0-15 min, 20% B; 15-25 min, 20-31% B; 25-35 min, 31-35% B; 35-40 min, 35-36% B; 40-45 min, 36-39% B; 45-75 min, 39-95%; 75-85 min, 95-20% B, 85-90 min, 20%B. The column temperature was set at 25 °C and the flow rate was 1 mL/min. The sample injection volume was 20 μL and the wavelength of detection was set at 203 nm.

7.3 Results and discussion

7.3.1 The color changes of ginseng leaves

From Figure 7.2, we can see that the color of the ginseng leaves gradually became darker with the increased steaming cycles. The leaf color was measured according to the color parameters L, a, and b, which are used by the International Commission on Illumination, in order quantify the changes of color during the steaming process. The L value corresponds to the lightness of color, with values ranging from 0 (black) to 100 (white). The a and b values are color coordinates expressed numerically, where a represents red or green color and b represents the yellow or blue color.

Table 7.1 shows that the L and b values of ginseng leaves decreased while the a value increased during the steaming process. A decrease in L value indicates color darkening during the steaming process. A decrease in negative a value, then an increase in positive a value indicates a decrease in the greenness and an increase in the redness of the leaves during steaming. A decreased positive b value indicates a

decrease in the yellowness. Therefore, the color measurements indicate some loss or change of color in ginseng leave during steaming, which provides an explanation for why it is called black ginseng or black ginseng leaf.

Table 7.1 Changes in color parameters of ginseng leaves during the steaming at different steaming cycles

Samples	**SGL0**	**SGL1**	**SGL2**	**SGL3**	**SGL4**
Time (min)	0	30	60	90	120
L	53.5 ±5.1	44.8 ± 1.1	40.0 ± 4.5	37.1 ± 3.7	32.3 ± 3.7
+a	-26.8 ± 3.8	-2.7 ± 1.1	-0.6 ± 0.1	1.0 ± 0.5	1.2 ± 0.3
+b	44.8 ± 6.8	15.2 ± 2.9	13.8 ± 2.3	13.2 ± 2.6	8.6 ± 2.1
ΔE		39.1 ± 6.2	42.7 ± 5.9	45.2 ± 5.5	50.4 ± 6.0
Samples	**SGL5**	**SGL6**	**SGL7**	**SGL8**	**SGL9**
Time (min)	150	180	210	240	270
L	33.6 ± 7.1	31.3 ± 1.8	28.7 ± 3.4	28.8 ± 3.2	28.0 ± 1.9
+a	1.5 ± 0.4	1.7 ± 0.6	2.1 ± 0.5	1.6 ± 0.3	1.7 ± 0.1
+b	11.5 ± 5.2	7.0 ± 2.1	6.7 ± 2.2	5.1 ± 4.9	2.5 ± 1.3
ΔE	48.0 ± 4.3	52.2 ± 6.6	53.8 ± 5.9	54.7 ± 4.4	57.0 ± 7.4

L indicates lightness, +a is the red coordinate, +b is the yellow coordinate, and ΔE indicates the total color change.

7.3.2 The contents of ginsenosides during different steaming times

The ginsenosides extracted from steamed ginseng leaves were well separated within 80 min by the RP-C18 column. From Figure 7.3, we can see that some paired isomers of less-polar ginsenosides were completely separated, such as Rk3 and Rh4, 20S-Rg3 and 20R-Rg3, 20S-PPT and 20R-PPT, 20S-Rh2 and 20R-Rh2, Rk2 and Rh3, and 20S-PPD and 20R-PPD. The peaks of the polar ginsenosides did not change much, but the peaks of the less-polar ginsenosides became more pronounced with each steam cycle

(Appendix 4 Figure S7.1), especially ginsenoside Rg3. There are relatively big peaks of Rg3 in the SGL9 sample, compared to no Rg3 peaks in the SGL0 sample.

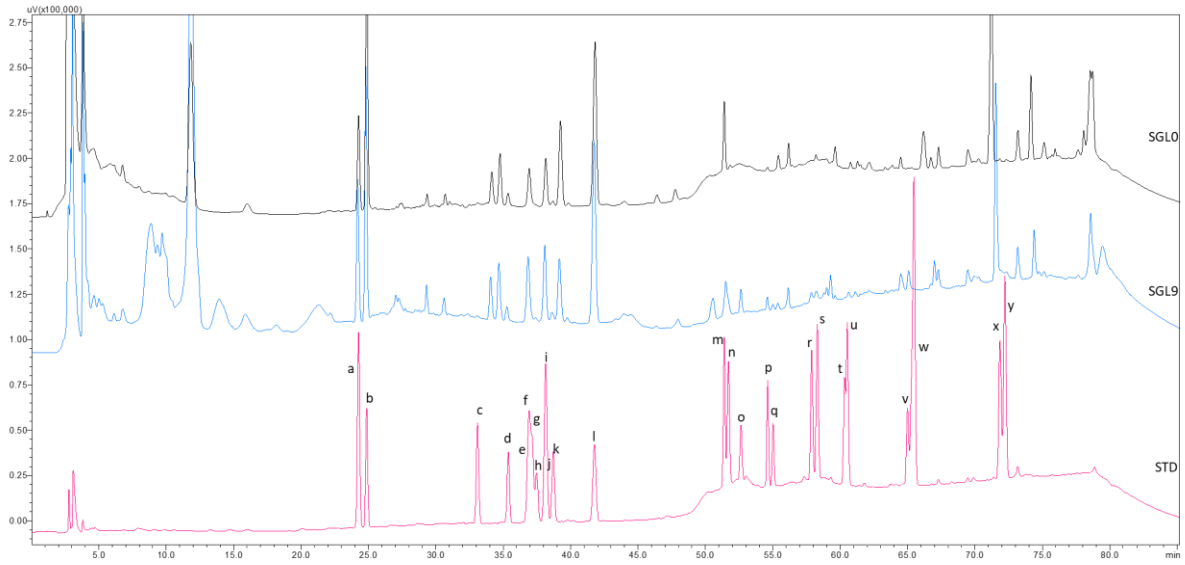


Figure 7.3 Off-set HPLC chromatograms of ginsenosides detected from the steamed ginseng leaves (SGL) as compared with the chromatogram of the ginsenoside standards. a, Rg1; b, Re; c, Rf; d, Rb1; e, 20S-Rg2; f, Rc; g, 20S-Rh1; h, 20R-Rg2; i, Rb2; j, 20R-Rh1; k, Rb3; l, Rd; m, F2; n, Rk3; o, Rh4; p, 20S-Rg3; q, 20R-Rg3; r, 20S-PPT; s, 20R-PPT; t, 20S-Rh2; u, 20R-Rh2; v, Rk2; w, Rh3; x, 20S-PPD; y, 20R-PPD.

Sixteen ginsenoside references were used to conduct the quantitative analysis on the ginsenosides changes during the leaf steaming process. The calibration curves were plotted based on the linear regression of the integrated peak areas (y) to concentrations (x, mg) of 16 ginsenoside references in the standard solution at five different concentrations. Appendix 4 Table S7.1 shows the regression equations of calibration curves, correlation coefficient, and linear ranges for the ginsenoside standards. The validations of intraday and inter-day precisions are from 0.1% to 4.9% and from 3.4% to 6.5%, respectively (Appendix 4 Table S7.2). A recovery experiment was carried out to evaluate the accuracy of the method, and the recoveries of 16

ginsenosides were between 91.3% and 106.1%, which are listed in Appendix 4 Table S7.3.

The contents of 16 ginsenosides from different batches of steamed ginseng leaves were quantified (Appendix 4 Table S7.4). The amount of less polar ginsenosides (F2, Rg3, Rk2, Rk3, Rh3, Rh4, and PPT), which were not present in the raw ginseng leaf, accounted for 10.4% of the quantified ginsenosides after steaming nine times. From Figure 7.4, we can see the changes of ginsenoside content in different steaming cycles. Overall, although the contents of the polar PPD ginsenosides fluctuated in different steamed batches, the content of Rd tended to decrease from 26.3 mg/g at the beginning to 22.9 mg/g in the SGL9. while the total content of ginsenoside Rb1, Rb2, Rb3, and Rc (these four ginsenosides were marked Sbc) showed an increasing trend with steaming. The less polar ginsenosides could not be quantified in the raw ginseng leaves, and ginsenosides F2, Rg3, and Rk2 appeared from SGL1, SGL4, and SGL5, respectively. After being produced, the contents of the less polar ginsenosides F2, Rg3, and Rk2 increased by 12.9 folds, 8.6 folds, and 2.6 folds, respectively, at the ninth steaming leaves (Figure 7.4A). The content change of PPT-type ginsenosides was similar to that of PPD-type ginsenosides with steaming (Figure 7.4B). The content of ginsenoside Re gradually decreased by 33.8% after being steaming nine times and ginsenoside Rg1 slightly increased with steaming. The less polar ginsenosides PPT and Rh4 appeared from SGL3 and accumulated relatively rapidly, and Rk3 emerged from SGL5 and increased weakly.

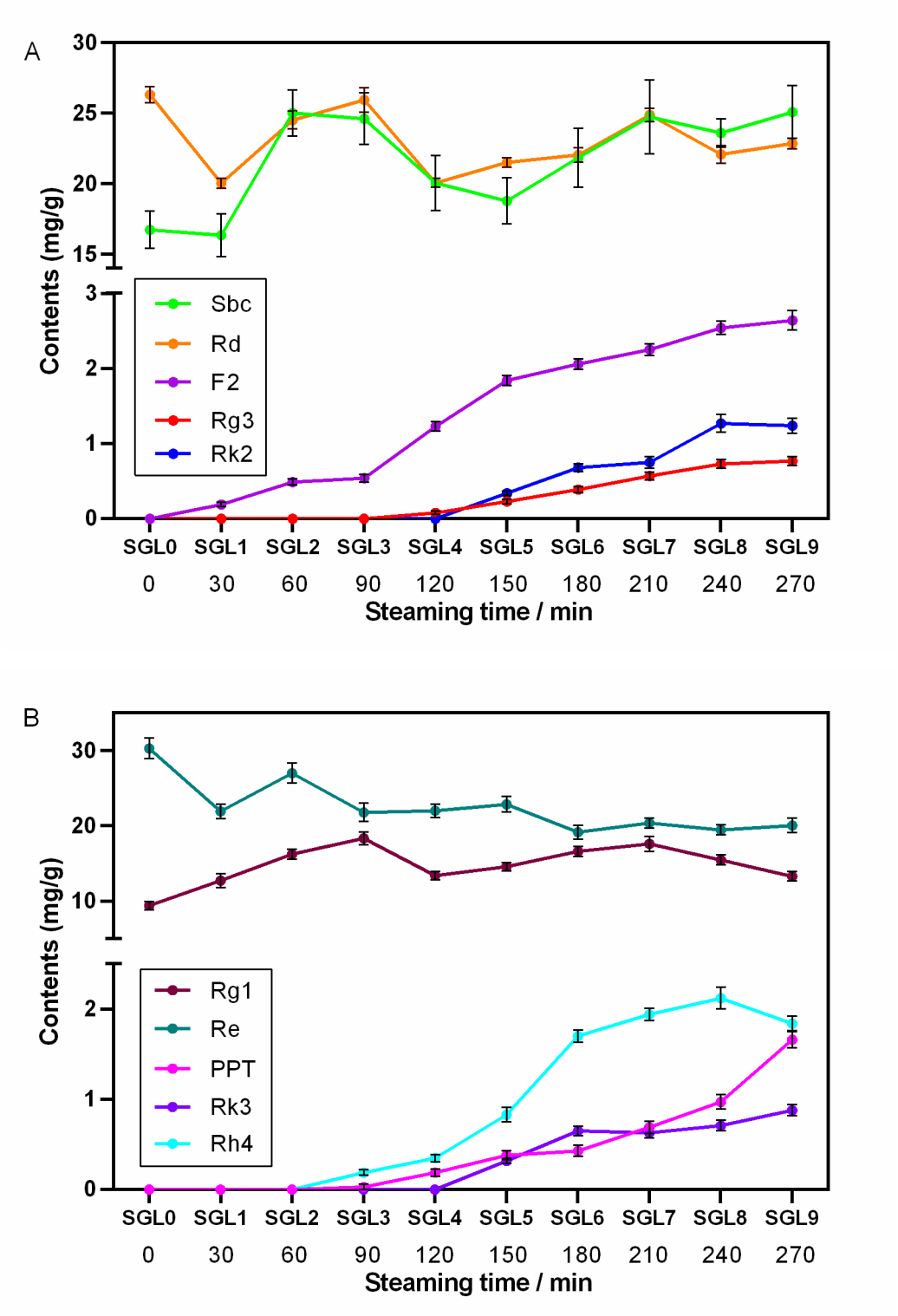


Figure 7.4 The contents of PPD-type ginsenosides (A) and PPT-type ginsenosides (B) during different steaming batches. Sbc refers to the sum of ginsenosides Rb1, Rb2, Rb3, and Rc. Rg3 refers to the 20S-Rg3 and 20R-Rg3.

From the above results, it showed that the less polar ginsenosides (both PPD-type and PPT-type), which were not present in the raw ginseng leaves, produced and accumulated through repeated steaming. The literature reported that the less polar ginsenosides can be transformed from the polar ginsenosides with heat process [12]. Briefly, the ginsenosides with big molecules, such as Rb1, Rb2, Rb3, and Rc, can transform to Rd by losing one sugar residue, and then ginsenoside Rd continues to lose sugars to from less polar ginsenosides. This would explain why the contents of the less polar PPD-type ginsenosides (F2, Rg3, and Rk2) increased as the amount of ginsenoside Rd decreased with steaming. If ginsenoside Rd is sourced from ginsenosides Sbc with heat process, the amount of Sbc should decrease with steaming. However, the amount of Sbc fluctuated and showed the opposite trend with steaming. It suggests that other compounds can transform into Sbc, leading to an increasing trend of Sbc in the heat process. While, there was an abundance of malonyl ginsenosides, especially mal-Rb2, mal-Rc and mal-Rd, in ginseng leaf in Chapter 4 [27]. The malonyl ginsenosides are thermally unstable and can be transformed into their neutral ginsenosides by losing the malonyl group [35].

Literature reported that the contents of malonyl ginsenosides (mal-Rb1, mal-Rb2, mal-Rb3, mal-Rc, and mal-Rd) decreased in the steamed ginseng and red ginseng when compared to the fresh ginseng, accordingly, their neutral ginsenosides enriched in the heat-treated ginseng [13]. Unsurprisingly, the malonyl ginsenosides would lose the malonyl group to produce ginsenosides Sbc and Rd and then lost some sugar groups to form the less polar PPD type ginsenosides during the steaming process of ginseng leaf. Apart from the demalonylation and deglycosylation, the less polar ginsenoside Rk2 and Rh3 can be produced from ginsenoside Rh2 by losing an H_2O to form a double bond between C-20 and C-21, and C-20 and C-22, respectively. The transformation pathway of PPD-type ginsenosides during the steaming process is displayed in Figure 7.5A based on my results and literature. Similarly, Figure 7.5B presents the relationship between PPT-type ginsenosides. Because there was little malonyl PPT-

type ginsenosides in the raw ginseng leaf [27], ginsenoside Re degraded to small ginsenosides during the steaming, but cannot be supplied from other compounds, leading to gradually decrease with steaming. Thus, steaming ginseng leaf can produce ginsenoside transformation similar to that of black ginseng.

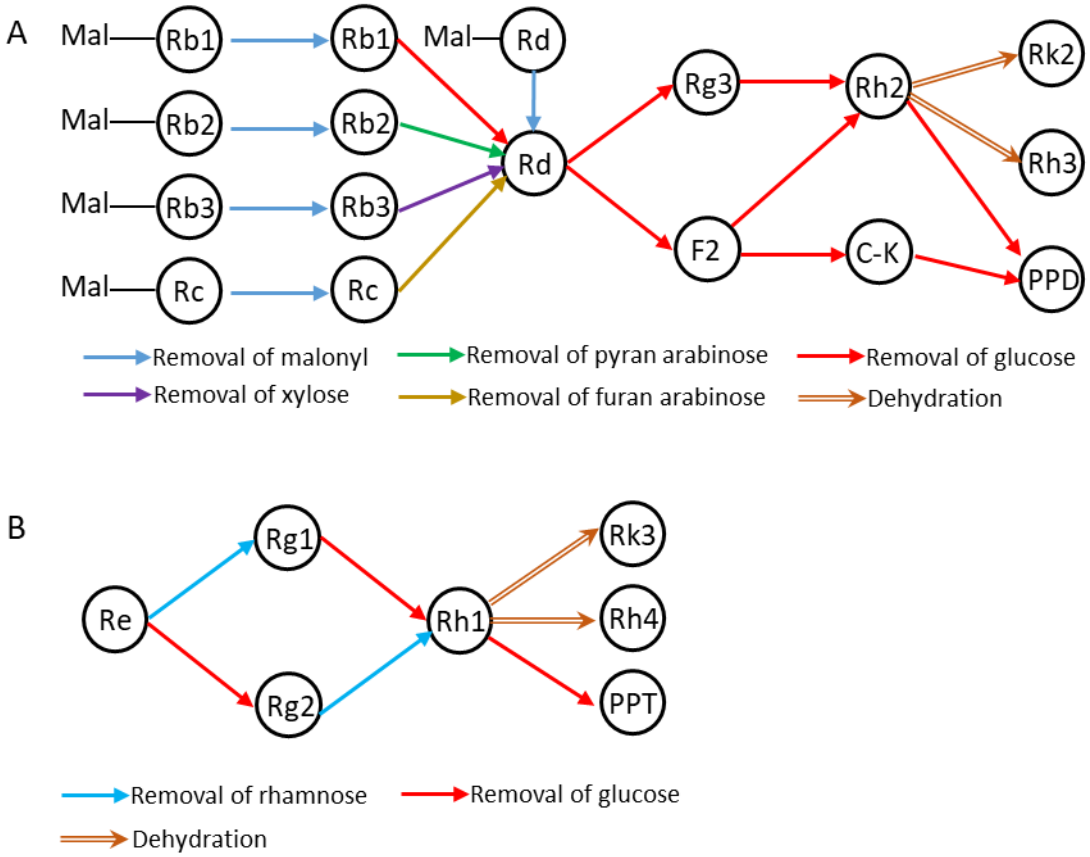


Figure 7.5 The proposed transformation pathway of PPD-type ginsenosides (A) and PPT-type ginsenosides (B) during the steaming process.

In the process of making black ginseng, Lee et al found that the less polar ginsenosides can be detected after steaming once and their amount increased with the number of steaming times and peaked in red ginseng steamed eight times [14]. While with these ginseng leaves, the less polar ginsenosides (except for F2) cannot be detected until steaming three times, some can be detected from four times or even from nine times. This is probably due to the short steaming time. In the preparation of black ginseng,

the steaming time is about 2 or 3 h each cycle [14, 36], while the ginseng leaf in this study was steamed for 30 min each cycle when considering that ginseng leaves are much thinner than ginseng root. This might be the same reason why the polar ginsenoside content in my steamed leaves is much higher than the less polar ginsenoside content after nine cycles of steaming treatment. It suggests that ginseng leaf needs to be steamed longer time each cycle (greater than 30 min) to produce black ginseng leaf. Another interesting thing was found that PPT-type ginsenosides losing sugars to yield the aglycones PPT seems easier than that PPD-type ginsenosides degrading to the aglycones PPD. Ginsenoside PPT was detected from SGL3 and its amount continued to increase to 1.66 mg/g at SGL9, while the aglycones PPD cannot be detected during the whole steaming treatment. In black ginseng root, the content of aglycones PPT was found 20 times more abundant than that of aglycones PPD (11.0 mg/g PPT vs 0.5 mg/g PPD) [18]. In addition, several studies found there were abundant of ginsenosides Rk1 and Rg5 [14, 18, 20, 22, 37, 38] and relatively content of ginsenosides Rk2 and Rh3 [20, 38] in the black ginseng or steamed ginseng. The polar PPD-type ginsenosides (Rb1, Rb2, Rc, Rd, etc) are more likely to be converted to ginsenosides Rk1 and Rg5 by dehydration from Rg3 and to ginsenosides Rk2 and Rh3 through losing an H_2O from Rh2 than to be completely degraded to the aglycones PPD during heat process.

In the application of ginseng or ginseng products, the residual pesticide is not only a safety issue but also reduces the value of the medicinal effect. Most ginseng is commercially planted at a high density under artificial shade nets in the field [39]. Pesticides (spray-on ginseng leaves) are frequently applied to control the various fungal diseases that affect ginseng growing at high planting densities [39]. Pesticides can remain in the ginseng plant. In particular, the amount of pesticide residues in ginseng leaves far exceed residues in ginseng roots [40, 41], which greatly affects the consumption of ginseng leaves. This might be the reason why ginseng leaf is not widely used. However, the ginseng leaves used in this study were from New Zealand

forest-grown ginseng, which was grown under the pine forest in an open wild environment as my previous reports [27, 34, 42]. No pesticides were used during the growing stage of ginseng. That means that New Zealand forest-grown ginseng leaves can be widely used to produce ginsenosides without pesticide residues. The steaming process of New Zealand forest-grown ginseng leaf will provide a source of less polar ginsenosides. As the first report of trying to produce black ginseng leaf, this proof-of-concept study used relatively short steaming times at each cycle since the used leaves are thin. I suggest that the steaming time could be appropriately extended to yield more less polar ginsenosides when the ginseng leaves are used as raw material to manufacture less polar ginsenosides.

7.4 Conclusion

This study provides the ginsenoside changes during nine cycles' steaming ginseng leaf (black ginseng leaf) for the first time. The major ginsenosides in the raw ginseng leaf converted into the less polar ginsenosides after nine cycles of steaming New Zealand forest-grown ginseng leaves. It suggests that ginseng leaf can be used as a source to manufacture the less polar ginsenosides by this heating process.

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Chapter Eight

Enrichment of the less polar ginsenosides (Rg3) by post-harvest processing and extraction

This chapter has been published in the following peer-reviewed open access publication.

Chen, W. Xu W. L.; Shi, D. X.; Balan, P.; Popovich, D. G. Enrichment of the less polar ginsenoside (Rg3) from ginseng grown in New Zealand by post-harvest processing and extraction. **Traditional Medicine Research**. 2021,6(4): 39. doi: 10.12032/TMR20210606233.

Previous studies found that NZ-grown ginseng contains abundant ginsenosides and that rare less polar ginsenosides, such as Rg3, exhibit more pharmacological activities than polar ginsenosides, which are the major components in ginseng. This chapter attempted to manipulate the ginsenoside profile of NZ-grown *Panax ginseng* and enrich the less polar ginsenosides through various treatments involving acetic acid (HAc), NaOH, pH, and high temperature. The contents of twenty-three ginsenoside extracts in different treatments were quantified using high-performance liquid chromatography (HPLC). The results showed that 0.5 mol/L HAc treatment can stimulate the degradation of polar ginsenosides to less polar ginsenosides (5.6% Rg3 was accumulated). Furthermore, when ginseng root was treated at 121°C for 100 min under a pH 3.0 HAc aqueous solution, the majority of the polar ginsenosides were converted into less polar ginsenosides. Specifically, 83.5 ± 3.7% of the less polar ginsenosides and 41.0 ± 2.4% of Rg3 were enriched, respectively. In contrast, alkali treatment could not convert the polar ginsenosides into less polar ginsenosides at a mild temperature and had less conversion than the acid treatment at high temperature. This is the first attempt to manipulate the ginsenoside profile of NZ-grown ginseng, and the conditions (high temperature with low pH) can be applied to produce and enrich the less polar ginsenosides (especially Rg3) from ginseng materials.

8.1 Introduction

Ginseng is a famous traditional medicinal herb and has been used in Eastern Asia (especially in China, Korea, and Japan) for thousands of years. Nowadays, ginseng is not just used as a medicinal source; it is increasingly being developed into health care products. It is widely planted, traded, and consumed throughout the world. Ginsenosides are a kind of triterpene saponin unique to ginseng species and are thought to be the most valuable and main bioactive constituents of ginseng [1]. Nearly 200 ginsenosides have been isolated and identified from ginseng plants and related ginseng products [2]. Traditionally, ginsenosides are classified into three main groups based on their skeleton of aglycones: protopanaxadiol type (PPD), protopanaxatriol type (PPT), and oleanane-type ginsenosides. According to the polarity of ginsenosides, the ginsenosides can be divided into two groups: polar ginsenosides and less polar ginsenosides. The major ginsenosides present in raw ginseng materials are the polar ginsenosides, such as ginsenosides Rb1, Rb2, Rb3, Rd, Re, Rg1, Rf, and malonyl ginsenosides. The less polar ginsenosides such as ginsenosides Rg3, Rh2, compound K, Rh3, Rh4, Rk1, Rk2, Rk3, Rg5, and so on, are rare in raw ginseng products and they are usually found in heat-processed ginseng products including red ginseng and black ginseng. Polar ginsenosides are the most abundant ginsenosides; they constitute more than 90% of the total ginsenosides in white ginseng and red ginseng [3].

However, these major ginsenosides contain multi-sugar moieties with larger molecule weight and cannot be fully absorbed by the human body [4]. They have low bioavailability and are not conducive to exerting pharmacological activities [5]. The less polar ginsenosides, such as Rg3 with its smaller molecule weight, can easily pass through the intestinal membrane and exhibits relatively higher biological functions and pharmacological activities compared to the polar ginsenosides [6]. For example, the less polar ginsenosides are superior to the polar ginsenosides in antitumor activity [7]. Moreover, after oral intake of ginseng, digestive enzymes and intestine bacteria

hydrolyze the major polar ginsenosides into the less polar ginsenosides (Rg3, Rh2, compound K, etc.) that are more easily absorbed by the human digestive system; however, the concentration of less polar ginsenosides remain low, even after the conversion process [8, 9] and can vary between people. Therefore, enriching less polar ginsenosides by transforming polar ginsenosides through post-harvest processing will significantly improve the oral bioavailability of ginseng.

Heat-processing is widely used to enrich the less polar ginsenosides. For example, two important commercial products of ginseng — red ginseng and black ginseng — are produced through steam treatment. The amount of the less polar ginsenosides in red ginseng is still very low [10, 11], although the concentration of the less polar ginsenosides has been largely improved in black ginseng [11, 12], the process of making black ginseng needs nine repeats of steaming and drying, which is complex and time-consuming. Also, some other degradation methods have been reported yielding the less polar ginsenosides. These methods mainly include high-temperature degradation [13], acid hydrolysis [14], alkaline treatment [15], enzymatic conversion [16], and microbial degradation [17]. There is no leading method to produce less polar ginsenosides because of the disadvantages during degradation of polar ginsenosides, such as the methods being time-consuming, having a high cost, or having a low conversion rate.

