

Comparison of antioxidant activity of ginseng root extracts obtained by pulsed electric field and hydrolytic enzyme processing

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Abstract. The antioxidant activity and biological compounds of ginseng root extracts were compared during pulsed electric field (PEF) and hydrolytic enzyme (HE) processing. The total ginsenoside content of ginseng root extracts by PEF (29.51 ± 1.67 mg/g) was higher than cellulase enzyme (HEC 23.74 ± 1.85 mg/g) and β -glucosidase enzyme (HEG 25.31 ± 2.34 mg/g) treatment. The ginseng root extracts obtained by PEF contained more total polyphenols (15.62 ± 1.07 mg/g) and flavonoids (16.58 ± 1.5 mg/g) than HEC (polyphenols 10.76 ± 1.22 mg/g; flavonoids 13.2 ± 1.63 mg/g) and HEG (polyphenols 14.09 ± 0.39 mg/g; flavonoids 15.37 ± 1.05 mg/g) treatment. Moreover, PEF processing showed more powerful scavenging activities against DPPH, ABTS radicals and FRAP activity of ginseng root extracts. Scanning electron microscopic examination of the ginseng root tissue with PEF processing provided direct evidence for the disruption of structure integrity. These results indicated that bioactive ingredients extraction with higher antioxidant activity could be obtained via PEF processing.

1. Introduction

As the healthy food and traditional medicine, ginseng was consumed widely in Asian countries. So far, more than 60 kinds of ginsenosides have been identified from ginseng plant [1]. Ginsenosides included 20(S)-protopapaxdiol and 20(S)-protopapaxtriol categories according to sugar moieties. For example, ginsenoside Rb1, Rc and Rg3 belonged to 20(S)-protopapaxdiol; ginsenoside Re, Rh1 and Rg1 belonged to 20(S)-protopapaxtriol [2]. The ginsenosides had various effects for human health, including antioxidant, immunomodulatory, neuro-protective, anticancer and antifatigue [3-5].



There are many methods for extracting ginsenoside from ginseng root, such as heat reflux [6], ultrasound extraction [7], microwave extraction [8], supercritical carbon dioxide extraction [9] and ultrahigh pressure extraction [10]. Pulsed electric field (PEF) belonged to a non-thermal technique for food processing [11]. The electrical damage of PEF caused reversible or irreversible pores formation of cell membrane. With the increasing intensity of PEF processing, the reversible pores formation converted into irreversible disruption in membrane. The PEF treatment had applied in the fields of bioactive ingredients extraction [12-14]. The PEF processing can enhance the extraction ration of polysaccharides from corn silk [15]. The maximum polyphenols yield of fresh tea leaves achieved 27% at intensity of electric field 0.9kV/cm [16]. A research reported the different conditions of pulsed electric field on chondroitin sulphate (CS) content in fish bone. These results indicated that the PEF processing enhanced the extraction yield of CS comparison with traditional methods [11]. Recent studies have focused the application of the enzyme hydrolysis on ginsenoside extraction. Lee *et al.* reported that single enzyme of Ultraflo L (UTGL) treatment showed higher levels of ginsenoside content (406.1 g/mg) from ginseng leaf than other enzymes (celluclast 1.5 L, cytolase PCL5 and viscozyme L) [17]. Compared with untreated ginseng leaves, the DPPH and ABTS antioxidant activities were significantly higher than those with UTGL treatment [17].

The ginseng root extract had important potential application on food industry and pharmaceutical industry. In the present study, we compared total ginsenoside content, antioxidant activity and biological compounds under PEF, HEC and HEG extraction.

2. Materials and methods

2.1. Materials

The ginseng roots (four year old) was obtained from Jilin province, dried in vacuum at 60°C and passed through an 80 mesh screen. The cellulase enzyme and β -glucosidase were purchased from Baoman Biology (Shanghai, China). The HPLC solvents were analytical grade and were purchased from J. T. Baker, (PA, USA). All other chemical reagents were analytical grade.

