

# Operation of an Electroporation Device for Grape Mash

M. Sack, J. Sigler, C. Eing, L. Stukenbrock, R. Stängle, A. Wolf, and G. Müller

**Abstract**— In the course of the production of red wine the electroporation of mash enables a fast extraction of the red pigments from the skin without remarkable heating of the mash. For white wine the formation of pores in the cells fosters among others the extraction of flavoring substances. During the harvest in 2008 the electroporation device KEA-WEIN has been operated successfully in two wineries. All together more than 5 m<sup>3</sup> of mash has been treated. During this year's experiments some experience in the operation of the electroporation device on-site in a winery has been gained. To facilitate the evaluation of the experimental results, the electroporation device has been equipped with different sensors and measurement systems, e.g. for the power drawn from the grid, and the temperature of the mash before and after the electroporation. The data logged during one exemplary run are presented in the paper. Based on the measurements an estimation about the energy consumption and efficiency of the device has been made. Must and wine made from the grape varieties Pinot Noir and Riesling has been chemically analyzed. Selected data is presented in the paper.

**Index Terms**—Electroporation, pulsed electric fields, PEF, grape mash, wine.

## I. INTRODUCTION

FOR the production of wine the extraction of valuable substances like for example flavoring substances or pigments from red wine grapes is one key issue. A good extraction requires opening the cells of the berries' tissue. Conventionally, the cells of the wine grapes are opened by fermentation, thermovinification, or by adding enzymes. Electroporation enables a fast and gentle cell opening at low temperature for the subsequent extraction [1].

Electroporation means the formation of pores in the cell membrane due to the application of an electric field to the membrane. Fig. 1 shows a model of a spherical cell between a pair of electrodes in an electrically conducting medium. After field application the membrane is charged resulting in a field enhancement across the membrane.

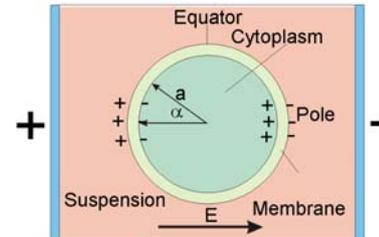


Fig. 1. Space charge configuration across the membrane of a ball-like biological cell.

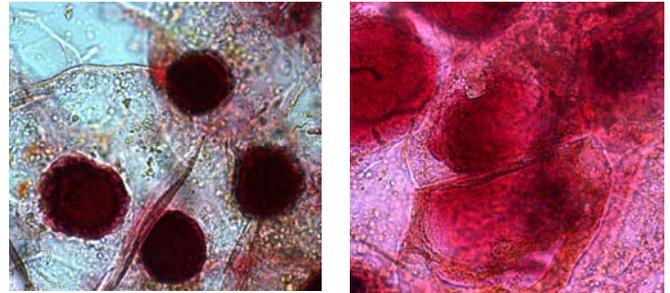


Fig. 2. Microscopic photos of peel tissue of Lemberger wine grapes before and after electroporation.



Fig. 3. Mash of red wine grapes (Pinot Noir) before and after electroporation.

The maximum transmembrane-voltage occurs at the poles of the cell. At a voltage in the order of 0.5 V to 1 V pore formation starts. After application of sufficient energy, the pore formation becomes irreversible. These pores foster the extraction. Fig. 2 shows two microscopic photos of peel tissue from Lemberger wine grapes before and after electroporation. Before the pulse application the red pigments are stored in vacuoles inside the cells. The applied electric field opens the membranes of cells and vacuoles. Subsequently, the pigments are extracted. In macroscopic scale the instantaneous red staining of the must shows to the beginning extraction of pigments from the peel tissue due to the effect of electroporation, Fig 3 [2], [3].

