

Processing of sugar beets assisted by pulsed electric fields

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Abstract: Pulsed electric fields (PEFs) are becoming more and more popular in different applications in processing agricultural products. The PEF technology is based on the application of high voltage short pulses that allow electroporation in the cell membranes. This review outlines the PEF applications used in processing sugar beets. New perspective technologies of the cold or warm aqueous extraction of sugar, cold pressing of sugar beet cossettes, and combined pressing-diffusion process are discussed. Electroporation devices for pilot and industrial applications in the sugar beet industry are also presented.

Keywords: aqueous extraction; diffusion; electroporation; pressing; pulsed electrotechnologies

The sugar beet (*Beta vulgaris*) is a very important agricultural crop in many countries in the world (Stevanato et al. 2019). It can be grown successfully outside tropical regions, and currently, it contributes more than 20% of the world's sugar production (the rest is produced from sugar cane; *Saccharum hybrids* L.). The sugar beet root contains ≈17.5% of sugar, ≈75% of water, ≈5% of insoluble solids (pectin, cellulose, hemicellulose, etc.), and ≈2.5% of minerals, as well as nitrogenous and non-nitrogenous substances (Figure 1) (van der Poel et al. 1998; Zicari et al. 2019).

The extraction method for obtaining sugar from sugar beets was firstly suggested by the German chemist Marggraf (1747). Conventional sugar production from the sugar beet is rather conservative and is based on aqueous extraction and juice purification processes (Figure 2) (McGinnis 1982; Asadi 2006; Schiweck et al. 2012). The aqueous extraction method utilises the hot water diffusion/extraction of sugar from sliced cossettes (i.e. long grated particles) at 70–75 °C. The hot water is slightly acidified water having a pH of about 5.5 to 5.8. In diffusers, the cossettes and hot water flow in opposite directions

(counter-current exchange). Typically, the diffusion process (to pass the cossettes through the diffuser) is time-consuming (≈90 min) as well as energy-consuming. For the juice purification, liming (addition of calcium hydroxide) is used. For the lime precipitation, carbonisation is used (carbon dioxide is bubbled through the sugar solution). Finally, the juice is filtered, demineralised, and concentrated via evaporation and vacuum pans and is fed to crystallisers.

However, the conventional technique of sugar production from sugar beets has many disadvantages related to the long extraction time, high power consumption, complexity of the purification procedures and related environmental problems. The conventional lime-carbonic purification method is rather complex. The purification operation is multi-staged with several liming and carbonisation steps. This process is accompanied by the consumption of a large quantity of lime (up to about 2.5% of the total weight of the processed sugar beets).

Typical by-products of the conventional techniques are a fibrous sugar beet pulp (≈70%), molasses or vinasse (concentrated impurities from the

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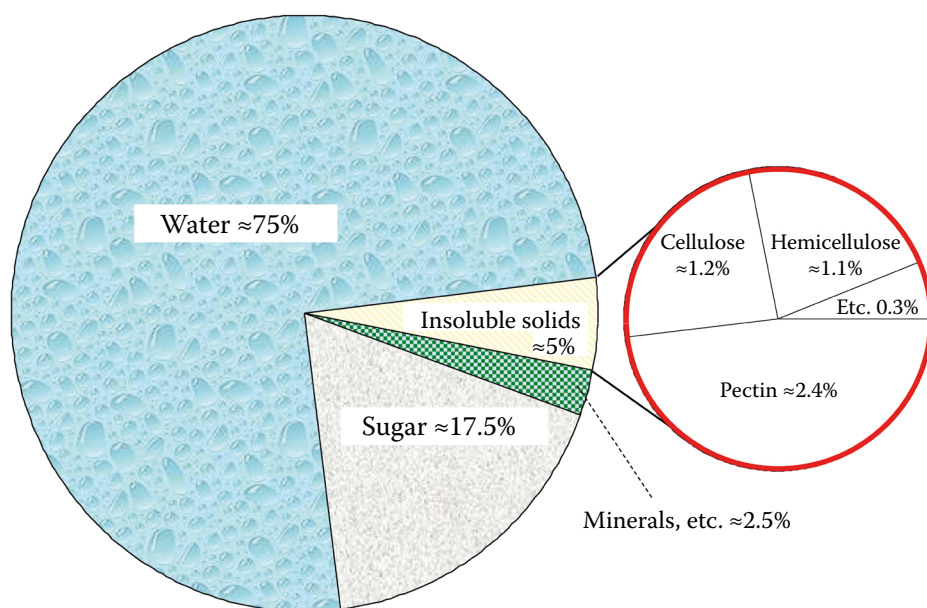


Figure 1. Composition of the root of the sugar beet (wet basis) (van der Poel et al. 1998; Zicari et al. 2019)

sugar refining) ($\approx 10\%$), and sugar beet tails (2–6%). Filter cakes rejected after the juice purification present an important environmental problem. The pressed and dried pulp (flakes or pellets) and molasses are traditionally used for animal feed. The molasses and beet tails can be used for fermentative biofuel and ethanol production, and the sugar crop residue is also suitable for biogas production (Zicari et al. 2019). The pectin from the beet pulp, as excellent gelling, thickening and emulsifying agents (Pacheco et al. 2019; Jafarzadeh-Moghaddam et al. 2021), and phenols, anthocyanins, and antioxidants from the beet molasses have also been demonstrated to have useful applications (Chen et al. 2015).

In recent decades, different pulsed electrotechnologies (moderate electric fields – MEF, pulsed electric fields – PEFs and high voltage electrical discharges – HVEDs) have actively been used for assisting food processing operations (Arshad et al. 2020; Dragomir et al. 2020; Vorobiev and Lebovka 2020; Arshad et al. 2021). Pulsed electrotechnologies assume the application of short duration pulses (from several ns to several ms) at a relatively high electric field strength, E (from $50\text{--}300\text{ V}\cdot\text{cm}^{-1}$ to $10\text{--}50\text{ kV}\cdot\text{cm}^{-1}$). For the MEF treatment mode at $E \leq 100\text{ V}\cdot\text{cm}^{-1}$ and at certain pulse parameters (such as frequency, pulse width, and delay time), a significant minimisation of the electrochemical reactions on electrodes has been observed (Pataro et al. 2014). For non-thermal PEF treatment mode, selective damage to the plasma membranes is expected without significant damage to the cell walls. In plant tissues, this mode can be realised at an

electric field strength of $E \approx 200\text{ V}\cdot\text{cm}^{-1} - 5\text{ kV}\cdot\text{cm}^{-1}$ and a PEF treatment time within $t_{PEF} \approx 10^{-4}\text{--}10^{-1}$ seconds (Vorobiev and Lebovka 2020). Moreover, the combination of MEF and PEF treatments allows one to obtain an electro-processing synergy (Lebovka 2005; Praporscic 2005; Shynkaryk 2006; Lebovka 2007a). Particularly, such combined treatments can be useful in applications for cooking foods (Blahovec et al. 2017). The HVED treatment mode is typically applied at $E = 40\text{--}60\text{ V}\cdot\text{cm}^{-1}$ with a pulse duration of several microseconds. For this mode, the electrical breakdown of water, propagation of streamers, shock waves, and cavitation of bubbles are typically observed. This mode can be useful in different bio-recovery applications (Li et al. 2019).

Application examples of different pulsed electrotechnologies have been demonstrated for assisting in the extraction and recovery of bioactive compounds, dehydration, juice expression, cooling, freezing, thawing and crystallisation, drying and freeze-drying, and inactivation microorganisms and enzymes (Vorobiev and Lebovka 2020). Various electrotechnologies were also applied for the effective valorisation of food waste and by-products (Arshad et al. 2021), bioethanol/biogas production (Kovačić et al. 2021), and the extraction of valuable compounds from microorganisms (Martínez et al. 2020).

This review focuses on the current status of applications of pulsed electrotechnologies, and, in particular, PEF treatments in processing sugar beets. A short overview of the electroporation phenomenon, the basic principles of pulsed electrotreatments, the ef-

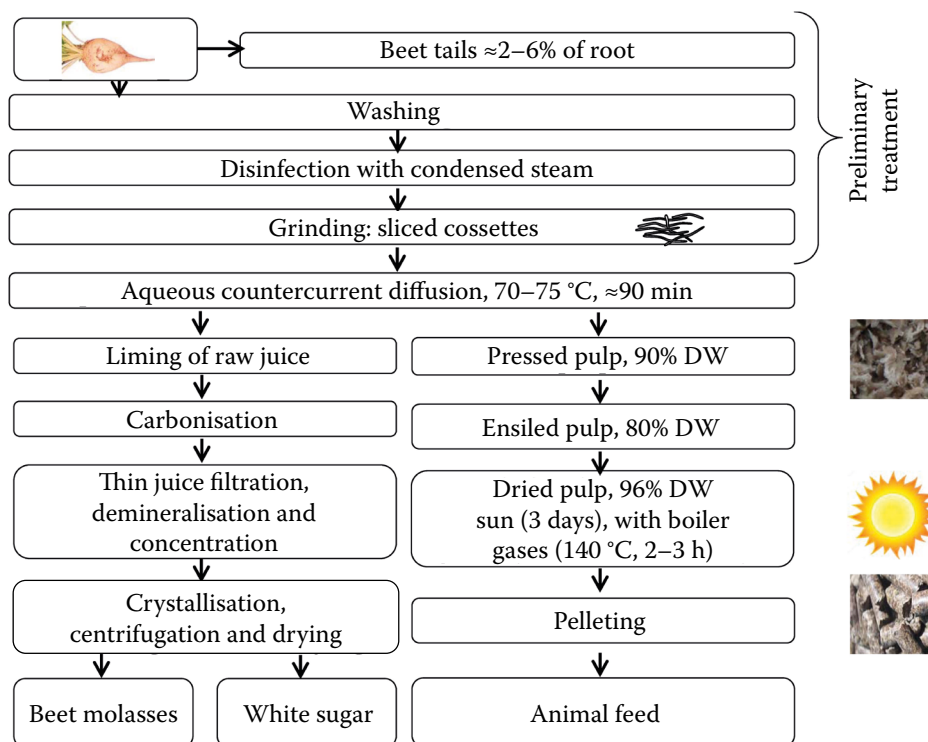


Figure 2. Schematic illustration of the conventional industrial process of sugar production from sugar beets (McGinnis 1982; van der Poel et al. 1998; Asadi 2006; Schiweck et al. 2012)

DW – dry weight

fects of electroporation in sugar beets, the application of electrofusion for sugar beet plant genetic transformation and gene expression, different extraction and expression operation variants assisted by PEF and examples of existing laboratory- and pilot-scale equipment are provided. Finally, the conclusions together with future perspectives and challenges are outlined.