In New Zealand (NZ), *Panax ginseng* (Asian ginseng) and *Panax quinquefolium* (American ginseng) have been grown as a second crop under pine forest for more than 15 years. Fifty and 43 ginsenosides have been identified from various tissues of NZ-grown Asian ginseng and NZ-grown American ginseng, respectively [18]. Among these ginsenosides, ginsenosides Rb1, Rb2, Rd, Re, Rg1, and corresponding malonyl ginsenosides account for the majority (more than 80%) [18]. Moreover, the average content of major ginsenosides in NZ is much higher than ginseng grown in China and Korea [19]. Thus, converting these major ginsenosides into the less polar ginsenosides through post-harvest processing is significant for improving the bioavailability and

pharmacological activity of NZ-grown ginseng. In this chapter, it is hypothesized that the ginsenoside profile of NZ-grown ginseng may be changed and less polar ginsenosides may be enriched through post-harvest processing and extractions. Different extraction conditions such as acidic extraction, alkali extraction, different pH extraction, and high-temperature extraction, were used to manipulate the ginsenoside profiles for enriching the less polar ginsenosides in NZ-grown ginseng extract. These studies will provide theoretical support for the application of NZ-grown ginseng in the food industry and healthcare product industry.

8.2 Materials and methods

8.2.1 Ginseng materials

Dried NZ-grown *P. ginseng* roots (oven-dried by Kiwiseng) were provided by Kiwiseng company (Rotorua, NZ). The ginseng samples were powdered using a CG2B coffee grinder (Breville, Sydney, Australia) and then passed through a 40-mesh sieve before extraction.

8.2.2 Ginsenoside standards, chemicals, and reagents

Twenty-three reference standards of ginsenosides (Rb1, Rb2, Rb3, Rc, Rd, Re, Rf, Rg1, Rg2, 20S-Rg3, 20R-Rg3, Rh1, 20S-Rh2, 20R-Rh2, Rh3, Rh4, Rk2, Rk3, F2, 20S-PPD, 20R-PPD, 20S-PPT, and 20R-PPT) were purchased from Star Ocean Ginseng Ltd (Suzhou, Jiangsu, China). The purities of all reference standards were no less than 98.0%. HPLC-grade methanol (MeOH) and acetonitrile (MeCN) were purchased from Fisher Chemical (Pittsburg, PA, USA). Water (deionized) was obtained from a Milli-Q Ultra-pure water system (Millipore, Billerica, MA, USA). Sodium hydroxide (NaOH), acetic acid (HAc), and *n*-butyl alcohol (*n*-BuOH) were purchased from Fisher Chemical (Pittsburg, PA, USA). The reagents used in this study were of analytical grade.

8.2.3 Preparation of ginseng extracts with acidic treatment

About 1 g ginseng powder was weighed and added to 20 mL 0.5 mol/L HAc aqueous solution. The sample was extracted in an ultrasonic bath at 50 ℃ for 1 h. The supernatant was collected after centrifugation at 1788 xg (Thermo Scientific Multifuge 1S-R Centrifuge, Marshall Scientific, Hampton, NH, USA) for 10 min and the sediments were extracted twice more with 20 mL 0.5 mol/L HAc aqueous solution. The three extracts of supernatant with the same HAc concentration were mixed and adjusted to neutral by NaOH aqueous solution. Then ginsenosides were extracted by liquid-liquid extraction using *n*-BuOH [20]. Briefly, the aqueous layer was extracted three times with 80 mL water-saturated *n*-BuOH. The resulting *n*-BuOH layer was washed three times with 80 mL distilled water to remove impurities. The remaining *n*-BuOH solution was transferred to a tared round-bottom flask, where the *n*-BuOH fraction was evaporated at 60 °C using a rotary vacuum evaporator. After evaporation, the concentrated extract was freeze-dried into powder. Another three experiments were designed and extracted by 0.25, 0.125, and 0 mol/L HAc aqueous solution as above, respectively.

8.2.4 Preparation of ginseng extracts with alkali treatment

The ginseng powder was extracted with 0.4, 0.2, 0.1 and 0 mol/L NaOH aqueous solution as described in section 2.3, except the alkali extraction solution were adjusted to neutral by HAc aqueous solution before liquid-liquid extraction using *n*-BuOH.

8.2.5 Preparation of ginseng extracts with high-temperature treatment

About 1 g ginseng powder was weighed and added to 40 mL deionized water in a 100 mL flask tube. The tube was closed by a filtered paper and extracted in an autoclave (121 ℃, 0.1 Mpa) for 10, 20, and 40 min, respectively. The extracts were filtered using

a filter paper and ginsenosides from the supernatant were extracted by liquid-liquid extraction using *n*-BuOH as described in section 2.3.

8.2.6 Preparation of ginseng extracts with high-temperature under different pH treatment

About 1 g ginseng powder was weighed and added to 40 mL of aqueous solutions with different pHs (pH 3.0, 5.0, 7.0, 9.0, 11.0) in a 100 mL flask tube. HAc was used for adjusting the pH of 3.0 and 5.0; NaOH was used for adjusting the pH of 9.0 and 11.0. The tube was closed by a filtered paper and extracted in an autoclave (121 ℃, 0.1 Mpa) for 100 min. The extracts were filtered using a filter paper and the supernatants were adjusted to neutral by NaOH or HAc aqueous solution. Ginsenoside from the aqueous layer was extracted by liquid-liquid extraction using *n*-BuOH as described in section 2.3.

8.2.7 HPLC analysis

The different ginseng extracts were prepared in about 1 mg/mL of 70% methanol aqueous solution and filtered through a 0.22-micron syringe filter before HPLC injection. The HPLC analysis including validated calibration curves was conducted as my previous methods (Chapter seven). The HPLC instrument used in this chapter was a Shimadzu prominence LC-20A UFLC stack HPLC system (Shimadzu, Kyoto, Japan) equipped with a DGU-20A3 degasser, LC-20AD pump, CTO column oven, SPD-20A detector, and SIL-20A autosampler. A Waters Symmetry Shield RP18 column (4.6 mm × 250 mm, 5 μm) was used. The mobile phase consisted of deionized water (A) and acetonitrile (B). The gradient elution program was as follows: 0-15 min, 20% B; 15-25 min, 20-31% B; 25-35 min, 31-35% B; 35-40 min, 35-36% B; 40-45 min, 36-39% B; 45-75 min, 39-95%; 75-85 min, 95-20% B, 85-90 min, 20%B. The column temperature was set

at 25 °C and the flow rate was 1 mL/min. The sample injection volume was 20 μL and the wavelength of detection was set at 203 nm.

The content of 23 individual ginsenosides in different extracts was quantified according to the 23 ginsenoside standard curves. The percentage of ginsenoside was calculated by the following formula: Percentage of ginsenoside X = content of ginsenoside X / total ginsenoside content × 100%, total ginsenoside content was the sum amount of 23 quantified ginsenosides.

8.2.8 Statistical analysis

Samples were determined three times and data were expressed as mean ± SD. One-way analysis of variance test and Sidak's multiple comparisons test were performed to analyze the experimental data using GraphPad Prism 8 software. Differences were considered significant if p-value < 0.05.

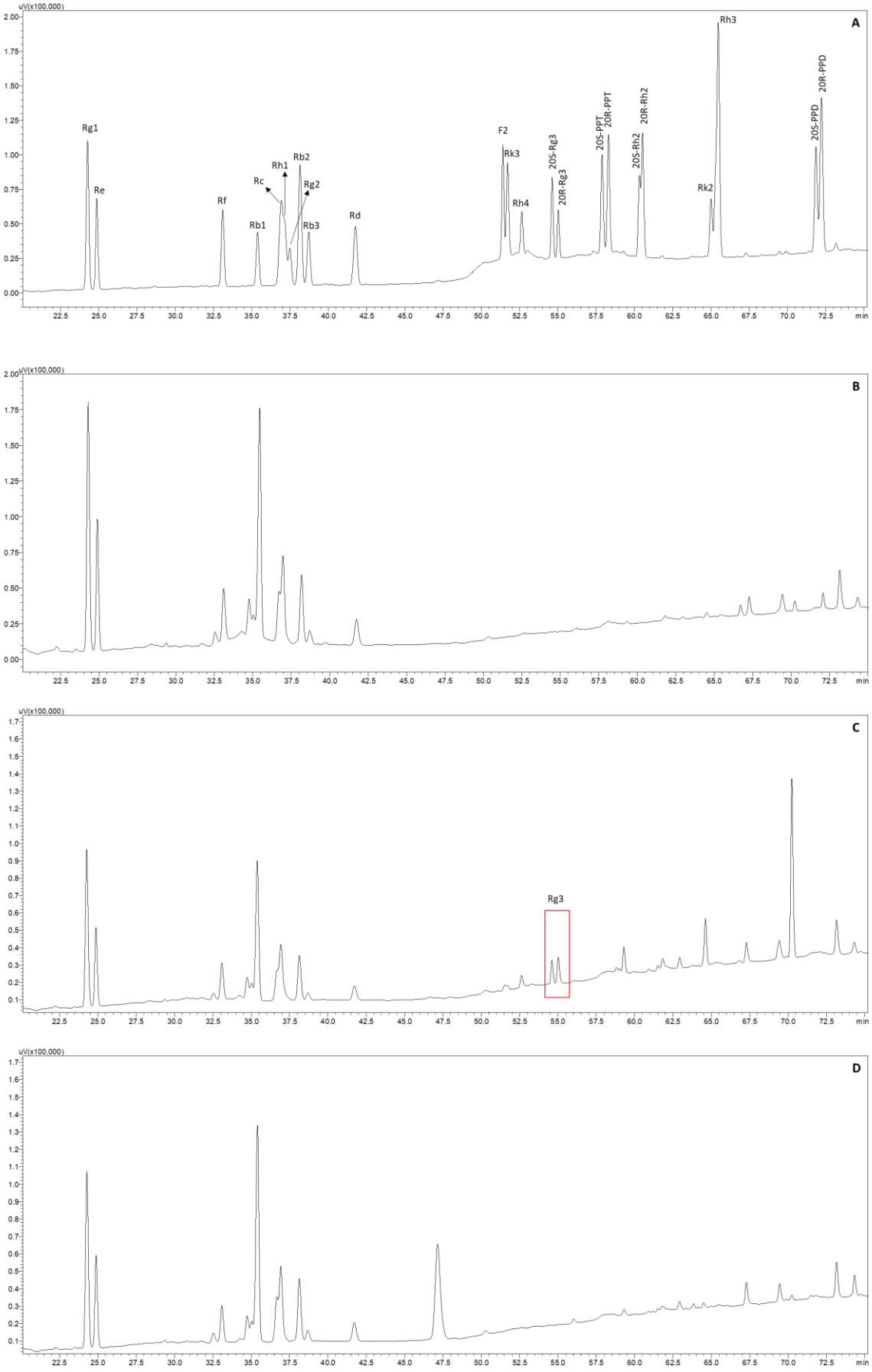
8.3 Results

8.3.1 Effects of different treatments on HPLC profiles of ginsenosides of ginseng root extracts

An HPLC-UV method was used to profile the ginsenoside constituents of different treatments. Twenty-three ginsenoside standards were employed to establish the HPLC method. The ginsenosides were well separated from each other during 20 and 75 min (Figure 8.1A); the peaks of polar ginsenosides appeared between 20 and 45 min and the peaks of less polar ginsenosides were distributed between 50 and 75 min. In the natural ginseng root (untreated, Figure 8.1B), the abundance of peaks came from the polar ginsenosides Rg1, Re, Rf, Rb1, Rc, Rb2, Rb3, and Rd; there were nearly no less-polar ginsenosides in the profile. Compared to the untreated ginseng root (Figure 8.1B), the HPLC profiles of all the treated ginseng root extracts changed to different extents (Figures 8.1C-H).

The profile of ginseng extracted with 0.5 mol/L HAc (Figure 8.1C) was similar to that of untreated ginseng in which ginsenosides appeared at retention time between 20 and 45 min (polar ginsenosides), but was different between 50 and 75 min (less polar ginsenosides). Between 50 and 75 min, the peaks of less polar ginsenosides, especially Rg3, appeared; they were not present in the naturally occurring ginseng root. This indicates that acidic treatment can stimulate less polar ginsenoside transformation, although the abundance of less polar ginsenosides was very low. When ginseng roots were extracted with 0.4 mol/L NaOH (Figure 8.1D), the profile was very similar to that of ginseng roots extracted with water between 20 and 45 min as well as between 50 and 75 min, indicating that alkali treatment did not involve many ginsenosides transformations. When ginseng was extracted at 121 ℃ for 40 min (Figure 8.1E), numbers and individual peak areas of the less polar ginsenosides between 50 and 75 min greatly increased, but the transformation was not complete, there were still certain abundances of polar ginsenosides displayed between 20 and 45 min.

Compared with the profile of ginseng extracted at 121 ℃ for 40 min, when ginseng roots were extracted at 121 ℃ for 100 min with pH 3.0 (Figure 8.1F), pH 7.0 (Figure 8.1G), and pH 11.0 (Figure 8.1H), the profiles changed to a greater extent: numbers and individual peak areas of the less polar ginsenosides between 50 and 75 min further increased, and those of the polar ginsenosides between 20 and 45 min further decreased. Particularly, the peaks of polar ginsenosides Rg1, Re, Rf, Rb1, and Rd disappeared when extracted at 121 ℃ with pH 3.0, suggesting that the polar ginsenosides degraded completely under this condition. However, the peaks of these polar ginsenosides emerged and the peak areas of the polar ginsenosides increased when the pH of the extraction solvent was increased to 7.0 and 11.0 respectively. Thus, compared to the neutral extraction solvent, the acidic solvent could enhance polar ginsenoside transformation into less polar ginsenosides, and the alkali solvent could reduce this transformation.


uV(x100,000)
A
Rg1
Re
Rf
Rb1
Rc
Rh1
Rb2
Rg2
Rb3
Rd
F2
Rk3
Rh4
20S-Rg3
20R-Rg3
20S-PPT
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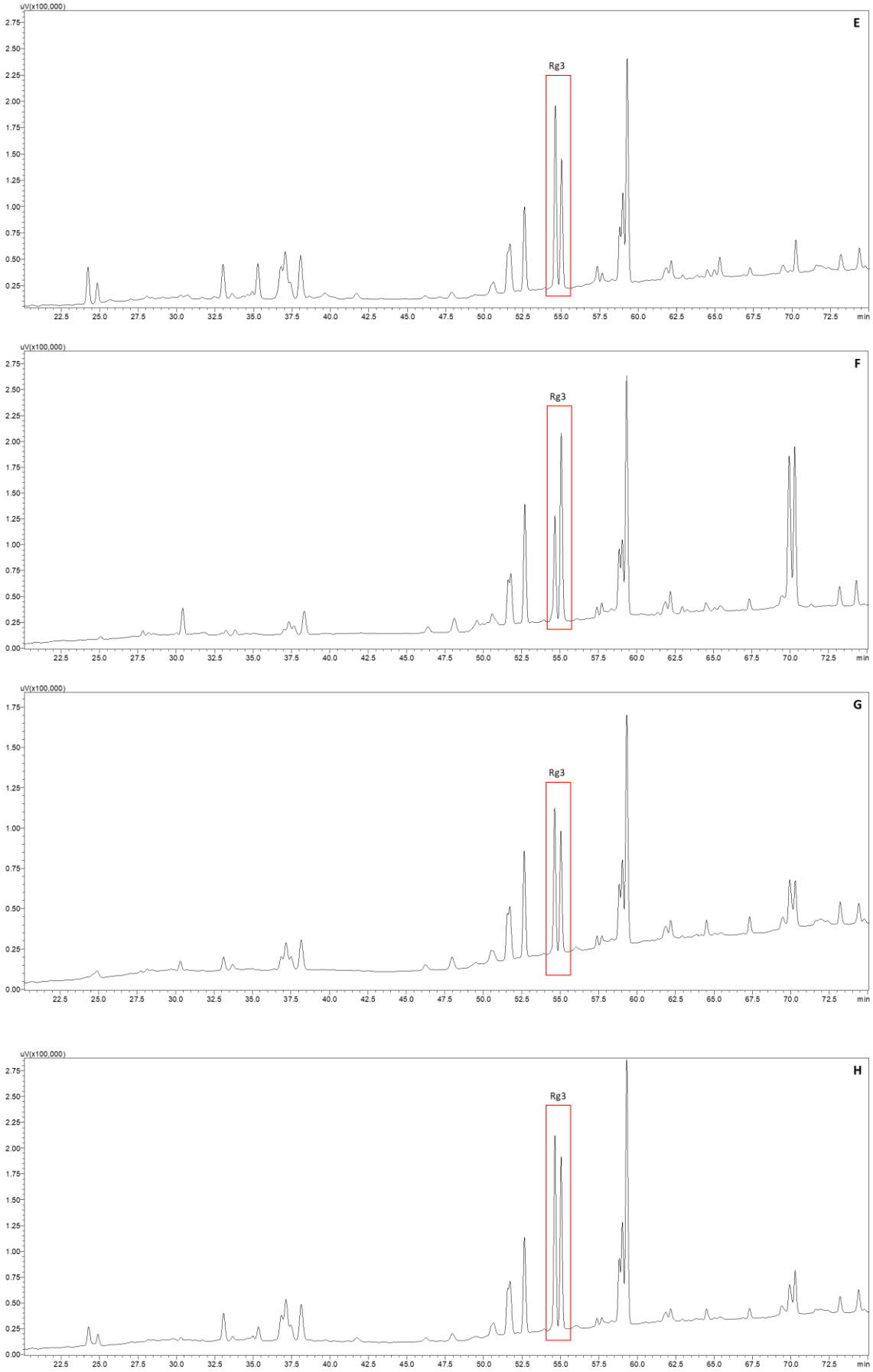
uV(x100,000)
E
Rg3
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Rg3
G
Rg3
H
Rg3
min

Figure 8.1 HPLC chromatograms of ginsenoside profiles at different extraction conditions. A, ginsenoside standard mixture; B, ultrasonically extracted with deionized water at 50 ℃ for 1 h; C, ultrasonically extracted with 0.5 mol/L HAc aqueous solution at 50 ℃ for 1 h; D, ultrasonically extracted with 0.4 mol/L NaOH aqueous solution at 50 ℃ for 1 h; E, extracted with deionized water at 121 ℃ (0.1 Mpa) for 40 min; F, extracted with pH 3.0 aqueous solution at 121 ℃ (0.1 Mpa) for 100 min; G, extracted with pH 7.0 aqueous solution at 121 ℃ (0.1 Mpa) for 100 min; H, extracted with pH 11.0 aqueous solution at 121 ℃ (0.1 Mpa) for 100 min.

8.3.2 Effect of ginsenoside conversion in different extraction conditions

To quantify the contents of ginsenosides in different treated extracts, calibration curves of ginsenoside standards were used to calculate each of the treated extracts (the HPLC validation was carried out in Chapter 7). The contents of individual ginsenosides can be seen in the supplementary data. In order to exhibit the changes to constituents during different treatments, some terms were defined as the following: Sbcd was the sum content of ginsenosides Rb1, Rb2, Rb3, Rc, and Rd, referring to the polar PPD type ginsenosides; Segf was the sum content of ginsenosides Re, Rg1, and Rf, referring to the polar PPT type ginsenosides; the amount of Rg3 was the contents of 20S-Rg3 plus 20R-Rg3; the polar ginsenoside amount was Sbcd plus Segf; the less polar ginsenoside amount was the sum content of ginsenosides Rg3, F2, Rk3, Rh4, Rh2, Rk2, Rh3, PPT, and PPD.

8.3.2.1 The effect of acidic treatment on ginsenosides conversion

To explore the effect of acidic treatment on ginsenoside transformation, ginseng root powder was extracted with 0, 0.125, 0.25, and 0.5 mol/L HAc, respectively. As shown in Figure 8.2A, the percent of polar ginsenosides in acidic extraction significantly ($p < 0.05$) decreased from 95.1 ± 0.1% to 88.6 ± 0.4% when the concentration of HAc

increased from 0 to 0.5 mol/L. Meanwhile, the concentration of Rg3 increased significantly with the increase of HAc concentration (Figure 8.2B). There was nearly no presence of Rg3 when water was used for extraction. However, when ginseng was treated and extracted with 0.5 mol/L HAc, the percentage of Rg3 increased to 5.6 ± 0.0%. These results indicate that acidic treatment can convert polar ginsenosides into less polar ginsenosides like Rg3, and this conversion showed an acid dose-dependent effect within the range of concentrations tested (0-0.5 mol/L). Although acid treatment can improve the transformation, the acid-stimulating conversion is very low, so it will be difficult to meet the needs of industrial production.

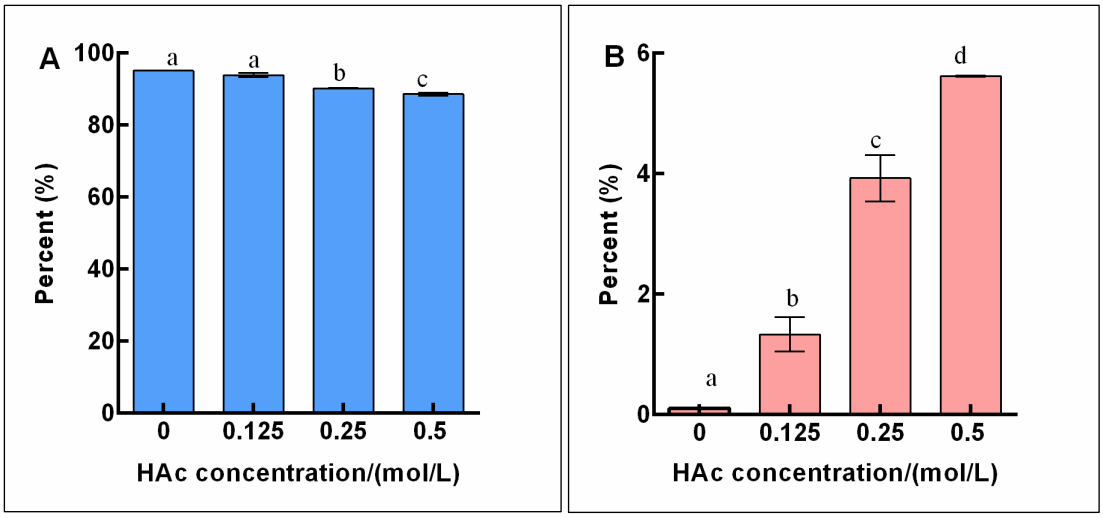

Figure 8.2 The polar ginsenosides percentage (A) and Rg3 percentage (B) in different HAc concentrations treatments. Different lowercase letters at the top of the columns indicate significant differences (p-value < 0.05).

8.3.2.2 The effect of alkali treatment on ginsenoside conversion

Compared to the low conversion induced by acid, the conversion performance of alkali treatment was worse. Ginseng roots were treated and extracted with different concentrations of NaOH, but there was no significant change in the concentrations of the polar ginsenosides, including Sbcd and Segf (Figure 8.3). The less polar

ginsenosides, including Rg3, were rare and hard to quantify in the extracts. These results suggest that few polar ginsenosides (neither Sbcd nor Segf) converted into less polar ginsenoside in the tested alkali solutions. Additionally, there is a big peak at 47 min in the HPLC profile of alkali-treated ginseng extract (Figure 8.1D) which was not detected in other treated ginseng extracts; this may be a byproduct generated during NaOH treatment.

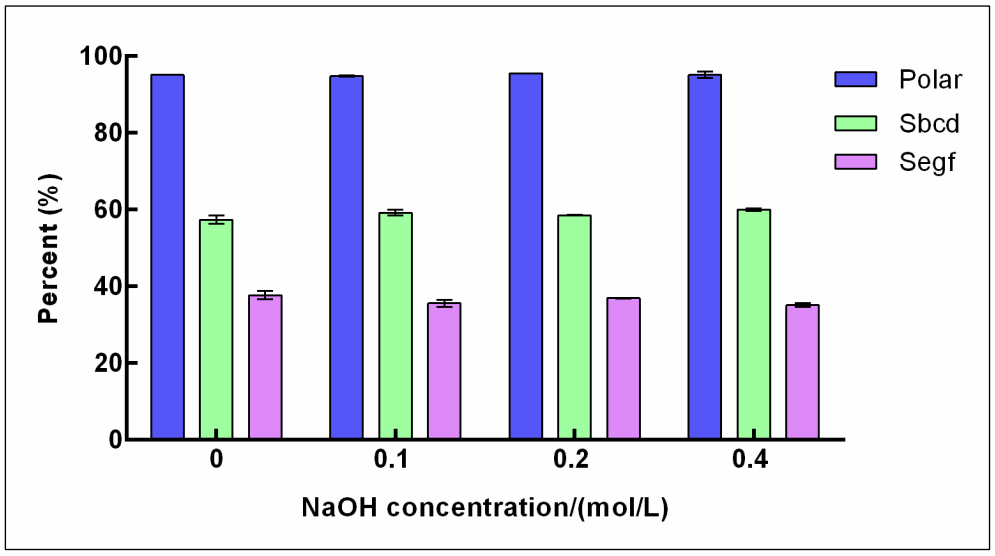

Figure 8.3 The percentage of the polar ginsenosides, Sbcd, and Segf in different NaOH concentration treatments. Sbcd is the sum saponin of ginsenosides Rb1, Rb2, Rb3, Rc, and Rd, referring to the polar PPD type ginsenosides; Segf was the sum saponin of ginsenosides Re, Rg1, and Rf, referring to the polar PPT type ginsenosides; Polar refers to the total polar ginsenosides, which is Sbcd plus Segf.

8.3.2.3 The effect of high-temperature extraction on ginsenoside conversion

Compared to the relatively mild extraction temperature with acid or alkali treatment, high-temperature treatment had a significant effect on the conversion of the polar ginsenosides into the less polar ginsenosides. As shown in Figure 8.4A, there was no significant change in the concentration of Sbcd between 10 min extraction and 20 min extraction. When the extraction lasted for 40 min, the percent of Sbcd decreased significantly from 34.7 ± 0.6% to 25.8 ± 1.4%. For the polar PPT type ginsenosides, the

content of Segf decreased significantly with extraction time. Specifically, Segf account for 34.2 ± 0.2% in the total saponins extracted for 10 min, and then remarkably reduced to 21.8 ± 0.2% at 20 min and 13.5 ± 0.8% at 40 min, respectively (Figure 8.4B). Conversely, the less polar ginsenosides significantly accumulated as the treatment time increased (Figure 8.4C). The percentage of the less polar ginsenosides at 40 min of treatment (54.4 ± 0.3%) is 2.3 times that at 10 min of treatment (23.2 ± 0.4%). Rg3 showed a consistent trend within the concentration of less polar ginsenosides during high-temperature treatment. Rg3 (13.0 ± 0.4%) was produced after incubating at 121 °C for 10 min, while this figure increased significantly by 1.2 times (28.4 ± 0.6%) when the treatment time reached 40 min (Figure 8.4D). Among the less polar ginsenosides, Rg3 accounted for more than half of the total amount of the less polar ginsenosides (55.8% at 10 min, 58.1% at 20 min, and 52.1% at 40 min), which were obtained by degrading from Sbcd. Also, Segf was degraded to form the less polar ginsenosides Rk3 and Rh4, and the proportion of both less polar ginsenosides reached 16.1 ± 0.5% when ginseng was incubated at 121 °C for 40 min.