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## II. TEST SET-UP

### A. The Electroporation Device KEA-WEIN

The mobile electroporation device KEA-WEIN, Fig. 4, is equipped with an electroporation reactor connected to a Marx generator, Fig. 5. It has been designed for a throughput of between 500 and 1000 kg mash per hour. The 6-stage Marx generator has a stage capacitance of 140 nF, maximum charging voltage of 50 kV per stage and can be operated up to a repetition rate of 20 Hz. The stored energy per pulse is 1 kJ. The spark gaps inside the spark gap tube are operated in nitrogen gas to prevent the production of nitric oxides. The gas serves as well as cooling medium for the spark gaps. It is circulated by a ventilator in a closed loop through a heat exchanger, which is cooled back alternatively by cooling water or a chiller or a combination of both. For the described experiments only the chiller has been used. The ignition voltage of the spark gaps is adjusted coarsely by varying the spark gap distance. The fine tuning is done by means of a pressure regulation inside the spark gap tube. The pressure is kept constant by a feedback control loop. The Marx generator is grounded at its center spark gap to enable a ground symmetric operation, Fig. 6 [4]. So the insulating distance to ground potential is reduced, and the center of the electroporation reactor is virtually near ground potential preventing a considerable current flow out of the electroporation area. The electroporation reactor is equipped with a plate electrode system and has a quasi homogeneous field distribution, Fig. 7 [5]. It has been designed for a maximum field strength of 60 kV/cm in the mash. The treatment area has a square cross-section of 12.5 cm<sup>2</sup>. Allowing a decrease of the electric field by 10 % compared to the maximum field strength along the center trajectory, the length of the treatment area is 4.7 cm resulting in a volume of 57.6 cm<sup>3</sup>.

The mash is intermingled with small air bubbles, which can cause a flash-over inside the reactor, if the applied electric field is above the breakdown strength. Therefore, an automated degassing valve has been mounted at the inlet of the electroporation reactor. But it cannot remove the air completely from the mash. To prevent a flash-over inside the reactor the mash is pressurized to 0.2-0.3 MPa<sub>abs</sub> (2 - 3 bar<sub>abs</sub>), i.e. 0.1-0.2 MPa above ambient pressure. The pressure causes the gas bubbles to shrink and increases the breakdown strength of the gas. Fig. 8 shows the hydraulic scheme. The pressure is applied by a pump. To prevent the mash from blocking, for pressure regulation instead of a throttling valve a second pump is used. Both pumps are equipped with speed variable drives enabling a speed control. Based on a pressure measurement at the inlet of the electroporation reactor a feedback control loop keeps the pressure constant. The air chamber decouples both pumps enabling the use of a rather slow control circuitry.

The pulse circuit has been designed for an aperiodically damped pulse. Typically, the conductivity of the mash is in the order of 3 mS/cm. The electroporation reactor has been designed to have a resistance of 35 Ohm at that conductivity.



Fig. 4. Mobile electroporation device KEA-WEIN on-site in a winery.

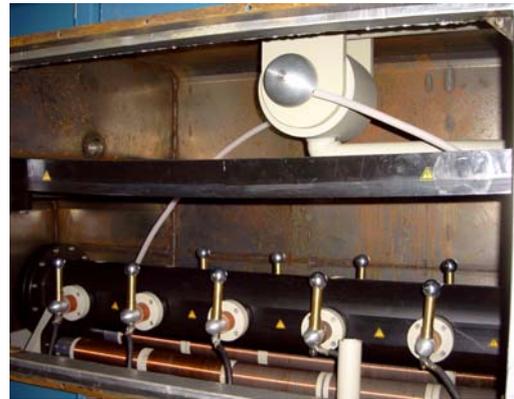


Fig. 5. Electroporation reactor and Marx generator.

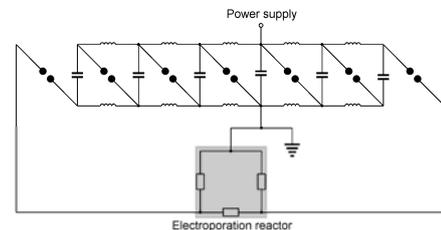


Fig. 6. Ground-symmetric operation of the Marx generator (fixed ground connection).

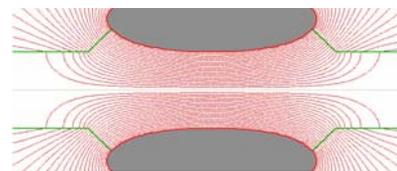


Fig. 7. Equipotential lines inside the EP-reactor.