2.2. Instruments and apparatus

The PEF treatment instrument was indicated in Figure 1(a). This apparatus consisted with material chamber, pump, pulse generator and sensing temperature device [12]. The following instruments and apparatus: electronic balance (JA10002, Shanghai, China); centrifuge biofuge heraeus (4000-40,000 r/min, Shanghai, China); rotary evaporator (RE-52A, Shanghai, China); the HPLC instrument (Agilent 1100, HP Technologies, USA); spectrophotometer (UV-VIS8500, Beijing, China); steam sterilizer (DSX-280B, Shanghai, China); freeze dryer (ZL-10TD, Shanghai, China); magnetic stirrer (85-2, Jintan, China) were used in the present study.

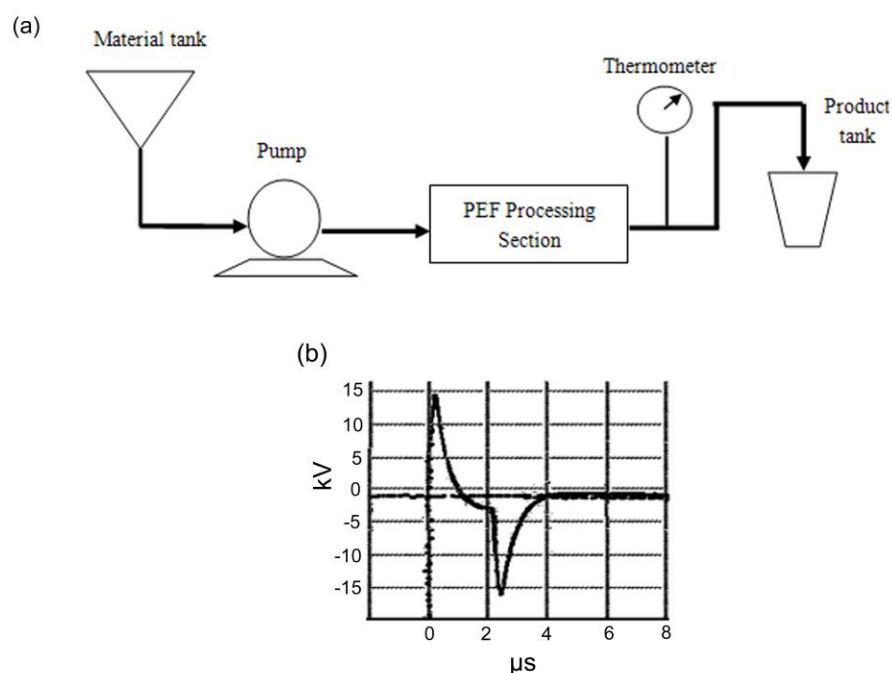


Figure 1. (a) Diagram of high intensity pulsed electric fields processing instrument. (b) The pulsed wave form.

2.3. Methods of extraction of ginsenosides from ginseng root

2.3.1. PEF extraction. The ginseng powder (1g) was treated with 70% ethanol solution (50mL) on the condition of pulsed electric field 10 kV cm^{-1} (PEF1), pulsed electric field 15 kV cm^{-1} (PEF2), pulsed electric field 20 kV cm^{-1} (PEF3) and pulse number 8, respectively [18]. The ginseng root extracts were centrifuged (5000 r/min for 20 min) after treatment. The extracts were freeze-dried and then dissolved in methanol as test solution.

2.3.2. Hydrolytic enzyme extraction. The ginseng powder (1g) was extracted with distilled water (50mL), adding commercial enzyme cellulose and β -glucosidase to the solution. The solutions were extracted at 50°C for 4 h (HEC1 and HEG1), 6 h (HEC2 and HEG2) and 8 h (HEC3 and HEG3). To inactivate the enzyme, the treated solutions were boiled for 10 min. Then, ginseng root extracts were centrifuged (5000 r/min for 20min). The freeze-dried extracts were dissolved in methanol as test solution.