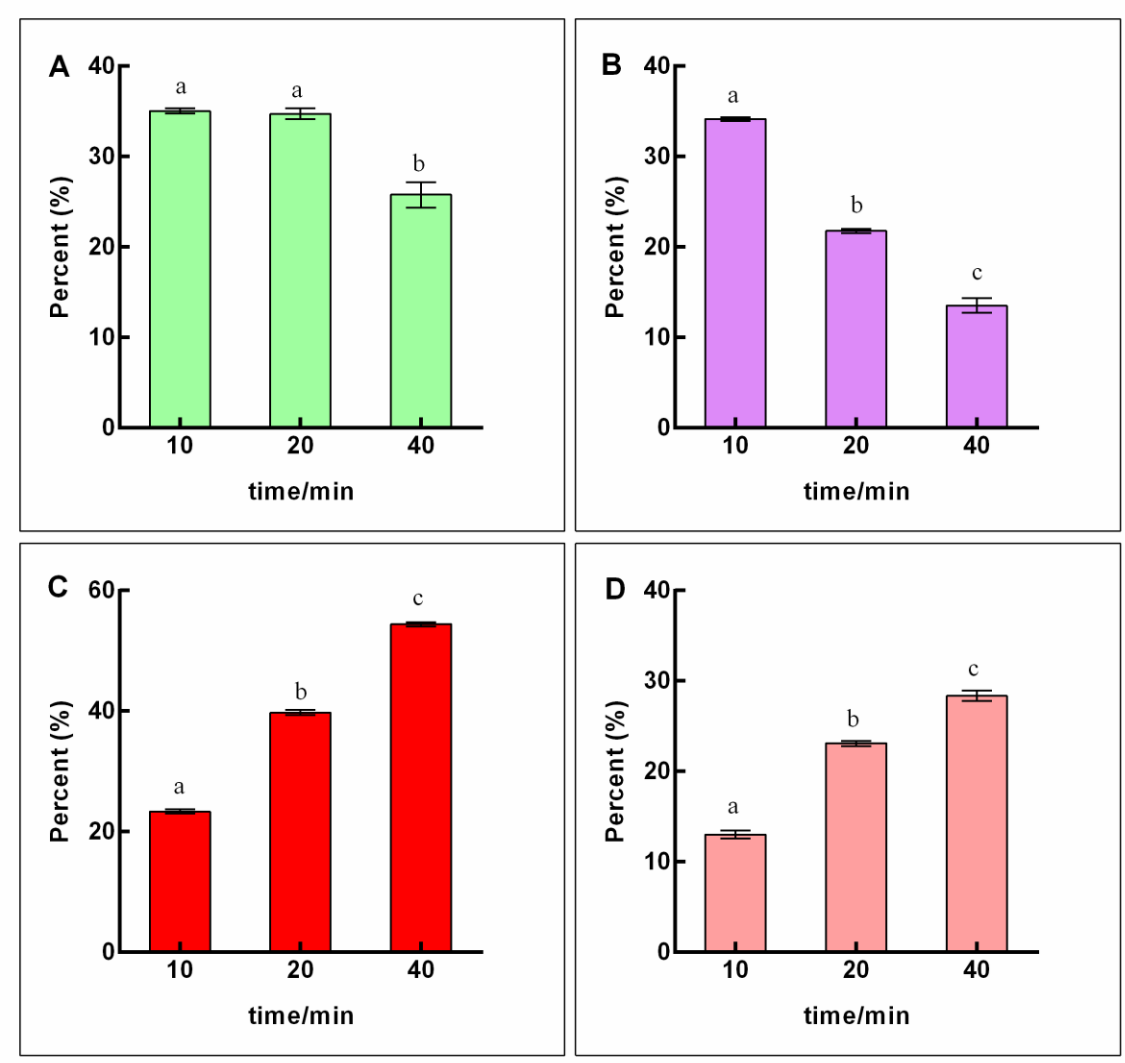

Figure 8.4 The percentage of Sbcd (A), Segf (B), less polar ginsenosides (C), and Rg3 (D) extracted at 121 °C (0.1 MPa) for different times. Sbcd is the sum saponin of ginsenosides Rb1, Rb2, Rb3, Rc, and Rd, referring to the polar PPD type ginsenosides; Segf was the sum saponin of ginsenosides Re, Rg1, and Rf, referring to the polar PPT type ginsenosides; the amount of Rg3 was the contents of 20S-Rg3 plus 20R-Rg3; The less polar ginsenoside amount was the sum content of ginsenosides Rg3, F2, Rk3, Rh4, Rh2, Rk2, Rh3, PPT, and PPD. Different lowercase letters at the top of columns indicate significant differences (p-value < 0.05).

8.3.2.4 The effect of pH on ginsenoside conversion at high temperature

The above results indicate that both acid treatment and high temperature can convert polar ginsenosides into less polar ginsenosides to some extent. However, it was not known whether different pH levels affect the conversion of ginsenosides during high-temperature extraction. Therefore, the ginseng roots were incubated with different pH solutions at 121 ℃ for 100 min. The results are shown in Figure 8.5. When ginseng was incubated with a neutral solution (pH 7.0) at 121 ℃ for 100 min, the polar ginsenosides Sbcd (12.2 ± 2.2%) and Segf (10.2 ± 0.7%) further degraded to form more less-polar ginsenosides (77.6 ± 2.9%), including Rg3 (33.7 ± 2.3%), compared to ginseng treated at 121 ℃ for 40 min. As the pH level of extraction solution increased, the concentration of polar ginsenosides (Sbcd and Segf) increased, while the concentration of less polar ginsenosides (including Rg3) decreased. However, there were no significant changes to the proportions of Sbcd, the less polar ginsenosides, and Rg3 that were incubated in the acidic conditions (pH 3.0 and pH 5.0) or in the alkaline conditions (pH 9.0 and pH 11.0) compared to when they were incubated in a neutral condition (pH 7.0). These figures showed significant differences between ginseng extracted with the pH 3.0 solution and ginseng extracted with the pH 11.0 solution. For example, more than 80% of the less polar ginsenosides were obtained through degrading the polar ginsenosides at pH 3.0 extraction, while about 70% of the less polar ginsenosides were produced at pH 11.0 extraction. These results suggest that high-temperature extraction is an effective method to convert polar ginsenosides into less polar ginsenosides; moreover, a certain acidic extraction solvent can promote this conversion, while an over-basic extraction condition has an opposite effect.

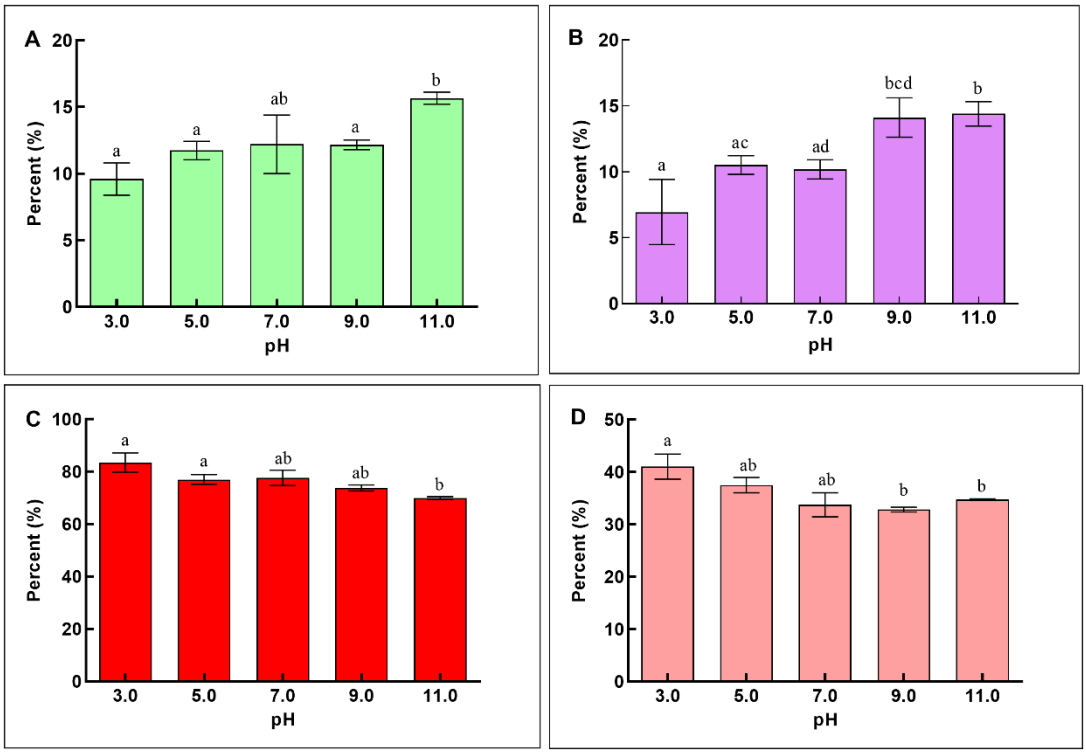

Figure 8.5 The percentage of Sbcd (A), Segf (B), less polar ginsenosides (C), and Rg3 (D) extracted at 121 °C (0.1 Mpa) for 100 min under different pH. Sbcd is the sum saponin of ginsenosides Rb1, Rb2, Rb3, Rc, and Rd, referring to the polar PPD type ginsenosides; Segf was the sum saponin of ginsenosides Re, Rg1, and Rf, referring to the polar PPT type ginsenosides; the amount of Rg3 was the contents of 20S-Rg3 plus 20R-Rg3; The less polar ginsenoside amount was the sum content of ginsenosides Rg3, F2, Rk3, Rh4, Rh2, Rk2, Rh3, PPT, and PPD. Different lowercase letters at the top of columns indicate significant differences (p-value < 0.05).

8.4 Discussion

Acid treatment and alkali treatment are the two most common methods for degrading large molecules. Major polar ginsenosides can convert into rare less polar ginsenosides under different acidic conditions. These acids include formic acid, acetic acid, citric acid, lactic acid, tartaric acid, and hydrochloric acid [21]. In one study, polar

PPD type ginsenosides Rb1, Rb2, and Rc were incubated at 60 °C in different acids including acetic acid, citric acid, lactic acid, tartaric acid, and hydrochloric acid [22]. It was found that hydrochloric acid could increase the yield of ginsenosides Rg3 and $\triangle^{20}$-ginsenosides Rg3 compared to lactic acid and citric acid. Additionally, the transformation of ginsenosides to $\triangle^{20}$-ginsenosides Rg3 by 1% acids was significantly greater than that by 0.1% acids [22]. Another study involved different concentrations of formic acid (0.01%, 0.1%, 0.5%, 2%, and 5%) and found that ginsenosides cannot be converted under neutral conditions, but that less polar ginsenosides can be produced under strong acidic conditions [23]. Less polar PPT type ginsenosides, including prosapogenin of Re and Rg1, were obtained when polar PPT type ginsenosides Re and Rg1 were treated with 0.1N-HCL at 37°C for 120 min [24]. Consistent with these results, this study found that the less polar ginsenosides especially Rg3, could not be detected in the *n*-BuOH extract from deionized water treatment and that its amount increased significantly with the increase of HAc concentrations. However, the yield of Rg3 was not high even when treated with 0.5 mol/L HAc for 3 h (3 times, each 1 h). This may be due to the insufficient treatment time or the mild temperature. As reported in the literature, the pH, time, and temperature are the significant factors that control the conversion of ginsenosides in acid conditions [22]. In the acidic environment, the polar ginsenosides could convert into less polar ginsenosides by losing the sugars at C3, C6, and C20 positions. Additionally, it seems that these ginsenosides are a priority to lose the sugar at the C20 position to form ginsenoside Rg3 (Figure 8.6).

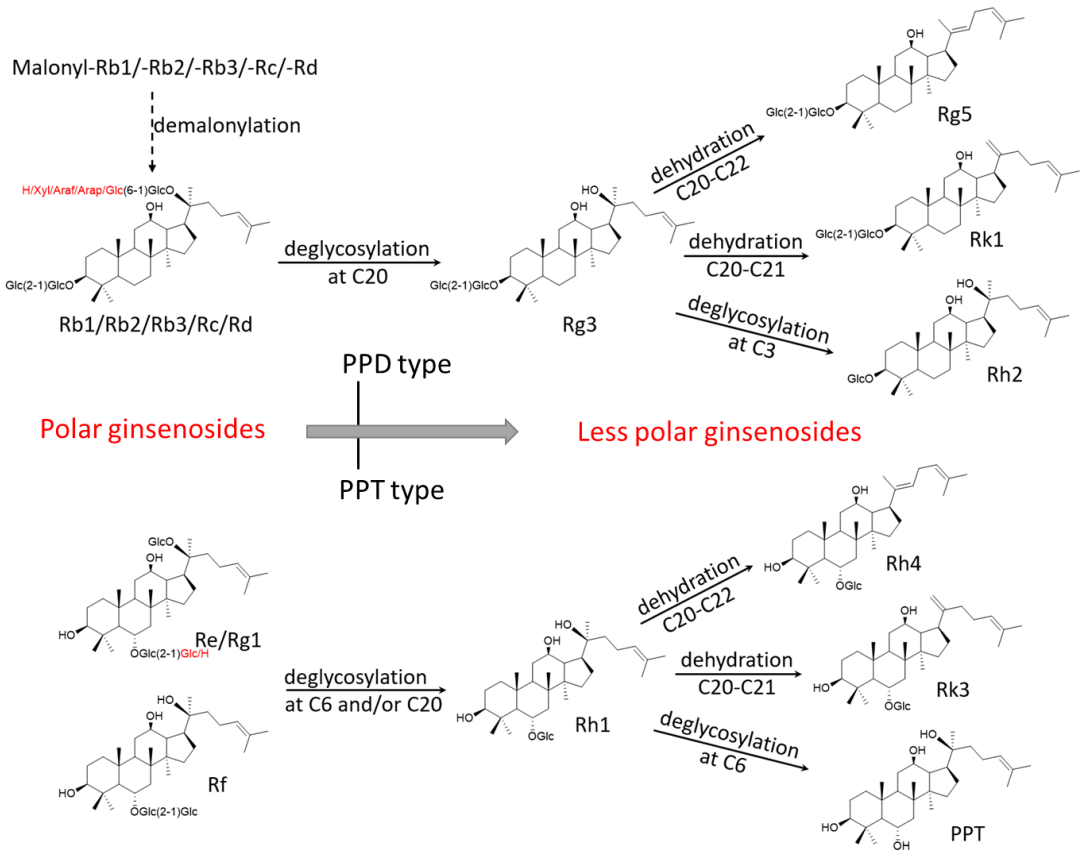

Figure 8.6 The proposed mechanism of polar ginsenosides converting into less polar ginsenosides at high temperature

The literature has reported that ginsenosides can also degrade into less polar ginsenosides under alkali conditions. A new compound (ginsenoside Rh19) and several rare ginsenosides were isolated and identified from ethyl acetate extraction after the total saponins were incubated in a boiling water bath with 2 mol/L NaOH aqueous solution for 8 h [25]. Compared to the aqueous solution, organic solutions such as glycerol, and isoamyl alcohol have a higher yield in the alkaline hydrolysis of ginsenosides. When isoamyl alcohol was used as the solvent, refluxing for 24 h at normal pressure, the yield of less polar PPD was highest under NaOH hydrolysis. However, the yield reduced when the reaction time was over 32 h in isoamyl alcohol [26]. Another study used glycerol as the solvent and explored the factors affecting the alkali hydrolysis of ginsenosides. The results showed that the amount of NaOH, reaction time, reaction temperature, and the volume of glycerol had important effects

on ginsenoside degradation. The optimal degradation conditions were when the weight of NaOH was 2 times that of the total saponins, the ratio of glycerol and total saponins were 15 mL/g, and the reaction was incubated at 235 °C for 200 min [27]. In a further study, the total saponins from the stem and leaf of *Panax quinquefolium* were converted into some new compounds and rare ginsenosides in a 10% NaOH glycerol solution at 235 °C for 2 h [28]. The literature reports that compared to acid hydrolysis, high temperatures can be used to hydrolyze ginsenosides under alkalic conditions; the less polar ginsenosides were not detected at relatively high temperature in this chapter. This suggests that ginsenoside conversion is more difficult under alkali conditions than acidic conditions. This may be related to the presence of large amounts of acidic ginsenosides such as malonyl ginsenosides.

Compared to acidic and alkali treatments, heat-treatment is widely used for manipulating the constituents of ginseng and has been for thousands of years. For example, steaming is a conventional application used to produce red ginseng and black ginseng. Raw ginseng is steamed at 95-100 °C for 2-3 h to produce red ginseng and steamed at 95-100 °C nine times (each for 2-3 h) to produce black ginseng [29]. Many less polar ginsenosides including Rg3, Rk1, and Rg5, which are absent in raw ginseng, can accumulate in high concentrations in black ginseng. Furthermore, these less polar ginsenosides have exhibited various bioactivity of anti-diabetes, anti-inflammation, and anti-cancer [30-32]. However, there is still a relatively high amount of polar ginsenosides (24.1 mg/g accounts for about 33% of the total saponins) in black ginseng [29]. The polar ginsenosides can be further transformed and the less polar ginsenosides can be further enriched when ginseng is treated at a temperature over 100 °C. One study showed that steaming raw ginseng at 120 °C for 2 h remarkably increased the radical-scavenging activity. Ginsenosides Rg3 and Rg5, which are not present in raw ginseng, are the most abundant ginsenosides in the ginseng steamed at 120 °C, accounting for 39% and 19% of all ginsenosides, respectively [13]. From this study, the profile of ginsenosides changed remarkably when the ginseng root was

heated at 121 °C using an autoclave. Although the less polar ginsenosides increased significantly at the first 20 min, especially Rg3 (which increased to 24% of total ginsenosides after steaming for 20 min), the concentration of Sbcd did not decrease at the first 20 min. The previous study found that NZ-grow ginseng contained abundant malonyl ginseng [19] and these malonyl ginsenosides (mal-Rb1, -Rb2, -Rb3, -Rc, -Rd) could degrade to corresponding neutral ginsenosides (Rb1, Rb2, Rb3, Rc, Rd) with high-temperature treatment [33]. This is likely because some malonyl ginsenosides converted into the corresponding neutral ginsenosides Sbcd, which compensated for the reduction of Sbcd, degrading to Rg3 (Figure 8.6).

The above results demonstrate that acidic conditions and high temperature could promote the conversion from polar ginsenosides to less polar ginsenosides. Moreover, the transformation could be further enhanced if ginseng material is treated at a high temperature coupled with an acidic condition. In Liu *et al.*'s study, individual ginsenosides Rb1 and Re were steamed at 120 °C under different pH (4.0 – 7.0) for 4 h. The content of Rb1 and Re decreased significantly and almost degraded completely after steaming for 2 h under acidic conditions (pH 4.0 – 6.0) [34]. This result agrees with these findings that the polar ginsenosides Sbcd and Segf converted into more less-polar ginsenosides at 121 °C under acidic conditions (pH 3.0 – 5.0) than under neutral conditions (pH 7.0). Contrary to these results, the content of ginsenosides Rb1 and Re remained almost constant under neutral conditions even after steaming at 120°C for 4 h [34]. This may be because, during the steaming process of ginseng extract, some of the thermally unstable acidic ginsenosides were degraded to neutral ginsenosides and produced small organic acids, such as malonic acid or acetic acid, which in turn promoted the further conversion of neutral polar ginsenosides to less polar ginsenosides; the organic acids cannot be produced when the neutral polar ginsenosides are steamed alone.

Additionally, the polar saponins can not only undergo deglycosylation at C20 to form the less polar ginsenosides Rg3 and Rh1 but can also further dehydrate to form double

bonds between C20 and C21 or C22. A large amount of Rk3 and Rh4, which are the products of Rh1 through losing an H_2O at C20-21 and C20-22, are quantified from the extract after high-temperature treatment. Besides ginsenoside Rg3, Rg5 and Rk1 are the major components in high temperature treated ginseng products [11, 35], they could be also enriched in this study and the peaks between 57.5 and 60.0 min maybe ginsenosides Rk1 and Rg5 according to their retention times and polarities [34, 36], though they were not quantified due to the shortage of reference standards Rk1 and Rg5. On the other hand, the less polar ginsenosides Rg3 and Rh1 continued to lose glucose at C3 and C6 to form ginsenoside Rh2 and PPT, respectively, except for forming double-bond saponins by dehydration (Figure 8.6). However, little amounts of ginseng Rh2 and PPT were determined in the extract. In contrast, the concentrations of Rk3 and Rh4 were larger compared to the concentrations of Rh2 and PPT. The results suggest that the glycosidic bonds of C3 (PPD type ginsenosides) and C6 (PPT type ginsenosides) are stable for ginsenosides Rg3 and Rh1, and Rg3 is a priority to lose an H_2O at C20 position to form Rg5 and Rk1, rather than losing a glucose at C3 position to form Rh2 at high temperature. Similarly, ginsenoside Rh1 is more likely to lose an H_2O at the C20 position to produce Rk3 and Rh4, instead of deglycosylating at the C6 position to obtain ginsenoside PPT at a high temperature.

In addition to the transformation at low pH, this study also explored the transformation of ginsenosides at high pH with a high temperature. Although the polar ginsenosides could be converted into less polar ginsenoside at 120 °C under alkali conditions (pH 11.0), this conversion efficiency was lower than that under acidic conditions (pH 3.0). The conversion efficiency of ginsenosides Segf under alkaline conditions was also lower than that under neutral conditions (pH 7.0). This suggests that alkali has a negative effect on polar ginsenoside conversion. However, some publications have reported that alkali could enhance the transformation of ginsenosides at high temperature [15, 25, 26]. This transformation is probably caused primarily by high temperatures, rather than by alkali according to these results.

8.5 Conclusion

This study investigated the effects of different treatments (including acid treatment, alkali treatment, different pH treatment, and high-temperature treatment) on the enrichment of less polar ginsenosides from NZ-grown ginseng. My results show that acidic treatment can stimulate the degradation of polar ginsenosides to less polar ginsenosides. Furthermore, the polar ginsenosides can be almost completely converted into less polar ginsenoside in a low pH condition at high temperature. In contrast, alkali treatment may have a negative effect on this conversion. This is the first attempt to manipulate the ginsenoside profile of NZ-grown ginseng, and the conditions (high temperature with low pH) can be applied to produce and enrich the less polar ginsenosides (especially Rg3) from ginseng materials.

8.6 References

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Chapter Nine

The effects of New Zealand grown ginseng fractions on cytokine production from human monocytic THP-1 cells

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Pro-inflammatory cytokines and anti-inflammatory cytokines are important mediators that regulate the inflammatory response in inflammation-related diseases. The aim of this study is to evaluate different New Zealand (NZ)-grown ginseng fractions on the productions of pro-inflammatory and anti-inflammatory cytokines in human monocytic THP-1 cells. Four NZ-grown ginseng fractions, including total ginseng extract (TGE), non-ginsenoside fraction extract (NGE), high-polar ginsenoside fraction extract (HPG), and less-polar ginsenoside fraction extract (LPG), were prepared and the ginsenoside compositions of extracts were analyzed by HPLC using 19 ginsenoside reference standards. The THP-1 cells were pre-treated with different concentrations of TGE, NGE, HPG, and LPG, and were then stimulated with lipopolysaccharide (LPS). The levels of pro-inflammatory cytokines, including tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), interleukin-6 (IL-6), interleukin-8 (IL-8), and anti-inflammatory cytokines, such as interleukin-10 (IL-10), and transforming growth factor beta-1 (TGF-β1), were determined by enzyme-linked immunosorbent assay (ELISA). TGE at 400 μg/mL significantly inhibited LPS-induced TNF-α and IL-6 production. NGE did not show any effects on inflammatory secretion except inhibited IL-6 production at a high dose. Furthermore, LPG displayed a stronger effect than HPG on inhibiting pro-inflammatory cytokine (TNF-α, IL-1β, and IL-6) production. LPG at 100 μg/mL not only significantly inhibited the production of pro-inflammatory cytokines TNF-α, IL-1β, and IL-6, but also enhanced the production of anti-inflammatory cytokine IL-10. NZ-grown ginseng exhibited anti-inflammatory effects *in vitro*, which is mainly attributed to ginsenoside fractions (particularly less-polar ginsenosides) rather than non-saponin fractions.

9.1 Introduction

Inflammation is a protective response of the human body to harmful stimuli, such as microbial infections and chemical toxins [1]. It is a complex biological reaction involving coordinating signaling pathways and producing inflammatory cytokines and chemokines [2]. Cytokines are small extracellular proteins that act as mediators in the immune response. They regulate the inflammatory process through highly complex pathways [3]. It has been reported that chronic low-grade inflammation and invigoration of the innate immune system are closely related to the pathogenesis of chronic diseases, such as diabetes [4], ageing [5], cancer [6], and cardiovascular disease [7]. Pro-inflammatory cytokines including interleukin-1 beta (IL-1β), interleukin-6 (IL-6), interleukin-8 (IL-8), and tumor necrosis factor-alpha (TNF-α), and anti-inflammatory cytokines such as interleukin-10 (IL-10), and transforming growth factor beta-1 (TGF-β1), have been reported to be involved in the events of inflammation-related diseases [8-10].

Ginseng is a popular Chinese herb. Traditionally, it is used for enhancing immunity, restoring homeostasis, treating illness, and helping longevity [11]. Ginsenosides, a type of triterpene saponins, are the unique and mainly active components of ginseng. Nearly two hundreds ginsenosides have been identified from ginseng plants and products [12]. Recently, ginsenosides have been reported to show various beneficial effects, including anti-ageing [13], anti-fatigue [14], anti-cancer [15], anti-diabetes [16], and anti-inflammatory effects[17]. In New Zealand (NZ), *Panax ginseng* has been grown under the open-wild pine forest with the volcanic pumice soil and high-intensity UV rays. From our previous studies, NZ-grown ginseng contains abundant ginsenosides; the average content of total ginsenosides in NZ is much higher than that in other countries [18]. However, there are no publications about any bioactive effects of NZ-grown ginseng. Whether NZ-grown ginseng affects the production of pro-inflammatory or anti-inflammatory cytokines is not known. It is hypothesized that

ginsenoside extracted from NZ-grown *P. ginseng* may be the active component of anti-inflammatory effect.

The human monocytic THP cell line is derived from the peripheral blood of a 1-year-old male patient with acute monocytic leukemia [19]. This cell line has been widely used as a model to study immune response and functions. Usually, lipopolysaccharide (LPS) is used as an initiator to activate immune cells *in vitro* and *in vivo* studies [20]. In this chapter, four NZ-grown ginseng fractions, including total ginseng extract (TGE), non-ginsenoside fraction extract (NGE), high-polar ginsenoside fraction extract (HPG), and less-polar ginsenoside fraction extract (LPG), were prepared and analyzed by HPLC. LPS-induced THP-1 cells were used to evaluate the effects of TGE, NGE, HPG, and LPG on pro-inflammatory and anti-inflammatory cytokine productions. The results show that the ginsenoside fraction, rather than the non-ginsenoside fraction, exhibits the anti-inflammatory activity. Furthermore, LPG displayed a stronger effect than HPG on inhibiting pro-inflammatory cytokine (TNF-α, IL-1β, and IL-6) production. Particularly, 100 μg/mL LPG not only significantly inhibited the production of pro-inflammatory cytokines TNF-α, IL-1β, and IL-6, but also enhanced the production of anti-inflammatory cytokine IL-10. This study provides a preliminary explanation that LPG from NZ-grown ginseng can regulate the production of pro- and anti-inflammatory cytokines, suggesting LPG may have some potential to treat inflammation-related diseases. This needs to be further investigated in the future.

