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Pulsed electric field extraction of polysaccharide from *Rana temporaria chensinensis* David

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Abstract

In order to develop and optimize a pulsed electric field (PEF) extraction method and evaluate it against conventional extraction methods for the extraction of polysaccharide from *Rana temporaria chensinensis* David, we have investigated various experimental conditions, respectively, such as electric field intensity (10–30 kV/cm), pulse duration (2–6 μs) and concentration of distilling solvent (0–1% KOH, v/v), and then optimized them by an orthogonal experiment. The result showed that the largest extraction ratio is 55.59% by PEF on the conditions of 0.5% KOH, 20 kV/cm electric field intensity and 6 μs pulse duration. Comparing it with the conventional extraction methods, such as alkali extraction method, enzyme extraction method and compound extraction method, the extraction ratio and polysaccharide content of PEF method are higher than the other three methods. The PEF extraction ratio for 6 μs is 1.77 times the compound extraction method for 6 h, the total sugar contents are more than 26.34% of that for the compound extraction method and the impurity of extraction material is less. So the PEF method is a novel and promising method to extract polysaccharide of *R. temporaria chensinensis* David.

Keywords: Pulsed electric field (PEF); Polysaccharide; *Rana temporaria chensinensis* David; Extraction

1. Introduction

Rana temporaria chensinensis David is a kind of frog with high commercial value. It has a long history of being used in traditional medicine and has been reported to improve health and immunity and prevent diseases such as antioxidant, antifatigue, inflammation, etc. (Wang et al., 2002a). Many bioactive compounds have been identified in different *R. temporaria chensinensis* David samples, including proteins, amino acids, fatty acids, polysaccharide, vitamins, incretion and so on (Wang et al., 2002a). In most of the reports, the biological or pharmacological activity was mainly associated with the polysaccharide (Sun et al., 2000; Wang et al., 2002b). The polysaccharide of *R. temporaria chensinensis* David is a kind of white power extracted from *R. temporaria chensinensis* David, which is a potent anticoagulant, anticancer, antioxidant and prevents diabetes (Sun et al., 2000; Wang et al., 2002b). These facts mean that there is renewed interest in the extraction of the polysaccharide from *R. temporaria chensinensis* David.

Pulsed electric field (PEF) extraction is a novel technique that is currently used for the non-thermal pasteurization of food (Zeng et al., 2005) in the extraction of active ingredients from natural biomaterials. The electric pressure ranges from 20 to 80 kV or more. High electric pressure can cause some structural changes in structurally fragile foods, such as cell deformation, cell membrane damage, protein denaturation and so on (Zeng et al., 2005). Under the process of PEF, the differential electric pressure between the cell interior and the exterior of cell membranes is so large that it will lead to rapid permeation. Consequently, the concentration between the cell interior and the exterior of cell membranes can reach equilibrium in an ultra-short time. The higher the electric pressure is, the more solvent can enter into the cell and the more compounds can permeate the cell membrane.

PEF extraction has several principal procedures. Firstly, the raw material is mixed with the solvent. Secondly, the mixture is treated with high-pulsed electric pressure. Lastly, the mixture, after processing, is filtered to remove the solid particles. Thus, the extraction solution of PEF can be prepared which contains the active ingredients that we need. PEF extraction is operated at room temperature without any heat-

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ing process, except for the rise in temperature resulting from compression.

There have been some reports about the extraction of polysaccharide from *R. temporaria chensinensis* David (Sun et al., 2000, 2001a; Wang et al., 2002b; Fan et al., 1994), such as the alkali extraction method, enzyme extraction method and compound extraction method. However, there have been no reports on the use of PEF for the extraction of polysaccharide from *R. temporaria chensinensis* David. So the purposes of this study were to develop and optimize a PEF extraction method and evaluate it and conventional extraction methods for the extraction of polysaccharide from *R. temporaria chensinensis* David.

