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SUMMIT
SOFIA UNIVERSITY MARKING MOMENTUM FOR INNOVATION AND TECHNOLOGICAL TRANSFER

SOFIA UNIVERSITY -
MARKING MOMENTUM
FOR INNOVATION AND TECHNOLOGICAL TRANSFER

National Recovery and Resilience Plan
OF THE REPUBLIC OF BULGARIA

research group:
Pulsed Electric Field Applications in Biotechnology
research area: Biophysics, Biotechnology, and Cell Biology

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Extraction of Bioactive Compounds from Yeast by Combining Pulsed Electric Field Treatment and Enzymatic Hydrolysis

INTRODUCTION

Spent brewer's yeast and baker's yeast represent valuable and abundant raw materials for the production of extracts with diverse composition and applications. Among the available approaches, enzymatic hydrolysis is one of the most effective methods for generating extracts rich in biologically active peptides. This process typically involves the use of exogenous proteolytic enzymes, applied either individually or in combination.

However, efficient extraction of intracellular compounds from yeast requires prolonged enzymatic treatment (12–48 h) at elevated temperatures (50–60 °C). This limitation is primarily due to the presence of the cell envelope, comprising the plasma membrane and the cell wall, which acts as a major barrier to mass transfer. In recent years, pulsed electric field (PEF) treatment has emerged as a rapid, non-thermal, and scalable method for cell permeabilization. PEF induces electropermeabilization of the plasma membrane through the generation of an additional transmembrane potential. Under appropriate conditions, this process can become irreversible, leading to a significant release of intracellular compounds.

OBJECTIVES

The aim of this study is to evaluate the potential of PEF pretreatment combined with enzymatic hydrolysis as a sustainable strategy for the valorization of spent brewer's yeast, enabling the conversion of waste biomass into high-value products.

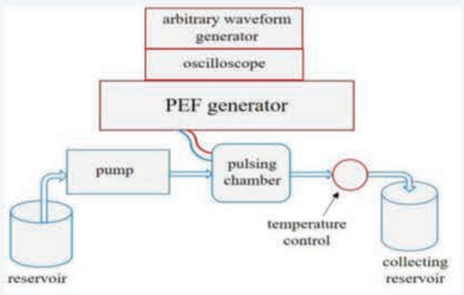
METHODOLOGY

Experiments were conducted using spent brewer's yeast (Kamenitza AD, Haskovo) and commercial fresh baker's yeast (VIVO, Lesaffre Magyarország).

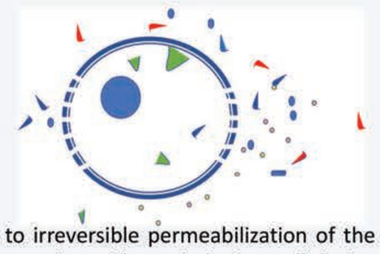
Irreversible electropermeabilization was induced by PEF treatment in a continuous-flow chamber using a generator of monopolar rectangular pulses (Hydropuls Mini, GBS-Elektronik, Germany; 2.3 kV, 10 A).

Cells were exposed to pulses with durations between 0.025–0.5 ms, resulting in a total treatment time of 2.25–10 ms, at electric field strengths of 3–5 kV/cm and flow rates between 35 and 140 mL/min.

Enzymatic hydrolysis of control and PEF-treated cells was performed using Alcalase (Novozymes, Denmark), an endoprotease (~27 kDa).



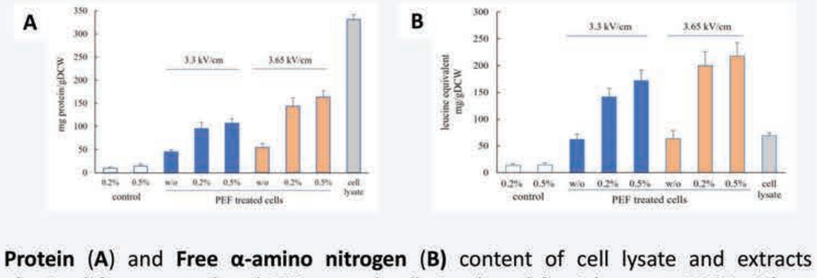
Scheme of the equipment used for continuous treatment with pulsed electric field



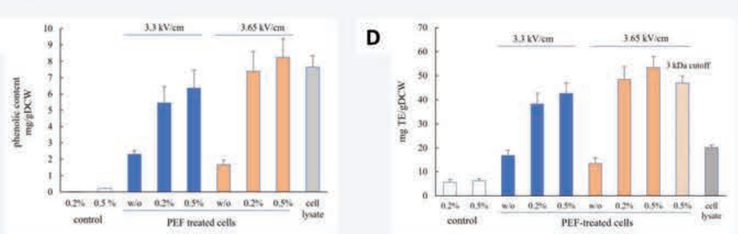
PEF treatment leads to irreversible permeabilization of the plasma membrane and increased cell wall porosity without inducing cell lysis, which is an important condition for the selective release of intracellular components.