PULSED TREATMENT AND ELECTROPORATION EFFECTS IN SUGAR BEETS

Basic principles of pulsed electrotreatments. Pulsed electrotreatments are aimed at the non-thermal processing of sugar beet tissue without significant ohmic heating. Numerous experiments have shown that PEF processing allows one to achieve significant electroporation (piercing, perforation or electropermeabilisation) of the cytoplasmic membranes in plant cells (Kotnik et al. 2019). The extent of the electroporation in plant materials may depend on the size of the cells, their orientation and spatial distribution in space, electro-physical properties, pH of the media, and presence of air and osmotic agents, etc. The permeability of the membrane can be loosed either temporarily or permanently, and resealing the membranes to the initial or a modified

state is possible with reversible electroporation (Kotnik et al. 2019). Typically, the electroporation of membranes is not accompanied by significant damage to the tissue matrix and, in special experimental conditions, the electroporation of sugar beet tissue can also be observed using a moderate PEF treatment at $E < 100 \text{ V}\cdot\text{cm}^{-1}$ (Lebovka et al. 2007b).

PEF generators include a power source, capacitors, inductance coils, resistors, and quick switches. Such generators can provide monopolar or bipolar pulses of rectangular, exponential decay or damped oscillating shapes (Vorobiev and Lebovka 2020). Bipolar pulses seem to be more advantageous as they demonstrate better electroporation efficiency, offer minimum energy consumption with reduced dissolution of the electrodes, and decreased electrolysis. Generators with rectangular pulses are more expensive than the generators with an exponential pulse shape, but they provide better control of treatment protocols in laboratory experiments.

The main electrotreatment variables are the electric field strength (E), the total treatment time, (t_{PEF}), and the total specific energy (energy input – W), (Arshad et al. 2020; Dragomir et al. 2020; Tylewicz 2020). The effective membrane disintegration in plant cells requires a relatively low specific energy input ($W \approx 1\text{--}15 \text{ kJ}\cdot\text{kg}^{-1}$). Different treatment

chambers for both batch and continuous processing have been developed. In laboratory-scale experiments, the batch plate-to-plate electrode geometries with a maximally homogeneous electric field are preferred. In pilot scale and industrial experiments, continuous flow chambers with parallel plates, and coaxial and collinear cylinder geometries are commonly used (Raso et al. 2016).

ELECTROPORATION OF SUGAR BEETS

For the estimation of the tissue electroporation degree, different methods can be used, e.g. electrical conductivity, electrokinetic, textural, acoustic, diffusion, image analyses, tomography, and dye uptake techniques (Vorobiev and Lebovka 2020). The sugar beet was used in the pioneering works of Zagorul'ko to study the effects of an electric treatment on the vegetable's tissue (Zagorul'ko and Myl'kov 1953; Zagorul'ko 1957). For better regulation of the treatment efficiency and in order to avoid ohmic heating, a PEF treatment was applied. The first PEF generator produced exponential pulses with a duration of 20 μ s and with an electric field strength up to $E = 20$ kV·cm⁻¹. The observed effects were explained by the selective injury (puncture) of the plasmatic membrane shells without any noticeable heating of surrounding medium. This phenomenon was called as electropasmolysis. Reversible (or unfinished) electropasmolysis has also been observed

with relatively small electric fields (at $E < 400$ V·cm⁻¹). The level of electropasmolysis (disintegration index) was characterised from the measurements of the electrical resistivity of the sugar beet tissue. The electrical potential on the plasmatic shells (transmembrane potential) required for the electropasmolysis of the sugar beet was estimated to be $u_m = 0.953$ V (Zagorul'ko and Myl'kov 1953). Moreover, the estimated energy consumption was relatively low, $W \approx 4\text{--}5$ kJ·kg⁻¹ (Zagorul'ko 1957). Particularly, empirical relationships were proposed to estimate the disintegration degree of the sugar beet as a function of electric field strength and treatment time.

Nowadays, a very popular method for estimation of the level of sugar beet tissue electroporation is based on the measurement of the electrical conductivity σ (Vorobiev and Lebovka 2020). In the so-called low-frequency technique, the electrical conductivity is measured at the low frequency at the boundary between the α - and β -dispersion ranges, $f \approx 1\text{--}10$ kHz. With an increase in the PEF treatment t_{PEF} time, the value of σ increases from σ_i (intact or untreated tissue) and saturates at a long treatment time at some level σ_d that corresponds to the conductivity of the completely disintegrated tissue.

The damage degree (or disintegration index) was defined as in Equation (1) (Lebovka et al. 2007a):

$$Z = \frac{\sigma - \sigma_i}{\sigma_d - \sigma_i} \quad (1)$$

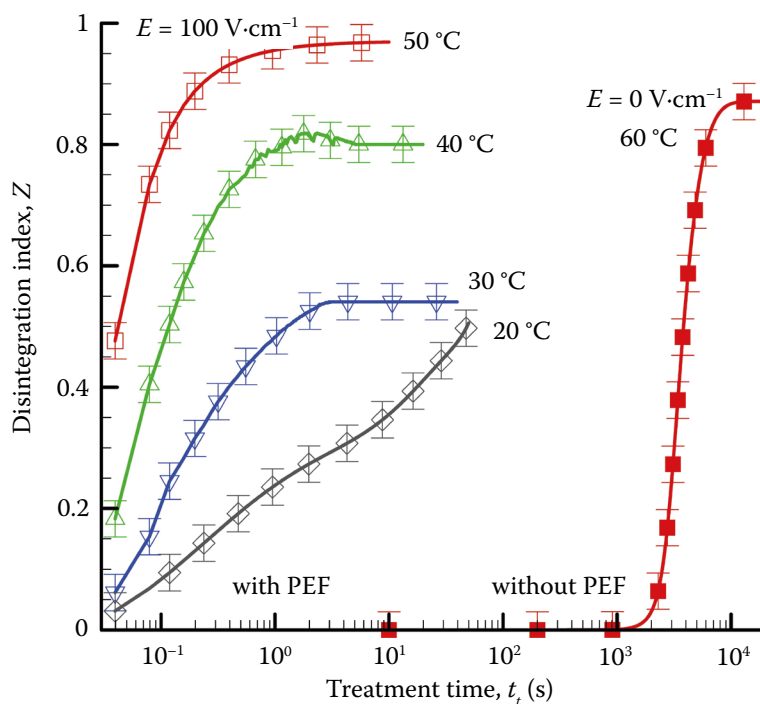


Figure 3. Damage degree Z versus the treatment time t_t for sugar beet tissues

PEF treatments were performed in near-isothermal conditions at different temperatures T and at a fixed electric field strength $E = 100$ V·cm⁻¹ (open symbols, $t_t = t_{PEF}$). The thermal treatment alone ($E = 0$ V·cm⁻¹) was performed at $T = 60$ °C (filled squares). Error bars are the standard deviation (compiled from Lebovka et al. 2007a)

Figure 3 shows typical changes of the damage degree Z versus the treatment time (t_p) for sugar beet tissues. The value of Z increased during the PEF treatment, and the effects were more pronounced at elevated temperatures. The value of Z also increased for thermal treatment even in the case when a PEF was not applied. Moreover, it was demonstrated that a significant amount of electroporation of the sugar beet tissue can be observed even during a moderate PEF treatment with a field strength under $100 \text{ V}\cdot\text{cm}^{-1}$, heating temperatures $50\text{--}60^\circ\text{C}$ and treatment times below 100 seconds (Lebovka et al. 2007b). The energy consumption during such treatments was mainly related to the temperature elevation by ohmic heating.

In early studies, the electroporation degree of sugar beet cells and protoplasts (plant cells lacking a cell wall) was determined by using the dye uptake technique (Lindsey and Jones 1987a). This technique was successfully applied to study electroporation after PEF treatments at different field strengths, number of pulses and pulse duration. The accumulation of the hydrophilic dye phenosafranine was also studied. The percentage of stained protoplasts of sugar beet suspension cells was evaluated at various electrical conditions (Joersbo et al. 1990), and an empirical equation for the correlation between the electroporation efficiency, electric field strength and pulse duration was proposed. It was suggested that the efficiency of the electroporation is closely related to the PEF energy delivered to the protoplasts.

The correlation between the level of electroporation and the textural properties of the sugar beet tissue was recently analysed (Rezaei et al. 2018). Stress–relaxation tests were performed and the viscoelastic parameters were estimated as a function of the cell disintegration index. The data were explained by accounting for the loss of turgor component of the sugar beet texture.

GENETIC TRANSFORMATIONS ASSISTED BY ELECTROPORATION

The production of sugar beet transgenic crops with increased tolerance to herbicides, salts, drought and fungi, increased resistance to diseases is very important. The production of such crops is also needed to maintain the competitiveness of the sugar beet industry (Joersbo 2007; Gurel et al. 2008). Electroporation and electrofusion applications for the genetic transformation of sugar beets started in the mid-1980s. The direct

transfer of foreign DNA in electroporated (rectangular pulses, E up to $255 \text{ V}\cdot\text{cm}^{-1}$) protoplasts and intact cells of sugar beets was studied (Lindsey and Jones 1987b; Eady et al. 1988). In these works, the first successful example of direct gene transfer in protoplasts was demonstrated. It was shown that mesophyll protoplasts were more susceptible to damage by electroporation than cell suspension protoplasts. The combination of chemical and electrical permeabilisation treatments was more effective than the application of the permeabilisation treatments alone (Lindsey et al. 1988). An improved PEF assisted protocol was reported to attain stable transformation, integration and expression of selectable foreign genes in the culture protoplasts of sugar beets (Lindsey and Jones 1989; Lindsey and Jones 1990). It was speculated that the obtained data can be useful for the production of transformed whole sugar beet plants. The efficiency of the electroporation of sugar beets and the direct gene transfer to plant protoplasts were studied at $E = 70\text{--}630 \text{ V}\cdot\text{cm}^{-1}$ using alternate, rectangular and exponentially decaying pulses (Joersbo and Brunstedt 1990a). It was demonstrated that a very cheap and easy to-use electroporator can be effectively used for the direct gene transfer. A significant stimulation of the protein synthesis (up to 240%) in electroporated sugar beet protoplasts was demonstrated (Joersbo and Brunstedt 1990b).