2.4. Determination of total ginsenoside content

The HPLC analysis was used Agilent 1100 system (reverse phase C_{18} column) and a UV spectrophotometric detector. The column temperature was maintained at 30°C . The mobile phase included solvent A (water) and solvent B (acetonitrile). The flow rate of solvent was 1 mL/min. The eluate was measured at 203 nm wavelength and 10 μL injection volume.

2.5. Analysis total polyphenol and flavonoid content

The modified method from Singleton and Lamuela-Raventos was used to determine total polyphenol content of ginseng root extraction [19]. The colorimetric assay was used to determine total flavonoid content of ginseng root extraction [20-21].

2.6. Analysis DPPH radical scavenging activity

The DPPH radical scavenging activity was measured by the method from Yang [22]. The DPPH power (2.5 mg) was dissolved in 10 mL ethanol and mixed completely. DPPH solution in ethanol (2 mL) was added to 2 mL sample solution. The absorbance of the extract was measured at 514 nm at room temperature, using ethanol as the blank.

$$SA(\%) = 1 - \frac{A_I - A_J}{A_0} \times 100$$

A_I indicated absorbance of DPPH and sample; A_J indicated absorbance of sample and ethanol; A_0 indicated absorbance of DPPH and ethanol.

2.7. Analysis ABTS radical scavenging activity

The ABTS antioxidant activity was analyzed using the method of Hu and Kitts [23]. The 7 mM ABTS stock solution was mixed with 2.45 mM potassium persulfate as ABTS radical cation in darkness for 14 h. The absorbance of ABTS solution was adjusted to 0.7 with distilled water. The ginsenoside root extract 50 μ L was added to 2 mL ABTS radical solution for reacting 6 min. The spectrophotometer was used to determine the ABTS antioxidant activity at 734 nm using trolox as positive control [23].

$$\text{Inhibition ratio (\%)} = (A_0 - A_1) / A_0 \times 100$$

where A_0 is the absorbance of the control, and A_1 is the absorbance of the test sample.

2.8. FRAP assay

The FRAP assay was determined following the method from Chen [24]. The ginseng root extracts were mixed with the FRAP solution for 1 h in dark condition. The absorbance was determined at 593 nm against water as a blank. The results were indicated by μ mol of Trolox equivalents (TE)/g FW.

2.9. Analysis structure changes of ginseng root tissue after extraction

To elucidate the effect of each extraction procedure on structure changes, ginseng root extraction with CPEF processing was analyzed by scanning electron microscopy (SSX-550 SEM). The sample particles were fixed on a specimen holder with aluminum tape and then sputter-coated with gold [10].

2.10. Statistical analysis

One way ANOVA and Duncan's multiple range tests were analyzed by SPSS 13.0 software, and statistical significance was showed as $P < 0.05$. The experiments were completed in three times and all data were presented as standard deviations (SD).

3. Results and discussion

3.1. Comparison of ginsenoside content between PEF and HE extraction

Total ginsenoside content of ginseng root extraction under PEF and HE treatment was shown in Figure 2. The extraction yield of ginsenoside by PEF2 processing was higher than for HEC2 and HEG2 treatment. Its content was 1.24 times higher than that with HEC2 treatment, and 1.17 times higher than that with HEG2 treatment. These results showed that the PEF processing enhanced the extractability of ginsenoside from ginseng root. Moreover, the extraction time of PEF2 processing was shorter than that with HE treatment. Therefore, the PEF processing was highly efficient to prepare ginsenoside from ginseng root.

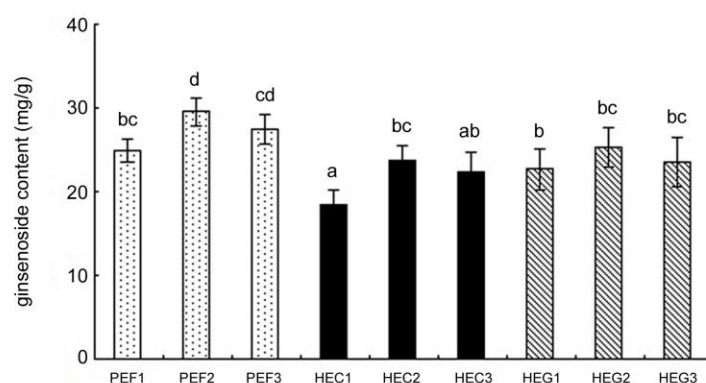


Figure 2. Changes of ginsenoside content in dry mass of ginseng root with different PEF treatment conditions.

