Research Article Open Access

Chengwen Lu, Yongguang Yin*

Optimum Conversion of Major Ginsenoside Rb1 to Minor Ginsenoside Rg3(S) by Pulsed Electric Field-Assisted Acid Hydrolysis Treatment

https://doi.org/10.1515/chem-2018-0031
received December 4, 2017; accepted February 6, 2018.

Abstract: Ginsenoside Rg3(*S*) is a primary bioactive component in ginseng, which has pharmacological effects and nutritional activities. In the present study, pulsed electric field (PEF)-assisted acid hydrolysis processing was used to convert major ginsenoside Rb1 to minor ginsenoside Rg3(*S*). The optimum parameters of PEF assisted acid hydrolysis were analyzed by response surface methodology (RSM). The optimum processing conditions were: electric field intensity, 20 $kVcm^{-1}$; acid concentration, 0.25 mol/L; pulse number, 10. The conversion rate of ginsenoside Rg3(*S*) achieved 68.58%, in accordance to the predicted value. The structure of hydrolyzed product was confirmed by ^{13}C-NMR. The results suggested that PEF-assisted acid hydrolysis significantly enhanced conversion rate of ginsenoside Rg3(*S*).

Keywords: Pulsed electric field; Ginsenoside Rb1; Ginsenoside Rg3(*S*); Acid hydrolysis.

1 Introduction

Ginseng has been used in a wide range of medicinal therapies and health food for thousands years in Eastern Asia. The major bioactive constituents in ginseng are ginsenosides, which demonstrated various pharmacological and therapeutic activities [1-3]. The minor ginsenoside Rg3(*S*) is one of the main bioactive components of ginsenosides. It is well known that ginsenoside Rg3(*S*) possessed an ability to inhibit the lung metastasis of tumor cells, suppressed the tumor cell invasion induced by 1-oleoyl-lysophosphatidic acid (LPA), and provided protection against the cerebral ischemia induced injury in a rat brain [4-6]. The ginsenoside Rg3 prevented cancer either singularly or synergistically, and promoted cisplatin efficacy through inhibiting HO-1 expression in cancer cells [7-8]. Moreover, ginsenoside Rg3 inhibited anti-inflammatory activity in a macrophage cell [9].

However, minor ginsenoside Rg3(*S*) is only present in red ginseng in small percentages (less than 0.003%) [10-12]. It is difficult to extract minor ginsenoside from ginseng. Up to now, many studies have aimed to produce Rg3(*S*) by chemical and biological methods. For example, the yield of ginsenoside Rg3(*S*) from red ginseng was enhanced by citric acid treatment [13]. The protopanaxadiol ginsenosides of ginseng water extraction were hydrolyzed to ginsenoside Rg3(*S*) under acidic conditions [11]. But the disadvantage of acid hydrolysis processing was high temperature and long hydrolysis time. Moreover, ginsenoside Rg3(*S*) can be produced by enzymatic and microbial treatment. For example, compared with traditional white ginseng extract, the quantity of ginsenoside Rg3(*S*) increased 4 times by Celluase-12T treatment [14]. The ginsenoside Rg3(*S*) was prepared by two glycoside hydrolases processing with high yield [15]. The *Microbacterium* sp. GS514 had an ability to transform ginsenoside Rb1 to Rg3 [16]. However, the cost of preparation ginsenoside by enzymatic or microbial treatment was high.

Pulsed electric field treatment was used in food sterilization, enzymatic reactions and extraction process [17-24]. Until recently, it was reported that PEF treatment can accelerate the rate of the chemical and enzymatic reactions with high efficiency and less energy consumption [21]. For example, PEF treatment accelerated the alkaline hydrolysis processing and enhanced the extraction ratio of chondroitin sulfate from fish bone [22]. Compared with untreated enzyme, the activity of pectinase increased 21.89 ± 1.67% under the electric field intensity 12 kV/cm [23]. Furthermore, the pulsed electric field combined with

***Corresponding author: Yongguang Yin,** College of Biological and Agricultural Engineering, Jilin University, Changchun 130022, PR China, E-mail: biofoodyyg@hotmail.com
Chengwen Lu: College of Life Science, Changchun Normal University, Changchun 130032, PR China

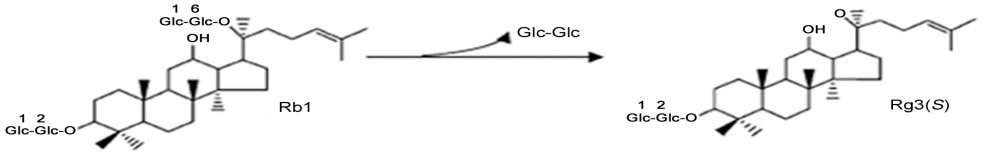

Figure 1: Transformation pathways from ginsenoside Rb1 into Rg3(*S*) by PEF assisted acid hydrolysis treatment.

enzyme hydrolysis transformed ginsenoside Rb1 to minor ginsenoside Rd [24].

