ELSEVIER

Trends in Food Science & Technology 12 (2001) 129–135

TRENDS IN FOOD SCIENCE & TECHNOLOGY

Review

Processing concepts based on high intensity electric field pulses†

Dietrich Knorr,* Alexander Angersbach, Mohamed N. Eshtiaghi, Volker Heinz and Dong-Un Lee

Department of Food Biotechnology and Food Process Engineering, Berlin University of Technology, Königin-Luise Str. 22, D-14195 Berlin, Germany (tel. +49-30-314-71250)

Emerging non-thermal food processing techniques are receiving considerable attention. This is because of their potential for quality and safety improvement of our food supply, for the ability to enhance conventional processing operations or to create alternative ones, as well as modify existing or non-conventional raw materials, food constituents or processed foods. High intensity electric field pulse technology is one of the most advanced emerging non-thermal processing methods and is currently undergoing intensive scientific and developmental evaluation. The objectives of this review are to summarize and identify the key advantages of this emerging technology and to convert it into optimum use. Those attempts are a combination of research on a kinetic basis and on a cellular level and the subsequent transfer of the knowledge gained into pilot scale.

* Corresponding author.

†This manuscript is based on the 2000 Division Lecture of the IFT Non-thermal Division, Annual IFT Meeting, Dallas, TX, USA.

Introduction

During the last decade an unprecedented quantity of research and development activities has been carried out regarding non-thermal processing of foods (Barbosa-Canovas, Pothakamury, Palov, & Swanson, 1997; Barsotti, Merle, & Gheftel, 1999; FDA, 2000; Hayashi & Balny, 1996; Hendrickx, van Loey, Ludikhuyze, Heinz, & Knorr, 2000; Isaacs, 1998; Mermelstein, 2000). Most of the work concentrated on high hydrostatic pressure (approx. 650 publications) and on high intensity electric field pulses (approx. 150 publications), with microbial inactivation, quality improvement and shelf life extension being the initial prime goals of those efforts (Palou, Lopez-Malo, Barbosa-Canovas, & Swanson, 1999; Vega-Mercado *et al.*, 1997). Gradually activities related to product modification (Balny & Masson 1993; Ohshima, Ushio, & Koizumo, 1993) and process development (Eshtiaghi & Knorr, 1993; Zhang, Barbosa-Canovas, & Swanson, 1995) also emerged and have been developed further. This paper intends to summarize the potential of high electric field pulse application for food process modification, development and the impact of the modification at the cellular level such as membrane permeabilization on product enhancement.

Membrane permeabilization by electric field pulses

Basic considerations

Membrane permeabilization

It is now generally accepted that the application of high intensity electric field pulses (HELP) with nanoseconds to microseconds duration leads to the permeabilization of biological membranes. Based on this phenomenon, many practical applications of high electric fields for reversible or irreversible permeabilization of various biological systems have been studied in the fields of medicine and bioscience (Chang, Chassy, Saunders, & Sowers, 1992; Ho & Mittal, 1996; Knorr & Angersbach, 1998; Lynch & Davey, 1996; Zimmermann & Neil, 1996). However, very little information is available regarding critical external electric field strength, breakdown voltage of the cell membrane, time sequence and the dynamics of the membrane permeabilization process, as well as on reversible–irreversible structural changes of cells in real food systems during and after

0924-2244/01/$ - see front matter
PII: S0924-2244(01)00069-3

HELP treatments. Fundamental understanding of these phenomena is essential for optimum process design and for characterization of critical process parameters of HELP in the food and biotechnology industry.

Basic consideration of HELP induced permeabilization

The presence of intact membranes with very low dielectric thickness and high resistance produces in a cellular probe (with conductive inner and outer phases) characteristic electrophysical behaviors. The application of an external electric field induces the charging process at the membrane interfaces (Fig. 1). The charging time constant, τ, is a cell specific parameter which depends on cellular size, membrane capacitance, the conductivity of the cell and the extracellular electrolyte. When an electric field with supercritical field strength is applied, a critical electrical potential can be induced across the cell membrane, which leads to rapid electrical breakdown and local structural changes of the cell membrane. At various field intensities for plant and animal the transmembrane potential reaches critical values of approx. 0.7–2.2 V within less than 1 μs after initiation of a pulse (Angersbach, Heinz, & Knorr, 2000).

