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RESEARCH

Valorization of Brewer's Yeast Biomass Through the Production of Extracts by Pulsed Electric Fields for Enhancing Microbial Growth and Wine Fermentation

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Abstract
Spent brewer's yeast (SBY) is an abundant by-product from beer production, rich in nitrogenous compounds. However, its valorization is hindered by the resistance of the yeast cell wall and the risk of co-extracting undesirable compounds. Pulsed electric fields (PEF) offer a selective and energy-efficient alternative to recover intracellular components. This study aimed to evaluate nitrogen-rich extracts obtained by PEF-assisted extraction of SBY as a sustainable nutrient source for microbial growth and wine fermentation. SBY biomass was treated using continuous-flow PEF (15 kV/cm, 150 µs) and incubated at 37 °C for 48 h to induce endogenous proteolysis. Moreover, a sequential extraction strategy was evaluated, allowing prior recovery of glutathione before proteolytic incubation. The resulting nitrogen-rich extracts were characterized for free α-amino nitrogen (FAN) and soluble protein content and evaluated as the sole nitrogen source in *Saccharomyces cerevisiae* growth media and white grape must fermentation. All SBY extracts showed FAN concentrations comparable to a commercial yeast extract (250 mg /g extract). Protein content was higher at pH 8, though this did not affect growth performance. The sequential strategy allowed glutathione recovery without altering nitrogen composition or microbial proliferation. Ten grams per liter of SBY extract was identified as optimal concentration for supporting microbial growth, matching the performance of a commercial yeast extract. In fermentation trials, the PEF-derived extract enhanced fermentative kinetics, allowing complete fermentation four days earlier than the unsupplemented control, and reduced residual sugars to levels similar to those achieved with a commercial enological nutrient (< 0.7 g/L). These findings validate the use of PEF to obtain functional nitrogen-rich extracts from SBY, supporting their application in microbial and enological processes within circular bioeconomy strategies.

Keywords Spent brewer's yeast · Yeast extract · Pulsed electric fields · Circular bioeconomy · Wine fermentation · *Saccharomyces cerevisiae*

Introduction

Spent brewer's yeast (SBY), the residual yeast biomass from beer fermentation, is produced in large quantities as a brewing industry by-product. For every 10,000 hL of beer, an estimated 20–40 tons of spent yeast can be generated, translating to a global annual production on the order of 0.7–1.3 million tons (Kruk et al., 2024). The majority of this biomass is currently discarded or utilized as low-value animal feed (Schlabitz et al., 2022). However, *Saccharomyces cerevisiae* (*S. cerevisiae*)—the predominant species in SBY—is a rich source of proteins, peptides, free amino acids, glutathione (GSH), and structural polysaccharides such as mannoproteins and β-glucans (Jaeger et al., 2020). These biomolecules have recognized nutritional, functional, and technological value, making SBY an increasingly attractive substrate for biotechnological valorization (Oliveira et al., 2022a, b; Podpora et al., 2015; Puligundla et al., 2020). The valorization of SBY thus aligns with the current shift towards circular bioeconomy models, which are gaining momentum as sustainable strategies to recover valuable compounds from industrial residues, reduce environmental burden, and promote the production of bio-based ingredients (Chae et al., 2001; Pasquet et al., 2024; Suphantharika et al., 2003; Wani et al., 2023; Yudhister et al., 2025).

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Conventional methods to extract bioactive compounds from SBY, such as thermal autolysis, enzymatic hydrolysis, or mechanical disruption, are often energy-intensive, time-consuming, or require the addition of costly reagents or downstream process (Chae et al., 2001; Ismail et al., 2024; Jacob et al., 2019; Oliveira et al., 2022a, b; Tanguler & Erten, 2008; Zarei et al., 2016). In this context, pulsed electric fields (PEF) have emerged as innovative and sustainable alternatives. PEF induces controlled electroporation of the cytoplasmic membrane through the application of high-intensity electric pulses of short duration (microseconds). This permeabilization enhances membrane permeability, facilitating the selective release of intracellular compounds while maintaining the structural integrity of the cell (Mahnič-Kalamiza & Miklavčič, 2022). This controlled permeabilization caused by PEF permits that the intracellular content can be released in stages rather than all at once. Previous studies have demonstrated that PEF-assisted extraction enables the sequential recovery of distinct fractions from the brewer's yeast biomass (Berzosa et al., 2024; Liu et al., 2013). This fractionation is driven by differences in molecular size and solubility, allowing for the separation of compound classes at each step. As a result, the process minimizes or even eliminates the need for extensive downstream purification, since it avoids the generation of a single, highly complex extract requiring further fractionation.

Due to its richness in nitrogenous compounds, which can represent up to 60% of the cell dry weight, yeast-derived extracts have gained attention for their ability to enhance microbial growth and fermentation performance across diverse bioprocesses (Tachibana et al., 2019; Zarei et al., 2016). In enology, nitrogen availability is a critical determinant of yeast metabolism, growth kinetics, and overall fermentation efficiency (Gobert et al., 2019; Lage et al., 2014; Mendes-Ferreira et al., 2011; Roca-Mesa et al., 2022). To avoid sluggish or stuck fermentations winemakers supplement nitrogen-deficient grape musts with inorganic nitrogen sources such as diammonium phosphate (DAP). However, organic nitrogen sources, particularly those derived from yeast, are gaining increasing interest due to their complex composition, sustained release, and potential to improve fermentation kinetics (Andújar-Ortiz et al., 2010; Balmaseda et al., 2024). Yeast autolysates and inactivated dry yeast (IDY) preparations have been employed to stimulate yeast activity during alcoholic fermentation and to support malolactic fermentation by lactic acid bacteria (Andújar-Ortiz et al., 2010; Guilloux-Benatier & Chassagne, 2003; Santamaría et al., 2020). However, the production of these preparations often relies on yeast biomass specifically grown for this purpose, which increases production costs and limits their sustainability compared to approaches based on industrial by-products.

The objective of this study was to evaluate the potential of a nitrogen-rich extract obtained through PEF-assisted cascade extraction of spent brewer's yeast as a sustainable alternative to conventional nitrogen supplements for microbial growth and enological fermentation. The extract, enriched in amino acids, peptides, and soluble proteins, was assessed for its ability to promote *S. cerevisiae* growth and sustain complete alcoholic fermentation in white grape must. This functional validation aimed to support the use of PEF-based extraction as a strategy for the biotechnological valorisation of yeast biomass in enological applications.

Materials and Methods

Preparation of Yeast Extracts from Electroporated Spent Brewer's Yeast

The preparation of yeast extracts from spent brewer's yeast via pulsed electric field (PEF) treatment was based on a previously described protocol (Berzosa et al., 2024) (Fig. 1).

SBY biomass was supplied by the brewery La Zaragozana (Zaragoza, Spain). Yeast cells were washed twice with distilled water by centrifugation (3000 g, 10 min, 20 °C) and resuspended in McIlvaine citrate–phosphate buffer (conductivity adjusted to 2 mS/cm) to yield two 12-L suspensions adjusted to pH 4 and pH 8, respectively. These conditions were previously shown to influence the extraction efficiency and nitrogen composition of PEF-treated yeast. The final cell concentration in each suspension was 10^8 cells/mL (48.3 ± 4.1 g dry weight/L).

PEF treatment was performed in continuous flow using a commercial unit (Vitave, Prague, Czech Republic). The yeast suspension was circulated through a titanium parallel-electrode chamber (0.56 cm electrode gap, 4 cm length, 0.5 cm width) using a pump at a flow rate of 10 L/h, corresponding to a residence time of 0.4 s in the treatment zone. Monopolar square-wave pulses of 3 µs were applied at a field strength of 15 kV/cm and a frequency of 123.5 Hz, resulting in a total treatment time of 150 µs. The specific energy input reached 97.5 ± 0.99 kJ/kg of yeast suspension, with outlet temperatures of 44.1 ± 0.3 °C. Prior to treatment, suspensions were tempered to 20 °C using a heat exchanger, and immediately post-treatment, were cooled to below 20 °C within 5 s. Under these processing conditions, over 90% of the yeast cells were electroporated, as determined by propidium iodide staining protocols previously described by Berzosa et al. (2024).

