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Review

Membrane Lipid Remodeling in Non-*Saccharomyces* Wine Yeasts Under Ethanol Stress: From Mechanism Toward a Predictive Lipid Signature

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Abstract

Non-*Saccharomyces* yeasts shape the aroma, freshness, and reduced-alcohol profiles of wines, yet their oenological contribution is curtailed by a poor tolerance to ethanol. Because ethanol acts on the plasma membrane, the cell's selective barrier, this limitation is rooted in the membrane lipidome, whose remodeling has been documented but never integrated into a wine-specific, predictive framework. This review provides that synthesis, keeping the non-*Saccharomyces* wine yeasts as the subject and *Saccharomyces cerevisiae* as a reference frame. It first describes how ethanol perturbs the bilayer and how the cell compensates through unsaturated fatty acids, ergosterol, phospholipid-class balance, and membrane fluidity. It then traces the concentration- and time-dependent remodeling of the lipidome, together with the medium-chain fatty-acid, temperature, and oxygen co-stressors accompanying it, and the comparative membrane biology distinguishing these yeasts from *S. cerevisiae*, including the markedly reduced uptake of exogenous sterols under oxygen limitation. Finally, it weighs the nutritional, process, and non-genetically modified strain-improvement strategies that reinforce them. The same lipid descriptors recur throughout, yet are seldom measured in improved wine strains. We argue that the membrane lipidome can be read as a candidate multivariate predictive signature of ethanol tolerance and of fermentation performance, and outline the validation it still requires.

Keywords: non-*Saccharomyces* yeasts; wine fermentation; membrane lipidome; ethanol tolerance; lipidomics; non-GMO strain improvement

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1. Introduction

Wine fermentation has long been understood as the work of *Saccharomyces cerevisiae*, yet the microbial reality of the grape must is far richer, and the past two decades have seen a deliberate return of the non-*Saccharomyces* yeasts (non-conventional yeasts, NCYs) that dominate its earliest stages [1]. These yeasts, native to the grape surface and the winery, are now inoculated on purpose to diversify the sensory outcome of fermentation [2,3]. The choice of starter culture is itself an oenological decision, since wine strains are selected for the fermentative and technological traits that shape wine quality and character [4]. Across native isolates assayed under osmotic, ethanol, and acidic-pH stress, several non-*Saccharomyces* species withstand the stresses of early fermentation, with species such as *Lachancea thermotolerans* (syn. *Kluyveromyces thermotolerans*) ranking among the more stress-resistant of the indigenous wine yeasts [5]. Their applied appeal is concrete: they broaden

aromatic complexity, sharpen freshness and acidity, and offer a biological route to lowering the final ethanol content of increasingly over-ripe, high-sugar musts [6]. This renewed interest has reframed the non-*Saccharomyces* not as spoilage organisms to be suppressed, but as functional partners in the modern winery.

The opportunity, however, is bounded by a persistent limitation. Against the *S. cerevisiae* reference frame, the non-*Saccharomyces* generally lack competitiveness under oenological conditions: they ferment less vigorously and show lower resistance to fermentation stress [7]. The decisive stressor is ethanol itself, produced by the cells during fermentation. As ethanol accumulates, the growth of native non-*Saccharomyces* is strongly curtailed or arrested, and at the higher concentrations reached late in fermentation only isolated species persist [5]. The result is the microbial succession in which the non-*Saccharomyces* drive the early, aroma-shaping phase but are progressively displaced as the alcohol rises, ceding the finish to *S. cerevisiae*; ethanol drives this displacement together with oxygen and nitrogen availability, temperature, pH, SO_2, interspecific competition, and secreted inhibitory metabolites, compounded by the weaker fermentative metabolism of many of these species [3]. Tolerating ethanol, surviving it, and adapting to it over time are likewise not the same as fermenting well or to completion, since sugar uptake, glycolytic flux, redox balance, and nutrient supply set limits of their own. Ethanol sensitivity constitutes one of the principal constraints on how far the oenological promise of these yeasts can be realized.

That constraint is, to a substantial degree, a problem of the plasma membrane (PM). Ethanol partitions into the lipid bilayer, and the cell's capacity to withstand it depends on how effectively it can reorganize its membrane lipids in response. In *S. cerevisiae*, where this response is best characterized, tolerance is read through four membrane descriptors: unsaturated fatty acids, ergosterol, phospholipid-class balance, and membrane fluidity. These descriptors already serve as predictive biomarkers in the reference yeast: a wine-relevant benchmark of *Saccharomyces* strains identifies low phosphatidylethanolamine (PE) together with high ethanol-induced fluidity and permeability as a lipid signature of the more tolerant strains [8]. The corresponding evidence in the non-*Saccharomyces* is thinner, and only partly concordant. A direct comparison of a wine *S. cerevisiae* with the non-*Saccharomyces Hanseniaspora uvarum* (syn. *Kloeckera apiculata*) associated ethanol tolerance with ergosterol enrichment, increased fatty-acid unsaturation, and maintained phospholipid biosynthesis, the ethanol-induced changes being most marked in the more tolerant strain [9]. Importantly, the lipid levers that confer tolerance are not universal: exogenous ergosterol and oleic acid help some non-*Saccharomyces* species but not others [10]. Ethanol tolerance itself is not governed by a single gene but is a complex polygenic trait, thought to involve more than 250 genes [7]. Membrane lipid remodeling is therefore one of the central, and most tractable, axes along which this trait is expressed. Ethanol, moreover, is not the only membrane stressor the cells encounter: the medium-chain fatty acids (MCFAs) they secrete during fermentation partition into the same bilayer and compound its effect, so that ethanol and MCFA tolerance are intertwined [11].

The membrane lipidome of the non-*Saccharomyces* wine yeasts is itself remodeled under ethanol, a phenomenon that is real but still only partly documented. Targeted lipidomic profiling of sterile grape juice fermented by indigenous non-*Saccharomyces* has shown species-dependent lipid profiles in the resulting wine [12]. The specificity can be pronounced: under ethanol, the wine yeast *Wickerhamomyces anomalus* (syn. *Pichia anomala*) inverts the canonical remodeling pattern of *S. cerevisiae*, undergoing a distinct rearrangement of its fatty-acid complement rather than reproducing the reference response [13]. Crucially, the magnitude of these membrane changes appears graded with phenotype: across a panel of *S. cerevisiae* and non-*Saccharomyces* wine yeasts, the extent of the ethanol-driven membrane response tracks each strain's tolerance and its fermentation performance,

whereas the least tolerant *Metschnikowia pulcherrima* responds the least—its membrane fluidity increases only marginally at low ethanol, and then falls and stiffens at higher concentrations [14]. The membrane lipidome, in other words, behaves as a readout of what the cell can withstand.

Yet this readout is not, in itself, a predictor. No measured lipid profile can say in advance whether a given non-*Saccharomyces* strain will tolerate ethanol and complete fermentation. The knowledge that could close this gap exists, but in three separate bodies of work that have never been brought together. First, a rich and quantitative literature already exists on ethanol and lipids in *S. cerevisiae*, where the descriptors introduced above function as predictive biomarkers of tolerance [8]. Second, the corresponding observations in the non-*Saccharomyces* are real but scattered and unsystematized: a multiple-regression analysis across a small mixed panel of wine yeasts, non-*Saccharomyces* together with *S. cerevisiae*, correlated PM oleic acid and ergosterol with ethanol tolerance [15], and chemometric analysis of fatty-acid profiles has been shown to separate *S. cerevisiae* from the less tolerant *Issatchenkia*, which reduces monounsaturated fatty acids (MUFAs) under ethanol in opposition to the reference [16]. Third, the broadest 2026 survey quantified total lipids together with fatty-acid and sterol composition in 29 isolates from thirteen species, and found high variability both between species and between strains of the same species [17], establishing species- and strain-specific baselines rather than a single non-*Saccharomyces* starting state. What is missing is the synthesis: to our knowledge, these three strands have not been combined into a framework that is at once wine-specific, focused on the non-*Saccharomyces*, and predictive.

The aim of this review is to provide that synthesis and to define the priorities for future research. We integrate the molecular basis of ethanol concentration- and time-dependent membrane lipid remodeling, including its modulation by co-stressors, and its relation to fermentation performance and to ethanol tolerance; the comparative lipid biology of the principal non-*Saccharomyces* wine yeasts measured against the *S. cerevisiae* reference frame; the nutritional and process interventions that act on those lipids; and the non-genetically modified (non-GMO) improvement strategies, such as adaptive laboratory evolution (ALE) and hybridization, together with an emerging framework that infers strain tolerance or fermentation performance from lipid profiles. We further identify the principal data gaps, chiefly the wine non-*Saccharomyces* whose membrane lipidome remains largely unmeasured, and specify the experimental steps required to make both strain tolerance and fermentation performance predictable from lipid composition.

The literature synthesized here was retrieved through iterative searches of Google Scholar, Scopus, and Web of Science, run in English and organized section by section, combining yeast species with membrane lipid descriptors, analytical techniques, and ethanol-tolerance and fermentation outcomes. Work from 2019 onward was prioritized for the comparative and predictive strands, and earlier studies were retained where they establish the biochemistry of the yeast membrane, so the evidence cited spans 1977 to 2026.

2. The Yeast Plasma Membrane Under Ethanol Stress

2.1. Architecture of the Yeast Plasma Membrane

The yeast PM is a fluid lipid bilayer built principally from glycerophospholipids, with their esterified fatty acids (FAs) and sterols [18]. The PM phospholipids (PLs) are phosphatidylcholine (PC), phosphatidylethanolamine, phosphatidylinositol (PI), phosphatidylserine (PS), and phosphatidic acid (PA), in proportions that vary across species (Section 4) [19]. The acyl chains esterified to these PLs are classified as saturated (SFA) or unsaturated (UFA), the latter subdivided into MUFA and poly-unsaturated (PUFA) species; chains of C22 and longer are termed very-long-chain fatty acids (VLCFAs) [13]. The ratio

of unsaturated to saturated chains (UFA:SFA), and the related unsaturation index (UI), are first-order determinants of bilayer order, because the proportion of saturated versus unsaturated acyl chains is a key determinant of lipid packing and the resulting membrane fluidity [20]. A defining metabolic constraint separates the reference yeast from its non-conventional relatives: *S. cerevisiae* possesses a single Δ9 fatty-acid desaturase (encoded by *OLE1*) and therefore synthesizes only MUFAs, namely oleic and palmitoleic acid, and cannot make PUFAs de novo, although it incorporates exogenous ones (Figure 1) [19].