The ginsenoside Rb1 is especially the most abundant ginsenoside (23%) in ginseng, and its structure can be easily converted to ginsenoside Rg3(*S*) by hydrolyzing the glcosidic-linkage at the C-20 position (Figure 1). The aim of this study was to optimize the conditions of PEF-assisted acid hydrolysis, which converted ginsenoside Rb1 to ginsenoside Rg3(*S*). The research data will provide a valuable method for preparing minor ginsenosides and promote the PEF technology application.

2 Materials and Methods

2.1 Materials and Instrumentation

Ginsenoside standards were purchased from Must Biotech Co. Ltd, Chengdu China. HPLC analytical grade solvents were purchased from J.T. Baker, USA. All other chemical reagents were analytical grade and deionized water was used to prepare sample solutions.

The PEF flowing system included a coaxial liquid material chamber, a high-voltage pulse generator, a pump and a fiber-optic temperature-sensing instrument [20]. The PEF processing apparatus was shown in Figure2. The PEF treatment procedure included a short burst of high voltage to materials between two stainless steel electrodes. The gap of two stainless steel electrodes was 0.1cm in treatment chamber. The pulse waveform was triangle wave. The PEF frequency was adjustable from 1000 to 3000 Hz, electric field strength ranged from 1 to 50 kV cm^{-1}, and pulse width was 2 μs. The inlet temperature was 20 and outlet temperature was 23 . A digital oscilloscope measured the input voltage and pulse waveform applied to the materials. The PEF system treated materials continuously.

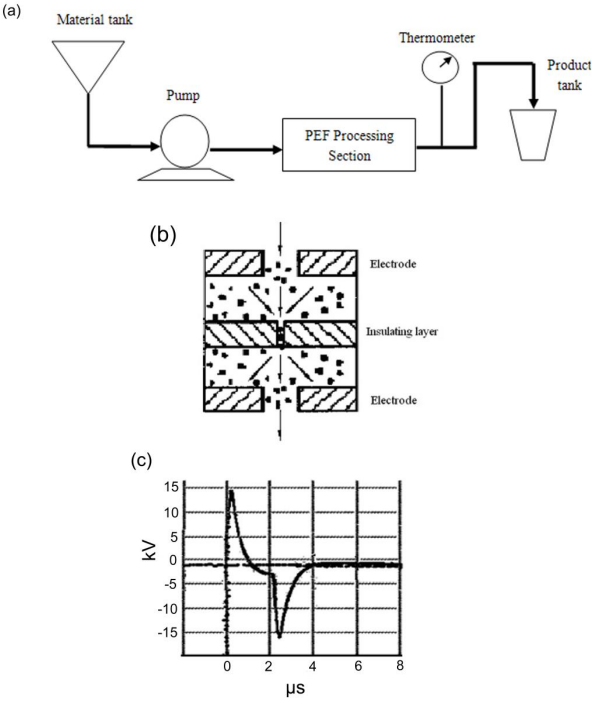

Figure 2: (a) Schematic of high-intensity pulsed electric fields processing apparatus (b) Structure diagram of PEF treatment chamber (c) Form of pulsed wave.

2.2 Preparation of Ginsenoside Rg3(*S*) with PEF Treatment

Standard ginsenoside Rb1 was dissolved in hydrochloric acid concentration from 0.15 mol/L to 0.35 mol/L. The sample solutions were then pumped into the treatment container and the high voltage pulse was turned on.. In order to optimize the condition, the pulse frequency and charge voltage were adjusted. The flow velocity was kept at 2 mL min^{-1}. The reaction mixtures were neutralized with sodium hydroxide, extracted with n-BuOH saturated with H_2O and measured by HPLC.

2.3 HPLC Analysis of Ginsenoside Rg3(*S*) Content

The HPLC analysis was performed using Agilent 1100 system with a reverse phase C_{18} column (4.6×150, 5 μm) and a UV detector. The hydrolyzed products were identified by retention times of the ginsenoside standards. The mobile phase consisted of solvent A (water) and solvent B (acetonitrile) were used for separation. The solvent gradient condition was 20% B (0-20 min), 20% B (20-31 min), 32% B (31-40 min), 43% B (40-70 min), 100% B (70-80 min) at a flow rate of 1mL/min. The column temperature was maintained at 30 C. The injection volume was 10 μL and the eluent was measured at a wavelength of 203 nm.

2.4 Single Factor Experiments

The PEF parameter: electric field intensity (10, 15, 20, 25, and 30 kV cm^{-1}), pulse number (6, 8, 10, 12 and 14) and acid concentration (0.15, 0.2, 0.25, 0.3 and 0.35 mol/L) were analyzed.