Electroporation was then used in many works to introduce a variety of exogenous molecules into sugar beet cells, gene transfer, and it has important applications in agriculture (Hall et al. 1994; Joersbo and Brunstedt 1996). Different transformation strategies assisted by electroporation, sonication, somatic hybridisation, and polyethylene glycol (PEG)-mediated have been attempted (Gurel et al. 2008; Rivera et al. 2012; Lal and Lal 2020). Recently, electrofusion of mesophyll protoplasts from two sugar beet varieties through the production of somatic hybrids was attempted (Al-Nema and Mozahim 2020). It was speculated that the somatic hybridisation technique can be useful for the production of sugar beet plants with a high sugar content.

EXTRACTION OF SUGAR

During the last few decades, different electroporation-assisted experiments with sugar beets aimed at the possibility of replacing or modifying the existing sugar technology, were performed. For sugar

extraction, different electroporation assisted approaches were tested:

- extraction by diffusion using cold (20–30 °C) or moderately heated water (up to 60 °C)
- extraction by pressing (expression);
- combined pressing-diffusion extraction;
- extraction by diffusion.

Different studies on the effects of alternating current (AC) and direct current (DC) electric fields on the sugar extraction from sugar beets were performed in the 1980s–1990s. These studies elucidated upon many interesting phenomena attractive for practical applications (Karpovich et al. 1981; Bazhal et al. 1983; Katroha et al. 1984; Kupchik et al. 1987; Dolinskaya et al. 1992).

Detailed studies on the PEF-assisted enhancement of sugar diffusion from sugar beet tissues were started in the 2000s. Particularly, the kinetics of diffusion extraction from electroporated ($E = 235\text{--}1\,180\text{ V}\cdot\text{cm}^{-1}$) sugar beet disks and cossettes has been studied in details (El-Belghiti et al. 2005b). The process was performed at an ambient temperature and continuous stirring. The extraction kinetics were described by a two-exponential kinetic model. The initial period of rapid extraction was explained by the formation of a thin layer of juice on the surface of sugar beet tissue immediately after the PEF treatment. The following prolonged period was explained by the diffusion of solutes from the interior of the sugar beet tissue. The mild heating conditions permitted an increase in the diffusion coefficient and the enhancement of the extraction rate from the electroporated tissue. A noticeable improvement in the juice quality (lower coloration, higher purity) was observed at optimal PEF parameters (El-Belghiti et al. 2005b).

The centrifugal aqueous extraction from electroporated ($E = 670\text{ V}\cdot\text{cm}^{-1}$ and $940\text{ V}\cdot\text{cm}^{-1}$) sugar beet tissues (disk samples and cossettes) was investigated at an ambient temperature (El-Belghiti et al. 2005a). The centrifugal field significantly enhanced the kinetics of the extraction from the electroporated samples. The implementation of four consecutive centrifugal steps permitted one to obtain a 97% sugar yield. Two-exponential extraction kinetics with a first rapid washing stage followed by a slow diffusion stage was observed. The impacts of the PEF ($E = 400\text{ V}\cdot\text{cm}^{-1}$ and $t_{PEF} = 0.1\text{ s}$) and heating on the sugar extraction from sugar beets were compared (Lebovka et al. 2007a). Both the heating and PEF treatment accelerated the extraction kinetics. However, the purity of a "cold" juice obtained at 20 °C ($P \approx 96\text{--}97\%$)

was noticeably higher for the electroporated samples when compared to that obtained at 70 °C for the untreated samples (Lebovka et al. 2007a).

The specific energy consumption needed for the thermal aqueous extraction from untreated cossettes at $T \approx 75\text{ °C}$ ($156\text{ J}\cdot\text{g}^{-1}$) was approximately 20 times higher than that required for "mild temperature" extraction at $T \approx 50\text{ °C}$ ($8\text{ J}\cdot\text{g}^{-1}$) from electroporated cossettes (Eshtiaghi and Maskooki 2009; Maskooki and Eshtiaghi 2011; Maskooki and Eshtiaghi 2012).

A scale-up study of PEF-assisted aqueous extraction from sugar beet cossettes was conducted in a pilot counter-current extractor with 14 extraction sections (Loginova et al. 2011b). The electric field strength was $E = 100\text{--}600\text{ V}\cdot\text{cm}^{-1}$, the total time of the PEF treatment was $t_{PEF} = 50\text{ ms}$, the extraction temperature was $T = 30\text{--}70\text{ °C}$, and the draught was 120–90%. The experiments evidenced that a combination of mild heating (for example, at 50 °C) and a PEF treatment can be useful for shortening the diffusion time. It was also shown that the juice extracted in the "cold" mode without heating is less coloured and purer in comparison to the juice derived using industrial thermal diffusion (Loginov et al. 2011; Loginova et al. 2011a; Loginova et al. 2012). The optimal values of the electric field intensity E were dependent upon the temperature: $E = 600\text{ V}\cdot\text{cm}^{-1}$ (at 30 °C) and $E = 260\text{ V}\cdot\text{cm}^{-1}$ (at 60 °C). It was speculated that this approach can simplify (or even eliminate) the very complicated and polluting carbonic purification process. The "cold" extraction resulted in a lower concentration of colloidal impurities (the obtained pulp was richer in pectin), lower colouration and better filterability of juice.

The value of the draught can be decreased from 120% to 100%, or even to 90%, for the mild temperature extraction from PEF treated cossettes. Table 1 presents the characteristics of the extracted juices.

In other experiments, the kinetics of sucrose extraction from electroporated sugar beets ($E = 1\text{--}7\text{ kV}\cdot\text{cm}^{-1}$ and $t_{PEF} = 40\text{--}100\text{ }\mu\text{s}$) was studied at different temperatures (20–70 °C) (Lopez et al. 2009). The sucrose yield increased with the field strength, time of extraction and temperature. The effects of the PEF were significant for low temperatures. The PEF treatment at $E = 7\text{ kV}\cdot\text{cm}^{-1}$ and a specific energy input $W = 3.9\text{ kJ}\cdot\text{kg}^{-1}$ allowed one to reduce the extraction temperature from 70 °C to 40 °C for 80%-sucrose extraction over 60 minutes.

The problems of energy optimisation in the extraction of sugar from sugar beets were recently discussed (Salehi and Omidvari 2016). The results of the

Table 1. Effects of electroporation and extraction temperature (T), on the characteristics of sugar beet juices

Characteristics	Temperature of extraction	
	50 °C, electroporated cossettes	70 °C, untreated cossettes
°Brix (%)	14.55 ± 0.07	14.8 ± 0.1
Sucrose (%)	13.58 ± 0.09	13.74 ± 0.08
Colloids (%)	0.112 ± 0.026	0.215 ± 0.029
Pectin (%)	0.0069 ± 0.0001	0.139 ± 0.030
Proteins (%)	0.0544 ± 0.0004	0.0565 ± 0.0017
Purity (%)	93.30 ± 0.26	92.54 ± 0.28

The PEF treatment of sugar beet cossettes was undertaken at $E = 600 \text{ V}\cdot\text{cm}^{-1}$ using 500 monopolar pulses of a near-rectangular shape with a pulse duration of $t_i = 100 \text{ }\mu\text{s}$ and a pulse repetition rate of $f = 200 \text{ Hz}$ (Loginov et al. 2011; Loginova et al. 2011a; Loginova et al. 2012)

statistical analysis evidenced a significant decrease in the temperature, time and energy consumption of the PEF treatment compared to the thermal treatment with the same extraction yields. The comparison of the efficiency of electric, thermal and combined treatments on solid-liquid extraction from sugar beets was recently presented (Ben-Ali 2018). PEF treatments at $E = 150, 250$ and $750 \text{ V}\cdot\text{cm}^{-1}$ were applied as a pre-treatment and an intermediate treatment during the extraction process, with temperatures in a range of $T = 25\text{--}60 \text{ }^\circ\text{C}$. The combined PEF treatment at moderate temperatures was shown to be the best in terms of the energy consumption. The energy saving diffusion at moderate temperatures ($T = 35\text{--}50 \text{ }^\circ\text{C}$) from electroporated sugar beet cossettes may also be attractive for the optimisation of pressed pulp drying (Figure 2) (Nakthong and Eshtiaghi 2020).

Extraction by pressing. In pioneering works, different multi-step pressing procedures were applied to extract sucrose from electroporated cossettes of sugar beets (Bouzzara and Vorobiev 2000; Eshtiaghi and Knorr 2002). The efficiency of cold pressing at $p = 5$ bars of electroporated ($E = 215\text{--}427 \text{ V}\cdot\text{cm}^{-1}$) sugar beet cossettes has been studied (Bouzzara and Vorobiev 2000). The multi-step process combined a first pressing stage without a PEF application with a juice yield of $\approx 19.1\%$ and a second pressing stage of electroporated cossettes. A total juice yield of 43%, 68% and 79% was attained for the corresponding electric field strengths of $E = 215 \text{ V}\cdot\text{cm}^{-1}$, $300 \text{ V}\cdot\text{cm}^{-1}$ and $427 \text{ V}\cdot\text{cm}^{-1}$, respectively. Thus,

a combination of pressing and a PEF treatment allowed a significant increase in the juice yield. Moreover, the juice obtained after the 2nd pressing step from the electroporated cossettes was noticeably less coloured when compared to the juice obtained from the untreated cossettes. For example, cold juices, expressed from the electroporated sample had a higher purity (95–98%) compared to those before a PEF application (90–93%) (Bouzzara and Vorobiev 2000).