2. Materials and methods

2.1. Materials and instrumentations

Live frogs (*R. temporaria chensinensis* David) that had been caught in Linjiang city of Jilin Province (China) were three instars. The frog body removed of purtenance was dried and comminuted.

Major components in the PEF system designed by Zhao et al. (2002) include a high-voltage repetitive pulse generator, a coaxial liquid materials treatment chamber, a fiber-optic temperature-sensing instrument, and a data acquisition system. The pulse duration (n) is calculated as

$$n = 2fL\nu^{-1}$$

where n is the pulse duration (μs), f the frequency (Hz), L the length of electrode (mm) and ν is the flow velocity (ml/s).

2.2. Pulsed electric field extraction

At the beginning of each experiment, we weighed exactly 2 g of frog powder and mixed it with a quantity of the appropriate solvent. Then, the mixture of frog powder and solvent was pumped through the PEF system with a flow velocity (ν) of 26 ml/min. A high voltage pulse was then turned on, and the pulse frequency and charge voltage were adjusted to the desired levels. After being processed for desired minutes, the high voltage pulse was turned off, and the mixture was filtered to remove the solid particles. The extraction solution was finished and prepared to determine the polysaccharide.

2.3. Conventional extraction methods

Traditional extraction methods are reported in the literature, such as alkali extraction method (Fan et al., 1994), enzyme extraction method (Sun et al., 2001a) and compound extraction method (Wang et al., 2002b). In the alkali extraction method, samples were prepared by mixing 2 g of frog powder with 5% KOH at 45 °C. After 2 h of moderate shaking, the extracts were filtered and prepared to determine the polysaccharide. In the enzyme extraction method, samples were prepared by mixing 2 g of frog powder with pepsin at 50 °C. After 5 h, the extracts were filtered and prepared to determine the polysaccharide. In the compound extraction method, samples were prepared by mixing 2 g of frog powder with 1% NaOH and compound enzymes for 6 h at 50 °C, then the extracts were filtered and prepared to determine the polysaccharide.

2.4. Estimation of total polysaccharide in R. temporaria chensinensis David

The extract solution was precipitated with 95% ethanol, dried to obtain the coarse polysaccharide and weighed. The content of total polysaccharide was measured with the phenol sulphate by colorimetry (Sun et al., 2001b). We selected sodium heparin as the standard sample, and an exactly weighed amount L (g) of coarse polysaccharide was mixed with a volume V (cm^3) of distilled water, and then the content of total polysaccharide was determined by colorimetry. The extraction ratio (ER, %) is the ratio of mass of pure polysaccharide to the mass of coarse polysaccharide.

$$\mathrm{ER} = CVL^{-1} \times 100\%$$

where C is the concentration of the solution used for colorimetric analysis according to the sodium heparin standard curve (g/cm^3), V the total volume of coarse polysaccharide solution (cm^3) and L is the mass of coarse polysaccharide (g).

3. Results and discussion

3.1. The effect of different electric field intensity on the ER of polysaccharide

Fig. 1 shows that the ER of polysaccharide was influenced by PEF intensity. When the PEF intensity was increased from 0 to 20 kV/cm, the ER of polysaccharide was increased from 17.11 to 26.87%. It is obvious that PEF intensity is useful for improving the ER of polysaccharide. The higher electric field intensity caused higher levels of extraction of polysaccharide. It is generally accepted that PEF exerts its effect primarily by causing the membranes of cell destroyed and the protein denatured (Zeng