9.2 Materials and methods

9.2.1 Chemicals

Twenty-one reference standards of ginsenosides, including Rg1, Re, Rf, Rb1, Rc, Rg2, Rh1, Rb2, Rb3, Rd, F2, Rk3, Rh4, 20(S)-Rg3, 20(R)-Rg3, 20(S)-PPT, 20(R)-PPT, 20(S)-

Rh2, 20(R)-Rh2, Rk2, and Rh3, were purchased from Star Ocean Ginseng Ltd (Suzhou, Jiangsu, China). The purities of all reference standards were no less than 98.0%. RPMI 1640 medium, fetal bovine serum (FBS), penicillin, and streptomycin were purchased from Gibco Life Technologies (Grand Island, NY, USA). The LPS (Escherichia coli 0127: B8), dimethyl sulfoxide (DMSO), 2-mercaptoethanol, were purchased from Sigma-Aldrich (St Louis, MO, USA). Enzyme-linked immunosorbent assay (ELISA) kits for human TNF-α, IL-1β, IL-6, IL-8, IL-10, and TGF-β1 were purchased from Thermo Fisher Scientific (Waltham, MA, USA). Acetonitrile (HPLC grade) and *n*-butanol were purchased from Merck Life Science (Auckland, New Zealand). Water (deionized) was obtained from a Milli-Q Ultra-pure water system (Millipore, Billerica, MA, USA).

9.2.2 Ginseng material

Ginseng extract powder (whole NZ-grown *Panax ginseng* plants were collected in May 2019 from pine forest around Taupo, NZ. After air-dried, the samples were extracted through water and the extract was filtered, concentrated, and dried into powder) was provided by Kiwiseng Co. Ltd (Rotorua, NZ) and marked as TGE (total ginseng extract).

9.2.3 Cells

The human monocytic THP-1 cell line was purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA).

9.2.4 Ginseng fractions preparation

TGE (4.85 g) was dissolved in 40 mL water. Then ginsenosides were extracted by liquid-liquid extraction using *n*-butanol (as in the previous chapter). The ginseng extract aqueous solution was extracted three times with 30 mL water-saturated n-butanol. The *n*-butanol layer fraction, which consists of high-polar ginsenosides

(HPG), and the water layer fraction (non-ginsenoside extract, NGE) were evaporated at 60 °C using a rotary vacuum evaporator, respectively.

TGE (9.76 g) was dissolved in 100 mL water and steamed in an autoclave (121 °C, 0.1 Mpa) for 100 min. Then the high temperature treated ginseng aqueous solution was extracted three times with 80 mL water-saturated n-butanol. The *n*-butanol layer fraction, containing less-polar ginsenosides (LPG), and the water layer fraction (NGE) were evaporated at 60 °C using a rotary vacuum evaporator, respectively. The concentrated extracts were freeze-dried into powder. Finally, 1.32 g HPG, 2.25 g LPG, and 9.49 g NGE (3.01 g N-W extract and 6.48 g H-W extract) were obtained and stored at – 20 °C before use. The 4 mg/mL of TGE, NGE, HPG, and LPG stock solutions were prepared using the complete RPMI 1640 medium and passed through 0.22 μm filter for sterilization. The stock solutions were stored at 4 °C and used within a week. The samples were diluted to needed doses using the complete RPMI 1640 medium before use. The flow chart of ginseng extract fraction preparation is shown in Figure 9.1.

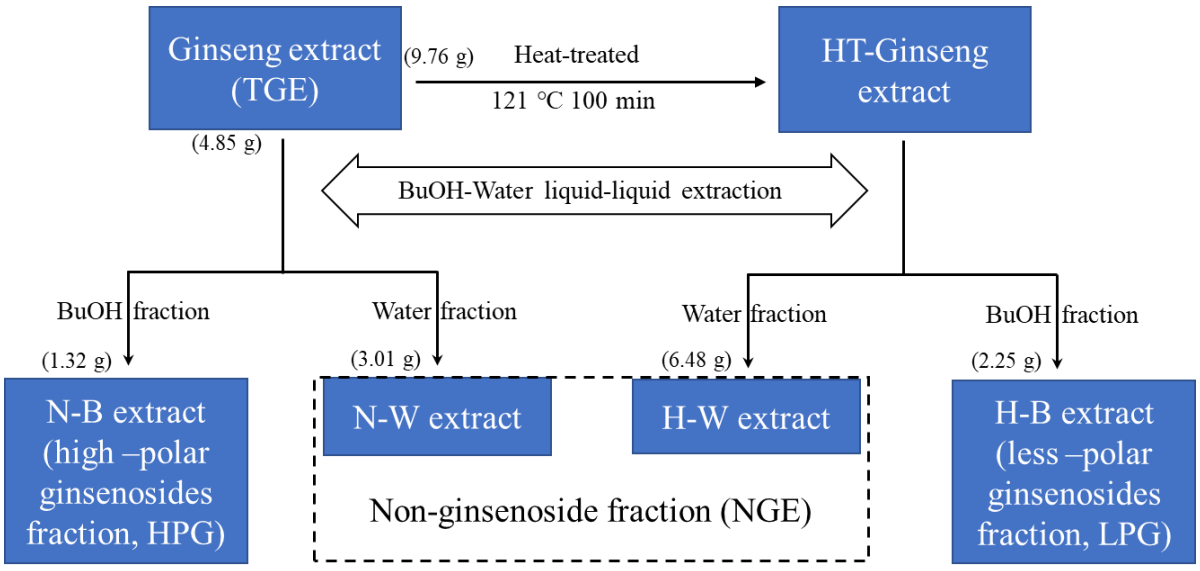


Figure 9.1 The flow chart of preparation of ginseng extract fractions

9.2.5 HPLC analysis

The different ginseng extracts (TGE, HPG, LPG, and NGE) and twenty-one ginsenoside reference standards were prepared about 1 mg/mL using deionized water or 70% methanol, and then filtered through a 0.22-mm syringe filter before HPLC injection. The HPLC analysis, including validated calibration curves, was conducted as a previous study [21]. The HPLC instrument used in this chapter was a Shimadzu Prominence LC-20A UFLC Stack HPLC system (Shimadzu, Kyoto, Japan) equipped with a DGU-20A3 degasser, LC-20AD pump, CTO column oven, SPD-20A detector, and SIL-20A autosampler. A Waters Symmetry Shield RP18 column (4.6 mm × 250 mm, 5 μm) was used. The mobile phase consisted of deionized water (A) and acetonitrile (B). The gradient elution program was as follows: 0–15 min, 20% B; 15–25 min, 20–31% B; 25–35 min, 31–35% B; 35–40 min, 35–36% B; 40–45 min, 36–39% B; 45–75 min, 39–95%; 75–85 min, 95–20% B, 85–90 min, 20% B. The column temperature was set at 25 °C and the flow rate was 1 mL/min. The sample injection volume was 20 μL and the wavelength of detection was set at 203 nm.

9.2.6 THP-1 cell culture and treatments

The THP-1 cells were cultured in RPMI 1640 medium containing 10% (v/v) FBS, 1% (v/v) antibiotics (100 U/mL penicillin and 100 μg/mL streptomycin), and 0.05 mM 2-mercaptoethanol in a humidified incubator at 37 °C containing 5% CO_2. Cells were subcultured every 3 days and maintained at a concentration between 1×10^5 and 1×10^6 cells/mL. The volume of 900 μL THP-1 cells (5×10^5/mL) was added into 24-well flat-bottomed plates and incubated for 24 h. After 24 h culturation, 100 μL TGE (400, and 4000 μg/mL), NGE (100, and 1000 μg/mL), HPG (10, 100, and 1000 μg/mL), LPG (10, 100, and 1000 μg/mL), or vehicle (complete RPMI 1640 medium) were separately added into wells, which make the ginseng extracts to the final concentrations of TGE (40, and 400 μg/mL), NGE (10, and 100 μg/mL), HPG (1, 10, and 100 μg/mL), and LPG (1, 10, and 100 μg/mL). The plates were incubated for 2 h, and then 10 μL 20 μg/mL

LPS was added into wells. The plates were transferred into a humidified incubator at 37 °C containing 5% CO_2. The cell-free supernatants were collected 24 h after LPS simulation [22].

9.2.7 Cell viability

Cell viability was evaluated using the 3-(4,5-dimethylthiazol-2-yl)- 2,5-diphenyltetrazolium bromide (MTT) assay [23]. Briefly, serial dilutions of ginseng extracts (TGE, NGE, HPG, and LPG) were added to each well of a 96-microwell plate. THP-1 cells were seeded to each well to a final density of 5×10^5 cells/mL. Control wells contained THP-1 cells and culture medium, but no tested extracts. Cells were incubated for 48, and 72 h before adding MTT (0.5 mg/mL) and incubated in the dark for 4 h. Formazan crystals were solubilized for 30 min with DMSO. Optical density measurements were recorded at 550 nm absorbance in a microplate reader for the 96-microwell plate.

9.2.8 Enzyme-linked immunosorbent assay (ELISA)

The concentrations of TNF-α, IL-1β, IL-6, IL-8, IL-10, and TGF-β1 in supernatants were determined by ELISA kits according to the manufacturer's instructions. A microplate reader was used to detect the optical density at 450 nm.

9.2.9 Statistical analysis

Statistical analyses were performed using GraphPad Prism 8 for Windows (GraphPad Software, Version 8.01, La Jolla, CA, USA). All data are expressed as means ± SD (n = 3). An unpaired t-test was used to analyze the experimental data. Differences were considered statistically significant at a p-value < 0.05.

9.3 Results

9.3.1 Ginsenoside profiles of ginseng extract fractions

The ginsenosides were separated within 70 min by the RP-C18 column using HPLC. The peaks of individual ginsenosides appeared between 20 min and 70 min in HPLC chromatograms. The ginsenoside profiles of TGE and different fraction extracts are shown in Figure 9.2. The main compositions of TGE are high-polar ginsenosides, such as ginsenosides Rg1, Re, Rc, and Rd (Figure 9.2B). Compared to the profile of TGE, the ginsenoside composition of HPE did not change after *n*-BuOH liquid-liquid extraction, they displayed the similar ginsenoside peaks, but the height and area of peaks increased after *n*-BuOH liquid-liquid extraction (Figure 9.2C). In the water fraction extract, no ginsenoside was detected (Figure 9.2E). It suggests that the ginsenoside composition and non-ginsenoside composition was effectively separated through *n*-BuOH liquid-liquid extraction. From the HPLC profile of LPG, we can see the peaks of high-polar ginsenosides (including Rg1, Re, Rb1) were disappeared, on the contrary, some peaks of less-polar ginsenosides (such as Rg3, Rk3, Rh4) emerged during the 50-70 min (Figure 9.2D). This means high-polar ginsenosides were converted into less-polar ginsenosides through high temperature treatment, which is also reported in pervious Chapter 8.

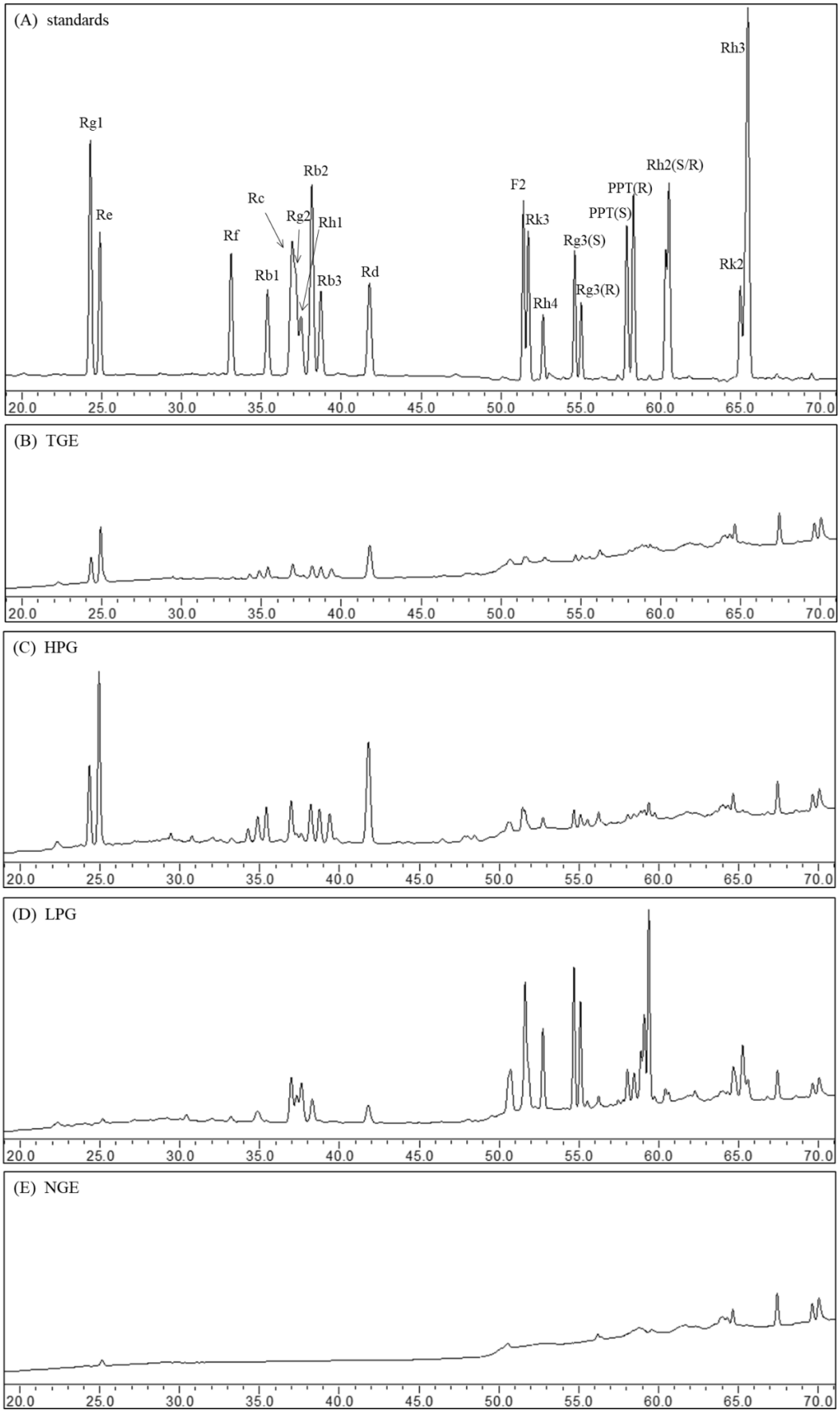


Figure 9.2 The HPLC chromatograms of ginsenoside standards (A), TGE (B), HPG (C), LPG (D), and NGE (E).

9.3.2 Quantification of ginsenosides in the ginseng fraction extracts

The concentrations of ginsenosides in different ginseng fraction extracts were quantified through HPLC (the method was validated in Chapter 7). The contents of 19 individual ginsenosides and the total ginsenosides are listed in Table 9.1.

Table 9.1 Ginsenoside content (mg/g) in the TGE, HPG, and LPG extracts.

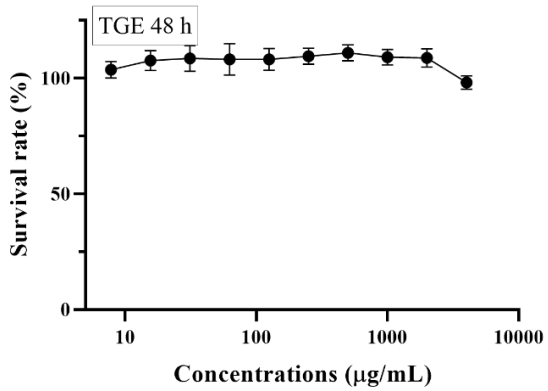
Ginsenosides	Ginsenoside content (mg/g) of ginseng extracts		
	TGE	HPG	LPG
Rg1	12.1 ± 1.6	45.0 ± 0.5	
Re	29.1 ± 2.2	97.3 ± 2.1	
Rf	1.1 ± 0.7	5.4 ± 0.7	
Rb1	7.5 ± 0.7	25.4 ± 1.9	
Rc	10.6 ± 0.6	37.4 ± 1.0	
Rg2			26.8 ± 1.2
Rh1			29.7 ± 1.0
Rb2	10.1 ± 1.4	35.9 ± 0.9	
Rb3	8.8 ± 1.3	30.0 ± 0.1	
Rd	26.2 ± 3.6	83.6 ± 10.3	18.1 ± 1.8
F2			74.3 ± 2.2
Rk3			32.0 ± 2.2
Rh4			53.0 ± 0.4
20(S)-Rg3			52.0 ± 0.1
20(R)-Rg3			65.5 ± 0.1
20(S)-PPT			15.6 ± 0.0
20(S)-Rh2			4.4 ± 0.4
Rk2			43.3 ± 0.3
Rh3			10.2 ± 0.2
Total	105.4 ± 12.0	360.0 ± 3.2	424.9 ± 2.6

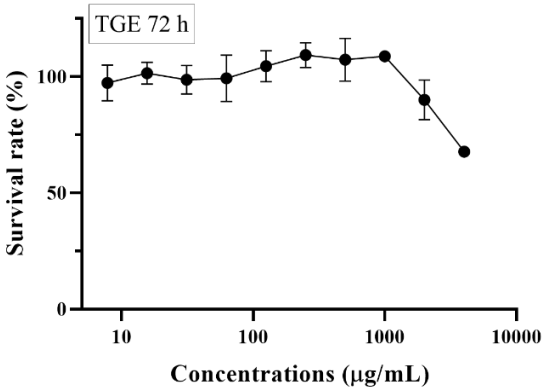
TGE and HPG contain the same compounds, and ginsenosides Re and Rd are the two most major compounds in both extracts, while HPG has much higher concentration of ginsenosides compared to TGE. The total content of ginsenosides in HPG (360.0 ±

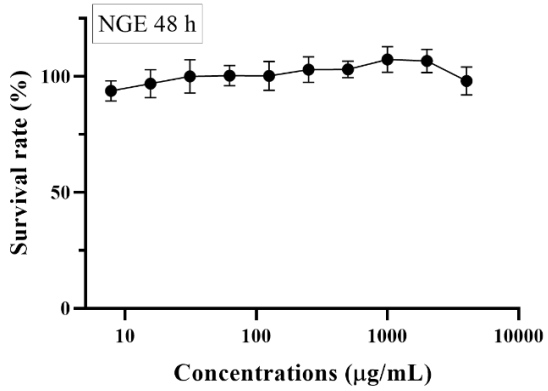
3.2 mg/g) increased 2.4 times after removing non-ginsenoside components from TGE (105.4 ± 12.0 mg/g) through liquid-liquid extraction. In LPG extract, the common high-polar ginsenosides such as Rb1, Rg1, Re, and Rc were not detected, majority of components are less-polar ginsenosides, its contents reached to 424.9 ± 2.6 mg/g. Ginsenoside Rg3 (20S/R Rg3), which is regarded as the unique active compound in ginseng products [24, 25], is enriched and accounts for 27.6% in LPG extract.

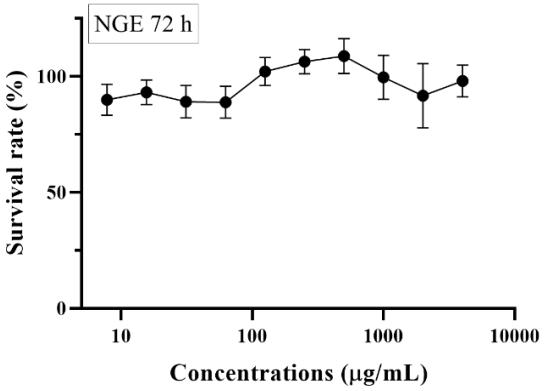
9.3.3 The effect of different ginseng fraction extracts on cell viability

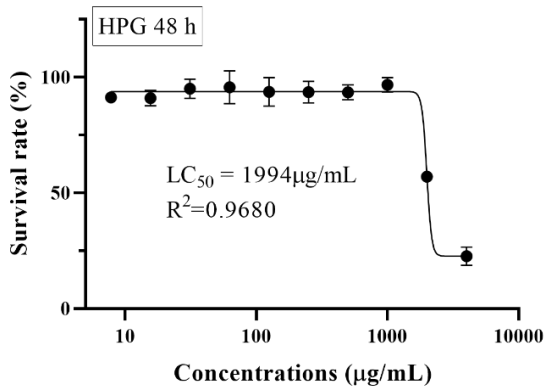
To evaluate the cytotoxic effect of fractioned ginseng extracts on Thp-1 cells, MTT assay was carried out, and dose-response curves are shown in Figure 9.3. Horizontal axis is concentrations of tested extract, and vertical axis is the survival rate of cells treated with different concentrations of tested extract for 48 h or 72 h. There is no obviously toxic effect of TGE and NGE on cell growth below 1000 μg/mL. At low concentrations, there is no obvious effect on cell growth, but when concentrations increased to some extent, HPG and LPG showed a dose-dependent response, and decreased cell growth as concentrations increased. A best fit curve (four parameter curve) was drawn. The LC_{50} (lethal concentration 50%) values were determined by plotting cell viabilities against concentrations. The LC_{50} of HPG for 48 h and 72 h is 1994 μg/mL ($r^2 = 0.9680$) and 1396 μg/mL ($r^2 = 0.9439$), respectively. While the LC_{50} of LPG for 48 h and 72 h is 713.1 μg/mL ($r^2 = 0.9833$) and 620.4 μg/mL ($r^2 = 0.9405$), respectively. The results show that the toxicity to Thp-1 cells increased when high-polar ginsenosides converted to less-polar ginsenosides. Cells seem more sensitive to the less-polar ginsenosides. Additionally, Figure 9.3 shows that the concentrations below 250 μg/mL of LPG and HPG, and dose under 1000 μg/mL of TGE and NGE are no toxic to Thp-1 cells treated for 48 h. Thus, advisable concentrations (1, 10, and 100 μg/mL for HPG and LPG; 10, and 100 μg/mL for NGE; 40, and 400 μg/mL for TGE) were used in the further assay.

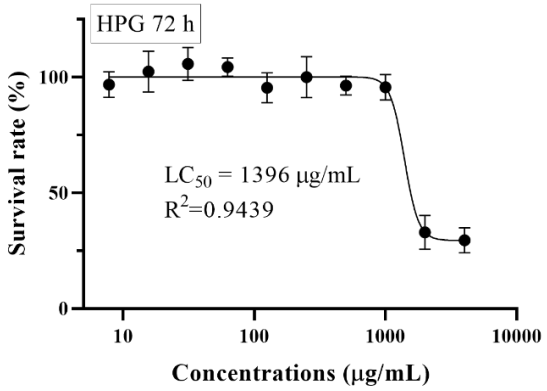

TGE 48 h
Survival rate (%)
0
50
100
10
100
1000
10000
Concentrations (μg/mL)

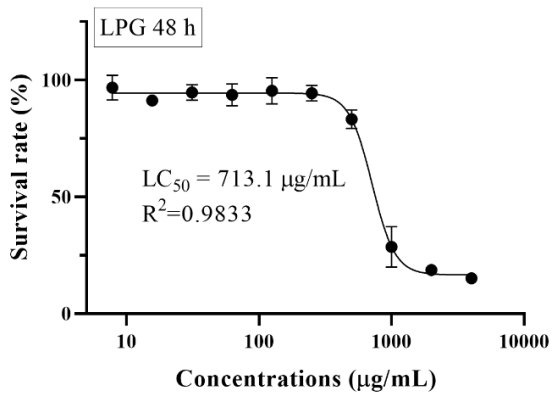

TGE 72 h
Survival rate (%)
0
50
100
10
100
1000
10000
Concentrations (μg/mL)


NGE 48 h
Survival rate (%)
0
50
100
10
100
1000
10000
Concentrations (μg/mL)


NGE 72 h
Survival rate (%)
0
50
100
10
100
1000
10000
Concentrations (μg/mL)


HPG 48 h
LC50 = 1994μg/mL
R2=0.9680
Survival rate (%)
0
50
100
10
100
1000
10000
Concentrations (μg/mL)


HPG 72 h
LC50 = 1396 μg/mL
R2=0.9439
Survival rate (%)
0
50
100
10
100
1000
10000
Concentrations (μg/mL)


LPG 48 h
LC50 = 713.1 μg/mL
R2=0.9833
Survival rate (%)
0
50
100
10
100
1000
10000
Concentrations (μg/mL)

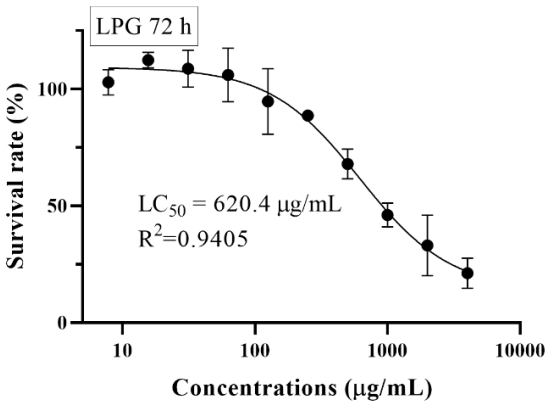

LPG 72 h
LC50 = 620.4 μg/mL
R2=0.9405
Survival rate (%)
0
50
100
10
100
1000
10000
Concentrations (μg/mL)

Figure 9.3 MTT dose-response relationship of ginseng fraction extracts after 48 and 72 h incubation with THP-1 cells assessed by an MTT viability assay. Values are expressed as a percentage of untreated control cells (mean ± SD).

9.3.4 Effects of total ginsenoside extract (TGE) on cytokine production

Based on the concentration of ginsenosides in TGE and MTT assay, 40 and 400 μg/mL TGE were used to evaluate the effect of total ginseng extract on cytokine production. TGE significantly inhibited LPS-stimulated TNF-α production from 1600 ± 38 pg/mL to 1180 ± 31 pg/mL at the concentration of 40 μg/mL, and to 978 ± 14 pg/mL at the centration of 400 μg/mL, respectively. There was an inhibited trend for IL-1β and IL-6 production in TGE treatment groups compared to control groups, but no statistical significance except IL-6 secretion treated with 400 μg/mL of TGE. There is no significant difference in other cytokine productions whether it is treated with TGE or not (Figure 9.4).