In another experiment, different multi-step pressing procedures (one-step, two-step and three step) were applied to electroporated sugar beet cossettes ($E = 1.2\text{--}2.5 \text{ kV}\cdot\text{cm}^{-1}$) with the addition of water at different stages (Eshtiaghi and Knorr 2002). The three-step pressing-extraction at 20 bars required ≈ 30 min for the PEF-treated cossettes compared to ≈ 70 min extraction needed for the untreated cossettes in the conventional thermal process. It provided a significant reduction in the energy requirement during the dehydration of the extracted cossettes (Eshtiaghi and Knorr 2002). The efficiency of "cold" pressing of the PEF-treated sugar beet cossettes was discussed.

The "cold" pressing process using a pilot scale multi-plate and frame pressing equipment (pressure of 5–15 bars) was realised to extract the juice from electroporated sugar beet cossettes (Jemai and Vorobiev 2003). The chamber consisted of a plate covered by filter cloth and a supporting flexible electrode (metallic grid) on one side and a rigid electrode on the other side. It was manually filled with grated cossettes. The process included two initial pressing steps with an intermediate PEF treatment followed by one or more washing steps and final pulp pressing. The purity of the 1st pressing juice was lower (90–93%) than the purity of the 2nd pressing juice obtained after the PEF application (96–98%) (Jemai and Vorobiev 2003). In addition, significant amounts of potassium, sodium and α -amino nitrogen were found to remain in the electroporated particles. The application of washing and final pressing operations allowed one to reduce the sugar losses in the pulp to about 3% of the initial sugar content. Additionally, the quantity of pectin was noticeably lower and the colour of the juice was systematically 3 to 4 times less intensive than the colour of factory juices (Jemai and Vorobiev 2006).

A similar two step pressing procedure at $p = 9.254$ bars was applied at ambient conditions to extract juice from electroporated ($E = 0.52\text{--}1.52 \text{ kV}\cdot\text{cm}^{-1}$) sugar beet cossettes (Gjörök

et al. 2016). The duration of the 1st period was 5 min then a PEF treatment was applied and the duration of the 2nd period was 25 minutes. The PEF treatment allowed one to significantly increase the maximum juice yield from 30.64% (untreated samples) to 80.32% (electroporated samples).

The effects of the combined ohmic heating (OH, 60 V·cm⁻¹, 50 Hz) and PEF treatment ($E = 600 \text{ V·cm}^{-1}$, $t_{PEF} = 0.04 \text{ s}$) on pressing of sugar beet cossettes in a vertical filter-press chamber were studied (Praporscic et al. 2005). The initial temperature ($T = 30\text{--}70 \text{ °C}$) was adjusted using OH and then the PEF treatment was applied. The results demonstrated the synergy of the combined OH + PEF treatments, leading to 85–87% juice extraction.

Filtration-consolidation behaviours during solid-liquid expression from untreated, electroporated and freeze-thawed sugar beet tissues were compared (Grimi et al. 2010). The electroporated and freeze-thaw tissues demonstrated two consolidation stages of expression (primary and secondary). A simplified semi-empirical consolidation model was proposed to describe the expression behaviour and to estimate the consolidation coefficients under different pressures. The filtration diffusivity and expression behaviour of thermally pre-treated (at $T = 50$ and 70 °C) and electroporated ($E = 600 \text{ V·cm}^{-1}$, $t_{PEF} = 10 \text{ ms}$) sugar beet tissues have been studied at constant pressure $p = 5 \text{ bar}$ (Mhemdi et al. 2012). Disk-like sample and cossettes were investigated. The consolidation coefficients that characterise the filtration diffusivity were estimated for different samples. The effects of different variants of the combined thermal and PEF treatments ($E = 600 \text{ V·cm}^{-1}$, $t_{PEF} = 10 \text{ ms}$ and $T = 20 \text{ °C}$) on the filtration-consolidation and solid-liquid expression for sugar beet disks and cossettes have been studied in details. For the untreated sugar beets, the pressure-induced rupture of the cells occurred under a pressure above 60 bars. It was demonstrated that after the sugar beet tissue treatment by heat (at 70 °C), PEF, and the combined (thermal and PEF) pre-treatments, the solid-liquid expression at $p = 5 \text{ bars}$ followed the filtration-consolidation behaviours for both the disk sample and cossettes.

The extraction by pressure was applied to produce a fermentable juice from electroporated sugar beet tails (Almohammed et al. 2016a). The process consisted of a PEF treatment of sliced sugar beet tails ($E = 450 \text{ V·cm}^{-1}$, $t_{PEF} = 10 \text{ ms}$ and specific energy input $W = 6.9 \text{ J·g}^{-1}$), the pressing of electroporated tails and batch fermentation of the recovered

juice for bioethanol production. The PEF treatment resulted in a significant increase in the yield of the solutes (from 16.8% to 79.85%), sucrose content (from 4.5 °S to 8.9 °S) and dryness of the pressed cake (from 15% to 24%) in comparison to the untreated tails. The batch fermentation and distillation of the juice from the electroporated sample allowed an increase in the ethanol content in the distillate (from 2.95% to 6.1% v/v).

Combined pressing-diffusion. In the combined pressing-diffusion technique, the juice from electroporated sugar beet cossettes ($E = 600 \text{ V·cm}^{-1}$ and $t_{PEF} = 10 \text{ ms}$) was expressed by rapid pressing (for 4 min at $p = 5 \text{ bar}$ and $T = 20 \text{ °C}$) and then the residual sucrose from the press-cake was extracted by diffusion (Mhemdi et al. 2012). Both juices were then mixed and the exhausted pulp was pressed. The mixed juice had better characteristics when compared to the characteristics of the conventional juice obtained by diffusion at 70 °C . It was more concentrated in sucrose, less coloured and purer. The direct pressing of cossettes allowed the expression of 50% of the undiluted juice (Brix 21.2%). The cossettes were better exhausted and the sucrose losses in pulp were reduced from 1.2% to 0.8%.

Later on, a more complicated combined pressing-diffusion technology was realised (Mhemdi et al. 2016). Sugar beet cossettes were first electroporated ($E = 600 \text{ V·cm}^{-1}$, $t_{PEF} = 10 \text{ ms}$), then they were pressed at $p = 5 \text{ bars}$ and $T = 20 \text{ °C}$ for 4 min, and afterward the pressed pulp was loaded to the perforated baskets of the laboratory counter-current extractor (Mhemdi et al. 2016). Extraction temperature was fixed at 70 °C . Finally, the diffusion juice was mixed with the pressing juice and the earlier exhausted pulp in the extractor was subjected to the pressing at $p = 5 \text{ bars}$. The results were compared with those obtained after the diffusion of the untreated cossettes using the same extractor at the same extraction temperature of 70 °C . It was demonstrated that the mixed juices obtained from the electroporated cossettes by cold pressing-diffusion technology were 30% less coloured than the juice obtained by conventional diffusion at 70 °C from the untreated cossettes. Moreover, the purity of the mixed juices (92.5–92.8%) was higher than that obtained by conventional diffusion (91.8%).

Combined liming and ultrafiltration techniques. It was demonstrated that the extraction from electroporated cossettes can be even more enhanced in the presence of lime (Arnold et al. 2012; Fren-

zel et al. 2012; Arnold et al. 2014). The sugar beets were limed directly during the slicing and diffusion was realised on the pilot counter-current extractor at the temperatures of 55–80 °C. The diffusion from the limed cossettes was effective even at $T = 60$ °C and the dryness of the pressed pulp obtained from the electroporated limed cossettes was higher on the 10% absolutes than that obtained from the untreated and non-limed cossettes. Moreover, the juice purity was higher and its colouration was lower when the electroporated/limed cossettes were processed.

The impacts of the electroporation ($E = 600 \text{ V}\cdot\text{cm}^{-1}$ and $t_{PEF} = 10 \text{ ms}$) and preheating on the purification of the raw juices expressed from the sugar beet have been studied (Mhemdi et al. 2014). Lime-carbonation was used to purify the raw expressed juices. The quality of the cold juice obtained by expression from PEF-treated cossettes (at $T = 20$ °C) was compared with that of the "thermal" juice obtained by expression from preheated cossettes (for 10 min at 80 °C). The filtration kinetics of the cold juice was faster than that of the "thermal" juice. Moreover, the purified cold juice was comparatively less coloured, less turbid and purer to the purified "thermal" juice.

The effects of the multistage liming on the quality of the juice expressed from the electroporated ($E = 600 \text{ V}\cdot\text{cm}^{-1}$, $t_{PEF} = 7 \text{ ms}$) sugar beet cossettes have been studied (Mhemdi et al. 2015). The cossettes were PEF treated, pressed at 5 bars during 15 min, and then the obtained juice was heated to 45 °C and subjected to a progressive pre-liming for 30 minutes. The pre-limed juice was heated to 85 °C for the main liming with a different quantity of lime. Then the juice of the 1st saturation ($T = 85$ °C, pH 11.2) was filtered at $T = 50$ °C and $p = 1$ bar, and the filtrate was subjected to a 2nd saturation ($T = 90$ °C, pH 9.2). Afterwards, the juice of the 2nd saturation was also filtered. The data evidenced that the juice of the 1st saturation obtained from the electroporated cossettes had better filtration properties than the juice of the 1st saturation obtained from the thermally treated cossettes (Mhemdi et al. 2015).

Application of different combined PEF-lime impregnation/extraction techniques for sugar beet cossettes has been discussed (Almohammed et al. 2016a). After the PEF treatment (for $t_{PEF} = 10 \text{ ms}$ at $E = 600 \text{ V}\cdot\text{cm}^{-1}$ and $T = 12$ °C), the cossettes were subjected to a double pressing with an intermediate lime impregnation (0.6 % g CaO per 100 g of fresh cossettes) by soaking the compressed cossettes in the cold juice mixed with the lime. The combined

PEF-lime impregnation technique allowed an increase in the dryness of the pulp (by 49.7%) and the juice yield (by 12% at 5 bars). The purity of the juice was higher (93.61% vs. 93.07%), its colouration was lower (by 39.7%), the concentration of the colloids was lower (by 28.7%), and the concentration of the proteins was lower (by 24.5%) when compared to the corresponding characteristics of the juice obtained by the PEF and one-step pressing with liming.