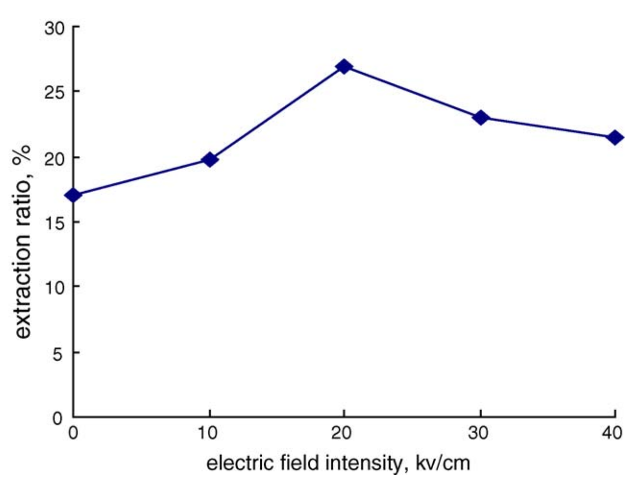

Fig. 1. Effect of electric field intensity on the extraction of polysaccharide. Solvent: water, frog powder: 2 g, frequency: 1200 Hz.

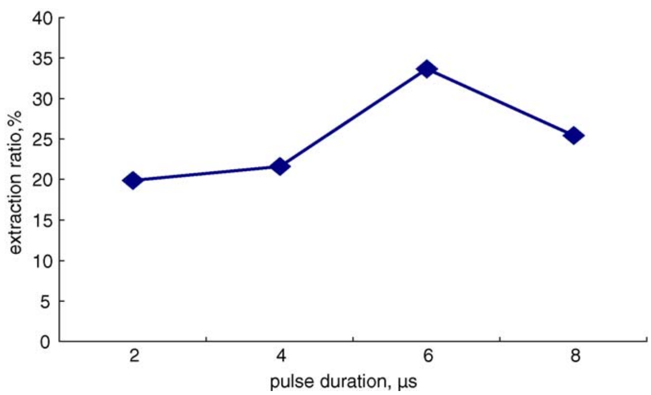

Fig. 2. Effect of pulse duration on the polysaccharide extraction. Solvent: water, electric field intensity: 20 kV/cm.

et al., 2005). Under high electric field strength, more solvent will enter into the cell and more compounds can permeate the cell membrane easily. Also the differential electric field strength between the inner and the exterior of the cell makes electropolar perforation of cell membrane. So increasing the PEF intensity could increase the ER of polysaccharide. However, with the increase in electric field intensity from 20 to 40 kV/cm, the ER of polysaccharide was decreased from 26.87 to 21.41%, causing decomposition of the polysaccharide. So 20 kV/cm PEF intensity was chosen to extract the polysaccharide from *R. temporaria chensinensis* David.

3.2. Effect of pulse duration on the ER of polysaccharide

Fig. 2 shows the effect of PEF pulse duration on the ER of polysaccharide. The results indicate that the largest ER of polysaccharide is 33.67% when the pulse duration is 6 μs. With the PEF electric pulse duration increasing from 2 to 6 μs, the ER of polysaccharide was increased from 19.82 to 33.67%. However, with the increase of electric pulse duration from 6 to 8 μs, the ER of polysaccharide decreased from 33.67 to 25.43%. Increasing the pulse duration led to permeation of a greater number of cells and at the same time led to decomposition of polysaccharide. So 6 μs PEF pulse duration was used in the following experiments.

3.3. The effect of different solvent concentration on the ER of polysaccharide

Fig. 3 shows that the ER of polysaccharide varies with the increase in the concentration of solvents. When the volume of KOH in the solvent was lower than 1% (v/v), the ER increased with the increase in solvent concentration. The reason is that the solubility of polysaccharide in KOH solution increases with the increasing concentration of KOH. When the volume of KOH in the solvent was higher than 1% (v/v), the ER decreased with the increase in KOH concentration. Furthermore, we observed in the experiments that the electric field intensity could not be increased any more and the electric field gave a spark of light when the KOH solution concentration was increased to 2% (v/v). This will be investigated in further research.