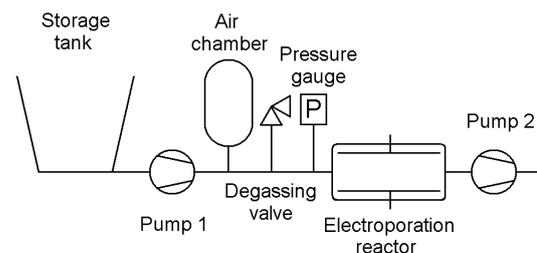


Fig. 8. Hydraulic scheme of the mash flow.

### B. Measurements

The electroporation device KEA-WEIN has been equipped with measurement devices for the nitrogen gas pressure and temperature, and the temperatures of the cooling water at the inlet and the outlet of the heat exchanger. The ignition of the 1<sup>st</sup> spark gap is sensed by means of a photo diode connected to the spark gap by a fiber-optic cable. To measure the ignition voltage the charging voltage of the capacitors is sampled at the moment of ignition. The repetition rate is calculated based on the ignition signal. The temperature of the mash is measured at the inlet and the outlet of the electroporation reactor. As described above, the measurement of the mash pressure is used for pressure regulation. Fig. 9 shows the measured signals of a typical run. The control signals for the speeds of the pumps are added to the pressure signal of the mash to demonstrate the operation of the pressure regulation. A power meter measures the total power drawn from the electric grid as active power  $P$ , reactive power  $Q$ , and apparent power  $S$ . This measurement includes the power required for the Marx generator, for the ventilation and cooling, for the pumps, and the measurement-, data acquisition- and control circuitry. The pulse current through the electroporation reactor is measured by means of a Rogowski coil connected to an oscilloscope. As due to limited space a high-voltage divider could not be integrated next to the electroporation reactor, the electric field inside the electroporation area has been calculated based on the current measurement, the circuit parameters and the geometry of the electroporation reactor, [5].

The specific energy applied to the mash can be calculated based on the increase of temperature ( $T_{out}-T_{in}$ ) and the specific heat capacity  $c$  according to equation 1.

$$W_{spec} = c \cdot (T_{out} - T_{in}) \quad (1)$$

The specific heat capacity of mash has been determined calorimetrically by measuring the energy required for warming up 1 kg of mash by 5 K. A value of  $c = 3.42$  kJ/kgK has been found. Due to the content of grape tissue it is less than the specific heat constant of water ( $c_{water} = 4.18$  kJ/kgK).

For the physical process of pore formation only a negligible amount of energy is required. But the electric field has to be established inside a conductive medium resulting in ohmic losses. Although these losses can be reduced by limiting the pulse length to the time required for the pore formation only, a thermal heating of the medium cannot be omitted. Apart from the pulse shape and other process parameters like conductivity and temperature the efficiency of the device is influenced by the losses of the apparatuses of the device, to which we refer by the term of technical efficiency. Variations of pulse shape and process parameters are beyond the scope of this paper.

The technical energy efficiency of the electroporation device can be estimated by comparing the active power drawn from the grid and the heat power applied to the mash. But for an improvement of the device it is interesting to get a more detailed insight into the energy consumption of different apparatuses of the device: the high-voltage power supply, the

pulse generation by the Marx generator including ohmic losses, the pumps for the mash, and the energy required for cooling the Marx generator and the power supply. The amount of energy required for the control circuitry is only small and, therefore, has not been measured separately. The energy required for the pumps has been acquired by the power converters of the drives. The power required for cooling has been calculated as difference of the power drawn from the grid with the high-voltage supply being switched on and switched off. To estimate the losses of the power supply for the high-voltage generation as well as the losses for the pulse generation, the power of the Marx generator has been calculated according equation (2).

$$P_{Marx} = 0.5 \cdot n \cdot C_{stage} \cdot V_{stage}^2 \cdot f_{rep} \quad (2)$$

$n$  is the number of stages,  $C_{stage}$  the capacitance per stage,  $V_{stage}$  the charging voltage per stage, and  $f_{rep}$  the repetition rate. The residual voltage of the Marx bank after the pulse is with less than 500 V negligible. The losses for the high-voltage generation can be calculated as difference of the input power of the power supply and the power of the Marx generator, the losses for the pulse generation as difference of the power of the Marx generator and the power for heating the mash. The thermal power  $P_{th}$  is calculated based on the measured throughput  $dm/dt$  according to equation (3).

$$P_{th} = c \cdot \frac{dm}{dt} \cdot (T_{out} - T_{in}) \quad (3)$$

Actually, the throughput has been measured volumetrically, and divided by the specific density of the mash.