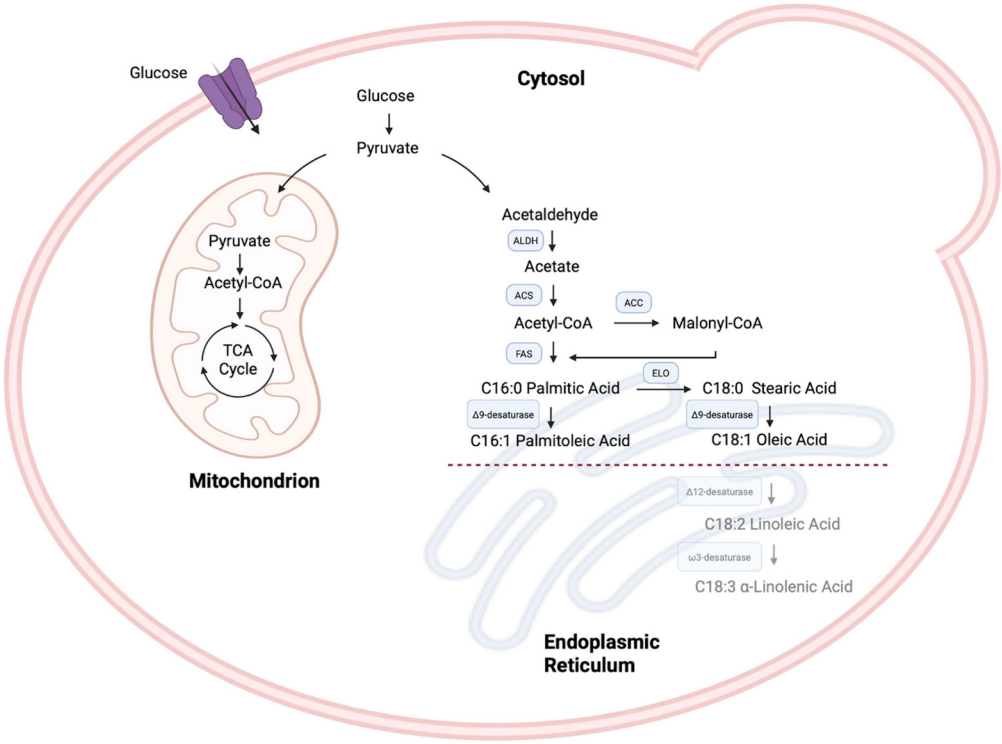

Figure 1. Shared and species-dependent fatty-acid desaturation in yeasts. The figure traces fatty-acid biosynthesis from glucose to the saturated C16 and C18 chains, then the desaturation steps that act on them. The solid upper branch depicts Δ9 desaturation to palmitoleic and oleic acids, a step encoded by *OLE1* in *Saccharomyces cerevisiae* [19]. The red dashed line marks the *S. cerevisiae* biosynthetic limit. The gray branch below it denotes an additional PUFA route absent from *S. cerevisiae* and present in some non-*Saccharomyces* yeasts. In *Kluyveromyces lactis*, a Δ12 desaturase converts oleic acid to linoleic acid, followed by an omega-3, or Δ15, desaturase that converts linoleic acid to alpha-linolenic acid [19]. *Metschnikowia pulcherrima*, *Torulaspora delbrueckii*, and *Kluyveromyces marxianus* synthesize PUFAs de novo [21]. Abbreviations: ALDH, aldehyde dehydrogenase; ACS, acetyl-CoA synthetase; ACC, acetyl-CoA carboxylase; FAS, fatty-acid synthase; ELO, fatty-acid elongase; TCA, tricarboxylic acid cycle. Created in BioRender. Aiello, E. (2026) https://BioRender.com/04x2qm1.

Interleaved among the PLs is ergosterol (Erg), the fungal counterpart of cholesterol and the end product of the ergosterol biosynthetic (ERG) pathway. Sterols modulate bilayer rigidity and help buffer the bilayer against fluidization; their abundance relative to PLs (the sterol:PL ratio) is a recurring index of membrane state [9]. Together, these saturated chains and sterols favor laterally ordered, tightly packed domains, the membrane analog of lipid rafts, whose degree of order can be read out biophysically (e.g., by Laurdan generalized polarization (GP) and 1,6-diphenyl-1,3,5-hexatriene (DPH) fluorescence anisotropy). Critically, no single number captures a biological membrane, and the indirect compositional indices (UI, sterol:PL) do not always track real fluidity, an important limitation outside *S. cerevisiae* [9]. In sum, the resting yeast membrane is a glycerophospholipid-based, ergosterol-buffered bilayer whose order is governed jointly by acyl-chain unsaturation and

sterol content, two distinct structural axes. Throughout this review, membrane lipidome denotes the lipid complement that builds and supplies this bilayer, read at the level each primary study reports. Three levels occur and are not interchangeable: whole-cell or dry-biomass composition, by far the commonest; direct biophysical readouts of bilayer order in living cells or in spheroplasts, by Laurdan-GP and DPH fluorescence anisotropy; and purified plasma-membrane fractions, reported here for a single dataset [15]. Some values are predicted by chemometric models rather than measured directly; the level of each dataset is given in the Method column of the tables in Sections 3.1 and 4.1.

2.2. Ethanol Perturbation of the Bilayer

Ethanol is an amphipathic small molecule that partitions into the membrane as a polar solvent and increases its fluidity [22], intercalating at the level of the polar head groups [23]. At low concentrations this fluidization stems from increased head-group spacing that depresses the lipid melting transition; at higher concentrations the bilayer can collapse into an interdigitated phase, with a reduction of up to 30% in membrane thickness in model PC bilayers [23]. The functional consequence is a loss of barrier integrity. Above a threshold concentration the membrane becomes markedly leaky to protons: passive H^+ influx rises steeply, aquaporin-mediated water transport is inhibited while passive water diffusion is stimulated, and the structure is interpreted as transitioning from a bilayer to non-bilayer structures. This proton leak directly opposes the PM proton-pumping ATPase (H^+-ATPase, Pma1), which maintains the transmembrane electrochemical gradient; under combined ethanol and thermal load, net proton efflux can be suppressed entirely, signaling failure of pH homeostasis [24]. Accordingly, in wine yeasts ethanol tolerance and H^+-ATPase activity each correlate with membrane oleic acid and ergosterol [15].

That the perturbation is governed by a threshold rather than a smooth gradient is a recurring theme. Biophysical measurements on whole cells show that head-group hydration and order are preserved across a wide range of exogenous ethanol and then break down abruptly at high concentrations, coinciding with loss of viability, evidence that the live cell actively defends its membrane state up to a limit beyond which the bilayer fails [25]. Sterols are central to where that limit lies: ergosterol at sufficient bilayer mole fractions strongly resists the induction of interdigitation, demanding far more ethanol before the membrane destabilizes [23]. The precise dose and time dependence of these changes is the subject of Section 3: how fluidity, UFA:SFA and ergosterol shift quantitatively as ethanol rises and as fermentation proceeds. Overall, ethanol fluidizes and ultimately permeabilizes the bilayer in a threshold-dependent manner, with proton leak and H^+-ATPase suppression as the proximate physiological lesions and ergosterol as the principal structural defense.

2.3. The Adaptive Remodeling Response

Yeasts counter membrane fluidization through homeoviscous adaptation: an active, sensor-driven adjustment of lipid composition that restores bilayer order (Figure 2). In *S. cerevisiae*, the central regulator is the OLE pathway, in which the membrane-bound transcription-factor precursors Mga2 and Spt23 sense the proportion of unsaturated lipids directly within the endoplasmic reticulum bilayer and accordingly regulate *OLE1*, the gene encoding the sole Δ9 desaturase. The system is tightly balanced: both deletion and overexpression of *OLE1* can be lethal, because an excess of saturated lipids triggers bilayer stress and the unfolded-protein response while overproduction of unsaturated lipids is equally harmful [20]. Because ethanol fluidizes the membrane, the cell opposes it by retuning the same two parameters, acyl-chain unsaturation and ergosterol. A widely reported adjustment is a rise in the proportion of UFAs and of ergosterol, but its direction and magnitude vary with strain, ethanol concentration, and growth conditions, and saturated-chain

accumulation has also been reported. The identity of the unsaturated chain matters: oleic acid (Δ9Z-C18:1) is the most efficacious UFA in overcoming ethanol toxicity, and tolerance is proposed to arise specifically from oleate incorporation effecting a compensatory decrease in fluidity, whereas the tested C16:1 isomers did not confer ethanol tolerance in *S. cerevisiae* [26].

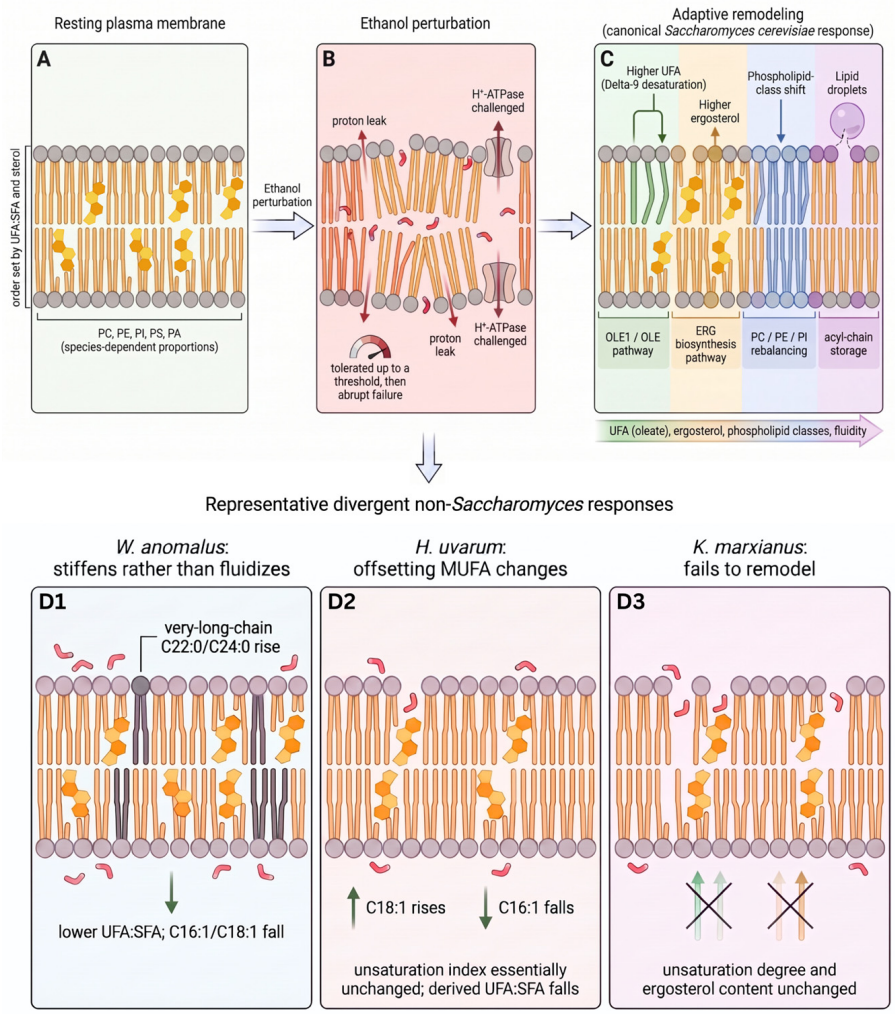

Figure 2. Conceptual schematic of the yeast PM lipidome, its compensatory remodeling under ethanol stress, and three representative divergent non-*Saccharomyces* responses. (**A**) The resting bilayer is built from the major glycerophospholipid classes, PC, PE, PI, PS, and PA, in species-dependent proportions, with ergosterol (orange ring structures) intercalated among the acyl chains; baseline order is set by the UFA:SFA balance together with sterol. (**B**) Ethanol (red curved shapes) partitions into the head-group region, fluidizing and, above a threshold, permeabilizing the bilayer: proton leak rises and the PM H^+-ATPase struggles to maintain the gradient. The perturbation is tolerated up to that threshold and then fails abruptly. (**C**) The cell responds by engaging four co-regulated compensatory mechanisms, each shown as a color-coded band: Δ9 desaturation via the *OLE1*/OLE pathway raises unsaturated, notably oleic, acyl chains; the ergosterol-biosynthesis (ERG) pathway enriches membrane sterol; phospholipid head-group classes are rebalanced across PC, PE, and PI; and surplus acyl chains are routed into lipid droplets for storage. The band below panel (**C**) carries the four descriptors these mechanisms converge on: UFA (oleate), ergosterol, phospholipid classes, and membrane fluidity [20,23,25]. Panels (**A**–**C**) depict the canonical *S. cerevisiae* response; panels (**D1**–**D3**) show three documented divergences from it, observed in separate studies under different ethanol doses and exposure conditions (4 to 9% *v*/*v*, growth or acute exposure), with the illustrated

changes drawn from whole-cell lipid composition data. (**D1**) *Wickerhamomyces anomalus* stiffens rather than fluidizes: UFA:SFA falls as C16:1/C18:1 decline while very-long-chain C22:0/C24:0 rise. (**D2**) *Hanseniaspora uvarum* raises C18:1 but lowers C16:1, leaving its unsaturation index essentially unchanged while the derived UFA:SFA falls. (**D3**) *K. marxianus* fails to remodel, leaving its unsaturated fatty-acid degree and ergosterol content unchanged where *S. cerevisiae* would raise both. Created in BioRender. Aiello, E. (2026) https://BioRender.com/6xrc1zw.