RESULTS

To validate the proposed approach under well-defined and reproducible conditions, the initial experiments were performed using commercially available **pressed baker's yeast.**



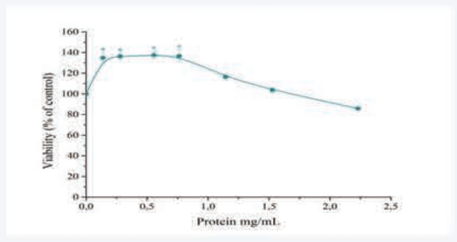
Protein (**A**) and **Free α-amino nitrogen** (**B**) content of cell lysate and extracts obtained from control and PEF-treated cells incubated for 4 hours at 48 °C with or without (w/o) Alcalase. The values represent the mean ± SD of three independent experiments.



Total phenolic content (**C**) and **antioxidant activity** (**D**) of 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 derived from three independent experiments.

RESULTS

The hydrolysates from PEF-pretreated cells demonstrated a positive effect on the proliferation of the human keratinocyte cell line HaCaT.

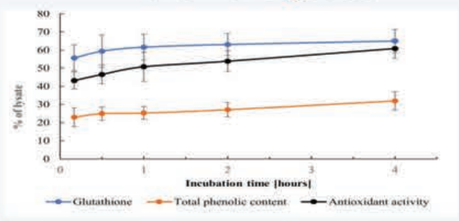


Effect of yeast extract obtained from PEF-treated cells incubated for 4 hours with 0.2% Alcalase on cell viability of HaCaT cells, treated for 24 hours. The data are presented as a mean value ± SE derived from three independent experiments, *P < 0.05.

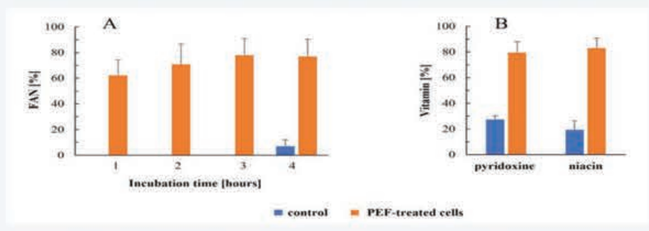
RESULTS

Following validation of the methodology, subsequent experiments were conducted using spent brewer's yeast as the target biomass.

Release of water-soluble substances from spent brewer's yeast

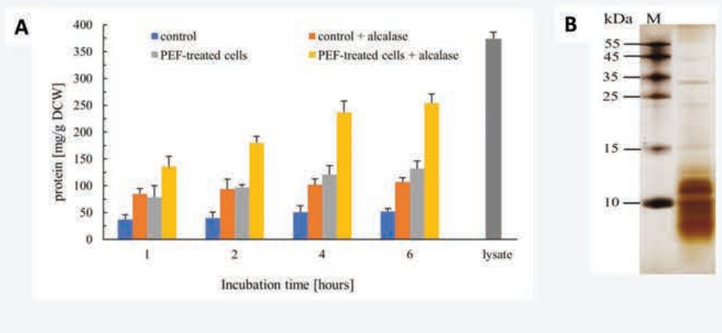


Release of **glutathione, phenolic compounds**, and **antioxidant activity** from PEF-treated cells. Electrical treatment conditions were as follows: electric field strength 3.2 kV/cm, 10 pulses of 0.5 ms duration, and pulse frequency of 7.1 Hz. Data represent mean ± SD (n = 3).



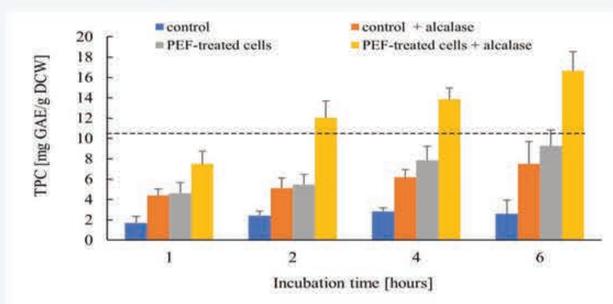
Free α-amino nitrogen (FAN) content of control and PEF-treated cells after 1–4 h of incubation at room temperature **(A)**; **vitamin content** of control and PEF-treated cells after 3 h of incubation at room temperature (**B**). Electrical treatment conditions were as follows: electric field strength 5.0 kV/cm, 90 pulses of 25 μs duration, and pulse frequency of 63.6 Hz. Data represent the mean ± SD (n = 3).

Enzymatic hydrolysis of control and PEF-treated cells

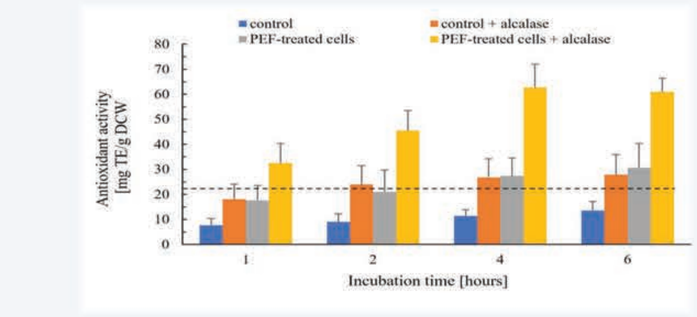


(**A**) **Protein content** of extracts obtained from control and PEF-treated cells incubated at 40 °C with or without (w/o) Alcalase. The protein content in the cell lysate obtained after mechanical disruption is shown as a reference. Data are mean ± SD (n = 4).

