Food Eng Rev (2010) 2:147–156
DOI 10.1007/s12393-010-9017-1

Research on Industrial-Scale Electroporation Devices Fostering the Extraction of Substances from Biological Tissue

M. Sack · J. Sigler · S. Frenzel · Chr. Eing · J. Arnold · Th. Michelberger · W. Frey · F. Attmann · L. Stukenbrock · G. Müller

Received: 7 January 2010 / Accepted: 4 March 2010 / Published online: 21 March 2010

Abstract Electroporation is an innovative method in food processing to support the extraction of substances from cells. By applying a pulsed electric field to the tissue, the cell membranes are charged and pores are formed in the membranes fostering the extraction. Although this principle is common to all electroporation devices, due to the different properties of each material, the devices and processes have to be designed for each application individually. The publication gives an overview on the advantages of electroporation for the processing of crushed grapes, sugar beets, and energy crop, and also on the design of electroporation devices for these applications.

Keywords Electroporation · Wine grapes · Apples · Sugar beets · Energy crop

The article is based on an invited talk on the research on industrial-scale electroporation devices at KIT (formerly Forschungszentrum Karlsruhe) and cooperation partners, given at the Conference on Bio and Food Electrotechnologies (BFE) which was held in October 2009 in Compiègne, France.

M. Sack (✉) · Chr. Eing · W. Frey · F. Attmann · G. Müller
Karlsruher Institut für Technologie, Institut für Hochleistungsimpuls- und Mikrowellentechnik, Hermann-von-Helmholtz-Platz 1, 76344 Eggenstein-Leopoldshafen, Germany
e-mail: martin.sack@kit.edu

J. Sigler · L. Stukenbrock
Staatliches Weinbauinstitut Freiburg, Merzhauser Str. 119, 79100 Freiburg, Germany

S. Frenzel · J. Arnold · Th. Michelberger
SÜDZUCKER AG Mannheim/Ochsenfurt, Wormser Str. 11, 67283 Obrigheim, Germany

Introduction

The extraction of substances from biological tissue is an important processing step in the preparation of many kinds of food. Sugar is extracted from sugar beets by means of an extraction process at 69–73 °C after a thermal denaturation at 70–78 °C. Unlike for the production of sugar from sugarcane, the required energy for heating is not delivered by the plants, instead oil or coal has to be burned for heating. The juice from fruits is extracted by pressing. But especially in the second pressing stage enzymes or thermal treatment may be used to foster the extraction. For the preparation of red wine the fermentation on skins is the classic way to open the cell membranes and to extract the pigments and valuable substances. But it requires 1–3 weeks for fermentation inclusive extraction. Another way is the thermovinification. The mash is heated up to approximately 80 °C and kept at this temperature for 2 min, before it is cooled back to less than 40 °C. This faster method consumes much energy, because especially in small heating devices the energy is not recuperated.

If an electric field is applied to a cell, the membrane is charged resulting in a field enhancement across the membrane, Fig. 1. At a voltage of ~0.5–1 V across the membrane, pores are formed. When applying a field-sensitive dye (ANNINE-6) to the cell, staining the bi-lipid layer of the cell membrane, the charging process of the cell membrane can be observed as a change in wave length of the fluorescent light from the dye which is transferred into a change of intensity by filtering [5]. Figure 2 shows a microscopic view of a protoplast before and during the application of an electric field with the cell membrane stained with ANNINE-6. In the experiment the pore formation is visible as a saturation effect limiting the voltage across the membrane. Due to the angular dependency of

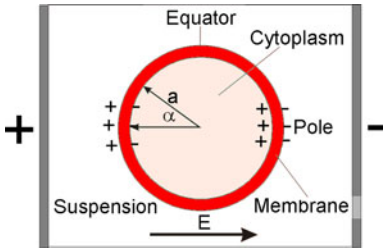

Fig. 1 Space charge configuration across the membrane of a ball-like biological cell

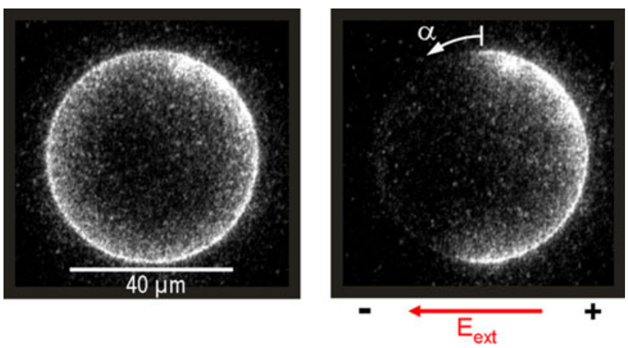

Fig. 2 BY2-Protoplast (tobacco) before and during the application of an electric field with the cell membrane stained with ANNINE-6

the field enhancement across the membrane and the natural DC potential across the cell membrane of −100 to −200 mV with rising voltage, the pore formation starts at the negative pole of the cell. Small pores may close after the pulse application. If sufficient energy is applied, the pores grow and irreversible pores are formed. Through these pores, substances may be extracted from the cell. Figure 3 shows microscopic pictures of cells from the skin tissue of Lemberger wine grapes before and after electroporation. The pigments are stored inside a vacuole. For an extraction both membranes of the cell and the vacuole have to be opened.