This homeoviscous logic has an important limit. Tolerance is not a monotonic function of the degree of unsaturation: in a lipid-auxotroph model, the most ethanol- and heat-tolerant cells were those enriched in oleate, with tolerance declining as unsaturation increased toward linoleate (C18:2) and linolenate (C18:3), the more unsaturated, more fluid and more oxidation-prone membranes being the most fragile [27]. In the same system ergosterol rose in step with supplement unsaturation yet did not by itself confer tolerance, behaving instead as a counterweight to the fluidizing supplement [27]. The desaturation and sterol arms are co-regulated rather than independent, and in a non-*Saccharomyces* wine yeast adapting to ethanol both are engaged together: up-regulation of *OLE1* alongside induction of ergosterol-biosynthesis genes [13]. A further asymmetry distinguishes the NCYs from the reference yeast, however: the six wine non-*Saccharomyces* surveyed lack functional homologues of the sterol-uptake transporters *AUS1* and *PDR11* and take up far less exogenous sterol than *S. cerevisiae*, depending instead on a conserved, active ergosterol-biosynthesis pathway [28,29]. Beyond fatty-acyl and sterol tuning, cells remodel PL head-group classes, rebalancing PC, PE and PI, and route surplus acyl chains into neutral-lipid stores within lipid droplets [30], with heat-shock proteins assisting [31]; lipid metabolism, cell-wall modification and intracellular homeostasis are among the main processes upregulated to restore ethanol damage [32]. That several non-*Saccharomyces* wine yeasts synthesize endogenous PUFAs at all, linoleate and linolenate that *S. cerevisiae* cannot make, already marks their remodeling repertoire as qualitatively distinct from the reference yeast, a distinction taken up quantitatively in Section 4 [21].

These compensatory moves do not translate uniformly into tolerance. Exogenous ergosterol and oleate raise ethanol tolerance in some yeasts but not others, and sterol and UFA levels rise sharply under active oxygenation while falling under oxygen limitation, so the capacity to remodel is conditioned by the environment [10]. Whether higher intrinsic fluidity is protective is genuinely contested: across a panel of *S. cerevisiae* strains the most tolerant become the most fluid and permeable under ethanol [8], yet on at least one wine strain lower intrinsic fluidity correlated with higher tolerance [22]. This unresolved tension, and the recurring failure of any single index to predict membrane behavior, is why a multivariate lipid signature, built on the same four descriptors introduced here (UFA, especially oleate; ergosterol; PL-class balance; fluidity), becomes the natural object of prediction in Section 7. Taken together, the cell defends its membrane through a co-regulated desaturation–sterol response, with desaturation governed by the OLE pathway and sterol remodeling constrained, in the species surveyed, by their reduced uptake of exogenous sterols under oxygen limitation; but no single descriptor maps cleanly onto tolerance, motivating a signature-level view.

3. Ethanol-Driven Lipid Remodeling Across Concentration and Time

3.1. Dose–Response: From Sub-Lethal to Lethal Ethanol

In *S. cerevisiae*, the canonical compensatory move is a rise in unsaturation with ethanol dose, though this response is not universal and varies with strain, ethanol concentration, and exposure time (Table 1). The phospholipid UFA:SFA ratio has been reported to climb with ethanol dose (0 to 1.5 M), measured 10 h after ethanol addition to growing cultures, as C18:1 rises and C16:0 falls [33]. Whole-cell UFA:SFA was reported to rise in parallel in cells

grown to mid-exponential phase in 10% *v*/*v* ethanol [9]. This composition partly offsets fluidization: DPH anisotropy of an ethanol-adapted *S. cerevisiae* stays nearly flat across 0–12% *v*/*v* (0.142 to 0.138), whereas an unadapted population fluidizes steadily (0.173 to 0.131) [9]. Whether this canonical rise reliably protects the membrane is not settled: the fluidity response has been reported inconsistently across studies, conditions, and methods (Section 2.3). In rice wine *S. cerevisiae*, the dose response proved non-monotonic: membrane fluidity rose only below approximately 4% *v*/*v* ethanol before falling at higher doses, relative UFA declined rather than climbed, and *OLE1* was repressed at high ethanol, with the most tolerant strain coming closest to holding UFA and fluidity constant [34].

The non-*Saccharomyces* do not track the *S. cerevisiae* pattern either; each remodels in its own, species-specific way (Table 1). *H. uvarum* raises C18:1 but lowers C16:1, so its unsaturation index is essentially unchanged and its derived UFA:SFA falls under a 4% *v*/*v* challenge [9]. *Issatchenkia* and related yeasts decrease, rather than increase, MUFA under ethanol, a divergence proposed to explain their inability to withstand more than 10% alcohol [16]. One of the most complete wine non-*Saccharomyces* datasets, on *W. anomalus*, is counterintuitive: under ethanol its Laurdan-GP rises and UFA:SFA falls, C16:1/C18:1 drops while very-long-chain C22:0/C24:0 rise even though *OLE1* is induced approximately 1.7-fold [13]. Independent transcriptomic and metabolomic profiling shows that ethanol also remodels its glycerophospholipid and fatty-acid metabolism [35,36]. Indirect indices thus do not always predict real fluidity outside *S. cerevisiae*.

Dose also orders these strains in a way that tracks the sterol pool more closely than the UFA pool. In one five-strain wine yeast panel, half-inhibitory ethanol concentrations (EC_{50}), defined as the added-ethanol concentration that halves the maximum specific growth rate, were 2.7% *v*/*v* for *Torulaspora delbrueckii* M1-20-4 and 6.5% *v*/*v* for *H. uvarum* B-10, below the 6.9% *v*/*v* of the designated *S. cerevisiae* reference strain, although a flor strain of the same species reached 9.1% *v*/*v*. After 4 h at 4% *v*/*v* ethanol, the same two non-*Saccharomyces* strains carried 10.1 and 9.4 compared with 67 µg of ergosterol per mg of purified plasma-membrane protein in the reference strain and 83.2 in the flor strain [15]. A multiple regression on oleic acid, palmitoleic acid and ergosterol closely tracks ethanol tolerance, with the authors identifying ergosterol, rather than UFA, as the key lipid [15]. Consistently, a higher ergosterol/phospholipid ratio (0.37 compared with 0.23 mg/mg) and more PC (43.5% compared with 35.5%) mark the more tolerant *S. cerevisiae* strain [37]; in the lager yeast *Saccharomyces pastorianus* CGMCC No. 4466, wheat-gluten peptide supplementation raises ethanol tolerance together with C18:1, UFA:SFA, and ergosterol [38]. The biophysics has an upper limit: the Laurdan-GP in *S. cerevisiae* holds constant up to 20% *v*/*v* and collapses irreversibly at or above 25% *v*/*v*, precisely where viability is lost [25]. Above approximately 10% *v*/*v* the bilayer becomes leaky to protons, marking the shift from adaptive to damaging perturbation [24]. One qualification frames the whole dose axis: ergosterol is protective under acute shock yet uncorrelated with completion under self-produced ethanol, and approximately 13 vol% already halves fermentative activity [23]. Direct fluidity data on the featured species remain scarce; a study of *T. delbrueckii* and *M. pulcherrima* reported a relative fluidity rise (Laurdan-GP) at 10% *v*/*v* that scales with tolerance, with the least-tolerant *M. pulcherrima* UMCC 15 showing the smallest rise and stiffening at 14–18% *v*/*v* [14]. On balance, unsaturation and ergosterol enrichment have been reported to defend fluidity in *S. cerevisiae*, though the response is strain- and dose-dependent, whereas the non-*Saccharomyces* often shift individual acyl-chain proportions with little net change in their summary indices (UI, UFA:SFA), and lose viability at lower ethanol.

Table 1. Ethanol-induced membrane lipid remodeling as a function of ethanol dose and exposure by organism.

Organism	Ethanol Dose	Ethanol Exposure	Observed Remodeling	Method	Ref.
S. cerevisiae	0–1.5 M	Ethanol added to growing culture, 10 h	UFA:SFA rises from 0.86 to 2.43 as C18:1 climbs from 16.7 to 33.5% and C16:0 falls.	GC-FAME (whole cell)	[33]
S. cerevisiae	10% *v*/*v* (growth); 0–12% *v*/*v* (acute DPH)	Growth in ethanol to mid-exponential phase	Whole-cell UFA:SFA rises from 1.09 to 2.87; DPH anisotropy stays near-flat once the cells are ethanol-adapted.	GC-FAME (whole cell); DPH (spheroplast plasma membranes)	[9]
S. cerevisiae	0–50% *v*/*v*	Acute ethanol exposure, 30 s	Laurdan-GP holds constant up to 20% *v*/*v*, then drops irreversibly at ≥25%, where viability is lost.	Laurdan-GP (living cells)	[25]
S. cerevisiae M	0–15% *v*/*v*	Acute ethanol exposure	Proton leak rises above 10% *v*/*v*, potentiated by temperature, with aquaporin *AQY1* inhibited.	stopped-flow; pH-metry (permeability, intact cells)	[24]
S. cerevisiae M	0–20% *v*/*v*	Ethanol exposure, 12 h	Fluidity rises below approximately 4% *v*/*v* then falls; relative UFA declines and *OLE1* is repressed at higher ethanol, while the most tolerant strain holds UFA and fluidity most nearly constant.	GC-FAME (whole cell); DPH (living cells); qPCR	[34]
S. cerevisiae	0–10% *v*/*v*	Stepwise adaptation by serial transfer	In adapted cells C16:0 falls while C18:1 shows no further increase over the non-adapted strain, the tolerant endpoint reached partly through saturated-chain reduction.	GC-FAME (whole cell)	[39]
S. cerevisiae	self-produced	Fermentation in synthetic juice, 1–20 days	UFA/TFA tracks viability, while ergosterol is inversely linked to final ethanol.	GC-FAME; HPLC-sterols (whole cell)	[40]
S. cerevisiae	self-produced	Fermentation in grape juice, 2–8 days	Long-chain unsaturated PC accumulating late marks fitter ferments, whereas high PI early marks failing ones.	HPLC-API-MS (whole cell); PLS	[41]
H. uvarum	4% *v*/*v* (growth); 0–12% *v*/*v* (acute DPH)	Growth in ethanol to mid-exponential phase	C18:1 rises but C16:1 falls, so the unsaturation index is essentially unchanged (0.77 to 0.73) and derived UFA:SFA falls from 3.42 to 2.76. Growth at 4% *v*/*v* nonetheless lowers DPH anisotropy from 0.161 to 0.151, indicating greater fluidity; acute ethanol lowers it further to 0.133 at 12% *v*/*v*, unlike the near-flat response of ethanol-adapted *S. cerevisiae*.	GC-FAME (whole cell); DPH (spheroplast plasma membranes)	[9]

