

Simultaneous Impact of High Pressures and Pulsed Electric field on *Saccharomyces cerevisiae* Model System

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Abstract

Results of simultaneous high pressure (HPP) and pulsed electric field (PEF) preservation treatments on *Saccharomyces cerevisiae* model suspension are presented. They are based on the novel implementation of PEF method, associated with the sequence of sine-wave pulses of the strong electric field. The simultaneous HPP + PEF enabled reaching the pasteurization threshold for pressure $P = 200$ MPa and the intensity of the electric field $E < 10$ kV/cm well below typical values for separate HPP and PEF treatments. Notable is the negligible parasitic heating for the new PEF implementation. The supplementary broad band dielectric spectroscopy (BDS) frequency scan for HPP, PEF and HPP + PEF revealed qualitative differences, particularly notable for the complex conductivity representation.

Keywords: Non-thermal preservation of foods and biomaterials; High pressures; Strong electric field; Broad band dielectric spectroscopy

Introduction

Innovative, non-thermal methods for foods preservation have reached a significant position in the modern food science and technology. They aim at maintaining the quality of fresh products, avoiding notable deterioration of relevant properties during processing and storage [1-5]. This is hardly, if at all, possible for basic preservation methods, such as the thermal pasteurization, addition of chemical agents or radiation. Amongst innovative methods of foods preservation the high pressure preservation/processing (HPP) has reached the most notable market position. In its basic implementation pressures between 400 MPa and 600 MPa, at near room temperatures, are exerted on already packed foods for few minutes time. For HPP the pasteurization threshold, i.e. 5 decades decrease of the total number of microorganisms, is most often linked to the pressure-induced break of cellular walls [1-8]. The technological sterilization target is reached for simultaneous actions of high pressure and temperature. This is known as the high pressure high temperature processing (HPHT). For this technology pressurization is carried out for temperatures $T \sim 120^\circ\text{C}$, below the value typically used for sterilization under atmospheric pressure but well above the thermal pasteurization threshold ($\sim 80^\circ\text{C}$), associated with proteins' denaturation. Consequently, for HPHT method basic advantages of HPP technology are partially lost. Notwithstanding, HPHT technology also indicates possible advantages of the simultaneous, common action of two different physical factors [2,4,8,9].

Increasing position within innovative non-thermal methods for foods preservation gains the application of pulsed electric fields (PEF), for which the strong electric field permeability of cellular is recognized as underlying phenomenon [10,11]. For this method different forms of pulses, described via electric field intensity vs. time dependences, are considered. However, in practice the discharge of energy collected in a set of capacitors is used. This can be associated with the necessity of the big energy output in a very short time.

The satisfactorily anti-microbial PEF action in foods is reached after a sequence of tens/hundreds of pulses, lasting at least tens of microseconds. Depending on the product, electric field intensity and

the total duration of pulses the number of microorganisms can decrease from 2 to 5 decades [10-13]. For PEF method the post-process increase of temperature, reaching even 40 K, is reported [12-14]. In the opinion of the authors the fact that the electric field act within a 'thin layer' in a very short time may indicate that the local heating can be even larger. It is notable that for PEF treatment some degree of denaturation and loss of microorganisms is reported, what may be considered as a possible aftermath of short-time local thermal pasteurization. The extensive resume of recent advances for HPP and PEF methods can be found in references [1-4,10-16]. This report presents studies focusing on the simultaneous impact of HPP + PEF on *Saccharomyces cerevisiae* model system. The behavior pre/post- HPP, PEF and HPP + PEF treatments was monitored via broad band dielectric spectroscopy (BDS) frequency scan. The research aimed on the preliminary answer to the question whether the hybrid, simultaneous action of strong electric fields and high pressures can yield any added value important for future implementations. Notable, are different physical origins of PEF and HPP antimicrobial action [14-16].