Applications of Electroporation

Processing of Sugar Beets, Wine Grapes, and Apples

Electroporation is considered to be an interesting alternative to open biological cells for an extraction process:

In first experiments on the electroporation of whole sugar beets an energy-efficient denaturation of the material with an applied specific energy of 2 kJ/kg could be achieved [6]. When cutting the beets into two halves, the juice started to drain out of the tissue forming drops. Due to the decreasing internal pressure of the cells the tissue became soft. Hence, in texture tests for slicing the electroporated beets a force of only 8 N has been required, which is only half on the force needed for cutting raw sugar beets. Accordingly, a decrease of the energy for subsequent slicing of the beets in a slicing machine and an extended time interval for changing the blades of the slicing machine has been expected. But the strong temperature dependence of the electroporation energy turned out to be a disadvantage. Example experiments made in November 2003 showed a significant decrease of the electroporation efficiency for an electric field strength of $\hat{E} = 6$ kV/cm below a temperature of 7 °C. As it was not possible to warm up the whole beets in the course of the processing fast enough, cossettes have been used instead.

In combination with the method of alkaline extraction [2, 4, 8] experiments on the electroporation of sugar beet cossettes on-site in a sugar factory demonstrated a more energy-efficient extraction of sugar from electroporated cossettes [6]. For the alkaline extraction, lime milk is added to the cossettes resulting in a strengthening of the cell walls and a chemical modification of the pectin as a component of beet tissue. As a consequence of combining the two processing steps the juice can better drain out of the cells after opening of the cell membranes. Due to the better extraction, the extraction temperature can be decreased from 72 to 60 °C without affecting the efficiency of the sugar extraction. A minimum temperature of 60 °C

Fig. 3 Microscopic photographs of peel tissue of Lemberger wine grapes before and after electroporation

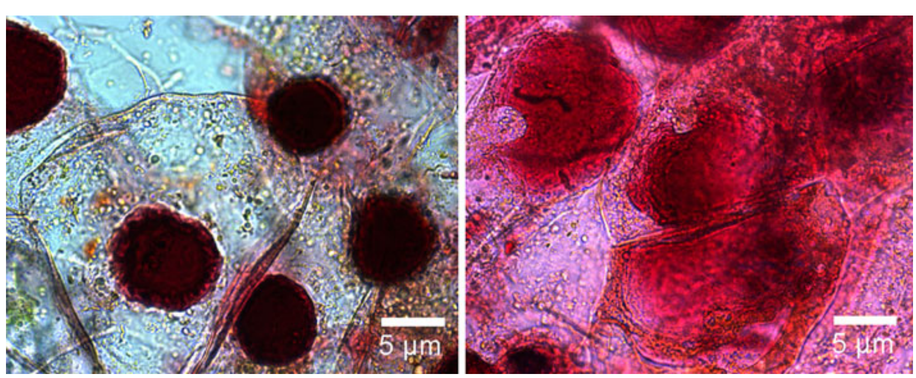

Table 1 Chemical analysis of must and wine [13, 18]

	Method of treatment	Specific electroporation energy [kJ/kg]	Must					Wine			
			Must sweetness [°Oe]	Total acid [g/l]	Yeast digestible nitrogen [mg/l]	Potassium [g/l]	Colour intensity	Alcohol [g/l]	Total dry extract [g/l]	Potassium [g/l]	Colour intensity
Pinot Noir 2008	Thermovinification	–	86.4	11.3	398	2.2	11.9	95.6	23.2	1.2	1.5
	Electroporation	49.5	84.8	10.4	394	2.0	12.9	98.1	23.7	1.1	1.5
Riesling 2008	Pumped only	0.0	89.9	7.5	300	1.7	1.1	96.3	23.5	1.0	0.1
	Electroporation	49.5	89.9	7.3	339	1.7	1.3	95.5	25.0	1.3	0.2

prevents the growth of mesophilic bacteria in the thin juice. Moreover, the amount of water for the extraction can be reduced resulting in a considerable reduction of evaporation energy for the subsequent concentration process of the extracted sugar solution. The strengthened cell walls and the chemical modification of beet tissue result in a better water extraction in the pressing stage for the extracted cossettes. For electroporated material a dry matter content of 40% could be achieved [6]. For thermally treated material the typical dry matter content is in the order of 35%. Consequently, for drying the cossettes less evaporation energy is necessary. Due to an improved purity of the extracted juice, less lime milk for purging is required. This results in additional savings for lime stone and coke for the production of the lime milk. The estimated consumption of electric energy for the electroporation is only 1–1.5 kWh per ton of sugar beets, which is only 3% of the total electric energy consumption of a sugar factory [6]. The results have been obtained by using the electroporation device KEA for whole sugar beets described later in paragraph “An electroporation device for large throughput”.

The electroporation of crushed grapes from red vine varieties combines the advantages of a fast processing and a gentle treatment at low temperature omitting an influence on the taste due to heating [7]. The pigments are extracted from the skin tissue within less than 24 h after electroporation by diffusion in an aquatic extraction process. Table 1 shows the results of the chemical analysis of differently treated variants of must and wines from Pinot Noir and Riesling grapes. The data of the heated (thermovinification) and electroporated variant from Pinot Noir grapes are comparable.