Following PEF treatment, two 12-L suspensions of pH 4 and pH 8 were divided in aliquots of 6 L each. One 6 L aliquot of pH 4 and other of pH 8 were incubated at 37 °C for 48 h. After incubation, suspensions were centrifuged (6000 g, 10 min, 20 °C), and the supernatants were

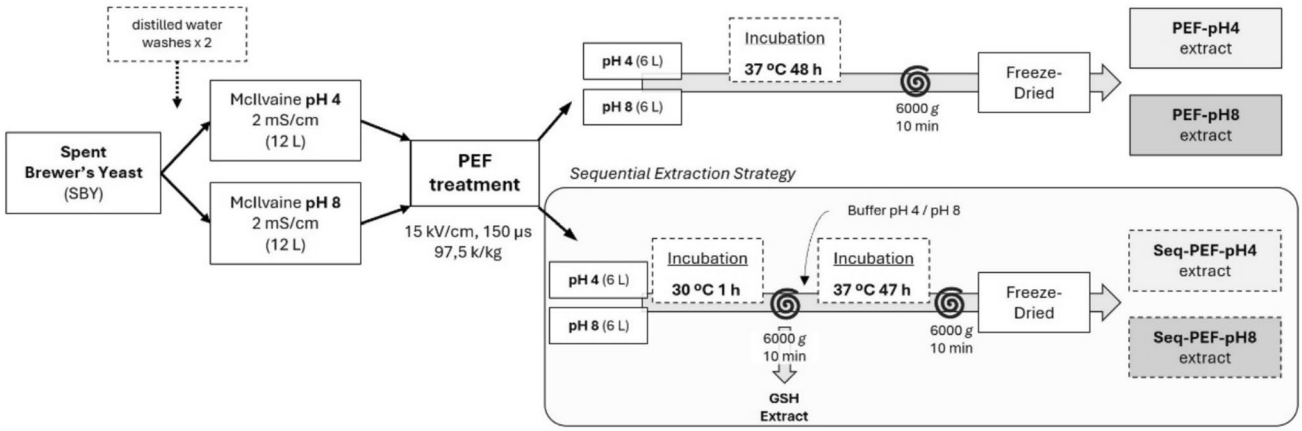

Fig. 1 Schematic overview of the experimental workflow for the preparation of nitrogen-rich extracts from spent brewer's yeast using PEF-assisted extraction. Two extraction strategies were followed: a conventional single-step incubation (37 °C 48 h) and a sequential extraction protocol involving prior glutathione recovery (30 °C 1 h)

freeze-dried, yielding two extracts referred to as PEF-pH4 and PEF-pH8 (Fig. 1).

Additionally, to confirm the feasibility of a PEF-mediated sequential extraction strategy, a cascade protocol was applied. After PEF treatment, the other 6 L aliquot of pH 4 and pH 8 were first incubated at 30 °C for 1 h to release a glutathione-enriched fraction. GSH is a tripeptide with strong antioxidant properties, playing a key role in redox balance and cellular protection. Its extraction from yeast is of great interest due to its potential applications as a natural antioxidant and functional ingredient in food and nutraceutical products (Li et al., 2004). Following removal of this initial extract, the residual biomass was resuspended in 6 L of fresh citrate–phosphate buffer at pH 4 and pH 8, respectively, and subjected to a second incubation at 37 °C for 47 h. The resulting supernatants were collected by centrifugation and subsequently freeze-dried, yielding two additional extracts—Seq-PEF-pH4 and Seq-PEF-pH8 (Fig. 1).

Saccharomyces Strains and Inoculum Preparation

Two commercial wine yeast strains of *S. cerevisiae* were used in different parts of this study: Viniferm 3D (Agrovin, Ciudad Real, Spain), characterized by a high demand for assimilable nitrogen, and Viacell® Rhône 4600 (Lallemand Inc., Montreal, Canada), known for its low nitrogen requirement. Precultures were prepared by inoculating a single colony of each strain into 10 mL of Sabouraud Dextrose Liquid Medium (Oxoid, Basingstoke, UK), followed by incubation at 25 °C for 24 h under static conditions. The cell concentration of each preculture was determined using a Thoma counting chamber. Precultures were subsequently used to inoculate the corresponding experimental media to assess the potential of the extracts as a nitrogen source for microbial growth and to enhance fermentation in grape must.

Microbial Growth Assessment

A defined growth medium was formulated using D-glucose anhydrous (20 g/L) (Scharlau, Barcelona, Spain) as the sole carbon source and yeast extracts obtained from SBY via pulsed electric field (PEF)-assisted extraction as the exclusive nitrogen source. The growth performance of *S. cerevisiae* Viniferm 3D was assessed using two complementary approaches: optical density measurements in microplates and viable cell counts on solid media.

Growth Monitoring via Optical Density in Microplates

Aliquots of 1.5 mL of the growth medium formulated with D-glucose (20 g/L) and varying concentrations (5, 10, 20, and 40 g/L) of the SBY extracts (PEF-pH4; PEF-pH8; Seq-PEF-pH4 and Seq-PEF-pH8) were dispensed into 24-well microplates (Nunc™ Multidish 24, Thermo Fisher Scientific, Denmark). All media were sterilized by filtration using 0.1 µm pore-size membrane filters. Cultures were inoculated with *S. cerevisiae* Viniferm 3D to an initial density of 10^4 cells/mL. Growth kinetics were recorded at 30 °C over 24 h using a CLARIOstar microplate reader (BMG Labtech, Germany) by measuring optical density at 600 nm (OD_{600}). Absorbance readings were taken every 3 min, with double orbital shaking at 700 rpm prior to each measurement to ensure homogenization. For optical density (OD_{600}) measurements, each medium was blanked against its corresponding uninoculated medium to correct for background absorbance due to color or turbidity, thereby ensuring accurate monitoring of yeast growth. Each experiment was performed

in three independent biological replicates conducted on different days, with two technical replicates (duplicate wells) per condition in each run.

Growth Quantification by Plate Count Method

To evaluate the ability to support microbial growth, the PEF-assisted SBY extracts were compared with a commercial yeast extract (LP0021B, Oxoid) and with a standard reference medium commonly used for yeast growth, Sabouraud Dextrose Liquid Medium (SD; Oxoid). Growth media containing D-glucose (20 g/L) and 10 g/L of either the SBY extract or the commercial yeast extract were prepared in 200 mL flasks with a working volume of 50 mL. The concentration of 10 g/L of yeast extract was selected according to the results described in the previous section (growth assessment via optical density in microplates). SD was also used as an additional reference medium. Before sterilization, a mild heat treatment (90 °C for 5 min) was applied to remove high-molecular-weight proteins (> 35 kDa). The applied mild heat treatment did not affect FAN levels but induced partial protein coagulation (20–30%) only in the extract obtained at pH 8. The coagulated proteins were subsequently removed by centrifugation. Preliminary tests indicated that the removal of these proteins did not affect microbial growth. Following this step, all media were sterilized by autoclaving at 121 °C for 15 min.

Media were inoculated with *S. cerevisiae* Viniferm 3D to an initial density of 10^4 cells/mL and incubated at 30 °C for 48 h. Samples were taken every hour, serially diluted in buffered peptone water (Oxoid), and 100 µL aliquots were spread in duplicate on potato dextrose agar (PDA; Oxoid). Plates were incubated at 25 °C for 48 h, and microbial growth was quantified by counting colony forming units (CFU/mL). All experiments were performed in three independent biological replicates conducted on different days.

