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Effect of pulsed electric field on functional and structural properties of canola protein by pretreating seeds to elevate oil yield

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ABSTRACT

Pulsed electric field (PEF) was applied to moisture-preconditioned canola seeds to evaluate the effect on oil yield. The effect of PEF on functional and structural properties of canola protein left over after oil extraction were also examined in albumin and globulin fractions obtained at optimal conditions for oil yield. Generally, PEF pretreatment significantly improved ($P < 0.01$) the following functional properties of canola protein and its fractions: solubility, water-holding capacity, emulsibility, emulsion stability, oil-holding capacity, foamability, and foam stability. Infrared spectrometry revealed that the secondary structure of the protein changed upon PEF, as shown by changes in the proportions of α-helices, β-sheets, and β-turns in the amide I region. An increased amount of free sulfhydryl groups and surface hydrophobicity suggested alterations in tertiary structure as well. PEF caused the formation of new protein aggregates with low molecular mass, demonstrated by sodium dodecyl sulfate polyacrylamide gel electrophoresis and changes in the number of total sulfhydryl groups.

1. Introduction

Pulsed electric field (PEF) is of scientific interest for its non-thermality, which helps to keep native flavor and nutrients intact. This technique was initially introduced in liquid food processing for microbial and enzyme inactivation, mainly in fruit juices, eggs, and milk, and in plant cell rupture to improve extraction of target substances such as vegetable oil. However, defects occur when the electrodes are immersed in the filling electrolyte in liquid systems. For example, the solution electrolyzes, the electrodes corrode, and small particles of electrode material shift into the liquid because of electrochemical reactions (Morren, Roodenburg, & de Haan, 2003). Additionally, high temperatures are observed at higher electric current settings (Heinz, Toepfl, & Knorr, 2003).

There is a need to extend the utilization of PEF into solid food processing and only a few attempts have been made to this effect. Fortunately, PEF is effective in treating low-moisture solid material (Qian, Gu, Jiang, & Chen, 2014) with no temperature variation, as the treatment chamber is a capacitor open to the air. Furthermore, the induced electric current remains low, which enables PEF to be utilized in raw material collection and pretreatment, semifinal product processing in-line, and/or final product storage. One study attempted to exploit PEF in solid food material by pretreating moisture pre-conditioned canola seeds to elevate oil yield, and a positive outcome was achieved (Wang & Qian, 2016).

Lower-temperature processing has been pursued in the oil industry, as it denatures the protein less; therefore, the proteins could retain their natural functions. One of the materials used for oil preparation is the seed of Brassicaceae (Cruciferae) plant, which contains significant amounts of stored oil and protein. Double low (low in erucic acid and low in glucosinolate) rapeseed cultivars (canola) have been developed to improve their edibility and reduce toxicity. The by-product left after oil extraction of rapeseed meal is the protein fraction, with protein content and amino acid composition similar to those of soybean. This rapeseed meal by-product can be used not only as forage for livestock, but also in the food industry because of its interesting functional properties (Aider & Barbana, 2011; Aluko & McIntosh, 2001). To fully utilize the proteins remaining after oil extraction, this present work aimed at shedding light on the effect of PEF on the functional and structural properties of canola protein and its albumin and globulin fractions, based on previous investigation in which PEF was successfully exploited to elevate oil yield.

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2. Material and methods

2.1. Materials and reagents

The double low rapeseed cultivar which is most extensively planted in China nationwide, Qinyou No. 7, grown in Yancheng, Jiangsu Province was purchased from an online shop; it contains 69.9 g/kg of moisture, 185.4 g/kg of protein (N × 6.25), 390.0 g/kg of crude lipids, and 29.59 mmol/kg of glucosinolate (spectrometric method). The soybean oil with added tertiary butylhydroquinone (TBHQ) was manufactured by Yihai Kerry Cereals & Oils Industry Co., Ltd., Taizhou, Jiangsu, and purchased from a local supermarket. Sodium dodecyl sulfate (SDS), glycine, tris[hydroxymethyl]aminomethane (Tris), and dialyzing tubes (molecular mass cutoff of 7000–14,000 Da) were produced by Shanghai Lanji Sci. & Tech. Development Company. Hydrogen chloride and sodium chloride were obtained from Hengli Reagents Factory. Urea, Coomassie brilliant blue G-250, 8-benzene amino-1-naphthalene sulfonic acid (ANS), and *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED) were purchased from Sigma-Aldrich, China. 5,5'-dithionitrobenzoate (DTNB) and protein markers (14.4–116.0 kDa) were manufactured by Shanghai Bio-Engineering Co. Ltd.

2.2. Pulsed electric field treatment and oil extraction

Canola seeds were pre-conditioned to moisture content of approximately 500 g/kg and applied to PEF treatment using the device and method described by Qian et al. (2014). After drying at 40 °C in a motor-driven air dryer (DGX9053B-2, Shanghai Fuma Experimental Equipment Co., Ltd., China) for about 10 h to a moisture of 60 g/kg, the seeds were ground and oil was extracted according to the Folch method, using isopropanol as the solvent at a material to liquid ratio of 1:20. The extracts of three repeating extractions were pooled and vaporized to remove the solvent in a rotation distillator (RE-52D, Shanghai Yarong BioChemical Instrument Factory, China). The oil yield was calculated as oil yield (%) = mass of extracted oil/mass of oil in the seeds (determined with Soxhlet extraction method).