2.5 Experimental Design of RSM

The Box-Behnken design (BBD) was applied to optimize conditions of three variables (X_1, electric field intensity; X_2, pulse number; X_3, acid concentration) [25, 26]. For statistical calculation, the independent variables were coded according to equation (1):

$$x_i = \frac{X_i - X_0}{\Delta X} \quad (1)$$

Where x_i represented the coded value of the variable X_i, X_0 was the actual value of X_i in the centre of the domain, and ΔX was the step change value. The three levels of independent variables were depicted in Table 1. The complete design of 17 experimental runs was employed in a random order as shown in Table 2. The polynomial second degreed model was used by the following equation.

$$Y = A_0 + \sum_{i=3}^{3} A_i X_i + \sum_{i=3}^{3} A_i X_i^2 + \sum \sum_{i<j=1}^{3} A_j X_i X_j \quad (2)$$

where Y represented the response variable, A_0, A_i, A_{ii}, and A_{ij} were regression coefficients of variables for intercept, linear, quadratic and interaction coefficients, respectively. X_i and X_j were the levels of the independent variables ($i \neq j$).

Table 1: Experimental range and levels of the independent variables used for Box-Behnken rotatable design.

	Level		
Independent variable	**-1**	**0**	**1**
Electric field intensity (kV cm^{-1}, X_1)	15	20	25
Pulse number (X_2)	8	10	12
Acid concentration (mol/L, X_3)	0.15	0.25	0.35

Table 2: Box-Behnken design matrix and response values showing the conversion rate of ginsenoside Rg3(*S*).

Standard order	X_1	X_2	X_3	Conversion rate (%)
1	1	-1	0	53.45
2	0	0	0	68.19
3	0	0	0	67.74
4	1	0	-1	56.81
5	-1	0	-1	41.43
6	0	-1	1	59.47
7	0	1	1	54.42
8	1	1	0	49.17
9	0	0	0	68.58
10	-1	1	0	48.39
11	0	-1	-1	50.27
12	-1	0	1	52.24
13	-1	-1	0	43.73
14	0	1	-1	58.31
15	0	0	0	67.36
16	1	0	1	51.28
17	0	0	0	68.35

2.6 Structural Identification

Ginsenoside Rb1 (1 mM) was hydrolyzed by PEF assisted acid hydrolysis treatment. The sample solutions were treated with n-BuOH and evaporated to dryness. The crude residues were dissolved with MeOH and separated on a silica gel column using $CHCl_3$/MeOH/H_2O (9:3:1). The fraction was evaporated in vacuum and dissolved in pyridine-d_5. The structure was analyzed via ^{13}C-NMR (a Bruker spectrometer at 500 MHz).

2.7 Statistical Analysis

All experiments were completed in triplicates. The response contour plots of experimental data were analyzed by Design Expert 7.0. The statistical significance was performed by one-way analysis of variance (ANOVA). For each analysis, the significance was considered by confidence level P<0.05.

Ethical approval: The conducted research is not related to either human or animals use.

3 Results and Discussion

3.1 Effect of Electric Field Intensity on Preparation of Ginsenoside Rg3(*S*)

In this study, the electric field intensity was an important variable in accelerating the hydrolysis of ginsenoside Rb1. The different electric field intensity was controlled by adjusting electric voltage. The HPLC profile of the reaction mixture was shown in Figure 3. The peak with retention time 45.88 corresponded to ginsenoside Rg3(*S*). The results demonstrated that the Rg3(*S*) content was significantly increased by PEF-assisted acid hydrolysis. It can be seen from Figure 4 that the hydrolysis of ginsenoside Rb1 enhanced gradually with the higher electric field intensity. The maximum conversion rate of Rg3(*S*) achieved 59.38% until the electric field intensity 20 kVcm^{-1}. When the electric field intensity achieved 25 kVcm^{-1}, the conversion rate of Rg3(*S*) reduced to 51.06%. Therefore, the electric field intensity 20 kV cm^{-1} was chosen to prepare ginsenoside Rg3(*S*). The conversion rate of Rg3(*S*) (59.38%) was higher than the organic synthesis of Rg3(*S*) (12.8%) [27]. While the electric field intensity exceeded 30 kVcm^{-1}, the electric field electrode caused sparks and wave fluctuation of PEF. This irreversible damage will cause the conversion loss with increased electric field intensity. Therefore, the selection of optimal electric field intensity for PEF processing was shown to be important.