In a laboratory-scale experiment, extraction curves of crushed grapes from electroporated and heated mash have been compared, Fig. 4, [12, 14]. One sample has been heated up to 80 °C for 2 min, to the other sample pulses have been applied. After this pre-treatment, both samples have been cooled down and the extraction has been performed at room temperature. The extraction curves of pigments and tannins for both samples exhibit the same behaviour: For the heated

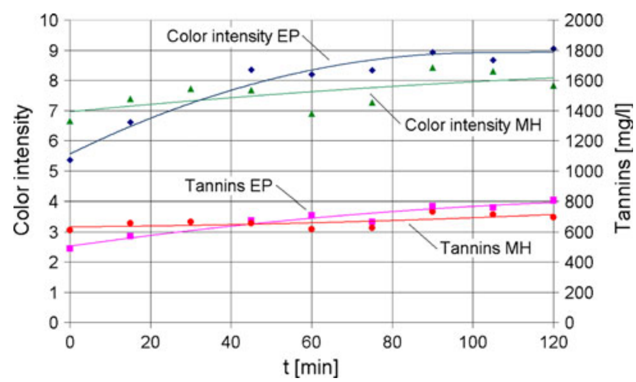

Fig. 4 Extraction curves for tannins and colour from Pinot noir mash after electroporation (EP) and mash heating (MH)

sample both curves start at larger initial values due to the accelerated diffusion at increased temperature. But both curves for the electroporated sample exhibit a steeper increase and, finally, the extraction result is even slightly better than for the heated sample. Measurements of the frequency dependence of the specific conductivity showed for the samples treated by a pulsed electric field (PEF) nearly a pure ohmic behaviour, while for the heated samples still some frequency-dependent components could be measured, Fig. 5. This result correlates with the slightly better extraction results of valuable substances for the PEF-treated sample. From the results it can be concluded that stable pores have been formed in the cell membranes by PEF treatment resulting in a better diffusion.

For Riesling grapes the electroporation has been compared to mash only pumped through the device. As the chemical analysis showed, the electroporation of mash from white wine grapes enables the extraction of more yeast digestible nitrogen which promises advantages to avoid the “atypical ageing note” of the wine. Furthermore, the content of total acid in the must and the wine is reduced. In a sensorial analysis, three wines made from the same mash have been evaluated by 50 experts. The only difference was the way of mash pre-treatment: whole-cluster pressing, use of enzymes, and electroporation. The

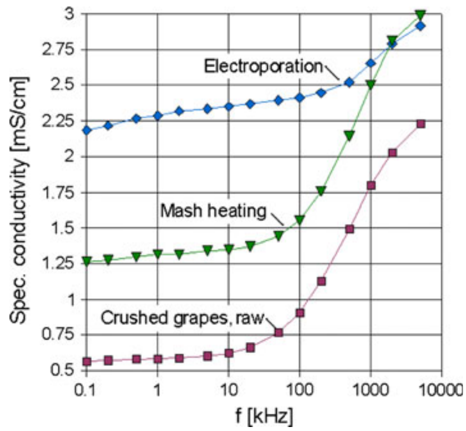

Fig. 5 Frequency dependence of the specific conductivity of crushed grapes: raw, after mash heating, and after electroporation

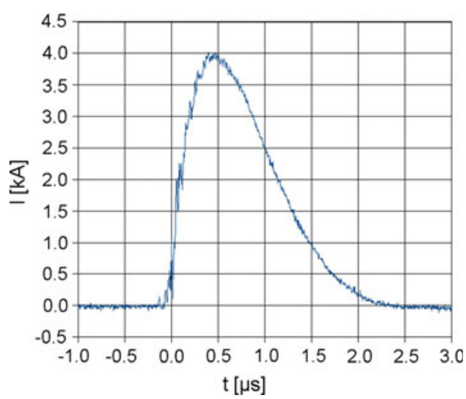

Fig. 6 Measured current shape during PEF treatment of Pinot Noir grape mash

wine made from electroporated mash has been put into the first rank.

Experiments showed that for a complete opening of the cells of Pinot Noir mash, a specific energy in the order of 35 kJ/kg is required [13]. The applied electric field strength was ~35–40 kV/cm at an aperiodically damped pulse shape of 1.5 µs pulse length. Figure 6 shows the measured pulse shape.

In combination with a subsequent fermentation even less energy for the processing is required. Puértolas et al. described a more intense colour of red wine after a combined process of PEF pre-treatment and subsequent fermentation when applying a specific energy of 5 kJ/kg to the mash at an electric field strength of 5 kV/cm only [9]. The alcohol produced during fermentation enables an alcoholic extraction of valuable substances. The extraction process after PEF treatment is much like the extraction after mash heating an aquatic extraction. The aquatic extraction results in a smaller content of polyphenolic substances in the mash than the alcoholic extraction. After blending the wines, anthocyanes–phenoles complexes are formed which is described to be the reason for a deeper colour [1]. Hence, the combination of both methods might be established as a new processing chain.

The electroporation of mash from apples enables an increased yield of high quality unfiltered juice in the first pressing stage, Table 2. In 2006, an electroporation device for apple mash with a throughput of 10 t/h has been installed on-site at a producer of apple juice [7]. In total, the increase by 6% of unfiltered juice results in an increased profit compensating the costs for the electroporation device after only 1.5 years. As the total yield of juice could be increased as well, there is a considerable decrease in energy required for drying the pressed material for producing animal food. Recently, Turk et al. reported about comparable results. An increase in the yield of apple juice by 5% has been obtained [20].