Determination of extent of membrane permeabilization

For investigations of the HELP process the fact that electric field-induced permeability variation of initially insulating biological membranes due to pore formation is reflected in large conductivity changes on cell membrane used. An electrophysical model of cell systems affected by the HELP process was developed which makes it possible to determine the extent of total or local disintegration of cell membranes (Angersbach *et al.*, 1999). Determination of the extent of the cell membrane permeabilization in a processed cell system is carried out by measuring the conductivity of the initially intact and of treated samples at characteristic low and high frequencies within the range of β-dispersion (for most plant and animal cells in suspension culture or tissue the characteristic low frequency range is in order of 10^3 Hz and high frequency range in order of 10^7 Hz) (Angersbach *et al.*, 1999). For determining the electric field effects directly on the cell membrane during the application of DC pulse (such as polarization effect at the intact membrane interfaces and field induced electropermeability changes) current and voltage measurements with high time resolution were used (Angersbach *et al.*, 2000).

For the post-pulse time (e.g. after HELP treatment) it is assumed that structural changes of biological membranes are responsible for the dependence of the complex conductivity of the cell system on the alternating current (AC) frequency.

Based on the results obtained in the authors laboratory, this method also proves to be an useful tool for the determination of the status of cellular materials and for the optimization of various processes regarding minimizing cell damage in various food processing operations; the monitoring of propagation of mass transfer during processing (i.e. dehydration); or for the evaluation of modified biosynthesis of metabolites in biological systems.

Reversible permeabilization

Based on our data the initiation of conductive channels across the membrane occurred within nanoseconds during the charging process of the membrane whereas the formation of a high conductance membrane due to pore expansion takes place within a few microseconds. The build up of the membrane potential and pore formation in cells of potato tissue within the first microsecond after the initiations of pulsing with supercritical

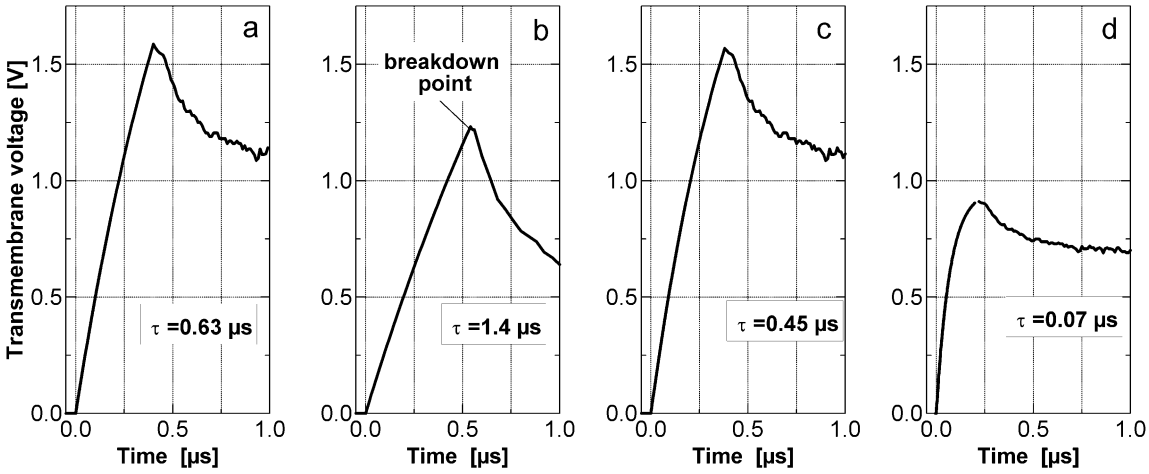

Fig. 1. Characteristic development of transmembrane voltage on the real biological cells obtained by application of one pulse with supercritical external field strength ($E = 1.0$ kV/cm for fish tissue (a), apple tissue (b) and plant cell suspension (c); $E = 1.7$ kV/cm for yeast suspension (d)). The breakdown point represent the critical transmembrane voltage. τ is the charging time constant of the cell membrane (after Angersbach *et al.*, unpublished data).

field strength is demonstrated in Fig. 2. Initially the transmembrane potential, π, increases exponentially with the time constant $\tau = 0.7$ μs, which is the charging time constant for intact membranes in potato cells. At approx. 0.4 μs, π reached 1.7 V and later decreased to a residual value of 0.1 V, consequent to pore formation and a drastic increase in membrane conductance.

It is important to note that the application of a single pulse even with supercritical field amplitude does not necessarily cause irreversible membrane rupture. The insulating properties of the cell membrane can be completely recovered within several seconds after termination of the pulse (Angersbach *et al.*, 2000).

The reversible–irreversible electrical permeabilization phenomena of cell membranes can be observed during an extended time period. Figure 3 provides a detailed insight into the build up and decrease of the membrane potential, the pore formation and the time dependent resealing after the application of a single pulse.