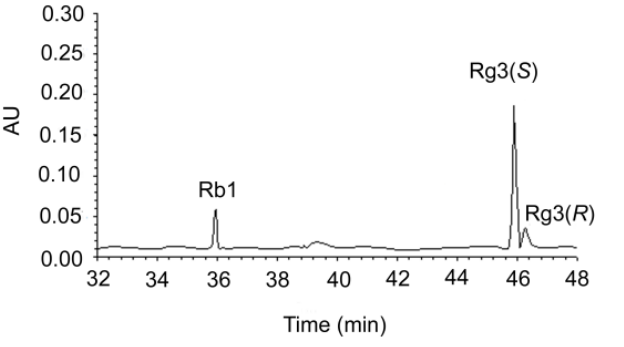

Figure 3: HPLC profile of the metabolites of ginsenoside Rb1 under PEF treatment (electric field intensity 20 kVcm^{-1}, pulse number 10 and acid concentration 0.2mol/L). The mixed reaction was extracted by n-BuOH, evaporated vacuo and analyzed by HPLC after dissolved in MeOH.

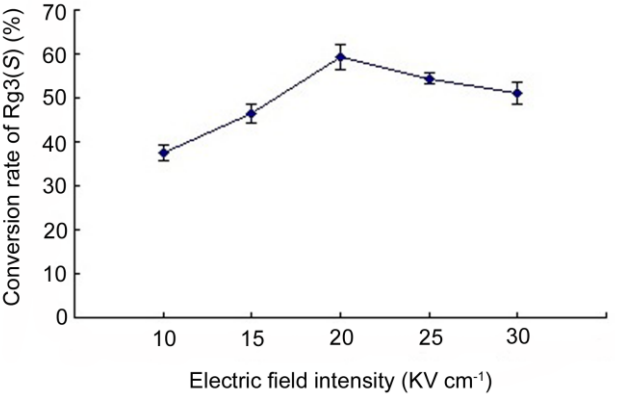

Figure 4: Effect of electric field intensity on the conversion rate of ginsenoside Rg3(*S*). Pulse number 8 and acid concentration 0.2 mol/L.

3.2 Effect of Pulse Number on Preparation of Ginsenoside Rg3(*S*)

Figure 5 showed the conversion rate of Rg3(*S*) by different pulse number treatment. When the pulse number increased from 6 to 12, the conversion rate of Rg3(*S*) increased from 47.56% to 63.94%. The results indicated that the maximum conversion rate of Rg3(*S*) was 63.94% on the condition of pulse number 12. Nevertheless, the conversion rate of Rg3(*S*) presented decreasing tendency when the pulse number increased from 12 to 14. Under different pulse number variables treatment, the large pulse frequency caused some irreversible changes occurred to molecular structure, leading to decrease the conversion rate of ginsenoside Rg3(*S*). Therefore, the pulse number 10 was chosen to convert ginsenoside Rb1 to ginsenoside Rg3(*S*).

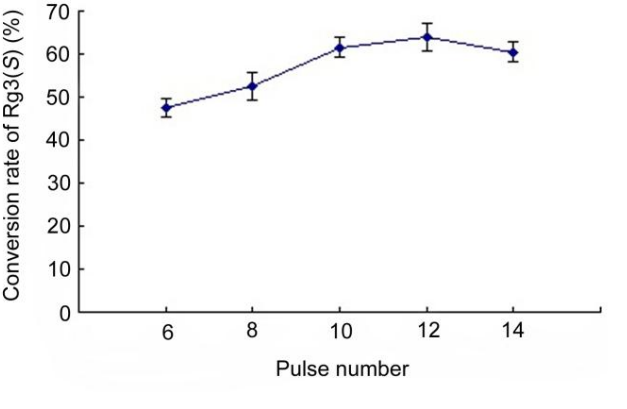

Figure 5: Effect of pulse number on the conversion rate of ginsenoside Rg3(*S*). Electric field intensity 20 kVcm^{-1} and acid concentration 0.2 mol/L.

Table 3: Regression coefficients estimate significance test for the quadratic polynomial model.

Model term	Coefficient Estimate	Standard error	Sum of square	Mean square	*F*-value	Probability (P)>F
Intercept	68.04	0.33	1283.16	142.57	267.21	< 0.0001
X_1	3.12	0.26	77.63	77.63	145.49	< 0.0001
X_2	0.42	0.26	1.42	1.42	2.66	0.1469
X_3	1.32	0.26	14.02	14.02	26.27	0.0014
X_1X_2	-2.24	0.37	19.98	19.98	37.45	0.0005
X_1X_3	-4.09	0.37	66.75	66.75	125.1	< 0.0001
X_2X_3	-3.27	0.37	42.84	42.84	80.28	< 0.0001
X_1X_1	-12.27	0.36	633.73	633.73	1187.72	< 0.0001
X_2X_2	-7.09	0.36	211.7	211.7	396.77	< 0.0001
X_3X_3	-5.34	0.36	119.87	119.87	224.67	< 0.0001

