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Extraction of yeast cell compounds: Comparing pulsed electric fields with traditional thermal autolysis

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ABSTRACT

Extracts from yeast cells (YE) are widely used in the food, nutraceutical, and pharmaceutical industries due to their rich composition of proteins, bioactive peptides, nucleotides, and free amino acids. Conventional heat-mediated autolysis is the predominant procedure to obtain YE but requires prolonged incubations at temperatures higher that 50 °C. Pulsed Electric Fields (PEF) have emerged as a promising non-thermal technology capable of enhancing intracellular compound release while preserving their bioactivity.

This study evaluates the efficiency of PEF-assisted extraction compared to conventional autolysis for obtaining extracts from *Saccharomyces cerevisiae*. Yeast suspensions were subjected to PEF treatments (20 kV/cm, 150 μs) followed by incubation at 25, 35, and 55 °C for up to 48 h. The extraction yields of glutathione (GSH), proteins, free amino nitrogen (FAN), nucleic acids, and individual amino acids were analyzed.

Results demonstrated that PEF significantly accelerated compound release, achieving over 70 % of total GSH extraction within 1 h, compared to 24 h for conventional autolysis. RNA was also released faster, reaching 80 % within 1 h at 55 °C, whereas heat-mediated autolysis required 48 h. Additionally, PEF-treated extracts exhibited enhanced proteolysis, yielding up to 10-fold higher concentrations of essential amino acids such as threonine, methionine, and leucine. Principal component analysis confirmed distinct amino acid profiles, indicating improved extraction efficiency.

These findings highlight PEF as a scalable, energy-efficient alternative to conventional autolysis, enabling faster processing at lower temperatures while preserving functionality. This innovative approach could, offering a more sustainable and efficient method for obtaining extracts from yeast.

1. Introduction

Extracts from yeast cells (YE) are the soluble fraction obtained from yeast cells after the removal of the cell wall, with applications across various industrial sectors depending on their specific composition.

Proteins constitute a major component of yeast biomass (40–60 % dry basis), being YE an excellent protein source, offering both functional and nutritional benefits (Bekatorou et al., 2006; Tomé, 2021). The gelation, water-binding, and emulsification properties of yeast proteins contribute to improved texture, stability, and mouthfeel in processed foods, making them highly valuable in the food industry (Ma et al., 2023). Moreover, yeast proteins are highly valued for their complete amino acid profile, containing all essential amino acids (Tao et al., 2023). This balanced composition supports the nutritional value of YE in dietary supplements (Ma et al., 2023).

YE also contain bioactive peptides naturally present in yeast, alongside those produced through proteolytic protein degradation during autolysis. These peptides offer antioxidant, immunomodulatory, and antihypertensive effects, making them highly valuable in nutraceuticals (Mirzaei et al., 2021), while also contributing to flavor enhancement in food applications (Alim et al., 2019; Liang et al., 2024; X. Zhao et al., 2025). Among these peptides, glutathione, a low-molecular-weight tripeptide stands out as a key bioactive peptide in yeast (Li et al., 2004). In the food industry, glutathione is valued for its antioxidant activity, helping preserve color, aroma, and taste (Binati et al., 2022; Giménez et al., 2023; Martínez & González-Arenzana, 2022; Tang et al., 2017; Yano, 2010). Glutathione is also valued in nutraceuticals and cosmetics for its free radical-neutralizing and cell health-enhancing properties (Al-Temimi et al., 2023; Gaucher et al., 2018).

Beyond their nutritional role, free amino acids, particularly glutamic

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acid, contribute significantly to the umami taste characteristic of YE (Tomé, 2021). Furthermore, other molecules present in YE, such as nucleotides, act synergistically to enhance this flavor. Nucleotides like 5′-guanosine monophosphate (5′-GMP) and 5′-inosine monophosphate (5′-IMP), produced through enzymatic RNA degradation during YE processing, intensify flavors in soups, sauces, and seasonings without high sodium levels and are 100 times more taste-active than monosodium glutamate, thus supporting the widespread use of yeast extracts as natural flavor enhancers (Chae et al., 2001; Şen Yılmaz, 2024; Tao et al., 2023; J. Zhao & Fleet, 2005). YE are classified as flavoring preparations, granting them the status of natural flavors (Regulation (EC) No 1334/2008 on Flavorings and Certain Food Ingredients with Flavoring Properties for Use in and on Foods, Pub. L. No. L 354, 2008). Meanwhile, in the U.S.A., the FDA designates YE as Generally Recognized as Safe (GRAS) (Food and Drug Administration (FDA), 2024).

Obtaining yeast extracts YE with a high concentration of target compounds requires to effectively release the intracellular content. Furthermore, the profile of functional compounds in these extracts, which determines their suitability for various industrial applications, is heavily influenced by the extraction methodologies (Tao et al., 2023). The conventional method for yeast extract YE production is autolysis, a process involving the self-degradation of yeast cells through their endogenous enzymes, releasing intracellular compounds (Tomé, 2021; Vieira et al., 2013). Typically, autolysis is induced by applying a controlled heat treatment at 50–70 °C for a period of 24–50 h (Tangüler & Erten, 2009). However, this method poses challenges with thermosensitive compounds, as prolonged heat exposure can degrade proteins and other bioactive compounds, reducing the extract quality and functionality (Tao et al., 2023).

Over the years, various techniques have been investigated to optimize the release of intracellular compounds from yeast cells. These include chemical methods, enzymatic methods, and physical approaches, as well as combinations of these techniques (Liu et al., 2016). However, each method presents limitations, including the potential loss of valuable compounds due to excessive hydrolysis, the formation of undesirable by-products, low extract purity, and significant energy requirements and costs (Tao et al., 2023). Consequently, these drawbacks have limited the industrial adoption of these methods, where heat-mediated autolysis remains the dominant process.

Among non-thermal extraction methods, Pulsed Electric Fields (PEF) have emerged as a promising technology to enhance compound release from microbial biomass (Martínez et al., 2020) gaining significant interest in the food industry due to its efficiency and sustainability (Barba et al., 2015). PEF technology induces cytoplasmic membrane permeabilization through electroporation, a process in which pores are formed by applying short, high-voltage pulses to a product positioned between two electrodes. This approach enables the selective release of bioactive compounds from yeast while minimizing thermal degradation (Berzosa et al., 2023; Ganeva et al., 2020; Liu et al., 2013; Yang et al., 2021). Electroporation also triggers endogenous enzymatic activity, offering the advantages of autolysis without the drawbacks associated with heat-mediated processes (Dimopoulos et al., 2018; Marín-Sánchez et al., 2024a; Martínez et al., 2016, 2019). Additionally, the scalability and adaptability of PEF technology make it well-suited for industrial applications.

Saccharomyces cerevisiae is a eukaryotic microorganism widely used in the food, biotechnology, and pharmaceutical industries (Zarei et al., 2016). The versatility of this yeast spans traditional roles in baking and alcoholic fermentation, as well as in the modern production of high-value bioactive compounds (Ballet et al., 2023; Que et al., 2024). Yeast extracts derived from *S. cerevisiae* serve as a rich source of functional molecules, including glutathione, nucleic acids, proteins, and amino acids.

This study aims to evaluate the efficacy of PEF as an alternative to the conventional heat-mediated autolysis for obtaining extracts from *S. cerevisiae*. By comparing PEF technology with conventional autolysis, the study assesses whether PEF can provide a more efficient method for obtaining YE rich in functional and nutritional compounds.

