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National Recovery and Resilience Plan
OF THE REPUBLIC OF BULGARIA

Pulsed Electric Field Pretreatment Enhances the Enzyme Hydrolysis of Baker's Yeast

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INTRODUCTION

Baker's yeast is a key starting material for producing extracts with diverse compositions and applications.

One of the most efficient methods for obtaining yeast extracts with a high content of biologically active peptides is enzymatic hydrolysis, using either a single exogenous endo-peptidase or a mixture of enzymes (endo- and exopeptidases, β-glucanase), applied simultaneously or sequentially. The release of significant intracellular content, from commercial baker's yeast usually requires, prolonged incubation (12–48 hours) with the enzymes at temperatures optimal for their activity, ranging between 50 and 60°C. The primary reason for this is the presence of the cell envelope – the plasma membrane and the cell wall, which acts as the main barrier hindering the extraction of intracellular components.

In recent years, pulsed electric field (PEF) treatment has gained popularity as a fast, non-destructive, and scalable method for permeabilization of microorganisms and plant cells, allowing the extraction of various intracellular components. The electrical treatment induces a change in plasma membrane integrity, known as electropermeabilization or electroporation by generating an additional transmembrane potential. Depending on the electrical parameters, pulsing media composition, cell concentration as well as the post-pulse incubation condition, the loss of membrane barrier functions can be irreversible (irreversible electropermeabilization) resulting in a massive release of intracellular content. PEF, which induces irreversible electropermeabilization, allows the entry of various compounds including macromolecules into cells with cell walls when they are added to the suspensions after electrical treatment.

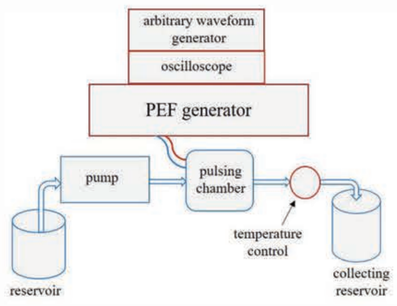
AIM

To evaluate the potential of PEF pretreatment of yeast cells, followed by their incubation with an exogenous protease, as an alternative method for producing yeast hydrolysates with diverse potential applications.

METHODOLOGY

The experiments were conducted using commercial fresh baker's yeast (VIVO, Lesaffre Magyarország).

The electric field treatment in a continuous-flow chamber was performed with a generator of monopolar rectangular pulses (2300V-10A), a Hydropuls mini (GBS-Elektronik, Germany). During the passage through the chamber, the cells received 20 pulses with a duration of 0.5 ms (total treatment time 10 ms) and electric field strength in the range of 3–3.7 kV/cm.



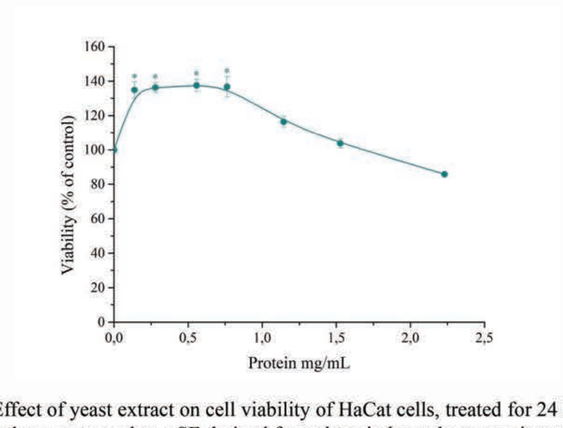


Figure 6. Effect of yeast extract on cell viability of HaCat cells, treated for 24 hours. The data are presented as a mean value ± SE derived from three independent experiments, *P < 0.05.

RESULTS

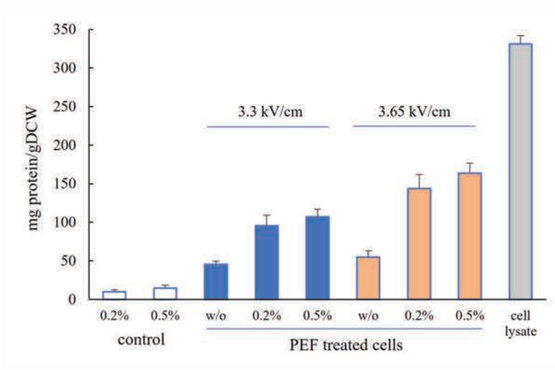


Figure 1. Protein content of cell lysates obtained after mechanical disruption and extracts obtained from control cells and cells subjected to PEF treatment at 3.3 kV/cm and 3.65 kV/cm after incubation for 4 hours at 48 °C with or without (w/o) Alcalase. The values represent the mean ± SD derived from three independent experiments.