Electroporation-Assisted Drying of Green Biomass

For the production of biofuel in a BTL process, dry biomass serves as source material. According to estimations, in Germany about 10% of fuel consumption could be covered by BTL-fuel from dry biomass. By the additional use of green biomass this fraction can be doubled. Crucial is an energy-efficient drying method for green biomass. The electroporation of the green plant material enables the extraction of a considerable amount of juice by pressing

Table 2 Electroporation of apple mash [7]

	Electroporation	Specific electroporation energy [kJ/kg]	Apple juice				
			Mass of raw material [kg]	Yield of juice in 1st pressing stage [l]	Yield of juice in 1st pressing stage [% of raw mass]	Yield of juice in 2nd pressing stage [% of raw mass]	Total yield of juice [% of raw mass]
Experiment #1	No	0	25,490	18,200	71.4	16.0	87.4
	Yes	4	24,680	18,350	74.4	19.4	93.8
Experiment #2	No	0	28,880	21,500	74.5	16.1	90.6
	Yes	6	28,930	23,500	81.2	15.3	96.5

omitting the evaporation energy [14]. Figure 7 shows the amount of extracted juice from grass after electroporation at $\hat{E} = 7$ kV/cm with different numbers of applied pulses and subsequent pressing at 48 daN/cm^2 for 15 min. The pulse shape of the applied electric field is shown on Fig. 8. After electroporation with sufficient energy the yield of juice could be doubled. Subsequently, the material has been dried in an oven at 105 °C. As an example, drying curves for young maize are shown. The electroporated material dried much faster than the only pressed material. As the different steepness of the curves for 20, 40, and 80 pulses demonstrates, this is not only because of smaller initial water content but as well due to a better diffusion of the vapour through the electroporated material. The electric energy required for the electroporation is only small compared to the evaporation energy, Fig. 9. With electroporation, approximately half of the evaporation energy for drying of non-electroporated material can be saved.

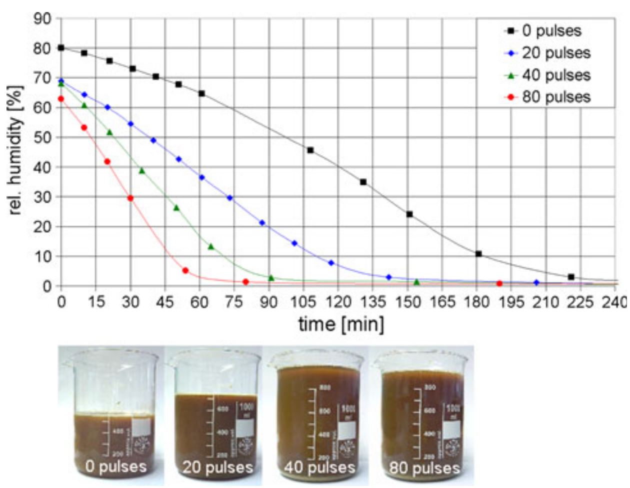

Fig. 7 Yield of juice after pressing (grass) and drying curves depending on the applied number of pulses (young maize)

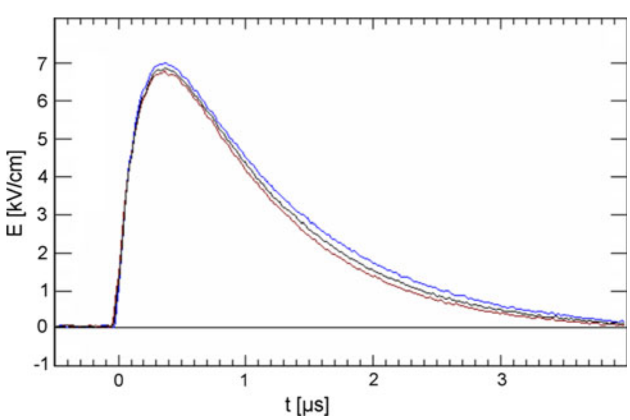

Fig. 8 Shape of the applied electric field for three subsequent pulses during PEF treatment of green biomass. The curves are based on a voltage measurement across the electrodes of the electroporation chamber

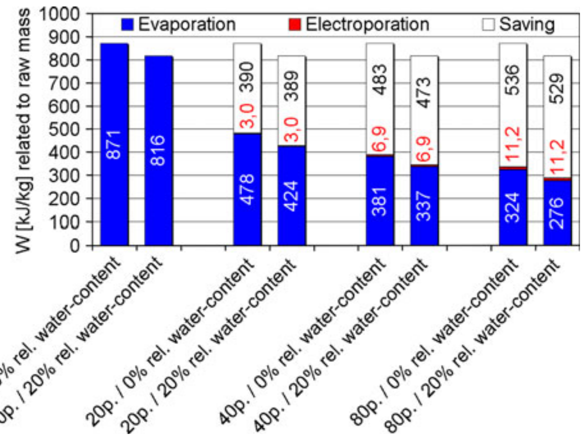

Fig. 9 Energy required for electroporation and evaporation (young maize, $\hat{E} = 7$ kV/cm)

Electroporation Devices

Batch-Processing of Energy Crop

When electroporating mash or whole sugar beets in a continuous flow, the electric contact to the electrodes is established by the juice or water, the plant material is immersed in. To omit the use of additional water for a drying process a portion of the juice from inside the plant material can be extracted by pressing to establish the electric contact to the electrodes [3]. To prevent air from being suck between the electrodes the pressure has to be maintained during the pulse application [15].

For field tests, the electroporation device KEA-MOBIL (Mobile Karlsruher Elektroporationsanlage, mobile Karlsruhe electroporation device) has been equipped with an electroporation reactor for batch-processing, Fig. 10. The system consists of a hydraulic press made from insulating material capable of applying a pressing force equivalent to up to 11 tons to the piston. The piston and the bottom of the test vessel both serve as electrodes. To enable a throughput of 50 kg per hour, the device has been equipped with an automated feeding system. One test vessel is manually emptied and filled outside the device, while the content of another test vessel is treated. The use of a hydraulic press and the manual handling of the material enables studying the influence of different parameters like pressing force, number of applied pulses, applied electric field and energy independent of each other for different sorts of plants like grass, green rye, maize, and lucerne, each having different properties for conveyance.