Mathematical Modelling

To enable quantitative comparisons under uniform experimental conditions, microbial growth kinetics were fitted using the modified Gompertz model, a well-established empirical approach for growth analysis across the biotechnological field (Wang & Guo, 2024). The equation used was:

$$N_t = N_0 + \left(N_{max} - N_0\right)e^{\left(-e^{(-b(t-M))}\right)} \quad (1)$$

where N_t is the cell concentration at time t; N_0 and N_{max} are the initial and maximum cell concentrations, respectively; b is the relative growth rate at the inflection point; and M is the time at which the maximum growth rate occurs. The lag phase duration (λ) was estimated using the expression:

$$\lambda = \mathrm{M} - \frac{1}{b} \quad (2)$$

Curve fitting was performed by non-linear regression using GraphPad Prism (v.8.4.2). The modified Gompertz model was manually implemented in the software by entering the corresponding Eq. (1), and parameters were estimated using the Levenberg–Marquardt algorithm by default. Model convergence and goodness of fit were automatically evaluated by the software based on residual minimization and the coefficient of determination (R^2).

Cell count data (CFU/mL) were $\log_{10}$-transformed prior to analysis, whereas optical density (OD_{600}) measurements were analyzed without transformation. Differences in Gompertz model parameters among treatments were assessed by one-way ANOVA using parameter estimates derived from biological replicates, and 95% confidence intervals were calculated from the variability of these replicate estimates.

White Must Fermentation

White grape must from *Vitis vinifera* cv. Chardonnay (2024 vintage) was provided by the Cooperativa San Juan Bautista (Fuendejalón, Aragón, Spain). The must had the following initial parameters: pH 3.3 ± 0.1 (Basic 20, Crison Instruments, Barcelona, Spain), °Brix 20.5 ± 0.05, and density 1.083 ± 0.0071 g/cm^3.

Fermentations were carried out in 500 mL Erlenmeyer flasks containing 200 mL of must. Two *S. cerevisiae* strains—Viniferm 3D and Viacell® Rhône 4600—were inoculated separately at an initial cell density of 10^6 cells/mL. The fermentation was conducted at 18 °C.

Upon reaching a residual density below 1.050 g/cm^3, fermentations were supplemented with either a SBY extract (PEF-pH8) or a commercial nitrogen supplement (Nutristart® ORG, Laffort SA, Bordeaux, France) composed of yeast autolysate (source of amino acids, vitamins, and minerals). Each supplement was added at two concentration levels (0.3 and 0.6 g/L), based on the manufacturer's dosage recommendations. Fermentation progression was monitored daily by recording weight loss of the flasks, used as an indirect measurement of CO_2 release. The percentage of weight loss was calculated using the following equation:

$$\%\text{Weight loss} = \frac{W_0 - W_x}{W_0} \times 100$$

where W_0 is the initial flask weight and W_x is the weight at each fermentation day.

Chemical Analysis

The quantification of soluble proteins and free α-amino nitrogen (FAN) was performed according to the methodologies described in Marín-Sánchez et al. (2024). Soluble protein content was measured using the BCA assay (Pierce™ BCA Protein Assay Kit, Thermo Fisher Scientific), while FAN was quantified using the ninhydrin colorimetric method with L-glycine as the standard.

The quantification of reduced glutathione (GSH) was conducted through a colorimetric method utilizing 5.5′-dithiobis-(2-nitrobenzoic acid) (DTNB) (Thermo Fisher Scientific), following the protocol described by Berzosa et al. (2024). Briefly, 960 µL of phosphate-buffered saline (PBS) at pH 7.5 containing 5.6 mM EDTA (Sigma-Aldrich) were mixed with 20 µL of a 0.4% DTNB solution in the same buffer, along with an additional 20 µL of the sample. After incubating for 2–10 min at room temperature, absorbance was measured at 412 nm. Glutathione concentrations were determined using a standard curve prepared with reduced L-glutathione (Sigma-Aldrich) ranging from 3.9 to 2000 µg/mL. The results were expressed as milligrams of reduced L-glutathione per gram of dry weight.

The main enological parameters of the resulting wines—including pH, total acidity, volatile acidity, remaining sugars (glucose + fructose) and malic acid—were determined at the end of alcoholic fermentation. All analyses were carried out at Cooperativa San Juan Bautista (Fuendejalón, Zaragoza, Spain), using validated methodologies commonly employed in the wine industry. Volatile acidity, malic acid, and residual sugars were quantified enzymatically using a Miura ONE autoanalyzer (I.S.E. Srl, Rome Italy). pH and total acidity were measured by potentiometry using pH-meter and acid–base titration with 0.1 M NaOH, respectively.

Statistical Analysis

Results are expressed as mean ± standard deviation from at least three independent experiments, each analyzed in duplicate. Statistical significance among treatments was assessed by one-way analysis of variance (ANOVA), followed by Tukey's post hoc test for multiple comparisons. All analyses were performed using IBM SPSS Statistics (Version 27, IBM Corp., Armonk, NY, USA), and differences were considered statistically significant at $p < 0.05$.

Results and Discussion

Composition of Nitrogenous Compounds in Extracts from Spent Brewer's Yeast Obtained by PEF-Assisted Extraction

Yeast extracts obtained from SBY treated by PEF were analyzed for their nitrogen content—specifically in the form of soluble proteins and free α-amino nitrogen (FAN)—to evaluate their potential as microbial nutrients (Fig. 2).

As shown in Fig. 2a, no statistically significant differences were observed in FAN concentrations between the PEF-pH8 and PEF-pH4 extracts. The release of amino acids, which constitute the majority of FAN, is primarily attributed to proteolytic activity triggered by membrane electroporation. Upon PEF application, the cytoplasmic membrane becomes permeable to small molecules, allowing water influx into the intracellular space and generating osmotic imbalance. This leads to the plasmolysis of vacuoles, where endogenous hydrolytic enzymes—including proteases—are

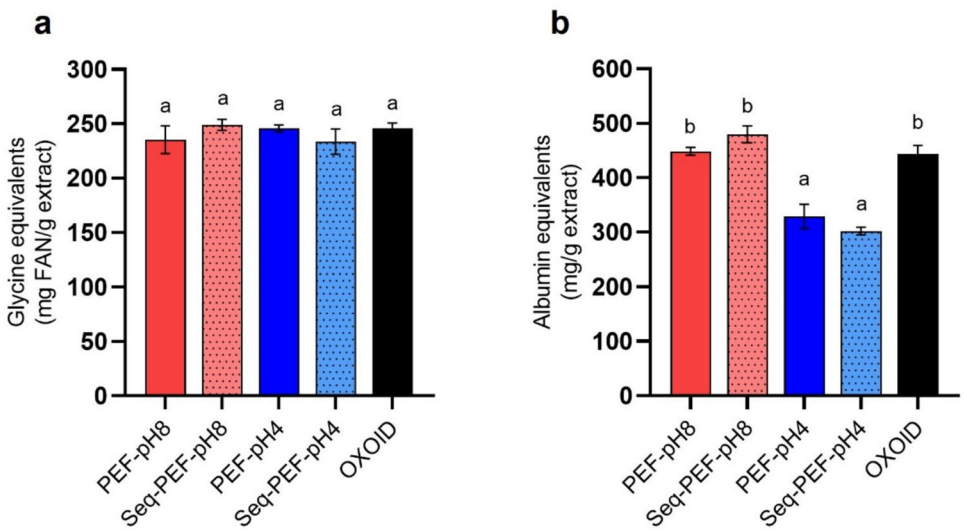

Fig. 2 Free α-amino nitrogen (FAN) (**a**) and soluble protein concentration (**b**) in yeast extracts obtained from spent brewer's yeast treated by PEF. Extracts were obtained either by direct incubation (PEF-pH4, PEF-pH8) or by a sequential strategy following prior glutathione extraction (Seq-PEF-pH4, Seq-PEF-pH8). A commercial yeast extract (OXOID) was included as reference. Values represent mean ± SD. Different letters indicate statistically significant differences ($p < 0.05$)

compartmentalized. As the pore size formed by electroporation is typically smaller than protease molecules, enzymatic hydrolysis takes place intracellularly, and the resulting low-molecular-weight amino acids subsequently diffuse out through the permeabilized membrane. This proteolytic activity occurred during the post-treatment incubation phase (37 °C, 48 h), where pH did not significantly influence FAN concentration, consistent with previous findings and attributable to the broad functional pH range of yeast proteases (Berzosa et al., 2024).