The purification of the juice expressed from the electroporated sugar beet cossettes was realised using dead-end ultrafiltration (Mhemdi et al. 2014). The effects of the electroporation and preheating on the qualitative characteristics of the juices have also been studied. A three-step extraction process was applied to obtain juice from electroporated ($E = 600 \text{ V}\cdot\text{cm}^{-1}$, $t_{PEF} = 5\text{--}20 \text{ ms}$) sugar beet cossettes. Cossettes were electroporated at the temperatures $T = 10$ °C (cold), 20 °C, 50 °C, 60 °C, 70 °C, and 80 °C (preheated); extraction of the juice by pressing at $p = 5$ bars for 15 min was undertaken; finally, purification of the juice by dead-end ultrafiltration (membrane of 30 kDa) was realised.

The juice yields were $Y = 66.5\%$ at 20 °C for the electroporated cossettes and $Y = 64\%$ at 80 °C for the untreated cossettes, i.e. they were comparable. However, the purity of the juice expressed at 20 °C (93.5%) was higher than that of the preheated juice at 80 °C (92.3%). The purity of the membrane filtered juices previously expressed at 20 °C (96%) was also higher than that of preheated and membrane filtered juice (95.3%). In addition, the quantity of proteins and colloids in the membrane filtered juice previously expressed at 20 °C was lower than that in the preheated and membrane filtered juice.

The juice expressed at 20 °C and $p = 5$ bars from the electroporated cossettes ($E = 600 \text{ V}\cdot\text{cm}^{-1}$, $t_{PEF} = 7 \text{ ms}$, $T = 20$ °C) can be easily purified by ultrafiltration (Mhemdi et al. 2014). The juice obtained from the electroporated cossettes was initially centrifuged (4 000 RPM for 15 min) to remove any suspended particles, and then the clarified juice was ultrafiltered at $T = 20$ °C and $p = 2$ bars. Polyether sulfone (PES) membranes with pore sizes of 30 kDa were used. The obtained filtrates demonstrated higher purity, lower colouration, and contained less colloids and proteins than the filtrate of the juice expressed from the preheated at 80 °C cossettes.

The purity of the sugar beet juice obtained by the PEF-assisted pressing can be noticeably increased

(from $93.6 \pm 0.5\%$ to $96.4 \pm 0.8\%$ by the dead-end ultrafiltration with 10 kDa membrane (Zhu et al. 2015). A higher ultrafiltration rate and better juice purity were obtained from the electroporated cossettes using a dynamic filter with a rotating disk.

Different multistep cold pressing techniques were applied for the electroporated sugar beet cossettes (Almohammed et al. 2015; Almohammed et al. 2016a; Almohammed et al. 2016b; Almohammed et al. 2017a; Almohammed et al. 2017b). The first pressing was undertaken at $p = 15$ bars. The 1st pulp was then impregnated by lime milk at $T = 10^\circ\text{C}$. After liming, the 1st pressed pulp was subjected to a 2nd, 3rd and 4th pressing with the addition of water. All the juices were mixed to obtain a mixed juice. The properties of this mixed juice were compared with the properties of the diffusion juice obtained from scalded cossettes. The mixed juice demonstrated a higher °Brix (18% instead of 14.5% for scalded cossettes), was purer (93.16% instead of 91.62%), contained a lower quantity of colloids ($9.94 \text{ mg}\cdot\text{g}^{-1}$ instead of $17.66 \text{ mg}\cdot\text{g}^{-1}$ of soluble matter), and a lower quantity of proteins ($0.92 \text{ mg}\cdot\text{g}^{-1}$ instead of $2.08 \text{ mg}\cdot\text{g}^{-1}$ of soluble matter), and it was less coloured. The filtration properties of the juice of the 1st saturation were significantly improved when the expression from the electroporated cossettes was performed with the lime addition. The purity of the thin juice obtained from electroporated and limed cossettes was approximately 95.5% when the total quantity of the lime used for the purification was equal to $6 \text{ kg CaO}\cdot\text{m}^{-3}$. It was higher than the purity of the thin juice obtained from the electroporated, but not that of the limed cossettes (94.3%) using the same total quantity of lime (Almohammed et al. 2017a; Almohammed et al. 2017b).

MODIFICATION OF SUGAR BEET PULP PECTIN

The effects of the PEF on the modification of the pectin presented in sugar beet pulp were studied (Ma et al. 2012; Ma and Wang 2013; Huang et al. 2018). The PEF treatment ($E = 18\text{--}30 \text{ kV}\cdot\text{cm}^{-1}$ and $t_{\text{PEF}} = 806\text{--}2\,418 \mu\text{s}$) allowed a significant decrease in the degree of esterification, molecular weight and particle size of the pectin (Ma et al. 2012). The PEF-assisted modification of the pectin with arachidic anhydride were investigated under solvent free conditions (Ma and Wang 2013). The PEF treatment significantly affected the properties of the pectin derivatives and increased the thermostability.

PRODUCERS OF EQUIPMENT, PATENTS AND COMMERCIAL APPLICATIONS

Producers of equipment. Nowadays, commercial-scale pulse generators and treatment devices are being developed and tested for different food applications (Vorobiev and Lebovka 2020). For example, large-scale systems for the treatment of sugar beets, apple mash, microalgae, yeast, seed samples have been fabricated by Basis EP (France). High voltage and high-power pulse PEF commercial systems for large-scale biomass processing are produced by Diversified Technologies (DTI) Inc. (USA). Different large- and small-scale PEF equipment and generators suitable for the treatment of vegetable materials (the cassava, the taro, carrots, potatoes and sweet potatoes, red beets and parsnips) are produced by Elea (Germany). Special PEF systems for the treatment of potatoes in the production of French fries and crisps have been fabricated by Pulsemaster, the Netherlands. A PEF pilot system for a grape mash treatment has been fabricated by Beta-tech (France). PEF treatment systems for the processing of potatoes and olives are being developed by Scandinova Systems AB (Sweden). Pilot scale generators and treatment machines have been designed and fabricated by the Institute of Pulsed Power of Karlsruhe Institute of Technology (KIT). Special PEF machines for extraction purposes in the batch and continuous modes have been fabricated by Vitave (Germany) and by Wek-Tec e.K. (Germany).

Patents. Table 2 presents some representative patents that have been issued during the last two decades. These inventions were related to the innovative methods and techniques for treating sugar beets, extraction of juices from the beet, different electroporation devices, and electroporation reactor systems.

Industrial scale devices. At the beginning of the 2000s, commercial pulsed power applications for food applications became increasingly significant (Loeffler 2002). Probably, the first mobile electroporation test device KEA (Karlsruher Elektroporations Anlage) was constructed for the treatment of sugar beets (Schultheiss et al. 2001; Schultheiss et al. 2002; Schultheiss et al. 2003). The device construction was based on the high repetition rate of the Karlsruhe Marx generator (300 kV) with the possibility of treating both whole sugar beets and cossettes. The reactor chamber volume was about 7 litres. The temperature increase of the tissue dur-

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Table 2. Some patents related to the application of PEFs for assisting the processing of sugar beets

Patent Date of publication	Title and reference	Current assignee
EP1086253B1 Jan 28, 2004	Process for treating sugar beets (Eshtiaghi and Knorr 2004)	Eridania Beghin Say SA
FR2805199B1 June 21, 2002	Method for extracting liquid from a cellular material, and devices for carrying out said method (Vorobiev et al. 2002)	Association Gradient
DE10144479C2 Sep 4, 2003	Electroporation reactor for the continuous processing of lumpy products (Schultheiss and Kern 2003)	KEA-TEC GmbH Forschungszentrum Karlsruhe GmbH
CA2460569C May 11, 2010	Electroporation reactor for continuously processing products in the form of pieces (Schultheiss and Kern 2010)	KEA-TEC GmbH Forschungszentrum Karlsruhe GmbH
DE102009011755A1 Sep 16, 2010	Electroporation reactor for pressured electroporation of biological commercial- and waste material as process mate- rial in continuous process throughput, comprises two roller groups with a roller and a fluid permeable transport device (Berghöfer et al. 2010)	Karlsruher Institut für Technologie (KIT)
EP1751860B1 May 9, 2012	Device for the electroporation of biologically vegetable processing material (Sack and Schultheiss 2012)	Karlsruher Institut für Technologie (KIT)
CA2722522A1 Oct 29, 2009	Process and device for the electroporation of beet cossettes (Arnold et al. 2009)	Südzucker AG
US8163091B2 Apr 24, 2012	Extraction of ingredients such as sugar from biological material (Frenzel et al. 2012)	Südzucker AG
CA2570475C May 1, 2012	Extraction of constituents from sugar beet chips (Arnold et al. 2012)	Südzucker AG
US8691306B2 Apr 8, 2014	Process for the electroporation of beet cossettes and device for carrying out this process (Arnold et al. 2014)	Südzucker AG
PL2515686T3 Sep 7, 2020	Reactor system for electroporation (Arnold et al. 2020)	Südzucker AG
FR2959399B1 Oct 12, 2012	Procede et installation de traitement des tissus vegetaux pour en extraire une substance vegetale, notamment un jus (Vidal and Vorobiev 2012)	Maguin SAS Comeca Power Université de Technologie de Compiègne (UTC)
US20130202751A1 Aug 8, 2013	Method and apparatus for treating vegetable tissues in order to extract therefrom a vegetable substance, in particular a juice (Vidal and Vorobiev 2013)	Maguin SAS
EP3783115A1 Feb 24, 2021	Sugar beet juice production and processing (Poiesz and Daniëls 2021)	Cooperatie Koninklijke Cosun U.A.
WO2012066144A1 May 24, 2012	Method for extracting molecules of interest from all or part of a plant matrix (Lanoiselle et al. 2012)	Université de Technologie de Compiègne (UTC) Société Sofralab

ing electroporation was less than 1 °C, and the specific energy input necessary for the reliable denaturation of the sugar beet tissue was 2–3 kJ·kg⁻¹. It was concluded that electroporation can replace the thermal denaturation of the sugar beet, it reduces energy cost and avoids the denaturing of vitamins, enzymes and proteins (Schultheiss et al. 2003).