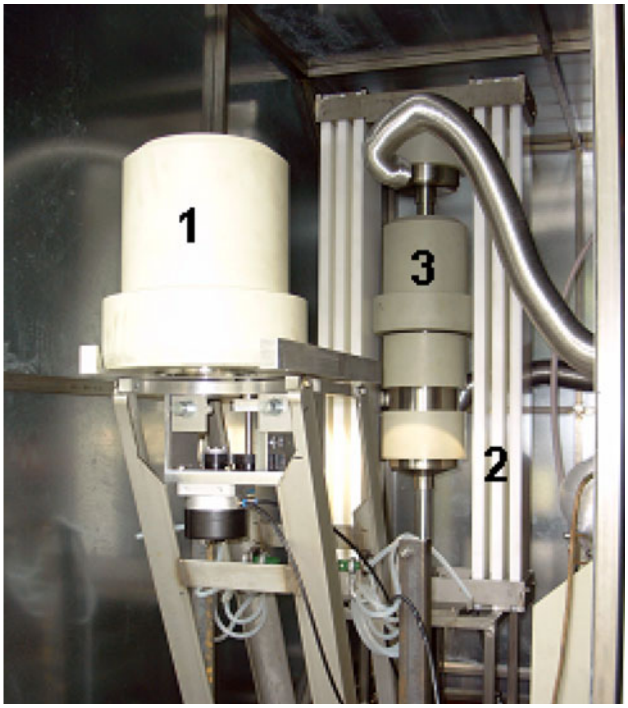

Fig. 10 Batch-processing of green biomass: Hydraulic press and automated feeding system; *1*: Test vessel on feeding system; *2*: hydraulic press; *3*: Test vessel inserted into press during processing

Fig. 11 Electroporation device for grape mash: *1*: High-voltage power supply; *2*: Control unit; *3*: Inlet and outlet for mash (with tubes disconnected); *4*: Marx generator and electroporation reactor (details see Fig. 13)

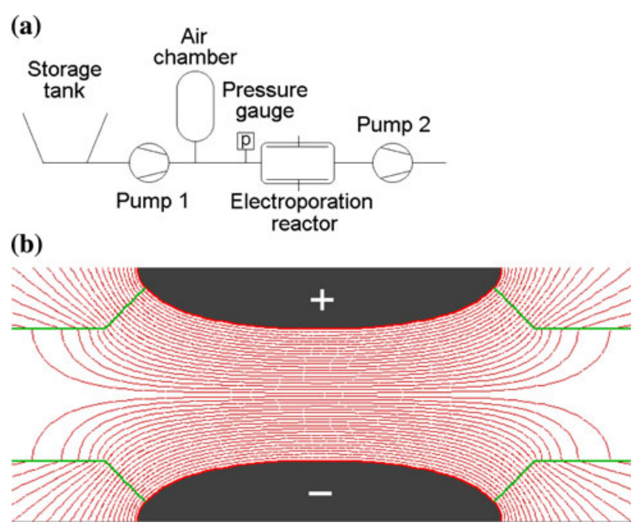

Fig. 12 **a** Hydraulic scheme and **b** equipotential lines inside the reactor

An Electroporation Device for Mash from Wine Grapes

With a diameter in the order of 10 µm the cells of wine grapes are approximately 5 times smaller than the cells of sugar beets with a diameter of ~50 µm. Moreover, the anthocyanes staining the red wine are stored in the cells of the skin tissue inside vacuoles with a diameter of approximately 5 µm. Hence, a larger electric field strength in combination with a faster rise is required to open the cell membranes and vacuoles. The electroporation of wine grapes requires an electric field strength in the order of 25–35 kV/cm. So the field strength may be higher than the breakdown strength of air under normal conditions (30 kV/cm). Figure 11 shows a photograph of the electroporation device for grape mash. Gas transported with the mash is partly removed by an automated de-gasing valve at the inlet of the electroporation reactor. To prevent a flashover inside the reactor caused by remaining gas bubbles in the grape mash, the mash is pressurized to ~0.2–0.3 MPa_{abs} (2–3 bar_{abs}). Due to the increased pressure, the gas bubbles shrink and the breakdown strength of the gas is increased. To prevent the mash from blocking for the pressure regulation a second pump is used rather than a throttling valve [16]. An air chamber decouples both pumps from each other, Fig. 12a. A quasi homogeneous field distribution inside the reactor guarantees a homogeneous treatment of the material, Fig. 12b. The shape of the electrodes has been calculated in such a way that no field enhancement at the borders of the electrodes occurs. The electroporation reactor is operated in ground-symmetric operation. So the voltage between the electrodes and ground is only half of the voltage with one electrode grounded. Hence, the insulation distance can be reduced and the device becomes more compact than in unsymmetrical operation. Moreover, the centre of the reactor is virtually nearly on ground potential omitting a considerable current flow out of the reactor. For safety reasons additional ground electrodes are provided in flow direction before and after the reactor safely preventing current from flowing out of the electroporation device. The electroporation reactor (Fig. 13) has been manufactured from polypropylene with electrodes