As previously described by Marín-Sánchez et al. (2024), the intracellular proteolysis induced by PEF represents a key technological advantage over mechanical disruption methods, such as high-pressure homogenization, which cause complete cell lysis and release proteases, proteins, and cell debris into the medium. This often results in more complex extracts that require additional purification steps to eliminate unwanted components (Oliveira et al., 2024).

To assess the feasibility of a PEF-assisted biorefinery approach, a sequential extraction strategy was also implemented. This involved an initial incubation step (30 °C, 1 h) targeting the recovery of intracellular glutathione, a high antioxidant compound of interest in food and biotechnological applications. This first extracts yielded GSH-reduced concentrations of 5.4 ± 0.38 mg/g and 4.6 ± 0.08 mg/g dry extract for the pH 8 and pH 4 conditions, respectively. The remaining biomass was then subjected to a second incubation (37 °C, 48 h), producing two additional nitrogenous extracts: Seq-PEF-pH8 and Seq-PEF-pH4 (Fig. 1). Importantly, the sequential extraction process did not result in a reduction of FAN content. No significant differences were found between sequential and non-sequential extracts at either pH (Fig. 2a) suggesting that early GSH extraction does not compromise the nitrogenous value of the final product in terms of free α-amino nitrogen. This is consistent with the fact that the initial 1-h extraction primarily allows for the diffusion of low-molecular-weight compounds present in the cytoplasm, such as GSH and a small pool of free amino acids naturally present in this compartment. In contrast, the majority of FAN is generated progressively during the subsequent 48-h incubation through intracellular proteolysis. Therefore, the amino acids recovered in the first extraction step represent only a minor fraction of the total FAN, and their removal does not significantly impact the overall amino acid content of the final extract (Yang et al., 2021).

With regard to protein concentration of the extracts (Fig. 2b), statistically significant differences were observed between pH conditions. Extracts obtained at pH 8 contained between 36 and 59% more soluble protein than extracts obtained at lower pH 4. This difference reflects enhanced protein solubilization under alkaline conditions, as previously reported by Ganeva et al. (2020), who also observed greater protein release at elevated pH following electroporation. However, within each pH, no significant differences in the extract protein concentration were observed between sequential and non-sequential extractions. These results, in conjunction with the FAN data, support the feasibility of a biorefinery cascade approach in which glutathione and nitrogen-rich fractions can be sequentially recovered from the same SBY biomass thereby enhancing both resource efficiency and process value.

The SBY extracts, particularly those obtained at pH 8, exhibit FAN and protein levels comparable to those of the commercial extract (OXOID) (Fig. 2). However, the nitrogen composition of yeast extracts is strongly influenced by the extraction method. Mechanical disruption techniques—such as high-pressure homogenization or ultrasonication—induce complete cell rupture, enabling rapid release of intracellular contents while preserving protein integrity. As a result, they typically yield extracts containing high total protein proportions (accounting for 50–64% of the extract composition) but low amino acid levels, due to limited proteolytic activity. In contrast, extraction strategies based on autolysis or enzymatic hydrolysis tend to favor the release of peptides and free amino acids (representing up to 30–40% of the extract composition) but often result in lower total protein proportions (usually below 35% of the extract) due to extensive proteolytic degradation (Jacob et al., 2019; Oliveira et al., 2022a, b; Vieira et al., 2016). In this context, the PEF-based process combined with moderate incubations offers a balanced compositional profile, allowing for extracts containing approximately 30–50% proteins and about 25% free amino acids relative to total solids. This selective outcome suggests that PEF induces a controlled enzymatic response mediated by endogenous yeast proteases, likely facilitated by electroporation. Unlike conventional strategies that rely on external enzymatic treatments or prolonged thermal autolysis, the PEF approach leverages the endogenous enzymatic potential of the yeast itself to promote protein hydrolysis, without the need for elevated temperatures. Indeed, recent findings have demonstrated that such PEF-triggered autolysis is significantly more efficient than heat-induced autolysis, achieving faster and higher liberation of nitrogen compounds (Berzosa et al., 2025).

The PEF-assisted extraction, applied under both direct and sequential processing protocols, yielded nitrogen-rich fractions with free amino nitrogen and protein contents comparable to those of commercial yeast extract. These compositional similarities, along with distinct nitrogen profiles, prompted further evaluation of their functional performance in microbial growth assays and fermentation trials, as described in the following sections.

