Australian Journal of Grape and Wine Research

AUSTRALIAN SOCIETY OF VITICULTURE AND OENOLOGY | ASVO

RESEARCH ARTICLE OPEN ACCESS

Low-Temperature Rehydration of Active Dry Wine Yeasts: Nutrient Effects on Fermentation Performance and Membrane Fluidity

Elisa Aiello[1] | Mattia Pia Arena[1] | Carlo Montanini[2] | Andrea Pulvirenti[1] | Maria Gullo[1]

[1]Department of Life Sciences, University of Modena and Reggio Emilia, Reggio Emilia, Italy | [2]AEB S.p.A., Brescia, Italy

Correspondence: Maria Gullo (maria.gullo@unimore.it)

Received: 24 February 2026 | **Revised:** 19 May 2026 | **Accepted:** 26 May 2026

Academic Editor: Paul Ramon Grbin

ABSTRACT

Active dry yeast rehydration is a critical step in winemaking, as suboptimal temperatures can impair membrane recovery and compromise fermentation performance. This study evaluated the effects of rehydration at 20°C on commercial *Saccharomyces cerevisiae* and *Torulaspora delbrueckii* strains and assessed the efficacy of two commercial rehydration nutrients on fermentation kinetics and membrane fluidity. Results showed that both nutrients enhanced early fermentation in *S. cerevisiae* strains, with increased CO_2 production after 24 h, whereas *T. delbrueckii* showed no significant response. Membrane fluidity analysis revealed that yeast cells rehydrated at 20°C exhibited high Laurdan generalized polarization (GP) values, indicating increased lipid order, which significantly decreased after 24 h of fermentation. FERMOPLUS Energy Glu 4.0 was associated with lower initial GP values and reduced variation between postrehydration and postfermentation measurements, suggesting a membrane-stabilizing effect that may help optimize rehydration protocols in commercial winemaking.

1 | Introduction

The use of active dry yeasts (ADYs) represents a convenient and economically advantageous method for preserving yeast cells with high fermentative activity, as these preparations can be stored for extended periods without requiring special conditions [1]. In winemaking, ADY starter cultures have become a consolidated practice, offering reliability and availability across a wide range of strains selected for key enological traits suited to different wine types and fermentation conditions [2, 3]. During the production and use of ADY, yeast cells undergo significant metabolic modifications in response to drastic environmental changes. During production, cells experience oxidative stress from aerobic propagation and nutrient limitation, specifically carbon and nitrogen depletion, in the final growth phases, followed by desiccation stress during drying; these conditions induce reactive oxygen species (ROS) generation, lipid peroxidation, and membrane reorganization [4–6]. Subsequently, during use, cells face the challenges of rehydration and inoculation into grape must, an environment characterized by high sugar concentrations, low oxygen availability, and nutrient profiles that differ substantially from industrial growth media [7]. The rehydration process constitutes a critical phase that, if not performed with appropriate care, can severely compromise yeast inoculum health. Active dried yeast cells exhibit significantly reduced volume, substantial plasma membrane reorganization, and considerable tension between the membrane and the cell wall [8, 9]. Several factors contribute to the recovery of viable yeast from dried cultures, including rehydration temperature, duration, solute concentration, and genetic background [10, 11]. Among these, temperature plays a particularly important role, with an optimal range generally reported between 35°C and 43°C [12, 13]. This is because dehydration causes a membrane transition from a liquid-crystalline state to a gel state, and rehydration at these temperatures, facilitated by intracellular trehalose, allows the membrane to

return to the liquid-crystalline state, minimizing leakage [14]. At temperatures below 30°C, this transition may be impaired or incomplete, leading to loss of intracellular components and permanent membrane damage [2]. More broadly, low temperature decreases membrane fluidity and increases lipid order, and this membrane rigidification is considered one of the primary signals involved in the yeast cold-shock response [15]. Yeasts counteract temperature-induced changes in membrane physical state through homeoviscous adaptation, a conserved process based on the remodeling of membrane lipid composition [16]. In wine yeasts, this adaptation involves changes in fatty acid unsaturation and medium-chain fatty acid (MCFA) content during low-temperature fermentation [17, 18]. These compositional changes, essential for growth following temperature downshift, also include increased triacylglyceride levels and decreased phosphatidic acid (PA) content and phosphatidylcholine/phosphatidylethanolamine (PC/PE) ratio [19]. As intracellular solutes can be lost during the initial phase of rehydration, it has been hypothesized that exogenous compounds might conversely be taken up by the cell in the same time window [20, 21]. Building on this rationale, supplementing the rehydration medium with commercial inactive dry yeast (IDY) preparations derived from *Saccharomyces cerevisiae* has become a key strategy to actively exploit this uptake window and support cell recovery [22, 23]. These preparations release cell-wall fragments that solubilize sterols and facilitate the repair of damaged yeast membranes [22]. This mechanism is particularly relevant under the anaerobic conditions of alcoholic fermentation, when *S. cerevisiae* cannot synthesize its own sterols and must assimilate them externally [24, 25]. IDY preparations also deliver bioactive compounds, such as glutathione, that are actively taken up during this short window and influence subsequent fermentation [26]. Consistently, transcriptional analyses of rehydrated ADY have shown that carbon and nitrogen signals supplied at this stage rapidly activate ribosomal biogenesis, fermentative pathways, and the nonoxidative branch of the pentose phosphate pathway [27]. These nutrient-based strategies become especially relevant at the winery scale, where rehydration conditions are often suboptimal. Although industrial yeast manufacturers recommend rehydration at 35°C–43°C to maximize cell viability and fermentation performance, in small- and medium-sized wineries, technical constraints such as the lack of dedicated heating equipment, the unavailability of temperature-controlled water, or the need for rapid operational workflows often lead to the use of ambient water temperatures. Evaluating commercial strains and rehydration nutrients under such realistic, suboptimal conditions can therefore provide practical guidance to winemakers, minimize the risk of fermentation failure, reduce energy demand associated with water heating, and support the development of more robust protocols, including the use of rehydration nutrient formulations. Based on these premises, we evaluated the effects of a suboptimal rehydration temperature (20°C) on selected *S. cerevisiae* and *Torulaspora delbrueckii* industrial enological yeast strains, preserved at the Unimore Microbial Culture Collection (UMCC) and commercialized as wine starters by AEB S.p.A., and assessed the efficacy of two rehydration nutrients, FERMOPLUS Energy Glu 3.0 and FERMOPLUS Energy Glu 4.0 (AEB S.p.A.), focusing on improving fermentation performance and stabilizing membrane fluidity. The parallel evaluation of two rehydration nutrients of different composition, tested on four industrial strains belonging to two different species, was intended to generate strain- and species-specific evidence, supporting the development of tailored rehydration protocols rather than broad generalizations across commercial preparations. These results may contribute to the optimization of rehydration protocols in commercial winemaking, with possible implications for operational flexibility, reduced production time, energy consumption, and costs, as well as the adoption of more targeted, strain-specific strategies, in line with the increasing demand for more sustainable practices in the wine sector.

2 | Materials and Methods

2.1 | ADY Strains and Rehydration Procedure

In this study, four strains of commercial ADY, produced and supplied by AEB S.p.A. (Brescia, Italy), were investigated. Three strains belonged to the species *S. cerevisiae*, whereas one strain belonged to the species *T. delbrueckii*. All strains were deposited at the UMCC of the University of Modena and Reggio Emilia, Italy. The yeast strain codes, species, and commercial names are listed in Table 1.

Before microvinification trials, the rehydration process was performed by suspending the ADY in a sucrose solution (50 g/L) at a ratio of 1:10 (g of yeast to mL of solution). The rehydration was carried out at 20°C for 30 min, either with or without the addition of rehydration nutrients. Two commercial rehydration nutrients were tested: FERMOPLUS Energy Glu 3.0 and FERMOPLUS Energy Glu 4.0 (AEB S.p.A, Brescia, Italy).

The composition and recommended dosage of these nutrients are reported in Table 2.

2.2 | Microvinification Trials

Microvinifications were performed in triplicate using 100-mL glass flasks filled with 90 mL of commercial red grape juice (Quargentan, Verona, Italy; pH 3.36; titratable acidity 5.7 g/L; total sugars 170.83; 1.55 g/L tartaric acid; 3.70 g/L malic acid; 0.16 g/L citric acid), sterilized by filtration through 0.2-μm membrane filters (Thermo Scientific, United States). Subsequently, 5 mL of the rehydrated ADY (corresponding to approximately 10^9 CFU/mL) was inoculated, and the flasks were

TABLE 1 | Yeast strains used in this study.

