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Effects of millisecond and microsecond pulsed electric fields on red beet cell disintegration and extraction of betanines

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ABSTRACT

The efficacy of PEF treatments—in the range of milliseconds at moderate electric field strengths (0.2–0.6 kV/cm) and in the range of microseconds at high electric field strengths (2–6 kV/cm)—has been compared in terms of red beet cell disintegration degree (*Zp*), extraction of betanines and total specific energy requirements.

In the range of treatment conditions investigated, the highest *Zp* value was larger for the treatments applied in the millisecond range than for the treatments applied in the microsecond range. While in the treatment applied at 0.6 kV achieved a *Zp* value higher than 0.95 after 60 ms of treatment, the highest *Zp* value obtained at 6 kV/cm in the microsecond range was 0.78 after 75 μs of treatment. In the range of 0–20 kJ/kg and *Zp* values among 0–0.75, the disintegration index mainly depended on the total specific energy applied, independently of the electric field strength or the duration of the treatment.

The comparison of the maximum betanine extraction yield (BEY_{max}) in the ms and μs ranges under the conditions in which the maximum extraction was observed revealed that the improvement in the betanine extraction was similar. The more intense treatment conditions applied in the ms range (0.6 kV/cm; 40 ms) and the μs range (6 kV/cm; 150 μs) increased the BEY_{max} 6.6 and 7.2 times, respectively, compared with the control. However, the comparison of the BEY_{max} in the ms and μs ranges under the same total specific energy revealed that the improvement in the betanine extraction was higher for the treatment applied in the μs range. For example, to obtain a BEY_{max} of 775 μg/g, a total specific energy of 6 kJ/kg was required when the treatment was applied in the μs range (75 μs; 4 kV/cm) and around 20 kJ/kg if the treatment was applied in the ms range (40 ms, 0.4 kV/cm).

1. Introduction

Mass transfer is a unit operation that happens in many processes of the food industry in which the aim is obtaining a given substance of interest (fruit juices, sugar, colorants, antioxidants, etc.), removing water from foods (drying), or introducing a given substance into the food matrix (osmotic dehydration, salting or curing).

The structure of most plant-based foods consists of tissues that are composed of cells with a basic eukaryotic organization (Aguilera and Stanley 1999). The cytoplasm of a eukaryotic cell is enclosed in a cytoplasmic membrane that defines its boundaries. This membrane consists of a phospholipid bilayer that contains proteins inserted within the lipid matrix. Mass transfer in food material composed by cells mainly depends on diffusion through the cell membranes. The presence of an intact cytoplasmic membrane, which acts as a semipermeable barrier, influences the migration of substances into or out of the food tissues (Donsi et al., 2010). It has been estimated that the average diffusion coefficient of a small solute in a membrane is often about a million times lower than that in adjacent aqueous solutions (Nobel, 1999). In order to increase the velocity of the mass transfer and reduce the operation time, food materials are pretreated in many cases by mechanical grinding, heat or enzymes to enhance the mass transfer rate by increasing the permeability of the cell membranes (Toepfl et al., 2006a). However, these techniques may require a significant amount of thermal or mechanical energy, can cause the loss of valuable food compounds, and may contaminate the extracts with undesirable compounds by disrupting the cells in small fragments.

Treatment of food material by pulsed electric fields (PEF) could replace these conventional techniques. This technology has been proven as an effective method for irreversible permeabilization of cell membranes in plant and animal tissues without increasing temperature and at low operating costs (Toepfl et al., 2006b). The use of pulsed electric fields is a treatment that involves the

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application of direct-current voltage pulses, ranging from microseconds to milliseconds, through a biological material placed between two electrodes. This voltage results in an electric field (E), the intensity of which depends on the gap between the electrodes and the voltage delivered. The application of the external electric field causes an increase of the permeability of the cytoplasmic membrane of the cell to ions and macromolecules as consequence of the formation of local defects of pores.

Although there is not a formal definition, electric field strengths of 0.1–1 kV/cm can be considered moderate electric fields and those with strengths of E > 1 kV/cm can be considered high-intensity electric fields (Asavasanti et al., 2010). Treatment duration—defined as the pulse duration multiplied by the number of pulses—and the strength of the electric field are the main processing parameters that determine the efficacy of PEF treatment. Although some studies for molecular biology and clinical biotechnological applications have been conducted in the nanosecond range (Son et al., 2014; Beebe et al., 2002), the duration ranges used in different studies related to improving the release of intracellular compounds from plant cells are in the millisecond to microsecond range (Vorobiev and Lebovka, 2006; Donsi et al., 2010; Puertolas et al., 2012). A reduction in the treatment duration from milliseconds to microseconds has to be compensated by an increase in the electric field intensity. The interdependencies of these two main processing parameters determines the total specific energy required to cause cell electroporation and depends on the voltage applied, total treatment time, and resistance of the treatment chamber, which varies according to the geometry and conductivity of the material treated (Heinz et al., 2001).