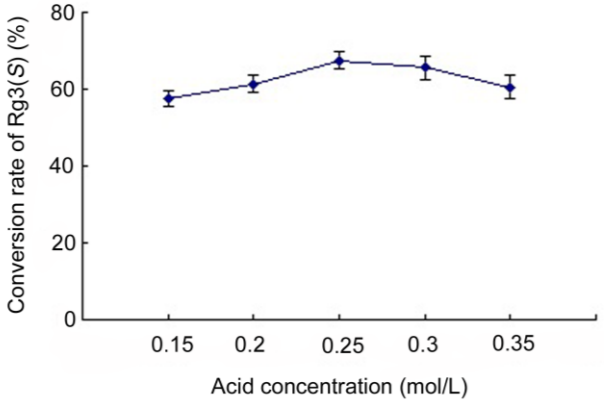

Figure 6: Effect of acid concentration on the conversion rate of ginsenoside Rg3(*S*). electric field intensity 20 kVcm^{-1} and pulse number 12.

3.3 Effect of Acid Concentration on Preparation of Ginsenoside Rg3(*S*)

Figure 6 showed the effect of acid concentration on PEF-assisted acid hydrolysis of ginsenoside Rb1. The conversion rate of Rg3(*S*) significantly accelerated during the acid concentration from 0.15 mol/L to 0.25 mol/L and the highest value was 67.53%, which was higher than acid-treated biotransformation (42.4%) [28]. However, the conversion rate of Rg3(*S*) decreased when the concentration increased from 0.25 to 0.35 mol/L. The conversion rate of Rg3(*S*) decreased under the higher acid concentration (0.35 mol/L) processing. These results might be from the interaction between PEF and high acid concentration. The hydrogen ions transferred with electrical pulses by PEF processing. When the PEF-assisted acid hydrolysis reached a balance, there was no significant increase with higher acid concentration. Therefore, the optimal parameter of acid concentration was 0.25 mol/L by PEF-assisted acid hydrolysis processing.

3.4 Optimization by Response Surface Methodology

To optimize hydrolysis parameters and understand relationships of the variables, the research data of PEF processing was analyzed by RSM. As shown in Table 2, the conversion rate of ginsenoside Rg3(*S*) under different experimental conditions was presented. The maximum conversion rate of Rg3(*S*) achieved 68.58% on the condition of electric field intensity 20 kVcm^{-1}, pulse number 10 and acid concentration 0.25 mol/L. Compared with the transformation of ginsenoside Rb1 into Rg3 (41.4%) by microbial strain GS514, the conversion rate of Rg3(*S*) (68.58%) by PEF-assisted hydrolysis was significantly increased [16] (Cheng *et al.*, 2008). The lowest conversion rate of Rg3(*S*) was 41.43% on the condition of electric field intensity 15 kVcm^{-1}, pulse number 10, and acid concentration 0.15 mol/L. The mathematical model that showed the relationship between the conversion rate of Rg3(*S*) and hydrolysis parameters was expressed by Eq.3.

$$Y=+68.04+3.12X_1+0.42X_2+1.32X_3-2.24X_1X_2-4.09X_1X_3-3.27X_2X_3-12.27X_1X_1$$
$$-7.09X_2X_2-5.34X_3X_3 \quad \text{.....Eq.3}$$

The value of variation coefficient (1.29) showed a high precision between experimental and predicted values. The coefficient of adjusted determination was 0.9934, indicating that the model was significantly accurate with predicted response. As can be seen in Table 3, the *P*-value of the model < 0.0001 and the *F*-value was 267.21.

The response surface was shown in Figure 7 and showed the relationship between conversion rate of Rg3(*S*) and experimental values of variables. The significant influence variables of the model were electric field intensity, electric field intensity squared, pulse number squared, and acid concentration squared. The interactive effect of electric field intensity and acid concentration, also the