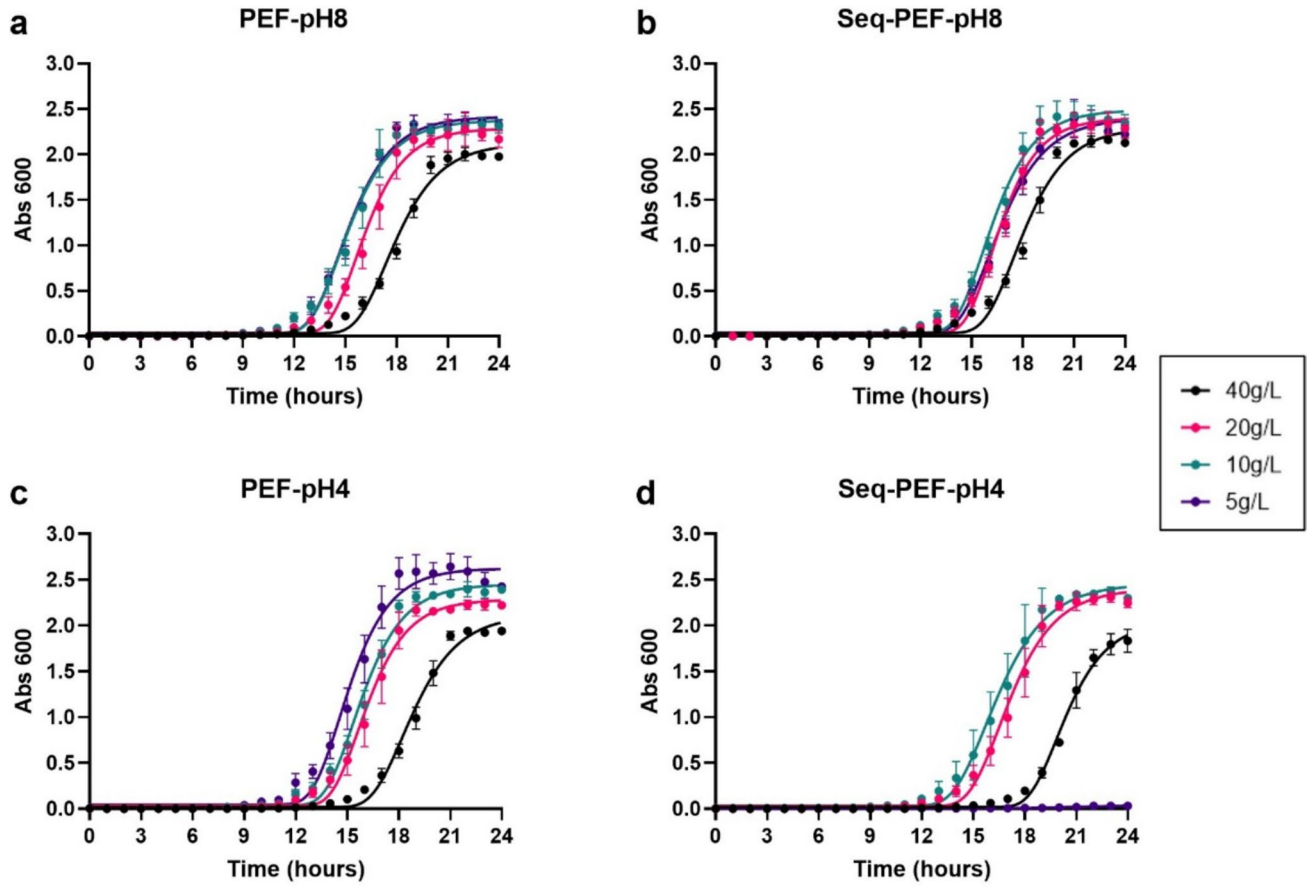

Fig. 3 Growth kinetics of *S. cerevisiae* Viniferm 3D in defined glucose (20 g/L) media supplemented with increasing concentrations (5–40 g/L) of nitrogen-rich extracts obtained from PEF-treated spent brewer's yeast. Growth was monitored by optical density (OD_{600} nm) at 30 °C for 48 h. Dots represent experimental measurements, and lines correspond to fitted Gompertz growth models. Results are shown for PEF-pH4 (**a**), PEF-pH8 (**b**), Seq-PEF-pH4 (**c**), and Seq-PEF-pH8 (**d**) extracts

Effect of SBY Extract Concentration on the Growth Kinetics of *Saccharomyces cerevisiae*

Figure 3 shows the growth curves of *S. cerevisiae* monitored by optical density in microplate assays under the tested conditions. All treatments exhibited a characteristic lag phase ranging from 12 to 18 h, followed by a rapid exponential growth phase. Most treatments reached the stationary phase between 18 and 19 h; however, at the highest concentration tested (40 g/L), the growth plateau was delayed to approximately 22–24 h. Additionally, no measurable growth was observed for the Seq-PEF-pH4 extract at 5 g/L, highlighting a concentration-dependent limitation specific to this fraction. A plausible explanation for this difference may be related to pH-dependent variations in the activity of endogenous yeast proteases, particularly proteases A and B, which are responsible for the majority of the overall proteolytic activity in *Saccharomyces cerevisiae*. These enzymes exhibit different pH optima: protease A is most active under acidic conditions (pH ~ 3.2), whereas protease B shows optimal activity near neutral pH (pH ~ 7.6) (Jones, 1991). It is therefore possible that proteolysis at pH 8 resulted in a more favorable release of amino acids for yeast growth, while proteolysis at pH 4 led to a less optimal composition, requiring higher extract concentrations to achieve similar effects.

To quantitatively assess growth behavior across the different extracts and concentrations, the curves were fitted using the modified Gompertz model (solid lines in Fig. 3), and the derived kinetic parameters are presented in Table 1. For most conditions, the specific growth rate (parameter *b*) did not differ significantly across concentrations or between extract types, indicating comparable metabolic capacity. However, biomass concentration (N_{max}), depended on the extract concentration. In fact, extract concentrations above 20 g/L led to a reduction in N_{max}, suggesting a potential inhibitory effect at elevated doses. This inhibitory trend at 40 g/L was supported by significantly prolonged lag phases (λ), which increased by 12 to 31% compared to the 5 g/L condition, and by 10 to 26% compared to 10 g/L. These effects were most pronounced in extracts obtained at pH 4. In both SBY extracts

Table 1 Estimated Gompertz model parameters for *S. cerevisiae* Viniferm 3D growth in media supplemented with different concentrations of PEF-derived SBY extracts

Yeast extract	Concentration (g/L)	N_0	N_{max}	b	M	λ	R^2
PEF-pH8	40	0.03 ± 0.00 **bcde**	2.12 ± 0.05 **bc**	0.57 ± 0.01 **bc**	17.43 ± 0.24 **de**	15.68 ± 0.24 **de**	0.989
	20	0.04 ± 0.01 **bcde**	2.27 ± 0.11 **bcd**	0.65 ± 0.05 **bc**	15.76 ± 0.46 **abc**	14.21 ± 0.48 **bc**	0.982
	10	0.04 ± 0.01 **bcde**	2.36 ± 0.07 **cde**	0.60 ± 0.03 **bc**	14.78 ± 0.46 **ab**	13.12 ± 0.44 **ab**	0.987
	5	0.04 ± 0.01 **bcde**	2.42 ± 0.07 **de**	0.59 ± 0.05 **bc**	14.64 ± 0.22 **a**	12.93 ± 0.36 **a**	0.991
Seq-PEF-pH8	40	0.04 ± 0.01 **cde**	2.30 ± 0.06 **bcd**	0.58 ± 0.03 **bc**	17.48 ± 0.32 **de**	15.74 ± 0.41 **de**	0.988
	20	0.04 ± 0.01 **de**	2.40 ± 0.12 **de**	0.67 ± 0.04 **c**	16.17 ± 0.23 **bcd**	14.67 ± 0.30 cd	0.989
	10	0.04 ± 0.01 **de**	2.49 ± 0.13 **de**	0.61 ± 0.04 **bc**	15.69 ± 0.19 **abc**	14.06 ± 0.18 **abc**	0.987
	5	0.03 ± 0.00 **bcde**	2.40 ± 0.18 **de**	0.56 ± 0.04 **bc**	16.06 ± 0.14 **abcd**	14.26 ± 0.26 **bc**	0.987
PEF-pH4	40	0.02 ± 0.00 **bc**	2.11 ± 0.02 **bc**	0.57 ± 0.01 **bc**	18.23 ± 0.30 **e**	16.47 ± 0.34 **ef**	0.990
	20	0.04 ± 0.01 **bcde**	2.28 ± 0.04 **bcd**	0.66 ± 0.02 **c**	15.69 ± 0.58 **abc**	14.18 ± 0.54 **bc**	0.986
	10	0.04 ± 0.01 **cde**	2.45 ± 0.05 **de**	0.63 ± 0.04 **bc**	15.32 ± 0.31 **abc**	13.73 ± 0.28 **abc**	0.992
	5	0.05 ± 0.00 **e**	2.63 ± 0.10 **e**	0.63 ± 0.05 **bc**	14.59 ± 0.44 **a**	13.01 ± 0.41 **a**	0.982
Seq-PEF-pH4	40	0.02 ± 0.00 **ab**	2.05 ± 0.17 **b**	0.62 ± 0.06 **bc**	19.87 ± 0.12 **f**	18.25 ± 0.08 g	0.988
	20	0.03 ± 0.00 **bcd**	2.40 ± 0.04 **de**	0.58 ± 0.03 **bc**	16.56 ± 0.62 cd	14.84 ± 0.56 cd	0.986
	10	0.03 ± 0.01 **bcde**	2.45 ± 0.01 **de**	0.54 ± 0.01 **bc**	15.79 ± 1.15 **abc**	13.93 ± 1.13 **abc**	0.984
	5	0.01 ± 0.00 **a**	0.07 ± 0.05 **a**	0.22 ± 0.12 **a**	21.97 ± 2.35 g	17.03 ± 0.26 **f**	0.840

Values represent means ± 95% CI. Different letters indicate statistically significant differences ($p < 0.05$)

obtained at this pH and at 40 g/L, the lag phase was significantly higher, and final cell density (N_{max}) was significantly reduced by 16% for PEF-pH4 and 19% for Seq-PEF-pH4, compared to the 10 g/L. Although a similar delay in growth onset was also observed for the pH 8 SBY extracts at 40 g/L, this did not result in a significant decrease in final biomass yield, suggesting that the lower efficacy of the extracts was more pronounced when the extraction was conducted under acidic conditions.

