



Full Length Article

Impact of Pulsed Electric Field Assisted with β -Glucosidase Processing on Bioactive Components and Antioxidant Capacity of Ginseng Root Extraction

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Abstract

The influence of pulsed electric field assisted with β -glucosidase (CPEF) processing on the bioactive components and antioxidant capacity of ginseng (*Panax ginseng* CA Meyer) root extraction were evaluated. The maximum total ginsenoside content from ginseng root achieved 38.15 mg/g by CPEF processing (electric field intensity of 15 kV/cm, enzyme concentration of 2% (w/w) and pulse number of 10), which was higher than heat reflux extraction (HRE) and pulsed electric field (PEF) extraction. Compared to HRE and PEF processing, CPEF extraction contained more total polyphenol (18.55 mg/g), flavonoid (19.94 mg/g) and total sugar content (47.35 mg/mL). Moreover, CPEF processing showed higher DPPH and ABTS radical scavenging activities, also enhanced FRAP activity from ginseng extracts. Morphology of the CPEF treated ginseng root structure was investigated by scanning electron microscopy, which provided evidence for the structural integrity disruption. The results demonstrated that CPEF processing at appropriate intensity had a great potential on bioactive ingredient extraction. © 2020 Friends Science Publishers

Keywords: Pulsed electric field; Ginseng; β -glucosidase; Bioactive components; Antioxidant activity

Introduction

As herbal medicines in eastern Asia, ginseng contains various bioactive constituents. Up to now more than 30 ginsenosides have been reported in ginseng plant (Qi *et al.* 2011). Depending on the type of skeletons and sugar moieties, ginsenosides have various biological functions, including anticancer, antioxidant, anti-cardiovascular and mental capacity improvement (Kim *et al.* 2011b; Karmazyn *et al.* 2011; Chen *et al.* 2016; Gu *et al.* 2019; Zhang *et al.* 2019). For example, ginsenoside Rd, a dammarane-type steroid glycoside extracted from ginseng plants, was an efficient agent for acute ischemic stroke treatment through inhibiting proteasome activity in microglial (Zhang *et al.* 2016). Ginsenoside Rh₂, a ginsenoside isolated from *Panax ginseng*, can inhibit human ovarian adenocarcinoma cells SKOV3 proliferation and lead to the apoptosis of cancer cells (Kim and Choi 2016). Moreover, Lee *et al.* (2012b) found that steaming process of ginseng converted some polar ginsenosides into less polar ginsenosides. Therefore, different treatment affected the antioxidant capacity of extracts from ginseng root. The traditional extraction methods for ginseng included heat reflux extraction (Gafner *et al.* 2004), carbon dioxide processing (Wang *et al.* 2001),

ultrasound extraction (Vongsangnak *et al.* 2004), microwave treatment (Shu *et al.* 2003), ultra-high-pressure treatment (Chen *et al.* 2009), hydrolytic enzymes extraction (Lee *et al.* 2012a). However, the traditional techniques are extremely time-consuming and inefficient in treatment.

Pulsed electric field (PEF) technology has applied in the field of active ingredients extraction (He *et al.* 2014; Zderic and Zondervan 2016; Wu *et al.* 2019; Andreou *et al.* 2020; Leong *et al.* 2020; Wu *et al.* 2020). PEF processing increased the extraction ratio of bioactive components from natural material. The PEF processing led to irreversible disruption of membrane with high electric field intensity. Additionally, optimum pulsed electric field intensity improved intracellular compounds extraction by lethal cell damage (Lohani and Muthukumarappan 2016). In this study, the CPEF processing was used to extract bioactive components of ginseng root. There are some researchers reported that the PEF processing can increase enzyme activity at moderate intensity, including peroxidase, β -galactosidase and β -glucosidase (Ohshima *et al.* 2007; Aguiló-Aguayo *et al.* 2008; Lu and Yin 2014). Zhang *et al.* (2017) reported that pulsed electric field enhanced pectinase activity $21.89 \pm 1.67\%$ on the condition of electric intensity 12 kV/cm. A possible explanation might be that PEF

treatment caused many new active sites or increased active sites size of enzyme. PEF treatment changed secondary or tertiary structures of pectinase molecular, but did not affect the primary structure (Zhao *et al.* 2012).