Experimental

The optimal electric 'pass through' into the interior of the pressure chamber should ensure high electric resistivity and the transfer of high voltage and high electric current signals. These should take place for hundreds of high pressure experiments. The 'pass through' element, developed in the Institute of High Pressure Physics Polish Academy

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Received November 10, 2015; **Accepted** December 03, 2015; **Published** December 09, 2015

Citation: Rzoska SJ, Musiał F, Rutkowska, Fonberg-Broczek M, Sokołowska B, et al. (2015) Simultaneous Impact of High Pressures and Pulsed Electric field on *Saccharomyces cerevisiae* Model System. J Food Process Technol 6: 518. doi:10.4172/2157-7110.1000518

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of Sciences (IHPP PAS) for nonlinear dielectric spectroscopy studies, is characterized by: resistivity $R \sim 1G\Omega$, $I_{max} \sim 0 A$ and $\Delta T < 1K$ [17-20]. The latter is well below typical 5 voltage used in PEF applications for foods: $E = 30 - 100 kV/cm$ [14-16]. Such intensities for $U \sim 1000V$ can be reached only for the distance between electrodes $0.3 mm < d < 0.1 mm$. However, this have to result in (i) undesired very small resistivity and consequently (ii) extra parasitic heating, (iii) increasing probability of electric breakdowns, figure 1 shows the new PEF apparatus developed for studies which results are presented below. The desired PEF impact on microorganisms was reached for notably lower intensities of the electric field than used so far. This means avoiding undesirable factors listed above ((i), (ii), (iii)).

It bases on high voltage amplifiers (up to 2000 V peak-peak), with the high energy output reaching 500 J. So far these parameter were obtained for the band up to $f = 20 kHz$. For higher frequency works are under development: max 500 V peak-peak was reached. It is notable that a similar design, although with lesser energy output, was used in dual field nonlinear dielectric effect (NDE) studies on super cooled liquids [17-20] and Electrooptic- Kerr Effect (EKE) investigations [21-24], also in water-based colloidal suspensions [22]. In these experiments changes of dielectric permittivity (NDE) or birefringence (EKE) vs. time are monitored. The undesired heating is detected on-line via the vertical shift of the baseline after strong electric field pulse action. However, these studies showed that the parasitic heating can be avoided if sine-wave pulses of high electric field with few kHz frequencies are applied [20,22]. This was explained by too large inertia of ionic species, responsible for heating, to be influenced by such form of the electric field. A similar effect was expected for *Saccharomyces cerevisiae* suspensions/colloids studies presented in this report in which the newPEF apparatus (Fig. 1) was used. As indicated below the implemented form of the strong electric field action decreased the voltage of the high electric to the level possible for application for the module with the sample placed within the high pressure chamber.

A notable feature of the design presented in figure 1 is also the flexibility in creating arbitrary forms and sequences of strong electric field pulses. For preliminary studies presented in this report, the strong electric field had the sine-wave form: each pulse contained ca. 10 periods of the electric field with $f = 1, 5$ and $10 kHz$ frequencies. Tests were carried out at room temperature using the flat-parallel capacitor with diameter $d = 40 mm$ and the distance up to 5 mm, made from Invar. No impact of PEF action on capacitor plates was observed. This factor is worth stressing, since such impact is often reported in PEF studies carried out so far. The on-line digital oscilloscope monitoring of the action of the strong electric field in the newPEF apparatus was possible: no electric bids associated with the strong electric field were detected. The shift of the baseline prior and after the sequence of strong electric field pulses was negligible. The copper - constantan thermocouple located within one of plates showed that heating during the treatment was lesser than $\Delta T < 1K$. Immediately prior and after the newPEF treatment broad band dielectric spectroscopy (BDS) scans were carried out. In high pressure studies of liquid and soft matter samples under pressure the total isolation from the impact of the pressure transmitting liquid (mixture of octane and silicone oil in our cases) constitute the key experimental problem [25-27]. figure 2 shows the module designed and build for given studies, avoiding such undesired impact. It enabled BDS monitoring immediately prior and after HPP, PEF and HPP+PEF. BDS scan was carried out using Alpha Novo control analyzer, between 100 Hz and 10 MHz, which enabled permanent 5 digits resolution.