This trend observed at the 40 g/L concentration can be attributed to the presence of co-extracted inhibitory compounds. Among these, hop-derived polyphenols (e.g., iso-α-acids, tannins), which accumulate on the yeast cell wall during brewing, may be released during extraction and negatively impact microbial viability (Jaeger et al., 2020; Kruk et al., 2024). Additionally, medium-chain fatty acids and small nitrogenous metabolites—such as 3-aminoisobutyric acid, succinate, and ethanolamine—have been reported to exert inhibitory effects at high concentrations (Andújar-Ortiz et al., 2010; Tachibana et al., 2019). Antimicrobial peptides of yeast origin may also contribute to growth suppression under these conditions (Branco et al., 2017; Rizk et al., 2016). The 48-h incubation required for extract preparation favors the release of this diverse set of compounds. The combined presence of these compounds likely accounts for the extended lag phases and diminished biomass yield observed at the highest extract concentration.

Results shown in Fig. 3 served to define a suitable working concentration for subsequent comparative studies involving alternative nitrogen sources and culture media. Based on the overall growth performance, 10 g/L was selected as the minimal concentration that ensured yeast proliferation across all extracts. Although some extracts supported similar growth at 5 g/L, the Seq-PEF-pH4 extract failed to promote cell growth at this concentration, indicating that 10 g/L was the minimum effective concentration for yeast growth.

Assessment of the SBY Extracts as a Nitrogen Source for *S. cerevisiae*: Comparison with Commercial Yeast Extract and Standard Media

To further evaluate the performance of PEF-derived extracts from spent brewer's yeast as nitrogen sources, their ability to sustain *S. cerevisiae* Viniferm 3D growth was benchmarked against two commercial references: a standard yeast extract (OXOID) and Sabouraud Dextrose (SD) medium, the latter widely used as a complete, complex medium for laboratory yeast cultivation. For direct comparison, experimental media were formulated using glucose (20 g/L) as the sole carbon source and each nitrogen source (PEF extract or OXOID extract) at 10 g/L. The SD medium was prepared according to manufacturer specifications and served as an optimized reference medium for maximal yeast growth performance.

Yeast proliferation was monitored by viable cell counts (CFU/mL) over time. Figure 4 shows the resulting growth curves, fitted using the modified Gompertz model, with corresponding kinetic parameters summarized in Table 2.

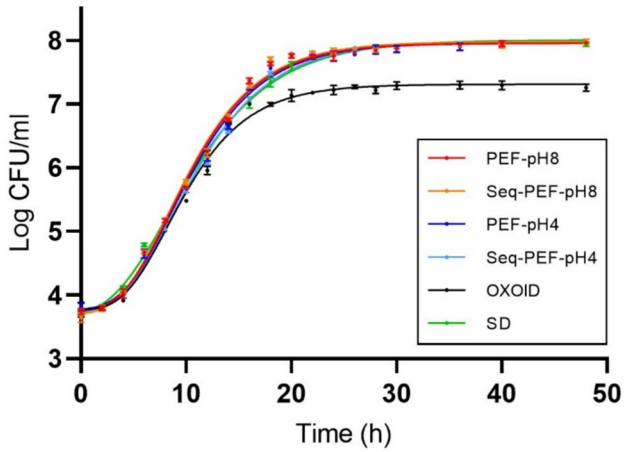

Fig. 4 Growth kinetics of *S. cerevisiae* Viniferm 3D in defined glucose media (20 g/L) supplemented with 10 g/L of PEF-derived SBY extracts, a commercial yeast extract (OXOID), or Sabouraud Dextrose (SD) medium. Growth was monitored by plate counts (log CFU/mL) at 30 °C for 48 h. Dots represent experimental values (mean ± SD) from replicates, and lines correspond to Gompertz model fits

Compared to the OD_{600}-based assays discussed in the "Effect of SBY Extract Concentration on the Growth Kinetics of *Saccharomyces cerevisiae*" section, all SBY extracts exhibited considerably shorter lag phases, ranging from 2.5 to 4 h, versus the 12–15 h previously reported. This reduction was also reflected in lower values of the model parameter *M*, indicating an earlier onset of exponential growth. These differences are attributable to the analytical methodology. Compared with spectrophotometric measurements (OD_{600}) that may overestimate lag duration at low biomass levels due to limited sensitivity, the colony-counting method provides more accurate quantification of early proliferative stages.

All SBY extracts supported robust and comparable growth kinetics, with no statistically significant differences observed in the estimated parameters (Table 2). Maximum cell densities (N_{max}) ranged from 7.96 to 7.98 log CFU/mL across all SBY extracts. Despite variations in protein concentration between extracts obtained at pH 4 and pH 8 (Fig. 2b), growth performance remained unaffected. Given that all extracts exhibited similar levels of FAN, these results suggest that FAN is a more critical determinant than total protein content for supporting yeast proliferation. This observation aligns with previous reports identifying free amino acids as the primary growth-promoting components in yeast extracts (Tachibana et al., 2019). Moreover, lag phase (λ) durations among SBY extracts were consistent (3.6–3.9 h), showing differences below ± 0.3 h, further support the equivalence in bioavailability of nitrogen sources obtained through both sequential and non-sequential extraction protocols, while demonstrating that the prior recovery of the glutathione-rich fraction does not impair the nutritional integrity of the final extract.

When compared to commercial products, no statistically significant differences in lag phase (λ) duration were observed among the PEF-derived and OXOID extracts. Only the SD medium showed a significantly shorter lag phase compared to all other conditions, reflecting its optimized composition for yeast cultivation. Despite this initial difference, the time required to reach stationary phase was similar across all conditions, including SD occurring between 19 and 20 h of incubation.

Moreover, while overall growth kinetics were comparable, and despite equivalent levels of FAN, the OXOID extract resulted in a significantly lower final cell density, approximately 0.6 log units below that achieved with the SBY extracts. This discrepancy indicates that total FAN content alone does not fully explain microbial growth performance, and that qualitative differences in amino acid composition are critical. Several studies have demonstrated that the amino acid profile of yeast extracts varies considerably depending on the origin of the biomass and the extraction technique employed (Jacob et al., 2019; Oliveira et al., 2022a, b; Podpora et al., 2016), which in turn directly impacts their effectiveness in supporting microbial proliferation. For instance, Andújar-Ortiz et al. (2010) observed that lactic acid bacteria displayed markedly different growth

Table 2 Growth parameters of *S. cerevisiae* Viniferm 3D fitted to the Gompertz model

Yeast extract	Log N_0	Log N_{max}	*b*	*M*	λ	R^2	RMSE	R^2_adj
PEF-pH8	3.75 ± 0.01 **a**	7.96 ± 0.01 **b**	0.22 ± 0.01 **c**	8.46 ± 0.08 **abc**	3.84 ± 0.08 **b**	0.996	0.092	0.995
Seq-PEF-pH8	3.77 ± 0.05 **a**	7.96 ± 0.05 **b**	0.21 ± 0.01 **bc**	8.64 ± 0.06 **bc**	3.91 ± 0.23 **b**	0.996	0.100	0.995
PEF-pH4	3.69 ± 0.08 **a**	7.98 ± 0.01 **b**	0.21 ± 0.01 **c**	8.29 ± 0.10 **ab**	3.60 ± 0.16 **b**	0.996	0.098	0.995
Seq-PEF-pH4	3.73 ± 0.01 **a**	7.98 ± 0.01 **b**	0.20 ± 0.01 **b**	8.82 ± 0.10 **c**	3.79 ± 0.14 **b**	0.997	0.091	0.996
OXOID	3.77 ± 0.08 **a**	7.31 ± 0.04 **a**	0.24 ± 0.01 **d**	8.20 ± 0.25 **a**	3.99 ± 0.35 **b**	0.994	0.099	0.993
SD	3.66 ± 0.07 **a**	8.00 ± 0.02 **b**	0.18 ± 0.01 **a**	8.30 ± 0.20 **ab**	2.77 ± 0.30 **a**	0.997	0.081	0.996

