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Total phenolic, isothiocyanate and radicle elongation determination of mung bean sprout during germination affected by different variables of pulsed electric field treatment

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Abstract

Mung bean germination started with moisture uptake, biochemical modification, interconversion and biosynthesis of metabolites. Mung bean sprout was commonly used a fresh salad vegetable with available amino acids, dietary fiber, minerals, vitamins and phytonutrients. Pulsed electric field (PEF) was a prominent technology as a green approach for improvement of seed germination. This research evaluated some technical parameters of PEF such as pulse electric field strength (300-1500 kV/cm), pulse number (50 -250) and pulse width (1.0-3.0 µs) at a stable frequency 1 Hz in ambient temperature on the total phenolic, isothiocyanate and radicle elongation of mung bean sprout. Results showed that pulse electric field strength and pulse number significantly increased isothiocyanate and total phenolic content while retardation of root length extension. Meanwhile pulse width showed a negative impact on total phenolic, isothiocyanate while extending root length extension. PEF should be conducted at pulse electric field strength (1200 kV/cm), pulse number (150), pulse width (1.5 µs) to retain the highest isothiocyanate (33.96±0.35 nmol/sprout) and total phenolic (221.30±1.46 mg GAE/100 g) while keeping a fair radicle elongation (8.39±0.17 mm). PEF treatment would be a promising non-lethal technology feasible for mung bean seed sprouting improvement.

Keywords

germination, isothiocyanate, mung bean sprout, total phenolic, pulsed electric field, radicle elongation

Introduction

Mung bean (*Vigna radiata* L.) was widely cultivated in tropical and subtropical regions (1). Seed germination began with moisture absorption and ended the elongation of the embryonic axis (2). This process lasted about 1-2 days depending on the incubation temperature (3). During sprouting, protein and starch were metabolized into amino acids and simple carbohydrates (4). The seed germination not only facilitated for the absorption of nutritional constituents but also degraded anti-nutritional elements (5, 6). Mung bean sprout was rich in proteins, vitamins, dietary fibers, minerals, polyphenols, polysaccharides and peptides with excellent antioxidant capability (7). Consumption of mung bean sprout was beneficial to prevent cancer, melanogenesis, hypertension, hyperglycemia, hyperlipemia (8-13). Isothiocyanate amassed in sprout greatly contributed to functional properties against cancer, tumor and inflammatory (14, 15).

Pulsed electric field (PEF) was one of the most prevalent non-thermal

processing technologies applied in agriculture and food industry due to its excellent proficiency, low-cost operation and versatile technique (16, 17). PEF supported for the extraction of phytochemical metabolites and improved shelf-life extension of materials without chemical residue (18, 19). PEF was believed to be a safe alternative that promoted cell membrane permeability, the ingredients of raw material exposing to the high field strength of narrow pulse width might induce provisional or eternal cavity in the cell membrane (20). The retarding and triggering germination validities induced by PEF were depended on the biochemical and physiological behavior of grains, electric field strength and magnitude (21). PEF interacted to the metabolism to release reactive oxygen species (ROS) and secondary metabolites (22, 23). Under PEF treatment, expanded holes in the plasma membrane led to the influx and efflux of polar molecules (24). Germinating barley treated by pulsed electric fields under pulse width 1 ms and field strength 1200 V/cm had the affected root length with exception of total metabolic activity of the seeds (3). Pulsed electric field was compared to UV- irradiation, hot water dip and ethanol vapours on the quality and stability of mung bean sprouts. There was no significant difference of different applications on sprout length and weight, except in ethanol treatment (25).

Purpose of our study examined the effect of some technical variable of PEF such as pulse electric field strength, pulse number and pulse width on the total phenolic, isothiocyanate and radicle elongation of mung bean sprout during germination.

Materials and Methods

Material and chemicals

Mung bean seeds were purchased from local market. All seeds were manually selected to ensure neither defect nor damage. They were set in wire mesh basket ready for water soaking. Chemical reagents were all HPLC grade such as acetonitrile, methanol from Merck (Germany); sulforaphane, 2-propanol, 1,2-benzenedithiol, phosphate buffer, Folin-Ciocalteu reagent, Na_2CO_3, gallic acid from Sigma Aldrich (USA). A pilot PEF equipment with 2 parallel electrodes was equipped in a chamber by 15 cm x 18 cm (length x width).

Researching method

600 gm of mung bean seeds was soaked in 2 l water at room temperature for 8 hr. They were lift out of water for 15 s to remove excess water. These seeds were treated by PEF under different field strength (300, 600, 900, 1200, 1500 V/cm), pulse number (50, 100, 150, 200, 250), pulse width (1.0, 1.5, 2.0, 2.5, 3.0 µs) at a fix frequency 1 Hz in ambient temperature. The PEF-treated mung bean seeds were wrapped by wet cloth and incubated at ambient condition. After 24 hr of incubation, the cloth wrapping mung bean seeds was wetted with 250 ml water. These seeds continued incubating for 24 hr. The sprouts collected from the germination process were ready for physicochemical analysis.