All measured or calculated values for the power have been averaged over two minutes of stable operation.

The experiments have been performed in four steps. First, the berries have been removed from the stalks to prepare the mash. This has been done by means of a machine mounted upon the tank of the first pump. Then, the mash has been pumped through the electroporation device with the high-voltage pulses being switched off to get a sample for reference. Next, the high-voltage has been switched on for electroporation, and, finally, the remaining mash has been removed from the device. To prevent the mash from demixing, it had to be stirred continuously while it is in the tank of the first pump. During the last three steps, the measurements shown on Fig. 9 have been performed.

The chemical analysis of must and wine has been performed according to regulations of the European Union [6], the color intensity especially as the sum of the absorbance at wavelengths of 420 nm, 520 nm, and 620 nm for radiation traversing a 1 cm optical path in the sample. For the measurements the must from the sampled mash has been passed through a sieve and centrifuged for purging. To check the validity of the analysis three separate samples have been analyzed. The average deviation from the mean value has been determined to be +/-5% with a maximum deviation of +13%/-9%.

III. EXPERIMENTAL RESULTS

A. Operation of the Electroporation Device

The graphs in Fig. 9 show the acquired operating parameters during one run.

The mash has been pumped through the device at a pressure of 0.3 MPa<sub>abs</sub> (3 bar<sub>abs</sub>). The speed of the second pump is kept constant for a constant throughput, while the speed of the first pump has been varied to keep the pressure constant. When the tank for the mash became empty, the speed of the second pump has been reduced automatically to keep the pressure still constant. During electroporation the device has been operated at a charging voltage of 30 kV per stage with a repetition rate of 19.5 Hz. The pressure of the nitrogen has been adjusted to 5 kPa (0.05 bar) above ambient pressure. Its temperature was kept constant at approximately 20 °C. The current pulse (Fig. 10) has a pulse length of  $t_h = 1.1 \mu s$  and a crest value of  $\hat{I} = 4 \text{ kA}$ . It is damped aperiodically. The resulting maximum electric field strength inside the electroporation area has been calculated to  $\hat{E} = 36 \text{ kV/cm}$ . Due to the applied energy the mash was warmed up by 10 K starting from an initial temperature of 19.5 °C. So during electroporation a specific energy of 34.2 kJ/kg has been applied to the mash. With the high-voltage supply switched on the device took an active power of 15.5 kW from the electric grid. While pumping without electroporation but with the chiller and the ventilation for cooling being switched on the active power was 4.7 kW.

Fig. 11 shows the losses of the device as percentage of each part. The data have been taken from a run at  $V_{\text{stage}} = 34.6 \text{ kV}$  and  $f_{\text{rep}} = 20 \text{ Hz}$ . At a throughput of 900 l/h the mash has been warmed up by 11 K resulting in a specific energy of 37.6 kJ/kg applied to the mash. Throughput and repetition rate result in the application of approximately 5 pulses per volume at a flow rate of 0.2 m/s. With an active power of 17.1 kW drawn from the grid, the technical efficiency of the device has been calculated to 55 %. With the mentioned operating parameters 25 % of the total power is required for cooling. The high-voltage generation causes 13 % of the losses, and the pulse generation 4 %. Both pumps together required 3 % of the total power only. As the speed of the ventilation and the temperature of the chiller are kept constant independent of the operating data of the Marx generator the efficiency increases with rising power applied to the mash.

In a conventional thermovinification process the mash is heated up to 80 °C, kept at that temperature for 2 minutes, and then cooled back to less than 40 °C. In small and medium size devices the heat is not recuperated. So the specific energy for thermovinification can be estimated to 205 kJ/kg for a temperature increase by 60 K. The energy applied to the mash during electroporation is less than 1/5<sup>th</sup> of that amount. Taking into account the efficiency of the electroporation device the electric power required for the operation of the electroporation device is approximately one third of the thermal power required for a conventional thermovinification process.