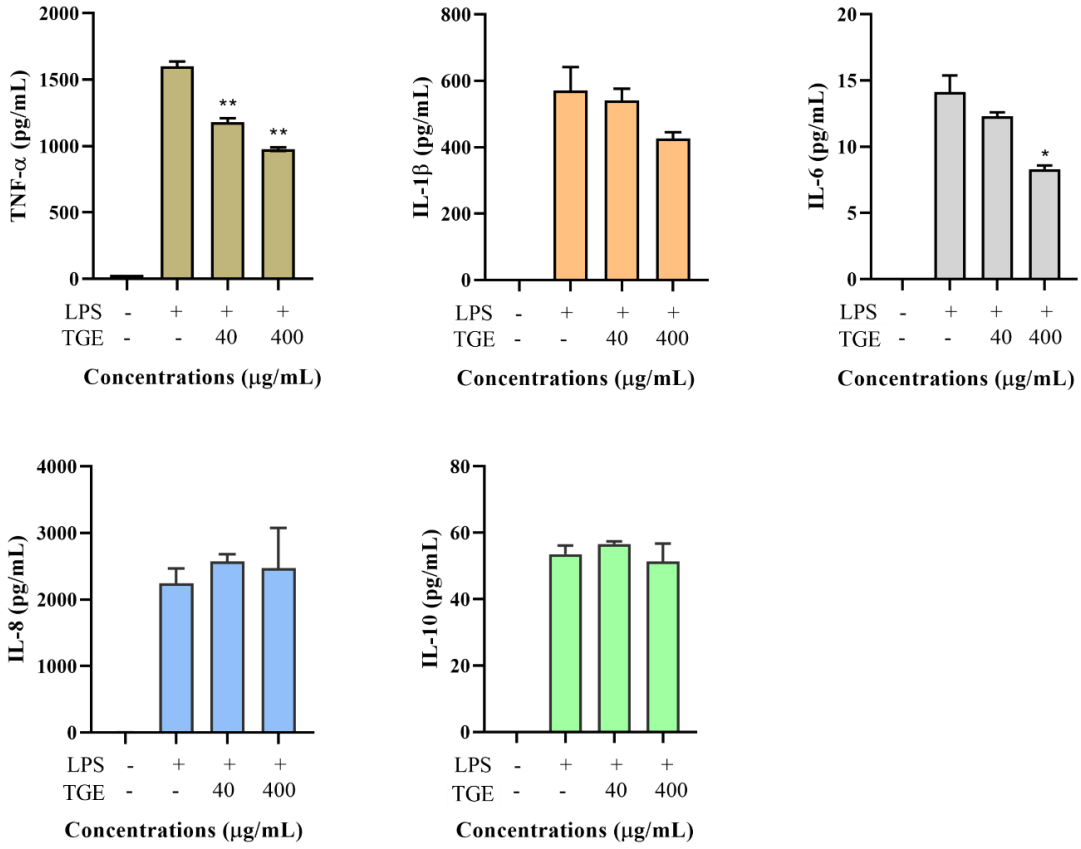


Figure 9.4 Effect of TGE on LPS-induced TNF-α, IL-1β, IL-6, IL-8, and IL-10 productions in the THP-1 cell culture medium. Data are shown as the mean ± SD of three repetitions. * $p < 0.05$, and ** $p < 0.01$ for comparison of LPS stimulation with and without ginseng extract pre-treatment.

9.3.5 Effects of non-ginsenoside fraction extract (NGE) on cytokine production

Total ginseng extract contains ginsenoside and non-ginsenoside fractions. We found the non-ginsenoside fraction accounts for about three-fourths of the total ginseng extract through separated process (see method section). However, it may not be the active ingredient of ginseng although its amount is large. As showed in Figure 9.5, NGE, the major component of ginseng extract, has no significant effect on the

production of various cytokines except for IL-6. The total ginseng extract can decrease TNF-α production by 26.3% - 38.9% ($p < 0.05$), but NGE, fractioned from the total ginseng extract, did not show any obvious effect on TNF-α production.

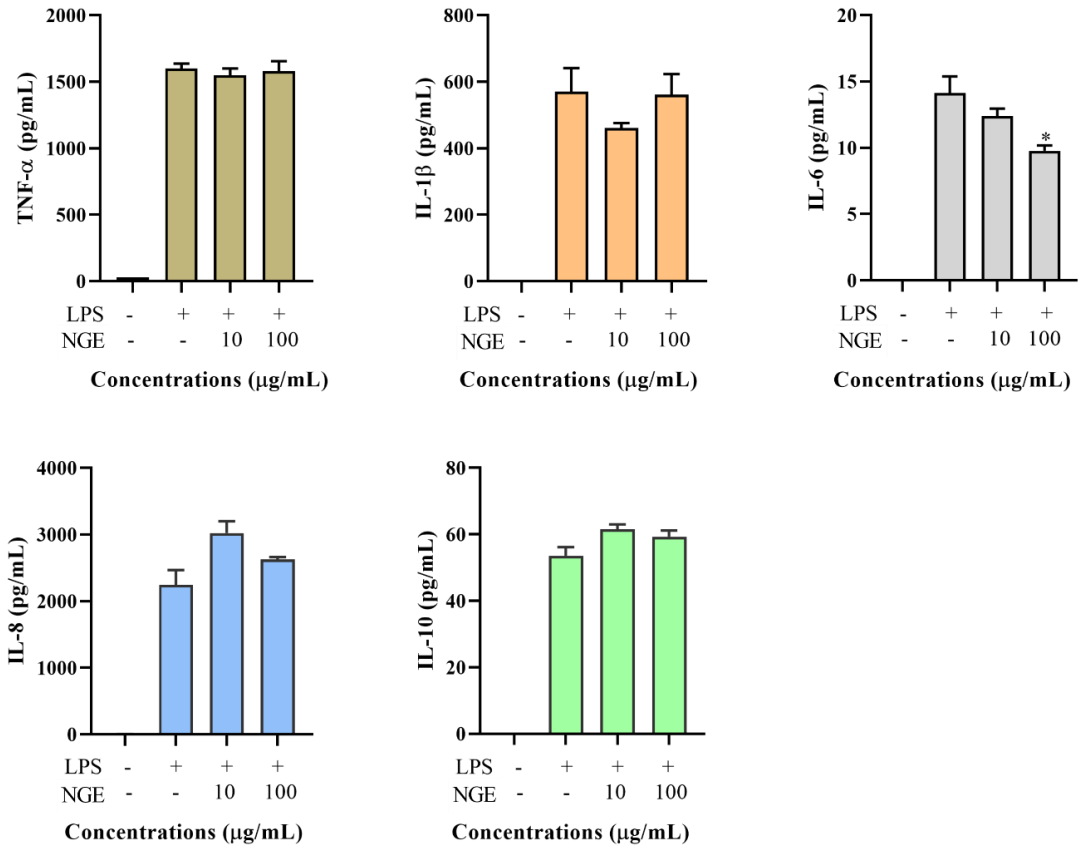
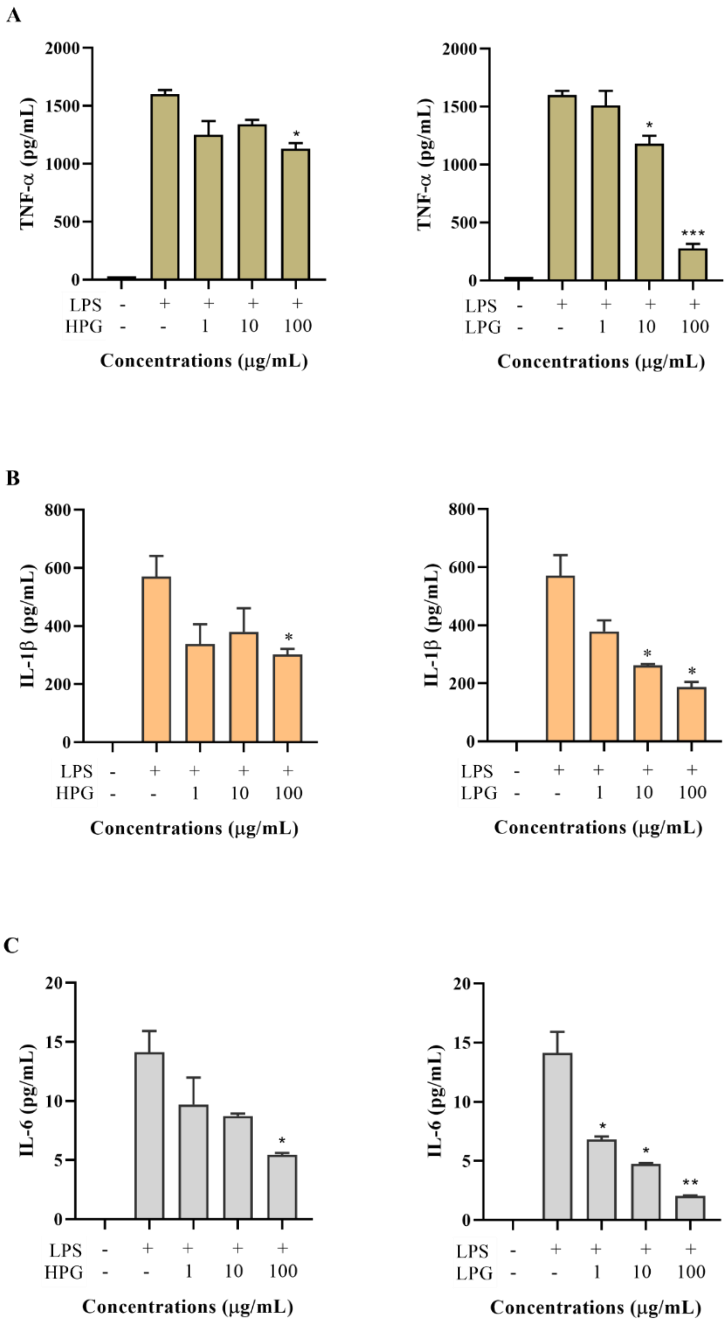


Figure 9.5 Effect of NGE on LPS-induced TNF-α, IL-1β, IL-6, IL-8, and IL-10 productions in the THP-1 cell culture medium. Data are shown as the mean ± SD of three repetitions. * $p < 0.05$ for comparison of LPS stimulation with and without ginseng extract pre-treatment.

9.3.6 Effects of ginsenoside fraction extracts on cytokine production

Ginsenosides are commonly regarded as the active ingredient of ginseng. High-polar ginsenoside fraction extract (HPG) and less-polar ginsenoside fraction extract (LPG) were separated from total ginseng extract. To evaluate the effect of HPG and LPG on

cytokine production, Thp-1 cells were treated with various concentrations of HPG and LPG for 2 h, followed by LPS (200 ng/mL) incubation. After 24 h treatment with LPS, cytokines including TNF-α, IL-1β, IL-6, IL-8, IL-10, and TGF-β1 in the culture medium were determined (standard curves were shown in the supplementary data). Results are shown in Figure 9.4.



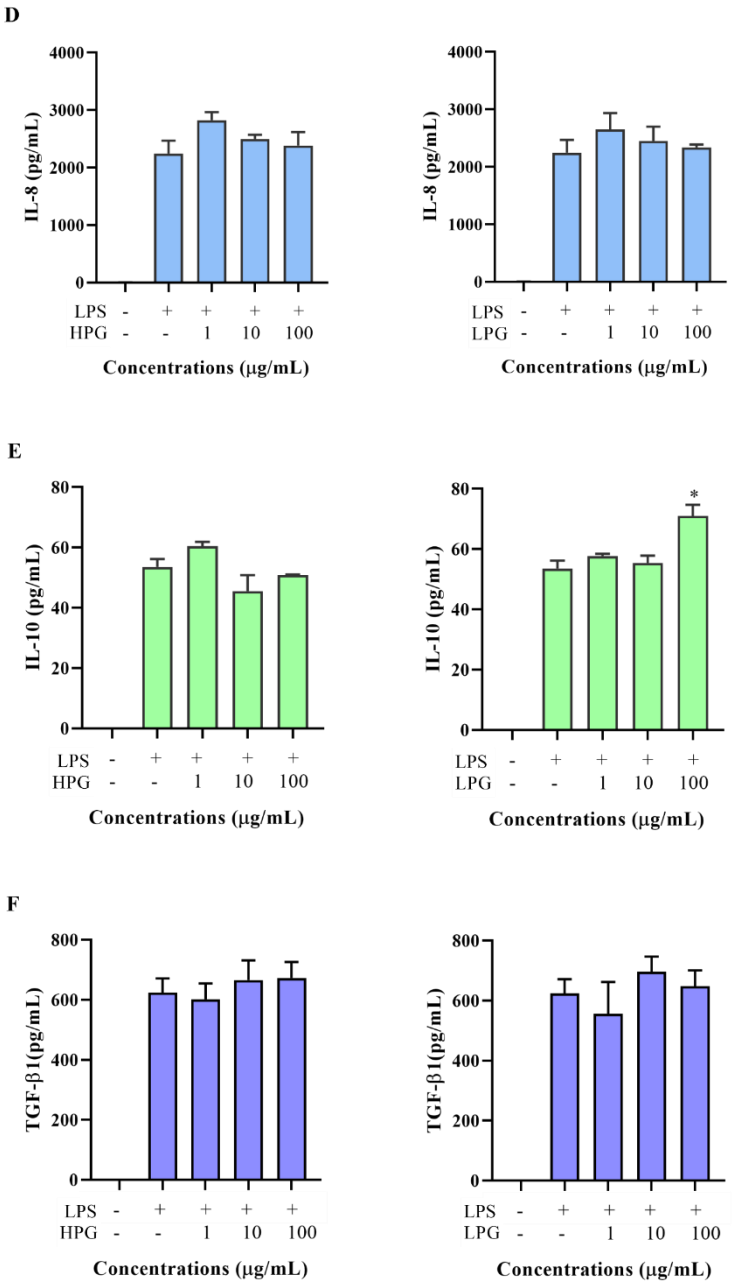


Figure 9.6 Effect of HPG and LPG on LPS-induced TNF-α (A), IL-1β (B), IL-6 (C), IL-8 (D), IL-10 (E), and TGF-β1 (F) production in the THP-1 cell culture medium. Data are shown as the mean ± SD of three repetitions. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ for comparison of LPS stimulation with and without ginseng extract pre-treatment.

Five cytokines (TNF-α, IL-1β, IL-6, IL-8, and IL-10) are almost undetectable in the culture medium without PLS treatment, but their levels increased dramatically after LPS stimulation. High dose (100 μg/mL) rather than low dose (10 or 1 μg/mL) of HPG

significantly inhibited LPS-induced TNF-α, IL-1β, IL-6 productions. For LPG, it shows a dose-dependent inhibitory effect on LPS-induced TNF-α, IL-1β, IL-6 productions. LPG at 100 μg/mL significantly decreased LPS-stimulated TNF-α from 1600 ± 38 pg/mL to 275 ± 41 pg/mL, IL-1β from 571 ± 50 pg/mL to 188 ± 12 pg/mL, and IL-6 from 14.1 ± 1.3 pg/mL to 2.1 ± 0.1 pg/mL, respectively (Figure 9.4A, 9.4B, and 9.4C). Both HPG and LPG did not show significant effect on LPS-induced IL-8 and TGF-β1 productions at different concentrations from 1 μg/mL to 100 μg/mL (Figure 9.4D and 9.4F). Interestingly, LPG at 100 μg/mL significantly enhanced LPS-induced IL-10 production, while HPG at different doses did not exhibit obvious effect on LPS-stimulated IL-10 production (Figure 9.4E).

9.4 Discussion

Pro-inflammatory or anti-inflammatory cytokines can be produced by leukocytes or endothelial cells based on their activities in regulating inflammation [26]. TNF-α and IL-1β, two pro-inflammatory cytokines, have been identified as playing a central role in acute and chronic inflammation [27, 28]. Anti-inflammatory cytokines, such as IL-10 and TGF-β, negatively regulate or inhibit over-activated inflammation [29]. Therefore, either suppressing the overproduction of pro-inflammatory cytokines or enhancing the production of anti-inflammatory cytokines is an effective strategy to reduce inflammation and its symptoms, and this method has been reported to successfully treat certain inflammatory diseases, such as diabetes, rheumatoid arthritis, and cancer [30, 31].

The total saponin extracts from *Panax ginseng* have shown anti-inflammatory effects through reducing productions of nitric oxide (NO) and LPS-induced TNF-α and IL-1β protein expression [31], which is in line with the present study that total extracts from NZ-grown ginseng suppressed LPS-induced TNF-α, IL-1β, and IL-6 production. Furthermore, in this work, it was demonstrated that ginsenoside fractions rather than non-ginsenoside fractions are the main component of ginseng to exert anti-

inflammatory activity. This result can be supported by previous publications. It has reported that the ginsenosides Rg1 [32], Re, Rb1 [33], Rc [34], Rh3 [35], and Rg3 [36] are able to inhibit the production of pro-inflammatory cytokines, including TNF-α, and IL-1β, in *in vitro* and *in vivo*. From the HPLC analysis, the main components of ginsenoside fractions from NZ-grown ginseng are ginsenoside Rb1, Rg1, Re, Rg3. This suggests that ginsenosides are the anti-inflammatory components of NZ-grown ginseng.

From present study, LPG exhibited more inhibition of pro-inflammatory cytokine secretion compared with HPG at the same dose. Suggesting that the anti-inflammatory activity of HPG is enhanced after transforming into LPG through heating process. The similar rules can be found in the literatures. It has been reported that black ginseng extract [37] and fermented red ginseng extract [38] possess more potent anti-inflammatory and anti-nociceptive activities than red ginseng extract. Interestingly, black ginseng and fermented red ginseng mainly contain LPG, while the majority component of red ginseng is HPG [39]. This may be due to smaller molecule structure of LPG compared to HPG, which makes it easier to pass through cell membrane to exhibit relatively higher biological activities [40]. Moreover, in present study, LPG on one hand inhibited production of LPS-induced pro-inflammatory cytokine of TNF-α, IL-1β, and IL-6, on the other hand enhanced LPS-induced anti-inflammatory cytokine of IL-10 secretion in Thp-1 cells. Indicating LPG possess a more effective anti-inflammatory effect than HPG, which shows inhibitory effect on pro-inflammatory cytokine production, but has no obvious effect on anti-inflammatory cytokine production. Together with previous studies, the results obtained with this work might indicate that ginseng extract from ginseng grown in NZ also show anti-inflammatory effect and processed ginseng extract (LPG) might be potential application to regulate inflammatory diseases.

Additionally, the amount of ginseng extract that do not contain ginsenosides (named non-ginsenosides fraction extract, NGE) was obtained from total ginseng extract in

this chapter. Although ginsenosides are commonly considered a unique component of ginseng and attract much more attention to study it, non-saponins account for most of the chemical component of ginseng [41]. Non-saponins of ginseng can be classified in to three categories: saccharides including monosaccharide, oligosaccharides, polysaccharides, fiber, and pectin; Nitrogen-containing compounds such as amino acid, peptide, protein, alkaloid, and nucleic acids; fat-soluble components including polyacetylenes, fatty acid, lipid, essential oil, organic oil, phenolic compounds, terpenoid, and phytosterols [41]. Considering the extraction method of this study, the major ingredient of NGE is saccharides, more likely ginseng polysaccharide. Kang *et al* found that pro-inflammatory cytokine including TNF-α, IL-6, and monocyte chemoattractant protein 1 (MCP-1) were significantly decreased by non-saponin fraction (NSF) from red ginseng [42]. Moreover, NSF successfully inhibited inflammatory responses through reducing productions of nitric oxide (NO), inducible nitric oxide synthase (iNOS), and cyclooxygenase-2 (COX-2). For example, 2000 μg/mL NSF reduced the levels of NO, iNOS, and COX-2 by 33, 83, and 64%, respectively [42]. Another study evaluated anti-diabetic effect of KGC05P0 that is a non-saponin fraction of Korean Red Ginseng. They found KGC05P0 could regulate diabetes related indicators including HbA1c, insulin, glucose tolerance, and fasting glucose level, via downregulating the PI3K/AKT pathway, inhibiting gluconeogenesis [43]. In addition, 400 mg/kg KGC05P0 significantly reduced TNF-α and IL-1β levels in blood from diabetic mice [43]. Beside the studied on non-saponin fraction, one study focuses on red ginseng acidic polysaccharide (RGAP) and found that RGAP treatment could rise the levels of IL-1, IL-6, and NO by macrophages, whereas TNF-α production were not different between RGAP treated or not [44], which is similar to the results in this study. Only 100 μg/mL NGE inhibited LPS-induced IL-6 production, NGE did not show effect on other cytokine productions in present study. This is likely because the concentration (10-100 μg/mL) in this chapter is relatively low, compared to 400 mg/kg [43]or 2000 μg/mL [42] used in other studies. A recent study found non-saponin fraction with rich polysaccharides of ginseng showed beneficial effect against

aging and Alzheimer's disease. The dose of non-saponin fraction used in that study were 500 and 1000 μg/mL. These suggest that a higher concentration of NGE should be used to explore bioactivities and functions of non-saponin fraction extract from NZ-grown ginseng in the future.

9.5 Conclusion

This is the first preliminary study on the activity of NZ-grown ginseng. This *in vitro* study suggests that ginsenoside fractions, rather than non-ginsenoside fractions, inhibit the productions of LPS-induced pro-inflammatory cytokines. This inhibitory effect is enhanced when HPG converts into LPG by heat-treatment. Furthermore, LPG can not only inhibit LPS-induced pro-inflammatory cytokine production but also improve LPS-induced anti-inflammatory cytokine secretion.

9.6 References

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Chapter Ten

General conclusion, highlights, limitations, and directions for further work

10.1 General conclusions of this thesis

In this thesis, the HPLC and LC-QTOF-MS/MS methods were established and validated for characterizing and quantifying ginsenoside components of New Zealand (NZ) grown ginseng. Ginsenosides extracted from NZ-grown *P. ginseng* were identified and quantified in different parts with different ages. Ginsenosides Rb1, mRb1, and Re were the main components in the underground parts. Furthermore, compared to ginseng grown in the Northern Hemisphere, ginseng grown in NZ contained more ginsenosides, specifically, the average content of total ginsenosides in NZ-grown ginseng was 40.1 ± 3.2 mg/g (n = 14), which showed significantly ($p < 0.05$) higher concentration than that of China-grown ginseng (16.5 ± 1.2 mg/g, n = 113) and Korea-grown ginseng (21.1 ± 1.6 mg/g, n = 106). Then, the ginsenoside profiles of two common ginseng species were compared; they contained similar components. Fifty and forty-three ginsenosides were identified from various tissues of NZ-grown *P. ginseng* and NZ-grown *P. quinquefolius* L., respectively. Twenty-nine ginsenosides were found in both ginseng species. Among these ginsenosides, the concentration of Rb1 was highest in the underground parts (fine root, rhizome, and main root), and ginsenoside Re was highest in the aboveground parts (stem and leaf). In terms of improving the use of ginseng resources, ginseng leaf can be made into black ginseng

leaf by steaming for ginsenosides transformation, and the less-polar ginsenoside (Rg3) can be greatly enriched through converting major ginsenosides at the post-harvest process, such as high-temperature treatment at low pH. Moreover, an *in vitro* study found that ginsenoside fractions from NZ-grown ginseng, especially less-polar ginsenoside fractions, exhibited anti-inflammatory effects through inhibiting the productions of pro-inflammatory cytokine (TNF-α, IL-1β, and IL-6) and enhancing the secretion of anti-inflammatory cytokine (IL-10). This thesis fills a gap in the chemical composition and biological activity of NZ-grown ginseng and provides methods for improving active components through post-harvest processes.

10.2 Highlights of this thesis

This thesis:

- systematically reviewed the ginsenosides derived from *P. ginseng* and *P. quinquefolius* L. Ginsenosides, for the first time, have been classified into 7 subtypes according to their structures. This is the first time that the relationships between ginsenosides of dammarane type have been established. (Chapter 2)
- provided the proposed mechanisms of ginseng's anti-diabetic effect by summarizing studies conducted in a cell model, animal studies, and human trials. (Chapter 3)
- developed a valid method for characterizing and quantifying ginsenoside compositions of NZ-grown ginseng. (Chapter 4)
- is the first report of a systematic analysis of saponin components of ginseng grown in NZ; nearly 100 ginsenosides were identified in NZ-grown ginseng. (Chapter 4)
- is the first report that compared ginsenoside contents between ginseng grown in NZ and ginseng grown in the Northern Hemisphere. NZ-grown ginseng has

a much higher average content of total ginsenosides than ginseng grown in the Northern Hemisphere, such as in China and Korea. (Chapter 5)

- is the first report of a comprehensive comparison of ginsenoside ingredients in various tissues of Asian ginseng and American ginseng. (Chapter 6)
- is the first report attempts to make black ginseng leaf, which can improve the sustainable use of ginseng resources. (Chapter 7)
- describes a method that can greatly enrich the active ginsenoside ingredient (Rg3) by the post-harvest process. (Chapter 8)
- contains the first report on a bioactivity study of NZ-grown ginseng. Specifically, the study found that it is ginsenosides, especially less-polar ginsenosides, not non-saponin fractions, that exert anti-inflammatory effects. (Chapter 9)

10.3 Limitations of this thesis

Although this thesis has produced eight publications including one book chapter, one journal published review, and six journal published research articles, there are some limitations need to be pointed out in this section.

In Chapter 3, I summarized the *in vivo* and *in vitro* anti-diabetic effects of ginsenosides and ginseng extracts. The limitation of the chapter is that there is neither discussion about how the different processed ginseng impact outcomes nor discussion about the effect of dose on the physiological results. Since different studies determined different parameters and not the same index of anti-diabetes. Some studies used the ginseng extracts (with different processing), and some studies used the individual ginsenosides (not the same ginsenosides). These factors make it difficult to discuss clearly how the different processed ginseng extract affected the outcomes of anti-diabetic effect. I listed the ginsenoside components of different processed ginseng extract, the outcomes of anti-diabetic effect of different processed ginseng extracts, and the dose used in each study.

In Chapter 5, the content of ginsenosides from NZ-grown *P. ginseng* was compared with that of Norther hemisphere grown *P. ginseng*. The limitation is that the sample size of NZ-grown ginseng is only 14, which is much smaller compared to 113 samples of China-grown ginseng and 106 samples of Korea-grown ginseng.

In Chapter 7, although the less polar ginsenosides were produced in the black ginseng leaf, the concentration of the less polar ginsenosides was very low, and most of the polar ginsenoside did not convert into the less polar ginsenoside, this is due to the short steaming time. The ginseng leaves were steamed for 30 min each cycle in this study. I think this time is a little short. Fresh ginseng roots were steamed for 3 h each cycle to make black ginseng root in some publications. The steaming time was shortened from 3 h to 30 min mainly considering ginseng leaf is flat and thin, while ginseng root is usually thick. It is suggested to steam a longer time (such as 1.5-2 h for each cycle) if ginseng leaf is used to produce black ginseng leaf or less polar ginsenosides.

In Chapter 9, the anti-inflammatory effect of different ginseng extract fractions was evaluated using *in vitro* cell model. The results indicated that 100 μg/mL ginsenoside fraction showed anti-inflammatory effect and 100 μg/mL non-ginsenoside fraction did not showed any inflammatory effect. The dose of 100 μg/mL may be too low for the non-ginsenoside fraction to show anti-inflammatory effect if non-ginsenoside fraction has anti-inflammatory activity. Another limitation may be that this study quantified the inflammatory cytokines in the cell medium using ELISA assay, it did not determine the mRNA expression of anti-inflammatory related genes. This can be explored in the further study.