The application of PEF in the range of milliseconds to microseconds as a permeabilization treatment to improve mass transfer of valuable compounds from cells of plant tissues has been demonstrated to be effective in the extraction of different compounds of interest, such as betanines from red beet. Betanines are water-soluble vacuolar pigments used as natural pigments in different products such as dairy products, fruit fillings, confectionary goods, meat substitutes and sausages (Stinzing and Carle, 2007). PEF-assisted aqueous extraction of betanine from red beet has been investigated by several authors (Chalermchat et al., 2004; Fincan et al., 2004; López et al., 2009; Loginova et al., 2011). However, the different experimental conditions and pulse generators used in these studies make it difficult to determine whether treatment is more effective for extracting this compound in the millisecond or microsecond range, in terms of total specific energy.

The objective of this investigation was to compare the efficacy of PEF treatments in the range of milliseconds at moderate electric field strengths (0.2–0.6 kV/cm) with treatments in the range of microseconds at high electric field strengths (2–6 kV/cm) in terms of the degree of cell disintegration, total specific energy and extraction of betanine.

2. Material and methods

2.1. Samples

Fresh red beet (*Beta vulgaris* L.) were purchased from a local market and stored at 4 °C until use. Different sizes of red beetroots samples were used for the PEF treatments in the millisecond and microsecond ranges in order to obtain the treatment chamber resistance required by the PEF equipment. For treatments in the millisecond range, one cylinder of 10 mm diameter and 30 mm length was cut using a cylindrical cutting device. The sample was placed in the treatment chamber and treated with PEF. The PEF-treated cylinder was then cut into three pieces (each 10 mm long), weighed and transferred to the extracting medium. For treatments in the microsecond range, three cylinders of 15 mm diameter and 10 mm length were cut using a cylindrical cutting device. The samples were placed in the treatment chamber and treated with PEF. PEF-treated cylinders were then cut in pieces of 10 mm diameter, weighed and transferred to the extracting medium to study the transient variation of betanine concentration. Although different sizes of red beetroots were used for the PEF treatments, betanine extraction was performed using samples with the same dimensions to avoid the influence of particle size.

2.2. PEF equipment

2.2.1. PEF equipment for the application of millisecond pulses

The PEF equipment that was used in this investigation was a Bio-Rad Gene Pulser Xcell Electroporation System (Bio-Rad, Hercules, CA, USA). The equipment consists of a set of capacitors, with a maximum capacitance of 3275 μF, that generates square waveform pulses ranging in duration from 0.05 to 5 ms with a maximum output voltage of 3000 V. A parallel electrode treatment chamber composed of a cylindrical methacrylate tube closed with two polished stainless steel cylinders was used to apply the PEF-treatments. The electrode diameter was 10 mm and the gap between the electrodes was 30 mm.

The PEF treatments ranged from 10 to 80 pulses of 1 ms (10–80 ms) with a frequency of 1 Hz at electric field strengths ranging from 0.2 to 0.6 kV/cm. The specific energy of these treatments ranged from 1.6 to 57.6 kJ/kg. Specific energy input (W) per treatment expressed in kJ/kg was calculated by the following equation:

$$W = \frac{1}{m} \times V \times I \times t \times \mathrm{Np} \quad (1)$$

where m is the mass of the red beetroot (kg), V is the input voltage (kV), I is the current intensity (A), and t is the treatment time (s) and Np is the number of applied pulses.

2.2.2. PEF equipment for the application of microsecond pulses

The PEF equipment that was used in this investigation was supplied by ScandiNova (Modulator PG, ScandiNova, Uppsala, Sweden). The apparatus generates square waveform pulses of 3 μs duration with a frequency of up to 300 Hz. The maximum output voltage and current were 30 kV and 200 A, respectively. The equipment consists of a direct current power supply that converts the 3-phase line voltage to a regulated DC voltage. It charges up 6 IGBT switching modules (high-power solid-state switches) to a primary voltage around 1000 V. An external trigger pulse gates all the modules and controls its discharge to a primary pulsed signal of around 1000 V. Finally, a pulse transformer converts this primary 1000 V pulse to the desired high-voltage pulse.

A parallel electrode treatment chamber consisted of a cylindrical methacrylate tube closed with two polished stainless steel cylinders was used to apply the PEF-treatments. The electrode diameter was 15 mm and the gap between the electrodes was 10 mm.

The PEF treatments ranged from 5 to 100 pulses of 3 μs (15–300 μs) with a frequency of 1 Hz, at electric field strengths ranging from 2 to 6 kV/cm. The specific energy of these treatments ranged from 0.04 to 74.16 kJ/kg. Specific energy input (W) per treatment was calculated by Eq. (1).

2.3. Cell disintegration index

The cell disintegration index (Zp) was used to identify the range of PEF treatment conditions for the pre-treatment of the red beet before the betanine extraction. This index characterizes the proportion of permeabilized cells based on the frequency dependence of conductivity of intact and permeabilized plant tissues (Angersbach et al., 1999).