2. Materials and methods

2.1. Yeast strain, growth conditions, and preparation of cell suspension

Saccharomyces cerevisiae strain 3D (Agrovin, Ciudad Real, Spain) was employed in this study. Precultures were prepared by inoculating a single colony into 10 mL of Sabouraud Dextrose Liquid Medium (Oxoid, Basingstoke, UK) in test tubes and incubating at 25 °C for 24 h. Subsequently, 1000 mL flasks containing 650 mL of Sabouraud Dextrose Liquid Medium (Oxoid) were inoculated with the precultures to an initial concentration of 10^4 CFU/mL. Cultures were incubated at 25 °C with orbital shaking until reaching the stationary growth phase after 48 h. For yeast suspension preparation, cultures were centrifuged at 3000 *g* for 10 min at 20 °C, and the resulting yeast cells were resuspended in citrate-phosphate McIlvaine buffer (pH 7, conductivity 2 mS/cm) to a final concentration of 10^9 CFU/mL (29.1 ± 1.6 g dry weight/L).

2.2. Heat-mediated autolysis

A temperature of 55 °C that is in the range of optimal temperatures (50–60 °C) reported in the literature (Jacob, Striegel, et al., 2019b; Takalloo et al., 2020; Tanguler & Erten, 2008) was selected for autolysis. Yeast suspensions (5 mL) were transferred to 15 mL Falcon tubes and incubated at 55 °C for different durations (see Section 2.5).

2.3. Pulsed electric fields (PEF) treatment

PEF treatment was conducted in a continuous flow chamber using a commercial PEF system (Vitave, Prague, Czech Republic), as previously described (Berzosa et al., 2024). For this study a titanium parallel-electrode chamber with a 0.56 cm gap, 4.0 cm length, and 0.5 cm width was employed, with yeast suspension flowing at 5.0 ± 0.1 L/h. The yeast suspension was pre-tempered to 20 °C using a heat exchanger located upstream of the treatment chamber. Electric field strengths of 10, 15, and 20 kV/cm, with frequencies ranging from 20.8 to 79 Hz, were applied using monopolar square wave pulses of 3 μs width, resulting in a total treatment duration between 50 and 192 μs. The total specific energy input ranged from 10.3 ± 2.1 to 207.5 ± 3.5 kJ/kg, resulting in exit temperatures of 22.5 ± 0.7 to 70.4 ± 0.8 °C (**Suppl. Table 1**). It is noteworthy that, the short residence time of 0.8 s within the treatment chamber, combined with rapid cooling to below 20 °C within 5 s through a second heat exchanger downstream, minimizing thermal damage.

2.4. Evaluation of the effects of the treatments on the cytoplasmic membrane permeability of S. cerevisiae yeast population

Cytoplasmic membrane permeabilization resulting from heat-mediated autolysis and PEF treatments was evaluated using propidium iodide (PI) uptake. Following treatments, 50 μL of PI solution (0.005 mg/mL) (Sigma-Aldrich, Saint Louis, MO, USA) was added to 450 μL of yeast cell suspension (10^6 CFU/mL), achieving a final PI concentration of 0.75 μM. Suspensions were incubated for 10 min at room temperature, then centrifuged at 3000 *g* for 2 min, and resuspended in 0.1 μm-filtered phosphate-buffered saline (PBS) (Sigma-Aldrich). PI, a hydrophilic molecule with a low molecular weight (660 Da), crosses only permeabilized cytoplasmic membranes and binds to intracellular nucleic acids, forming a fluorescent complex with excitation and emission peaks at 535 nm and 617 nm, respectively. Fluorescence measurements were obtained by flow cytometry using a Guava® easyCyte™ system (Luminex®, Tokyo, Japan), with excitation provided by a 488 nm laser and PI fluorescence detected through a RED-B filter (695/50 nm).

Samples were analyzed in duplicate, with 5000 events recorded per sample at a flow rate of 0.59 μL/s. Cells emitting red fluorescence above $4.5{\cdot}10^1$ a.u. were considered as permeable, and the percentage of permeabilization was calculated based on the proportion of cells exceeding this fluorescence threshold.

2.5. Experimental setup

To determine the benefits of PEF-induced electroporation for extracting bioactive compounds from *S. cerevisiae*, various temperatures (25, 35, and 55 °C) and incubation times (1, 6, 24, and 48 h) were tested. After PEF treatment or heat-mediated autolysis (55 °C), samples were placed in an incubator at the designated temperature for the corresponding time intervals. At specified incubation times of 1, 6, 24, and 48 h, yeast extracts were collected and analyzed to quantify the concentrations of target compounds. All incubations were carried out under sterile conditions. Specifically, for each time point, a separate sterile 15 mL Falcon tube with 5 mL of yeast suspension was used, and all handling steps—including sample preparation, transfers, and incubations—were conducted under aseptic conditions to prevent microbial contamination.

2.6. Determination of the total concentration of compounds in the yeast

The total concentrations of compounds (glutathione reduced, FAN (free α-amino nitrogen), proteins and nucleic acids) in the yeast cells were quantified following complete cell disruption achieved by bead milling (Mini-Beadbeater-Plus; BioSpec, Bartlesville, USA). For cell lysis, 1.5 mL of the yeast suspension was combined with 0.5 mm glass beads at a 1:5 weight ratio (glass bead/yeast suspension) in a 2.0 mL screw-capped tube. The progress of cell disruption was monitored microscopically (Eclipse E400, Nikon, Tokyo, Japan). Cell disintegration above 90 % was obtained after fourteen cycles of 70 s each, with cooling intervals in an ice water bath. Following disruption, the suspensions were centrifuged at 3000 *g* for 10 min, and the supernatant was collected for compound quantification. Extraction yields using the different methodologies and incubation conditions were expressed as a percentage of the total concentration present in the *S. cerevisiae* cells (Table 1).

2.7. Analytical methods

2.7.1. Glutathione reduced concentration

Reduced glutathione (GSH) quantification was performed using a colorimetric method with 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) (Thermo Fisher Scientific,Waltham, MA, USA), following a modified protocol based on Ganeva et al. (2020) as previous described by (Berzosa et al., 2024). In brief, 960 μL of phosphate-buffered saline (PBS) at pH 7.5 containing 5.6 mM EDTA (Sigma-Aldrich) was combined with 20 μL of a 0.4 % DTNB solution and 20 μL of the sample. After incubating at room temperature for 2–10 min, absorbance was read at 412 nm. Glutathione concentrations were calculated from a standard curve prepared with reduced L-glutathione (Sigma-Aldrich), ranging from 3.9 to 2000 μg/mL. Results were expressed in milligrams of reduced L-glutathione per gram of dry weight.

Table 1
Total concentrations of reduced glutathione, DNA, RNA, free α-amino nitrogen, and proteins in *S. cerevisiae* 3D cells following complete cell disruption using a bead mill.