This is essential for process development as optimum repetitive pulse sequences for irreversible permeabilization can be developed based on these data. Furthermore, the increase of the sample conductivity within a few hours after HELP treatment ultimately resulting in irreversible permeabilization, offers the potential for unique and lower energy process and product development concepts (e.g. an increase in stress-induced metabolite production and concurrently improved extractability).

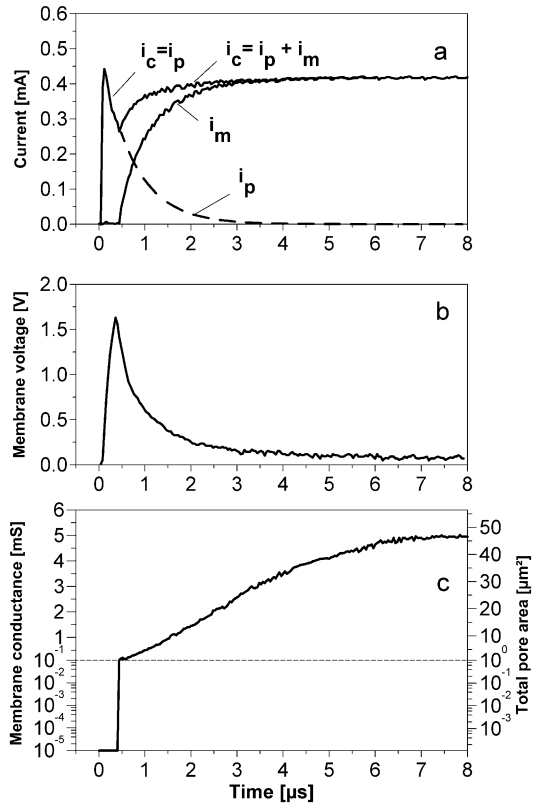

Fig. 2. Typical current, voltage and membrane property changes in an individual cell (e.g. a potato cell in tissue) induced from an external electrical field with supercritical field strength of 0.88 kV/cm: (a) currents and (b) transmembrane potential in average cell. The membrane breakdown voltage is 1.7 V. (c) membrane conductance and total pore area increase in the membrane surface in the field direction. i_c, intracellular current; i_p, polarization current; i_m, membrane current (after Angersbach *et al.*, 2000).

Irreversible permeabilization

The irreversibility of membrane permeabilization depends on the intensity of external electric fields and the number of pulses applied. Data in Fig. 4 show the characteristic evaluation of cell membrane permeabilization by repeated pulse application. Monitoring of the current during the first 8 μs after starting of repeated supercritical pulse applications resulted in reversibility after the first pulses and irreversible cell membrane rupture after several pulses. At repeated pulses with intervals of 1 s between them, the oscillographic tracking of the charging current of the cell membranes for the first and the second pulse were reproduced until the repeated breaking point of the membrane, i.e. approx. 0.5 μs, with high accuracy. This means that structural properties of the membrane after the first pulse were identical to those of an intact membrane. This finding is essential for the appropriate use of pulsed electric fields.

Only if the membrane structure is restored the induction of critical membrane voltage and membrane breakdown occurs again. Also, for the third pulse, the current course showed only a slight deviation from the exponential decaying process. This means that the

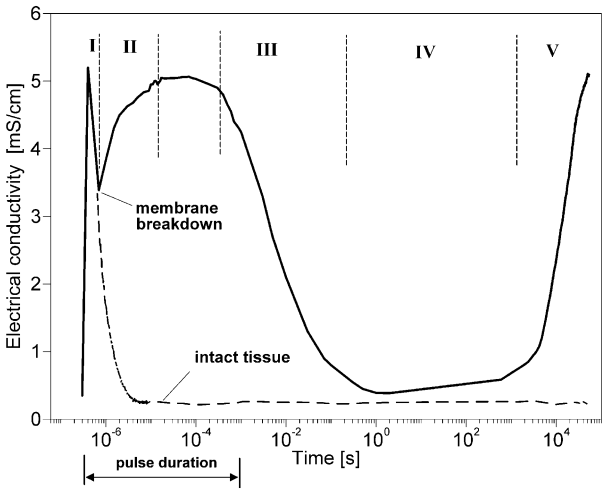

Fig. 3. Permeabilization characteristics in cells of potato tissue during and after pulse application. Peak field strength and duration of the exponential pulse was 0.88 kV/cm and 1 ms, respectively. The conductivity changes of intact tissue (broken line) is obtained by application of pulse at subcritical field strength of 0.1 kV/cm. I, membrane charging process; II, development of conductance membrane due to pore formation; III, resealing of the pore; IV, irreversible pore formation; V, stress induced indigenous (possibly enzymatic) membrane permeabilization.