UMCC code	Species	Commercial name	Commercial code
UMCC 3066	*Saccharomyces cerevisiae*	Fermol Fleur	PB2171
UMCC 2592	*Saccharomyces cerevisiae*	Fermol Red Fruit	PB2018
UMCC 19	*Saccharomyces cerevisiae*	Fermol Blanc	PB2019
UMCC 5	*Torulaspora delbrueckii*	Levulia Torula	BBMV3FA5

TABLE 2 | Composition and dosage of rehydration nutrients used in this study.

Product	Composition	Dosage
FERMOPLUS Energy Glu 3.0	Yeast hulls, yeast autolysates, thiamine hydrochloride (vitamin B1)	1:4 (nutrient:yeast)
FERMOPLUS Energy Glu 4.0	Yeast hulls, yeast autolysates, diammonium hydrogen phosphate (DAP), thiamine hydrochloride (vitamin B1)	1:4 (nutrient:yeast)

sealed with 5 mL of sterile paraffin oil (Carlo Erba, Milan, Italy) to ensure anaerobic conditions. Uninoculated filtered grape juice served as a negative control. Fermentation was conducted at 25°C under static conditions for 12 days. The progress of fermentation was monitored daily by measuring the weight loss of each flask, which corresponds directly to the amount of CO_2 released per flask (g) during alcoholic fermentation. Weight loss was monitored until a constant weight was maintained for three consecutive days [28]. The fermentative vigor (FV) was determined as the cumulative CO_2 production (g) after 48 h of fermentation [29]. Additionally, CO_2 production after 24 h was recorded as an early indicator of fermentation kinetics. At the end of fermentation, samples were collected and immediately analyzed for pH and titratable acidity. Aliquots for high-performance liquid chromatography (HPLC) analysis were stored at −20°C until analysis.

2.3 | Analytical Methods

The pH and titratable acidity of the samples were measured using an XS pH 80 PRO STIRRER (Securlab, Rome, Italy). Titratable acidity was determined by titration with 0.1 M NaOH solution and expressed as grams of tartaric acid per liter. Sugars (glucose and fructose), ethanol, glycerol, and organic acids (succinic, acetic, citric, tartaric, and malic acids) were quantified by HPLC [29]. Briefly, samples collected at the end of fermentation were filtered through 0.45-μm nitrocellulose membranes before analysis. About 20 μL of each filtered sample was injected into a Jasco LC-Net II/ADC HPLC system (Jasco Inc., Hachioji, Japan) equipped with a Jasco PU-2080 Plus pump. Isocratic elution was performed using an Aminex HPX-87H column (300 × 7.8 mm; Bio-Rad Laboratories, Segrate, Italy) maintained at 40°C with an Eldex CH-150 oven (Eldex Corp., Napa, CA, USA). The mobile phase consisted of 0.005 N H_2SO_4 and 5% (v/v) acetonitrile, at a flow rate of 0.6 mL/min. Calibration curves were generated using external standards, and peak integration was performed using Jasco ChromNav software v. 1.18.03 (Tokyo, Japan).

2.4 | Membrane Fluidity Assessment by Laurdan Generalized Polarization (GP)

Membrane fluidity was assessed using the Laurdan (6-dodecanoyl-2-dimethylaminonaphthalene) GP assay, following the protocol described by [30]. Samples were collected at two time points: immediately after the 30 min of rehydration (T0) and after 24 h of fermentation at 25°C (T24). A cell aliquot was standardized by diluting to 0.4 OD_{600} and harvested by centrifugation (8800 × g for 5 min), washed with phosphate-buffered saline (PBS; pH 7.4; Sigma-Aldrich, St. Louis, MO, USA), and resuspended in fresh PBS. To incorporate the fluorescent probe, samples were incubated with 5 μM Laurdan (dissolved in absolute ethanol) (Cayman Chemical, Ann Arbor, MI, USA) for 1 h at 30°C [31].

Membrane fluidity measurements were performed using a Jasco FP-6200 spectrofluorometer (Jasco Inc., Hachioji, Japan). A cuvette containing an unlabeled cell suspension at the same cell density was used to measure background fluorescence, which was subtracted from the fluorescence readings obtained from the labeled cell suspension. The experiment was carried out in triplicate. The results were expressed as GP, calculated using the following equation:

$$GP = \frac{(I_{440} - I_{490})}{I_{440} - I_{490}}, \quad (1)$$

where I_{440} and I_{490} represent the emission intensities at 440 nm and 490 nm, respectively. To better understand the membrane fluidity variations between the two time points, results were also expressed as *ΔGP (GP_T0–GP_T24)*.

2.5 | Statistical Analysis

All experiments were performed in triplicate. Results are expressed as mean ± standard deviation (SD). Data were analyzed by one-way and two-way ANOVA, followed by Tukey's HSD post hoc test for mean comparisons, with significance set at $p < 0.05$. Statistical analyses were performed using SPSS software v. 20.0.0 (IBM, Chicago, IL, USA). Principal component analysis (PCA) was performed using Python (version 3.12.3) with the scikit-learn library (version 1.7.2) on a dataset of 12 phenotypic variables: residual glucose, residual fructose, ethanol yield, glycerol yield, succinic acid yield, acetic acid yield, citric acid yield, tartaric acid, titratable acidity, malic acid consumption, CO_2 production at 24 h, and FV. Scatterplots with 95% confidence ellipses were generated using Matplotlib (version 3.10.7).

3 | Results and Discussion

3.1 | Evaluation of Fermentative Performance With Rehydration Nutrients

In this study, the fermentative performance of commercially available yeast strains was evaluated to better understand the effectiveness of rehydration nutrients under suboptimal conditions. The differences between the two nutrient treatments and the control are illustrated in Figure 1, which shows the CO_2 production kinetics, and in Table 3, which summarizes both the CO_2 produced after 24 h and the FV (CO_2 after 48 h).

The results showed an increase in CO_2 production after 24 h for all *S. cerevisiae* strains (UMCC 19, UMCC 3066, and UMCC 2592) when rehydrated with either FERMOPLUS Energy Glu 3.0 or 4.0, compared to the control without nutrients. Additionally, a trend toward higher FV values at 48 h was observed for all strains treated with rehydration nutrients, although this increase was not statistically significant for all strains. However, this enhanced early fermentative activity did not translate into differences in

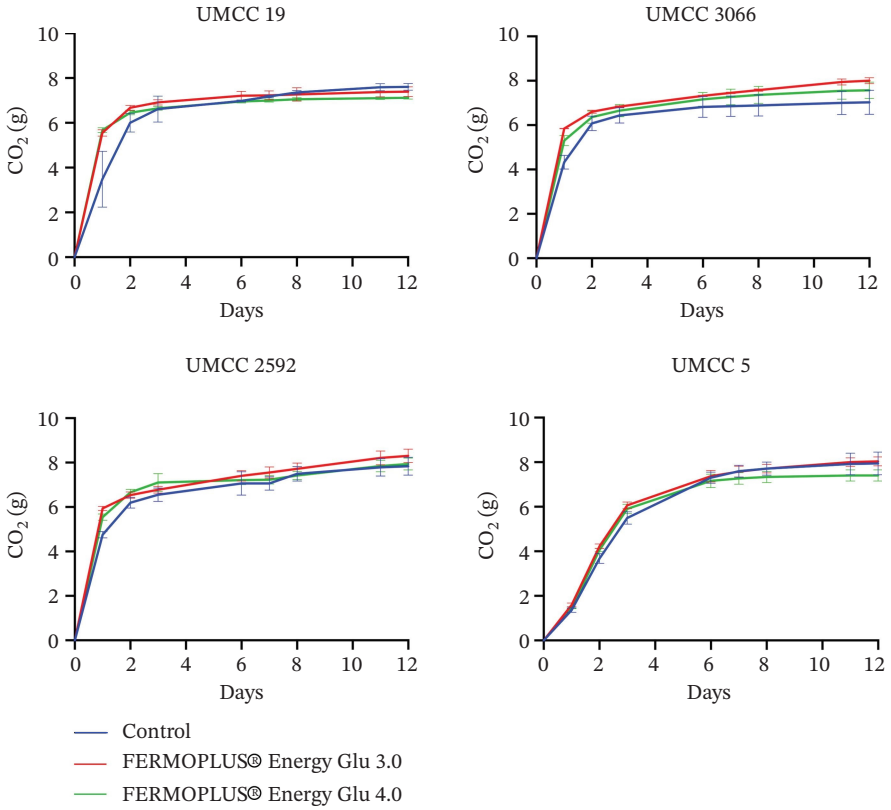

FIGURE 1 | CO_2 evolution over a 12-day microfermentation trial at 25°C in 0.2-μm-filtered grape juice for *S. cerevisiae* strains (UMCC 19, UMCC 3066, and UMCC 2592) and *T. delbrueckii* strain (UMCC 5). The control (revitalized strain without nutrients) is compared with two nutrient treatments, FERMOPLUS Energy Glu 3.0 and FERMOPLUS Energy Glu 4.0. Curves show mean ± standard deviation ($n = 3$).