Cells were grown in defined glucose medium (20 g/L) supplemented with 10 g/L of PEF-derived yeast extracts, commercial yeast extract (OXOID), or in Sabouraud Dextrose (SD) medium. Values represent means ± 95% confidence interval. Different letters within a row indicate statistically significant differences ($p < 0.05$)

behaviors—ranging from stimulation to inhibition—when cultured with extracts derived from various inactivated dry yeasts (IDY), attributing these effects, among other factors, to differences in amino acid profiles. Likewise, Kruk et al. (2024) showed that SBY extracts obtained by sonication from different brewing strains yielded distinct amino acid compositions, which directly influenced the growth of *Propionibacterium* cultures. The superior growth observed with SBY extracts obtained from electroporated yeast biomass may thus be attributed to a more favorable amino acid composition. Although individual amino acids were not quantified in this study, previous results by Berzosa (2025) demonstrated that electroporation enhances proteolysis, leading to higher overall levels of free amino acids in the extracts. This suggests that PEF-assisted extraction may improve the amino acid composition compared to conventional heat-mediated autolysis commonly used in yeast extract production. Building on this, it is plausible that compositional differences in amino acid profiles also exist among the PEF-derived extracts themselves. In particular, the absence of growth at 5 g/L for the Seq-PEF-pH4 (Fig. 3d) extract may reflect a suboptimal spectrum or concentration of essential amino acids—insufficient to sustain *S. cerevisiae* proliferation at low supplementation levels.

Furthermore, the growth performance observed with SBY extracts—used as the sole nitrogen source—was comparable to that of SD, a complex medium specifically formulated for yeast cultivation. This highlights the ability of these nitrogen-rich fractions, obtained from an industrial by-product through PEF, to functionally match the performance of standard complex media.

Effect of SBY Extracts to Enhance Nitrogen Content of White Grape Must for Supporting Alcoholic Fermentation

Nitrogen is the main limiting nutrient for microbial growth under enological conditions, critically influencing fermentation kinetics (Dewan et al., 2025; Gobert et al., 2019; Sica et al., 2025). Nitrogen deficiency in grape musts, especially in the form of yeast assimilable nitrogen (YAN), is the primary cause of sluggish or stuck fermentations, as it reduces the biomass yield and delays sugar consumption (Varela et al., 2004). To counteract this limitation, nitrogen supplementation is a common practice during winemaking.

In this context, the enological potential of a nitrogen-rich extract obtained from SBY treated by PEF was evaluated in fermentation trials using white grape must. Based on previous results—where no significant differences were observed among the SBY extracts in terms of *S. cerevisiae* growth—the PEF-pH8 extract was arbitrarily selected to enhance nitrogen content of white grape must. Its fermentative performance was compared against a commercial yeast-derived nutrient commonly used in winemaking (ORG).

Since nitrogen uptake and utilization are strongly strain-dependent (Gobert et al., 2019), two *S. cerevisiae* strains with contrasting nitrogen requirements were selected for the study: Viniferm 3D (high nitrogen demand) and Viacell® Rhône 3600 (low nitrogen demand), according to supplier specifications. Fermentation kinetics, expressed as cumulative weight loss (reflecting CO_2 release during alcoholic fermentation), are shown in Fig. 5 for both strains, with and without (control) nutrient supplementation.

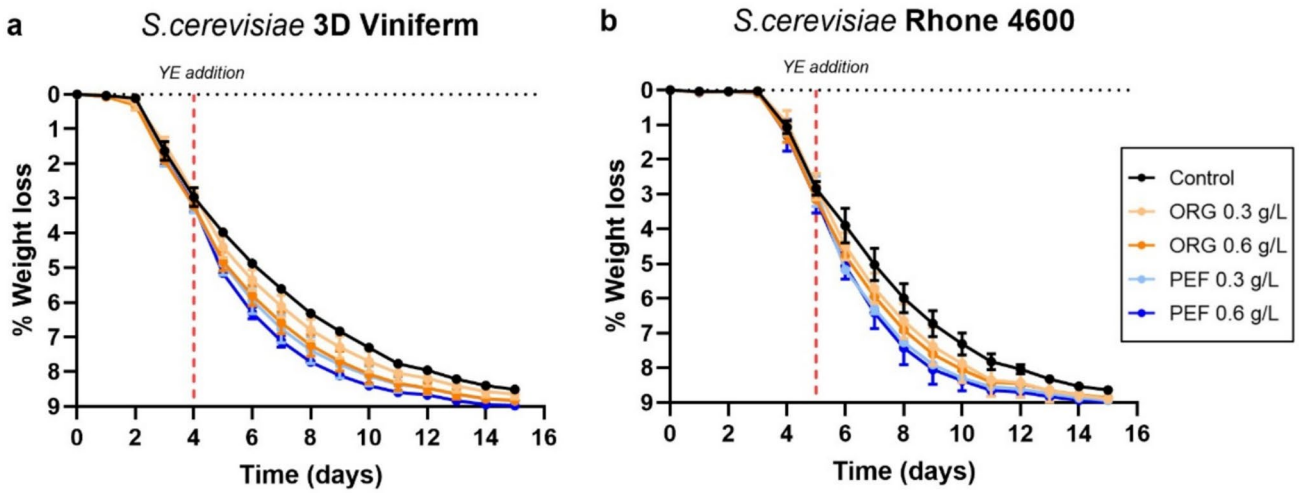

Fig. 5 Fermentation kinetics, measured as weight loss, of white grape must supplemented with the PEF-pH8 extract compared to a commercial yeast-derived nutrient (ORG) at two concentrations (0.3 and 0.6 g/L). Fermentations were carried out at 18 °C using two *S. cerevisiae* wine strains: Viniferm 3D (**a**) and Viacell® Rhône 3600 (**b**). A control condition without nutrient supplementation was included for each strain (control). The vertical dashed line indicates the time of yeast nutrient addition. Values represent mean ± SD from triplicate fermentations

Both strains exhibited an initial period of low fermentative activity during the first 3–4 days, with Viacell® Rhône 3600 initiating fermentation slightly later than Viniferm 3D. This delay corresponds to the period in which yeast cells were actively multiplying but had not yet reached the biomass threshold required to initiate measurable fermentation. Nutrient supplementation (PEF-pH8 or ORG) was performed when the must density had decreased by approximately 30 units (i.e., to < 1052) and in accordance with the ORG application protocol. This occurred on day 4 for Viniferm 3D (density: 1053) and on day 5 for Viacell® Rhône 3600 (density: 1050).

Following nutrient addition, a clear acceleration of fermentation kinetics was observed in all supplemented treatments compared to their respective controls. The enhanced CO_2 release, evidenced by a sharper rise in cumulative weight loss, confirmed the stimulatory effect of both PEF-pH8 and ORG extracts. Fermentations supplemented with the highest concentration (0.6 g/L) reached final weight loss values comparable to those of the controls, but in a significantly shorter time, up to four days earlier, indicating a faster progression toward fermentation completion. Similar results were reported by Seguinot et al. (2018), who observed an immediate increase in fermentative activity following nitrogen addition during the stationary phase, resulting in a comparable reduction in fermentation duration. However, other studies have reported no significant improvements in fermentation kinetics under similar conditions, despite observing enhancements in the aromatic profile of the resulting wines (Santamaría et al., 2020). As highlighted by Gobert (2019), the impact of nitrogen supplementation on fermentation dynamics is highly context-dependent, being strongly influenced by the yeast strain's nitrogen metabolism and the chemical composition of the must. Although FAN, amino acid profile, and micronutrient composition of the must were not analyzed in this study, such information would help better determine the contribution of nitrogenous compounds to the observed effects. Nevertheless, under the experimental conditions evaluated, the addition of yeast-derived extracts proved effective in enhancing fermentation kinetics and reducing total fermentation time, likely due to the presence of assimilable nitrogen compounds, as previously described in the literature.