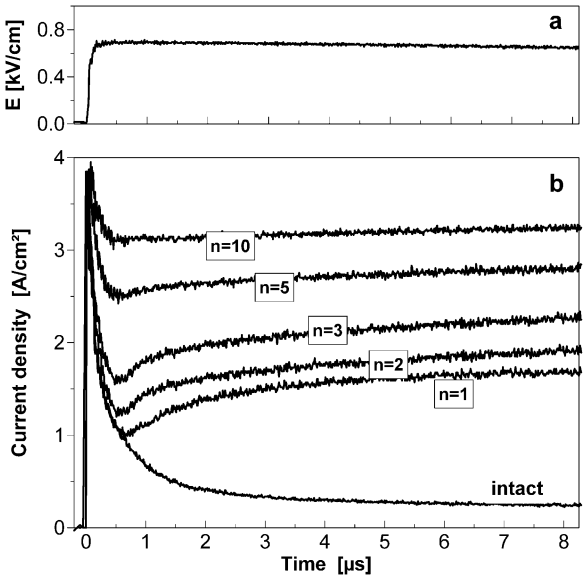

Fig. 4. Kinetics of permeabilization of potato cells in tissue during the application of 10 pulses with constant amplitude of field strength $E_o = 0.8$ kV/cm. (a) time dependency of field strength; (b) current density in the tissue in the first 8 μs after pulse start. Pulse frequency: 1 s^{-1}; *n*, pulse number. Current density trace for example without membrane permeabilization (intact) is reconstructed from relation obtained by application in the same sample of a single pulse with subcritical field strength level, $E_{sub} = 80$ V/cm (Angersbach *et al.*, 2000).

membrane structure was restored to a greater extend within 1 s after the second pulse. The membrane current was observed for pulse number 5 in addition to the polarization current at the start of the pulse. At 0.5 μs, a slight increase in the current course (caused by increase in the membrane conductance) was also observed. The current course of the pulse indicated that an irreversible membrane permeabilization occurred.

Applications

Stress reactions

Pressure-induced stress reactions of plant systems especially regarding the biosynthesis of desirable food constituents such as phenols have been described previously (Dörnenburg & Knorr, 1999). For the study of sublethal stress of electric field pulses on microbial, animal, and plant cells an airlift reactor equipped with an coaxial electrode configuration has been developed (Fig. 5).

Mass transfer improvements

The impact of irreversible membrane permeabilization by electric field pulses on mass transfer improvements is exemplified by providing data regarding pretreatment prior to osmotic dehydration or expression of plant materials. Further, an example is provided how conventional processing of foods can be supported or changed by the application of high electric field pulses. Pretreatment of plant tissues with high pressure prior to osmotic dehydration led to significant changes in the plant tissue architecture and increased mass transfer rates during osmotic dehydration due to the combined effect of high pressure induced membrane permeabilization and subsequent osmotic stress (Rastogi, Angersbach, & Knorr, 2000). When applying electric field pulses as pre-treatment an increase in mass transfer coefficients as compared to untreated controls could be observed (Table 1).

Based on the possibility of HELP treatment to allow stress induction as well as reversible or irreversible permeabilization with defined degrees of permeabilization, biological systems can either be stimulated to act as 'bioreactors' (i.e. induction of stress responses or infusion of constituents to affect endogenous biosynthetic

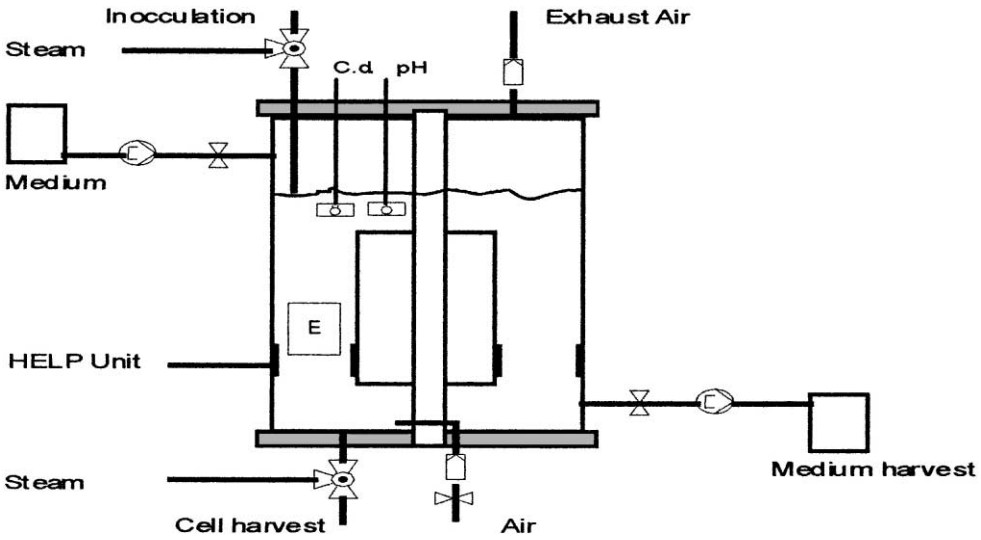

Fig. 5. Air-lift reactor with coaxial stainless steel electrodes (E) for the study of stress responses of biological systems to electric field pulses (after Mönch & Heinz, unpublished data).