With the described parameter adjustment the electro-

poration device has been operated with nearly no flash-over inside the electroporation reactor. More than 5 m<sup>3</sup> of mash from different sorts of grapes (Pinot Noir, Chardonnay, Alicante, Riesling, and Muskateller) have been treated.

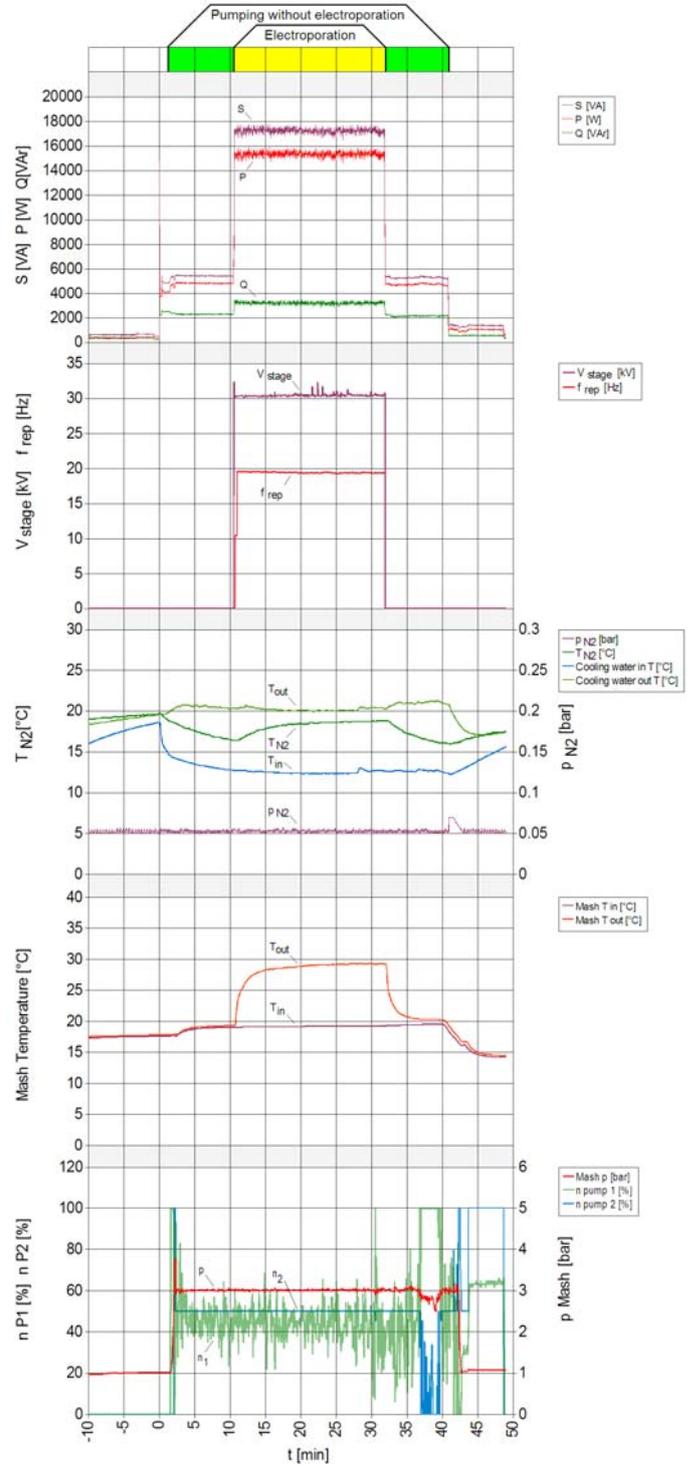


Fig. 9. Measurement of operating parameters: active power P, reactive power Q, and apparent power S drawn from the electric grid, ignition voltage per stage  $V_{\text{stage}}$ , and repetition rate  $f_{\text{rep}}$ , temperature  $T_{N_2}$  and pressure  $p_{N_2}$  of the nitrogen gas and cooling water (cooling water  $T_{\text{in}}$ ,  $T_{\text{out}}$ ), mash temperature before and after the electroporation reactor (mash  $T_{\text{in}}$ ,  $T_{\text{out}}$ ), pressure of the mash p and control signal for the pumps ( $n_1$ ,  $n_2$ ).