3.2. Comparison of total polyphenol content between PEF and HE extraction

As important secondary metabolites in plant, polyphenol compounds had antioxidative effect and preventive effect on cardiovascular diseases [25]. The total phenolic content by PEF2 processing was 15.62 mg/g, which was 45.3 % higher compared to that with HEC2 treatment and 10.86 % higher compared to that with HEG2 treatment (Figure 3). The accumulation of total phenolic compounds may affect the biological activities of ginseng root extraction [26].

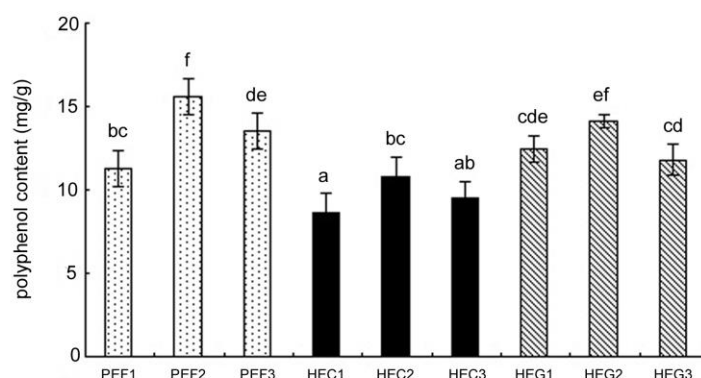


Figure 3. Changes of polyphenol content in dry mass of ginseng root extracts with different PEF treatment conditions.

3.3. Comparison of total flavonoid content between PEF and HE extraction

As complex phenolic molecules, the flavonoid compounds have distinctive antioxidative characteristics in plant [27]. The total flavonoid is important antioxidant ingredients of ginseng root. Total flavonoid contents of ginseng root extract were summarized in Figure 4. The PEF2 treatment contained higher content of total flavonoid (16.58 mg/g) than that with HEC2 (13.19 mg/g) and HEG2 treatment (15.37 mg/g).

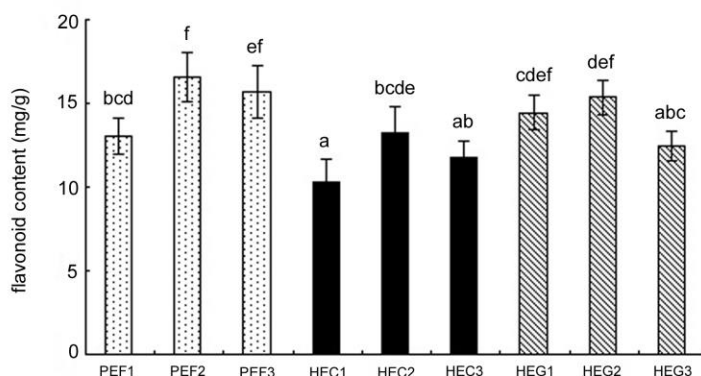


Figure 4. Changes of flavonoid content in dry mass of ginseng root extracts with different PEF treatment conditions.

3.4. Compared with DPPH radical scavenging activity between PEF and HE extraction

There is an interdependent relationship between structure and radical scavenging activity of ginsenoside [28]. We compared the radical scavenging activity of ginseng root extracts obtained with PEF and HE treatment. Figure 5 showed the DPPH radical scavenging activity of ginseng root extracts produced with different methods. Among the different methods, PEF2 method (54.89 %) indicated the maximum efficiency on DPPH scavenging activity, followed by HEG2 (53.59 %) and HEC2 method (46.23 %). Therefore, the ginseng root extracts by PEF processing showed significant capacity of scavenging the free DPPH radicals.