Table 1. Processing criteria of untreated, pre-treated and dehydrated paprika tissues (after Ade-Omowaye *et al.*, 2000a)

Pre-treatments	Critical moisture content (kg/kg ds)	Constant drying rate $R_{dc} \times 10^4$ (kg/m^2 s)	Heat transfer coefficient, h (W/m^2 K)	Mass transfer coefficient, k (kg/m^2 s)	Cell disintegration index, Z_p*
Control	6.10±0.20	9.68±0.15	73.13±0.10	0.043±0.005	0.00
Blanched (99°C, 3 min)	5.75±0.10[b]	13.20±0.21[b]	99.72±0.96[b]	0.059±0.007[b]	0.88±0.04
5%NaOH (25°C, 20 min)	5.66±0.11[b]	11.03±0.31[b]	83.29±0.61[b]	0.049±0.008[b]	0.00
5% NaOH (35°C, 20 min)	4.70±0.08[b]	12.25±0.13[b]	92.54±0.72[b]	0.054±0.003[b]	0.00
5% HCl (25°C, 20 min)	6.09±0.10[a]	10.04±0.31[b]	75.81±0.55[b]	0.044±0.005[a]	0.00
5% HCl (35°C, 20 min)	5.21±0.16[b]	10.50±0.45[b]	79.32±0.85[b]	0.047±0.002[b]	0.00
High pressure (400 MPa, 10 min)	5.18±0.13[b]	11.07±0.54[b]	83.61±0.78[b]	0.049±0.003[b]	0.58±0.02
HELP (2.4 kV/cm, 300 μs, 10 pulses)	5.16±0.05[b]	13.02±0.35[b]	98.36±0.93[b]	0.058±0.001[b]	0.61±0.03

[a] Insignificant difference to control at $P \leq 0.05$.
[b] Significant difference to control at $P \leq 0.05$.
* 0 = Intact membrane; 1 = fully disintegrated membrane.

reactions) or can be utilized to affect the recovery of constituents. The latter effect is exemplified in Table 2 where the potentials of HELP treatment on the composition of fruit juices is demonstrated.

For example, the fact that mineral concentration could be increased consistently is noteworthy in the context of functional foods (Watzke, 1999). The increase in juice yields due to HELP treatments reaching values similar to products treated with commercial enzymes but being closer related to the composition of freshly pressed ones has been reported previously (Eshtiaghi & Knorr, 2000). The short treatment times required for HELP treatment also allow subsequent continuous processing (i.e. decanter centrifuge). A flow chart of such processes performed at pilot plant level is shown in Fig. 6.

It should also be mentioned that with increasing degree of permeabilization of coconuts as obtained by various pre-treatments including high pressure and HELP the concentration of proteins and fat as well as milk yield after pressing could be increased (Ade-Omowaye, Angersbach, Esthiahgi, & Knorr, 2001). The impact non-thermal membrane permeabilization techniques such as high pressure, electric field pulses or dense gases can have on process modification and development is further demonstrated using beet sugar processing (Eshtiaghi & Knorr, 1999). The experimental design for the different process variables tested is outlined in Fig. 7.

Table 3 summarizes the key results regarding sugar beet juice yields, purity and also importantly total solids of the extracted cossets, an important energy related factor regarding their required subsequent dehydration.

From the data provided in Table 3, it can be concluded that after HELP treatment of (whole or size reduced) sugar beets ambient temperature processing (i.e. extraction or pressing) could become attractive alternatives to conventional sugar processing. For the

Table 2. Effect of HELP pre-treatment on the composition of freshly pressed fruit juices (after Eshtiaghi & Knorr, unpublished data)

	Grapes	Apple	Black-currant
Total solids (°B)	↑	↑	→
Density (g/ml)	↑	→	→
Acidity (mval/l)	↑	↑	↑
PH value	↓	↑	→
Conductivity (mS/cm)	↑	↑	↑
Turbidity	↓	↑	↓
Saccharose		↑	↑
Glucose	↑	→	→
Fructose	↑	→	↓
Minerals (Ca, K)	↑	↑	↑
Pectins	↓	↑	↑
Protein	↑	→	↑
Ascorbic acid			↑
Tartaric acid	↑		

HELP parameter: electrical field strength 2–3 kV/cm; pulse duration 0.7 ms; pulse number 20–40; repetitions frequency 1 Hz.
↑: increase; →: no change; ↓: decrease.