TABLE 3 | CO_2 production after 24 h (g) and fermentative vigor (FV; g CO_2 after 48 h) for *S. cerevisiae* strains (UMCC 19, UMCC 3066, and UMCC 2592) and *T. delbrueckii* strain (UMCC 5) under different rehydration conditions.

Strain	CO_2 after 24 h (g)			FV (g CO_2 after 48 h)		
	Control	Energy Glu 3.0	Energy Glu 4.0	Control	Energy Glu 3.0	Energy Glu 4.0
UMCC 19	3.49 ± 1.26^{d}	5.55 ± 0.15^{ab}	5.67 ± 0.10^{ab}	6.00 ± 0.41^{e}	6.68 ± 0.11^{d}	6.45 ± 0.10^{de}
UMCC 3066	4.33 ± 0.31^{cd}	5.85 ± 0.01^{ab}	5.31 ± 0.23^{abc}	6.08 ± 0.32^{de}	6.60 ± 0.07^{de}	6.36 ± 0.27^{de}
UMCC 2592	4.75 ± 0.14^{bc}	5.94 ± 0.10^{a}	5.55 ± 0.16^{ab}	6.20 ± 0.25^{de}	6.54 ± 0.15^{de}	6.66 ± 0.14^{d}
UMCC 5	1.37 ± 0.10^{e}	1.59 ± 0.09^{e}	1.43 ± 0.01^{e}	3.68 ± 0.22^{f}	4.23 ± 0.10^{f}	4.06 ± 0.08^{f}

Note: Values are expressed as mean ± SD ($n = 3$). Different superscript letters within the same parameter indicate statistically significant differences ($p < 0.05$, Tukey's test). Control = revitalized strain without nutrients; Energy Glu 3.0 = revitalized strain with FERMOPLUS Energy Glu 3.0; Energy Glu 4.0 = revitalized strain with FERMOPLUS Energy Glu 4.0.

terms of CO_2 production at the end of fermentation. The lag phase represents a critical period during which yeast cells undergo substantial metabolic reorganization to adapt to the grape must environment, facing osmotic, pH, temperature, and sulfur dioxide stresses. During this phase, transcriptional and proteomic changes involving nitrogen and carbon metabolism enzymes may determine proper adaptation and could potentially affect fermentative capacity and performance [32]. From a technological perspective, enhanced vigor at the onset of fermentation may facilitate the prevalence of the inoculated starter over competing indigenous microbial populations. According to the manufacturer's technical sheet, FERMOPLUS formulations contain readily assimilable amino acids, sterols, fatty acids, and glutathione. By providing these compounds directly, cells might allocate more energy to multiplication during rehydration, when energy expenditure is presumably greatest. The importance of nutrient availability for yeast recovery after desiccation has been demonstrated by [33], who reported that reactivation in a nutrient-rich growth medium was more efficient than rehydration in water alone. In contrast, *T. delbrueckii* UMCC 5 did not show significant differences between nutrient treatments and control, neither at 24 nor at 48 h. The lack of a significant response of *T. delbrueckii* UMCC 5 to nutrient supplementation is consistent with the well-established slower fermentative kinetics of this non-*Saccharomyces* species, which could make differences less pronounced [34–37].

To assess the fermentative performance of the four yeast strains under the different rehydration conditions, the final fermentation products were characterized for the main enological parameters at the end of the process (Table 4). The effects of strain, rehydration treatment, and their interaction were evaluated by two-way ANOVA, considering strain and treatment as fixed factors. Residual sugar concentrations were influenced only by strain, with a significant effect on glucose ($p < 0.05$) and a more pronounced effect on fructose ($p < 0.001$). In all cases, fructose remained higher than glucose, a pattern consistent with the preferential consumption of glucose by both *S. cerevisiae* and *T. delbrueckii*, which has been related to differences in the affinity of hexose transport systems [38]. Conversely, neither ethanol yield nor final ethanol concentration was significantly affected by strain, treatment, or their interaction, indicating that the use of FERMOPLUS Energy Glu 3.0 and 4.0 during rehydration did not modify the final ethanol production. Glycerol yield was markedly influenced by strain ($p < 0.001$), treatment ($p < 0.001$), and the strain × treatment interaction ($p < 0.01$). In yeast alcoholic fermentation, glycerol is produced through the glyceropyruvic pathway, where part of the carbon flux derived from glucose is redirected from ethanol formation toward glycerol synthesis. This process involves the reduction of dihydroxyacetone phosphate by glycerol-3-phosphate dehydrogenase, encoded by *GPD1* and *GPD2*. Under oxygen-limited fermentative conditions, this pathway contributes to the reoxidation of cytosolic NADH generated during metabolism [39]. The increased glycerol yields observed in the presence of FERMOPLUS Energy Glu 3.0 and 4.0 may therefore indicate a supplementation-dependent shift in carbon and redox metabolism during fermentation. Among the tested strains, *S. cerevisiae* UMCC 3066 consistently showed the highest glycerol yields, supporting the view that the effect of nutrient additions can vary according to both yeast species and the metabolic product considered [40]. From an enological perspective, glycerol is relevant because it can contribute to wine mouthfeel, flavor persistence, and perceived sweetness [41], with sensory effects on sweetness generally reported in the range of 5–12 g/L and effects on viscosity at concentrations equal to or above 28 g/L [42]. However, the glycerol concentrations measured in the present study were below these sensory thresholds. Succinic acid yield showed a similar response pattern, being significantly affected by strain ($p < 0.001$), treatment ($p < 0.05$), and their interaction ($p < 0.01$). During alcoholic fermentation, succinate production has been mainly associated with the reductive branch of the tricarboxylic acid (TCA) pathway, whereas the succinate dehydrogenase complex is considered inactive under anoxic fermentative conditions [43]. The concomitant increase in glycerol and succinate, especially in UMCC 3066 supplemented with FERMOPLUS Energy Glu 3.0, suggests that nutrient supplementation may have promoted a broader metabolic response involving both redox balancing and organic acid formation.

Acetic acid is another by-product of yeast fermentative metabolism. In *S. cerevisiae*, it is formed in the cytosol from the oxidation of acetaldehyde by aldehyde dehydrogenases as part of the pyruvate dehydrogenase bypass, with the Mg^{2+}-activated, $NADP^{+}$-dependent Ald6p playing a major role [43]. In the present study, acetic acid yield was significantly affected by strain ($p < 0.001$), whereas no significant treatment effect was detected. In all conditions, acetic acid concentrations remained well below the 0.7–1.0 g/L range commonly associated with the perception of vinegar-like off-flavors in wine [44–46]. Citric acid yield was influenced only by strain ($p < 0.001$). During alcoholic fermentation, citrate release into the medium can derive from residual activity of the TCA pathway [47], and the final concentration may therefore reflect strain-specific differences in this metabolic activity. *T. delbrueckii* UMCC 5 showed the lowest citrate yields under all tested conditions, clearly separating from the three *S. cerevisiae* strains. This result is consistent with previous observations indicating that *T. delbrueckii* only slightly modifies citric acid concentration during fermentation [35].