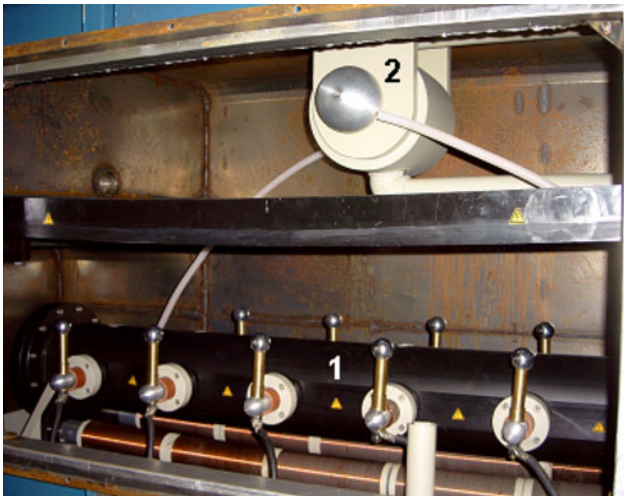

Fig. 13 Electroporation reactor for grape mash: *1*: Marx generator; *2*: Electroporation reactor

Fig. 14 Electroporation-device for whole sugar beets. The photograph has been taken during construction of the device. *1*: Power supplies; *2*: Shielding cabin for Marx generators; *3*: Electroporation reactor (details see Figs. 15, 16); *4*: Slicing machine

from stainless steel. The electroporation reactor is fed by a 6-stage Marx generator with a stage capacitance of 140 nF and a charging voltage of 50 kV/stage. So the total energy per pulse is 1.05 J. Due to the ground-symmetric operation it is grounded at its centre. For a repetition rate up to 20 Hz the spark gaps are cooled by a flow of nitrogen gas. During the harvests in 2008 and 2009, the electroporation device has been operated successfully on-site at three wineries, and more than 5 m^3 of grape mash has been treated each year.

For a reliable operation according to the standards for electromagnetic compatibility, the pulse circuit is shielded by a metal housing. The mains and leads to the control circuitry are protected against over-voltage by varistors and EMC-filters.

When operating electrical devices in wet environment, protection against electric shock in case of insulation failure is crucial. Hence, wineries are equipped with residual current devices (RCD), sensitive to a leakage current of typically 30 mA. The EMC-filters contain capacitors connected to ground. In a symmetric three-phase system there is no leakage current to ground due to such a filter. But an unsymmetric voltage causes a current to ground. Especially when switching the voltage, a pulse current may flow to ground, which may cause the RCD to trip [16]. The amplitude of the current depends on the phase angle. Current pulses with peak values of up to 20 A and a pulse length in the millisecond range have been measured. To limit these current pulses the switched devices have been divided into several groups, which are switched with a small delay of less than 1 s one after the other. So each pulse current is not sufficient for tripping the RCD.

Fig. 15 Wheel equipped with insulating rods for transporting the sugar beets through the PEF-treatment area. The photograph has been taken during assembly of the device

An Electroporation Device for Large Throughput

In a sugar factory a large throughput of sugar beets of up to the order of 15,000 t/d has to be treated. Figure 14 shows the test-setup KEA (Karlsruher Elektroporationsanlage, Karlsruhe electroporation device) for electroporation at a throughput of 10 t beets per hour [19]. The whole sugar beets are transported via a conveyor belt to the top of a wheel equipped with rods for transporting the beets through a basin filled with water, Fig. 15. The electric field for the electroporation is established by a set of high-voltage electrodes at the ground of the basin, Fig. 16. Grounded metallic belts around the wheel serve as ground electrode. Although due to the larger size of the cells of sugar beets, only a specific energy of ~1.0–1.5 kWh/t at a field strength in the order of 3–5 kV/cm is required; the estimated total current for a large electroporation device would be 72 kA [17]. Such a high current cannot be delivered by a single Marx generator in repetitive operation. The inductivity of the pulse circuit, which is typically in the order of 10–25 µH, would limit the current. Hence, the electroporation reactor has to be fed by several Marx generators. Moreover, the wear of the spark gaps is not in proportion to the current. Experiments showed that the wear of the spark gaps is increased by the factor of 4 when doubling the current [10, 11]. So the decrease of current per Marx generator by using several Marx generators reduces the total wear. The device KEA is equipped with two free running seven-stage Marx generators with a total charging voltage of 350 kV and an energy of 1.2 kJ per pulse operated at ~20 Hz repetition rate. Each generator is connected to one round-shaped high-voltage electrode. Figure 17 shows a superposition of 30 acquired pulse currents delivered by one Marx generator. The variation of current shape and peak current is caused by the varying electric resistance of the electroporation reactor due to inhomogeneities of the stream of whole sugar beets. The implemented electrode arrangement results in an inhomogeneous field distribution with a high-field region in the neighbourhood of the electrode and a considerable large low-field area resulting in an ineffective operation. An electrode configuration resulting in a homogeneous field configuration with small low-field regions at the input and output of the electroporation area can be set up as a plate electrode system with each pair of electrode connected to one Marx generator, which are triggered simultaneously [21]. During a seasonal campaign one Marx generator has to pulse up to 200 million times. For a homogeneous burn-up of the spark gap switches, they are equipped with a homogeneous field profile. To omit an additional trigger