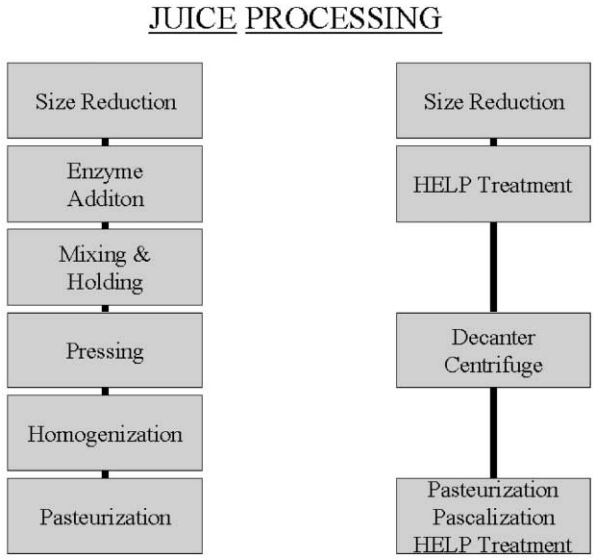

Fig. 6. Simplified flow diagram for conventional and proposed continuous processing of fruit and vegetable juices.

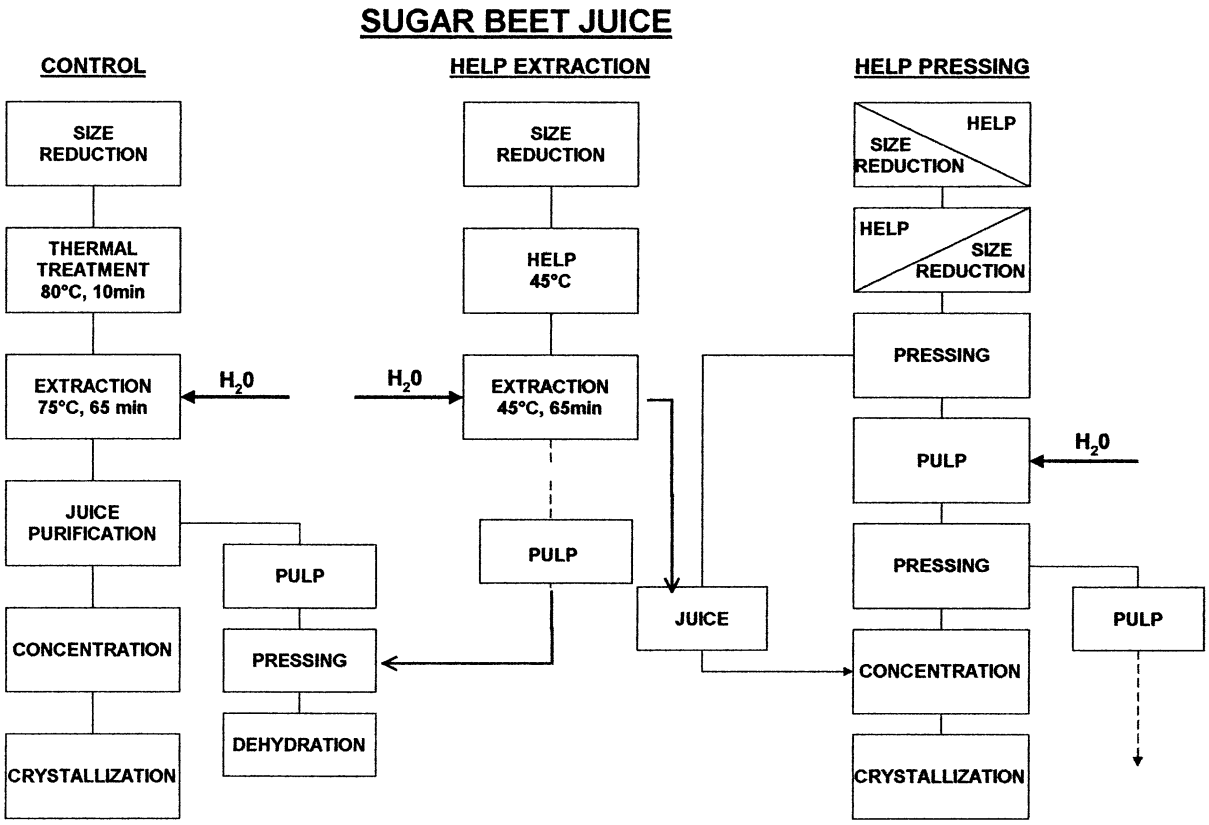

Fig. 7. Simplified flow diagram of the experimental design for comparison of different beet sugar processing options (after Eshtiaghi & Knorr, 2000).

control of microbial growth during such processing it might be necessary to identify temperature optima and to achieve maximum process time reductions.