10.4 Directions of further work for New Zealand grown ginseng

This thesis well-characterized the ginsenoside components of NZ-grown ginseng, including qualitative and quantitative analysis of ginsenosides, comparison of ginsenoside contents from different growing regions, comparative analysis of ginsenosides between ginseng species, improved utilization of ginseng leaves, enrichment of active ginsenoside ingredients, and evaluation of the anti-inflammatory effect of ginseng extracts. As a promising potential medicinal herb, and a potential new, valuable crop for NZ, there are two aspects worthy of further study: chemical composition study and biological activity study.

Chemical composition study

Although ginsenosides are thought to be the main active and unique components in ginseng, there are other ingredients in ginseng, such as ginseng polysaccharides, alkaloids, polyacetylenes, and phenolic acids [1-3]. These components can be studied further, particularly ginseng polysaccharides, which have been reported to account for nearly 40% of ginseng by weight [1]. Ginseng polysaccharides have immunostimulatory effects and contribute to the bioactivity of herbal medicines, providing great potential applications in food, pharmaceuticals, and other industries [4-7]. Therefore, ginseng polysaccharides are worthy of further study on their constituents and chemical structures.

Biological activity study

Ginseng has become one of the most important commodities for health care and disease treatment in Asian countries, such as Korea and China. Ginseng is widely distributed in 35 countries around the world and it can be used for medicines, food,

dietary supplements, health supplements, and agricultural products based on the characteristics of different consumers [8]. Modern pharmacological studies have found that ginseng has various pharmacological effects. For example, American ginseng has effects on the central nervous system, cardiovascular system, endocrine system, and cancer [9]. Ginseng contains more than 200 different compounds (Chapter 2), and different compounds exert different biological activities. Furthermore, the concentration of the active components is affected by growing surroundings, harvest time, and other factors. Even in the same batch of ginseng product, different extracts can be obtained that can have a different pharmacological effect due to the different method of extraction and the solvents used [10]. This thesis found that NZ-grown ginseng has abundant ginsenoside composition (Chapter 4 and 6) and the average content of total ginsenosides from NZ-grown ginseng is significantly higher than that of ginseng grown in the Northern Hemisphere (Chapter 5). However, there is no research on the biological activity of NZ-grown ginseng; it is uncertain whether ginseng grown in NZ still has these pharmacological activities or has stronger bioactivities than ginseng grown in the Northern Hemisphere. Although my study suggests that NZ-grown ginseng has anti-inflammatory activity *in vitro*, this is a preliminary study. More research needs to be done to provide evidence of the quality, efficacy, and safety of NZ-grown ginseng before it is consumed in the market.

Besides the ginsenosides, some other potential bioactive components may play a key role in the health benefits of ginseng. For example, the polyphenols play an important role in anti-inflammatory and anti-oxidative effects of ginseng [11]. Some studies showed that ginseng polysaccharides could regulate the blood glucose and may be the bioactive components of ginseng to assist the anti-diabetic effect [12-13]. Gintonin, a complex compound, was reported to have anti-inflammatory effect and inhibit the Aβ protein production [14]. Polyacetylenes showed anti-proliferative effect in the cell study [15].

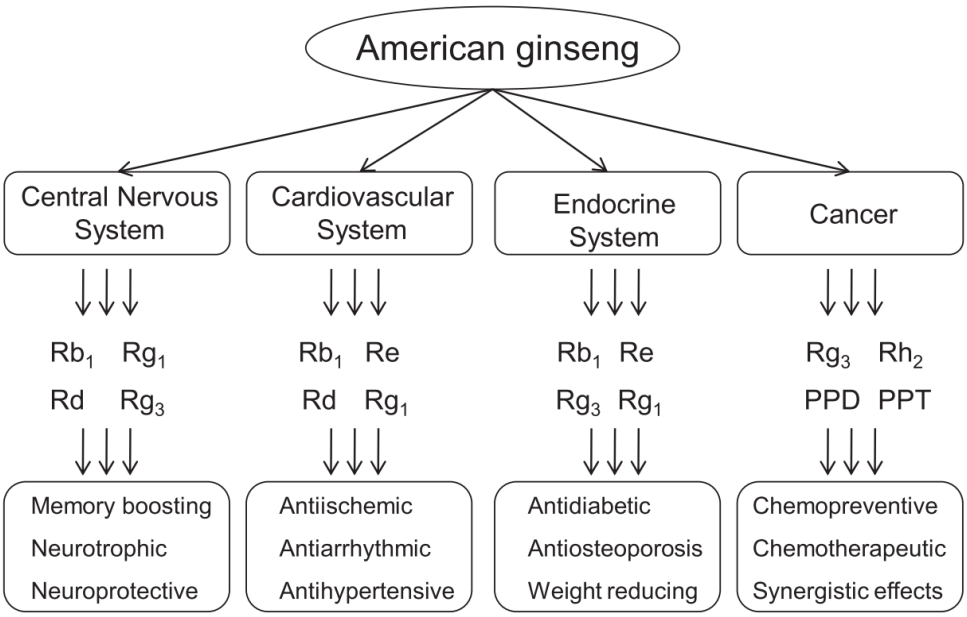

Figure 10.1 Biological and pharmacological activities of American ginseng and ginsenosides cited from Qi et al (2011, phytochemistry).

Among the various pharmacological effects of ginseng, anti-diabetic activity has been widely studied. Ginseng is regarded as a promising medicinal herb intervention for diabetes [16]. Actually, ginseng has been used to treat diabetes since the Song Dynasty (about 1078 A.D.), which was known as Xiaoke disease [17]. As reviewed in Chapter 3, ginsenosides and ginseng extracts, particularly Korean red ginseng extracts, showed anti-diabetic effects in animal studies and human trials. There is some evidence that ginsenoside Rg3 can alleviate hyperglycaemia through affecting the incretin hormone glucagon-like peptide-1 (GLP-1) [18]. But it is present at a very low concentration in ginseng. In this thesis, ginsenoside Rg3 was greatly enriched by post-harvest processing (Chapter 8), which can provide the material (Rg3) for further anti-diabetic studies in cell or animal models.

10.5 References

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Appendix 1 Supplementary data of Chapter four

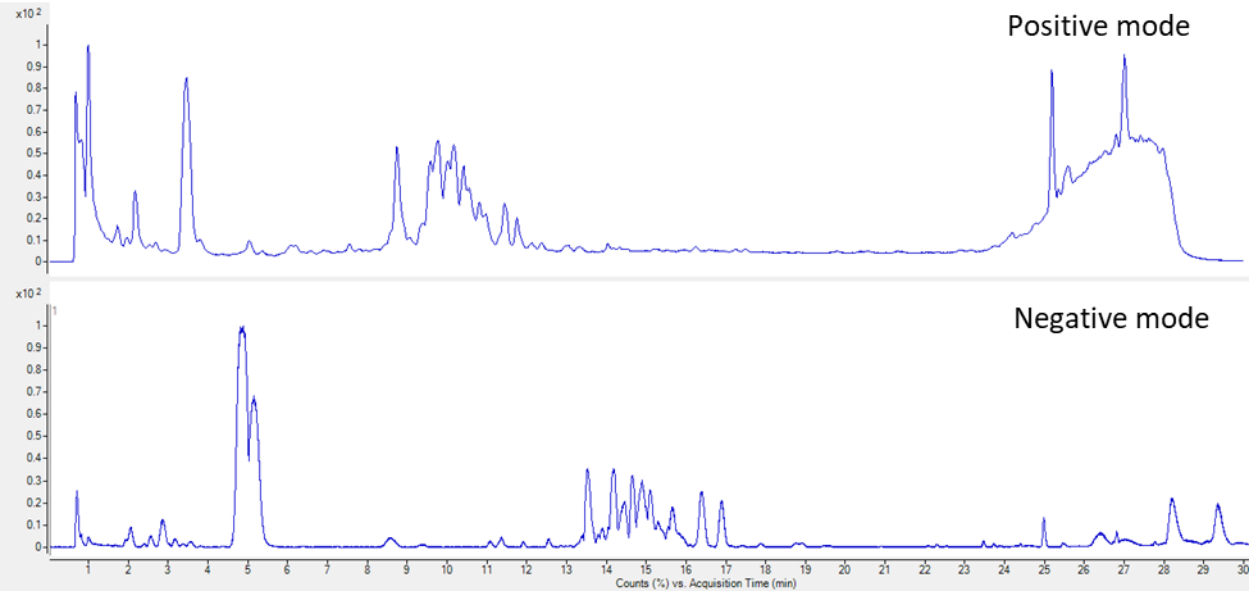
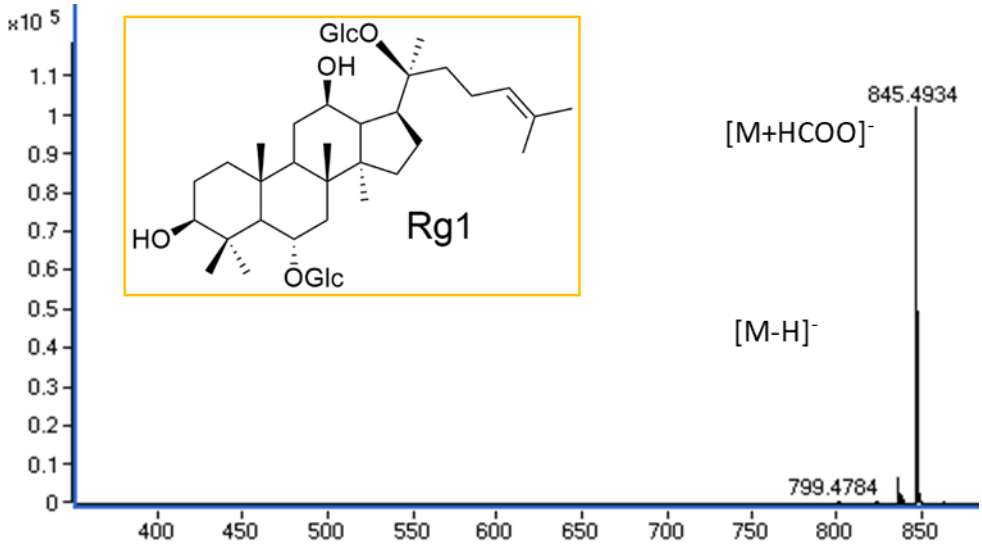
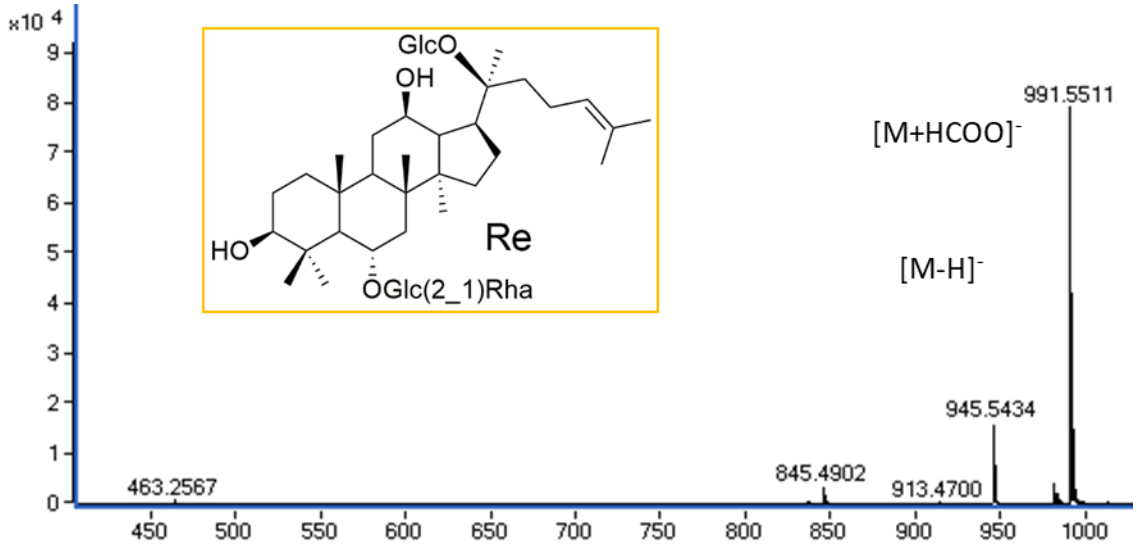


Figure S4.1 The LC-MS chromatograms of positive and negative mode

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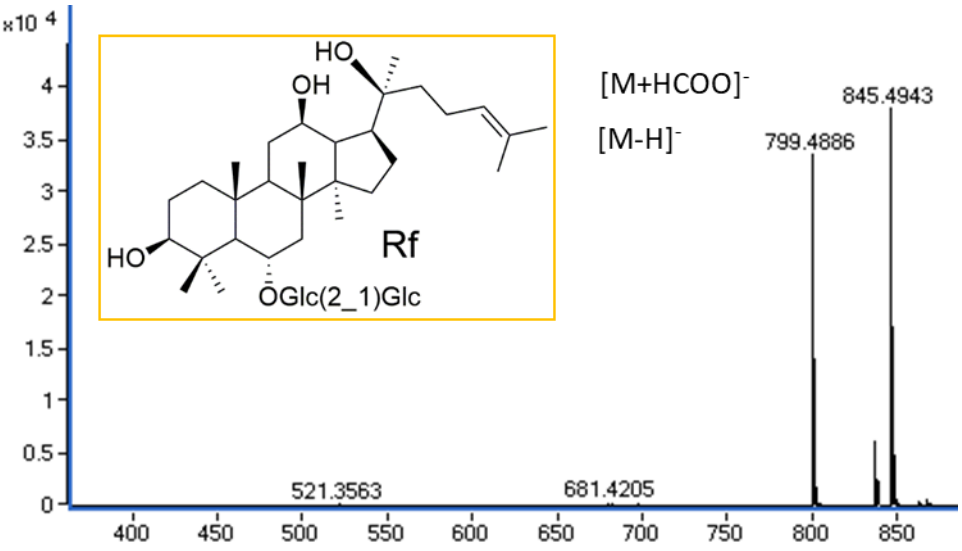
913.4700
463.2567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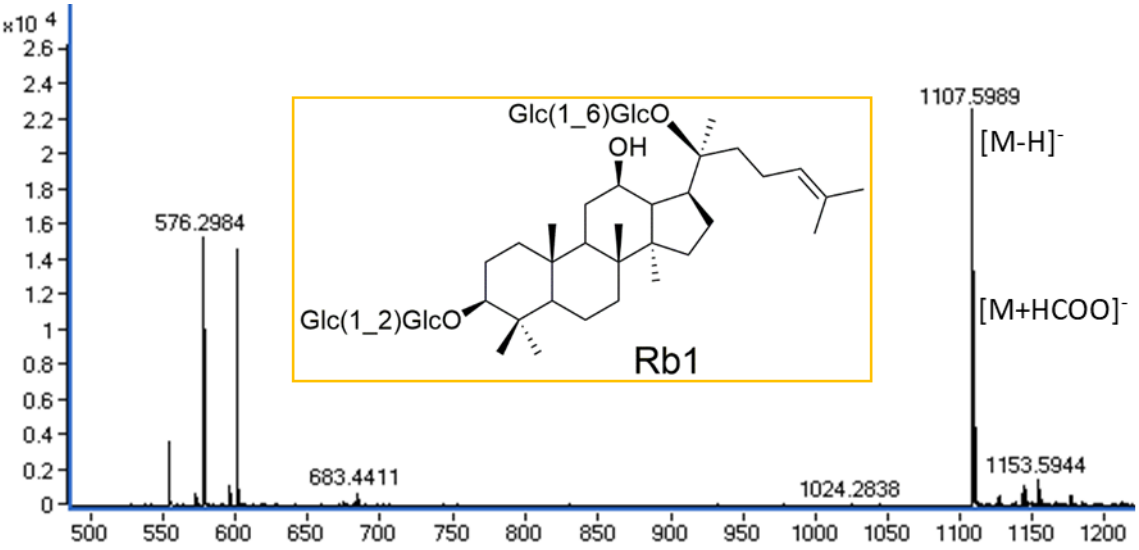
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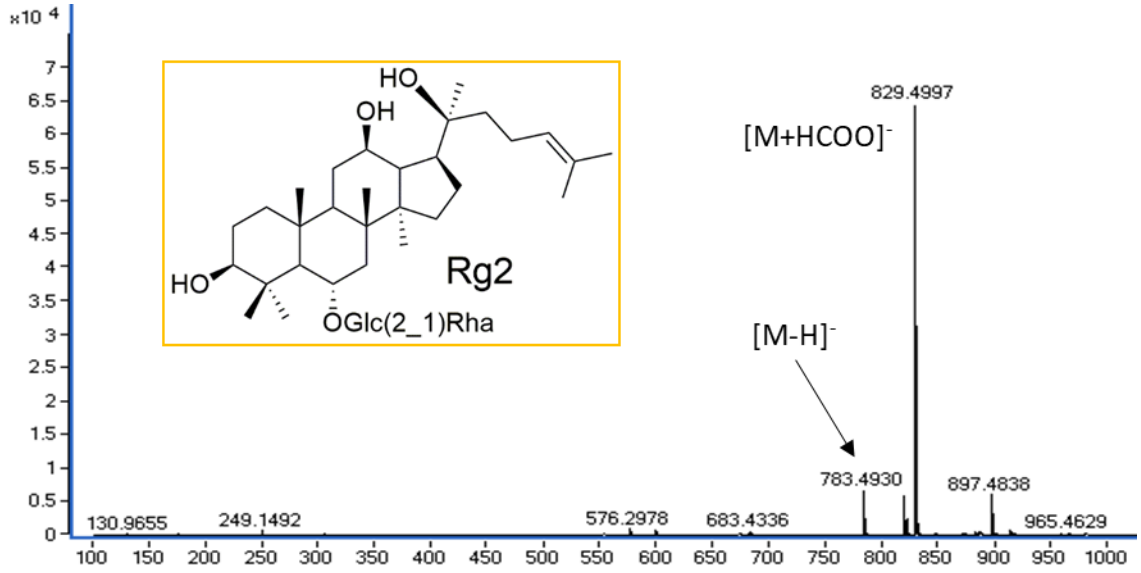
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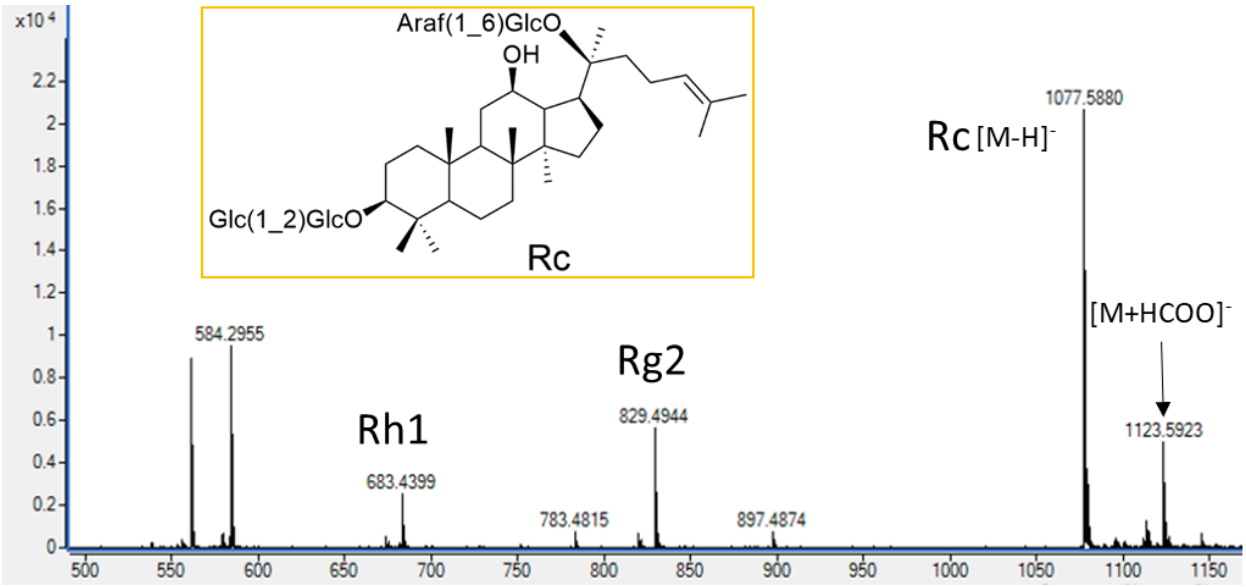
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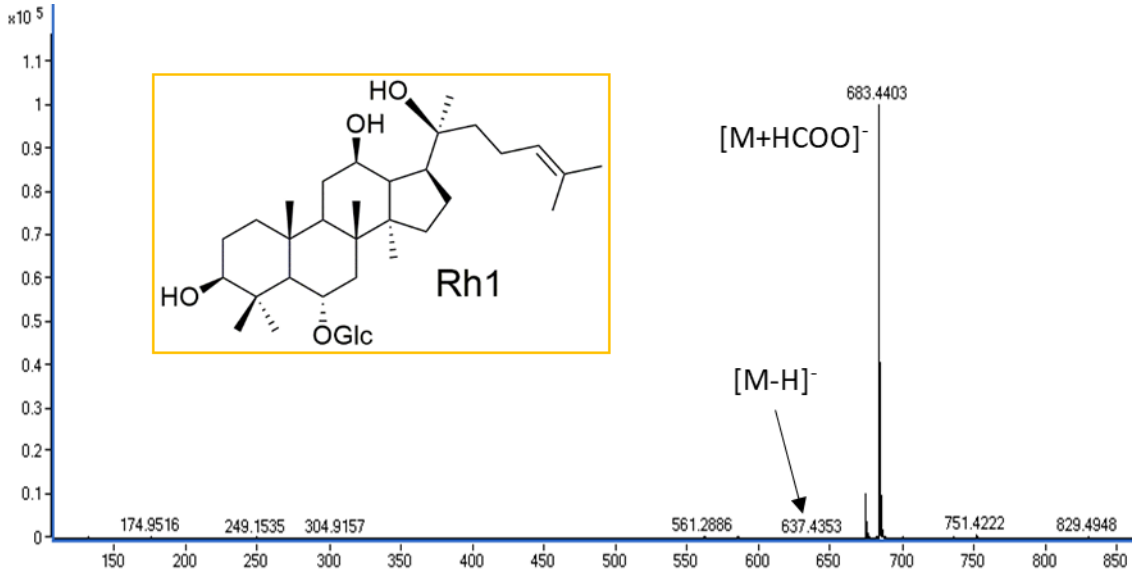
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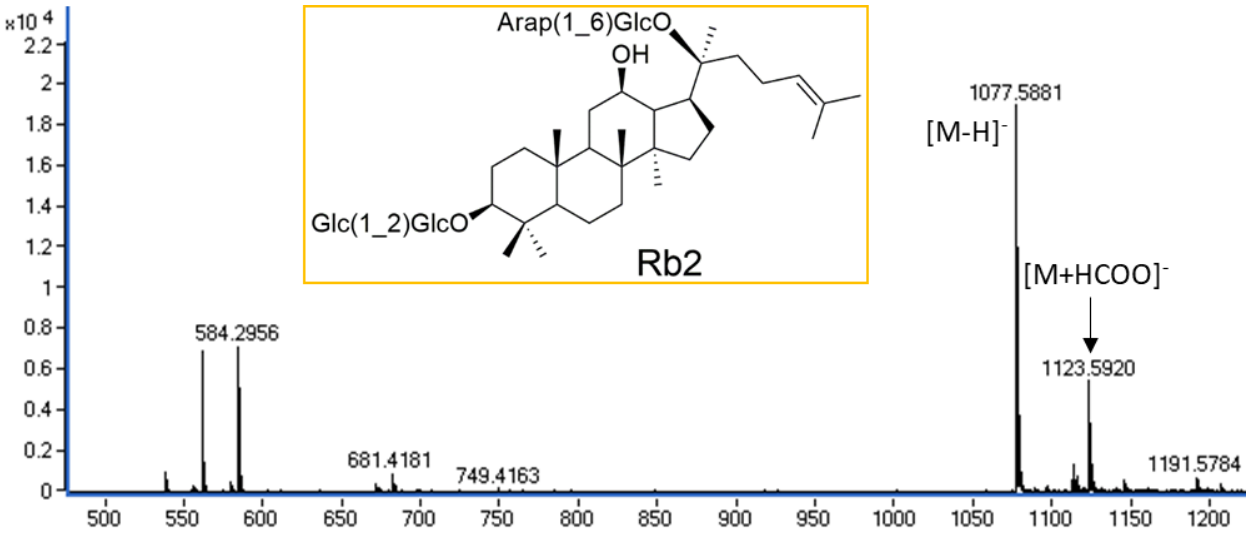
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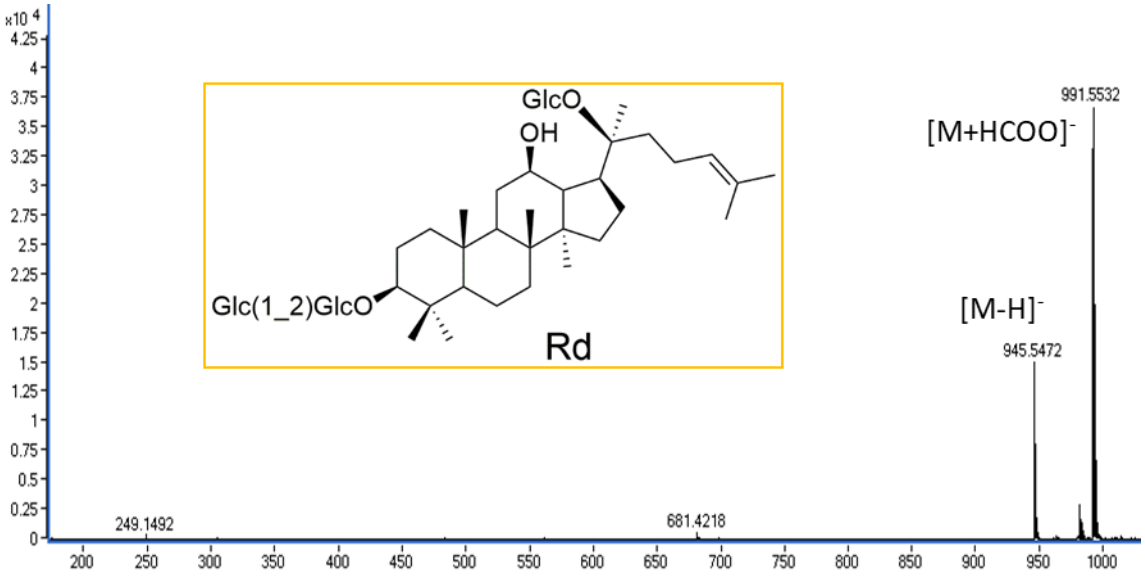
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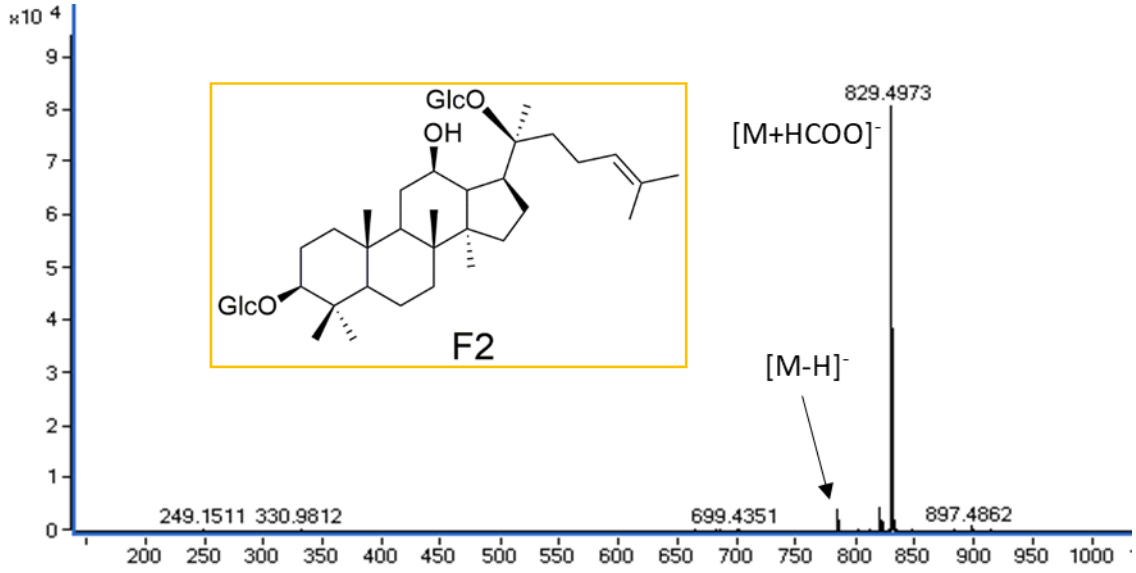
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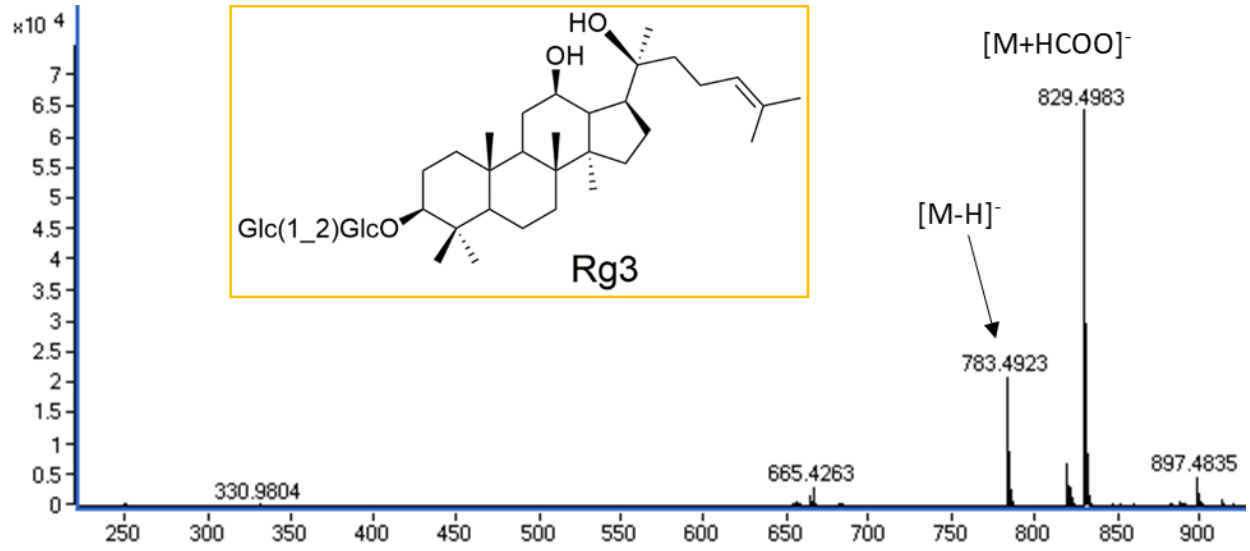
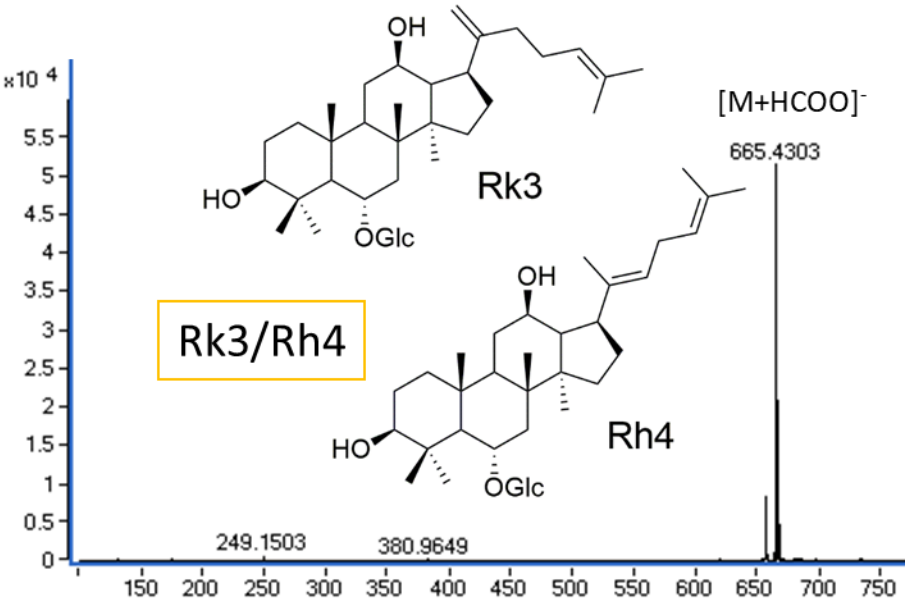
897.4862