Table 1. *Cont.*

Organism	Ethanol Dose	Ethanol Exposure	Observed Remodeling	Method	Ref.
T. delbrueckii, *H. uvarum*	4% *v*/*v* (composition); 0–10% *v*/*v* (EC_{50} growth series)	4 h (composition); 48 h (EC_{50} growth series)	Ergosterol runs about 7-fold lower than in *S. cerevisiae*, with EC_{50} values of 2.7–6.5% *v*/*v*.	GC-FAME; GC-sterols (purified plasma membrane)	[15]
T. delbrueckii, *M. pulcherrima*	0–18% *v*/*v*	Growth in ethanol, 24 h	Relative fluidity rises at 10% *v*/*v* in proportion to tolerance; the least-tolerant *M. pulcherrima* UMCC 15 stiffens at 14–18%.	Laurdan-GP (living cells)	[14]
W. anomalus	9% *v*/*v*	Acute exposure, 6 h	Laurdan-GP rises 2.19-fold (the membrane stiffens) and UFA:SFA falls; MUFA decline while VLCFA rise, even as *OLE1* is induced.	GC-MS (whole cell); Laurdan-GP (living cells)	[13]
Issatchenkia spp.	up to 10% *v*/*v*	Growth in ethanol, 72 h	MUFA decrease under ethanol, the opposite of *S. cerevisiae*.	GC-FAME (whole cell); PCA/LDA	[16]

Abbreviations: GC-FAME, gas chromatography of fatty-acid methyl esters; qPCR, quantitative polymerase chain reaction; HPLC, high-performance liquid chromatography; API-MS, atmospheric-pressure-ionization mass spectrometry; PLS, partial least squares; GC-MS, gas chromatography–mass spectrometry; PCA, principal component analysis; LDA, linear discriminant analysis; TFA, total fatty acids. ^{M} cross-beverage/model.

3.2. Temporal Dynamics: From the Adaptive Early Phase to Late Fermentation

Fermentation time defines a second trajectory, alongside the ethanol-dose axis of Section 3.1, along which composition shifts the other way. Without added ethanol, *Saccharomyces* phospholipid unsaturation actually falls during growth (0.70 at 8 h to 0.41 at 32 h): the basal tendency is toward saturation as cultures age [33]. During fermentation, UFA declines and SFA plus MCFA rise in all strains, mostly in the exponential phase, as oxygen and lipid precursors deplete [42]. Against this baseline, the adaptive lipidome that predicts success resists the late stiffening: in a 61-strain *S. cerevisiae* screen the most tolerant strains mount the largest Laurdan-GP fluidity increases at 10% ethanol, while the least-tolerant fail to modulate fluidity and carry significantly elevated PE, with low PE being beneficial [8].

Lipid profiling resolved by fermentation phase connects specific compositional shifts to fermentation outcome (Table 1). In 22 industrial *S. cerevisiae* strains (primarily wine strains), high PI early in fermentation correlates negatively with biomass and final ethanol, with failed ferments being PI-rich, whereas long-chain unsaturated PC accumulating in the stationary phase correlates positively with both by blunting ethanol fluidization [41]. Ergosterol shows only modest association with cell density and no correlation with ethanol over the time course [41], and in self-fermenting strains is even inversely linked to final ethanol [40]. What tracks viability and membrane integrity over time are the C16/TFA and UFA/TFA ratios, not total lipid or ergosterol content [40]. Adaptation can also be stepwise: raising ethanol incrementally to 10% v/v yields an adapted *S. cerevisiae* with lower C16:0 and little further C18:1 gain, reaching the tolerant endpoint partly through saturated-chain reduction [39]. Cross-domain and cross-beverage analogs reinforce this: a bioethanol-adapted strain accumulates more ergosterol, trehalose, and UFA, with ergosterol increasing by 107.8% at 10% v/v [43], and a tolerance-evolved rice-wine strain raises oleic acid and the C18/C16 ratio from 34.68 to 40.30% [44]. Comparable time-resolved data for the wine non-*Saccharomyces* are largely missing, a gap revisited in Section 7. Over the time course, tolerance is signaled less by a single late composition than by the capacity to keep the membrane appropriately fluid through stationary phase, PI-early and PC-late acting as opposing temporal markers.

3.3. Co-Stressors Subordinate to Ethanol

Ethanol never acts alone. Three co-stressors track it through fermentation (Table 2): the MCFA the yeast secretes, the fermentation temperature, and the oxygen that gates sterol and UFA synthesis. Each pushes on the same four descriptors (UFA:SFA, ergosterol, PL classes, fluidity) that shaped the dose and time responses above. They are treated here not as a separate catalog of stresses but as modulators that sharpen or blunt the ethanol signal and, in mixed fermentation, as the route by which one species' metabolism reaches another's membrane.

Table 2. Co-stressors of ethanol (MCFA, temperature, oxygen/sterol) and their membrane lipid effects in wine yeasts.

Co-Stressor	Organism	Membrane Lipid Effect	Ref.
MCFA (C8/C10)	*S. cerevisiae*	undissociated C8/C10 insert into PL, reducing organization and raising permeability; synergize with ethanol	[11]
MCFA (C8/C10)	*S. cerevisiae*	C8 exported by Pdr12/Tpo1 efflux and wall-sequestered; C10 diffuses in, esterified to ethyl decanoate	[45,46]

Table 2. *Cont.*

Co-Stressor	Organism	Membrane Lipid Effect	Ref.
MCFA (C10, decanoic)	*S. cerevisiae* (wine)	oleic acid rises and palmitoleic falls, ergosterol nearly doubles, and H^+-ATPase activity rises about fourfold	[47]
MCFA + ethanol/pH	*S. cerevisiae*	C10 a stronger H^+-influx enhancer than C8; toxicity rises with the undissociated fraction	[48,49]
MCFA (C8)	*S. cerevisiae*	C8 adaptation raises C18:1 and chain length; exogenous oleate protects; fluidity unchanged	[50]
MCFA + SO_2	*S. cerevisiae*	viability falls from 74.4 to 45.9% with 10 mg/L MCFA, and to 3.1% with a further 60 mg/L SO_2	[51]
MCFA + ethanol	*Starmerella bacillaris*	most MCFA-sensitive of the NCYs tested; as a sole agent inhibits at 5 vol% ethanol only at high MCFA (complete at 80 mg/L)	[52]
Cold (13 °C)	*S. cerevisiae*; *Saccharomyces bayanus*	unsaturation roughly doubles (*S. cerevisiae*); the strain reported as *S. bayanus* raises MCFA instead (ΣMCFA 36.09% at 13 °C, compared with 22.13% at 25 °C) and did not complete fermentation at 13 °C	[42]
Cold/heat (15–35 °C)	*S. cerevisiae*	15 °C raises PE and short-chain PC; 35 °C strongly raises PI; DPH anisotropy unchanged	[53]
O_2 depletion	*S. cerevisiae*; *T. delbrueckii*	unsaturation index crashes in both species; ergosterol and oleate rescue *S. cerevisiae* but not *T. delbrueckii*	[54]
O_2/sterol uptake	non-*Saccharomyces* (6 spp.)	lack *AUS1*/*PDR11* (6 genomes searched); sterol uptake far below *Saccharomyces* across 12 species (300–4800 compared with 868–73,000 AU)	[28]
O_2/aeration	*S. cerevisiae*; non-*Saccharomyces*	secreted C8/C10 decrease with O_2; anaerobiosis favors MCFA over long-chain unsaturated FA	[55,56]

3.3.1. Medium-Chain Fatty Acids (C8/C10)

MCFAs are the co-stressors most strongly amplified by ethanol. Octanoic (C8) and decanoic (C10) acids reach inhibitory levels during fermentation (0.7–23 mg/L) and, undissociated, insert into PL to lower organization and raise permeability, synergizing with ethanol leakage. The synergy is explicit: 6% *v*/*v* ethanol halves growth, and 8 mg/L octanoic adds a further 26–33% inhibition [11]. Decanoic is the more toxic: more liposoluble and, acting as a proton carrier, a stronger enhancer of passive H^+ influx that uncouples the gradient [48,49]. The two acids are handled by distinct routes: C8 is detoxified by Pdr12/Tpo1 export and retention in the cell wall, where about 80% of it is recovered, whereas C10 enters by passive diffusion and is esterified to ethyl decanoate [45,46]. Low pH and ethanol jointly potentiate this toxicity by favoring the undissociated species [46,49]. These mechanisms are best resolved in *S. cerevisiae*; among the non-*Saccharomyces* tested, *Starmerella bacillaris* (syn. *Candida zemplinina*) was the most MCFA-sensitive; as a sole inhibitor MCFA suppresses its growth even at 5 vol% ethanol, but only at higher MCFA doses, with complete inhibition at 80 mg/L [52]. The interaction is therefore intrinsic to mixed fermentation: C8/C10 accumulate as fermentation by-products and become inhibitory to the wine non-*Saccharomyces* specifically at high ethanol, so rising ethanol and a growing MCFA pool act on their membranes together rather than in isolation. In a non-engineered *S. cerevisiae*, octanoic adaptation raises C18:1 and chain length without altering fluidity, and exogenous oleate protects against C8-induced leakage [50]. Decanoic acid itself remodels a wine *S. cerevisiae* membrane, raising C18:1 by 65% and lowering C16:1 by 70%, nearly

doubling ergosterol (from 40 to 77.7%), and activating the H^+-ATPase fourfold [47]. In a real *S. cerevisiae* fermentation, MCFA toxicity compounds with SO_2: 10 mg/L MCFA cut viability to 45.9%, compared with 74.35% in the control, with 60 mg/L SO_2 driving it to 3.1% [51].