Factory scaling problems were discussed (Schultheiss et al. 2002; Schultheiss et al. 2003). For the PEF treatment of sugar beets, an industrial machine with a throughput of 10 000 t·d⁻¹ (115 kg·s⁻¹) with a special treatment chamber construction, special electrode configuration and the possibility of simultaneously running several generators connected to one electroporation chamber are required. Moreover, important problems related to the treatment of the whole beet and the slicing step were also discussed.

Detailed laboratory experiments on the electroporation efficiency of sugar beets have been carried out to optimise the operations of an industrial device (Sack et al. 2004), where the dependence of the electroporation efficiency on the beets' quality was discussed.

The operational experience of the industrial-scale device KEA-ZAR (Karlsruhe, Electroporation plant-cell denaturation reactor) to daily treat several tonnes of beets (operation in the 2003 campaign) was described (Bluhm et al. 2004; Schultheiss et al. 2004). The device included two 7-stage Marx generators (350 kV, 6 kA) with transportation of the whole roots by means of a roll through an electrode area. In order to improve the energy-efficient processing, a long-term test of a triggered Marx-generator in repetitive operation was performed (Sack and Bluhm 2005).

The small-scale electroporation device KEA-MOBIL (mobile Karlsruhe electroporation device) with a two-stage Marx generator was designed for a batch-wise throughput of 50 kg·h⁻¹ and equipped with a combined electroporation reactor and press was presented in Sack et al. (2009).

An industrial pilot device with capacity at 10 t·h⁻¹ (Maguin, Hazemeyer and UTC, France) was developed in France and tested for cold extraction of juice from sugar beet cossettes (Vidal 2014; Vorobiev and Maishak 2018). The extracted cold juice was then ultra-filtered, concentrated by evaporation so the sugar crystallisation could proceed. The ultra-filtered PEF juice had much lower colour in comparison to the traditional cleared thin juice. The PEF treated cossettes were pressed and the pulp rich in sucrose was used as animal feed.

The status of PEF applications for sugar beet electroporation, different generator configurations, the technical requirements and perspectives for industrial-scale implementation have been reviewed in different works (Bluhm and Sack 2009; Sack et al. 2010; Sack and Mueller 2016; Sack and Mueller 2017). Electroporated sugar beets can be sliced with lower energy consumption, but additional sugar losses are induced. The scaled design of PEF treatment reactors for electroporation-assisted extraction processes for sugar beet tissue was discussed (Sack and Mueller 2016). For the effective electroporation of sugar beets, a specific energy amount in the order of 5 kJ·kg⁻¹ was proposed. The PEF treatment protocols also influence any device's design. For example, the pulse shape, duration and pulse repetition rate may influence the electrode corrosion inside the treatment zone. For large-scale processing, a pulse repetition rate of approximately 20 Hz was chosen as optimal. The electrode design for the energy efficient operation is also a very important point (Sack and Mueller 2017). The optimal design of systems with parallel-plate and collinear electrodes was analysed. For effective treatments, it is desirable to use electrodes with rounded edges. A non-pumpable lumpy material like sugar beets can be transported either by a conveyor belt or a wheel equipped with rods.

CONCLUSION

The analysis of the current literature shows great interest in the electroporation phenomenon of plant foods and particularly in the electroporation of sugar beets. PEF treatments can be used for the genetic transformation and production of sugar beet transgenic crops with increased tolerance to herbicides, salts, drought and fungi, and disease resistance. Assistance by PEF allows one to improve the diffusion, pressing, combined pressing-diffusion, liming and juice ultrafiltration processes. PEF treatments can be integrated in the green or multistep extraction of pure and high-quality biomolecules. During the last two decades, the essential steps have been completed for the industrial implementation of PEFs in sugar beet processing. The drawbacks of PEF techniques include the high capital investment, limiting the availability of commercial units, and complexities in the contraction of large-scale design of PEF treatment reactors.

REFERENCES

- Almohammed F., Mhemdi H., Grimi N., Vorobiev E. (2015): Alkaline pressing of electroporated sugar beet tissue: Process behavior and qualitative characteristics of raw juice. *Food and Bioprocess Technology*, 8: 1947–1957.
- Almohammed F., Mhemdi H., Vorobiev E. (2016a): Pulsed electric field treatment of sugar beet tails as a sustainable feedstock for bioethanol production. *Applied Energy*, 162: 49–57.
- Almohammed F., Mhemdi H., Vorobiev E. (2016b): Several-staged alkaline pressing-soaking of electroporated sugar beet slices for minimization of sucrose loss. *Innovative Food Science & Emerging Technologies*, 36: 18–25.
- Almohammed F., Koubaa M., Khelfa A., Nakaya M., Mhemdi H., Vorobiev E. (2017a): Pectin recovery from sugar beet pulp enhanced by high-voltage electrical discharges. *Food and Bioprocess Technology*, 103: 95–103.
- Almohammed F., Mhemdi H., Vorobiev E. (2017b): Purification of juices obtained with innovative pulsed electric field and alkaline pressing of sugar beet tissue. *Separation and Purification Technology*, 173: 156–164.
- Al-Nema Q., Mozahim A.M. (2020): Electrofusion of mesophyll protoplasts from two varieties of sugar beet (*Beta vulgaris* L.). *Journal of Life and Bio Sciences Research*, 1: 22–25.
- Arnold J., Frenzel S., Michelberger T., Scherer P., Scheuer T., Weibel M. (2009): Process and device for the electroporation of beet cossettes. Patent No. CA2722522A1, Südzucker AG.
- Arnold J., Frenzel S., Michelberger T., Scherer P., Scheuer T. (2012): Extraction of constituents from sugar beet chips. Patent No. CA2570475C, Südzucker AG.
- Arnold J., Frenzel S., Michelberger T., Scherer P., Scheuer T., Weibel M. (2014): Process for the electroporation of beet cossettes and device for carrying out this process. Patent No. US8691306B2. Südzucker AG.
- Arnold J., Sack M., Schmidt G., Epperlein D. (2020): Reactor system for electroporation. Patent No. PL2515686T3, Südzucker AG.
- Arshad R.N., Abdul-Malek Z., Munir A., Buntat Z., Ahmad M.H., Jusoh Y.M.M., Bekhit A.E.D., Roobab U., Manzoor M.F., Aadil R.M. (2020): Electrical systems for pulsed electric field applications in the food industry: An engineering perspective. *Trends in Food Science & Technology*, 104: 1–13.
- Arshad R.N., Abdul-Malek Z., Roobab U., Munir M.A., Naderipour A., Qureshi M.I., Bekhit A.E.D., Liu Z.W., Aadil R.M. (2021): Pulsed electric field: A potential alternative towards a sustainable food processing. *Trends in Food Science & Technology*, 111: 43–54.
- Arshad R.N., Abdul-Malek Z., Roobab U., Qureshi M.I., Khan N., Ahmad M.H., Liu Z.W., Aadil R.M. (2021): Effective valorization of food wastes and by-products through pulsed electric field: A systematic review. *Journal of Food Process Engineering*, 44: e13629.
- Asadi M. (2006): *Beet-sugar Handbook*. Hoboken, New Jersey: John Wiley & Sons, Inc.
- Bazhal I.G., Kupchik M.P., Gul, I.S. (1983): Desugaring of sugar beet slices in an electric field. *Sakharnaya Promyshlennost – Sugar Industry*, 3: 28–30. (in Russian)
- Ben-Ali S. (2018): Comparison of electric, thermal and combined treatment effect on solid-liquid extraction. *International Journal of Engineering and Technology*, 10: 44–52.
- Berghöfer T., Bluhm H., Eing C., Sack M. (2010): Electroporation reactor for pressured electroporation of biological commercial- and waste material as process material in continuous process throughput, comprises two roller groups with a roller and a fluid permeable transport device. Patent No. DE102009011755A1, Karlsruher Institut für Technologie – KIT.
- Blahovec J., Vorobiev E., Lebovka N. (2017): Pulsed electric fields pretreatments for the cooking of foods. *Food Engineering Reviews*, 9: 226–236.
- Bluhm H., Schultheiss C., Frey W., Gusbeth C., Sack M., Strissner R. (2004): Industrial scale treatment of biological cells with pulsed electric fields. In: *Proceedings of Conference Record of the Twenty-Sixth International Power Modulator Symposium, 2004 and 2004 High-Voltage Workshop*, May 23–26, 2004, San Francisco, USA: 8–14.
- Bluhm H., Sack M. (2009): Industrial-scale treatment of biological tissues with pulsed electric fields. In: Vorobiev E., Lebovka N. (eds): *Electrotechnologies for Extraction from Food Plants and Biomaterials*. Food Engineering Series. New York, Springer: 237–269.
- Bouzzara H., Vorobiev E. (2000): Beet juice extraction by pressing and pulsed electric fields. *International Sugar Journal*, 102: 194–200.
- Chen M., Zhao Y., Yu S. (2015): Optimisation of ultrasonic-assisted extraction of phenolic compounds, antioxidants, and anthocyanins from sugar beet molasses. *Food Chemistry*, 172: 543–550.
- Dolinskaya I.N., Dan'kevich G.N., Gulyj I.S., Kupchik M.P., Matvienko A.B., Katrokha I.M. (1992): The influence of electrical and thermal factors on efficiency of processes to extract soluble substances from plant raw materials. *Elektronnaya Obrabotka Materialov (Surface Engineering and Applied Electrochemistry)*, N1: 66–69. (in Russian)
- Dragomir M.C.B., Zeca E.D., Ivan A.S., Stoica M. (2020): Pulsed electric field and high voltage electrical discharge-innovative food electrotechnologies. A review. *Journal of Agroalimentary Processes and Technologies*, 26: 34–39.