Total concentration in *S.cerevisiae* cells (mg/g)	
Reduced glutathione	7.82 ± 0.09
DNA	0.80 ± 0.01
RNA	75.62 ± 4.70
Free α-amino nitrogen (FAN)	40.80 ± 4.47
Proteins	708.93 ± 32.13

2.7.2. Antioxidant capacity

The antioxidant capacity of yeast extracts was quantified using the DPPH (2,2-Diphenyl-1-picrylhydrazyl) radical scavenging assay, which relies on the reduction-induced color change of DPPH from purple to yellow. Samples, appropriately diluted in distilled water, were mixed at a 1:1 ratio with DPPH solution (0.04 g/L in methanol; Sigma-Aldrich). After 30 min incubation in the dark, absorbance was measured at 516 nm, with a methanol–water (1:1) mixture as the blank and a DPPH–water (1:1) solution as the control. A standard curve was generated using Trolox (0–10 μg/mL in water, Sigma-Aldrich). Antioxidant capacity was reported as mg of Trolox equivalents per gram of dry weight. DPPH radical inhibition (%) was calculated using the equation:

$$\textit{DPPH radical inhibition}\ (\%) = \frac{A_C - A_S}{A_C} \times 100 \quad (1)$$

where A_C represents the absorbance of the control and A_S denotes the absorbance of the sample at 516 nm.

2.7.3. Nucleic acids: DNA and RNA

Both long-stranded DNA and RNA quantifications were carried out according to the protocols provided by the respective commercial kits. DNA concentration was measured using the Qubit™ dsDNA BR Assay Kit, while RNA quantification was performed with the Qubit™ RNA BR Assay Kit (Thermo Fisher Scientific, Waltham, USA). Following the manufacturer's instructions, fluorescence readings were obtained, and concentrations were determined using standard curves generated from the assay standards. All measurements were conducted using the DeNovix DS-11 FX (DeNovix Inc., Wilmington, DE, USA).

RNA fragments analysis was performed following the method described by Herbert et al. (1971). Briefly, RNA was extracted using 0.5 M perchloric acid at 37 °C for 2 h, followed by hydrolysis with 0.5 M perchloric acid at 100 °C for 15 min. Quantification was conducted using the orcinol reagent, which produces a greenish color with an absorbance peak at 670 nm. The resulting readings were compared against a standard curve generated with purified yeast RNA (Sigma) to determine RNA concentration.

2.7.4. Protein concentration

Protein extraction was quantified using the Pierce BCA Protein Assay Kit (Thermo Fisher Scientific, Rockford, IL, USA), which employs the Biuret reaction. Briefly, 200 μL of the working reagent was combined with 25 μL of the sample, appropriately diluted in distilled water. The mixture was then incubated at 37 °C for 30 min, after which absorbance was measured at 562 nm. A standard curve was constructed using albumin standards ranging from 2.0 to 0.06 mg/mL, and results were expressed as milligrams of albumin equivalents per gram of dry weigh.

2.7.5. Free α-amino nitrogen (FAN) concentration

Free α-amino nitrogen (FAN) quantification was conducted using a ninhydrin-based colorimetric assay, adapted from Dimopoulos et al. (2018). For the assay, 500 μL of appropriately diluted extract in distilled water was mixed with 250 μL of Ninhydrin Reagent (Sigma-Aldrich) and incubated at 100 °C for 15 min. Following incubation, samples were cooled in an ice-water bath for 5 min, after which 1.25 mL of stop solution (0.2 % (*w/v*) potassium iodate (KIO_3) prepared in a 40 % ethanol–water solution was added to halt color development. Absorbance was then measured at 570 nm against a blank containing distilled water instead of the sample. Results were expressed as grams of L-glycine equivalents per gram of dry weigh.

2.7.6. Individual free amino acids concentration

The determination of the concentration of individual free amino acids in the samples was performed using a Biochrom 30 autoanalyzer (Biochrom Limited, Cambridge, England), which performs cation-exchange chromatography followed by post-column derivatization

with ninhydrin for detection. Prior to analysis, samples were pre-treated to remove proteins using 10 kDa molecular weight cut-off filters and then the remaining proteins were precipitated with sulfosalicylic acid (SSA). 150 mg of SSA was added to 2 mL of sample. The mixture was incubated at 4 °C for 1 h, centrifuged at 11.000 *g* for 15 min at the same temperature, and the resulting supernatant was adjusted to pH 2.2 using 0.3 M lithium hydroxide. Prepared supernatants were directly injected into the autoanalyzer, where amino acids were separated and quantified based on their absorbance at 570 nm (or 440 nm for proline) after ninhydrin derivatization.

2.7.7. Dry weight determination

Dry weight of the samples was obtained by drying them to a constant weight at 30 °C for 15 h in a centrifugal concentrator (miVac DNA-23050-B00, Ipswich, England).

2.8. Statistical analysis

Results are expressed as mean ± standard deviation, calculated from two replicates of three independent experiments ($n = 6$). Statistical significance was evaluated using a one-way analysis of variance (ANOVA) followed by Tukey's post hoc test, performed with GraphPad Software (GraphPad Software Inc., San Diego, CA, USA), with a significance threshold set at $p < 0.05$. The Pearson correlation coefficient was computed to assess the linear relationship between variables, with a 95 % confidence interval, using Graph-Pad Software. Additionally, principal component analysis (PCA) was conducted in software R version 4.4.1 (R Core Team) to analyze the amino acid composition across samples.

3. Results and discussion

3.1. PEF treatment parameters selection for the electroporation of S. cerevisiae cells

Efficient extraction of intracellular compounds from *S. cerevisiae* using PEF depends on the permeabilization of the cytoplasmic membrane through electroporation. This phenomenon occurs when the external electric field applied generates a transmembrane potential that exceeds a critical threshold, resulting in membrane pore formation. The degree of electroporation is influenced by specific PEF parameters, such as electric field strength and treatment time (Mahnič-Kalamiza & Miklavčič, 2022).

To identify the optimal PEF conditions for achieving electroporation in *S. cerevisiae* cells, various electric field strengths and treatment times were evaluated (**Suppl. Table 1**). Electroporation was assessed using flow cytometry measuring red fluorescence intensity at the single-cell level to enable precise quantification of dye uptake on a per-cell basis. Fig. 1 shows the density plots of the fluorescence intensity and electroporation percentages as a function of electric field (10, 15, and 20 kV/cm) and total treatment time (50, 100, 150, and 192 μs) for PEF-treated cells, compared to an untreated cell (control). PI red fluorescence emission above the defined threshold ($>4.5 \times 10^1$ a.u.) was used as an indicator of electroporation. Only 4.0 % of the untreated *S. cerevisiae* cells (control) were stained by PI, suggesting intact membranes in most of the population. At 10 kV/cm, electroporation was limited, peaking at 26.1 % with the longest treatment time. In contrast, at 15 kV/cm, electroporation increased significantly with prolonged exposure time, ranging from 18.4 % to 79.3 %. This trend was further intensified at 20 kV/cm, where electroporation rates varied from 41.9 % at 50 μs to more than 99.9 % at 150 μs or higher.

The distribution patterns in the density plot showed that, except for the two most intense treatments (20 kV/cm for 150 and 192 μs), all other conditions resulted in the appearance of two distinct subpopulations among cells emitting red fluorescence above the defined threshold. This effect was particularly pronounced in treatments at 15 kV/cm, where one subpopulation exhibited fluorescence emission slightly above the threshold ($>4.5 \times 10^1$ a.u.), while the other displayed values exceeding 1.0×10^3 a.u. This pattern suggests the presence of a fraction of the population experiencing lower-intensity electroporation, resulting in lower PI uptake.

When the applied PEF intensity exceeded a critical threshold (20 kV/cm for 150 μs), the more resistant subpopulation was no longer detected. The presence of this resistant fraction, which exhibited lower susceptibility to electroporation, may be associated with variations in cell size, as smaller cells generally require more intense PEF treatments to achieve electroporation (Agarwal et al., 2007).