3.3.2. Temperature

Temperature modulates the same lipid levers in opposite directions, with low temperature reducing membrane fluidity and raising lipid order [57], the baseline on which ethanol then acts. The evidence is divided by design: fermentations combine temperature and self-produced ethanol, while growth experiments probe thermal adaptation alone. In fermentations at 15, 25, and 35 °C sampled at matched ethanol stages, low temperature elevated PE and short-chain unsaturated PC while 35 °C drove PI extremely high, yet DPH anisotropy stayed essentially constant, consistent with homeoviscous buffering, and membrane ergosterol was highest in the strain that exhibited stuck fermentation at all three temperatures [53]. In fermentations at 13 and 25 °C, *S. cerevisiae* roughly doubles its unsaturation degree at the lower temperature, whereas Uvaferm UVA (Lallemand), reported as *Saccharomyces bayanus* and marketed at the time as *Saccharomyces uvarum* [42,58], left UFA unchanged and raised MCFA instead; its fermentation at 13 °C became stuck, an association proposed rather than demonstrated, since strains with similar profiles at 25 °C fermented very differently [42]. The response is also time-resolved: *S. cerevisiae* QA23 fermenting at 13 °C raised unsaturation early and shortened chain length as fermentation progressed [59]. Under thermal adaptation alone, the cold-induced MCFA rise is general across the *Saccharomyces* strains tested, not the trait of one species [60], and in the cryotolerant *Saccharomyces kudriavzevii* more MCFA and shorter chains accompany better cold adaptation, whereas at 28 °C its fermentations took approximately twice as long to finish as those of *S. cerevisiae*, a delay the authors relate to its lower tolerance to the accumulating ethanol [61]. Cooler starts also prolong survival: non-*Saccharomyces* inoculated into must held at 15–17.5 °C for its first three days stayed detectable to day 13, even after the must warmed to 25 °C, whereas in 25 °C controls the indigenous non-*Saccharomyces* were undetectable by day 8, an indirect lever on the ethanol-tolerance window [62].

3.3.3. Oxygen and Sterol Availability

Oxygen gates both arms of the response, since ergosterol and UFA biosynthesis are O_2-dependent. Under anaerobiosis the unsaturation index crashes in both *S. cerevisiae* (from 0.6 to 0.2) and *T. delbrueckii* (from 0.5 to 0.2), and ergosterol and oleate fully rescue *S. cerevisiae* but not *T. delbrueckii*, in which ergosterol excess instead inhibits fermentation [54]. The reason is that wine non-*Saccharomyces*, namely *H. uvarum*, *Hanseniaspora guilliermondii*, *L. thermotolerans*, *T. delbrueckii*, *M. pulcherrima*, and *S. bacillaris*, lack the sterol-uptake transporters *AUS1*/*PDR11* and depend on their own O_2-requiring synthesis [28]. Anaerobiosis thus both starves them of sterols and favors toxic C8/C10, whose secretion conversely decreases with oxygenation as they are channeled into long-chain biosynthesis [55,56]. Oxygen demand rises in the order *L. thermotolerans*, then *T. delbrueckii*, then *M. pulcherrima*, the last being an obligate aerobe that drives dissolved O_2 to zero within hours [56,63], and *Metschnikowia* may stop growing at zero oxygen because it cannot import membrane lipids [64]. Oxygen limitation can itself be lethal in mixed culture: *T. delbrueckii* and *L. thermotolerans* survive approximately 65–70 g/L self-produced ethanol in single culture yet begin to die at approximately 35 g/L in mixed culture, with their death attributed to oxygen deficit rather than toxic metabolites [65]. For these non-*Saccharomyces*, their markedly reduced sterol uptake turns oxygen limitation into a direct constraint on the ethanol-tolerance lipidome.

Taken together with the dose- and time-resolved responses established largely in *S. cerevisiae*, these co-stressor effects bring into focus how lipid composition itself differs between *S. cerevisiae* and the non-*Saccharomyces* wine yeasts.

4. Comparative Membrane Lipid Composition of *S. cerevisiae* and the Non-*Saccharomyces*

4.1. The S. cerevisiae Reference

The *S. cerevisiae* membrane provides a consolidated, reproducible baseline, albeit one that varies with strain and growth conditions. In rehydrated wine active dry yeast, SFAs hold near 25.4–25.9% and UFAs near 74.1–74.6%, resulting in a derived UFA:SFA ratio of approximately 2.9, with mean chain lengths around 16.4–16.9 carbons on a strain-dependent C16/C18 backbone [66]. The decisive trait is biosynthetic. As introduced in Section 2.1, *S. cerevisiae* possesses the single Δ9 desaturase *OLE1* [19] and therefore yields only MUFAs (oleic, palmitoleic) and no de novo PUFAs [21]. Its glycerophospholipids are dominated by PI, then PC, PE, and PS (Table 3), the major acyl species capped at two double bonds and totaling 32–34 carbons across both chains [67]. This compact, PI-rich membrane, whose unsaturated chains are exclusively mono-unsaturated (no PUFA), is the reference against which the NCYs are compared.

4.2. The Non-Saccharomyces Wine Yeasts

Against that frame, the best-characterized NCYs tend to be more unsaturated and biosynthetically more capable. Two of the best-resolved species, *T. delbrueckii* and *M. pulcherrima*, were profiled directly alongside *S. cerevisiae* under the same experimental conditions, *T. delbrueckii* carrying the highest pre-stress UFA:SFA of the species (Table 3) [19]. Crucially, both NCYs synthesize PUFAs that *S. cerevisiae* cannot: linoleic acid (C18:2) is present in *T. delbrueckii* and *M. pulcherrima*, while linolenic acid (C18:3) is detected only in *M. pulcherrima*, which therefore carries the highest unsaturation index [19]. That this is an endogenous capacity, not a medium artifact, was confirmed in synthetic must without lipids, where *M. pulcherrima*, *T. delbrueckii*, and *Kluyveromyces marxianus* all produced PUFAs whereas *S. cerevisiae* produced only MUFAs [21]. On a cellular-mass basis, total UFA ranked *M. pulcherrima* (1402.3 μg/g), then *K. marxianus* (929.5), *T. delbrueckii* (566.9), and *S. cerevisiae* (316.9) [30]. A Fourier-transform near-infrared (FT-NIR) survey of five wine NCYs, although inactivated at different growth stages, predicted *T. delbrueckii* at the top for both MUFA and ergosterol, above the *S. cerevisiae* values of 2.03 and 0.37 g/100 g dried yeast (Table 3) [68].

Table 3. Lipid composition of *S. cerevisiae* (reference frame) and the principal non-*Saccharomyces* wine yeasts, organized per species. Units and reference bases are given in each cell and differ across sources, so values are directly comparable only when they come from the same source.

Species	Phospholipid Composition	Fatty-Acid Composition	Sterol Composition	Method	Culture Condition or Sample Basis	Ref.
S. cerevisiae (reference)	PC 31.5, PE 17.4, PI 39.9, PS 4.8 (% of total phospholipids) [a]	UFA:SFA 1.91–2.01; UI 0.66–0.67 [c]; SFA 1.04, MUFA 2.03 (g/100 g dried yeast) [g]; no PUFA (only saturated and mono-unsaturated chains; two-acyl-chain phospholipid species carry at most two double bonds) [a,d]	ergosterol 0.37, total sterols 0.84 (g/100 g dried yeast) [g]; ergosterol 53–71 (% of total sterols) [c]; total sterols 16.5–26.9 µg/mg protein [c]	LC-FTICR-MS [a] (whole cell); GC-FAME/GC-MS [c] (whole cell); FT-NIR/PLS [g] (predicted, dried biomass)	shake-flask culture, no ethanol [a]; YPD, 6 h of growth, before stress exposure [c]; lipid-free defined medium, semi-aerobic, 25 °C [d]; thermally inactivated dried yeast [g]	[67] [a], [19] [c], [21] [d], [68] [g]
T. delbrueckii	PC approximately 45, PE approximately 24 (% of total phospholipids) [e]	UFA:SFA 2.45–2.61; UI 0.97–0.99 [e]; SFA 1.50, MUFA 6.18 (g/100 g dried yeast) [g]; contains linoleic acid (C18:2), a PUFA [h]	ergosterol 38–41, squalene 33–36 (% of total sterols) [e]; total sterols 25.0–27.9 µg/mg protein [e]; ergosterol 0.50, total sterols 1.14 (g/100 g dried yeast) [g]; cellular ergosterol 33.8 µg/g biomass [f]	GC-FAME/GC-MS [e] (whole cell); FT-NIR/PLS [g] (predicted, dried biomass); GC-MS [f] (whole cell)	YPD, 6 h of growth, before stress exposure [e]; end of alcoholic fermentation, lipid-free synthetic grape must [f]; thermally inactivated dried yeast [g]; lipid-free defined medium, semi-aerobic, 25 °C [h]	[19] [e], [30] [f], [68] [g], [21] [h]
M. pulcherrima	PC and PE the major classes; PI and PS minor [e]	UFA:SFA 1.98–2.20; UI 1.11–1.19 [e]; SFA 1.42, MUFA 3.16 (g/100 g dried yeast) [g]; contains linoleic (C18:2) and linolenic (C18:3) acids, both PUFA [e]; cellular UFA 1402 µg/g biomass [f]	ergosterol 89–97 (% of total sterols); total sterols 3.78–8.16 µg/mg protein [e]; ergosterol 0.46, total sterols 1.02 (g/100 g dried yeast) [g]; cellular ergosterol 48.3 µg/g biomass [f]	GC-FAME/GC-MS [e] (whole cell); FT-NIR/PLS [g] (predicted, dried biomass); GC-MS [f] (whole cell)	YPD, 6 h of growth, before stress exposure [e]; end of alcoholic fermentation, lipid-free synthetic grape must [f]; thermally inactivated dried yeast [g]	[19] [e], [30] [f], [68] [g]

Table 3. *Cont.*

Species	Phospholipid Composition	Fatty-Acid Composition	Sterol Composition	Method	Culture Condition or Sample Basis	Ref.
H. uvarum/*H. guilliermondii*	N.M.	*H. guilliermondii*: oleic acid 14 (% of total fatty acids and sterols, aerobic) [j]; no linolenic acid (C18:3) [k]	*H. uvarum* ergosterol 46.1 (% of total sterols) [i]; *H. guilliermondii* ergosterol 71.74 (% of total fatty acids and sterols, aerobic) [j]; *H. uvarum* 49.57 (% of total fatty acids and sterols, aerobic) [j]; ergosterol per dry biomass: *H. uvarum* 5.69, *H. guilliermondii* 4.65 mg/g [k]	GC-FAME [i] (whole cell); GC-FAME (aerobic/anaerobic) [j] (whole cell); GC-MS [k] (whole cell)	aerobic YPD [i]; aerobic YM broth, before the ethanol challenge [j]; modified YNB medium with a high carbon/nitrogen ratio, selected to prompt lipid biosynthesis, 72 h [k]	[9] [i], [10] [j], [17] [k]
L. thermotolerans	PC 26.3, PE 14.9, PI 49.5, PS 4.9 (% of total phospholipids) [a]	SFA 1.10, MUFA 3.20 (g/100 g dried yeast) [g]; PUFA present [a]; PE, PC, and PI species reach up to five double bonds across both chains, versus at most two in the same classes of *S. cerevisiae* [a]	ergosterol 0.40, total sterols 0.91 (g/100 g dried yeast) [g]	LC-FTICR-MS [a] (whole cell); FT-NIR/PLS [g] (predicted, dried biomass)	shake-flask culture, no ethanol [a]; thermally inactivated dried yeast [g]	[67] [a], [68] [g]
W. anomalus	PC 37, PE 31, PS and PI 17 (% of total phospholipids) [q]	contains linoleic (C18:2) and linolenic (C18:3) acids, both PUFA; oleic acid (C18:1) 27, C18:2 37, C18:3 13 (% of total fatty acids) [q]; MUFA 39–56, odd-chain C17:1 6–30 (% of total fatty acids) [r]	ergosterol 10, lanosterol 3 (% of neutral lipids) [q]	TLC densitometry/GLC [q] (whole cell); GC-FAME [r] (whole cell)	fermentor culture, lactate-based medium, 27 °C, onset of stationary phase [q]; YPD, 24 h at 15 to 30 °C [r]	[69] [q], [70] [r]

Table 3. *Cont.*

Species	Phospholipid Composition	Fatty-Acid Composition	Sterol Composition	Method	Culture Condition or Sample Basis	Ref.
S. bacillaris	N.M.	MUFA 5.67 (g/100 g dried yeast) [m]; PUFA status conflicting (linoleic and linolenic acids present in strain CBS 2649 [o] versus both absent in the two tested isolates [k])	total sterols 0.98 (g/100 g dried yeast) [m]; ergosterol per dry biomass: 5.29 and 4.15 mg/g in two isolates [k]	FT-NIR/PLS [m] (predicted, dried biomass); GC-MS [k] (whole cell)	modified YNB medium with a high carbon/nitrogen ratio, selected to prompt lipid biosynthesis, 72 h [k]; freeze-dried biomass from fed-batch propagation [m]; defined liquid medium, 48 h at 25 °C, aerated [o]	[71] [m], [17] [k], [72] [o]

Symbols and notes: N.M., not measured quantitatively. Superscript letters map each cell to its source. Values from FT-NIR/PLS are chemometric predictions; the others are direct measurements by the methods listed. Abbreviations: GC, gas chromatography; LC-FTICR-MS, liquid chromatography–Fourier-transform ion cyclotron resonance mass spectrometry; TLC, thin-layer chromatography; GLC, gas–liquid chromatography; FT-NIR, Fourier-transform near-infrared.