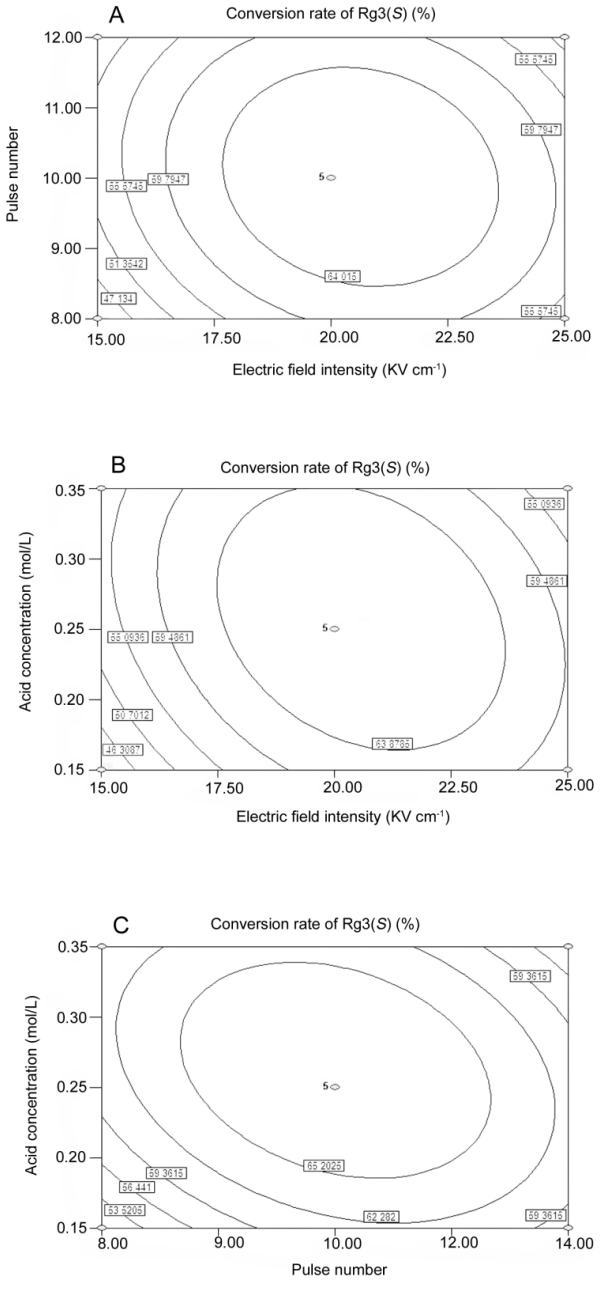

Figure 7: Contour plot of electric field intensity, pulse duration and acid concentration for producing ginsenoside Rg3(*S*) under PEF treatment (a) Electric field intensity and pulse number (b) Electric field intensity and acid concentration (c) Pulse number and acid concentration.

interactive effect of pulse number and acid concentration exerted remarkable effects. Figure 7c showed that electric field intensity (X_1) kept on 20 kVcm^{-1}, the pulse number (X_2) and acid concentration (X_3) demonstrated quadratic effects on the response conversion rate. When the acid concentration was 0.15 mol/L, the conversion rate of Rg3(*S*) gradually increased, then had decreased tendency with higher pulse number. The maximum conversion rate reached 68.58% at optimal processing parameters of the electric field intensity 20 kVcm^{-1}, pulse number 10 and acid concentration 0.25 mol/L. The data was closed to predicted values 68.04%.

Table 4: ^{13}C-NMR chemical shifts of ginsenoside Rg3 in pyridine-d_5.

Carbon site	Rg3(R)	Rg3(S)	1 (ppm)	Carbon site	Rg3(R)	Rg3(S)	1 (ppm)
C-1	39.2	39.2	39.1	C-22	43.3	36.0	36.0
C-2	26.7	26.8	26.8	C-23	22.7	23.1	23.0
C-3	89.0	89.0	88.9	C-24	126.1	126.4	126.3
C-4	39.8	39.8	39.8	C-25	130.8	130.8	130.8
C-5	56.4	56.5	56.4	C-26	25.9	25.9	25.9
C-6	18.5	18.5	18.5	C-27	17.7	17.1	17.1
C-7	35.2	35.3	35.2	C-28	28.2	28.2	28.2
C-8	40.0	40.1	40.1	C-29	16.6	16.7	16.7
C-9	50.4	50.5	50.4	C-30	17.4	17.8	17.4
C-10	37.0	37.0	37.0	1′	105.2	105.2	105.2
C-11	32.2	32.1	32.1	2′	83.5	83.5	83.5
C-12	70.9	71.0	71.0	3′	78.0	78.0	78.0
C-13	49.3	48.7	48.8	4′	71.8	71.8	71.6
C-14	51.9	51.8	51.8	5′	78.1	78.3	78.3
C-15	31.5	31.4	31.4	6′	62.9	62.9	62.8
C-16	26.8	26.9	27.1	1″	106.1	106.1	106.1
C-17	50.7	54.9	54.9	2″	77.2	77.2	77.2
C-18	15.9	15.9	15.9	3″	78.4	78.4	78.4
C-19	16.4	16.4	16.5	4″	71.7	71.7	71.6
C-20	73.0	73.0	73.0	5″	78.3	78.2	78.2
C-21	22.8	28.2	28.0	6″	62.8	62.8	62.7

3.5 Structural Identification

The ^{13}C- NMR data were shown in Table 4. The signals for C-17, C-21 and C-22 of the samples emerged at δ 50.7, δ 28.0 and δ 36.0 respectively. These results were consistent with

carbon positions of ginsenoside Rg3(*S*) [29]. The sample was confirmed as 3-*O*-[*β*-D-glucopyranosyl-(1,2)-*β*-D-glucopyranosyl]-20(*S*)-protopanaxadiol, the similar to standard ginsenoside Rg3(*S*). The ^{13}C- NMR data confirmed that the metabolite of ginsenoside Rb1 which hydrolyzed under PEF treatment was ginsenoside Rg3(*S*). Therefore, PEF treatment-assisted acid hydrolysis can convert ginsenoside Rb1 to ginsenoside Rg3(*S*).