In our previous study, pulsed electric field assisted with β -glucosidase (CPEF) processing showed high efficiency for ginsenosides extraction from ginseng root (Lu *et al.* 2017). The study of CPEF processing on bioactive components and antioxidant activity of ginseng extraction is lacking. The aim of this study was to evaluate the effects of CPEF treatment on changes of total polyphenol, flavonoid and antioxidant activity of ginseng extracts.

Materials and Methods

Materials

Four years ginseng (*Panax ginseng* CA Meyer) was grown in pots in Jilin province, dried at 60°C until reaching a constant weight, ground to fine particle and screened through an 80-mesh screen. The β -glucosidase was purchased from Baoman Biology Co. (Shanghai, China). Other chemical reagents were analytical grade and solvents were of HPLC grade.

Instruments

PEF instruments were mainly consisted of material chamber, temperature sensing instrument and pump (Yin *et al.* 2008). As represented in Fig. 1, the PEF processing depended upon high voltage induced by two stainless steel electrodes. The PEF pulse width was 2 μ s, frequency ranged from 1000 to 3000 Hz, electric field intensity ranged from 1 to 50 kV/cm. Electronic balance; Magnetic stirrer; Portable steam sterilizer; HPLC system (Agilent 1100, USA).

Methods of extracting ginsenosides from ginseng root

CPEF processing: The ginseng root powder (1 g) was extracted by deionized water and β -glucosidase. The extracts were pumped into the material chamber with 2 mL/min flow rate. The optimized operating parameters included CPEF1, pulse number 10 and electric field intensity 10 kV/cm; CPEF2, pulse number 10 and electric field intensity 15 kV/cm; CPEF3, pulse number 10 and electric field intensity 20 kV/cm; CPEF4, pulse number 8 and electric field intensity 10 kV/cm. The extracts were boiled to inactivate the enzyme and centrifuged for 20 min (5000 \times g). Then samples were freeze-dried and dissolved in 5 mL methanol.

PEF processing: The ginseng root powder was extracted by 70% ethanol on the PEF condition of pulse number 8 and electric field intensity 10 kV/cm. Then samples were freeze-dried and dissolved in 5 mL methanol.

HRE processing: The ginseng root powder was extracted by 70% ethanol and incubated at a temperature of 70°C for 6 h. Then samples were freeze-dried and used as test sample.

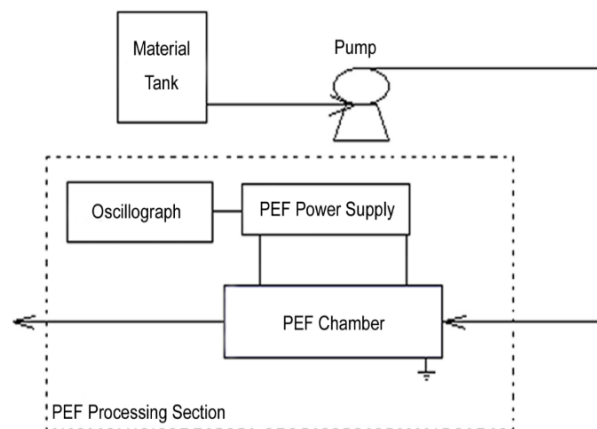


Fig. 1: Schematic of high intensity pulsed electric fields processing apparatus

Analysis of total ginsenoside content

Total ginsenoside content was measured by Agilent 1100 system and UV spectrophotometric detector. The mobile phase was water (A) and acetonitrile (B) with gradient procedure of 20% B at 0–20 min, 20% B at 20–31 min, 32% B at 31–40 min, 43% B at 40–70 min, 100% B at 70–80 min. The column temperature kept at 30°C and flow rate was 1 mL/min. The eluate was measured with wavelength 203 nm. The chromatographic peaks were determined by retention times of ginsenoside standards.