Malic acid consumption was strongly affected by strain ($p < 0.001$) and treatment ($p < 0.001$), whereas the interaction between these factors was not significant. Among the *S. cerevisiae* strains, UMCC 2592 showed the greatest malate degradation, reaching 34% in the presence of FERMOPLUS Energy Glu 4.0. This agrees with the known strain-dependent variability of malic acid degradation within *Saccharomyces* yeasts [48]. By contrast, *T. delbrueckii* UMCC 5 showed the lowest malate consumption, ranging from 3.4% to 11.7%, in line with the description of *T. delbrueckii* as a moderate consumer of must malic acid [35]. The higher malate degradation observed under nutrient-supplemented conditions also agrees with the stimulatory effect of nutrient availability on malic acid consumption previously reported for *T. delbrueckii* [35]. Tartaric acid content and pH were not significantly affected by either strain or treatment, whereas titratable acidity showed a significant strain-dependent variation ($p < 0.001$).

Afterward, PCA was applied to a subset of 12 phenotypic variables (see Section 2.5). The first and second PCA components together explain 54.5% of the overall variability. PC1 accounted for 35.4% of the variance and showed strong positive loadings for CO_2 production at 24 h (0.973) and FV (0.962), as well as for malic acid consumption (0.842), succinic acid yield (0.753), and citric acid yield (0.734). Accordingly, PC1 captured variation primarily associated with early fermentation kinetics and overall fermentative performance and was also linked to a greater degradation of malic acid. PC2 accounted for 19.1% of the variance and was mainly influenced by titratable acidity (loading = 0.826), tartaric acid (loading = 0.777), and acetic acid yield (loading = 0.729). The PCA score plot grouped by yeast strain (Figure 2) revealed a separation of *T. delbrueckii* UMCC 5 from the three *S. cerevisiae* strains along PC1. UMCC 5 was positioned on the negative side of this component, consistent with the significantly lower CO_2 release at 24 h and reduced FV observed in Table 3. This pattern reflects the slower early fermentation kinetics commonly reported for *T. delbrueckii* compared to *S. cerevisiae* [49]. Additionally, UMCC 5 exhibited significantly lower citric acid values compared to *S. cerevisiae* strains (Table 4), a trait previously reported for *T. delbrueckii* under similar fermentation conditions [30], as well as a significantly lower malic acid consumption (Table 4). The *S. cerevisiae* strains (UMCC 19, UMCC 3066, and UMCC 2592) clustered on the positive side of PC1, indicating overall comparable fermentation profiles under the tested conditions; a moderate separation along PC2 was nevertheless observed, reflecting the differences detected in the fermentation metabolites (Table 4). When samples were grouped by rehydration treatment (Figure S1), the three rehydration conditions (Control, FERMOPLUS Energy Glu 3.0, and FERMOPLUS Energy Glu 4.0) showed largely overlapping 95%

TABLE 4 | Values of residual sugars (glucose and fructose, g/L), ethanol yield (g per 100 g of consumed sugar), ethanol concentration (% v/v), glycerol, succinic acid, acetic acid, and citric acid yields (g per 100 g of consumed sugar), malic acid consumption (%), tartaric acid (g/L), titratable acidity (TA; g/L), and pH for the four yeast strains under the three rehydration conditions.

Strain	Treatment	Glucose (g/L)	Fructose (g/L)	Ethanol yield (%)	Ethanol (% v/v)	Glycerol yield (%)	Succinic acid yield (%)	Acetic acid yield (%)	Citric acid yield (%)	Malic acid consumption (%)	Tartaric acid (g/L)	TA (g/L)	pH
UMCC 19	Control	1.87 ± 0.39^{a}	3.67 ± 0.16^{ab}	45.41 ± 1.01^{a}	9.14 ± 0.18^{a}	2.44 ± 0.09^{f}	1.59 ± 0.07^{bc}	0.10 ± 0.01^{abc}	0.37 ± 0.02^{ab}	15.60 ± 3.86^{efg}	1.70 ± 0.19^{a}	7.63 ± 0.06^{ab}	3.21 ± 0.08^{a}
	Energy Glu 3.0	2.19 ± 0.44^{a}	4.08 ± 0.28^{ab}	44.27 ± 5.74^{a}	8.87 ± 1.16^{a}	2.74 ± 0.01^{e}	1.86 ± 0.04^{ab}	0.11 ± 0.01^{abc}	0.26 ± 0.06^{ab}	23.47 ± 3.33^{bcde}	1.48 ± 0.37^{a}	7.47 ± 0.21^{abc}	3.17 ± 0.14^{a}
	Energy Glu 4.0	2.00 ± 0.00^{a}	4.17 ± 0.47^{ab}	47.74 ± 0.13^{a}	9.58 ± 0.00^{a}	2.91 ± 0.10^{de}	1.87 ± 0.05^{ab}	0.16 ± 0.02^{abc}	0.20 ± 0.10^{ab}	28.41 ± 0.84^{abc}	1.76 ± 0.02^{a}	7.57 ± 0.06^{abc}	3.19 ± 0.05^{a}
UMCC 3066	Control	2.41 ± 0.49^{a}	3.69 ± 0.06^{ab}	46.85 ± 3.24^{a}	9.40 ± 0.62^{a}	3.40 ± 0.21^{ab}	1.75 ± 0.04^{abc}	0.20 ± 0.09^{a}	0.26 ± 0.22^{ab}	17.26 ± 5.31^{defg}	1.60 ± 0.35^{a}	7.67 ± 0.06^{ab}	3.20 ± 0.05^{a}
	Energy Glu 3.0	2.29 ± 0.65^{a}	3.79 ± 0.23^{ab}	45.15 ± 2.05^{a}	9.06 ± 0.41^{a}	3.69 ± 0.08^{a}	2.10 ± 0.37^{a}	0.17 ± 0.00^{ab}	0.32 ± 0.28^{ab}	22.53 ± 0.46^{bcde}	1.61 ± 0.38^{a}	7.70 ± 0.26^{a}	3.27 ± 0.10^{a}
	Energy Glu 4.0	2.27 ± 0.45^{a}	3.91 ± 0.22^{ab}	44.46 ± 1.06^{a}	8.92 ± 0.24^{a}	3.45 ± 0.01^{ab}	1.67 ± 0.04^{bc}	0.16 ± 0.01^{abc}	0.35 ± 0.06^{ab}	20.73 ± 5.06^{cdef}	1.74 ± 0.10^{a}	7.63 ± 0.15^{ab}	3.29 ± 0.02^{a}
UMCC 2592	Control	2.79 ± 0.01^{a}	3.91 ± 0.34^{ab}	44.52 ± 1.87^{a}	8.90 ± 0.39^{a}	2.93 ± 0.10^{de}	1.73 ± 0.03^{bc}	0.06 ± 0.03^{c}	0.30 ± 0.05^{ab}	26.06 ± 3.07^{abcd}	1.24 ± 0.31^{a}	7.23 ± 0.15^{c}	3.12 ± 0.09^{a}
	Energy Glu 3.0	2.26 ± 0.35^{a}	3.46 ± 0.12^{b}	45.62 ± 3.75^{a}	9.18 ± 0.73^{a}	3.30 ± 0.16^{bc}	1.81 ± 0.04^{ab}	0.07 ± 0.02^{bc}	0.47 ± 0.02^{a}	31.06 ± 1.87^{ab}	1.19 ± 0.45^{a}	7.40 ± 0.10^{abc}	3.19 ± 0.17^{a}
	Energy Glu 4.0	1.95 ± 0.19^{a}	3.47 ± 0.26^{b}	47.05 ± 1.58^{a}	9.48 ± 0.34^{a}	3.07 ± 0.01^{cd}	1.70 ± 0.02^{bc}	0.12 ± 0.08^{abc}	0.35 ± 0.11^{ab}	34.38 ± 1.83^{a}	1.74 ± 0.12^{a}	7.37 ± 0.12^{abc}	3.24 ± 0.14^{a}
UMCC 5	Control	1.86 ± 0.24^{a}	4.13 ± 0.06^{ab}	46.36 ± 0.17^{a}	9.31 ± 0.04^{a}	2.90 ± 0.13^{de}	1.59 ± 0.14^{bc}	0.13 ± 0.01^{abc}	0.10 ± 0.03^{b}	3.37 ± 3.36^{h}	1.66 ± 0.31^{a}	7.63 ± 0.06^{ab}	3.24 ± 0.15^{a}
	Energy Glu 3.0	1.82 ± 0.27^{a}	4.09 ± 0.09^{ab}	45.12 ± 1.20^{a}	9.06 ± 0.23^{a}	3.06 ± 0.06^{cd}	1.44 ± 0.06^{c}	0.11 ± 0.01^{abc}	0.10 ± 0.01^{b}	9.51 ± 4.54^{gh}	1.40 ± 0.06^{a}	7.37 ± 0.12^{abc}	3.12 ± 0.02^{a}
	Energy Glu 4.0	1.84 ± 0.07^{a}	4.30 ± 0.25^{a}	44.28 ± 2.03^{a}	8.88 ± 0.43^{a}	3.24 ± 0.00^{bc}	1.44 ± 0.03^{c}	0.10 ± 0.03^{abc}	0.10 ± 0.01^{b}	11.70 ± 2.38^{fgh}	1.54 ± 0.07^{a}	7.30 ± 0.10^{bc}	3.11 ± 0.06^{a}
Strain		*	***	ns	ns	***	***	***	***	***	ns	***	ns
Treatment		ns	ns	ns	ns	***	*	ns	ns	***	ns	ns	ns
Strain × treatment		ns	ns	ns	ns	**	**	ns	ns	ns	ns	ns	ns