These findings indicate that, in the *S. cerevisiae* strain used in this

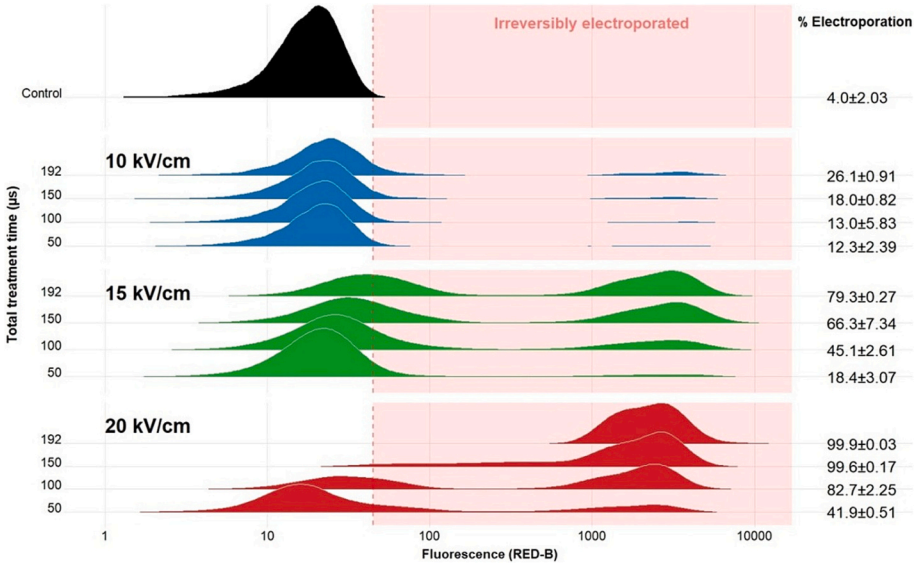

Fig. 1. Density plot showing the fluorescence distribution (RED—B) in *S. cerevisiae* 3D populations, including untreated cells (Control) and PEF-treated cells with different electric field intensities (10, 15, and 20 kV/cm) and total treatment times (50, 100, 150, and 192 μs). The shaded red region marks the threshold (>4.5 × 101 a.u.) above which cells were considered permeable to PI, indicating irreversible electroporation. Electroporation percentages for each treatment condition are displayed on the right as mean ± standard deviation. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

study, a PEF intensity of 20 kV/cm for 150 μs is required for the entire electroporated population to display fluorescence intensities distributed around the detected maximum. This observation suggests a higher resistance to PEF of the strain used in this investigation compared to previous studies, which report that an electric field strength of approximately 10 kV/cm, or slightly higher, is typically sufficient to inactivate 90 % of the yeast population (Aronsson et al., 2005; Cserhalmi et al., 2002; Zhang et al., 1994). Consequently, the PEF treatment condition of 20 kV/cm for 150 μs (149.1 ± 2.97 kJ/kg, 56.5 ± 0.7 °C), which resulted in more than 90 % electroporation in the yeast population, was selected for further investigation. This condition will be used to compare PEF-assisted extraction with conventional heat-mediated autolysis for the recovery of bioactive compounds from yeast extracts.

3.2. Extraction of glutathione

Reduced glutathione is a critical antioxidant tripeptide in *S. cerevisiae*, playing a vital role in cellular defense against oxidative stress. Due to its functional properties, GSH is a valuable target in YE, especially for applications in the food and nutraceutical industries (Li et al., 2004). Fig. 2 presents the concentration of reduced glutathione (Fig. 2A) and the antioxidant capacity of the extracts (Fig. 2B) obtained through heat-mediated autolysis at 55 °C and PEF pretreatment, followed by incubation at 25 °C, 35 °C, or 55 °C for different durations (1, 6, 24, and 48 h). The results indicate that electroporation induced by PEF enabled the release of 70–75 % of the total GSH content within the first hour of incubation, regardless of the incubation temperature. At 25 °C, GSH concentrations increased progressively over time, achieving maximum extraction yield at 24 h, beyond which further incubation did not result in significant enhancement ($p > 0.05$). In contrast, GSH levels remained stable throughout the incubation period at 35 °C, while at 55 °C, a decline in GSH concentration was observed.

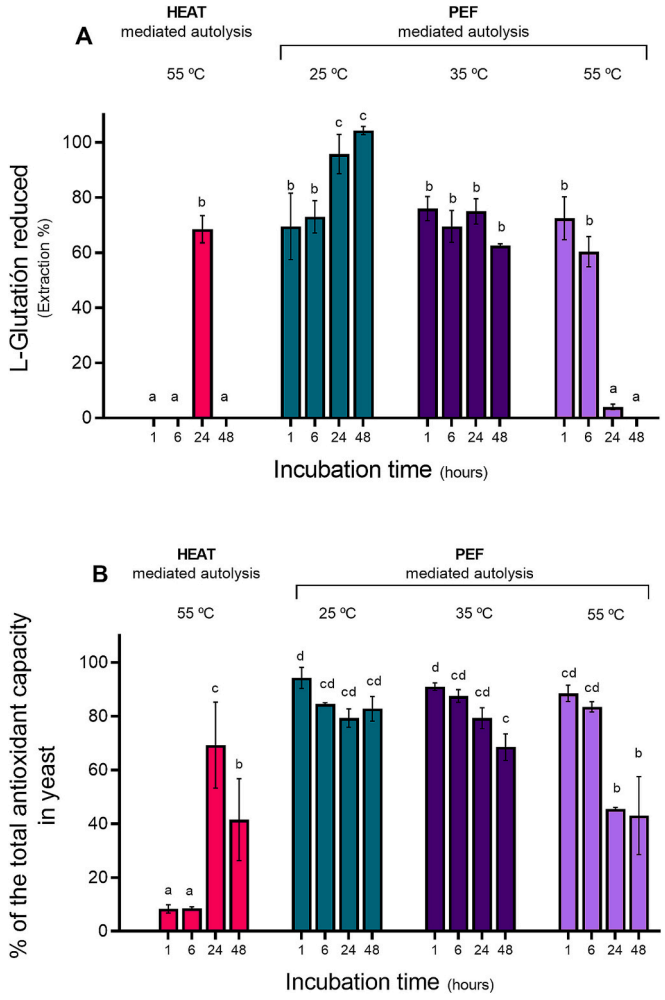

Fig. 2. Percentage of reduced glutathione (A) and antioxidant capacity (B) extracted from yeast cells via heat-mediated autolysis (55 °C) and PEF-mediated autolysis (20 kV/cm, 150 μs) at varying incubation temperatures (25, 35, and 55 °C) across different incubation times (1, 6, 24, and 48 h). Different letters denote statistically significant differences ($p < 0.05$).

The rapid extraction observed within the first hour of incubation can be attributed to the low molecular weight of glutathione (307 Da), which facilitates its diffusion across the permeabilized cytoplasmic membrane following PEF treatment. Similar studies have reported GSH extraction yields of 60–78 % within the first 10 min of incubation post-PEF in different *S. cerevisiae* strains (Berzosa et al., 2024; Ganeva et al., 2020). On the other hand, the reductions in GSH concentration observed at temperatures higher that 25 °C may be attributed to the accelerated oxidation of reduced glutathione (GSH) into glutathione disulfide (GSSG) under higher temperatures (Wang et al., 2010).