Analytical methods

The polyphenol content of ginseng extracts was measured according to Singleton and Lamuela-Raventos (1999). The flavonoid content of ginseng extracts was measured by a colorimetric assay (Woisky and Salatino 1998). Total sugar content was carried out using the phenol–H₂SO₄ methods. The glucose standards were used for quantification (Dubois *et al.* 1951).

Analysis of antioxidant activity

DPPH antioxidant activity was analyzed according to Yang *et al.* (2006). DPPH was dissolved by ethanol, and then DPPH solution (2 mL) was mixed with sample solution (2 mL). The absorbance was determined at 514 nm against ethanol as blank.

ABTS radical scavenging activity was measured according to Hu and Kitts (2001). Stable ABTS radical cation consisted of 7 mM ABTS solution and 2.45 mM potassium persulfate. Adding 50 μ L of ginsenoside extracts into 2 mL of ABTS radical solution reacted for 6 min. The absorbance was determined at 734 nm by the spectrophotometer.

FRAP measurement was similar with the method of Chen *et al.* (2010). The samples reacted with FRAP solution

for 1 h in darkness. The absorbance of the extracts was measured at 593 nm. Results for FRAP activity were expressed as $\mu\text{mol Trolox equivalents (TE)}/\text{g FW}$.

Morphology of ginseng extracts structure

The structure changes of ginseng extracts were detected by scanning electron microscopy (SEM). Samples were fixed to a specimen holder and sputter-coated with gold. The extracts were measured by high vacuum SEM (SSX-550, Shimadzu, Japan) as described by Chen *et al.* (2009).

Statistical analysis

All experiments were analyzed by one-way ANOVA and Duncan's multiple range tests using SPSS software (Version 13.0). Each experiment (optimized treatment conditions by Response Surface Methodology in previous study) was tested in triplicates and data was expressed as mean \pm SD. For each analysis, the level of significance was considered at 5%.

Results

Effect of extraction methods on ginsenoside content

The calibration curves were generated by concentrations and plotting peak areas from HPLC chromatograms. The retention time of the standard was used to identify each ginsenoside (Rb1, Re, Rb2, Rc, Rg1) in the sample. Total ginsenoside content of extracts under different processing are shown in Fig. 2. The maximum ginsenoside content of 38.15 mg/g was obtained as extracted by using electric field intensity 15 kV/cm, pulse number 10 and 2% (w/w) enzyme concentration (CPEF2). Compared with HRE and PEF treatment, CPEF processing improved the total saponin content and shortened treatment time. Meanwhile, the total ginsenoside content of CPEF2 processing (10 min) was higher than HRE treatment (6 h). Compared with HRE and PEF treatment, the ginsenoside content enhanced 1.19 times and 1.29 times respectively. Therefore, PEF treatment combined with β -glucosidase increased total ginsenoside content of ginseng extracts.

Changes in total polyphenol content of ginseng extracts

The polyphenol compounds are the important antioxidant compound in plant, and recent researches indicated that phenolic compounds exhibited antioxidative. The total phenolic content of the CPEF2 processing (18.55 mg/g) was higher than 44.81 and 18.76% as compared to HRE and PEF, respectively. The highest total polyphenol content of ginseng extracts was detected by CPEF treatment at 15 kV/cm electric field intensity, pulse number 10 and 2% (w/w) enzyme concentration (Fig. 3).