Note: Values are expressed as mean ± SD ($n = 3$). Different superscript letters within the same parameter (column) indicate statistically significant differences ($p < 0.05$) according to Tukey's HSD post hoc test applied to all 12 strain × treatment cell means following two-way ANOVA. The bottom three rows of the table report the significance of the two-way ANOVA fixed effects (strain, treatment, and strain × treatment interaction). Control = revitalized strain without nutrients; Energy Glu 3.0 = revitalized strain with FERMOPLUS Energy Glu 3.0; Energy Glu 4.0 = revitalized strain with FERMOPLUS Energy Glu 4.0.

Abbreviation: ns = not significant.

* $p < 0.05$.

** $p < 0.01$.

*** $p < 0.001$.

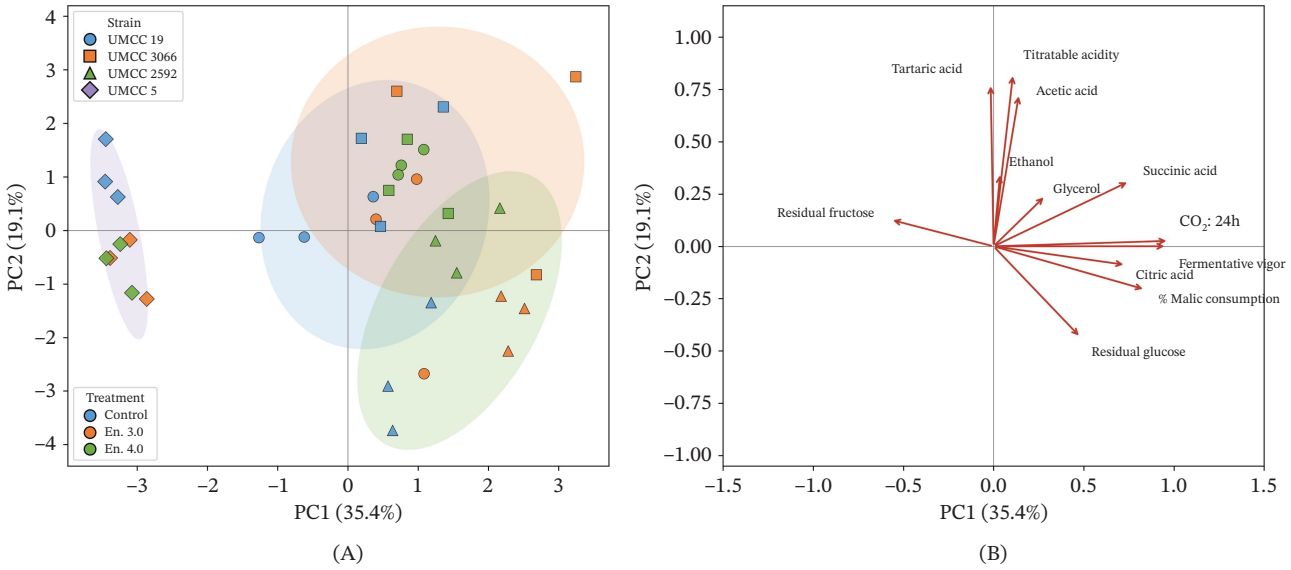

FIGURE 2 | Principal component analysis (PCA) of 12 phenotypic parameters measured in *S. cerevisiae* UMCC 19, UMCC 3066, UMCC 2592 and *T. delbrueckii* UMCC 5 under three rehydration conditions (Control = revitalized strain without nutrient; En. 3.0 = revitalized strain with FERMOPLUS Energy Glu 3.0; En. 4.0 = revitalized strain with FERMOPLUS Energy Glu 4.0). (A) Score plot showing the distribution of samples in the PC1–PC2 plane; strains are represented by different symbols and treatments by different colors, according to the legend. Shaded ellipses represent 95% confidence regions for each strain. (B) Loading plot showing the contribution of each variable to PC1 and PC2.

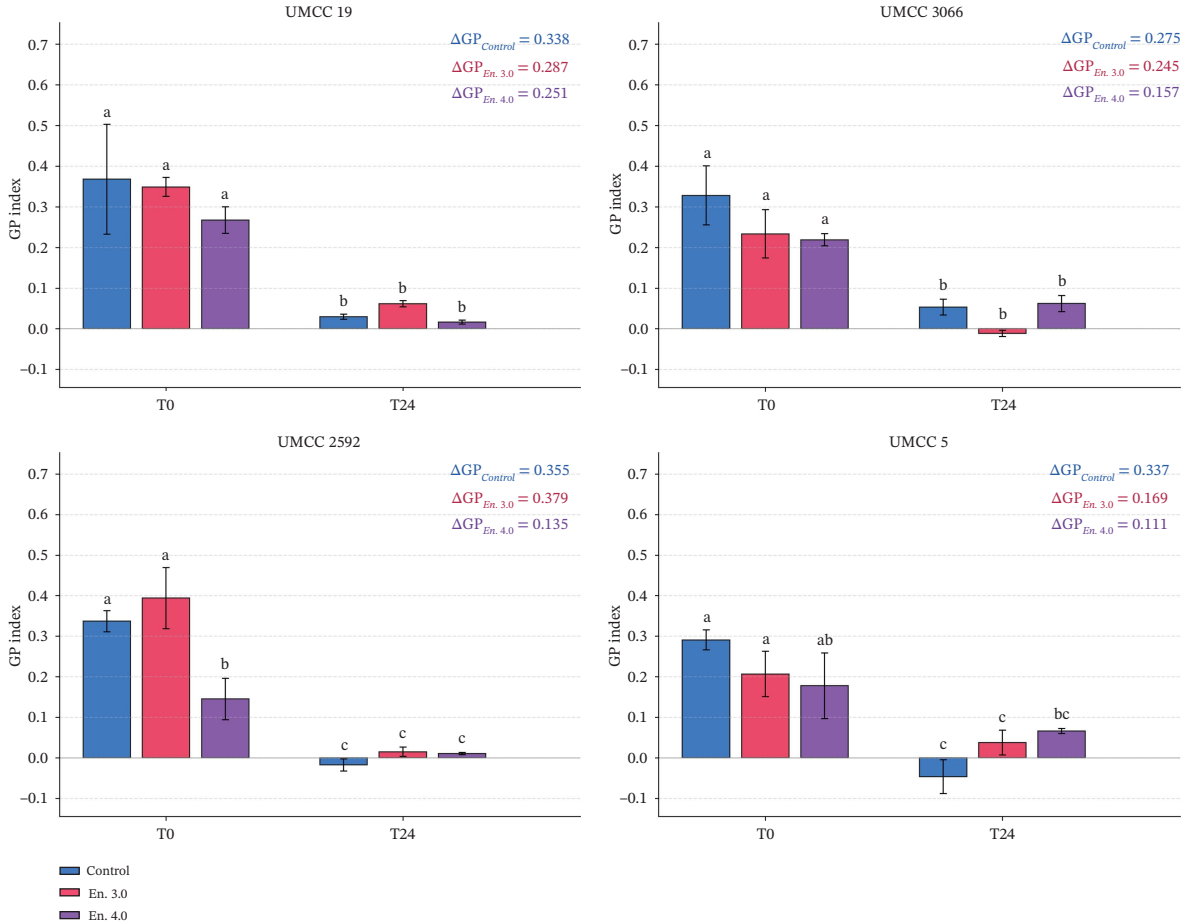

FIGURE 3 | Membrane fluidity (GP index) in *S. cerevisiae* strains (UMCC 19, UMCC 3066, UMCC 2592) and *T. delbrueckii* strain (UMCC 5). GP values at T0 (immediately after rehydration) and T24 (24-h fermentation) under Control, En. 3.0, and En. 4.0 treatments. The variation in GP between the two timepoints is also reported. Different letters indicate significant differences ($p < 0.05$).

confidence ellipses in the PC1–PC2 plane. Overall, these results suggest that, under the present experimental conditions, rehydration nutrients may exert effects that are more evident on early fermentation kinetics, whereas their impact on the final wine composition appeared more limited, with malic acid consumption being the parameter most consistently influenced by the treatment across the tested strains (Table 4). This corroborates the unchanged CO_2 production at the end of fermentation and suggests that rehydration supplements primarily influence yeast performance during the fermentation phase rather than the final product, which maintained optimal parameters. However, fermentative responses were largely strain-dependent, indicating that the efficacy of a given rehydration strategy is strongly influenced by intrinsic yeast traits [50].