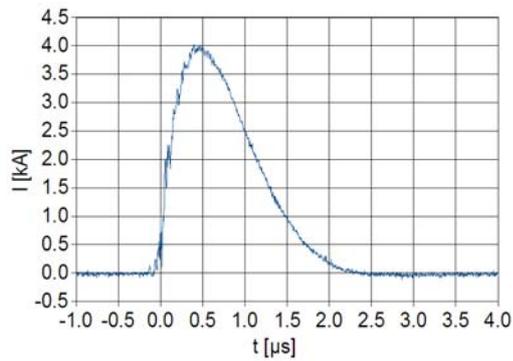


Fig. 10. Pulse shape: Current through the electroporation reactor.

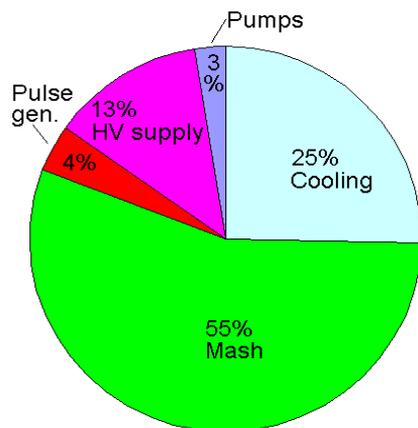


Fig. 11. Distribution of losses during operation at  $V_{\text{stage}} = 34.6 \text{ kV}$  and  $f_{\text{rep}} = 20 \text{ Hz}$ .

### B. Chemical Analysis of Must and Wine

The must and the produced wine have been chemically analyzed. Tab. 1 shows selected data for must and wine from Pinot Noir and Riesling grapes. For the red wine a thermovinification process served as reference, because it involves an aquatic extraction as it has been applied as well to the mash after electroporation. The extraction time after opening the cells for both processes was 12 hours. The data of both variants made from Pinot Noir grapes are comparable. Nearly the same amount of pigments has been extracted from the peel tissue in both processes, as the data for the color intensity indicate. So electroporation might be an energy efficient alternative to thermovinification.

For the white wine electroporation enabled the extraction of more valuable substances from the cells compared to only pumping the mash through the device. So a higher content of yeast digestible nitrogen in the must after electroporation has been observed. But the amount of total acid in the electroporated must is slightly reduced due to a buffer action. In average over all experiments on mash from white grape varieties in 2008 the total acid of the electroporated samples is reduced by 3 % compared to samples taken from mash which has been only pumped through the device. The content of

yeast digestible nitrogen could be increased by 10 %. A higher content of yeast digestible nitrogen helps against the "atypical ageing note" in the wine. As the cells of the berries are opened partly as well by pressure and sheer forces inside the pumps, the described differences are due to the field application only.

TABLE I

CHEMICAL ANALYSIS OF MUST AND WINE.

	Method of treatment	Specific electroporation energy [kJ/kg]	Must				Wine		
			Must sweetness [°Oe]	Total acid [g/l]	Yeast digestible nitrogen [mg/l]	Color intensity	Alcohol [g/l]	Total dry extract [g/l]	Color intensity
Pinot Noir 2008	Thermovinification	..	86.4	11.3	398	11.9	95.6	23.2	1.5
	Electroporation	34.2	84.8	10.4	384	12.9	98.1	23.7	1.5
Riesling 2008	Pumped only	0.0	89.9	7.5	300	1.1	96.3	23.5	0.1
	Electroporation	34.2	89.9	7.3	339	1.3	95.5	25.0	0.2

## IV. CONCLUSION

During the harvest in 2008 the electroporation device KEA-WEIN has been operated successfully on-site in two wineries. During the tests typical operating data have been collected. A comparison of the chemical analysis of must and wine produced from electroporated mash to the characteristics of conventionally treated mash and wine showed for red wine a comparable result. For white wine an improvement in the extraction of yeast digestible nitrogen and other valuable substances has been achieved.

## V. ACKNOWLEDGMENT

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