Root length or radicle elongation (mm) was examined by micrometer. Isothiocyanate (nmol/sprout) was determined according to the standard procedure (26). Sprouts were finely crushed and mixed with water (1:4, w/v) and kept at room temperature for 60 min. The mixture was centrifugated at 4000 rpm for 3 min. 100 µl of supernatant was diluted with 100 µl of phosphate buffer (0.05 mol/l, pH 8.5) and 200 µl of 2-propanol of 1,2-benzenedithiol (5 mmol/l). The blend was kept warm at 60±2 °C for 90 min. Isothiocyanate (nmol/sprout) was determined by high-performance liquid chromatography method (Techno, model: HPLC 580) using a polymeric C18 column at 368 nm, sulforaphane as standard reagent, 75% acetonitrile as mobile phase at a flow rate of 0.8 ml/min. Total phenolic content or TPC (mg GAE/100 g) was quantified by Folin-Ciocalteu reagent procedure (27). Extract was diluted with 90% methanol (v/v) in a 10 ml test tube and centrifuged at 5000 rpm for 3 min. Aliquot 1 ml of the extract was reacted with 1 ml Folin-Ciocalteu reagent 12% (w/v). After 15 min of reaction, 3.0 ml of sodium carbonate (10% w/v) was added. Reaction occurred for 45 min in dark place, the absorbance was measured at 760 nm by spectrophotometer (Shimazu, UV-1800) and compared with a pure linear of gallic acid (0-450 mg/l). R^2 of the calibration curve was presented at 0.83.

Statistical analysis

The experiments were run in triplicate with different groups of samples. The data were presented as mean±standard deviation. Statistical analysis was performed by the Statgraphics Centurion version XVI.

Results and Discussion

Pulsed electric field strength

The pulsed electric field strength significantly increased isothiocyanate (27.19±0.26 to 35.51±0.34 nmol/sprout), total phenolic (129.43±1.43 to 238.19±1.79 mg GAE/100 g) in mung bean sprout while radicle elongation was reduced (9.52±0.14 down to 8.01±0.10) (Table 1). The pulsed electric field strength retarded the extension of root length that facilitating for the accumulation of phytonutrients. There was no significant difference of radicle elongation, isothiocyanate and total phenolic between the mung bean treated by field strength 1200 kV/cm and 1500 kV/cm. The field strength 1200 Kv/cm was selected for further experiments. The triggering validity of PEF on germination might be due to water absorption related to electric field

Table 1. Pulsed electric field strength of PEF to isothiocyanate, radicle elongation and total phenolic in mung bean sprout after 2 days of germination

Field strength (V/cm)	300	600	900	1200	1500
Radicle elongation (mm)	9.52± 0.14^{a}	9.14± 0.15^{ab}	8.78± 0.11^{b}	8.36± 0.13^{bc}	8.01± 0.10^{c}
Isothiocyanate (nmol/sprout)	27.19± 0.26^{c}	28.68± 0.31^{bc}	30.04± 0.23^{b}	32.69± 0.30^{ab}	35.51± 0.34^{a}
Total phenolic (mg GAE/100 g)	129.43± 1.43^{c}	157.21± 1.36^{bc}	189.83± 1.84^{b}	207.51± 1.63^{ab}	238.19± 1.79^{a}

Figures are the mean of three replications; Figures in row followed by the same letter/s are not differed significantly (α = P=0.05)

strength (28). PEF promoted metabolic reaction and respiration via releasing oxygen during the catalase scavenging of hydroxyl peroxide. Moisture was deeply diffused into seed kernel, the trypsin and tyrosinase inhibitors were also metabolized (29). The PEF provoked the production of reactive species which stimulated germination (30). PEF induced a 50% increment in the germination rate of lentil seeds (31). Root length of barley sprout was decreased by high pulsed electric field strength (3). Insufficiency of radicle elongation could be the aftermath of the accelerated cell wall integrity associated to the establishment of oxidative internal-bonds in the apoplast. Reduced influx speed of simple particles from the kernel endosperm could be reason of the short radicle (32). PEF at 6 kV/cm with 50 pulses increased from 3% to 9% of the length, 8% to 42% of weight of wheat plantlets (33).