x10 4
HO
OH
Glc(1_2)GlcO
Rg3
[M+HCOO]-
829.4983
[M-H]-
783.4923
330.9804
665.4263
897.4835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5.5
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
OH
HO
OGlc
Rk3
[M+HCOO]-
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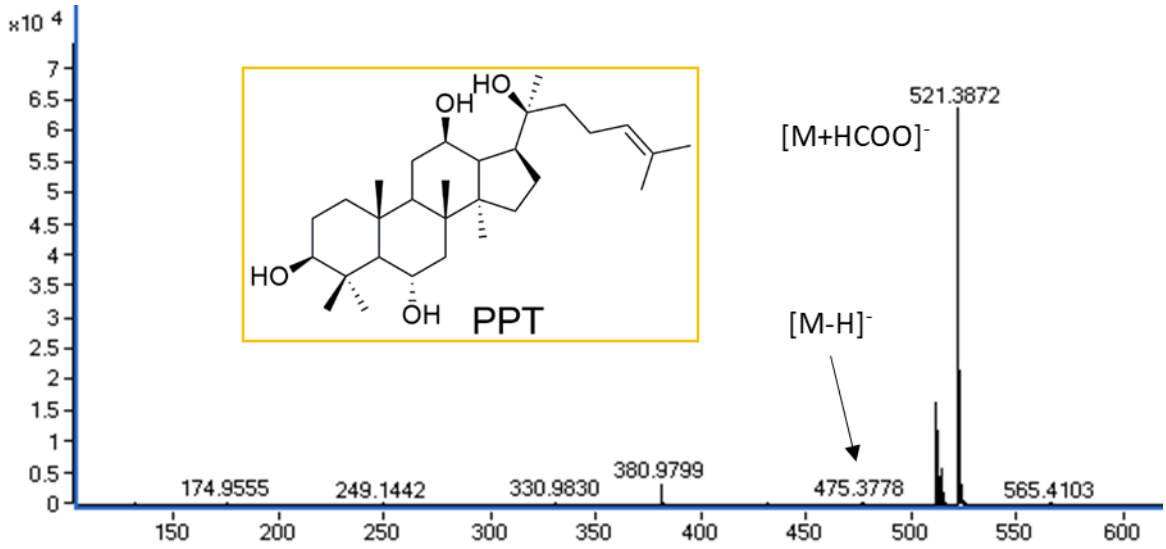
Rk3/Rh4
OH
HO
OGlc
Rh4
249.1503
380.9649
150 200 250 300 350 400 450 500 550 600 650 700 750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7
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6
5.5
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
HO
OH
HO
OH
PPT
521.3872
[M+HCOO]-
[M-H]-
174.9555
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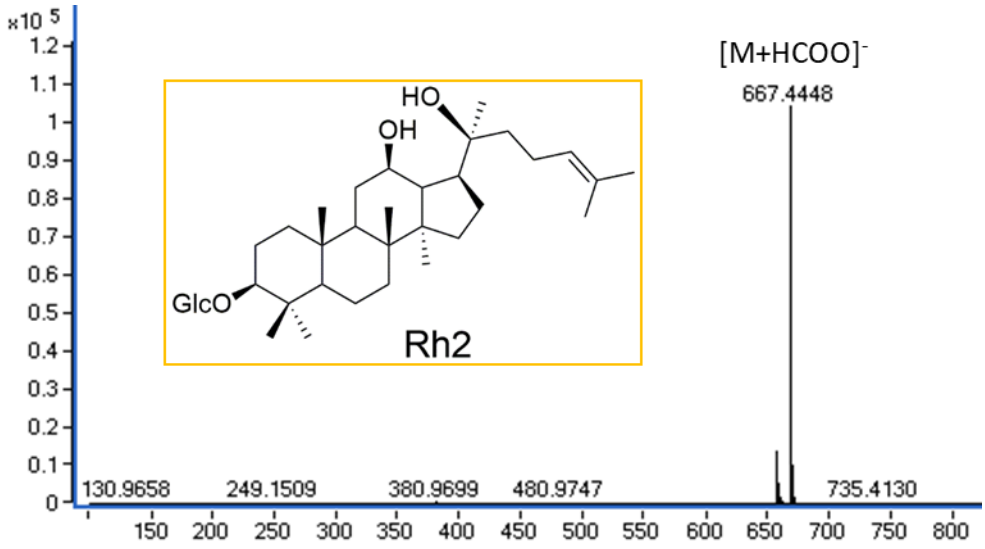
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1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
HO
OH
GlcO
Rh2
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735.4130
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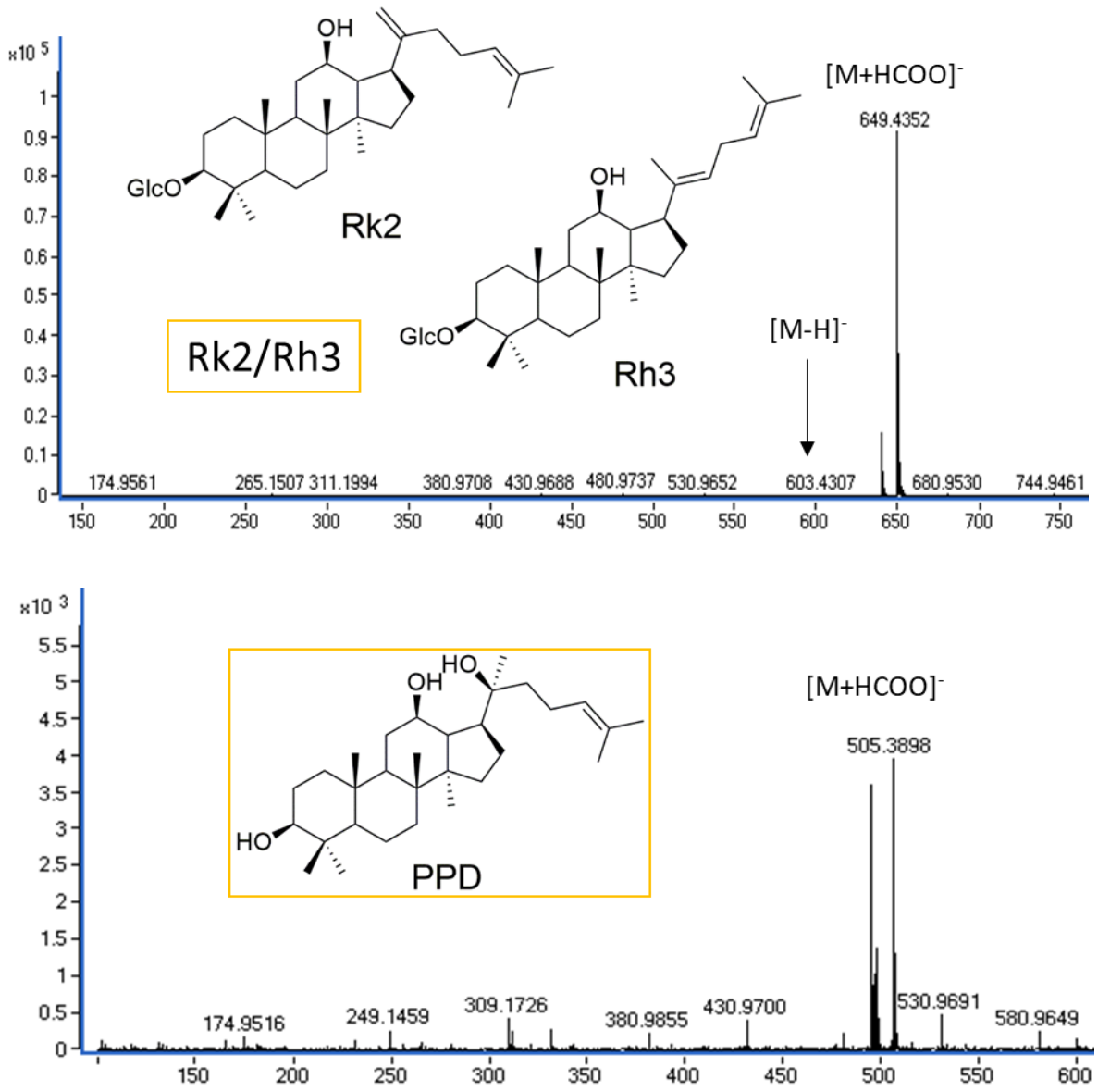


Figure S4.2 The MS peaks of ginsenoside standards in the negative mode

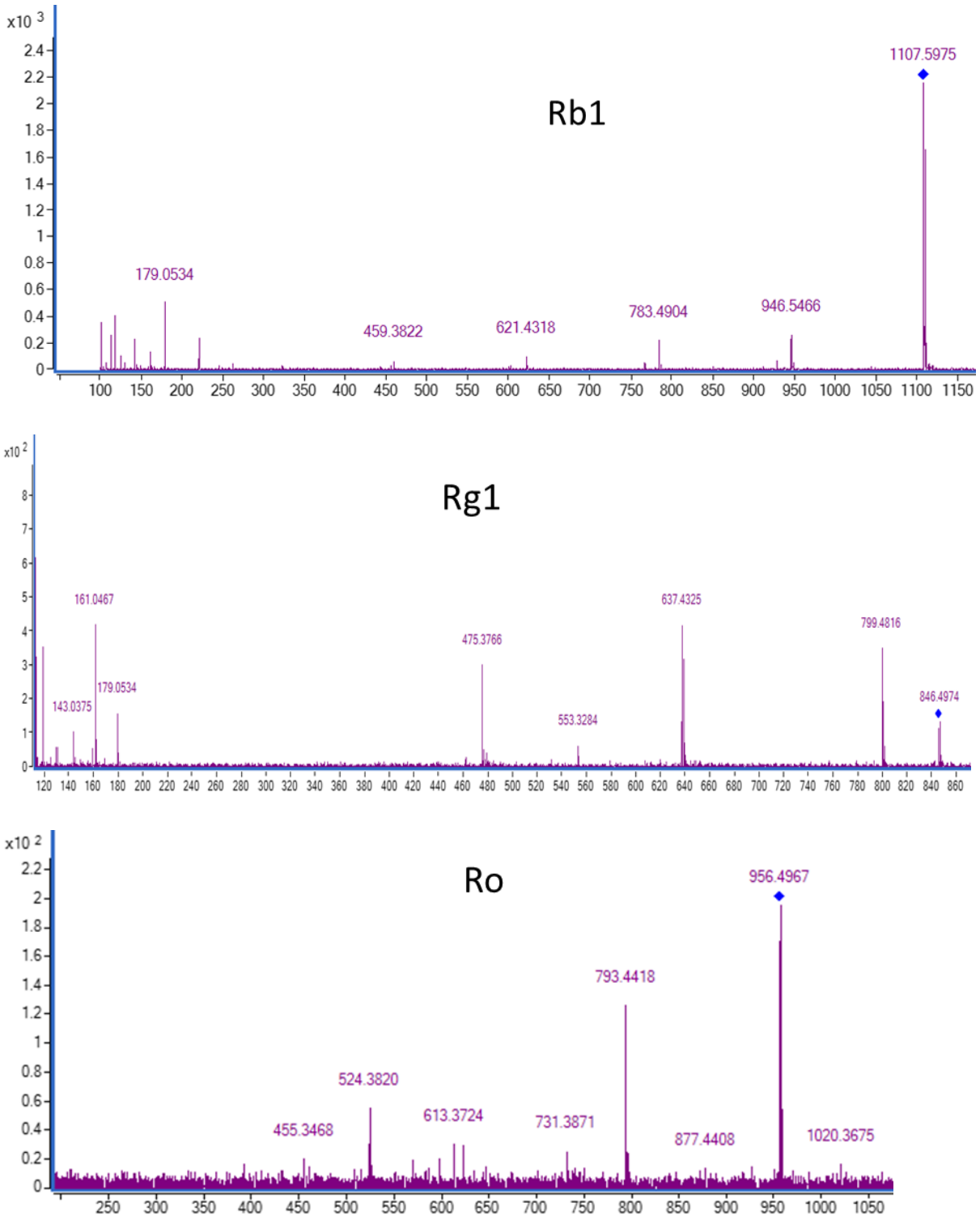
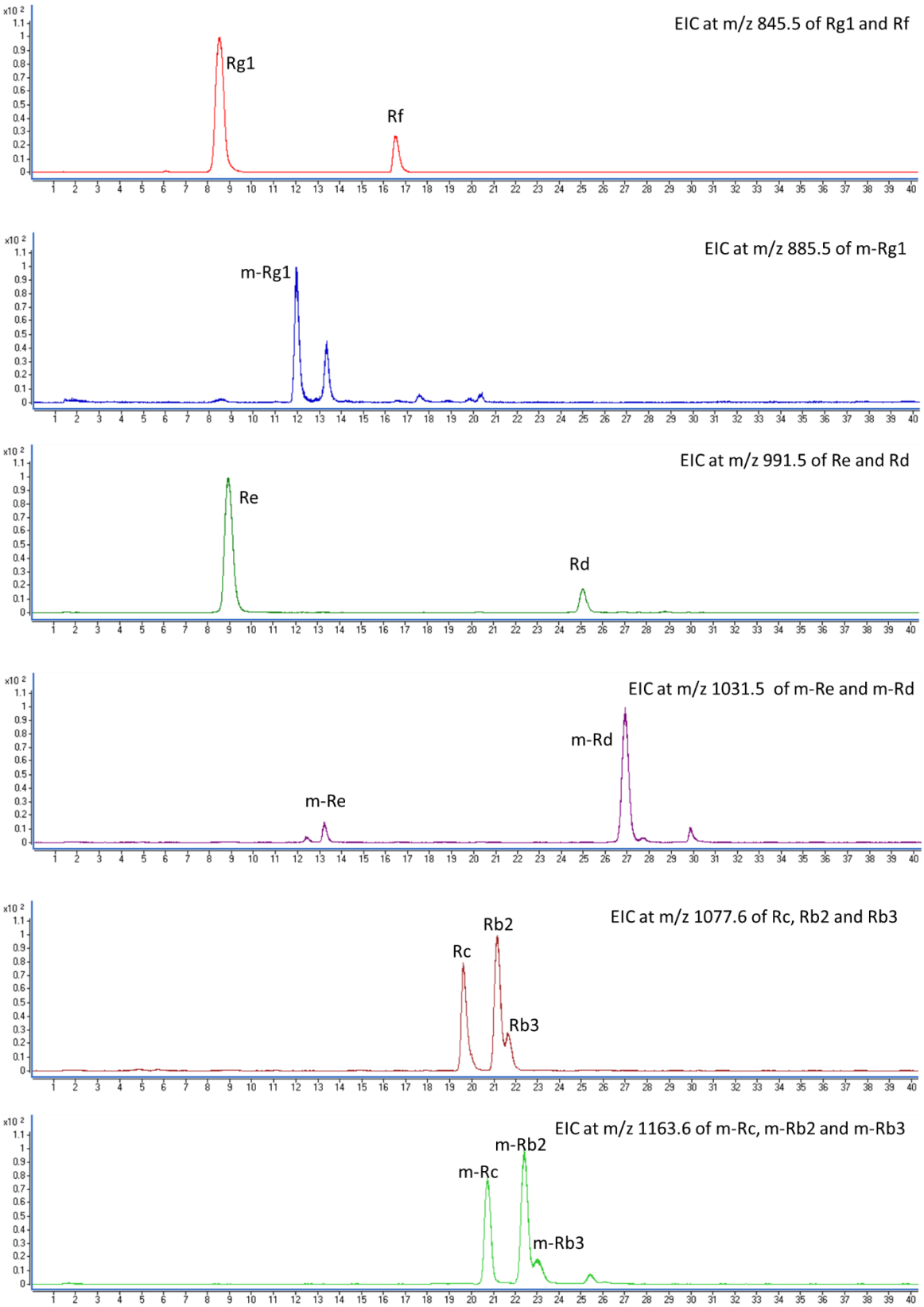


Figure S4.3 The MS/MS peaks of ginsenosides Rb1 (PPD-type), Rg1 (PPT-type), and Ro (OA-type)


EIC at m/z 845.5 of Rg1 and Rf
Rg1
Rf
EIC at m/z 885.5 of m-Rg1
m-Rg1
EIC at m/z 991.5 of Re and Rd
Re
Rd
EIC at m/z 1031.5 of m-Re and m-Rd
m-Rd
m-Re
EIC at m/z 1077.6 of Rc, Rb2 and Rb3
Rb2
Rc
Rb3
EIC at m/z 1163.6 of m-Rc, m-Rb2 and m-Rb3
m-Rb2
m-Rc
m-Rb3

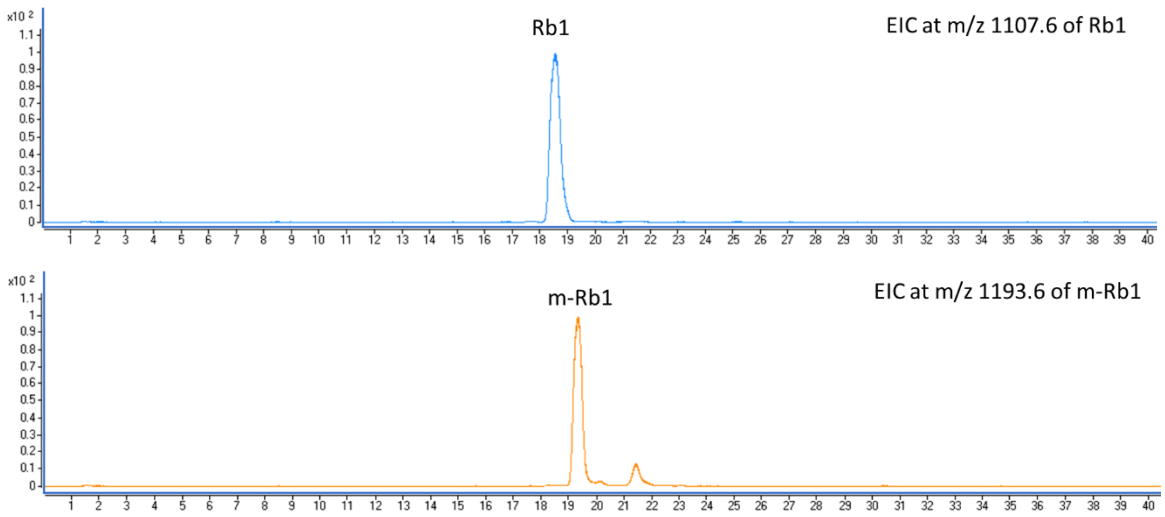


Figure S4.4 The extracted ion chromatograms (EICs) of quantified ginsenosides

Appendix 2 Supplementary data of Chapter five

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Appendix 3 Supplementary data of Chapter six

Table S6.1 The regression equations, linear ranges, limits of detection and limits of quantification of 14 ginsenosides

Ginsenosides	Calibration curve	r^2	Concentration range (mg/L)	LOD (mg/L)	LOQ (mg/L)
Rb1	y=40414x+29985	0.9986	2.0-128.2	2.1	6.9
Rb2	y=42370x-36168	0.9994	2.2-141.0	2.1	6.9
Rb3	y=41463x-24949	0.9998	1.8-57.7	1.1	3.7
Rc	y=36973x-30003	0.9995	1.9-119.7	1.6	5.5
Rd	y=76766x+18313	0.9988	2.1-67.3	2.9	9.5
Re	y=74382x-12033	0.9999	2.4-153.8	0.9	3.0
Rf	y=68030x+39879	0.9996	2.5-162.4	2.4	6.8
p-F11	y=153686x+46754	0.9998	3.6-57.7	1.0	3.4
F2	y=114456x+3865.7	0.9995	2.4-38.4	1.2	3.9
Rg1	y=117288x+46652	0.9994	3.0-192.3	2.8	9.5
Rg2	y=142443x+52824	0.9997	1.6-102.5	1.0	3.4
Rg3	y=100975x+2937.7	0.9998	2.0-32.1	0.6	2.0
Rh1	y=161372x+41690	0.9994	1.7-27.8	0.9	2.9
Rh2	y=194885x-27449	0.9999	2.3-18.7	0.3	1.0

y is the integrated peak area and x is the amount of analyte (ng). LOD: limit of detection; LOQ: limit of quantification.

