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The Effect of Pulsed Electric Fields on the Antioxidant Activity of Polyphenol Components in Moxa Leaves

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Abstract In condition that a maximum isolation was achieved when moxa materials suspended was treated for 1μs pulse width at 25kV, 300Hz, flow velocity 2mL/min, in monopolar mode, PEF treatment did not influence on structural stability of polyphenol components contained in moxa leaves. The antioxidant activity of the extract is 1.91 times (DPPH), 10.04 times (·OH), 1.76 times (O_2^-) and 2.56 times (lipid radical scavenging activity) as many as Vit C. This work clearly demonstrated that the PEF treatment was an effective method to achieve a high throughput of the natural polyphenol components extraction.

Key words pulsed electric field (PEF), moxa leaves, polyphenol components, antioxidant activity

Introduction

The great leader Comrade **Kim Il Sung** said as follows.

"Further, good research should be undertaken in Korean medicine which has a long tradition and has become indispensable to our people, and its merits should be assimilated and introduced into our public health service for the masses."("**KIM IL SUNG** WORKS" Vol. 10 P. 209)

The research on the nonthermal bactericidal effect of PEF, conducted in 1967[1], became the basis for the development of PEF technology as a food preservation process. From the technical standpoint, PEF is promising because of technical improvement in high voltage switching, allowing high frequency signal to treat thousands of times per second a small volume chamber, finally being green technical that allow very high throughput [3, 4]. The polyphenol supplement is beneficial to the improvement of cell's activity; to the increase of immunity; to the advance of metabolisms; to the prevention of senescence; to the improvement of brain cell's ability and to the defending and treatment of other diseases in the human health. So it is important to find out the resources of the polyphenol components (e.g. the available resources to extract polyphenol in plant tissues throw out besides food source) and to increase the extraction efficiency. Moxa leaves are known to be a very good source of many biological components. For developing a high throughput extraction of polyphenol and studying PEF influence on structural stability of polyphenol contained by moxa leaves, PEF was used. As far as we know, no reference about the effects of PEF on antioxidant activity of polyphenol in moxa leaves has been found in the literature.

The goal of this research is to establish the effects of the PEF optimum conditions of polyphenol components extraction from moxa leaves on its antioxidant activity.

1. Materials and Methods

1.1. Preparation of sample

Fresh moxa leaves (*Artemisia asiatica* Nak.) from a local market was used for this work. The samples were sieved (≤ 1mm) through a sieve after smashing, and then immediately used for the PEF treatment to separate polyphenol components from moxa leaves.

1.2. Quantification of polyphenol components

Quantification of polyphenol components is conducted by Folin-Dennis method.

Standard value for quantifying polyphenol components is measured by using catechin(Fig.).

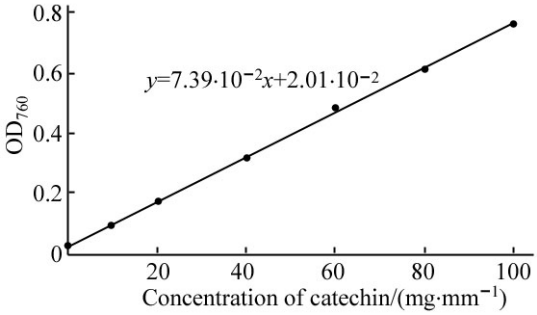

Fig. Standard curve for quantifying polyphenol

1.3. Extraction of polyphenol components by hot water and PEF treatment

We solved sample 0.5g into hot distilled water(100℃) 100mL, extracted it for 2h in bath at 100 ℃ and quantified polyphenol components extraction by PEF.

We solved pulverized 0.5g of samples into 10mL of water and supplied into PEF device. Electrode space and pulse frequency is 2mm and 300Hz as to optimizing condition for the extraction of polyphenol components. After PEF treatment, we extracted in hot water(100℃) for 1h and decided the content of polyphenol components.

1.4. Measurement of antioxidant activity

We calculated linear equation between radical scavenging rate and concentration and IC_{50}. Antioxidant activity is measured by DPPH, ·OH, O_2^- scavenging activity.

1.5. PEF generating device

The technical characteristics of the device are as follows.(table 1)

Table 1. The technical characteristics of device

Parameter	Value	Parameter	Value
Pulse voltage/kV	25	Flow velocity /(mL·min^{-1})	0.5～5.0
Electrode space/mm	2～8	Frequency/Hz	25～1 000
Pulse length / μs	1	Electrode area/mm^2	6.25

1.6. PEF treatment

Before PEF treatment, moxa leaves were pulverzed during a short time at room temperature, filtered through a sieve(≤1mm), and resuspended in solution and the suspended samples were transferred to variable speed pump("Model D100A"). PEF treatments were carried out in a continuous flow bench scale system using sawtooth-wave pulses. The moxa leaves solution was treated in a parallel-electrode treatment chamber on condition of table 1. Temperature may be raised when PEF treatment[1], temperature above 50℃ make PEF treatment more effective because of the greater

fragility of the lipid bilayer above the phase transition temperature[1, 7, 8]. But some experiments showed that temperature rise after PEF treatment was about (10±1) ℃, compatible with a theoretical increase of 11 ℃. Under these conditions, the final temperature was about 33℃, below the temperature range of possible combined lethal effects of PEF[4]. The temperature rise was therefore not implicated in cell lethality. The temperature range 25～35℃ was not considered to reduce the membrane resistance, and thus to have no consequences on the survival rates[4]. At intermediate temperatures(24～45℃), there is no evidence of a synergetic effect between PEF treatment and temperature[4].

So we have not considered the effect of temperature at PEF treatment and only considered it at polyphenol components extraction after PEF treatment.