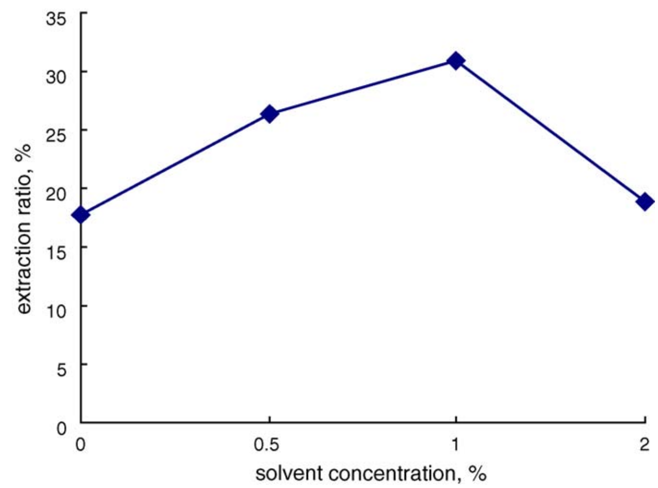

Fig. 3. Effect of extraction solvent concentration on the polysaccharide extraction. Electric field intensity: 20 kV/cm, pulse duration: 6 μs.

3.4. The effect of different combinations of PEF conditions on the ER of polysaccharide

Table 1 shows that the ER of polysaccharide varies for the different combinations of PEF experimental conditions. The largest ER is 55.59% for PEF combination conditions of 20 kV/cm electric field intensity and 0.5% KOH and 6 μs pulse duration. The ER of this combination condition is higher than its value for any single condition. So the correct choice of experiment parameters, as predicted by the experiment described here, can considerably improve the PEF process. The effect of electric field intensity on the ER in this experiment is the largest, and the effect of pulse duration takes second place, and the least effective factor is the extraction solution concentration.

3.5. Comparison of PEF and conventional extraction methods

In order to compare the results of PEF with other traditional extraction methods, we performed all experiments using raw materials from the same batch, and the technology of extraction method is exactly the same as that given in the literature.

Table 1
Results of the orthogonal experiment

Treatment	Electric field intensity (kV/cm)	Extraction solution concentration	Pulse duration (μs)	Extraction percentage (%)
1	10	Water	2	18.94
2	10	0.5% KOH	4	11.00
3	10	1% KOH	6	26.87
4	20	Water	4	15.90
5	20	0.5% KOH	6	55.59
6	20	1% KOH	2	28.00
7	30	Water	6	18.56
8	30	0.5% KOH	2	12.51
9	30	1% KOH	4	37.83
Range	14.22	13.10	13.85	

Table 2
Compare of PEF and conventional extraction methods

Method	Alkali extraction	Enzyme extraction	Compound extraction	PEF extraction
Extraction conditions	5% KOH, 2 h	Pepsin, 5 h	1% NaOH + compound enzymes, 6 h	0.5% KOH, 20 kV/cm, 6 μs
ER (%)	19.78	24.44	31.38	55.59
Total sugar content (μg/ml)	10.78	14.93	16.81	43.15

Table 2 shows that the PEF extraction time is ultrashort. The PEF for 6 μs gave higher ER and extraction yield of polysaccharide than the other three traditional extractions for 2, 5 and 6 h, respectively. The PEF ER for 6 μs is 1.77 times of the value obtained by compound extraction method for 6 h, and total sugar content is more than 26.34% than that obtained by compound extraction method. There are only a few impurities in the extracting solution of PEF since PEF extraction is homogenization and every part of material on the electric field is treated by the same field strength.

4. Conclusion

Conditions for PEF extraction of polysaccharide from *R. temporaria chensinensis* David have been studied. PEF has been shown to be an efficient method for extraction of polysaccharide from *R. temporaria chensinensis* David. Compared with the conventional extraction methods, the PEF procedure provided higher extraction yield, higher ER, less impurities, and it required an ultrashort time.

PEF is suitable for fast extraction of polysaccharide from *R. temporaria chensinensis* David. Food and drug industries will benefit from this emerging technology, for it is more rapid, highly efficient and saves energy compared with conventional extraction methods.

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