The sterol axis, however, decouples from unsaturation. *M. pulcherrima* concentrates its sterol pool almost entirely as ergosterol yet carries the lowest total sterol content, whereas *T. delbrueckii* shows the opposite emphasis, with the lowest ergosterol fraction and the highest squalene (Table 3) [19]. Across whole fermentations, *M. pulcherrima* nonetheless accumulates the highest ergosterol of the species compared [21], and on a dry-mass basis its cellular ergosterol (48.3 μg/g) likewise exceeds that of *T. delbrueckii* (Table 3) [30]. The resident fatty-acid composition may also shape how each species responds to externally supplied lipids, with the UFA-rich *M. pulcherrima* and the MUFA-rich *K. marxianus* behaving differently from one another and from *S. cerevisiae* [73]. The highest unsaturation index and the highest ergosterol both occur in *M. pulcherrima*, one of the least ethanol-tolerant species (Section 3.1), showing that neither property by itself confers ethanol tolerance.

H. uvarum is one of the few wine NCYs whose membrane fluidity has been measured directly against *S. cerevisiae* under ethanol, together with *T. delbrueckii* and *M. pulcherrima* (Section 3.1). In *H. uvarum* ethanol raised oleic acid but lowered palmitoleic acid, so its unsaturation index barely moved (its dose response is resolved in Section 3.1) even as ergosterol rose from 46.1% to 50.9% of total sterols [9]. Within the genus, ethanol tolerance is species-specific: *H. guilliermondii* approaches *S. cerevisiae*, carrying the highest ergosterol fraction of total fatty acids and sterols but the lowest oleic acid, unlike the more sensitive *H. uvarum* (Table 3) [10]. The broadest 2026 wine yeast lipid survey quantified ergosterol per dry mass for these isolates (Table 3) and confirmed that *H. guilliermondii*, like *S. cerevisiae*, produces no linolenic acid (C18:3) [17]. For *L. thermotolerans* a rare quantitative glycerophospholipid baseline keeps the PI-rich pattern, yet its PE, PC, and PI molecular species carry up to five double bonds in total across both acyl chains, compared with at most two in the corresponding classes of *S. cerevisiae*, a pattern consistent with additional desaturase activity (Table 3) [67]. Consistent with this, its lipid metabolism is part of the transcriptional program that repairs ethanol damage under oxygen depletion [32].

Under ethanol, *W. anomalus* stiffens rather than fluidizes, accumulating very-long-chain C22:0/C24:0 at the expense of monounsaturated C16:1/C18:1, the opposite of the canonical *S. cerevisiae* response, as detailed in Section 3.1 [13]. Yet in sterile grape juice fermented by indigenous non-*Saccharomyces*, unsaturated-fatty-acid biosynthesis was the metabolic pathway most affecting the wine lipid profile across the isolates [12], confirming that lipid change is not uniform across the NCYs.

The secondary taxa add to this heterogeneity. *K. marxianus* is a clear counterexample: a poorly ethanol-tolerant yeast (growth inhibited above 6% *v*/*v*) that fails to remodel, leaving its unsaturated fatty-acid degree and ergosterol content unchanged under ethanol stress, exactly where *S. cerevisiae* would raise both [74]. A comparative untargeted lipidomic panel of grape-associated yeasts likewise resolved *H. uvarum*, *M. pulcherrima*, and *Pichia terricola* (syn. *Issatchenkia terricola*) into discrete clusters and confirmed the absence of PUFA in *S. cerevisiae*, their low abundance in *H. uvarum*, and the species-level ranking of ergosterol [75].

The most consequential gap concerns *S. bacillaris*, an ecologically valuable, fructophilic, ethanol-persistent wine yeast whose isolates tolerate up to 14% *v*/*v* and produce mostly 8.0–9.5% *v*/*v* ethanol in pure culture, with production reaching as high as approximately 14% *v*/*v* [76], yet whose complete membrane lipidome has, to our knowledge, not yet been resolved. Reports even conflict on its most basic feature: a GC analysis quantified linoleic and linolenic acids in strain CBS 2649 [72], classified at the time as *Candida (Torulopsis) stellata* and now assigned to *S. bacillaris*, whereas a recent GC-MS screening of wine yeast lipid composition found neither acid in the two *S. bacillaris* isolates tested [17]. Both measurements therefore concern the same species, and the discrepancy remains open. Available data for *S. bacillaris* remain incomplete: FT-NIR/PLS provides predictions of

MUFA and total sterols [71], cellular fatty acids and ergosterol were quantified by GC-MS after growth in a common medium selected to prompt increased lipid biosynthesis [17], and the *Starmerella* clade's lipid metabolism has been reviewed as biosynthetic rather than membrane-remodeling [77]. None of these resolves PL-class composition or a membrane-fractionated profile, and a mixed-fermentation study that lowered wine ethanol with this species reports phenotype only, without membrane data [78]. This data gap, together with the fragmentary data available for the other species, is what a predictive lipid signature would have to fill, and Section 8 returns to it as the priority target.

4.3. Transferability of S. cerevisiae Lipid Principles to the Non-Saccharomyces

Not all of the lipid principles established in *S. cerevisiae* transfer to the NCYs. Three rules transfer cleanly. First, the capacity for endogenous ergosterol synthesis is conserved: *ERG6*, a marker gene of the ergosterol biosynthetic pathway, retains 66–83% sequence identity across *Hanseniaspora*, *L. thermotolerans*, *T. delbrueckii*, *M. pulcherrima*, and *S. bacillaris* [28]. Second, the oleic-acid-and-ergosterol pairing remains a useful, though not universal, tolerance correlate: across a mixed panel of wine yeasts (non-*Saccharomyces* together with *S. cerevisiae*), ethanol tolerance and proton-ATPase activity co-vary with combined oleic acid and ergosterol [15]. Third, the unsaturation lever is engaged: the wine non-*Saccharomyces* remodel their fatty-acyl unsaturation under ethanol (Section 3.1), and, at the wine-lipidome level, unsaturated-fatty-acid biosynthesis is the pathway most affecting composition across indigenous non-*Saccharomyces* fermentations [12].

Three others do not carry over to the non-*Saccharomyces*, marking the principal gaps in current knowledge. The most consequential is exogenous sterol uptake: the *AUS1*/*PDR11* transporters that let *S. cerevisiae* import sterols under hypoxia have no functional homologues in any of the six wine NCYs surveyed, whose sterol uptake falls far below that of *Saccharomyces* [28]. The NCYs can thus make ergosterol but most cannot rescue their membranes by scavenging it. The second failure is the chain that runs from unsaturation through membrane fluidity to tolerance. The very metric that statistically separates tolerant from sensitive *S. cerevisiae* is built entirely on *Saccharomyces*: low PE coupled to high fluidity, with the unsaturation index alone being insufficient [8]. Even within *Saccharomyces*, the homeoviscous logic is not simple: in an *S. cerevisiae* × *S. uvarum* hybrid adapted by ALE, increased ethanol tolerance tracked reduced PE and a more fluid (lower-GP) membrane rather than any change in mean chain length, and the authors stress that saturation shifts alone cannot fully account for tolerance [79]. In the NCYs the link can break outright: *W. anomalus* stiffens under ethanol while accumulating VLCFA rather than MUFA [13], and the poorly tolerant *K. marxianus* fails to raise its unsaturation or ergosterol at all (Section 4.2). The third reservation is interpretive: indices derived from sterol:PL or unsaturation can misrepresent the actual membrane state, particularly in species other than *S. cerevisiae* [9], so a higher proportion of C18:2 does not assure greater ethanol tolerance in the NCYs [19].

Collectively, these NCYs share *S. cerevisiae*'s biosynthetic capacity but lack its sterol-import route and any single transferable fluidity rule. Where these compositional differences expose vulnerabilities, targeted interventions may close the gap, provided that what works in *S. cerevisiae* transfers.

4.4. Ethanol Tolerance and Lipid Composition in Saccharomyces Species Other than S. cerevisiae

Within the genus itself, the same comparison tests whether these descriptors are conserved or specific to *S. cerevisiae*. Across 26 strains of seven *Saccharomyces* species, the minimum inhibitory ethanol concentration (MIC) for growth ranked *S. cerevisiae* highest (average 112.5 g/L), then *Saccharomyces paradoxus* (88.7 g/L) and *S. uvarum* (as *S. bayanus* var. *uvarum*; 80.5 g/L), with *S. kudriavzevii* lowest (59.3 g/L); in the three strains profiled,

the basal UFA:SFA ratio followed the same order while ergosterol did not differ significantly [80]. Patagonian *S. uvarum* and *Saccharomyces eubayanus* isolates grew at up to 8% *v*/*v* exogenous ethanol and not above [81]. Part of the signature carries over: across eight brewing strains including the *S. eubayanus* type strain, fermented at 10 and 20 °C with and without 8% *v*/*v* ethanol, oleic acid and ergosterol favored growth at the higher ethanol level [82]. Composition diverges elsewhere: *S. kudriavzevii* carries more MCFAs, shorter chains, and more squalene than *S. cerevisiae* regardless of growth temperature [61], and during wine fermentation short-chain fatty acids accumulate in *S. cerevisiae* and its hybrids but not in the *S. uvarum* or *S. kudriavzevii* parents [83], a difference too narrowly documented to make any particular MCFA a taxonomic or phylogenetic trait. Hybrids inherit lipid regulation by class: in ethanol-free cultures the lager hybrid *S. pastorianus* pairs *S. cerevisiae*-like control of storage lipids with *S. eubayanus*-like control of cardiolipin [84], while wine hybrids show no common parental pattern [83]. Studies measuring the membrane lipidome, membrane fluidity, and ethanol tolerance together are as scarce within the genus as in the NCYs; the one dataset combining all three concerns a constructed *S. cerevisiae* × *S. uvarum* hybrid [79]. Whether the proposed descriptors are phylogenetically conserved therefore remains open: an indicator that fails at this evolutionary distance cannot be assumed to transfer beyond it.