- Eady C., Warren G., Lindsey K., Jones M.G.K. (1988): Electrofusion and electroporation of sugar beet (*Beta vulgaris* L.) protoplasts. In: Puite K.J., Dons J.J.M., Huizing H.J., Kool A.J., Koornneef M., Krens F.A. (eds): Progress in Plant Protoplast Research. Dordrecht, Springer: 261–262.
- El-Belghiti K., Rabhi Z., Vorobiev E. (2005a): Effect of centrifugal force on the aqueous extraction of solute from sugar beet tissue pretreated by a pulsed electric field. Journal of Food Process Engineering, 28: 346–358.
- El-Belghiti K., Rabhi Z., Vorobiev E. (2005b): Kinetic model of sugar diffusion from sugar beet tissue treated by pulsed electric field. Journal of the Science of Food and Agriculture, 85: 213–218.
- Eshtiaghi M.N., Knorr D. (2002): High electric field pulse pretreatment: Potential for sugar beet processing. Journal of Food Engineering, 52: 265–272.
- Eshtiaghi M.N., Knorr D. (2004): Process for treating sugar beets. Patent No. EP1086253B1, Tereos SA.
- Eshtiaghi M.N., Maskooki A. (2009): Effect of various pulsed electric fields conditions on extraction of sugar from sugar beet (Persian). Iranian Journal Food Science and Technology Research, 5: 151–162.
- Frenzel S., Michelberger T., Witte G. (2012): Extraction of ingredients such as sugar from biological material. Patent No. US8163091B2, Südzucker AG.
- Gjorek J., Flisar K., Miklavčič D., Poklar N.U., Golob J. (2016): Extraction of sugar solution from sugar beet cossettes by electroporation and compressive load. In: Proceedings of the 1st World Congress on Electroporation and Pulsed Electric Fields in Biology, Medicine and Food & Environmental Technologies, Sept 6–10, 2015, Portorož, Slovenia: 384–387.
- Grimi N., Vorobiev E., Lebovka N., Vaxelaire J. (2010): Solid–liquid expression from denaturated plant tissue: Filtration–Consolidation behaviour. Journal of Food Engineering, 96: 29–36.
- Gurel E., Gurel S., Lemaux P.G. (2008): Biotechnology applications for sugar beet. Critical Reviews in Plant Sciences, 27: 108–140.
- Hall R.D., Pedersen C., Krens F.A. (1994): Regeneration of plants from protoplasts of *Beta vulgaris* (sugar beet). In: Bajaj J.P.S. (ed.): Biotechnology in Agriculture and Forestry, 29: Plant Protoplasts and Genetic Engineering V. Berlin and Heidelberg, Springer-Verlag GmbH: 16–37.
- Huang X., Li D., Wang L. (2018): Effect of particle size of sugar beet pulp on the extraction and property of pectin. Journal of Food Engineering, 218: 44–49.
- Jafarzadeh-Moghadda M., Shadde R., Peighambarous S.H. (2021): Sugar beet pectin extracted by ultrasound or conventional heating: A comparison. Journal of Food Science and Technology, 58: 2567–2578.
- Jemai A.B., Vorobiev E. (2003): Enhanced leaching from sugar beet cossettes by pulsed electric field. Journal of Food Engineering, 59: 405–412.
- Jemai A.B., Vorobiev E. (2006): Pulsed electric field assisted pressing of sugar beet slices: Towards a novel process of cold juice extraction. Biosystems Engineering, 93: 57–68.
- Joersbo M. (2007): Sugar beet. In: Pua E.C., Davey M.R. (eds): Transgenic Crops IV. Biotechnology in Agriculture and Forestry, 59, Berlin and Heidelberg, Springer: 355–379.
- Joersbo M., Brunstedt J. (1996): Electroporation and transgenic plant production. In: Lynch P.T., Davey M.R. (eds): Electrical Manipulation of Cells. Boston, Springer: 201–222.
- Joersbo M., Brunstedt J. (1990a): Direct gene transfer to plant protoplasts by electroporation by alternating, rectangular and exponentially decaying pulses. Plant Cell Reports, 8: 701–705.
- Joersbo M., Brunstedt J. (1990b): Stimulation of protein synthesis in electroporated plant protoplasts. Journal of Plant Physiology, 136: 464–467.
- Joersbo M., Brunstedt J., Floto F. (1990): Quantitative relationship between parameters of electroporation. Journal of Plant Physiology, 137: 169–174.
- Karpovich N.S., Bazhal I.G., Gulyi I.S., Bobrovnik L.D., Totnaylo M.A. (1981): The behavior of the structural elements of a plant cell in an electric field. Sakharnaya Promyshlennost – Sugar Industry, 10: 32–35. (in Russian)
- Katroha I.M., Matvienko A.B., Vorona A.G., Kupchik M.P. (1984): Intensification of sugar extraction from sugar beet slices in an electric field. Sakharnaya Promyshlennost – Sugar Industry, 7: 28–31. (in Russian)
- Kotnik T., Rems L., Tarek M., Miklavčič D. (2019): Membrane electroporation and electroporabilization: Mechanisms and models. Annual Review of Biophysics, 48: 63–91.
- Kovačić Đ., Rupčić S., Kralik D., Jovičić D., Spajić R., Tišma M. (2021): Pulsed electric field: An emerging pretreatment technology in a biogas production. Waste Management, 120: 467–483.
- Kupchik M.P., Matvienko A.B., Mank V.V. (1987): Changes in the ultrastructure of beet cells during diffusion under the influence of temperature and electric field. Sakharnaya Promyshlennost – Sugar Industry, 5: 25–27. (in Russian)
- Lal R., Lal S. (2020): Genetic Engineering of Plants for Crop Improvement. London, CRC Press.
- Lanoiselle J.L., Vorobiev E., Bousseta N., Manteau S., Logeat M. (2012): Method for extracting molecules of interest from all or part of a plant matrix. Patent, No. WO2012066144A1, Universite de Technologie de Compiègne UTC and Societe Francaise De Laboratoires D'oenologie Sofralab.

<https://doi.org/10.17221/91/2021-RAE>

- Lebovka N.I., Praporscic I., Ghnimi S., Vorobiev E. (2005): Does electroporation occur during the ohmic heating of food? *Journal of Food Science*, 70: 308–311.
- Lebovka N.I., Shynkaryk M.V., El-Belghiti K., Benjelloun H., Vorobiev E. (2007a): Plasmolysis of sugarbeet: Pulsed electric fields and thermal treatment. *Journal of Food Engineering*, 80: 639–644.
- Lebovka N.I., Shynkaryk M., Vorobiev E. (2007b): Moderate electric field treatment of sugarbeet tissues. *Biosystems Engineering*, 96: 47–56.
- Li Z., Fan Y., Xi J. (2019): Recent advances in high voltage electric discharge extraction of bioactive ingredients from plant materials. *Food Chemistry*, 277: 246–260.
- Lindsey K., Jones M.G.K. (1987a): The permeability of electroporated cells and protoplasts of sugar beet. *Planta*, 172: 346–355.
- Lindsey K., Jones M.G.K. (1987b): Transient gene expression in electroporated protoplasts and intact cells of sugar beet. *Plant Molecular Biology*, 10: 43–52.
- Lindsey K., Jones M.G.K., Fish N. (1988): Direct gene transfer into plant protoplasts. In: Walker J.M. (ed.): *New Nucleic Acid Techniques. Methods in Molecular Biology*, 4. Totowa, Humana Press: 519–536.
- Lindsey K., Jones M.G.K. (1989): Stable transformation of sugarbeet protoplasts by electroporation. *Plant Cell Reports*, 8: 71–74.
- Lindsey K., Jones M.G.K. (1990): Electroporation of cells. *Physiologia Plantarum*, 79: 168–172.
- Loeffler M.J. (2002): Commercial pulsed power applications in Germany. In: *Proceedings of Symposium on Pulsed Power and Plasma Applications*. Kailua-Kona, Hawaii.
- Loginov M., Loginova K., Lebovka N., Vorobiev E. (2011): Comparison of dead-end ultrafiltration behaviour and filtrate quality of sugar beet juices obtained by conventional and "cold" PEF-assisted diffusion. *Journal of Membrane Science*, 377: 273–283.
- Loginova K., Loginov M., Vorobiev E., Lebovka N.I. (2011a): Quality and filtration characteristics of sugar beet juice obtained by "cold" extraction assisted by pulsed electric field. *Journal of Food Engineering*, 106: 144–151.
- Loginova K., Vorobiev E., Bals O., Lebovka N. (2011b): Pilot study of countercurrent cold and mild heat extraction of sugar from sugar beets, assisted by pulsed electric fields. *Journal of Food Engineering*, 102: 340–347.
- Loginova K., Loginov M., Vorobiev E., Lebovka N.I. (2012): Better lime purification of sugar beet juice obtained by low temperature aqueous extraction assisted by pulsed electric field. *LWT – Food Science and Technology*, 46: 371–374.
- Lopez N., Puertolas E., Condon S., Raso J., Alvarez I. (2009): Enhancement of the solid-liquid extraction of sucrose from sugar beet (*Beta vulgaris*) by pulsed electric fields. *LWT – Food Science and Technology*, 42: 1674–1680.
- Ma S., Wang Z. (2013): Pulsed electric field-assisted modification of pectin from sugar beet pulp. *Carbohydrate Polymers*, 92: 1700–1704.
- Ma S., Yu S., Zhang B., Wang Z. (2012): Physicochemical properties of sugar beet pulp pectin by pulsed electric field treatment. *International Journal of Food Science & Technology*, 47: 2538–2544.
- Marggraf A.S. (1747): *Histoire de l'Academie Royale des Sciences et Belles Lettres de Berlin*.
- Martínez J.M., Delso C., Álvarez I., Raso J. (2020): Pulsed electric field-assisted extraction of valuable compounds from microorganisms. *Comprehensive Reviews in Food Science and Food Safety*, 19: 530–552.
- Maskooki A.M., Eshtiaghi M.N. (2011): Effects of various pulsed electric field conditions on cell disintegration and mass transfer of sugar beet. *Journal of Food Science and Engineering*, 1: 67.
- Maskooki A., Eshtiaghi M.N. (2012): Impact of pulsed electric field on cell disintegration and mass transfer in sugar beet. *Food and Bioproducts Processing*, 90: 377–384.
- McGinnis R.A. (1982): *Beet-sugar technology*. Beet Sugar Development Foundation. Fort Collins, Colorado: 265–274.
- Mhemdi H., Almohammed F., Bals O., Grimi N., Vorobiev E. (2015): Impact of pulsed electric field and preheating on the lime purification of raw sugar beet expressed juices. *Food and Bioproducts Processing*, 95: 323–331.
- Mhemdi H., Bals O., Grimi N., Vorobiev E. (2012): Filtration diffusivity and expression behaviour of thermally and electrically pretreated sugar beet tissue and press-cake. *Separation and Purification Technology*, 95: 118–125.
- Mhemdi H., Bals O., Grimi N., Vorobiev E. (2014): Alternative pressing/ultrafiltration process for sugar beet valorization: Impact of pulsed electric field and cossettes preheating on the qualitative characteristics of juices. *Food and Bioprocess Technology*, 7: 795–805.
- Mhemdi H., Bals O., Vorobiev E. (2016): Combined pressing-diffusion technology for sugar beets pretreated by pulsed electric field. *Journal of Food Engineering*, 168: 166–172.
- Nakthong N., Eshtiaghi M.N. (2020): Pulsed electric field treatment of sugar beet. In: *Proceeding of the 6th International Conference on Environment and Renewable Energy* Feb 24–26, 2020, Hanoi, Vietnam: 1–8.
- Pacheco M.T., Villamiel M., Moreno R., Moreno F.J. (2019): Structural and rheological properties of pectins extracted from industrial sugar beet by-products. *Molecules*, 24: 392.
- Pataro G., Barca G.M.J., Pereira R.N., Vicente A.A., Teixeira J.A., Ferrari G. (2014): Quantification of metal release from stainless steel electrodes during conventional and