4 Conclusion

In the present study, PEF processing was applied to prepare ginsenoside Rg3(*S*) by hydrolysis of ginsenoside Rb1 under acidic conditions. The results indicated that PEF treatment accelerated the conversion rate of ginsenoside Rb1 to Rg3(*S*). The metabolite of ginsenoside Rb1 with PEF treatment was identified as ginsenoside Rg3(*S*) by ^{13}C-NMR. RSM was used to obtain a desirable quadratic polynomial mathematical model for producing ginsenoside Rg3(*S*) under PEF treatment. The maximum conversion rate of ginsenoside Rg3(*S*) reached 68.58% under the optimal conditions. These findings suggested that PEF-assisted acid hydrolysis can be used to prepare ginsenoside Rg3(*S*) with high efficiency and provided data for preparing a bioactive compound by PEF processing.

Acknowledgements: This research project was financed by National Natural Science Foundation of China (31601400) and Natural Science Foundation from Jilin Province Department of Science and Technology (20140101040JC).

Conflict of interest: Authors state no conflict of interest.

References

[1] Siddiqi M.Z., Siddiqi M.H., Kim Y.J., Jin Y., Huq M.A., Yang D.C., Effect of fermented red ginseng extract enriched in ginsenoside Rg3 on the differentiation and mineralization of preosteoblastic MC3T3-E1 cells. J. Med. Food, 2015, 18, 542–548

[2] Kim J.H., Choi J.S., Effect of ginsenoside Rh-2 via activation of caspase-3 and Bcl-2-insensitive pathway in ovarian cancer cells. Physiol. Res., 2016, 65, 1031-1037.

[3] Zhou T.T., Zu G., Zhang X.G., Wang X., Li S., Gong X.Y., Liang Z.H., Zhao J., Neuroprotective effects of ginsenoside Rg1 through the Wnt/β-catenin signaling pathway in both in vivo and in vitro models of Parkinson's disease. Neuropharmacology, 2016, 101, 480-489

[4] Mochizuki M., Yoo Y.C., Matsuzawa K., Sato K., Saiki I., Tono-oka S., Inhibitory effect of tumor metastasis in mice by saponins, ginsenoside Rb2, 20(*R*)- and 20(*S*)-ginsenoside Rg3 of red ginseng. Biol. Pharm. Bull., 1995, 18, 1197-1202

[5] Tian J.W., Fu F.H., Geng M.Y., Jiang Y.T., Yang J.X., Jiang W.L., Neuroprotective effect of 20(*S*)-ginsenoside Rg3 on cerebral ischemia in rats. Neurosci. Lett., 2005, 374, 92-97

[6] Joo S.S., Won T.J., Lee Y.J., Hwang K.W., Lee S.G., Yoo Y.M., Lee D.I., Ginsenoside Rg3 from red ginseng prevents damage of neuronal cells through the phosphorylation of the cell survival protein akt. Food Sci. Biotechnol. 2006, 15, 244-247

[7] Yun T.K., Lee Y.S., Lee Y.H., Kim S.L., Yun H.Y., Anticarcinogenic effect of *Panax ginseng* C.A. Meyer and identification of active compounds. J. Korean Med. Sci., 2001, 16, 6-18

[8] Lee C.K., Park K.K., Chung A.S., Chung W.Y., Ginsenoside Rg3 enhances the chemosensitivity of tumors to cisplatin by reducing the basal level of nuclear factor erythroid 2-related factor 2-mediated heme oxygenase-1/NAD(P)H quinone oxidoreductase-1 and prevents normal tissue damage by scavenging cisplatin-induced intracellular reactive oxygen species. Food Chem. Toxicol., 2012, 50, 2565-2574

[9] Shin Y.M., Jung H.J., Choi W.Y., Lim C.J., Antioxidative, anti-inflammatory, and matrix metalloproteinase inhibitory activities of 20(*S*)-ginsenoside Rg3 in cultured mammalian cell lines. Mol. Biol. Rep., 2013, 40, 269-279

[10] Quan L.H., Min J.W., Yang D.U., Kim Y.J., Yang D.C., Enzymatic biotransformation of ginsenoside Rb1 to 20(*S*)-Rg3 by recombinant β-glucosidase from *Microbacterium esteraromaticum*. Appl. Microbiol. Biot., 2012, 94, 377-384