Suspensions of *Saccharomyces cerevisiae* NCFB 3191 strain were used. Twenty-four hour culture in YPG-broth (1% yeast extract-Difco, 1% peptone-Difco, 2% glucose-POCh) was centrifuged at $4^{\circ}C$ for 10 min at $6000 \times g$, and sedimented cells were aseptically re-suspended in phosphate-buffered saline (PBS, pH 7.2) and again centrifuged. The washing procedure was repeated twice more. The final suspensions

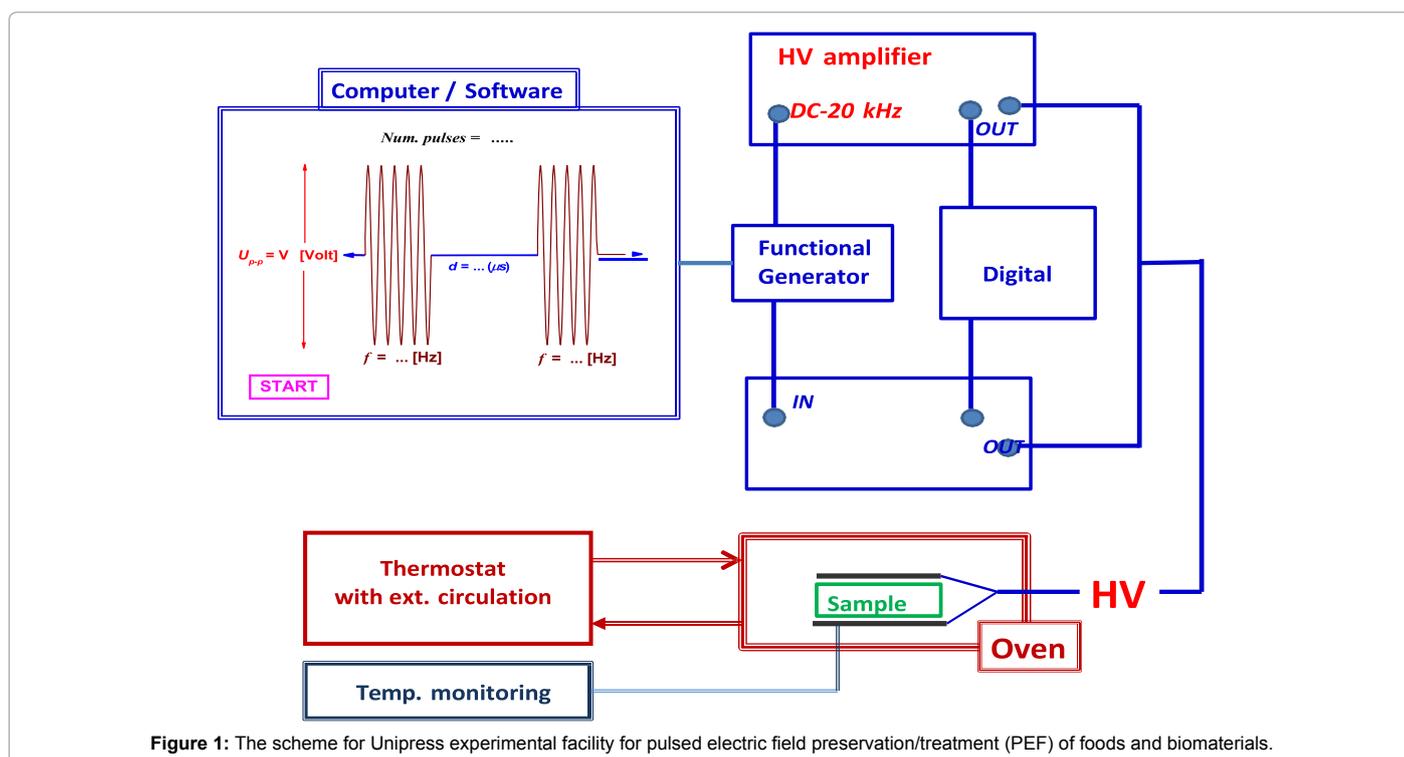
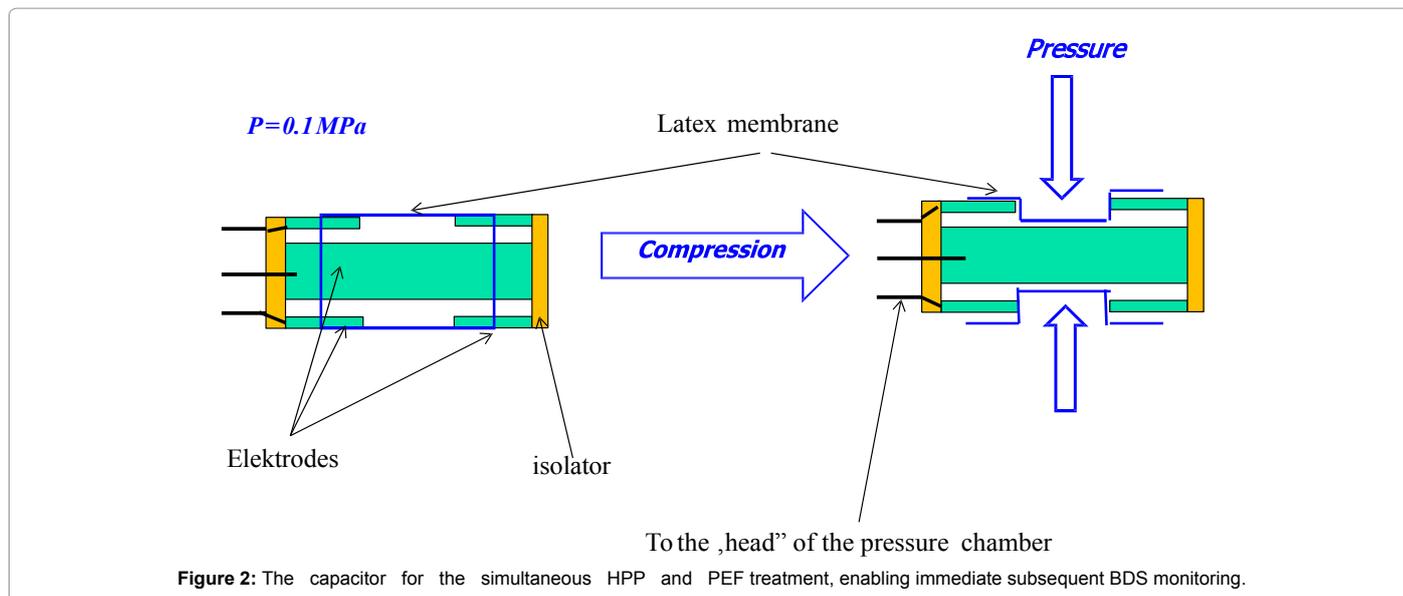