Factorial trials were performed to investigate the influence of PEF on oil yield and proteins. The voltage was set from 10 to 35 kV at 5 kV intervals at a pulse frequency of 600 Hz, pulse width of 8 μs, and residence time of 180 s; the residence time was set from 60 to 210 s at intervals of 30 s at the voltage of 30 kV, pulse frequency of 600 Hz, and pulse width of 8 μs; the frequency was set at 100 and 200–1000 Hz at intervals of 200 Hz, at the voltage of 30 kV, residence time of 180 s, pulse width of 8 μs; and the pulse width was set at 1 and 2–10 μs at intervals of 2 μs, at the voltage of 30 kV, pulse frequency of 600 Hz, and residence time of 180 s, when the voltage, residence time, pulse frequency, and pulse width were taken as the subject factorial variables, respectively. The optimal conditions for maximum oil yield were obtained using response surface methodology according to the Box-Behnken design, with the four variables at three levels based on the above factorial trials.

2.3. Preparation of canola protein isolate

The defatted meal of PEF-treated canola seed was extracted with alkaline distilled water, followed by precipitation with acid to obtain canola protein isolate (CPI) according to methods described by Yoshie-Stark, Wada, & Wasche (2008). Briefly, ten volumes of distilled water adjusted to pH 12.0 with 1 mol/L NaOH were added to canola meal. The slurry was stirred for 1 h at room temperature, and then centrifuged at 3000 × *g* for 20 min. The pellet was re-extracted two more times. The supernatants of the three extractions were pooled, adjusted to pH 6.0 with 1 mol/L HCl, and incubated for 1 h at room temperature, followed by centrifugation at 3000 × *g* for 20 min. The precipitate was re-dissolved in distilled water, adjusted to pH 7.0 with 1 mol/L NaOH, dialyzed against distilled water for 6 h, and finally freeze-dried to obtain CPI.

2.4. Fractionation of albumin and globulin

Canola protein was fractionated to obtain albumin and globulin fractions according to the solubility. Briefly, 20 g of defatted canola meal was dispersed in 200 mL of distilled water adjusted to pH 11.0. This mixture was stirred at 30 °C for 2 h, and then the dispersion was centrifuged at 3000 × *g* for 20 min to obtain the supernatant and pellet. The supernatant was incubated at room temperature for 2 h, adjusted to pH 6.0, and centrifuged at 3000 × *g* for 20 min. The precipitated albumin was freeze-dried. The pellet was dispersed in 100 mL of NaCl solution at a concentration of 20 g/L and stirred at room temperature for 2 h, then centrifuged at 3000 × *g* for 20 min. The supernatant was adjusted to pH 7.2, and centrifuged at 3000 × *g* for 20 min; the precipitated globulin was dialyzed and freeze-dried.

2.5. Determination of functional properties of protein

2.5.1. Determination of solubility

Protein solubility was determined according to methods described by Molina, Papadopoulou, & Ledward (2001). Protein (10 g/L) dissolved in distilled water was stirred at room temperature for 1 h and centrifuged at 3000 × *g* for 20 min. The solubility (S) was calculated as S = A/B × 100%, where A is the amount of dissolved protein in the supernatant (g) and B is the total protein (g).

2.5.2. Determination of water-holding capacity

Protein (0.1 g) (M) was transferred to a centrifuge tube and weighed (B, g). Next, 5 mL of distilled water was added, and the solution was vortexed. The tube was centrifuged at 3000 × *g* for 15 min followed by incubation for 20 min, then the supernatant was discarded and the tube was weighed again (A, g). The water-holding capacity (WHC) was calculated by WHC = (A-B)/M.

2.5.3. Determination of oil-holding capacity

Protein (0.1 g) (M) was transferred to a 10-mL graduated centrifuge tube, and 5 mL of soybean oil was added. After vortexing thoroughly, the tube was centrifuged (3000 × *g*, 20 min), followed by standing for 20 min at room temperature. The volume of oil supernatant was read as V in mL, and the oil-holding capacity (OHC) in mL/g was calculated by OHC = (5-V)/M.

2.5.4. Determination of foaming capacity and foam stability

A dispersion of 1.0 g of protein in 100 mL distilled water was stirred at 10,000 rpm for 1 min with a Warring blender and transferred to a volumetric cylinder. The volume was measured immediately (V_0) and after standing for 30 min (V). The foaming capacity (FC, %) and foam stability (FS, %) were calculated as FC = V_0/100 and FS = V/V_0, respectively.

2.5.5. Determination of emulsifying capacity and emulsion stability

A centrifugation method was utilized to determine the emulsifying capacity (EC) and emulsion stability (ES) according to procedures described by Sathe (1982). Protein (0.2 g) was dispersed in 5 mL of distilled water in a 100-mL graduated centrifuge tube. After 5 mL of soybean oil was added, the tube was centrifuged at 195 × *g* for 3 min, and the height of the emulsion (H_1) and liquid (H_2) layers was noted. The tube was then heated at 80 °C for 20 min in a water bath and cooled down to room temperature with tap water. The