5. Interventions to Improve Non-*Saccharomyces* Fermentation via Membrane Lipids

5.1. Nutritional and Process Levers

The most direct lever is lipid supply, because the same clarification that produces clean musts strips them of the very nutrients the membrane needs. Grape-must clarification depletes musts of sterols and UFA, which are critical nutrients in alcoholic fermentation, and under high nitrogen this micronutrient starvation actively triggers nitrogen-dependent yeast cell death [85]. Restoring that lipid fraction is correspondingly protective: supplying 1.5% (*v*/*v*) grape solids as a source of non-soluble phytosterols and long-chain fatty acids keeps end-of-fermentation viability above 85%, compared to below 60% in clarified must, and a delipidification control, in which solids stripped of their lipids lose the benefit, shows the effect is attributable principally to lipid content rather than to nitrogen or polysaccharide [86]. The choice and timing of the supplement are decisive in their own right: under lipid limitation an excess of nitrogen accelerates rather than prevents death, and adding ammonium early in fermentation triggers cell death whereas the same addition once cells have entered stationary phase does not [85]. These outcomes, all obtained in *S. cerevisiae*, establish nutritional rules that extend unevenly to the non-*Saccharomyces*, within the transfer limits set out in Section 5.2.

Sterol identity is a second lever, and its effect is species-dependent. Reviewing oenological thresholds, 2–8 mg/L phytosterols suffice for normal early growth and 15 mg/L ergosterol supports complete fermentations, while 150 g/L of inactivated dry yeast (IDY) delivers roughly 40 µg/L ergosterol [87]. Yet the type of sterol supplied affects the outcome: under sterol limitation native ergosterol preserves more *S. cerevisiae* viability than phytosterols (49.0% compared with 40.9%) and gives faster ferments under high sugar [88]. The intrinsic lipid profile of each species further conditions the response to any exogenous mixture: supplied fatty-acid/sterol blends improve the three wine non-*Saccharomyces* far more than the near-insensitive *S. cerevisiae* EC1118, with *M. pulcherrima*, *T. delbrueckii*, and *K. marxianus* each favoring a different blend [73]. Inactivated non-*Saccharomyces* biomass differs in sterol content, with *T. delbrueckii* derivatives carrying the highest ergosterol of the wine species surveyed and *M. pulcherrima* among the next richest [68]; this compositional ranking applies to the inactivated biomass, not to the donor strain's own ethanol tolerance.

Oxygenation is the third lever, and its timing is the most consequential, because ergosterol and UFA synthesis are O_2-dependent (the mechanism is described in Section 3.3.3). Where phytosterols are absent, 5–10 mg/L O_2 restores fermentation rate [87], and an early aeration remodels the non-*Saccharomyces* membrane directly: in *Hanseniaspora vineae* it raises the UFA:SFA ratio 3.6-fold and 2.2-fold (after one and two days of aeration) and cuts ethanol by 16.9% in mixed fermentation while prolonging the non-*Saccharomyces* persistence [89]. One-percent dissolved oxygen sustains *L. thermotolerans*, *T. delbrueckii*, and *M. pulcherrima* while lowering ethanol without loss of quality, although *M. pulcherrima* benefits from lower oxygen to avoid excess acetic acid [56]. The trade-off sets a practical limit: an aerated *M. pulcherrima*/*S. cerevisiae* co-fermentation can lower alcohol by up to 3.7% (*v*/*v*), but only at the cost of unacceptable volatile acidity, the practical optimum at 25% air giving a 2.2% (*v*/*v*) reduction [63]. Temperature is a milder, indirect lever on the same window: a cold start at 17.5 °C lowers final ethanol by about 0.8% (*v*/*v*) and keeps the non-*Saccharomyces* detectable to day 12–13 (the lipid mechanism is described in Section 3.3.2) [62].

Nitrogen, finally, is necessary but rarely sufficient on its own. In a sequential *T. delbrueckii*/*S. cerevisiae* fermentation without cell-to-cell contact, nitrogen alone leaves the ferment stuck even at 600 mg nitrogen/L, and only the combined addition of thiamine, zinc, and amino acids lets *S. cerevisiae* complete it [90]. Process designs based on persistence extend the time the non-*Saccharomyces* remain active: a sequential inoculation lengthens the ferment, with the native non-*Saccharomyces* persisting to the end of fermentation [1], and oxygenation extends *S. bacillaris* persistence while modulating mainly the acetic acid content [91]. In practice, what sustains the non-*Saccharomyces* membrane through fermentation is lipid restitution, with grape solids or ergosterol, together with correctly timed oxygen and a complete micronutrient package, not nitrogen alone (Table 4).

Table 4. Nutritional and process interventions, their membrane lipid bases, and reported outcomes from wine fermentations and complementary assays.

Intervention	Organism	Membrane Lipid Basis	Reported Outcome	Ref.
Grape solids 1.5% (*v*/*v*)	*S. cerevisiae*	non-soluble phytosterols + LCFA restore lipid status; delipidified control fails	end-ferment viability above 85% versus below 60% in clarified must	[86]
Nitrogen and lipid timing	*S. cerevisiae* (EC1118)	sterol/UFA depletion from clarification; N excess accelerates death	early N triggers death; late (stationary) addition does not	[85]
Phytosterol versus ergosterol	*S. cerevisiae*	native ergosterol preserves membrane better than phytosterols under limitation	viability 49.0% versus 40.9%; faster high-sugar ferments	[88]
Exogenous FA/sterol blends	*M. pulcherrima*, *T. delbrueckii*, *K. marxianus*	response set by intrinsic FA profile (*AUS1*/*PDR11* homologues reported absent in all three species)	favored blend raises CO_2 production, slightly in *M. pulcherrima* and significantly in *K. marxianus* and *T. delbrueckii*; no significant EC1118 response	[73,92]
Inactivated NCY biomass	*T. delbrueckii*, *M. pulcherrima*	derivatives contain ergosterol (*T. delbrueckii* highest of wine species)	iron- and copper-enriched wine-like solution: *M. pulcherrima* derivative has the highest initial O_2 consumption rate (0.84 mg/L/day); *T. delbrueckii* derivative has the highest total O_2 consumption capacity among the non-*Saccharomyces* (4.06 mg/L)	[68]
Early oxygenation	*H. vineae*	UFA:SFA rises 3.6-fold/2.2-fold (palmitoleate-driven)	ethanol −2.4% (*v*/*v*), −16.9% relative; persistence prolonged	[89]
Oxygenated pre-culture	*H. guilliermondii*	O_2-built sterol/UFA pool exceeds anaerobic ergosterol/Tween 80	viability rises at 25% (*v*/*v*) ethanol	[93]

Table 4. *Cont.*

Intervention	Organism	Membrane Lipid Basis	Reported Outcome	Ref.
Aeration (25% air)	*M. pulcherrima*	respiratory carbon diversion (aerated co-fermentation with *S. cerevisiae*)	alcohol −2.2% (*v*/*v*) at 25% air; up to −3.7% only at high (100%) aeration, with volatile-acidity penalty	[63]
Cold start (17.5 °C)	*M. pulcherrima*	low-temperature fluidity defense extends survival window	ethanol −0.8% (*v*/*v*); NCYs to day 12–13	[62]
N + thiamine/Zn/amino acids	*T. delbrueckii* then *S. cerevisiae*	non-lipidic; micronutrient co-limitation gates completion	N alone stuck at 600 mg/L; full package completes	[90]
Sequential inoculation	*M. pulcherrima*	phytosterols modulate ester/MCFA output	ethanol about −8 g/L (−1% vol)	[94]
O_2 and strain combination	*S. bacillaris*	O_2 extends NCY persistence	with O_2, acetic acid is almost two-fold higher in mixed cultures except with MUT5705; day-14 dominance only with IONYS WF	[91]
Sequential co-fermentation	non-*Saccharomyces* (native)	environmental persistence, not membrane remodeling	synthetic must: mixed fermentation finishes within 8 days versus 4 days with *S. cerevisiae* alone; natural must: NCYs detected to end by qPCR	[1]
Ergosterol versus oleate in lipid-limited must	*S. cerevisiae* (EC1118, wild-type)	ergosterol the limiting lipid (restores viability to approximately 100%; oleate does not); N excess accelerates death	viability 70% (low N) versus 45% (high N) at 70 g/L CO_2	[95]
Ergosterol + oleate (anaerobic)	*S. cerevisiae* versus *T. delbrueckii*	full rescue only in *S. cerevisiae*; in *T. delbrueckii* ergosterol excess instead inhibits fermentation, a transfer ceiling	*T. delbrueckii* sugar fermented/ethanol (g/L): 75/35.9 (no addition), 59/20.5 (ergosterol), 106/45.0 (ergosterol + oleate)	[54]
Exogenous sterol uptake	non-*Saccharomyces* (6 spp.)	lacking *AUS1*/*PDR11*, sterol uptake far below *Saccharomyces*, a transfer ceiling	sterol feeding ineffective under hypoxia	[28]
Laboratory-evolved (non-GMO) *S. cerevisiae*	*S. cerevisiae* (IONYS WF)	laboratory evolution, not a membrane lipid intervention	ethanol −1.0% (*v*/*v*); raised glycerol	[91]

Abbreviations: LCFA, long-chain fatty acid; N, nitrogen; Zn, zinc.

5.2. Transferability of S. cerevisiae Interventions to the Non-Saccharomyces

The most efficacious *S. cerevisiae* intervention is also the one that does not transfer to the non-*Saccharomyces*: exogenous sterol supply presumes the *AUS1*/*PDR11* import route that the wine non-*Saccharomyces* surveyed lack (Section 4.3) [28], as the oenological-threshold literature also notes [87].

Two controlled comparisons quantify this transfer ceiling. Ergosterol (25 mg/L) plus oleic acid (31 mg/L) fully rescues anaerobic growth and fermentation in *S. cerevisiae* but not in *T. delbrueckii*; indeed, in the latter, ergosterol excess instead inhibits fermentation, the strain producing 35.9 g/L with no addition, 20.5 g/L with ergosterol, and 45.0 g/L only when oleate is added back (the underlying anaerobic unsaturation index crash is presented in Section 3.3.3) [54]. Extending the panel, exogenous ergosterol plus oleate raise ethanol tolerance only in *S. cerevisiae* and *H. guilliermondii*, and not in *H. uvarum* or *T. delbrueckii* despite measurable incorporation, while *Debaryomyces hansenii* does not even incorporate them—a species-dependent transfer ceiling rather than a dosing failure [10]. Its companion study shows the membrane route that does help is upstream: an oxygen-supplied pre-culture, more than anaerobic ergosterol/Tween 80, raises viability at 25% (*v*/*v*) ethanol in *S. cerevisiae* and *H. guilliermondii* [93]. Anticipating a strain's response to sterol feeding would require reading its measured lipid signature together with its sterol-uptake capacity and oxygen regime (Section 7). In the end, the most effective *S. cerevisiae* lever, exogenous sterol rescue, transfers to at most one *Hanseniaspora* species; for the others, oxygen availability

instead supports endogenous sterol and UFA synthesis, but the resulting ethanol-tolerance benefit is species-dependent.