Table 3 shows typical enological parameters of the resulting wines—including total and volatile acidity, malic acid, pH, and residual sugars at the end of the alcoholic fermentation (15 days). As previously reported by other studies (Santamaría et al., 2020), no statistically significant differences were observed in total acidity, volatile acidity, malic acid concentration, or pH between the supplemented and control wines, indicating that the addition of yeast extracts did not adversely affect the acid–base balance relevant attributes of the wine. Minor variations observed between yeast strains are likely attributable to inherent metabolic differences.

Importantly, statistically significant differences were observed in residual sugar content. All supplemented fermentations resulted in significantly lower residual sugar levels (< 2 g/L) compared to the controls (2.22–2.51 g/L), confirming a clear improvement in fermentative performance. Moreover, wines supplemented with the PEF-pH8 extract exhibited slightly greater sugar consumption than those treated with ORG at equivalent concentrations. Notably, supplementation with the lowest SBY extract dose (0.3 g/L) resulted in final residual sugar levels (0.67 ± 0.07 g/L) comparable to those achieved with the highest ORG dose (0.6 g/L) for both yeast strains.

According to the classification established by the International Organization of Vine and Wine, wines containing less than 4 g/L of residual sugars are considered dry. Based on this criterion, all fermentations—both supplemented and

Table 3 Oenological parameters of wines fermented with or without nutrient supplementation

S. cerevisiae strain	Yeast extract (g/L)	Volatile acidity (g/L)	Total acidity (g/L)	Malic acid (g/L)	pH	Remaining sugars (g/L)
Viniferm 3D	- (Control)	0.14 ± 0.01 **ab**	5.89 ± 0.01 **b**	1.65 ± 0.07 **b**	3.15 ± 0.01 **a**	2.51 ± 0.41 **e**
	ORG (0.3)	0.13 ± 0.00 **ab**	5.88 ± 0.01 **b**	1.62 ± 0.08 **b**	3.15 ± 0.01 **a**	1.60 ± 0.28 cd
	ORG (0.6)	0.12 ± 0.02 **a**	5.88 ± 0.03 **b**	1.63 ± 0.07 **b**	3.15 ± 0.00 **a**	0.73 ± 0.11 **ab**
	PEF-pH8 (0.3)	0.18 ± 0.06 **bc**	5.89 ± 0.01 **b**	1.60 ± 0.07 **b**	3.15 ± 0.02 **a**	0.57 ± 0.10 **ab**
	PEF-pH8 (0.6)	0.18 ± 0.02 **abc**	5.88 ± 0.01 **b**	1.61 ± 0.03 **b**	3.15 ± 0.01 **a**	0.53 ± 0.08 **a**
Viacell® Rhône 3600	- (Control)	0.21 ± 0.01 **cde**	5.25 ± 0.01 **a**	1.15 ± 0.02 **a**	3.19 ± 0.01 **b**	2.22 ± 0.40 **de**
	ORG (0.3)	0.24 ± 0.01 **de**	5.27 ± 0.02 **a**	1.16 ± 0.02 **a**	3.20 ± 0.03 **b**	1.21 ± 0.18 **bc**
	ORG (0.6)	0.23 ± 0.01 **de**	5.24 ± 0.01 **a**	1.16 ± 0.02 **a**	3.19 ± 0.01 **b**	0.71 ± 0.11 **ab**
	PEF-pH8 (0.3)	0.25 ± 0.02 **e**	5.25 ± 0.01 **a**	1.11 ± 0.02 **a**	3.19 ± 0.01 **b**	0.68 ± 0.18 **ab**
	PEF-pH8 (0.6)	0.25 ± 0.02 **e**	5.24 ± 0.03 **a**	1.12 ± 0.04 **a**	3.19 ± 0.01 **b**	0.39 ± 0.05 **a**

Fermentations were carried out at 18 °C for 15 days using *S. cerevisiae* strains Viniferm 3D and Viacell® Rhône 3600. Treatments included supplementation with PEF-pH8 extract or a commercial yeast-derived nutrient (ORG) at 0.3 and 0.6 g/L. A control without nutrient addition was included for each strain. Values represent means ± 95% confidence interval. Different letters within a row indicate statistically significant differences ($p < 0.05$)

non-supplemented—can be regarded as completed within the 15-day period. Based on the initial must density and °Brix measurements, the estimated sugar concentration was approximately 195 g/L, a value not indicative of a high fermentative burden. It is generally accepted that a YAN concentration of 120–140 mg N/L is sufficient to complete the fermentation of approximately 200 g/L of sugar (Bell & Henschke, 2005; Gobert et al., 2019). As the minimum nitrogen requirement for complete alcoholic fermentation is intrinsically linked to the initial sugar concentration (Martínez-Moreno et al., 2012), it is reasonable to assume that the must used in this study did not present a limiting nitrogen deficiency to complete fermentation. Nevertheless, the significantly lower residual sugar levels observed in the supplemented treatments suggest that nutrient addition enhanced fermentative performance, even under non-limiting nitrogen conditions, thereby demonstrating a technological advantage.

These results demonstrate that the PEF-derived extract from spent brewer's yeast not only supports complete alcoholic fermentation but performs comparably, or even slightly better, than a commercial enological nutrient. This highlights its potential as a sustainable and effective alternative for nitrogen supplementation in winemaking, particularly considering its origin from an abundant agro-industrial by-product.

Conclusions

The valorization of spent brewer's yeast aligns with the principles of a circular and sustainable agri-food system, offering a means to recover high-value compounds from an abundant brewing by-product. However, efficient extraction remains challenging due to the structural rigidity of the yeast cell wall and the risk of co-extracting undesirable compounds. Conventional disruption methods often suffer from poor selectivity or high processing demands.

This study demonstrates that PEF technology provides a more selective alternative for nitrogen recovery from SBY. By inducing controlled membrane permeabilization, PEF enables the release of soluble proteins and free amino acids while preserving overall cell structure and leveraging the yeast's own enzymatic machinery.

A major contribution of this work is demonstrating the feasibility of a sequential extraction strategy, enabling the recovery of a glutathione-rich antioxidant fraction followed by a nitrogen-rich extract without compromising nutrient quality. Importantly, following these extractions, the remaining yeast cell walls remain almost intact, offering further potential for downstream valorization, such as mannoprotein or β-glucan recovery. Both sequential and non-sequential nitrogen-rich extracts showed equivalent FAN levels and supported similar growth kinetics in *S. cerevisiae*.

Growth trials demonstrated that PEF-derived extracts matched the performance of commercial yeast extract in supporting yeast proliferation, with 10 g/L identified as the optimal concentration. In white grape must fermentations, the selected PEF extract performed comparably—or slightly better—than a commercial enological nutrient, accelerating fermentation and reducing residual sugar content.

Altogether, these results help to validate the potential of PEF as a platform technology for the valorization of SBY, enabling the sustainable production of both antioxidant and nitrogen-rich fractions. The proposed approach provides a scalable and modular solution for the generation of microbial nutrients from brewing residues, contributing to waste reduction, process efficiency, and resource circularity within the agro-industrial sector.

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Data Availability The authors declare that the data supporting the findings of this study are available within the paper. Should any data files be needed they are available from the corresponding author upon reasonable request.

Declarations

Competing interests The authors declare no competing interests.

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