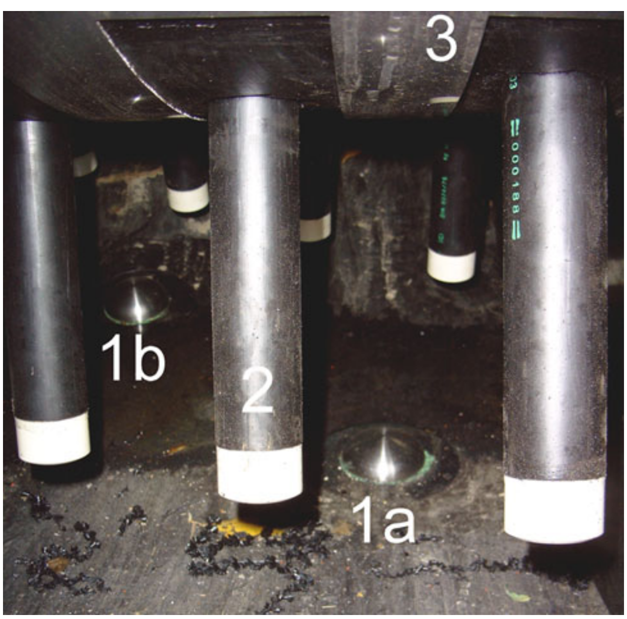

Fig. 16 View inside the PEF-treatment area: *1a*/*1b*: High-voltage electrodes; *2*: Insulating rod; *3*: Metallic belt around the wheel as ground electrode

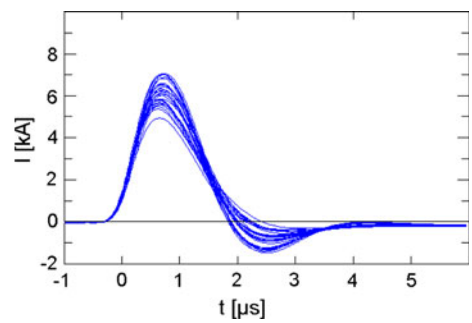

Fig. 17 Measured current shape (30 acquisitions). The variation in amplitude and shape is caused by inhomogeneities when transporting the whole sugar beets through the PEF-treatment area

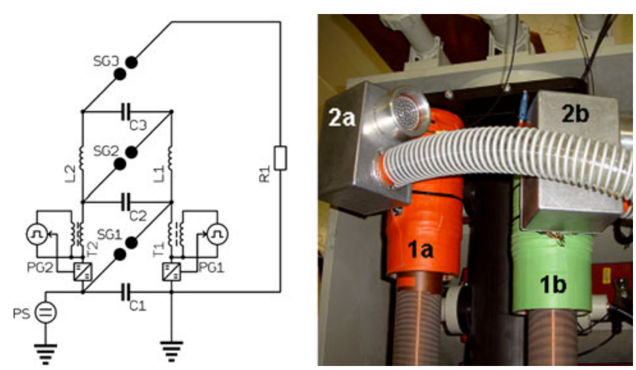

Fig. 18 Over-voltage triggering: Equivalent circuit and implementation into a Marx generator. *T1*/*T2* and *1a*/*1b* Trigger transformers; *PG1*/*PG2* and *2a*/*2b*: Trigger pulse generators and trigger power supplies; *C1*…*C3*: Stage capacitors; *PS*: High-voltage power supply; *L1*/*L2*: Charging coils; *SG1*… *SG3*: Spark gaps

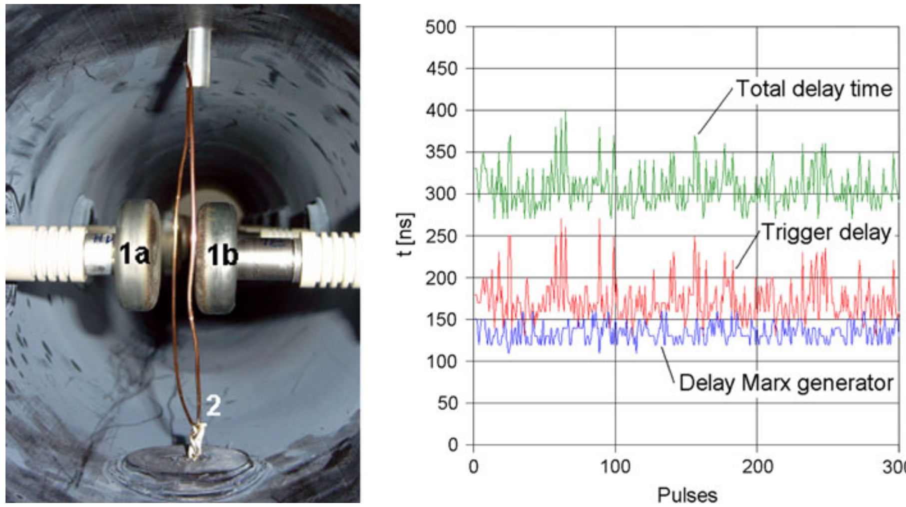

Fig. 19 **a** Spark gap with corona wire and **b** delay times measured at a 7-stage Marx generator: *1a*/*1b*: Spark gap electrodes; *2*: Corona wire