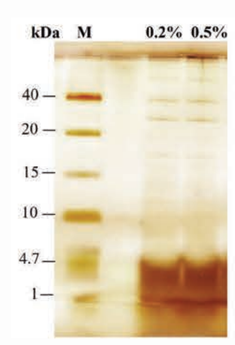


Figure 2. Tricine-SDS-PAGE analysis of the extract obtained from PEF treated cells (3.65 kV/cm) after 4 hours of incubation with 0.2% and 0.5% Alcalase.

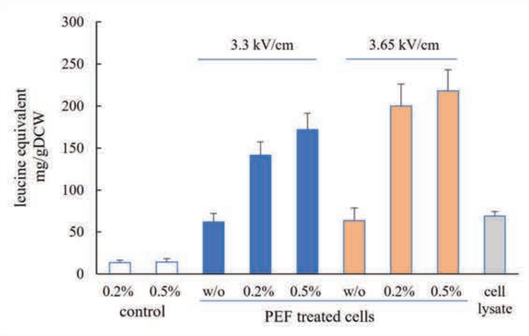


Figure 3. Free α-amino nitrogen (FAN) content of cell lysate and extracts obtained from control and PEF treated cells incubated for 4 hours at 48 °C with or without (w/o) Alcalase. The values represent the mean ± SD of three different experiments.

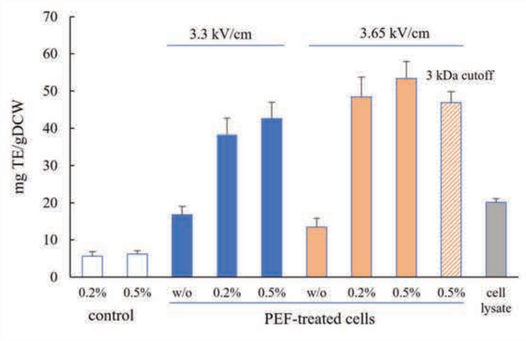


Figure 4. Antioxidant activity of cell lysates and extracts obtained from control cells and PEF treated cells incubated with or without (w/o) Alcalase. The values represent the mean ± SD derived from three independent experiments.

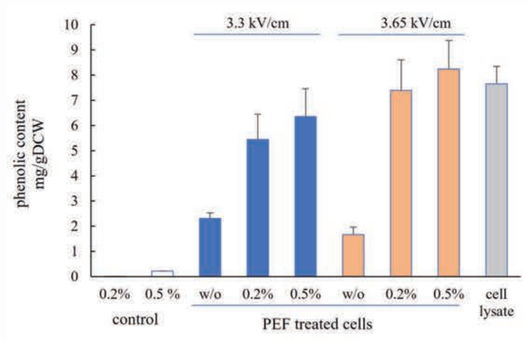


Figure 5. Phenolic content of cell lysate and extracts obtained from control and PEF treated cells after 4 hours incubation at 48°C with and without Alcalase. The values represent the mean ± SD derived from three independent experiments.

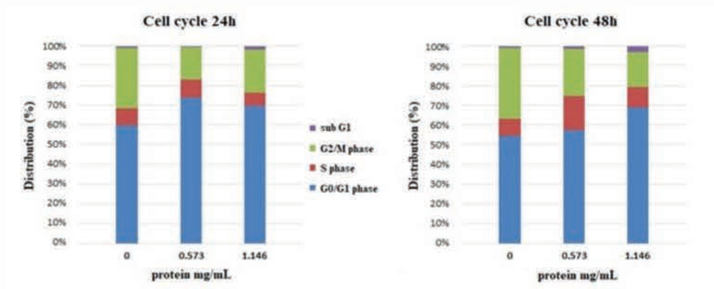


Figure 7. Effect of yeast extract on the cell cycle of HaCat cells, treated for 24 and 48 hours. The data are presented as redistribution of cells into the different phases of the cell cycle in percentages.

CONCLUSIONS

This study demonstrates the potential of PEF pretreatment to facilitate the enzymatic hydrolysis of fresh baker's yeast, resulting in the production of extracts with high antioxidant activity after a relatively short enzyme incubation. The combination of irreversible electropermeabilization and a brief heat shock under specific electrical conditions significantly enhances enzymatic hydrolysis and improves the recovery of intracellular content. During hydrolysis, the cell wall acts as a selective filter, enabling the fractionation of peptides. The resulting hydrolysates stimulate the proliferation of human keratinocytes, even without additional purification. Further studies could optimize the individual steps of the procedure described here and confirm its scalability for the production of enzyme hydrolysates from baker's yeast and other yeast species.

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