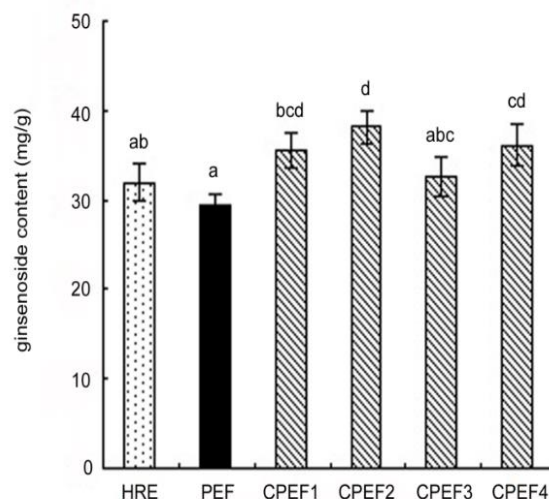


Fig. 2: Changes of ginsenoside content in ginseng root under different extraction conditions. Different letters indicate significant differences ($p < 0.05$)

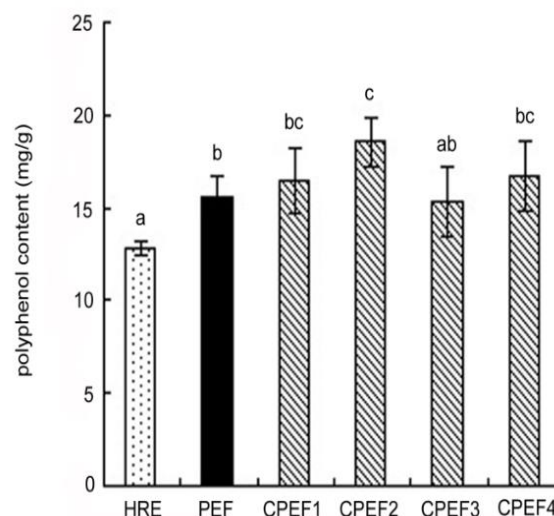


Fig. 3: Changes of polyphenol content in ginseng root extraction under different extraction conditions. Different letters indicate significant differences ($p < 0.05$)

Changes in total flavonoid content of ginseng extracts

The flavonoid compounds are complex phenolic molecules and play an important role on antioxidant activity of plant. In this study, compared to the HRE (14.69 mg/g) and PEF (16.58 mg/g) processing, total flavonoid content (19.94 mg/g) with CPEF2 processing enhanced significantly. The CPEF processing showed an increasing trend in total flavonoid content with higher pulsed electric field intensity. As pulsed electric field intensity reached 20 kV/cm, total flavonoid content decreased gradually (Fig. 4).

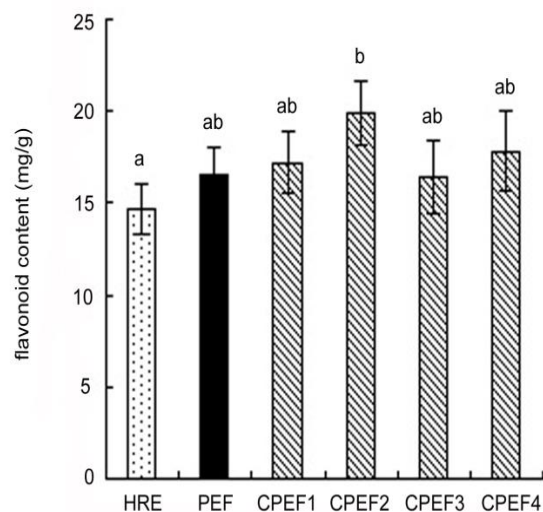


Fig. 4: Changes of flavonoid content in ginseng root extraction under different extraction conditions. Different letters indicate significant differences ($p < 0.05$)

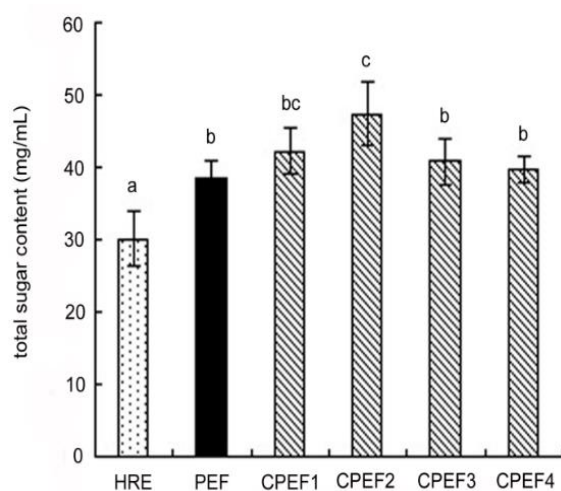


Fig. 5: Changes of total sugar content in ginseng root extraction under different extraction conditions. Different letters indicate significant differences ($p < 0.05$)