<https://doi.org/10.17221/91/2021-RAE>

- pulsed ohmic heating. *Innovative Food Science & Emerging Technologies*, 21: 66–73.
- Poiesz E.G., Daniëls A.C.P.H. (2021): Sugar beet juice production and processing, Patent No. EP3783115A1, Cooperatie Koninklijke Cosun U.A.
- Praporscic I. (2005): Influence du traitement combine par champ electrique pulse et chauffage modere sur les proprietes physiques et sur le comportement au pressage de produits vegetaux [PhD Thesis]. Compiègne, Université de Technologie de Compiègne (in French).
- Praporscic I., Ghnimi S., Vorobiev E. (2005): Enhancement of pressing of sugar beet cuts by combined ohmic heating and pulsed electric field treatment. *Journal of Food Processing and Preservation*, 29: 378–389.
- Raso J., Frey W., Ferrari G., Pataro G., Knorr D., Teissie J., Miklavčič D. (2016): Recommendations guidelines on the key information to be reported in studies of application of PEF technology in food and biotechnological processes. *Innovative Food Science & Emerging Technologies*, 37: 312–321.
- Rezaei K., Shahidi Noghabi M., Behzad K., Maskooki A. (2018): Evaluation of the effect of pulsed electric field (PEF) treatment on the quality of raw syrup extracted from sugar beet. *Journal of Food Science and Technology*, 15: 267–276. (in Iranian)
- Rivera A.L., Gómez-Lim M., Fernández F., Loske A.M. (2012): Physical methods for genetic transformation in plants. *Physics of Life Reviews*, 9: 352.
- Sack M., Attmann F., Stangle R., Wolf A., Frey W., Muller G. (2009): Upgrade of the electroporation device KEA-MOBIL. *Acta Physica Polonica-Series A General Physics*, 115: 1081.
- Sack M., Bluhm H. (2005): Long-term test of a triggered Marx-generator in repetitive operation. In: *Proceedings of the 2005 IEEE Pulsed Power Conference*, June 13–15, 2005, Monterrey, USA: 1113–1116.
- Sack M., Schultheiss C. (2012): Device for the electroporation of biologically vegetable processing material, Patent No. EP1751860B1, Karlsruher Institut fuer Technologie KIT.
- Sack M., Schultheiss C., Bluhm H. (2004): Parameter studies on the electroporation efficiency of sugar beets. In: *The Proceedings of the 31st IEEE International Conference on Plasma Science*, Jun 28–Jul 1, 2004, Baltimore, USA: 196.
- Sack M., Sigler J., Frenzel S., Eing C., Arnold J., Michelberger T., Frey W., Attmann F., Stukenbrock L., Müller G. (2010). Research on industrial-scale electroporation devices fostering the extraction of substances from biological tissue. *Food Engineering Reviews*, 2: 147–156.
- Sack M., Mueller G. (2016): Scaled design of PEF treatment reactors for electroporation-assisted extraction processes. *Innovative Food Science & Emerging Technologies*, 37: 400–406.
- Sack M., Mueller G. (2017): Design considerations for electroporation reactors. *IEEE Transactions on Dielectrics and Electrical Insulation*, 24: 1992–2000.
- Salehi M., Omidvari A. (2016): PEF application on optimization of energy consumption in extraction of sugar from sugar beet. *Journal of Energy Management*, 5: 26–33.
- Schiweck H., Clarke M., Pollach G. (2012): Sugar. In: *Ullmann's Encyclopedia of Industrial Chemistry*. Wiley-VCH. 34: 558–624.
- Schultheiss C., Bluhm H.J., Mayer H.G., Sack M., Kern M. (2003): Electroporation for the treatment of sugar beet cells. Part 1: Principle of electroporation and development of industrial devices. In: *Proceedings of the 22nd General Assembly of the International Commission for Sugar Technology*, May 18–21, 2003, Madrid, Spain: 209–215.
- Schultheiss C., Bluhm H.J., Mayer H.G., Kern M. (2001): Industrial-scale electroporation of plant material using high repetition rate marx generators. In: *Proceedings of the 28th IEEE International Conference on Plasma Science and 13th IEEE International Pulsed Power Conference*, June 17–21, 2001, Las Vegas, USA: 207–210.
- Schultheiss C., Sack M., Bluhm H., Mayer H.G., Kern M. (2004): Operational experience of industrial scale electroporation devices. In: *The Proceedings of the 31st IEEE International Conference on Plasma Science*, June 28–July 1, 2004, Baltimore, USA: 114.
- Schultheiss C., Bluhm H., Mayer H.G., Kern M., Michelberger T., Witte G. (2002): Processing of sugar beets with pulsed-electric fields. *IEEE Transactions on Plasma Science*, 30: 1547–1551.
- Schultheiss C., Kern M. (2003): Electroporation reactor for the continuous processing of lumpy products, Patent No. DE10144479C2, KEA TEC GmbH Forschungszentrum Karlsruhe GmbH.
- Schultheiss C., Kern M. (2010): Electroporation reactor for the continuous processing of lumpy products, Patent No. CA2460569C, KEA TEC GmbH Forschungszentrum Karlsruhe GmbH.
- Shynkaryk M. (2006): Influence de la perméabilisation membranaire par champ electrique sur la performance de sechage des vegetaux [PhD Thesis]. Compiègne, Université de Technologie de Compiègne (in French).
- Stevanato P., Chiodi C., Broccanello C., Concheri G., Biancardi E., Pavli O., Skaracis G. (2019): Sustainability of the sugar beet crop. *Sugar Tech*, 21: 703–716.
- Tylewicz U. (2020): How does pulsed electric field work? In: Barba F.J., Parniakov O., Wiktorin A. (eds): *Pulsed Electric Fields to Obtain Healthier and Sustainable Food for Tomorrow*. Cambridge, Elsevier Inc: 3–21.

<https://doi.org/10.17221/91/2021-RAE>

- van der Poel P.W., Schiweck H., Schwartz T. (1998): Sugar Technology. Beet and Cane Sugar Manufacture. Berlin, Verlag Dr. Albert Vartens KG.
- Vidal O.P. (2014): First pulsed electric field (PEF) application at industrial scale in beet sugar industry. *Sugar Industry/Zuckerindustrie*, 139: 37–39.
- Vidal O.P., Vorobiev E. (2012): Procédé et installation de traitement des tissus végétaux pour en extraire une substance végétale, notamment un jus. Patent No. FR2959399B1, Comeca Power, FR, Maguin SAS, FR, Université de Technologie de Compiègne UTC.
- Vidal O.P., Vorobiev E. (2013): Method and apparatus for treating vegetable tissues in order to extract therefrom a vegetable substance, in particular a juice, Patent No. US20130202751A1, Maguin Sas.
- Vorobiev E., Andre A., Bouzrara H., Bazhal M. (2002): Method for extracting liquid from a cellular material, and devices for carrying out said method, Patent No. FR2805199B1, Association Gradient.
- Vorobiev E., Lebovka N. (2020): *Processing of Foods and Biomass Feedstocks by Pulsed Electric Energy*. Cham, Springer.
- Vorobiev E., Maishak F. (2018): Selective extraction of sucrose from sugar beet by electroporation and its influence on the technology of sugar production. *Sahar (Sugar)*, 3: 19–29 (part 1) and 4: 28–37 (part 2) (in Russian).
- Zagorul'ko A.Y., Myl'kov M.N. (1953): Production of juice at low temperature using electroporation. *Sakharnaya Promyshlennost – Sugar Industry*, 10: 15–18. (in Russian)
- Zagorul'ko A.Y. (1957): Impact of thermal plasmolysis and selective electroporation on the structure of the plasma cell membrane and permeability of beet tissues. *Sakharnaya Promyshlennost – Sugar Industry*, 11: 67–71. (in Russian)
- Zhu Z., Mhemdi H., Ding L., Bals O., Jaffrin M.Y., Grimi N., Vorobiev E. (2015): Dead-end dynamic ultrafiltration of juice expressed from electroporated sugar beets. *Food and Bioprocess Technology*, 8: 615–622.
- Zicari S., Zhang R., Kaffka S. (2019): Sugar beet. In: Pan Z., Zhang R., Zicari S. (eds): *Integrated Processing Technologies for Food and Agricultural By-Products*. Cambridge, Elsevier: 331–351.

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