In contrast, heat-mediated autolysis at 55 °C did not yield detectable levels of GSH until 24 h of incubation, at which point approximately 68 % of the total GSH was recovered. However, prolonging the incubation to 48 h resulted in a complete depletion of detectable GSH. Compared to PEF treatment, which induces immediate cytoplasmic membrane permeabilization in 99 % of the yeast population, membrane permeabilization during heat-mediated autolysis followed a markedly different progression. Within the first 6 h of incubation at 55 °C, the proportion of cells permeable to PI remained at approximately 30 %, only reaching 99 % after 24 h (data not shown). This initially limited membrane permeability, combined with the accelerated oxidation of GSH at elevated temperatures, likely accounts for the lower peak GSH extraction observed at 24 h compared to electroporated cells incubated at 25 °C. Moreover, the subsequent depletion of GSH after 48 h suggests its progressive degradation under prolonged thermal exposure.

The high antioxidant capacity of GSH, which surpasses that of ascorbic acid (Foyer & Noctor, 2011), represents its most significant attribute in YE. Fig. 2B illustrates the antioxidant capacity of the obtained extracts, revealing a trend that closely follows GSH concentrations with minor variations. A strong and significant positive correlation was observed between GSH content and antioxidant capacity (Pearson's $r = 0.882$, $p < 0.0001$), with a 95 % confidence interval of 0.697 to 0.957. This correlation suggests that approximately 77.8 % of the variation in antioxidant capacity ($R^2 = 0.778$) can be attributed to GSH levels. In addition to GSH, other bioactive compounds, including phenolic compounds, antioxidant enzymes, amino acids, and vitamins, contribute to the antioxidant capacity of ~~yeast extracts~~ YE (Mirzaei et al., 2021; Vieira et al., 2016). These components may account for the residual antioxidant activity observed in extracts obtained through both heat-mediated autolysis and PEF after prolonged incubation at elevated temperatures, even when GSH was no longer detectable.

Our findings align with previous studies suggesting that GSH plays an important role as an antioxidant in YE (Berzosa et al., 2023; Ganeva et al., 2020; Lee et al., 2005). As seen in our study, a reduction in antioxidant capacity has also been reported in heat-mediated yeast autolysates obtained prolonging incubation in contrast to extracts obtained through mechanical disruption methods (Jacob, Striegel, et al., 2019c; Vieira et al., 2017). This decline is likely attributable to the degradation of antioxidative compounds, including GSH, as reflected in its reduced concentrations in thermally treated autolysates. These results underscore the importance of extraction techniques that minimize incubation time and limit thermal exposure to preserve antioxidant capacity. In this context, PEF emerges as a particularly promising approach, as demonstrated by our findings.

3.3. Extraction of nucleic acids: DNA and RNA

Nucleic acids constitute a critical component in YE, with their significance largely contingent upon the specific application of the extract. For instance, the flavor-enhancing properties of yeast extracts are primarily linked to nucleotides derived from RNA degradation. In contrast, for other applications such as supplements in microorganism culture media, the presence of DNA is undesirable and should be minimized to prevent interference with downstream analyses or experimental processes (Jacob, Striegel, et al., 2019c). Yeast cells inherently contain relatively low levels of DNA, constituting less than 1.5 % of their dry weight, while RNA content is considerably higher, typically ranging from 5 % to 15 % (Zhao & Fleet, 2005).

Fig. 3 presents the extraction of DNA (Fig. 3A) and RNA (Fig. 3B) from electroporated and heat-treated cells. Unlike glutathione, DNA release was not significantly enhanced by the electroporation caused by PEF, resulting in extraction yields of only 2 %, 4 %, and 15 % after 1 h of incubation at 25 °C, 35 °C, and 55 °C, respectively (Fig. 3A). The organized structure and large molecular size of DNA likely limit its diffusion across the electroporated membrane or yeast cell wall. Increasing the incubation time and temperature did not cause an important release of DNA from electroporated yeasts reaching 26 % after 48 h of incubation at 55 °C. In comparison, heat-mediated autolysis did not yield significant DNA extraction until 24 h of incubation, consistent

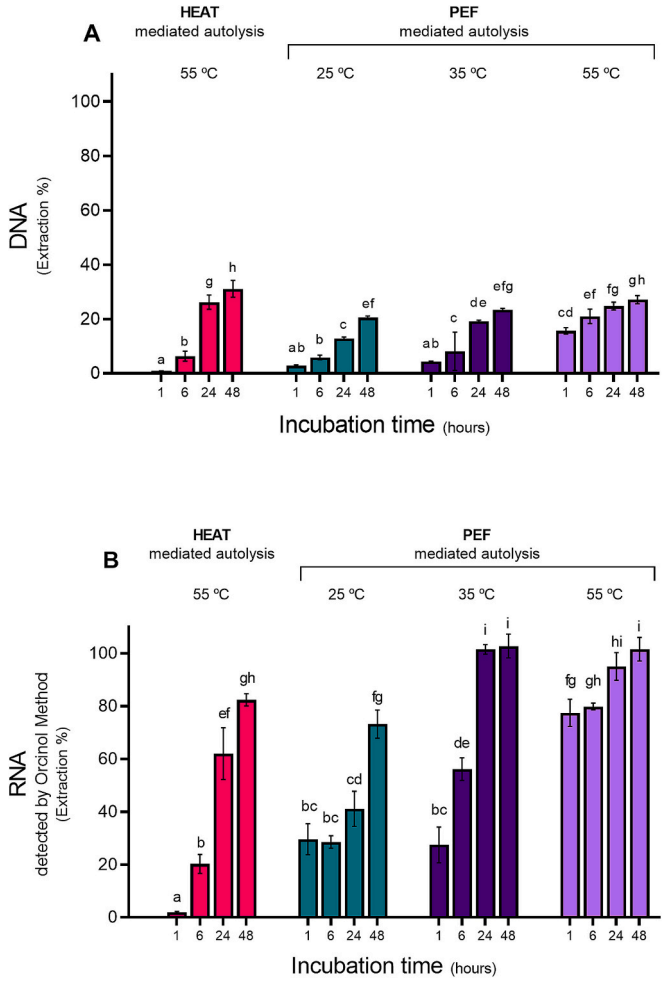

Fig. 3. Percentage of DNA (A) and RNA detected by orcinol method (B) extracted from yeast cells via heat-mediated autolysis (55 °C) and PEF-mediated autolysis (20 kV/cm, 150 μs) at varying incubation temperatures (25, 35, and 55 °C) across different incubation times (1, 6, 24, and 48 h. Different letters denote statistically significant differences ($p < 0.05$).

with the degree of membrane permeabilization determined by PI uptake. After 48 h, DNA extraction via heat-mediated autolysis reached 31 %, slightly surpassing the yields obtained from electroporated yeast under comparable incubation conditions.

The low DNA extraction yields during heat-mediated autolysis processes are well-documented in literature. Zhao and Fleet (2003) conducted a detailed study on the effects of autolysis conditions of *S. cerevisiae* on DNA degradation and extraction, demonstrating that a proportion of DNA remains largely intact within cells even after extended incubation periods. Specifically, after 10 days of autolysis only 55 % of the total DNA was released, indicating substantial intracellular retention. Moreover, under optimal autolysis conditions (40 °C, pH 7), less than 40 % of DNA was extracted within the first 48 h. These findings are consistent with the results of the present study. After staining with PI, the yeast population subjected to PEF treatment exhibited red fluorescence in more than 95 % of the cells following 48 h of incubation (data not shown). This observation indirectly suggests the retention of intracellular DNA, as PI fluorescence requires binding DNA. These results align with those reported by Jacob, Striegel, et al. (2019c), who found that heat-mediated autolysis yields lower concentrations of long DNA fragments compared to mechanical disruption methods. The selective retention of DNA during autolysis may be advantageous for applications requiring YE with minimal DNA content. Furthermore, the findings of this study indicate that combining PEF treatment with incubation at 25–35 °C could further reduce DNA concentrations in yeast extracts compared to conventional autolysis.