3.2 | Effect of Rehydration Nutrients on Yeast Membrane Fluidity

Membrane fluidity was assessed at two time points: immediately after the 30 min of rehydration at 20°C (T0) and after 24 h of fermentation at 25°C (T24), with the aim of evaluating whether rehydration nutrients could influence membrane physical properties during yeast reactivation under suboptimal temperature conditions.

The GP values obtained are presented in Figure 3. At T0, all strains displayed elevated Laurdan GP values, consistent with increased membrane lipid order immediately following rehydration. This observation is consistent with the literature, as it is well established that dehydration induces a membrane phase transition from liquid-crystalline state to gel state and that rehydration at suboptimal temperatures (< 30°C) impairs the restoration of membrane fluidity, leading to increased permeability and loss of intracellular components [51]. Conversely, it is known that rehydration at 35°C–43°C facilitates the transition back to a liquid-crystalline state and minimizes cellular damage [14].

After 24 h of fermentation at 25°C (T24), GP values decreased significantly ($p < 0.05$) for all strains and treatments, indicating increased membrane fluidity. This shift reflects the adaptation of yeast cells to fermentation conditions through homeoviscous adaptation. As reported in the literature, yeast cells modulate their membrane lipid composition to maintain optimal fluidity [17]. Analysis of the change in GP values (ΔGP) revealed differences among treatments (Figure 3). Although GP differences at T0 did not reach statistical significance in most strains, a reproducible pattern was evident across all tested strains. FERMOPLUS Energy Glu 4.0 treatment was associated with lower initial GP values and, notably, with a reduction in GP variation (ΔGP) over the 24-h fermentation period. This suggests that the additive may prime membrane properties already during rehydration, resulting in enhanced membrane stability during subsequent fermentation stress. A plausible explanation is that membranes in their transitional state during rehydration allow uptake of exogenous lipids and sterols from yeast derivative-based additives, supporting membrane recovery [21]. Additionally, the DAP in FERMOPLUS Energy Glu 4.0 may contribute through early availability of assimilable nitrogen and phosphate [52].

4 | Conclusions

This study investigated the effects of suboptimal rehydration temperature (20°C) on commercial ADY strains and evaluated the efficacy of two rehydration nutrients, FERMOPLUS Energy Glu 3.0 and FERMOPLUS Energy Glu 4.0, in enhancing fermentation performance and membrane stability. The results demonstrated that both rehydration nutrients significantly improved early fermentation kinetics in *S. cerevisiae* strains, as evidenced by increased CO_2 production after 24 h compared to the control. However, *T. delbrueckii* showed no significant response to nutrient supplementation, likely due to its inherently slower fermentation kinetics as a non-*Saccharomyces* species. Notably, these early-phase improvements were only partially reflected in the final wine composition, indicating that rehydration nutrients act predominantly on yeast performance during the initial fermentation stages. A notable exception was malic acid consumption, which emerged as the parameter most consistently affected by nutrient supplementation, particularly with FERMOPLUS Energy Glu 4.0, whereas ethanol yield and the overall wine composition remained largely strain-driven. Regarding membrane fluidity, yeast cells rehydrated at 20°C exhibited high Laurdan GP values, consistent with increased membrane lipid order and incomplete recovery from the dehydration-associated gel-like membrane organization. After 24 h of fermentation, GP values decreased markedly, reflecting homeoviscous adaptation. FERMOPLUS Energy Glu 4.0 was associated with lower GP values at T0 and reduced ΔGP values compared to the control, suggesting a potential stabilizing effect on membrane properties. This effect may be hypothetically attributed to the uptake of exogenous lipids and sterols, as well as to the early availability of nitrogen and phosphate from its DAP component. These findings may contribute to the optimization of rehydration protocols in commercial winemaking, offering potential benefits in terms of operational flexibility, as well as reduction in production time and costs. Further studies could explore the expression of genes related to membrane fluidity modulation and low-temperature rehydration stress response, to elucidate the molecular basis of yeast adaptation under suboptimal rehydration conditions.

Author Contributions

Elisa Aiello: methodology; writing–original draft and preparation; writing–reviewing, and editing; data curation; and formal analysis. Mattia Pia Arena: writing–original draft and preparation and writing, reviewing, and editing. Carlo Montanini: writing–reviewing and editing. Andrea Pulvirenti: resources. Maria Gullo: supervision and funding acquisition.

Acknowledgments

We acknowledge the contribution and support from the Italian national node (MIRRI-IT) of the European Research Infrastructure MIRRI-ERIC.

Funding

This research was funded by the European Commission–NextGenerationEU, Project "Strengthening the MIRRI Italian Research Infrastructure for Sustainable Bioscience and Bioeconomy" (SUS-MIRRI.IT) code *n*. IR0000005, and by the European Union–NextGenerationEU Grant, CN_00000033, Project "National Biodiversity

Future Center (NBFC),” CUP E93C22001090001. Part of the work was granted by AEB S.p.A.

Conflicts of Interest

Author Carlo Montanini was employed by the company AEB S.p.A. The remaining authors declare no conflicts of interest.

Data Availability Statement

The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding author.

References

1. A. Peña, S. Uribe, M. Clemente, and N. Sánchez, “Rehydration Temperature is Critical for Metabolic Competence and for Membrane Integrity in Active Dry Yeast (ADY),” *Archives of Microbiology* 158, no. 2 (1992): 75–80, https://doi.org/10.1007/bf00245208.

2. S. A. Schmidt and P. A. Henschke, “Production, Reactivation and Nutrient Requirements of Active Dried Yeast in Winemaking: Theory and Practice,” *Australian Journal of Grape and Wine Research* 21 (2015): 651–662, https://doi.org/10.1111/ajgw.12189.

3. A. Pulvirenti, L. De Vero, G. Blaiotta, et al., “Selection of Wine *Saccharomyces cerevisiae* Strains and Their Screening for the Adsorption Activity of Pigments, Phenolics and Ochratoxin A,” *Fermentation* 6, no. 3 (2020): 80, https://doi.org/10.3390/fermentation6030080.

4. R. Pérez-Torrado, R. Gómez-Pastor, C. Larsson, and E. Matallana, “Fermentative Capacity of Dry Active Wine Yeast Requires a Specific Oxidative Stress Response During Industrial Biomass Growth,” *Applied Microbiology and Biotechnology* 81, no. 5 (2009): 951–960, https://doi.org/10.1007/s00253-008-1722-9.

5. E. Garre, F. Raginel, A. Palacios, A. Julien, and E. Matallana, “Oxidative Stress Responses and Lipid Peroxidation Damage are Induced During Dehydration in the Production of Dry Active Wine Yeasts,” *International Journal of Food Microbiology* 136, no. 3 (2010): 295–303, https://doi.org/10.1016/j.ijfoodmicro.2009.10.018.

6. M. B. França, A. D. Panek, and E. C. A. Eleutherio, “Oxidative Stress and Its Effects During Dehydration,” *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* 146, no. 4 (2007): 621–631, https://doi.org/10.1016/j.cbpa.2006.02.030.