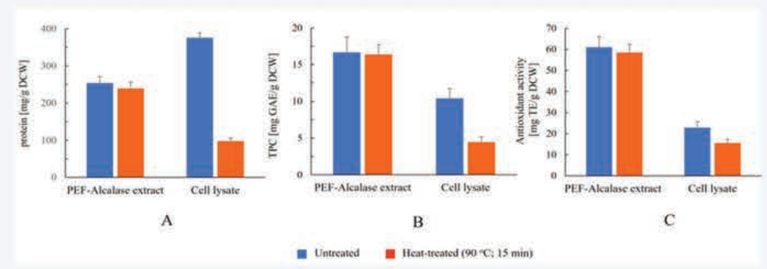
(**B**) **Tricine-SDS-PAGE analysis** of the protein released from PEF-treated cells after 6 hours incubation with Alcalase. Lane M: molecular weight marker.



Total phenolic content (TPC) of extracts obtained from control and PEF treated cells incubated at 40 °C with or without (w/o) Alcalase. Total phenolic content in the cell lysate obtained after mechanical disruption is shown as a dashed line. Data are mean ± SD (n = 4).



Effect of Alcalase on the **antioxidant activity** released from control and PEF-treated cells. Control and PEF-treated cell suspensions were incubated in 50 mM PPB (pH 8.5) with or without 0.2% Alcalase for up to 6 h at 40 °C, and the released antioxidant acidity was quantified at the indicated time points. The antioxidant activity in the cell lysate obtained after mechanical disruption is shown as a dashed line.



Effect of **heat treatment** on **protein (A)**, **total phenolic content (B)**, and **antioxidant activity (C)** in extracts obtained from PEF-treated cells after 6 h of incubation with Alcalase and cell lysates. Data represent mean ± SD (n = 3).

CONCLUSION

In this study, we present a simple approach for the valorization of spent brewer's yeast based on continuous-flow pulsed electric field (PEF) treatment. The method relies on irreversible electropermeabilization of yeast cells suspended in water, which is used as both treatment and post-pulse incubation medium, thereby reducing process complexity.

Under optimal conditions (>98% irreversible electropermeabilization), the outlet temperature remained within 36–38 °C, and no temperature control was required. The specific energy input (62.9 kJ/L; ~1 MJ/kg DCW) was substantially lower than that of mechanical disintegration methods.

Post-PEF incubation enabled rapid release of low-molecular-weight, water-soluble intracellular compounds without the use of buffers or added chemicals, facilitating downstream applications. The resulting extracts are characterized by low purine nucleotide content, which is advantageous for nutritional use.

PEF treatment also acts as a non-thermal inactivation method, stabilizing the biomass while preserving heat-sensitive compounds. In addition, it promotes water release, thereby reducing the energy demand for subsequent drying.

PEF pretreatment significantly enhanced enzymatic hydrolysis with Alcalase, yielding extracts with >51% soluble solids after 6 h. This effect is attributed to the loss of membrane integrity and increased cell wall permeability, facilitating enzyme access to intracellular proteins.

Furthermore, the combined approach provides a highly efficient, solvent-free route for the recovery of both soluble and bound phenolic compounds. Together with the peptide fraction, the resulting extracts exhibit high antioxidant activity, supporting their potential use in food, nutraceutical, and cosmetic applications.

A cascade processing approach can be applied, in which low-molecular-weight compounds are first recovered during aqueous incubation (within 1–2 h), followed by enzymatic hydrolysis of the remaining biomass. This enables selective recovery of different fractions and the production of extracts with tailored composition.

Overall, the combined PEF–enzymatic strategy provides an efficient, solvent-free, and scalable route for yeast valorization, with potential applications in food, nutraceutical, and cosmetic industries.

More detailed characterization of the composition and bioactivity of both aqueous extracts and enzymatic hydrolysates could further demonstrate their functional value and practical potential as dietary supplements and functional ingredients for cosmetic and pharmaceutical applications.

Veleva, R.; Ganeva, V.; Zhiponova, M. Pulsed Electric Field Pretreatment Enhances the Enzyme Hydrolysis of Baker's Yeast. Microorganisms 2024, 12, 2470. https://doi.org/10.3390/microorganisms12122470

Ganeva, V.; Angelova, B. Valorization of Spent Brewer's Yeast by Pulsed Electric Field Treatment Combined with Enzymatic Hydrolysis. J. Fungi 2026, 12, 250. https://doi.org/10.3390/jof12040250

Veleva , R.; Ganeva V. Cascade Extraction of Bioactive Compounds from Spent Brewer's Yeast via Pulsed Electric Field Treatment and Enzymatic Hydrolysis (research in progress)

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