In summary, based on the data accumulated on HELP so far and based on information accumulating on cell membrane permeabilization, it appears possible to take advantage of the key features of HELP processes such as controlled membrane permeabilization, low energy requirements (in the range of 6–10 kJ kg^{-1} of plant tissue to achieve complete membrane permeabilization) and the short processing times (in the seconds range) to modify existing processes or to develop new, energy efficient, waste free and very targeted process options for the food and drinks industry as well as for cosmetics, pharmaceutical or biotechnology applications.

Table 3. Sugar beet juice yields, purity, sugar recovery rates and total solids of extracted cassettes after treatment with various processing options (see Fig. 7)

	Thermal control 80°C	HELP extraction 45°C	HELP pressing
Juice purity (%)	93	93	93
Pressed pulp			
Dry matter	15.25	17.7	24.91
Total relative yield (%)	96.94	97.22	97.40
Sucrose loss (%)	0.62	0.57	0.56

Future considerations

Some aspects of the implications of membrane permeabilization of plant or microbial systems by electric field pulses have been highlighted. Further data are needed on the consequences of membrane permeabilization (i.e. effects on cell walls and microbial death) and on biosynthetic reactions induced by HELP treatment. There are clear indications that non-linear inactivation kinetics are dominant for non-thermal processes and should be considered for thermal processes (Casolari, 1981; Heinz & Knorr, 1998, 2000; Peleg, 1995; Peleg & Cole, 1999; Smelt & Wouters, 1988) which also requires more systematic studies with a wide variety of microorganisms. Interestingly the data by Stumbo (1948) which were the basis for the establishment of log linear inactivation kinetics of thermal processes can also be evaluated as non-linear. Further Casolari's (1981, 1994) single hit theory offers one explanation for the tailing of inactivation curves. Consequently, non-linear inactivation kinetics of thermal and non-thermal processes also need to be investigated in more detail.

There is also a real need for understanding kinetics of recovery of injured microorganisms after treatment, of changes in food quality and nutritional value during post treatment storage and on aspects required by the European novel food legislation (i.e. inactivation of toxins, inactivation/generation of allergens). Other factors not considered by the novel food regulation such as the potential for generation of free radicals needs to be assessed to assure product safety and consumer confidence.

There is a tremendous innovative potential in utilizing the benefits and advantages of emerging technologies to develop new processes and products or to improve existing ones. Modifications such as membrane permeabilization (i.e. structure engineering) and induction of stress response (i.e. metabolite production) are just a few examples of such exiting future applications of HELP.

Acknowledgements

The authors wish to acknowledge that parts of this work have been supported by EC-projects (AIR1.CT92 0296, FAIR CT96-1175, FAIR CT97-3044), the German Research Foundation (DFG Kn260/6-1), the German Ministery for Education and Research (BMBF No.0339598/5) and by the FEI (Forschungskreis der Ernährungsindustrie e.V., Bonn), the AiF and the German Ministry of Economics (Project No. 10611 N).

References

Ade-Omowaye, B. I. O., Angersbach, A., Esthiahgi, M. N., & Knorr, D. (2001). Impact pf high intensity electric field pulses on cell permeabilisation and as pre-processing steps in coconut processing. *Innovative Food Science and Emerging Technology*, *1*, 203–209.

Angersbach, A., Heinz, V., & Knorr, D. (1999). Electrophysiological model of intact and processed plant tissues: cell disintegration criteria. *Biotechn. Prog*, *15*, 753–762.

Angersbach, A., Heinz, V., & Knorr, D. (2000). Effects of pulsed electric fields on cell membranes in real food systems. *Innovative Food Sci. & Emerging Technol*, *1*, 135–149.

Balny, C., & Masson, P. (1993). Effects of high pressure on proteins. *Food Rev. Int*, *9*, 611–628.

Barbosa-Canovas, G. V., Pothakamury, V. R., Palou, E., & Swanson, B. (1997). *Non-thermal preservation of foods*. New York: Marcel Dekker.