7. E. Vaudano, O. Noti, A. Costantini, and E. García-Moruno, “Effect of Additives on the Rehydration of *Saccharomyces cerevisiae* Wine Strains in Active Dry Form: Influence on Viability and Performance in the Early Fermentation Phase,” *Journal of the Institute of Brewing* 120, no. 1 (2014): 71–76, https://doi.org/10.1002/jib.114.

8. I. Slaninová, S. Šesták, A. Svoboda, and V. Farkaš, “Cell Wall and Cytoskeleton Reorganization as the Response to Hyperosmotic Shock in *Saccharomyces cerevisiae*,” *Archives of Microbiology* 173, no. 4 (2000): 245–252, https://doi.org/10.1007/s002030000136.

9. S. Dupont, L. Beney, J. F. Ritt, J. Lherminier, and P. Gervais, “Lateral Reorganization of Plasma Membrane is Involved in the Yeast Resistance to Severe Dehydration,” *Biochimica et Biophysica Acta (BBA)–Biomembranes* 1798, no. 5 (2010): 975–985, https://doi.org/10.1016/j.bbamem.2010.01.015.

10. I. Poirier, P. A. Maréchal, S. Richard, and P. Gervais, “*Saccharomyces cerevisiae* Viability is Strongly Dependent on Rehydration Kinetics and the Temperature of Dried Cells,” *Journal of Applied Microbiology* 86, no. 1 (1999): 87–92, https://doi.org/10.1046/j.1365-2672.1999.00638.x.

11. J. M. Llauradó, N. Rozès, M. Constantí, and A. Mas, “Study of Some *Saccharomyces cerevisiae* Strains for Winemaking After Preadaptation at Low Temperatures,” *Journal of Agricultural and Food Chemistry* 53, no. 4 (2005): 1003–1011, https://doi.org/10.1021/jf049324n.

12. F. F. Bauer and I. S. Pretorius, “Yeast Stress Response and Fermentation Efficiency: How to Survive the Making of Wine—A Review,” *South African Journal of Enology and Viticulture* 21, no. 1 (2000): 27–46, https://doi.org/10.21548/21-1-3557.

13. B. Rodríguez-Porrata, M. Novo, J. Guillamón, N. Rozès, A. Mas, and R. C. Otero, “Vitality Enhancement of the Rehydrated Active Dry Wine Yeast,” *International Journal of Food Microbiology* 126, no. 1–2 (2008): 116–122, https://doi.org/10.1016/j.ijfoodmicro.2008.05.016.

14. S. B. Leslie, S. A. Teter, L. M. Crowe, and J. H. Crowe, “Trehalose Lowers Membrane Phase Transitions in Dry Yeast Cells,” *Biochimica et Biophysica Acta (BBA) – Biomembranes* 1192, no. 1 (1994): 7–13, https://doi.org/10.1016/0005-2736(94)90136-8.

15. J. Aguilera, F. Randez-Gil, and J. A. Prieto, “Cold Response in *Saccharomyces Cerevisiae*: New Functions for Old Mechanisms,” *FEMS Microbiology Reviews* 31, no. 3 (2007): 327–341, https://doi.org/10.1111/j.1574-6976.2007.00066.x.

16. R. Ernst, C. S. Ejsing, and B. Antonny, “Homeoviscous Adaptation and the Regulation of Membrane Lipids,” *Journal of Molecular Biology* 428, no. 24 (2016): 4776–4791, https://doi.org/10.1016/j.jmb.2016.08.013.

17. M. J. Torija, G. Beltran, M. Novo, et al., “Effects of Fermentation Temperature and *Saccharomyces* Species on the Cell Fatty Acid Composition and Presence of Volatile Compounds in Wine,” *International Journal of Food Microbiology* 85, no. 1–2 (2003): 127–136, https://doi.org/10.1016/s0168-1605(02)00506-8.

18. G. Beltran, M. Novo, J. M. Guillamón, A. Mas, and N. Rozès, “Effect of Fermentation Temperature and Culture Media on the Yeast Lipid Composition and Wine Volatile Compounds,” *International Journal of Food Microbiology* 121, no. 2 (2008): 169–177, https://doi.org/10.1016/j.ijfoodmicro.2007.11.030.

19. M. López-Malo, R. Chiva, N. Rozès, and J. M. Guillamón, “Phenotypic Analysis of Mutant and Overexpressing Strains of Lipid Metabolism Genes in *Saccharomyces cerevisiae*: Implication in Growth at Low Temperatures,” *International Journal of Food Microbiology* 162, no. 1 (2013): 26–36, https://doi.org/10.1016/j.ijfoodmicro.2012.12.020.

20. M. J. Beker, J. E. Blumbergs, E. J. Ventina, and A. I. Rapoport, “Characteristics of Cellular Membranes at Rehydration of Dehydrated Yeast *Saccharomyces cerevisiae*,” *Applied Microbiology and Biotechnology* 19, no. 5 (1984): 347–352, https://doi.org/10.1007/bf00253783.

21. V. Soubeyrand, V. Luparia, P. Williams, et al., “Formation of Micella Containing Solubilized Sterols During Rehydration of Active Dry Yeasts Improves Their Fermenting Capacity,” *Journal of Agricultural and Food Chemistry* 53, no. 20 (2005): 8025–8032, https://doi.org/10.1021/jf050907m.

22. M. Á. Pozo-Bayón, I. Andújar-Ortiz, and M. V. Moreno-Arribas, “Scientific Evidences Beyond the Application of Inactive Dry Yeast Preparations in Winemaking,” *Food Research International* 42, no. 7 (2009): 754–761, https://doi.org/10.1016/j.foodres.2009.03.004.

23. M. Guilloux-Benatier and D. Chassagne, “Comparison of Components Released by Fermented or Active Dried Yeasts After Aging on Lees in a Model Wine,” *Journal of Agricultural and Food Chemistry* 51, no. 3 (2003): 746–751, https://doi.org/10.1021/jf020135j.

24. M. E. Van der Rest, A. H. Kamminga, A. Nakano, Y. Anraku, B. Poolman, and W. N. Konings, “The Plasma Membrane of *Saccharomyces cerevisiae*: Structure, Function, and Biogenesis,” *Microbiological Reviews* 59, no. 2 (1995): 304–322, https://doi.org/10.1128/mr.59.2.304-322.1995.

25. G. Girardi Piva, E. Casalta, J. L. Legras, et al., “Characterization and Role of Sterols in *Saccharomyces cerevisiae* During White Wine Alcoholic Fermentation,” *Fermentation* 8, no. 2 (2022): 90, https://doi.org/10.3390/fermentation8020090.

26. G. Winter, P. A. Henschke, V. J. Higgins, M. Ugliano, and C. D. Curtin, “Effects of Rehydration Nutrients on H2S Metabolism and Formation of Volatile Sulfur Compounds by the Wine Yeast VL3,” *AMB Express* 1, no. 1 (2011): 36, https://doi.org/10.1186/2191-0855-1-36.

27. M. Novo, G. Beltran, N. Rozès, et al., "Early Transcriptional Response of Wine Yeast After Rehydration: Osmotic Shock and Metabolic Activation," *FEMS Yeast Research* 7, no. 2 (2007): 304–316, https://doi.org/10.1111/j.1567-1364.2006.00175.x.

28. F. Mezzetti, L. De Vero, and P. Giudici, "Evolved *Saccharomyces cerevisiae* Wine Strains With Enhanced Glutathione Production Obtained by an Evolution-Based Strategy," *FEMS Yeast Research* 14, no. 6 (2014): 977–987, https://doi.org/10.1111/1567-1364.12186.

29. L. Signorello, M. P. Arena, M. Brugnoli, F. V. Romeo, and M. Gullo, "Valorization of Olive Mill Wastewater by Selective Sequential Fermentation," *Foods* 14, no. 13 (2025): 2170, https://doi.org/10.3390/foods14132170.

30. E. Aiello, M. P. Arena, L. De Vero, et al., "Wine Yeast Strains Under Ethanol-Induced Stress: Morphological and Physiological Responses," *Fermentation* 10, no. 12 (2024): 631, https://doi.org/10.3390/fermentation10120631.

31. S. Ishmayana, U. J. Kennedy, and R. P. Learmonth, "Further Investigation of Relationships Between Membrane Fluidity and Ethanol Tolerance in *Saccharomyces cerevisiae*," *World Journal of Microbiology and Biotechnology* 33, no. 12 (2017): 218, https://doi.org/10.1007/s11274-017-2380-9.