electrode, which would be subjected to an increased wear, the Marx generators are triggered by over-volting their first spark gaps. According to Fig. 18 the over-voltage is coupled into the circuit by pulse transformers connected to trigger pulse generators replacing the ground-side charging coils. The polarity of the pulse is chosen in such a way that the induced pulse is added to the charging voltage of the stage capacitor. For the initialization of the breakdown of the spark gap, seed electrons are required. The time scatter until their appearance determines the jitter. To keep the jitter low, seed electrons can be generated by illuminating the electrodes with ultraviolet light. This light can be easily generated by a corona ring surrounding the electrodes, Fig. 19a. The ring is designed in such a way that a corona discharge in a small high-field area around the ring emits light to the electrodes. To obtain the stable light emission of a negative glow discharge, the ring is electrically connected to the negative switching electrode. The electric field strength in the inter-electrode space between the ring and the switching electrodes has been designed to be sufficiently small to prevent a flashover. Figure 19b shows the measured switching times of a 7-stage Marx generator. For a charging voltage of 93% of the self-breakdown voltage the jitter of the total switching time has been measured to 55 ns. Nevertheless, in some few cases the total switching delay is more than 150 ns. To prevent energy oscillations between the Marx generators due to delayed switching, every Marx generator is connected to a separate pair of electrodes inside the electroporation reactor rather than connecting all generators to one common electrode. Then the oscillations are damped by means of the resistance between neighboured electrode systems. This resistance is defined by the shape of the area between two neighboured electrode systems and the specific conductivity of the material filling this area. The energy due to losses in this resistance is transferred to the mash.

Conclusion

Electroporation in industrial scale enables an efficient and gentle way to open cells for an extraction process. While an electroporation device for apple mash is already in operation at a producer for apple juice, the electroporation devices for mash from wine grapes and sugar beets require still some research and development for an optimized and reliable operation in an industrial environment.

References

1. Blankenhorn D (2009) Thermische Verfahren zur Rotweinbereitung, LVWO Weinsberg. Online available at http://www.landwirtschaft-mlr.baden-wuerttemberg.de, accessed Aug 2009
2. Bliesener KM, Miehe D, Buchholz K (1991) Assem Comm Int Techn Sucr 19:393–406
3. Bouzrara H, Vorobiev E (2000) Beet juice extraction by pressing and pulsed electric fields. Int Sugar J 102(1216):194–200
4. Broughton NW, Sargent D, Okojie NF (1992) British Sugar Technical Conference, Eastbourne
5. Flickinger B, Berghöfer T, Hohenberger P, Eing C, Frey W (2010) Transmembrane potential measurements on plant cells using the voltage-sensitive dye ANNINE-6, protoplasma. Springer, NewYork
6. Frenzel S, Michelberger T, Bluhm H, Sack M, Kern M (2005) Entwicklung und Bau einer Elektroimpuls-Pilotanlage. Final Report, FKZ 0330434, TIB Hannover
7. Müller G et al (2007) Karlsruher Elektroporationsanlage KEA—Die Erfolgsgeschichte eines Technologietransfers in die Industrie. Nachrichten des Forschungszentrums Karlsruhe, Forschungszentrum Karlsruhe 39:153–158
8. Ponant J, Foissac S, Esnault A (1988) Zuckerindustrie 113:665–676
9. Puértolas E, Saldana G, Condón S, Álvarez I, Raso J (2009) Evolution of polyphenolic compounds in red wine from Cabernet Sauvignon grapes processed by pulsed electric fields during aging in bottle. Food Chem. doi:10.1016/j.foodchem.2009.08.018
10. Sack M, Bluhm H (2005) Long-term test of a triggered Marx-generator in repetitive operation. In: Proceedings of PPC, pp 1113–1116

11. Sack M, Bluhm H, Stängle R, Müller G (2007) Technische Elektroporation von Pflanzenzellen bei großen Massenströmen. Oral talk, DPG-Tagung, Düsseldorf, Germany, 17 March 2007
12. Sack M, Eing C, Stängle R, Wolf A, Müller G, Sigler J, Stukenbrock L (2008) On the electroporation of mash for the production of red wine. ICOPS 2008, Karlsruhe, Germany, 15–19 June 2008
13. Sack M, Eing C, Stängle R, Wolf A, Müller G, Sigler J, Stukenbrock L (2009) Electric measurement of the electroporation efficiency of mash from wine grapes. Trans DEI 16(5): 1329–1337
14. Sack M, Eing C, Berghöfer T, Buth L, Stängle R, Frey W, Bluhm H (2008) Electroporation-assisted dewatering as an alternative method for drying plants. IEEE Trans Plasma-Sci 36(5):2577–2585
15. Sack M, Attmann F, Stängle R, Wolf A, Frey W, Müller G (2009) Upgrade of the electroporation device KEA-MOBIL. Acta Phys Polonica A 115(6):953–955
16. Sack M, Müller G (2008) Optimisation of an electroporation device for mash. In: Proceedings of OPTIM2008, Brasov, Romania, 22–24 May 2008, pp 113–118
17. Sack M, Schultheiss C, Bluhm H (2005) Triggered Marx generators for the industrial-scale electroporation of sugar beets. IEEE Trans Ind Appl 41(3):707–714
18. Sack M, Sigler J, Eing C, Stukenbrock L, Stängle R, Wolf A, Müller G (2009) Operation of an electroporation device for mash. In: Proc. PPC2009, Washington, DC, pp 1104–1107
19. Schultheiss C, Sack M, Bluhm H, Mayer HG, Kern M, Lutz W (2003) Operation of 20 Hz Marx generators on a common electrolytic load in an electroporation chamber. In: Proceedings of PPC03, Dallas, pp 669–672
20. Turk M, Vorobiev E, Baron A (2009) Pulsed electric field assisted pressing of apple mash on a continuous pilot-scale plant: extraction yield and qualitative characteristics of cider juice. In: Proceedings of BFE 2009, Compiègne, France, pp 114–119
21. Vorobiev E, Lebovka NI (2008) Electrotechnologies for extraction from food plants and biomaterials. Springer, NewYork ISBN 978-0-387-79373-3