2. Results and Discussion

2.1. DPPH·scavenging activity

Table 2 shows the DPPH·scavenging activity of polyphenol components of moxa leaves.

As shown in table 2, we can know clear dependence DPPH·scavenging activity on the radical concentration.

Table 2. DPPH scavenging rate as to the concentration

Division	Concentration /(μg·mL^{-1})	Scavenging rate/%	Division	Concentration /(μg·mL^{-1})	Scavenging rate/%
Extract by hot water	10	41.67±0.95	Extract by PEF	40	57.55±0.51
	20	47.58±1.08		50	61.91±0.72
	30	52.56±1.45	Vit C	10	6.77±0.29
	40	57.69±0.77		30	26.87±0.78
	50	61.60±0.49		50	48.52±1.47
Extract by PEF	10	40.25±0.22		70	72.68±1.10
	20	47.76±0.64		90	98.38±1.60
	30	52.75±0.92			

$p<0.05$

From table 2, the IC_{50} of extracts are 25.61μg/mL by hot water, 26.15μg/mL by PEF treatment, 49.43μg/mL of Vit C. The result shows that the conformation of polyphenol components in moxa leaves were not affected by PEF treatment and the DPPH scavening activity of it were 1.91 times as much as Vit C.

2.2. ·OH scavenging activity

·OH radical is very dangerous due to the highest activity and there is no enzymes to dissolve it in living cells. Therefore, it is an important index to estimate the antioxidant activity. Table 3 shows the comparative results between Vit C and the extracts of moxa leaves.

·OH radical has the highest activity in living cells. ·OH scavenging activity is an important index to estimate the antioxidant activity. Table 3 shows the comparative results between Vit C and the extracts of moxa leaves.

Table 3. ·OH scavenging rate as to the concentration

Division	Concentration /(μg·mL^{-1})	Scavenging rate/%	Division	Concentration /(μg·mL^{-1})	Scavenging rate/%
Extract by hot water	5	48.43±0.81	Extract by PEF	15	69.18±0.70
	10	60.30±0.68		20	80.57±0.57
	15	68.73±0.97	Vit C	30	38.50±0.74
	20	79.47±0.30		50	46.74±1.08
Extract by PEF	5	48.77±0.91		70	55.52±0.82
	10	59.61±0.75		90	67.75±0.83

$p<0.05$

As shown in table 3, ·OH scavenging rate increased as the concentration of polyphenol components rise. There is the linear relationship between the concentration in 5.0～20.0μg/mL and ·OH scavenging rate, when IC_{50} was calculated from regression equation, the IC_{50} of the extracts are 25.61μg/mL by hot water, 26.15μg/mL by PEF treatment, 49.43μg/mL of Vit C and ·OH scavenging activity was 10.04 times as much as Vit C.

2.3. $O_2^{\cdot-}$ scavenging activity

Table 4 shows the linear relationship between the concentration of polyphenol components and $O_2^{\cdot-}$ radical elimination rate. We calculated regression equation and IC_{50} of the extracts.

Table 4. $O_2^{\cdot-}$ scavenging rate as to the concentration

Division	Concentration /(μg·mL^{-1})	Scavenging rate/%	Division	Concentration /(μg·mL^{-1})	Scavenging rate/%
Extract by hot water	5	49.62±1.07	Extract by HIPEF	15	63.45±1.15
	10	57.35±0.85		20	71.19±0.98
	15	64.58±0.46	Vit C	5	38.39±0.96
	20	70.19±0.80		10	53.55±1.20
Extract by HIPEF	5	48.76±0.66		15	61.46±0.18
	10	56.30±1.20		20	74.56±1.03

The IC_{50} of the extracts are 4.94μg/mL by hot water, 5.83μg/mL by PEF treatment, and $O_2^{\cdot-}$ scavenging activity was 1.76 times as much as Vit C.

2.4. Lipid radical scavenging activity

Table 5 shows the influence of polyphenol components on the lipid radical scavenging activity *in vitro*.

Table 5. The influence of polyphenol components concentration on the lipid radical scavenging activity

Division	Concentration /(μg·mL^{-1})	Elimination rate/%	Division	Concentration /(μg·mL^{-1})	Elimination rate/%
Hot water	10	42.65±0.43	PEF	10	43.51±1.30
	20	53.47±0.99		20	51.28±1.15
	30	68.52±1.22		30	68.44±1.28
	40	83.70±1.17		40	83.59±1.40

$p<0.05$

As shown in table 5, the elimination rate was increased when the concentration rises. The IC_{50} of extracts are 16.26μg/mL by hot water, 16.48μg/mL by PEF treatment, and lipd radical scavenging activity was 2.56 times as much as Vit C.

The investigators have reported that the higher electric field strengths and pulse number (treatment time, μs) were, the more extraction rate increases, but when PEF treatment is excessive the limitation, the extraction rate decreases reversely, because of denaturation and conformation variation of the biological components in the molecular level [2, 5, 6].

But, our results show that the antioxidant activity of moxa leaves is much higher than Vit C, thus standard PEF treatment does not influence on structural stability of polyphenol components and can use effectively in extraction of useful components in animal and plant.

Conclusion

Pulsed electric fields were studied to extract polyphenol components from moxa leaves. This work clearly has been demonstrated that the combination of polyphenol components extraction method and PEF treatment techniques was an effective method in order to achieve a higher level of polyphenol components extraction. PEF treatment conditions does not influence on structural stability of polyphenol components contained by moxa leaves and the antioxidant activity of polyphenol components. The antioxidant activity of the extract is 1.91 times (DPPH), 10.04 times (·OH), 1.76 times (O_2^-) 2.56 times (lipid radical scavenging activity) as much as Vit C.

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