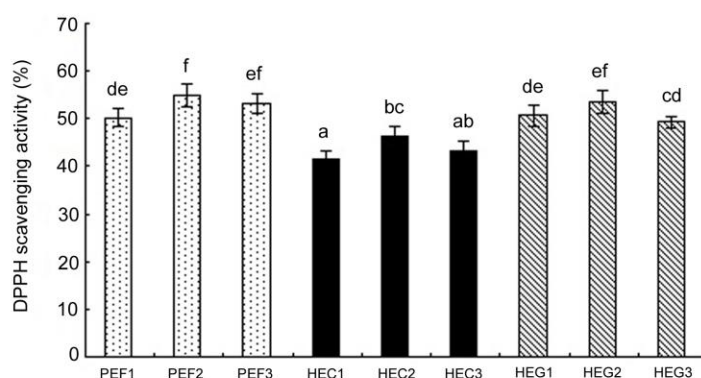


Figure 5. Changes of DPPH scavenging activity of ginseng root extraction with different PEF treatment conditions.

3.5. Comparison of ABTS radical scavenging activity between PEF and HE extraction

The antioxidant capacity of plant can be analyzed by ABTS radical scavenging activity according to the ability of reducing radical cations [29]. The ABTS radical scavenging activity of ginseng root extract was shown in Figure 6. Compared with HEG2 treatment (44.28 %) and HEC2 treatment (40.49 %), the PEF2 processing had significantly higher ABTS radical scavenging activity (45.83 %). From these results, it is confirmed that PEF processing showed higher antioxidant capacity of gradients. These results might be related with the altered position of phenolic group.

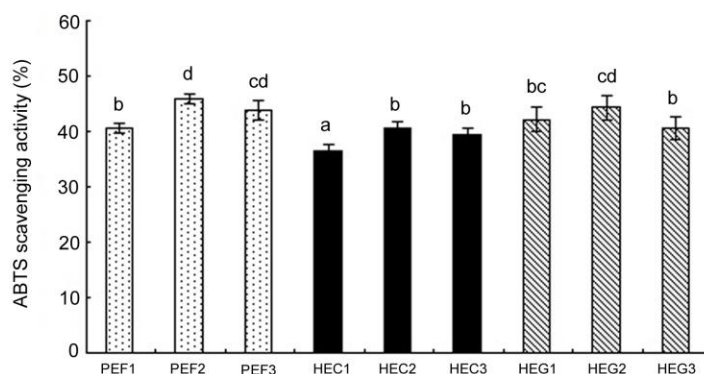


Figure 6. Changes of ABTS scavenging activity of ginseng root extracts with different PEF treatment conditions.

3.6. Comparison of FRAP activity between PEF and HE extraction

Antioxidant capacity of ginseng root extracts as evaluated by FRAP was summarized in Figure 7. The ginseng root extracts via PEF2 processing showed higher FRAP values than that via HEC2 and HEG2 processing. The FRAP values of PEF extraction showed a rapid increase until electric field intensity 15 kV cm^{-1} , then decreased with higher electric field intensity. The maximum FRAP values achieved $7.22 \text{ } \mu\text{mol (TE)/g FW}$ at the PEF parameters of electric field intensity 15 kV cm^{-1} and pulse number 8. The analysis clearly demonstrated that PEF processing was capable of improving the antioxidant capacity of ginseng root extracts.

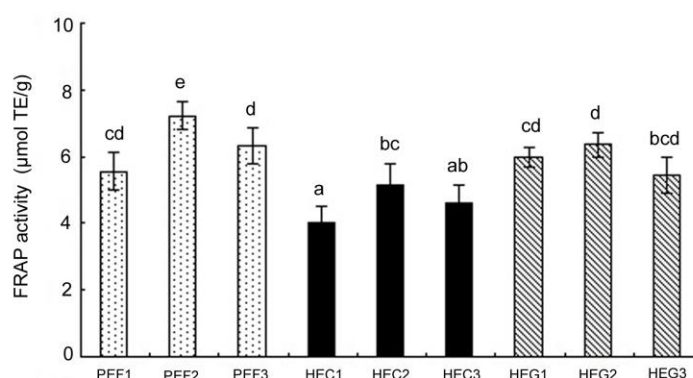


Figure 7. Changes of FRAP activity of ginseng root extracts under different extraction conditions.