Unlike DNA, long-stranded RNA fragments were undetectable in YE obtained through either heat or PEF-mediated autolysis when analyzed by the Qubit RNA BR assay, which is specifically designed to quantify high-molecular-weight RNA strands (**Suppl. Fig. 1**). This lack of detectable long-stranded RNA likely results from the activity of nucleic acid-hydrolyzing enzymes in *S. cerevisiae*, such as endoribonucleases, exoribonucleases, nucleotidases, and nucleosidases, which degrade RNA into fragments too small to be detected by the Qubit assay or even by electrophoresis gels (Zhao & Fleet, 2005). However, RNA was detected in the extract using the orcinol assay (Fig. 3B). This technique, based on the assumption that ribose in yeast cells primarily originates from RNA, quantifies RNA by measuring the ribose released during its acid hydrolysis (Herbert et al., 1971). Therefore, this method accounts for both intact RNA and its degradation products. The presence of RNA degradation products, rather than intact long-stranded RNA, is beneficial, as YE are highly valued for their elevated nucleotide content, which contributes umami and savory characteristics to their flavor profile (Sombutyanuchit et al., 2001).

Assuming that RNA hydrolysis occurs in the cytoplasm of the cells during heat-mediated autolysis or after electroporation, Fig. 3B illustrates the progressive release of RNA fragments throughout incubation. In heat-mediated autolysis, significant extraction was observed from 6 h onwards, reaching a maximum yield of 82 % after 48 h. These findings are consistent with those reported by Zhao and Fleet (2005), who documented RNA release primarily in the form of degradation products, with yields of 70–80 % after 48 h of autolysis at temperatures ranging from 30 °C to 60 °C.

In contrast to DNA extraction, Jacob, Striegel, et al. (2019c) reported that RNA concentrations in extracts obtained through autolysis at 50 °C for 24 h were comparable to those achieved via mechanical disruption. However, autolysis offers a key advantage over mechanical methods, as it leverages endogenous enzymatic activity for nucleic acid degradation, a process that facilitates the efficient release of nucleotides (Tao et al., 2023). Nonetheless, the extended incubation times and elevated temperatures required for conventional heat-mediated autolysis will likely reduce its economic feasibility due to increased energy consumption and prolonged processing durations, underscoring the need for more efficient alternative approaches. Conversely, PEF-treated samples exhibited significantly faster RNA degradation and release, with approximately 30 % of the total RNA extracted after just 1 h of incubation at 25 °C and

35 °C (Fig. 3B). This rapid release is likely driven by autolytic processes induced by PEF, which facilitate the accelerated release of endogenous enzymes that catalyze RNA degradation.

RNA extraction in PEF-treated suspensions continued to increase with prolonged incubation, reaching 73 % at 25 °C after 48 h and achieving complete extraction (100 %) at both 35 °C and 55 °C within 24 h. This trend aligns with previous reports indicating that RNase activity in yeast is highest between 30 and 50 °C (Zhao & Fleet, 2005). Notably, PEF treatment enabled a RNA extraction yield of 80 % within just 1 h at 55 °C, whereas heat-mediated autolysis required 48 h to reach similar yields at the same temperature. Shorter autolysis periods are preferable for maximizing 5′-nucleotide production, as prolonged autolysis can lead to RNA degradation into 2′- and 3′-nucleotides through nuclease activity. Unlike 5′-nucleotides, these derivatives have no impact on flavor (Sombutyanuchit et al., 2001). Therefore, these findings highlight the efficiency of PEF-induced autolysis in achieving high RNA yields in significantly shorter times and at lower temperatures than conventional heat-mediated autolysis. Nevertheless, if the goal is to produce flavor-enhancing yeast extracts, high incubation temperatures remain essential to promote Maillard-type reactions, which are responsible for generating key volatile compounds with meat-like sensory attributes, as demonstrated by Raza et al. (2020).

3.4. Extraction of proteins and total amino acids

Proteins and amino acids represent essential components of YE due to their nutritional and functional importance. These biomolecules play an important role in defining the quality and potential applications of YE, making their efficient recovery a crucial aspect.

Fig. 4A illustrates the protein extraction over the time achieved through heat and PEF-mediated autolysis. Consistent with the trends observed for glutathione and nucleic acid extraction, heat-mediated autolysis exhibited limited effectiveness for protein release with less than 24 h of incubation, reaching a maximum yield of 35 % only thereafter. In contrast, PEF treatment significantly enhanced protein extraction, achieving yields of 25–30 % within 1 h of incubation at 25 °C and 35 °C, and up to 40 % at 55 °C. This extraction efficiency is likely attributed to the release of low-molecular-weight peptides that readily diffuse across the permeabilized cytoplasmic membrane (Marín-Sánchez et al., 2024b). PEF treatment at 55 °C enabled the extraction of higher protein concentrations within 1 h than those obtained via heat-mediated autolysis after 24 h, underscoring its potential to substantially reduce production times and associated costs in industrial applications. Protein extraction in PEF-treated samples continued to increase with extended incubation, reaching a plateau of 50 % after 24 h. These findings align with previous studies reporting a rapid release of proteins within the initial hours of incubation, followed by a plateau after 20 h in both electroporated yeast (Dimopoulos et al., 2018) and microalgae (Scherer et al., 2019). This further supports the effectiveness of PEF in enhancing protein recovery compared to conventional heat-mediated autolysis.

The absence of increased protein extraction beyond 24 h in PEF-treated samples can be attributed to the enzymatic hydrolysis of proteins into smaller peptides and free amino acids by proteases released from intracellular organelles due to electroporation. These enzymes likely catalyze the hydrolysis of proteins, as indicated by the release of free amino nitrogen (FAN) (Fig. 4B) which reflects the concentration of free amino acids and small peptides and serves as a reliable marker of proteolytic activity. The low molecular weight of amino acids facilitated their rapid extraction through the electroporated cytoplasmatic membrane, with nearly 100 % of the FAN being extracted within 1 h of incubation following PEF treatment, irrespective of the incubation temperature. Furthermore, ongoing proteolysis during incubation led to a progressive increase in amino acid concentrations beyond 100 %, with higher yields observed at elevated incubation temperatures. Heat-mediated autolysis, however, was ineffective in extracting amino acids during the initial hours of incubation. After 24 h, FAN levels exhibited a