Pulse number

Pulse number (50-250) of PEF treatment caused a slight reduction of root length (8.36±0.13 down to 7.96±0.12 mm). Meanwhile, isothiocyanate and total phenolic gradually increased (32.69±0.30 to 37.25±0.30 nmol/sprout, 207.51±1.63 to 290.26±1.75 mg GAE/100 g, respectively). At pulse number 150, the isothiocyanate, total phenolic and radicle elongation were no significant difference with values in the samples treated by pulse number 200 and 250; hence pulse number 150 was chosen for next experiment (Table 2). The PEF created numerous holes on seed surface which enabled the mass transfer of external elements into the core kernel. Radicle elongation of barley sprout was impaired by field strength over 10000 V/cm and pulse number over 30 pulses (3). PEF exposure at field strength 6 kV/cm and pulse number 50 pulses improved moisture absorption, seed sprouting performance (33). Isothiocyanate was originated from enzymatic myrosinase breakdown of glucosinolate. Isothiocyanate was accounted for the strong taste of mustard, radish or broccoli sprouts (34). Isothiocyanate was important in food processing due to its antimicrobial, neuroprotective and anticarcinogenic characteristics (35). This substance was water soluble and thermal sensitive during processing (36).

Table 2. Pulse number (n) of PEF to isothiocyanate, radicle elongation and total phenolic in mung bean sprout after 2 days of germination

Pulse number	50	100	150	200	250
Radicle elongation (mm)	8.36± 0.13^{a}	8.31± 0.11^{a}	8.13± 0.16^{ab}	8.01± 0.13^{b}	7.96± 0.12^{b}
Isothiocyanate (nmol/sprout)	32.69± 0.30^{b}	32.95± 0.25^{b}	34.72± 0.33^{ab}	37.19± 0.29^{a}	37.25± 0.30^{a}
Total phenolic (mg GAE/100 g)	207.51± 1.63^{b}	213.18± 1.68^{b}	246.52± 1.63^{ab}	287.64± 1.59^{a}	290.26± 1.75^{a}

Figures are the mean of three replications; Figures in row followed by the same letter/s are not differed significantly (α = P=0.05).

Pulse width

Pulse width (1.0-3.0 µs) caused a negative impact by decreasing isothiocyanate (34.72±0.33 down to 31.69±0.34 nmol/sprout) and total phenolic (246.52±1.63 down to 136.70±1.50 mg GAE/100 g) while increasing radicle elongation (8.13±0.16 to 8.97±0.11 mm). There were no significant difference of radicle elongation, isothiocyanate and total phenolic on sampled treated by pulse width 1.0 µs and 1.5 µs (Table 3). Pulse width should be controlled not over 1.5 µs to retain the highest isothiocyanate (33.96±0.35 nmol/sprout) and total phenolic (221.30±1.46 mg GAE/100 g) while achieving a medium radicle elongation (8.39±0.17 mm). Pulse width 1 ms and field strength 1200 V/cm affected to root length meanwhile there was no significant difference in the total metabolic activity of the seeds (3). The accumulation in phenolic content in sprout from PEF-treatment might be due to the interaction between PEF and cells leading to the cell wall fracture, DNA vulnerability, modification of enzymatic activity, protein structure and activation of calcium channel and growth constituents (37). PEF-treatment induced the increment of total phenolic in seed wheat plantlets (33).

Table 3. Pulse width (µs) of PEF to isothiocyanate, radicle elongation and total phenolic in mung bean sprout after 2 days of germination

Pulse width (µs)	1.0	1.5	2.0	2.5	3.0
Radicle elongation (mm)	8.13± 0.16^{c}	8.39± 0.17^{bc}	8.65± 0.12^{b}	8.83± 0.14^{ab}	8.97± 0.11^{a}
Isothiocyanate (nmol/sprout)	34.72± 0.33^{a}	33.96± 0.35^{ab}	33.04± 0.29^{b}	32.37± 0.31^{bc}	31.69± 0.34^{c}
Total phenolic (mg GAE/100 g)	246.52± 1.63^{a}	221.30± 1.46^{ab}	201.54± 1.59^{b}	175.98± 1.57^{bc}	136.70± 1.50^{c}

Figures are the mean of three replications; Figures in row followed by the same letter/s are not differed significantly (α = P=0.05).

Conclusion

Mung bean sprout was a popular healthy food. During germination, the most valuable nutritional components in the mung bean seed were retained, while a great amount of bioactive constituents significantly accumulated. We have successfully examined several technical parameters of PEF on the germination of mung bean seed. Findings of this research found that pulsed electric field strength, pulse number showed a positive effect on physicochemical and phyto-nutritional properties like radicle elongation, isothiocyanate and total phenolic of mung bean sprout. On contrary, pulse width revealed a negative impact on mung bean sprout during germination. PEF treatment should be utilized in mung bean seed sprouting to improve its germination efficiency.

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Authors contributions

NPM arranged the experiments and also wrote the manuscript.

Compliance with ethical standards

Conflict of interest: The author strongly confirmed that this research is conducted with no conflict of interest.

Ethical issues: None.

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