3.7. Comparison of structure changes of ginseng particles between PEF and HE extraction

The samples treated with PEF and HE processing were analyzed by scanning electron microscopy. There were significant structural changes of ginseng root powder under PEF and HE treatment. As can be seen from Figure 8a, the ginseng root structures of untreated samples were kept intact, described by SEM micrograph. However, the ginseng root structure of PEF2 processing generated hollow openings and smaller particles (Figure 8b). The samples of HEC2 and HEG2 treatment observed only puny damage of structure (Figure 8c and Figure 8d). These results indicated that the surface tension of ginseng root structure were destroyed by PEF processing and induced many small particles generation.

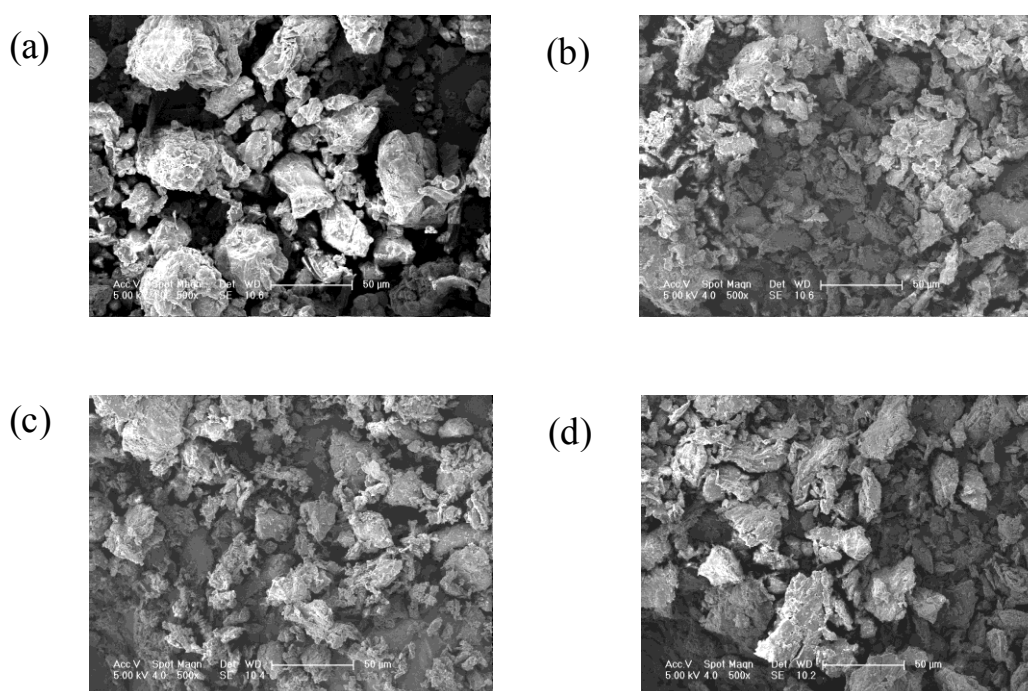


Figure 8. Electron micrograph of samples. (a) untreated sample; (b) sample treated by PEF (pulsed electric field 10kV/cm, pulse number 8); (c) sample treated by HEC; (d) sample treated by HEG.

4. Conclusion

In this study, total biological compounds and antioxidant activity of ginseng root extractions obtained by PEF, HEC and HEG methods were investigated. Compared with the HEC and HEG methods, the PEF processing had higher ginsenoside extraction efficiency, higher total polyphenol and flavonoid content. The process indicated higher antioxidant activity of ginseng root extractions. The PEF treatment as a non-thermal technology was high yield, high speed and low energy consumption, compared to the traditional methods. Therefore, PEF processing was capable to extract bioactive ingredients from plant materials with high quality.

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