Changes in total sugar content of ginseng extracts

The total sugar content of ginseng extracts with HRE, PEF and CPEF treatment were shown in Fig. 5. The total sugar presented higher levels by CPEF treatment compared to HRE and PEF processing. However, total sugar content of ginseng extracts increased (47.35 mg/mL) until pulsed electric field intensity 15 kV/cm and then showed decreasing tendencies under CPEF treatment. The pulsed electric field intensity might be the most important factors on CPEF processing. Also it was directly correlated to the ginsenoside content of ginseng root extraction.

Changes in antioxidant activity of ginseng extracts

Table 1 showed the DPPH antioxidant activities of ginseng extracts with HRE, PEF and CPEF treatment. The results indicated that CPEF2 treatment (61.11%) showed a high efficiency in scavenging activity, followed by PEF (54.89%) and HRE (51.63%) method. The ABTS radical scavenging capacities of ginseng extracts with different treatment was shown in Table 1. The ABTS antioxidant activity of CPEF2 processing (52.16%) was higher than PEF (45.83%) and HRE (43.06%) processing. When the pulsed electric field achieved 20 kV/cm, the ABTS scavenging activity of ginseng extract decreased to 46.39%. The FRAP antioxidant activity of ginseng extracts was analyzed under different processing. PEF and CPEF extraction showed higher FRAP values than HRE processing. The FRAP activity of extracts increased significantly, while the electric field intensity achieved 15 kV/cm. However, the FRAP activity of extracts decreased gradually with higher electric field intensity. On the CPEF conditions of electric field intensity 15 kV/cm and pulse number 10, the FRAP activity of ginseng extracts achieved maximum 9.61 $\mu\text{mol TE/g}$. It was indicated that CPEF processing enhanced FRAP activity of ginseng extracts (Table 1).

Changes in structure of ginseng particles

The ginseng particles by CPEF treatment was detected under scanning electron microscopy. The SEM images revealed that the significant structure changes were caused by different extraction processing. From the micrographs of the untreated samples, it can be observed that the structures of ginseng extracts were kept intact (Fig. 6A). In the case of PEF processing, the ginseng particles had puny damage (Fig. 6B). Under the CPEF processing, the ginseng particles generated hollow openings and many small particles (Fig. 6C).

Discussion

In our study, PEF treatment combined with β -glucosidase increased total ginsenoside content of ginseng extracts. Hou *et al.* (2010) also found that PEF was highly efficient on ginsenoside extraction from *Panax ginseng*. The advantages of CPEF treatment were mild reaction, speediness and low power. Moreover, the CPEF treatment used the aqueous medium as solvent reduced the purified procedures of ginsenoside root extraction. These results presented a promising way to extract bioactive compounds.

Results of this study indicated that CPEF extraction enhanced total polyphenol (18.55 mg/g), flavonoid (19.94 mg/g) and total sugar content (47.35 mg/mL). Lee *et al.* (2011) described that the high hydrostatic pressure processing increased polyphenol amounts of red ginseng until 30 MPa of pressure (Lee *et al.* 2011). The polyphenols

Table 1: Changes of antioxidant activity in ginseng root extraction under different extraction conditions

Treatment	HRE	PEF	CPEF1	CPEF2	CPEF3	CPEF4
DPPH scavenging activity (%)	51.63±4.51a	54.89±2.50ab	56.56±4.12ab	61.11±4.90b	55.72±3.81ab	58.40±4.97ab
ABTS scavenging activity (%)	43.06±3.09a	45.83±0.87ab	47.56±2.95ab	52.16±4.24b	46.39±3.99ab	50.30±4.14b
FRAP (μmol TE/g)	3.96±0.91a	7.22±0.42b	8.03±0.52bc	9.61±0.60d	8.69±0.77cd	8.30±0.69bc