Appendix 4 Supplementary data of Chapter seven

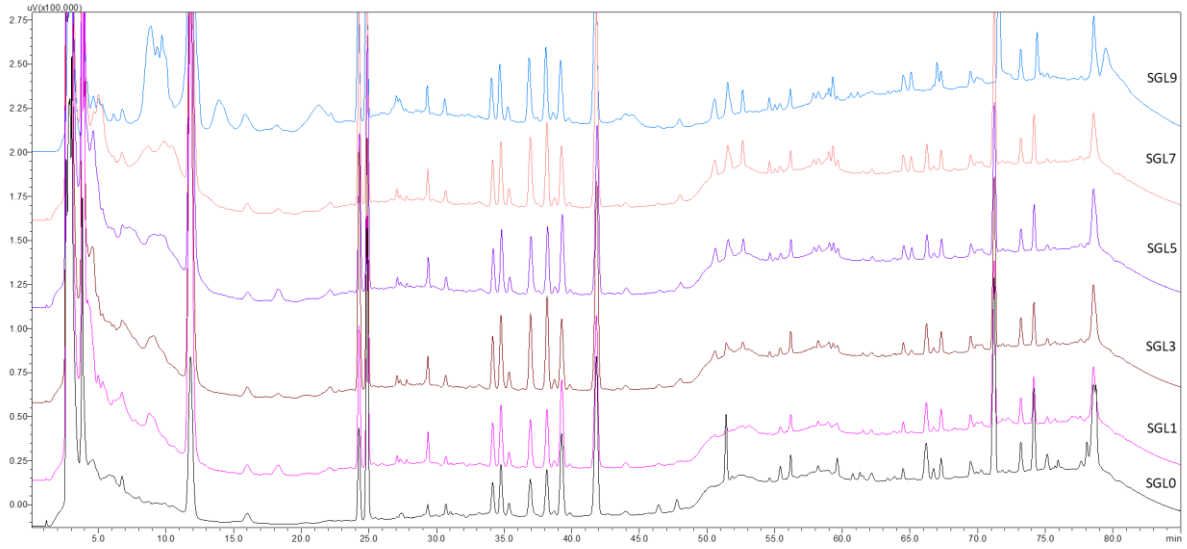

Figure S7.1 Shifted HPLC chromatogram of ginseng leaves at different steaming cycles

Table S7.1 The regression equations, linear ranges, limits of detection and limits of quantification of ginsenoside standards

Ginsenosides	Calibration curve	r^2	Concentration range (mg/L)	LOD (mg/L)	LOQ (mg/L)
Rg1	y=6650.9x+20748	0.9999	20.6-1029.4	13.4	44.5
Re	y=6049.6x+21738	0.9999	12.4-617.7	6.4	21.2
Rf	y=7658.4x+3491	0.9996	10.4-517.7	2.5	8.4
Rb1	y=6022.2x+2221	0.9999	9.5-476.5	1.8	6.0
Rg2	y=8133.7x+52824	0.9997	8.5-481.8	1.0	3.4
Rh1	y=7597.5x+10594	0.9999	9.2-458.8	6.2	20.7
Rc	y=5720.8x-2278	0.9995	11.3-564.7	1.4	4.6
Rb2	y=5228.0x+2227	0.9994	11.9-594.2	2.1	7.0
Rb3	y=5480.0x+2626	0.9998	11.1-552.9	2.4	7.9
Rd	y=6204.5x+2683	0.9998	12.9-647.1	2.5	8.2
F2	y=6957.8x+23124	0.9995	2.4-485.4	1.7	5.6
Rg3	y=8331.3x+28160	0.9998	7.7-382.4	1.8	6.0
20R-Rg3	y=5313.9x+6110.3	0.9981	8.4-323.5	2.2	7.3
Rk3	y=5950.8x+18810	0.9993	5.5-386.2	1.9	6.2
Rh4	y=5656.1x+27710	0.9989	4.7-412.3	3.0	9.9
Rk2	y=4676.8x+70784	0.9991	6.3-389.3	4.7	15.6
Rh3	y=4909.8x+45090	0.9994	5.8-436.9	2.4	7.9
Rh2	y=12634x+1084	0.9999	6.0-300.0	1.0	3.3
PPT	y=9181.3x+6620	0.9998	8.2-405.9	2.6	8.6
PPD	y=14733x+11324	0.9999	6.7-335.3	3.4	11.2

y is the integrated peak area and x is the concentration of analyte (mg/L). LOD, limit of detection; LOQ: limit of quantification.

Table S7.2 Inter-day variation and intra-day variation (RSD) of the ginsenoside standards. Intra-day and inter-day variations were used to evaluate the precision of this HPLC method for determining ginsenoside. Ginsenoside standards with the known concentrations were mixed together and tested. In the intra-day variability experiment, the test was conducted within one day and three parallel experiments were carried out. While for the inter-day precision, the mixed standard solutions were examined in three different days. The relative standard deviation was used to describe variations.

Name	Inter-day		Intra-day	
	Con.(mg/L)	RSD	Con.(mg/L)	RSD
Rg1	134.7	5.5%	134.0	1.9%
Re	76.2	4.3%	77.2	2.3%
Rf	63.8	6.3%	64.9	1.2%
Rb1	59.6	2.6%	59.7	0.1%
Rg2	51.4	5.6%	52.0	2.7%
Rh1	55.3	4.8%	54.5	1.4%
Rc	69.2	4.1%	70.3	0.7%
Rb2	74.7	2.6%	75.3	1.3%
Rb3	69.8	5.3%	68.6	0.4%
Rd	80.8	6.1%	81.4	2.4%
F2	56.7	4.7%	57.1	3.6%
Rg3	46.8	5.3%	47.8	4.4%
20R-Rg3	40.5	6.5%	41.3	4.9%
Rk3	76.9	3.6%	77.6	1.3%
Rh4	72.3	4.4%	73.3	2.5%
Rk2	57.6	5.7%	58.0	1.4%
Rh3	64.1	4.2%	64.9	2.6%
Rh2	68.7	4.7%	69.0	1.1%
PPT	49.7	3.6%	50.2	2.3%
PPD	41.7	3.9%	42.0	1.8%

Table S7.3 The recovery of ginsenoside standards. The recoveries were expressed according to the following formula: recovery (%) = (found amount original amount)/added amount X100. Ginsenoside standards with known amount were added into a certain amount (0.5 g) of the sample.

Ginsenosides	Original (μg)	Added (μg)	Found (μg)	Recovery (%)	RSD (%)
Rg1	222.6	11.0	232.6 ± 1.1	91.4	10.2
Re	169.6	10.6	179.3 ± 1.0	91.3	9.5
Rf	69.3	4.4	73.8 ± 0.3	103.0	6.4
Rb1	211.1	16.5	226.8 ± 1.3	94.9	8.1
Rg2	15.3	1.5	16.9 ± 0.2	106.5	11.0
Rh1	2.7	1.0	3.8 ± 0.0	104.0	3.0
Rc	75.0	5.4	80.3 ± 0.2	96.9	4.1
Rb2	72.1	6.4	78.4 ± 0.1	99.4	1.7
Rb3	19.8	1.7	21.6 ± 0.1	105.8	3.5
Rd	41.1	2.6	43.7 ± 0.2	100.4	8.1
F2	0.1	1.4	1.6 ± 0.0	106.5	1.5
Rg3	0.7	1.2	2.0 ± 0.0	107.8	2.6
20R-Rg3	0.0	1.3	1.2 ± 0.1	91.4	3.9
Rk3	0.0	1.0	0.9 ± 0.0	96.9	4.2
Rh4	0.0	0.9	0.8 ± 0.1	93.2	8.0
Rk2	0.0	1.3	1.3 ± 0.1	101.6	5.6
Rh3	0.0	1.0	0.9 ± 0.1	97.9	6.3
Rh2	0.1	1.4	1.4 ± 0.0	92.0	1.1
PPT	0.0	1.3	1.3 ± 0.0	97.0	3.0
PPD	0.0	1.2	1.3 ± 0.1	105.0	5.9

Table S7.4 The sixteen ginsenoside contents (mg/g) in the ginseng leaves with different steamed cycles.

Ginsenosides	SGL0	SGL1	SGL2	SGL3	SGL4	SGL5	SGL6	SGL7	SGL8	SGL9
Rg1	9.4 ± 0.1	12.7 ± 0.2	16.2 ± 0.1	18.4 ± 0.2	13.4 ± 0.2	14.6 ± 0.2	16.6 ± 0.2	17.6 ± 0.3	15.5 ± 0.3	13.3 ± 0.1
Re	30.3 ± 0.5	22.0 ± 0.3	27.0 ± 0.4	21.8 ± 0.2	22.0 ± 0.3	22.9 ± 0.2	19.2 ± 0.2	20.4 ± 0.2	19.5 ± 0.2	20.1 ± 0.3
Rb1	1.4 ± 0.0	1.4 ± 0.0	3.0 ± 0.1	1.4 ± 0.1	1.5 ± 0.0	1.4 ± 0.1	1.4 ± 0.1	1.5 ± 0.0	1.5 ± 0.0	1.3 ± 0.0
Rc	6.9 ± 0.1	6.5 ± 0.1	9.2 ± 0.1	9.8 ± 0.0	8.2 ± 0.1	7.7 ± 0.2	9.1 ± 0.2	10.3 ± 0.1	10.3 ± 0.2	10.9 ± 0.2
Rb2	8.0 ± 0.2	7.9 ± 0.2	11.6 ± 0.3	12.3 ± 0.2	9.3 ± 0.2	8.7 ± 0.3	10.0 ± 0.4	11.4 ± 0.3	10.5 ± 0.1	11.1 ± 0.2
Rb3	0.4 ± 0.0	0.6 ± 0.0	1.2 ± 0.0	1.2 ± 0.0	1.2 ± 0.0	1.0 ± 0.0	1.3 ± 0.0	1.5 ± 0.1	1.4 ± 0.0	1.9 ± 0.0
Rd	26.3 ± 0.6	19.2 ± 0.3	24.5 ± 0.6	26.0 ± 0.9	20.1 ± 0.3	21.5 ± 0.3	22.0 ± 0.5	24.9 ± 0.5	22.1 ± 0.6	22.9 ± 0.4
F2	#	0.2 ± 0.0	0.5 ± 0.0	0.5 ± 0.0	1.2 ± 0.0	1.8 ± 0.0	2.1 ± 0.0	2.3 ± 0.0	2.5 ± 0.0	2.6 ± 0.1
20S-Rg3	#	#	#	#	#	0.1 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.4 ± 0.0	0.4 ± 0.0
20R-Rg3	#	#	#	#	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.4 ± 0.1
PPT	#	#	#	#	0.2 ± 0.0	0.4 ± 0.1	0.4 ± 0.0	0.7 ± 0.0	1.0 ± 0.0	1.7 ± 0.0
Rk3	#	#	#	#	#	0.3 ± 0.0	0.7 ± 0.0	0.6 ± 0.1	0.7 ± 0.0	0.9 ± 0.1
Rh4	#	#	#	0.2 ± 0.0	0.4 ± 0.0	0.8 ± 0.1	1.7 ± 0.0	1.9 ± 0.0	2.1 ± 0.1	1.8 ± 0.1
Rk2	#	#	#	#	#	0.3 ± 0.0	0.7 ± 0.0	0.8 ± 0.0	1.3 ± 0.1	1.2 ± 0.0
Sbc	16.7 ± 1.3	16.4 ± 1.5	25.0 ± 1.6	24.6 ± 1.9	20.1 ± 2.0	18.8 ± 1.6	21.9 ± 2.1	24.8 ± 2.6	23.6 ± 1.0	25.1 ± 1.9
Rg3	#	#	#	#	0.1 ± 0.0	0.2 ± 0.0	0.4 ± 0.0	0.6 ± 0.0	0.7 ± 0.0	0.8 ± 0.0

refers to not quantifiable. Sbc refers to the sum of ginsenosides Rb1, Rb2, Rb3, and Rc. Rg3 refers to the 20S-Rg3 and 20R-Rg3.

Appendix 5 Supplementary data of Chapter eight

Table S8.1 The percentage (%) of ginsenosides in the HAc treatments (mean ± SD)

Ginsenosides	0 M HAc	0.125 M HAc	0.25 M HAc	0.5 M HAc
Rg1	20.8 ± 0.3	20.8 ± 0.0	23.1 ± 0.4	20.0 ± 0.0
Re	11.1 ± 0.2	10.7 ± 0.3	11.5 ± 0.4	10.5 ± 0.2
Rf	5.9 ± 0.6	5.7 ± 1.0	6.8 ± 1.3	6.3 ± 0.1
Rb1	27.1 ± 1.2	26.4 ± 0.8	21.5 ± 0.5	23.3 ± 1.0
Rc	13.0 ± 0.7	13.1 ± 1.0	12.0 ± 0.3	14.1 ± 0.1
Rg2	4.0 ± 0.1	3.7 ± 0.1	3.7 ± 0.9	2.8 ± 0.3
Rb2	10.7 ± 0.7	10.3 ± 0.1	9.3 ± 0.5	9.6 ± 0.0
Rb3	3.0 ± 0.6	3.5 ± 1.6	2.5 ± 0.5	2.1 ± 0.2
Rd	3.7 ± 0.3	3.4 ± 0.2	3.6 ± 0.5	2.9 ± 0.3
F2	0.2 ± 0.2	0.1 ± 0.0	0.3 ± 0.0	0.4 ± 0.1
Rk3	0	0.1 ± 0.0	0.3 ± 0.0	0.5 ± 0.0
Rh4	0.6 ± 0.3	0.8 ± 0.2	1.5 ± 0.3	2.1 ± 0.2
20S-Rg3	0	0	1.3 ± 0.2	2.0 ± 0.0
20R-Rg3	0.1 ± 0.0	0.8 ± 0.1	2.7 ± 0.2	3.7 ± 0.0
Polar	95.1 ± 0.1	93.9 ± 0.6	90.2 ± 0.1	88.6 ± 0.4
Rg3	0.1 ± 0.0	1.3 ± 0.3	3.9 ± 0.4	5.6 ± 0.0

Table S8.2 The percentage (%) of ginsenosides in the NaOH treatments (mean ± SD)

Ginsenosides	0 M NaOH	0.1 NaOH	0.2 M NaOH	0.4 M NaOH
Rg1	20.8 ± 0.3	20.0 ± 0.5	20.6 ± 0.0	19.3 ± 0.4
Re	11.1 ± 0.2	10.7 ± 0.6	11.1 ± 0.0	10.7 ± 0.2
Rf	5.9 ± 0.6	4.9 ± 0.2	5.2 ± 0.0	5.1 ± 0.1
Rb1	27.1 ± 1.2	29.2 ± 0.2	28.4 ± 0.0	29.2 ± 0.5
Rc	13.0 ± 0.7	12.5 ± 0.3	12.9 ± 0.3	13.0 ± 0.2
Rg2	4.0 ± 0.1	4.2 ± 0.1	3.8 ± 0.2	4.2 ± 0.2
Rb2	10.7 ± 0.7	11.2 ± 0.2	11.1 ± 0.1	11.3 ± 0.1
Rb3	3.0 ± 0.6	2.4 ± 0.1	2.5 ± 0.0	2.6 ± 0.1
Rd	3.7 ± 0.3	3.9 ± 0.4	3.7 ± 0.2	3.8 ± 0.4
F2	0.2 ± 0.2	0.1 ± 0.1	0	0.1± 0.1
Rk3	0	0.2 ± 0.0	0.3 ± 0.4	0.1 ± 0.2
Rh4	0.6 ± 0.3	0.5 ± 0.1	0.3 ± 0.1	0.4 ± 0.3
20S-Rg3	0	0	0	0
20R-Rg3	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.1	0.2 ± 0.1
Polar	95.1 ± 0.1	94.8 ± 0.1	95.5 ± 0.0	95.1 ± 0.9
Rg3	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.1	0.2 ± 0.1

Table S8.3 The percentage (%) of ginsenosides in the high temperature (121 ℃, 0.1 MPa) extraction with different time (mean ± SD)

Ginsenosides	10 min	20 min	40 min
Rg1	18.8 ± 0.1	10.1 ± 0.1	5.2 ± 0.2
Re	9.7 ± 0.0	6.2 ± 0.0	3.7 ± 0.3
Rf	5.7 ± 0.1	5.4 ± 0.1	5.1 ± 0.2
Rb1	16.1 ± 0.2	10.7 ± 0.6	6.8 ± 0.8
Rc	4.9 ± 0.8	10.4 ± 0.0	7.0 ± 0.9
Rg2	7.5 ± 0.5	3.8 ± 0.1	6.5 ± 0.1
Rh1	1.1 ± 0.2	1.7 ± 0.1	2.3 ± 0.0
Rb2	8.5 ± 0.0	9.0 ± 0.2	9.8 ± 0.2
Rb3	2.1 ± 0.2	2.0 ± 0.0	1.3 ± 0.3
Rd	3.4 ± 0.2	2.7 ± 0.2	1.7 ± 0.4
F2	1.1 ± 0.2	2.2 ± 0.0	3.7 ± 0.6
Rk3	1.8 ± 0.1	3.7 ± 0.1	5.9 ± 0.4
Rh4	3.3 ± 0.4	6.5 ± 0.3	10.7 ± 0.5
20S-Rg3	5.7 ± 0.3	9.8 ± 0.2	13.9 ± 0.3
20R-Rg3	7.3 ± 0.2	13.3 ± 0.1	15.3 ± 0.3
20S-PPT	0	0.4 ± 0.1	0.7 ± 1.0
20R-PPT	1.0 ± 0.3	0	0.4 ± 0.3
Rk2	0.1 ± 0.2	0.2 ± 0.3	0.3 ± 0.1
Rh3	1.9 ± 0.6	1.8 ± 1.3	2.8 ± 0.6
Sbcd	35.0 ± 0.3	34.7 ± 0.6	25.8 ± 1.4
Segf	34.2 ± 0.2	21.8 ± 0.2	13.5 ± 0.8
Less polar	23.3 ± 0.4	39.8 ± 0.4	54.4 ± 0.3
Rg3	13.0 ± 0.4	23.1 ± 0.3	28.4 ± 0.6

Table S8.4 The percentage (%) of ginsenosides in the high temperature (121 ℃, 0.1 MPa) extraction for 100 min with different pH (mean ± SD)

Ginsenosides	pH = 3.0	pH = 5.0	pH = 7.0	pH = 9.0	pH = 11.0
Rg1	2.1 ± 1.4	2.7 ± 1.7	1.4 ± 0.4	5.1 ± 2.3	5.2 ± 0.3
Re	1.9 ± 0.4	1.6 ± 0.4	4.6 ± 0.2	3.2 ± 1.1	2.7 ± 0.5
Rf	23.0 ± 1.5	6.3 ± 1.3	4.2 ± 0.1	5.8 ± 1.9	6.5 ± 0.1
Rb1	2.1 ± 0.5	1.6 ± 0.1	2.2 ± 0.6	2.0 ± 0.6	3.8 ± 0.2
Rc	2.1 ± 0.2	4.2 ± 0.0	4.0 ± 0.7	4.5 ± 0.3	5.7 ± 0.0
Rg2	2.4 ± 0.1	5.3 ± 0.0	4.6 ± 0.4	6.4 ± 2.6	5.8 ± 0.1
Rh1	5.4 ± 0.3	6.4 ± 0.0	6.2 ± 0.3	6.3 ± 0.2	6.0 ± 0.4
Rb2	3.4 ± 0.3	4.0 ± 0.1	4.5 ± 0.8	3.8 ± 0.7	3.6 ± 0.4
Rb3	0.9 ± 0.2	0.9 ± 0.3	0.9 ± 0.7	1.1 ± 0.3	1.1 ± 0.1
Rd	1.2 ± 0.0	1.1 ± 0.1	0.6 ± 0.6	0.8 ± 0.1	1.5 ± 0.2
F2	5.4 ± 0.5	4.4 ± 0.1	5.3 ± 0.3	5.3 ± 0.8	3.9 ± 0.1
Rk3	8.5 ± 0.5	7.8 ± 0.0	9.0 ± 0.2	7.1 ± 0.9	6.7 ± 0.01
Rh4	18.9 ± 1.0	14.5 ± 0.3	17.4 ± 0.7	14.6 ± 0.5	11.8 ± 0.0
20S-Rg3	10.9 ± 0.6	14.8 ± 0.6	14.6 ± 1.0	13.9 ± 0.3	14.7 ± 0.1
20R-Rg3	30.1 ± 1.8	22.7 ± 0.9	19.1 ± 1.3	18.9 ± 0.2	20.0 ± 0.0
20S-PPT	0	0.8 ± 0.2	0	0	0
20R-PPT	0.6 ± 0.4	0.9 ± 0.1	1.4 ± 0.1	1.3 ± 0.1	0.7 ± 0.0
20S-Rh2	0.1 ± 0.0	0.1 ± 0.0	0	0	0
20R-Rh2	0.1 ± 0.1	0.1 ± 0.1	0	0	0.1 ± 0.0
Rk2	0.2 ± 0.1	0	0	0	0
Rh3	0.6 ± 0.0	0.2 ± 0.0	0	0	0.2 ± 0.1
Sbcd	9.6 ± 1.2	11.7 ± 0.7	12.2 ± 2.2	12.2 ± 0.4	15.7 ± 0.4
Segf	7.0 ± 2.5	10.5 ± 0.0	10.2 ± 0.7	14.1 ± 1.5	14.4 ± 0.9
Less polar	83.5 ± 3.7	76.9 ± 1.9	77.6 ± 2.9	73.7 ± 1.1	69.9 ± 0.5
Rg3	41.0 ± 2.4	37.5 ± 1.5	33.7 ± 2.3	32.8 ± 0.5	34.7 ± 0.2

Appendix 6 Supplementary data of Chapter nine

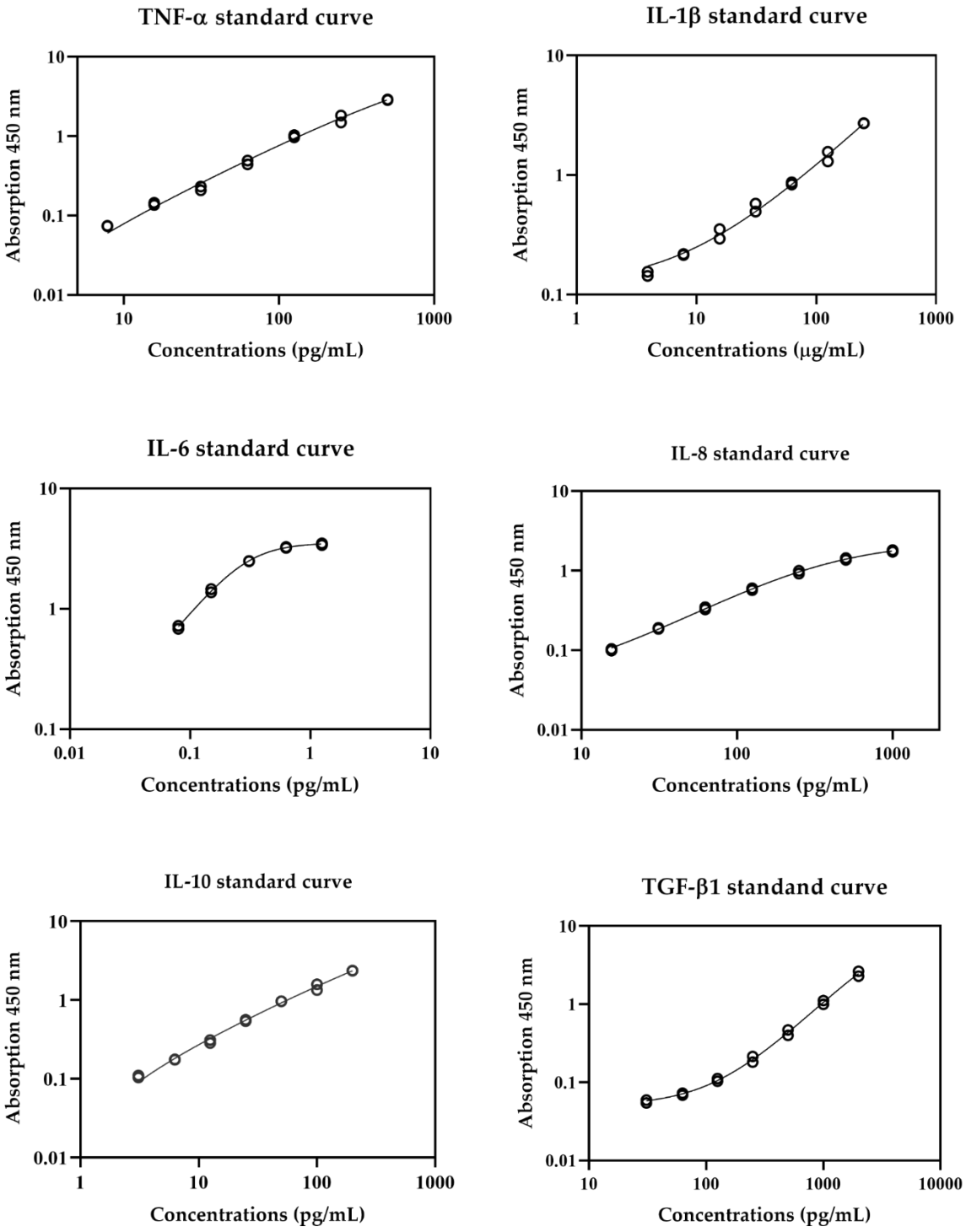

Figure S9.1 The standard curves of TNF-α, IL-1β, IL-6, IL-8, IL-10, and TGF-β1.

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Chen W, Balan P, and Popovich DG (2019) Review of ginseng anti-diabetic studies. Molecules, 24 (24), 4501.

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Chen W, Balan P, and Popovich DG (2020) Ginsenosides analysis of New Zealand–grown forest Panax ginseng by LC-QTOF-MS/MS. J Ginseng Res, 44(4): 552-562.

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Journal of Ginseng Research

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Chen W, Balan P, and Popovich DG (2019) Analysis of ginsenoside content (Panax ginseng) from different regions. Molecules, 24,3491

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Chen W, Balan P, and Popovich DG (2020) Comparison of ginsenoside components of various tissues of New Zealand forest-grown Asian ginseng (Panax ginseng) and American ginseng (Panax quinquefolium L.). Biomolecules, 10, 372.

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We, the candidate and the candidate's Primary Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated below in the *Statement of Originality*.

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Name/title of Primary Supervisor:	David Popovich / Dr
In which chapter is the manuscript /published work:	Chapter 7

Please select one of the following three options:

(●) The manuscript/published work is published or in press

- Please provide the full reference of the Research Output:

Chen W, Balan P, and Popovich DG (2020) Changes of ginsenoside composition in the creation of black ginseng leaf. Molecules, 25, 2809.

( ) The manuscript is currently under review for publication – please indicate:

- The name of the journal:

Molecules

- The percentage of the manuscript/published work that was contributed by the candidate: 95.00

- Describe the contribution that the candidate has made to the manuscript/published work:

The candidate carried out all the experiments, data analysis, made all the figures and tables, prepared the manuscript, revised and responded according to the reviewers' comments.

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- Please provide the full reference of the Research Output:

Chen W, Xu WL, Shi DX, Balan P, Popovich D. Enrichment of the less polar ginsenoside (Rg3) from ginseng grown in New Zealand by post-harvest processing and extraction. Traditional Medicine Research. 2021;6(4):39. doi:10.12032/TMR20210606233.

( ) The manuscript is currently under review for publication – please indicate:

- The name of the journal:

Traditional Medicine Research

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Chen W, Balan P, and Popovich DG. The Effects of New Zealand Grown Ginseng Fractions on Cytokine Production from Human Monocytic THP-1 Cells. Molecules 2021, 26, 1158. https://doi.org/10.3390/molecules26041158

( ) The manuscript is currently under review for publication – please indicate:

- The name of the journal:

Molecules

- The percentage of the manuscript/published work that was contributed by the candidate: 95.00

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The candidate carried out all the experiments, data analysis, made all the figures and tables, prepared the manuscript, revised and responded according to the reviewers' comments.

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