Barsotti, L., Merle, P., & Cheftel, J. C. (1999). Food processing by pulsed electric fields: I. Physical aspects. *Food Rev. Int*, *15*, 163–180.

Casolari, A. (1981). A model describing microbial inactivation and growth kinetics. *Journal of Theoretical Biology*, *88*, 1–34.

Chang, D.C., Chassy, B.M., Saunders, J.A., & Sowers, A.E. (Eds.) (1992). *Guide to electroporation and electrofusion*. San Diego: Academic Press

Dörnenburg, H., & Knorr, D. (1999). Monitoring the impact of high pressure processing on the biosynthesis of plant metabolites using plant cell cultures. *Trends in Food Science & Technology*, *9*, 355–361.

Eshtiaghi, M. N., & Knorr, D. (1993). Potato cube response to water blanching and high hydrostatic pressure. *Journal of Food Science*, *58*, 1371–1374.

Eshtiaghi, M. N., & Knorr, D. (1999). Method for treating sugar beet. International patent WO 99/64634.

Eshtiaghi, M. N., & Knorr, D. (2000). Anwendung elektrischer Hochspannungsimpulse zum Zellaufschluss bei der Saftgewinnung am Beispiel von Weintrauben. *Food Eng. Packaging Technol. (LVT)*, *45*, 23–27.

FDA. (2000, June 2). *Kinetics of microbial inactivation for alternative processing technologies*. US Food and Drug Administration, Center for Food Safety and Applied Nutrition.

Hayashi, R., & Balny, C. (1996). *High pressure bioscience and biotechnology*. Amsterdam: Elsevier Science.

Heinz, V., & Knorr, D. (1998). High pressure germination and inactivation kinetics of bacterial spores. In N. S. Isaacs (Ed.), *High pressure food science, bioscience and chemistry* (pp. 435–441). Cambridge: The Royal Society of Chemistry.

Hendrickx, M., van Loey, Ludikhuyze, I. R., Heinz, V., & Knorr, D. (in press). *High pressure treatment of foods*. Gaithersburg, MD: Aspen

Ho, S. Y., & Mittal, G. S. (1996). Electroporation of cell membranes: a review. *Critical Reviews in Biotechnology*, *16*, 349–362.

Isaacs, N. S. (1998). *High pressure food science, bioscience and chemistry*. Cambridge: The Royal Society of Chemistry.

Knorr, D., & Angersbach, A. (1998). Impact of high-intensity electrical field pulses on plant membrane permeabilisation. *Trends in Food Science & Technology*, *9*, 185–191.

Lynch, P. T., & Davey, M. R. (1996). *Electrical manipulation of cells*. New York: Chapman and Hall.

Mermelstein, N. H. (2000). Annual meeting papers address nonthermal processing methods. *Food Technol*, *54*, 184–192.

Ohshima, T., Ushio, H., & Koizumo, C. (1993). High-pressure processing of fish and fish products. *Trends in Food Science & Technology*, *4*, 370–375.

Palou, E., Lopez-Malo, A., Barbosa-Canovas, G. V., & Swanson B. (1999). High-pressure treatment in food preservation. In M.S. Rahman (Ed.), *Handbook of food preservation*. New York: Marcel Decker

Peleg, M. (1995). A model of microbial survival after exposure to pulsed electric fields. *Journal of the Science of Food and Agriculture*, *67*, 93–99.

Peleg, M., & Cole, M. B. (1999). Reinterpretation of microbial survival curves. *Critical Reviews in Food Science*, *38*, 353–380.

Rastogi, N. K., Angersbach, A., & Knorr, D. (2000). Combined effect of high hydrostatic pressure pretreatment and osmotic stress on mass transfer during osmotic dehydration. In *Proceedings of the International Congress Engineering Foods*, Puebla, Mexico.

Stumbo, C. R. (1948). Bacteriological considerations relating to process evaluation. *Food Technology*, *2*, 115–132.

Vega-Mercado, H., Martin-Belloso, O., Qin, B., Chang, F. J., Gongora-Nieto, M. M., Barbosa-Canovas, G. V., & Swanson, B. (1997). Non-thermal food preservation: pulsed electric fields. *Trends in Food Science & Technology*, *8*, 151–157.

Watzke, H. J. (1999). Impact of processing on bioavailability examples of minerals in foods. *Trends in Food Science & Technology*, *9*, 320–327.

Zhang, Q., Barbosa-Canovas, G. V., & Swanson, B. (1995). Engineering aspects of pulsed electric field pasteurization. *J. Food Eng.*, *25*, 261–281.

Zimmermann, U., & Neil, G.A. (Eds.). (1996). *Electromanipulation of cells*. New York: CRC Press.