Different small letters indicate significant differences among treatment means ($p < 0.05$)

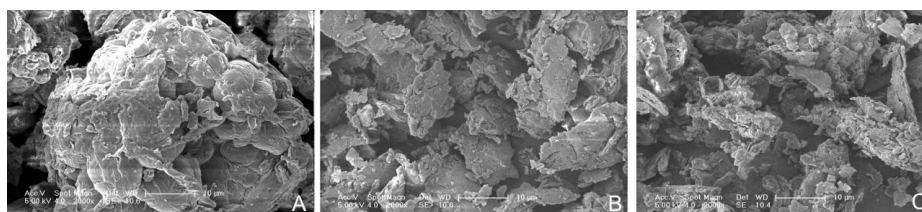


Fig. 6: Electron micrograph of samples. (A) untreated sample; (B) sample treated by PEF (electric field intensity 10 kV/cm, pulsed number 8); (C) sample treated by CPEF (electric field intensity 15 kV/cm, pulsed number 10)

content of fresh tea leaves was determined by pulsed electric field intensity and processing time (Zderic and Zondervan 2016). Kim *et al.* (2011a) reported that polyphenol content was enhanced with higher antioxidant activity of ginseng extracts. It has been reported that PEF extraction method significantly increased the total flavonoid content of two grape varieties (Vicas *et al.* 2016). Under optimum PEF treatment conditions, the total flavonoid contents from defatted seed cake achieved maximum yield (Teh *et al.* 2015a). Previous studies have shown that total sugar content from *Rana temporaria chensinensis* by PEF method was 26.34% higher than compound extraction method (Yin *et al.* 2006). Therefore, CPEF processing enhanced extraction efficiencies of total sugar content from ginseng root extracts.

It was evident from the results that CPEF processing showed higher DPPH antioxidant activities (61.11%) and ABTS antioxidant activities (52.16%), also higher FRAP activity from ginseng extraction (9.61 μmol TE/g). Chen *et al.* (2009) reported that ultrahigh pressure extraction showed significantly higher radical scavenging activity of extracts from ginseng root. Moreover, pulsed electric field assisted extraction enhanced the DPPH scavenging activity in the extract from defatted canola seed cake (Teh *et al.* 2015b). Therefore, CPEF treatment significantly enhanced anti-radical activity (DPPH antioxidant activities 11.33%, ABTS antioxidant activities 13.81% and FRAP activity 33.1%) of ginseng root extracts compared with PEF treatment. With ultrasound assisted extraction, the higher ABTS scavenging activity of rapeseed extracts was positive correlation with total polyphenol (Szydłowska-Czerniak and Tułodziecka 2014). Compared with untreated samples, the FRAP antioxidant capacity of homogenized grapes increased significantly with PEF processing (Vicas *et al.* 2016). It was reported that PEF processing (30 V voltage and 5% ethanol) enhanced the FRAP activity of extracts from defatted flax seed cake (Teh *et al.* 2015b). Therefore, the higher antioxidant capacity of ginseng root extraction might be

controlled by extraction process. In the case of CPEF processing, the ginseng particle generated hollow openings and many small particles. These results might be related with the high pulsed electric field intensity, causing the ginseng particle broken and appearance of small particles. These results indicated that CPEF processing induced structural crack in the surface of ginseng particles and released more bioactive components with disrupted structure.

Conclusion

Compared to HRE and PEF processing, the CPEF treatment provided higher ginsenoside content, biological compound, radical scavenging capacity and shorter extraction time. Under CPEF processing, the important factors in ginseng root extraction were pulsed number and electric field intensity. This technology might be applied in the nutraceutical industry that enhanced the bioactive components content of ginseng extracts. Further study may facilitate the large-scale application of PEF technology on bioactive ingredients extraction.

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