32. Z. Salvadó, R. Chiva, S. Rodríguez-Vargas, F. Rández-Gil, A. Mas, and J. M. Guillamón, "Proteomic Evolution of a Wine Yeast During the First Hours of Fermentation," *FEMS Yeast Research* 8, no. 7 (2008): 1137–1146, https://doi.org/10.1111/j.1567-1364.2008.00389.x.

33. N. Kuliešienė, R. Žūkienė, G. Khroustalyova, C.-R. Chang, A. Rapoport, and R. Daugelavičius, "Changes in Energy Status of *Saccharomyces cerevisiae* Cells During Dehydration and Rehydration," *Microorganisms* 9, no. 2 (2021): 444, https://doi.org/10.3390/microorganisms9020444.

34. R. Velázquez, E. Zamora, M. L. Álvarez, L. M. Hernández, and M. Ramírez, "Effects of New *Torulaspora delbrueckii* Killer Yeasts on the Must Fermentation Kinetics and Aroma Compounds of White Table Wine," *Frontiers in Microbiology* 6 (2015): 1222, https://doi.org/10.3389/fmicb.2015.01222.

35. D. Mecca, S. Benito, B. Beisert, et al., "Influence of Nutrient Supplementation on *Torulaspora delbrueckii* Wine Fermentation Aroma," *Fermentation* 6, no. 1 (2020): 35, https://doi.org/10.3390/fermentation6010035.

36. N. P. Jolly, O. P. H. Augustyn, and I. S. Pretorius, "The Role and Use of Non-*Saccharomyces* Yeasts in Wine Production," *South African Journal of Enology and Viticulture* 27, no. 1 (2006): 15–39, https://doi.org/10.21548/27-1-1475.

37. M. Bely, P. Stoeckle, I. Masneuf-Pomarède, and D. Dubourdieu, "Impact of Mixed *Torulaspora delbrueckii–Saccharomyces Cerevisiae* Culture on High-Sugar Fermentation," *International Journal of Food Microbiology* 122, no. 3 (2008): 312–320, https://doi.org/10.1016/j.ijfoodmicro.2007.12.023.

38. F. Silva-Sousa, T. Fernandes, F. Pereira, et al., "*Torulaspora delbrueckii* Phenotypic and Metabolic Profiling Towards Its Biotechnological Exploitation," *Journal of Fungi* 8, no. 6 (2022): 569, https://doi.org/10.3390/jof8060569.

39. R. Ansell, K. Granath, S. Hohmann, J. M. Thevelein, and L. Adler, "The Two Isoenzymes for Yeast NAD+-Dependent Glycerol 3-Phosphate Dehydrogenase Encoded by *GPD1* and *GPD2* Have Distinct Roles in Osmoadaptation and Redox Regulation," *EMBO Journal* 16, no. 9 (1997): 2179–2187, https://doi.org/10.1093/emboj/16.9.2179.

40. G. Zara, I. Mannazzu, A. Del Caro, et al., "Wine Quality Improvement Through the Combined Utilisation of Yeast Hulls and *Candida zemplinina/Saccharomyces Cerevisiae* Mixed Starter Cultures," *Australian Journal of Grape and Wine Research* 20, no. 2 (2014): 199–207, https://doi.org/10.1111/ajgw.12078.

41. L. Laguna, B. Bartolomé, M. V. Moreno-Arribas, A. R. Beadling, B. Bartolomé, and M. Victoria Moreno-Arribas, "Exploring Mouthfeel in Model Wines: Sensory-to-Instrumental Approaches," *Food Research International* 102 (2017): 478–486, https://doi.org/10.1016/j.foodres.2017.09.009.

42. N. N. Ivit, R. Longo, and B. Kemp, "The Effect of Non-*Saccharomyces* and *Saccharomyces* Non-*Cerevisiae* Yeasts on Ethanol and Glycerol Levels in Wine," *Fermentation* 6, no. 3 (2020): 77, https://doi.org/10.3390/fermentation6030077.

43. F. Remize, E. Andrieu, and S. Dequin, "Engineering of the Pyruvate Dehydrogenase Bypass in *Saccharomyces cerevisiae*: Role of the Cytosolic Mg2+ and Mitochondrial K+ Acetaldehyde Dehydrogenases Ald6p and Ald4p in Acetate Formation During Alcoholic Fermentation," *Applied and Environmental Microbiology* 66, no. 8 (2000): 3151–3159, https://doi.org/10.1128/AEM.66.8.3151-3159.2000.

44. J. H. Swiegers, E. J. Bartowsky, P. A. Henschke, and I. S. Pretorius, "Yeast and Bacterial Modulation of Wine Aroma and Flavour," *Australian Journal of Grape and Wine Research* 11, no. 2 (2005): 139–173, https://doi.org/10.1111/j.1755-0238.2005.tb00285.x.

45. E. J. Bartowsky and P. A. Henschke, "Acetic Acid Bacteria Spoilage of Bottled Red Wine—A Review," *International Journal of Food Microbiology* 125, no. 1 (2008): 60–70, https://doi.org/10.1016/j.ijfoodmicro.2007.10.016.

46. C. A. Corison, C. S. Ough, H. W. Berg, and K. E. Nelson, "Must Acetic Acid and Ethyl Acetate as Mold and Rot Indicators in Grapes," *American Journal of Enology and Viticulture* 30, no. 2 (1979): 130–134, https://doi.org/10.5344/ajev.1979.30.2.130.

47. C. Camarasa, J. P. Grivet, and S. Dequin, "Investigation by 13C-NMR and Tricarboxylic Acid (TCA) Deletion Mutant Analysis of Pathways for Succinate Formation in *Saccharomyces cerevisiae* During Anaerobic Fermentation," *Microbiology* 149, no. 9 (2003): 2669–2678, https://doi.org/10.1099/mic.0.26007-0.

48. S. Redzepovic, S. Orlic, A. Majdak, B. Kozina, H. Volschenk, and M. Viljoen-Bloom, "Differential Malic Acid Degradation by Selected Strains of *Saccharomyces* During Alcoholic Fermentation," *International Journal of Food Microbiology* 83, no. 1 (2003): 49–61, https://doi.org/10.1016/S0168-1605(02)00320-3.

49. S. Benito, "The Impact of *Torulaspora delbrueckii* Yeast in Winemaking," *Applied Microbiology and Biotechnology* 102, no. 7 (2018): 3081–3094, https://doi.org/10.1007/s00253-018-8849-0.

50. P. Díaz-Hellín, J. Úbeda, and A. Briones, "Improving Alcoholic Fermentation by Activation of *Saccharomyces* Species During the Rehydration Stage," *LWT–Food Science and Technology* 50, no. 1 (2013): 126–131, https://doi.org/10.1016/j.lwt.2012.06.011.

51. J. H. Crowe, L. M. Crowe, and F. A. Hoekstra, "Phase Transitions and Permeability Changes in Dry Membranes During Rehydration," *Journal of Bioenergetics and Biomembranes* 21, no. 1 (1989): 77–91, https://doi.org/10.1007/BF00762213.

52. V. D. Marks, G. K. van der Merwe, and H. J. J. van Vuuren, "Transcriptional Profiling of Wine Yeast in Fermenting Grape Juice: Regulatory Effect of Diammonium Phosphate," *FEMS Yeast Research* 3, no. 3 (2003): 269–287, https://doi.org/10.1016/s1567-1356(02)00201-5.

Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Supporting Information.** Figure S1. Principal component analysis (PCA) of 12 phenotypic parameters (residual glucose, residual fructose, ethanol yield, glycerol yield, succinic acid yield, acetic acid yield, citric acid yield, tartaric acid, titratable acidity, CO_2 after 24 h, fermentative vigor, and malic acid consumption) measured in *S. cerevisiae* UMCC 19, UMCC 3066, UMCC 2592, and *T. delbrueckii* UMCC 5 under three rehydration conditions (Control = revitalized strain without nutrient; En. 3.0 = revitalized strain with FERMOPLUS Energy Glu 3.0; En. 4.0 = revitalized strain with FERMOPLUS Energy Glu 4.0). (A) Score plot showing the distribution of samples in the PC1–PC2 plane; strains are represented by different symbols and treatments by different colors, according to the legend. Shaded ellipses represent 95% confidence regions for each rehydration treatment. (B) Loadings plot showing the contribution of each variable to PC1 and PC2.