[11] Choi K.T., Botanical characteristics, pharmacological effects and medicinal components of Korean *Panax ginseng* C.A. *Meyer.* Acta Pharmacol. Sin., 2008, 29, 1109-1118

[12] Kitagawa I., Yoshikawa M., Yoshihara M., Hayashi T., Taniyama T., Chemical studies of crude drugs. Constituents of *Ginseng radix rubra*. Yakugaku Zasshi, 1983, 103, 612-622

[13] Yi J.H., Kim M.Y., Kim Y.C., Jeong W.S., Bae D.W., Hur J.M., Jun M., Chang of ginsenoside composition in red ginseng processed with citric acid. Food Sci. Biotechnol., 2010, 19, 647-653

[14] Chang K.H., Jee H.S., Lee N.K., Park S.H., Lee N.W., Paik H.D., Optimization of the enzymatic production of 20(*S*)-ginsenoside Rg3 from white ginseng extract using response surface methodology. New Biotechnol., 2009, 26, 181-186

[15] Kim, J.K., C.H. Cui, Q.M. Liu, M.H. Yoon, S.C. Kim and W.T. Im, Mass production of the ginsenoside Rg3(S) through the combinative use of two glycoside hydrolases. Food Chem., 2013, 141, 1369-1377

[16] Cheng L.Q., J.R. Na, M.H. Bang, M.K. Kim and D.C. Yang, Conversion of major ginsenoside Rb1 to 20(*S*)-ginsenoside Rg3 by *Microbacterium* sp. GS514. Phytochemistry, 2008, 69, 218-224.

[17] Yang N.N., Huang K., Lyu C., Wang J.P., Pulsed electric field technology in the manufacturing processes of wine, beer, and rice wine: A review. Food Control, 2016, 61, 28-38

[18] Zhao W., Yang R.J., Effect of high-intensity pulsed electric fields on the activity, conformation and self-aggregation of pepsin. Food Chem., 2009, 114, 777-781

[19] Abdullah S.H., Zhao S., Mittal G.S., Baik O.D., Extraction of podophyllotoxin from *Podophyllum peltatum* using pulsed electric field treatment. Sep. Purif. Technol., 2012, 93, 92-97

[20] Yin, Y.G., Y.R. Cui and H.W. Ding, Optimization of betulin extraction process from *Inonotus obliquus* with pulsed electric fields. Innov. Food Sci. Emerg. Technol., 2008, 9, 306-310
[21] He G.D., Yin Y.G., Meng L., Yan L.N., Yu Q.Y., Enzyme method extraction of collagen from bovine bone by high intensity pulsed electric fields. Nongye Jixie Xuebao, 2010, 11, 124-128
[22] He G.D., Yin Y.G., Yan X.X., Yu Q.Y., Optimisation extraction of chondroitin sulfate from fish bone by high intensity pulsed electric fields. Food Chem., 2014, 164, 205-210
[23] Zhang F.S., Tian M.L., Du M.Y., Fang T., Enhancing the activity of pectinase using pulsed electric field (PEF) treatment. J. Food Eng., 2017, 205, 56-63
[24] Lu C.W., Yin Y.G., Pulsed electric field treatment combined with commercial enzymes converts major ginsenoside Rb1 to minor ginsenoside Rd. Innov. Food Sci. Emerg., 2014, 22, 95-101
[25] Zhao W.Z., Yu Z.P., Liu J.B., Yu Y.D., Yin Y.G., Lin S.Y., Chen F., Optimized extraction of polysaccharides from corn silk by pulsed electric field and response surface quadratic design. J. Sci. Food Agr., 2011, 91, 2201-2209.
[26] Guo X., Zou X., Sun M., Optimization of extraction process by response surface methodology and preliminary characterization of polysaccharides from Phellinus igniarius. Carbohyd. Polym., 2010, 80, 344–349.
[27] Anufriev V.P., Malinovskaya G.V., Denisenko V.A., Uvarova N.I., Elyakov G.B., Kim S.I., Baek N.I., Synthesis of ginsenoside Rg3, a minor constituent of Ginseng radix. Carbohyd. Res., 1997, 304, 179-182
[28] Bae E.A., Han M.J., Choo M.K., Park S.Y., Kim D.H., Metabolism of 20(*S*)- and 20(*R*)-ginsenoside Rg3 by human intestinal bacteria and its relation to in vitro biological activities. Biol. Pharm. Bull., 2002, 25, 58-63
[29] Sun C.P., Gao W.P., Zhao B.Z., Cheng L.Q., Optimization of the selective preparation of 20(R)-ginsenoside Rg3 catalyzed by D, L-tartaric acid using response surface methodology. Fitoterapia, 2013, 84, 213-221.