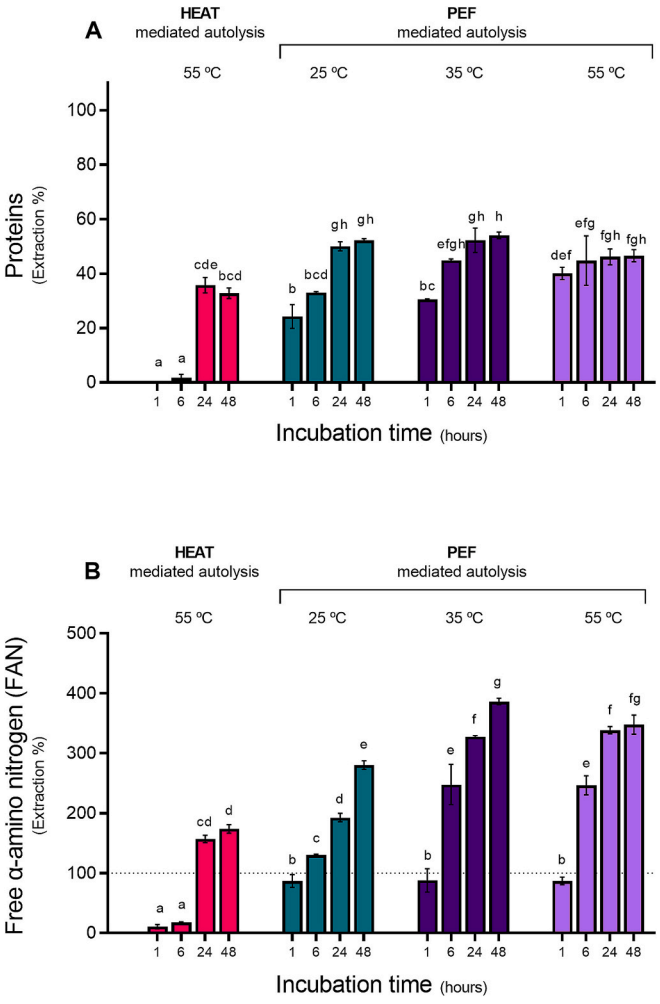

Fig. 4. Percentage of proteins (A) and free α-amino nitrogen (FAN) (B) extracted from yeast cells via heat-mediated autolysis (55 °C) and PEF-mediated autolysis (20 kV/cm, 150 μs) at varying incubation temperatures (25, 35, and 55 °C) across different incubation times (1, 6, 24, and 48 h. Different letters denote statistically significant differences ($p < 0.05$).

substantial increase, exceeding 100 % and suggesting the presence of proteolytic activity as well. Nevertheless, the maximum FAN values in samples subjected to heat-induced autolysis were 0.6 and 1.2 times lower than those obtained from PEF-treated samples incubated at 25 °C and 35 °C, respectively, further demonstrating the potential of PEF to enable efficient extraction at lower temperatures. This increase in amino acid and peptide concentrations as a consequence of proteolysis enhanced by electroporation has been previously described in the literature, highlighting the role of PEF in accelerating protein hydrolysis and promoting the release of low-molecular-weight nitrogenous compounds (Dimopoulos et al., 2018; Ganeva et al., 2020; Yang et al., 2021).

3.5. Free amino acid composition in the extracts obtained from heat- and PEF-mediated autolysis samples

To further elucidate the autolysis mechanisms induced by these technologies, a comprehensive analysis of the free amino acid composition in the extracts obtained from heat- and PEF-mediated autolysis samples was performed (Fig. 5). Given the absence of statistically significant differences in FAN concentrations between PEF-treated samples incubated at 35 °C and 55 °C, the extracts from 35 °C incubations were selected for free amino acid profiling to prioritize the lower temperature condition.

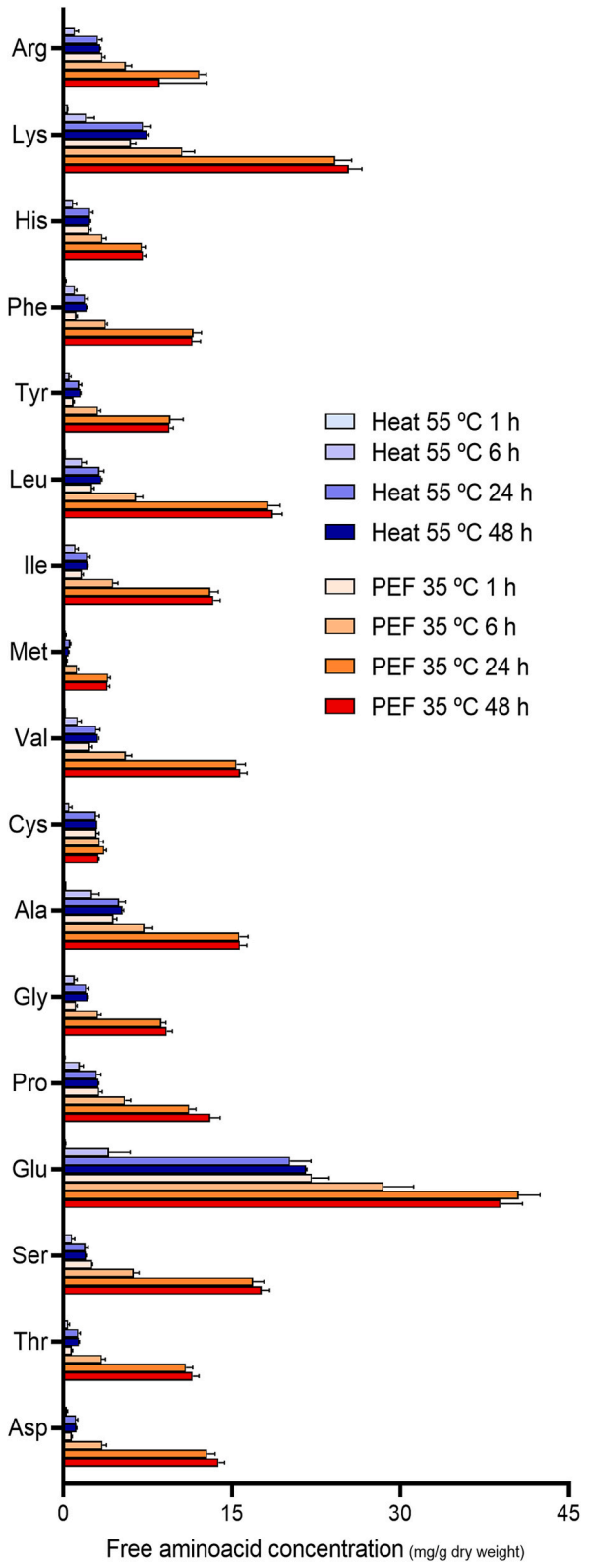

Fig. 5. Free amino acid concentration (mg/g dry weight) in YE obtained through heat-mediated autolysis at 55 °C and PEF-mediated autolysis at 35 °C over different incubation times (1 h, 6 h, 24 h, and 48 h). Heat treatments are represented in shades of blue, while PEF treatments are shown in shades of orange. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Similar to the trends observed for FAN, the analysis of free amino acids demonstrated that PEF treatment at 35 °C significantly enhanced extraction efficiency compared to thermal autolysis at 55 °C. The concentrations of all amino acids, with the exception of cysteine, were notably higher in PEF-treated samples than in those subjected to thermal treatment at the same incubation times. As previously mentioned, PEF promotes autolytic processes mediated by vacuolar proteinases A and B, as well as carboxypeptidase Y, which catalyze the degradation of peptides and proteins into their constituent amino acids, thereby facilitating their release (Jacob, Hutzler, & Methner, 2019).