Figure 1: The scheme for Unipress experimental facility for pulsed electric field preservation/treatment (PEF) of foods and biomaterials.



of *S. Cerevisiae* were prepared in PBS, reaching cell concentration of about 10^{17} - 10^{18} cfu/g (colony forming units/g, i.e. living cells/g) and in agarose gel, with the concentration of about 10^7 - 10^8 cfu/g). The suspension was prepared in water and in gel (agar) to reduce electric conductivity. Samples were centrifuged for 10 min at $3500 \times g$ and prepared for the electron microscopy. The cells were fixed with 2.5% glutaraldehyde cacodylic buffer and incubated overnight, then washed in 0.1 M cacodylic buffer (pH 7.2). Next the material was post-fixed in 1% OsO_4 in ddH_2O for 3h and washed three times in ddH_2O . After post-fixation, samples were dehydrated through a graded series of EtOH (50% - 10 min, 70% - 24 h, 90% - 10 min, 96% - 10 min,) and dried in the open air. Next, dry samples were mounted on aluminum stubs in different positions and coated with gold in sputter coater (POLARON SC7620) and were examined in LEO 1430VP scanning electron microscope produced by Zeiss(Germany).

Results and Conclusions

Results of *newPEF* action on *Saccharomyces cerevisiae* suspension under atmospheric pressure are shown in figure 3. It is related to following conditions: $E = 20 \text{ kV/cm}$ with, 50 sine-wave pulses ($f = 5 \text{ kHz}$, 10 cycles each) and 1 second delay between pulses it is possible to crossover the pasteurization threshold. The photo shows the strong destruction of intracellular organelles and the visible impact on the cell wall along with the reduction of living yeast cell number from 5.6×10^7 to 3.5×10^2 , i.e. 5.2 decade (hence the pasteurization threshold - 5 decades - was reached). The increase of temperature was only 0.5 K during processing. For other tested frequencies at one decade smaller decrease of the number of microorganisms was noted. Results of the hybrid, simultaneous actions of HPP and PEF are shown in figure 4. They are related to following condition $E = 5 \text{ kV/cm}$, $f = 5 \text{ kHz}$, 10 cycles in each pulse with 15 pulses and 1 s distance between them for PEF and $P = 200 \text{ MPa}$ and $t = 5 \text{ min}$ for HPP. Under such mild (P , E) separate PEF or HPP can yield only a sub-lethal effect, with microorganism revitalizing after treatment. The hybrid PEF+HPP treatment resulted in 5.8 log units' reduction of the number of living cells. figure 5 Presents results of BDS scans of tested yeast samples: (i) native (prior to HPP/PEF treatments), (ii) after HPP treatment, (iii) after PEF action and (iv) after simultaneous PEF+HPP, treatments. BDS experimental results are



Figure 3: TEM picture of *Saccharomyces cerevisiae* cell after PEF treatment, basing on apparatus from Figure 1. Conditions: $E = 20 \text{ kV/cm}$ with, 50 sine-wave pulses ($f = 5 \text{ kHz}$, 10 cycles each) and 1 second delay between pulses.

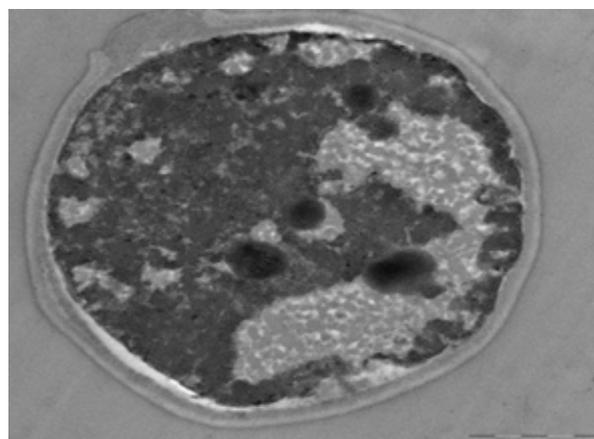


Figure 4: TEM picture of *Saccharomyces cerevisiae* cell after PEF treatment. Conditions: $E = 5 \text{ kV/cm}$, $f = 5 \text{ kHz}$, 10 cycles in each pulse with 15 pulses and 1s distance between them for PEF and $P = 200 \text{ MPa}$ and $t = 5 \text{ min}$ for HPP.

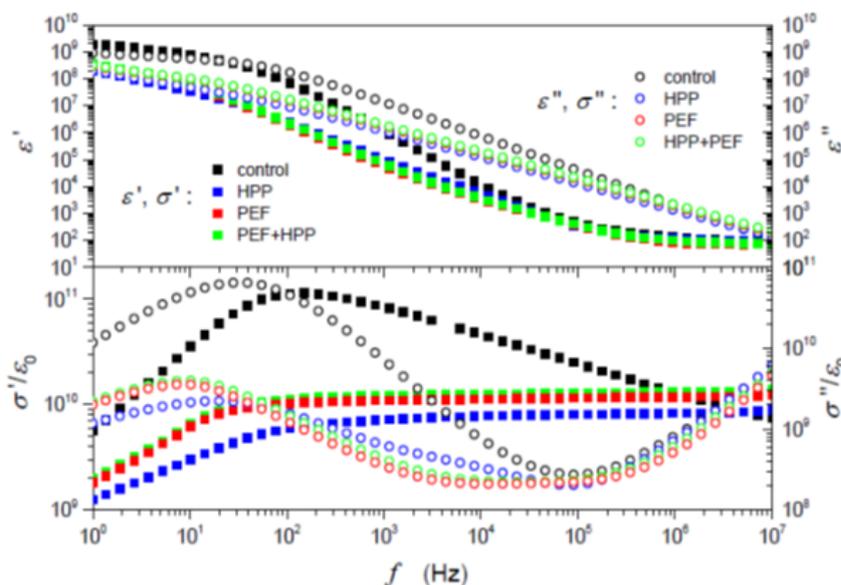


Figure 5: The BDS scan of dielectric properties for the basic yeast colloid/suspension and the same system after PEF, HPP and hybrid PEF + HPP treatments as discussed above. The upper part is for the complex dielectric permittivity and the lower one for the complex electric conductivity representation.

presented in two representations. First, in most common the complex dielectric permittivity representation [28].

$$\varepsilon^*(f) = \varepsilon'(f) - i\varepsilon''(f) = \frac{C}{C_0} - \frac{iG}{\omega C_0} \quad (1)$$

Where C and G are for capacitance and conductance, C_0 is for the empty capacitor and

$\omega = 2\pi f$ is for the angular frequency. However for highlighting features appearing in highly conductive media electric conductivity representation is often advised [28, 29]:

$$\sigma^*(f) = i\varepsilon_0 \omega \varepsilon^* = i\varepsilon_0 \omega (\varepsilon' - i\varepsilon'') = \varepsilon_0 \omega \varepsilon' + i\varepsilon_0 \omega \varepsilon'' = \sigma' + i\sigma'' \quad (2)$$

Jonscher [28, 30] showed that the real part of electric conductivity, often recalled simply as the conductivity, can be splitted into two terms: $\sigma = \sigma'(f) = \varepsilon_0 \omega \varepsilon'' = \sigma_{DC} + \sigma_{AC} \approx \sigma_{DC} + A\omega^S$. The component σ_{DC} is for the 'static', i.e. the frequency independent component of electric conductivity.

Regarding the behavior of $\varepsilon''(f)$, it first increases following the dependence $\varepsilon''(f) \propto f^{-k}$. For HPP, *new*PEF and HPP + *new*PEF treated samples the behavior is related to $k \approx 1$, what correlates with the constant $\sigma''(f) = \sigma_{DC}$ values from 10 MHz to ca. 100 Hz in the electric conductivity representation (the lower part of figure 2). Such behavior is observed also in 'classical' fluids. It is associated with the fact that the constant number of ionic species can be gradually more 'slowly' moved by the electric field when decreasing its frequency. However, in the same range of frequencies for the native 'vivid' sample of yeast the coefficient $k \approx 1.25$, what is reflected by the linear increase of $\sigma'(f)$ in the log-log scale $\sigma'(f) \propto f^{1-k} \approx f^{-0.25}$. To the best of the authors knowledge this is the first ever evidence of such behavior in BDS

studies in fluid samples. It may suggest that on decreasing frequency of the electric field the number of interacting with the field ionic species increases (!). On reaching the threshold frequency, equal to ca. 100 Hz for the control sample and 10-20Hz for samples after 'cold pasteurizations', the exponent notably decreases and $k < 1$. This can be explained as the results of the final polarization of capacitors plates via the Maxwell-Wegner effect [28]. The evolution of the real part of dielectric permittivity $\varepsilon'(f)$ and the imaginary part of the conductivity $\sigma''(f) = \varepsilon_0 \omega \varepsilon''(f)$ show changes of polarization ability of ionic species. It is notable that for $f \rightarrow 10\text{MHz}$ the permittivity $\varepsilon' \rightarrow 80$, i.e. the value characteristic for dielectric constant of water [28].

Concluding, results presented show that new developments for PEF treatments for foods and biomaterial make it possible to reduce qualitatively the parasitic heating. The simultaneous action of high pressures and strong electric fields (HPP + *new*PEF) caused the pasteurization threshold can be reached for notable lower pressures and weaker electric field than for separate HPP and PEF treatments. This can indicate an interesting perspective for the development of innovative, non-thermal methods for preservation of foods, pharmaceuticals, etc. Finally, it has been noted that the BDS frequency scan can serve as the convenient tool for on-line tests of features and 'quality' estimation of foods or biomaterials after HHP or PEF treatments.

Acknowledgments

This research was supported by National Centre for Science (NCN, Poland) via grant 2011/01/B/NZ9/02537.

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