Amino acid concentrations in PEF-treated samples reached levels comparable to those obtained after 24 h of heat-induced autolysis within only a few hours of incubation. Moreover, based on the results of this study, the autolysis is not only faster but also more efficient in PEF-treated samples compared to heat-mediated autolysis. Remarkably, after 24 h of incubation the autolysis induced by PEF yields 1 to 10 times more for all amino acids compared to heat-mediated autolysis. However, extending the incubation time from 24 to 48 h did not enhance amino acid yields. Previous studies have reported that protease activity peaks during the first 24 h of incubation (Yang et al., 2021), which may explain why most protocols for producing proteolyzed yeast extracts typically limit the incubation period to 24 h or less (Jacob, Hutzler, & Methner, 2019; Oliveira et al., 2022; Saksinchai et al., 2001; Tangüler & Erten, 2009). This agrees with our findings and further underscores the accelerated kinetics of autolysis in PEF-treated samples. This increased efficiency is further supported by the PCA biplot analysis of amino acid profiles (Fig. 6), which compares the amino acid composition of yeast extracts obtained through heat- and PEF-mediated autolysis over the incubation time. The size and dispersion of the ellipses represent the variability within each group. The larger ellipse for the PEF-treated samples indicates a greater variation in amino acid concentrations over the incubation period compared to the heat-treated group, whose ellipse is smaller and more concentrated. This suggests that amino acid release evolves differently in PEF-treated samples, likely due to the rapid and efficient initiation of autolytic processes triggered by PEF. In contrast, the narrower ellipse observed for the heat-treated samples suggests a more uniform and less pronounced progression of autolysis over time.

Moreover, the quantification of individual amino acids (Fig. 5) reveals significant differences, particularly for serine (Ser), threonine (Thr), aspartic acid (Asp), methionine (Met), isoleucine (Ile), and leucine (Leu), whose extraction yields were 5 to 10 times higher in PEF-treated samples compared to those obtained through heat-mediated autolysis. These results underscore the potential nutritional benefit of yeast extracts obtained through PEF-mediated autolysis, as they exhibit significantly higher concentrations of essential amino acids such as Thr, Met, Ile, and Leu.

Glutamic acid plays a crucial role as a natural flavor enhancer, contributing to the umami taste and enhancing the sensory characteristics of yeast extracts. This amino acid was the most abundant in the analyzed extracts, exhibited concentrations twice as high in PEF-treated samples compared to those obtained through heat-mediated autolysis. In contrast, cysteine stands out as the only amino acid whose concentration did not increase during the incubation period, likely due to its involvement in disulfide bond formation, which stabilizes protein structures and complicates its hydrolysis. The PCA analysis revealed that glutamic acid and cysteine were the primary contributors to principal component 2 (PC2) (**Suppl. Fig. 2**), emphasizing their role as key differentiators among the samples.

While the free amino acid levels measured in this study fall within the ranges reported by other authors (Jacob, Hutzler, & Methner, 2019), a direct comparison should be approached with caution due to the considerable influence of variables such as yeast strain, cultivation conditions, growth medium composition, and the physiological state of the cells (Sirisena et al., 2024). These authors also reported that autolytic processes were more efficient for amino acid extraction than

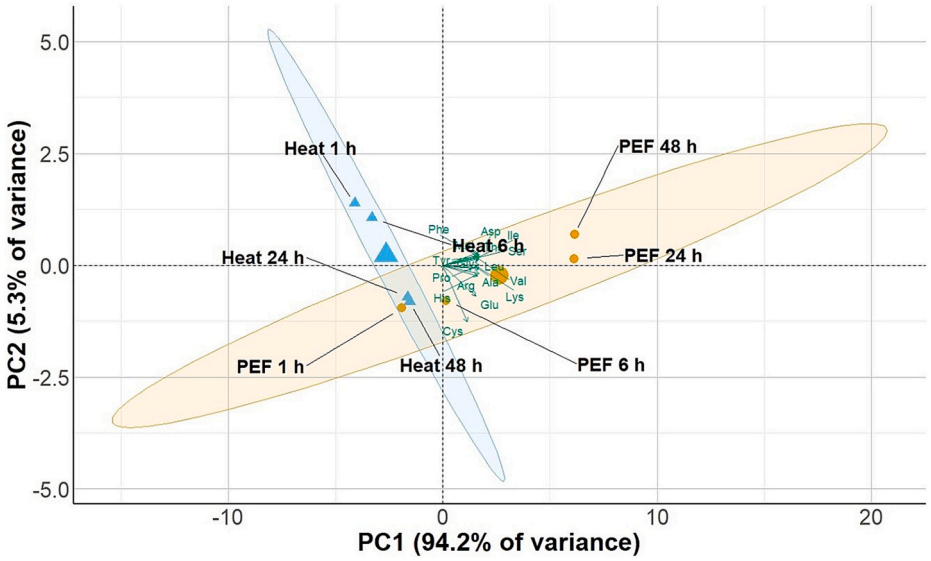

Fig. 6. Principal Component Analysis (PCA) biplot of free amino acid profiles in YE obtained through heat-mediated autolysis at 55 °C (▲) and PEF-mediated autolysis at 35 °C (●) over different incubation times (1 h, 6 h, 24 h, and 48 h). The ellipses represent the confidence intervals for each treatment. PC1 explains 94.2 % of the variance, while PC2 accounts for 5.3 %.

mechanical procedures such as sonication or cell mill disruption. This observation further emphasizes the relevance of our findings, wherein PEF-mediated autolysis demonstrated superior efficacy in amino acid extraction than conventional heat-mediated autolysis. Furthermore, since the cellular structure remains intact after treatment, PEF enables the production of more purified extracts (Marín-Sánchez et al., 2024b) compared to other techniques used to induce autolysis, such as high-pressure homogenization (Dimopoulos et al., 2020; Oliveira et al., 2022).

From an application standpoint, Fig. 6 further highlights the efficiency of PEF treatment, as the sample distribution indicates that PEF-treated extracts obtained after just 1 and 6 h of incubation closely resemble those produced through heat-mediated autolysis after 24 and 48 h. This demonstrates the potential of PEF to substantially reduce both incubation time and temperature in industrial processes, providing a more efficient alternative without compromising yield or quality.

4. Conclusions

PEF-pretreatment of yeast has demonstrated their potential as an innovative and efficient alternative to conventional heat-mediated autolysis for obtaining extracts from yeast. By inducing electroporation, PEF facilitates the rapid release of intracellular bioactive compounds, reducing the time and temperature required for extraction. This study highlights that PEF technology enhances the extraction of key YE components, including proteins, amino acids, nucleotides, and glutathione, improving the overall yield and quality of the final product. The findings suggest that PEF can significantly accelerate proteolysis and nucleic acid degradation, leading to distinct amino acid profiles that may have applications in functional food formulations. Taken together, these results lay the groundwork for the guided development of YE with customizable compositions and functionalities, depending on the intended end use.

Beyond improving extraction efficiency, PEF reduced duration and temperature of incubation aligns with industrial efforts to develop more environmentally friendly and cost-effective extraction technologies. Additionally, the selective permeabilization induced by PEF allows for a more controlled and targeted extraction process, which may be beneficial for producing YE tailored to specific industrial applications.

Future studies should focus on optimizing process parameters for large-scale applications and further investigating the functional properties of PEF-extracted yeast derivatives in various food and nutraceutical applications.

CRediT authorship contribution statement

Alejandro Berzosa: Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Javier Marín-Sánchez:** Visualization, Validation, Investigation. **Ignacio Álvarez:** Writing – review & editing, Visualization. **Cristina Sánchez-Gimeno:** Writing – review & editing, Visualization, Supervision, Funding acquisition. **Javier Raso:** Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used ChatGPT 4.0 in order to readability. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

Declaration of competing interest

The authors affirm that the research was carried out without any affiliations or financial ties that might be perceived as potential conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2025.116852.

Data availability

Data will be made available on request.

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