5.3. Reported Ethanol Reduction Outcomes

The ethanol reduction achievable with these non-*Saccharomyces* strategies ranges from 0.25 to 3.7% (*v*/*v*) across inoculation strategies, varying with species and fermentation conditions [62]. These reductions were obtained with heterogeneous strategies, most involving mixed or sequential fermentations. Sequential *M. pulcherrima*/*S. cerevisiae* lowers ethanol by about 8 g/L (approximately −1% vol) independently of must composition, with phytosterols modulating the ester and MCFA profile [94], while the two aeration outcomes quantified in Section 5.1 span the rest of the range, with aeration of *H. vineae* cutting final ethanol by 2.4% (*v*/*v*) [89] and respiratory *M. pulcherrima* reaching the upper bound only with the volatile-acidity penalty [63]. A laboratory-evolved *S. cerevisiae* (IONYS WF) reduces ethanol by 1.0% (*v*/*v*) and raises glycerol, complementing the non-*Saccharomyces* lever without genetic modification [91]. Some co-starters work through metabolism rather than membrane lipids: *S. bacillaris* and *Zygosaccharomyces bailii* co-starters lower wine ethanol (0.45% and 0.80% *v*/*v* in sequential and simultaneous inoculation, respectively), a metabolic, non-lipidic mechanism proposed at the laboratory scale [96].

Beyond modulating the fermentation environment, the cell itself can be improved through non-GMO strain development.

6. Non-GMO Strategies for Ethanol Tolerance in Wine Non-*Saccharomyces*

When environmental levers reach their ceiling, improving the strain itself, without genetic modification, becomes the route. Four non-transgenic strategies upgrade a wine yeast: clonal selection, random mutagenesis, hybridization, and ALE [7]. Their appeal in oenology is partly regulatory: each acts on natural or induced genetic variation rather than recombinant DNA, so the resulting strains escape the consumer-acceptance barriers that constrain transgenic routes in winemaking. The biology, however, is more complex: ethanol tolerance is the quantitative, polygenic trait introduced in Section 1, so what is selected is not a single allele but a whole-genome configuration. Because the membrane lipidome is one system that configuration reshapes (Section 5.2), what matters is whether the lipid signature of the improved strain is ever measured. For the wine non-*Saccharomyces*, it almost never is (Table 5). Alongside strain improvement, oenology also selects and combines existing strains, from native-collection screening to multispecies consortia (Section 6.3).

Table 5. Non-GMO strain-improvement studies for ethanol tolerance and ethanol-yield reduction in non-*Saccharomyces* yeasts, organized per study.

Method	Organism	Trait Gained	Lipid Signature	Ref.
ALE (serial batch, 6 to 12% *v*/*v*)	*T. delbrueckii* (wine)	Ethanol tolerance from 9 to 11.5–12% *v*/*v*; SO_2 cross-protection; co-inoculum to 10.8% *v*/*v*	Not measured	[97]
ALE (200 gen., L-Phe selection)	*L. thermotolerans* (wine)	Ethanol tolerance (biomass 2.26-fold at 8% *v*/*v*); high-sugar/SO_2/pH	Not measured	[98]
ARTP mutagenesis + selection	*Hanseniaspora* sp. (wine)	Ethanol-yield reduction (−1.4% *v*/*v* in Merlot, sequential)	Not measured	[99]
Random mutagenesis (UV/DES)	*P. terricola* (wine)	Ethanol tolerance from 8 to 10–11% *v*/*v*; aroma modulation	Not measured	[100]
Spontaneous-mutant selection (SO_2, then ethanol, then CO_2)	*T. delbrueckii* (wine)	Growth on 10% *v*/*v* ethanol; stable after 100 doublings	Not measured	[101]

Table 5. *Cont.*

Method	Organism	Trait Gained	Lipid Signature	Ref.
Cell mixing (Td × Sc) + spontaneous mutants resistant to high CO_2 pressure	*T. delbrueckii* (sparkling wine)	Intermediate ethanol/SO_2/Cu resistance; near-*S.c.* CO_2 pressure	Not measured	[102]
Hybridization (protoplast fusion, Td × Sc)	*T. delbrueckii* × *S. cerevisiae* clone F1-11 (wine)	Survival above the *S. cerevisiae* parent in a 2 h ethanol and acetic-acid challenge; fructose consumption similar	Not measured	[103]
Hybridization (rare-mating + sporulation)	*S. cerevisiae* × *S. uvarum* allotriploid	Intermediate ethanol tolerance (MIC 11.44–12.16%); ergosterol-pathway over-representation (transcriptomic)	Not measured	[104]
Controlled multistarter consortium (10,000:1)	*S. bacillaris*, *L.t.*, *T.d.*, *M.p.* + *S.c.*	Significant ethanol reduction (*S. bacillaris*, *M. pulcherrima*)	Not measured	[105]
Native-collection screening (co-starter selection)	*S. bacillaris*, *L. thermotolerans*, *Metschnikowia* (wine)	Ethanol-tolerance ranking for co-starter choice	Not measured	[2]
Co-starter pairing with *S. cerevisiae*	*S. bacillaris*, *Z. bailii* (wine)	Wine ethanol lowered (sequential to 11.34% *v*/*v*)	Not measured	[96]
Bioprospecting (extreme oenological niches)	*Hanseniaspora opuntiae*, *Z. bailii* (wine)	Native ethanol tolerance above 10% *v*/*v*	Not measured	[106]
Stepwise ethanol adaptation	*S. cerevisiae* (lab strain, FY834)	Tolerance via lower C16:0 (saturated-chain reduction)	Measured	[39]
ALE-adapted interspecies hybrid	*Saccharomyces* hybrid	Tolerance with reduced PE, more fluid (lower-GP) membrane	Measured	[79]
Bioethanol adaptation [M]	*S. cerevisiae* (bioethanol)	Tolerance with higher ergosterol, UFA, and trehalose	Measured [M]	[43]
Tolerance-evolved strain [M]	rice-wine *S. cerevisiae*	Tolerance with higher oleic acid and C18:C16 ratio	Measured [M]	[44]
De novo hybrids (rare-mating + meiotic segregation) [M]	lager *Saccharomyces* hybrid	High-ethanol growth; higher UFA, oleic acid, and ergosterol; PLS-DA	Measured [M]	[82]
ALE (transcriptional reprogramming) [M]	*K. marxianus* (bioethanol)	Ethanol tolerance from 7 to 10% *v*/*v*; UFA- and sterol-biosynthesis genes up-regulated (transcriptomic)	Not measured	[107]
ALE (serial batch, 4% *v*/*v*) [M]	*K. marxianus* (bioethanol)	Tolerance with higher FAME and ergosterol than the parent	Measured [M]	[108]
Ion-beam mutagenesis [M]	*Kazachstania* sp. (cross-beverage)	Tolerance to 20% *v*/*v*; TFA increased by 105.48% with no UFA:SFA shift	Measured [M]	[109]

Symbols and notes: "Measured" means the study characterized the membrane lipid composition of the improved strain; "not measured" means it did not. [M] cross-beverage/domain. Abbreviations: ARTP, atmospheric and room-temperature plasma; PLS-DA, partial least squares–discriminant analysis; L-Phe, L-phenylalanine; *S.c.*, *Saccharomyces cerevisiae*; *L.t.*, *Lachancea thermotolerans*; *T.d.*, *Torulaspora delbrueckii*; *M.p.*, *Metschnikowia pulcherrima*.

6.1. Adaptive Laboratory Evolution

ALE carries the clearest wine non-*Saccharomyces* proof of concept. In the first wine case, a native *T. delbrueckii* from Cabernet Sauvignon must was serially batch-cultured in rising ethanol, from 6% to 12% *v*/*v* over roughly 300 generations and 114 days, yielding the clone YCPUC10-F, selected for growth at 11.5% *v*/*v* against a parental ceiling of 9% *v*/*v* [97]. In a real fermentation, co-inoculated with *S. cerevisiae*, the evolved-strain pairing reached 10.8% *v*/*v* ethanol versus 11.9% *v*/*v* for the pure *S. cerevisiae* control, though it did not significantly outperform the parental co-culture in final ethanol or end-of-fermentation viability [97]. For a fermentation that must survive sulfite, the clone also gained ethanol-to-SO_2 cross-protection, shortening its lag 3.4-fold and accelerating growth 1.9-fold under 50 mg/L free SO_2 [97]. A second wine case spans another genus: a *L. thermotolerans* evolved under L-phenylalanine as sole nitrogen source over 200 generations gained ethanol tolerance as a correlated response, derivative AE200-27 reaching 2.26-fold the parental

biomass at 8% *v*/*v* while also improving high-sugar, SO_2, and pH tolerance [98]. ALE thus delivers multi-stress robustness from one selective pressure, but in neither wine case was the membrane interrogated.

Mutagenesis-assisted selection sits alongside ALE here. Screening indigenous wine *Hanseniaspora* spp. identified low-ethanol-yield isolates, and atmospheric and room-temperature plasma (ARTP) mutagenesis then produced HuC32-2-72, a novel oenological ARTP mutant, which in sequential inoculation cut final ethanol by 1.4% *v*/*v* in Merlot versus the *S. cerevisiae* monoculture [99]. Where an ALE-improved non-*Saccharomyces* strain has been probed for its lipid program at all, it is cross-beverage: in bioethanol *K. marxianus*, ALE raised tolerance from 7% to 10% *v*/*v* through transcriptional reprogramming rather than ploidy change or fixed mutations, up-regulating UFA-biosynthesis and, under high ethanol, sterol-biosynthesis genes, a transcriptional readout rather than a measured lipidome [107].

6.2. Hybridization and Breeding

Hybridization recombines parental tolerance phenotypes and, like ALE, points to a lipid axis. It has also been attempted across the genus boundary: protoplast fusion of *T. delbrueckii* and *S. cerevisiae*, framed as traditional strain breeding, yielded four clones with intermediate fructose consumption; the best-performing clone, F1-11, showed fructose consumption similar to that of the *S. cerevisiae* parent and higher survival than that parent during a 2 h challenge with ethanol and acetic acid [103]. Membrane lipids were not measured, although ethanol inhibition of sugar transport was [103]. A later study considered true genetically stable hybrids of these species unlikely, describing wine *T. delbrueckii* strains as haploid, with eight chromosomes compared with 16 in haploid *S. cerevisiae* [102]. An interspecific *S. cerevisiae* × *S. uvarum* hybrid built by rare-mating, an explicitly non-GMO procedure, and sporulation produced the allotriploid H14A7, with ethanol tolerance intermediate between its parents (MIC 11.44–12.16% *v*/*v*) [104]. The bridge toward a lipid signature is the transcriptomic asymmetry behind that phenotype: ergosterol biosynthesis (*ERG1*, *ERG3*, *ERG5*, *ERG6*, *ERG27*) was over-represented in the *S. uvarum* subgenome during fermentation, alongside fatty-acid catabolic and short-chain fatty-acid terms linked to membrane composition [104]. The improved tolerance thus tracks an ergosterol-and-membrane program, inferred from expression rather than from measured ergosterol or fatty-acid content. Cross-beverage breeding shows the measured version: de novo *S. cerevisiae* × *S. eubayanus* lager hybrids made by the same rare-mating-plus-meiotic-segregation logic carried higher oleic acid and ergosterol favoring high-ethanol growth, with partial least squares–discriminant analysis (PLS-DA) cleanly separating stress conditions on the lipid data [82].

The genetic architecture breeding must navigate has been mapped most thoroughly in engineered *Saccharomyces* hybrids. Tetraploidization restores fertility to otherwise sterile interspecies hybrids (spore viability up to 98%), enabling multigenerational intercrossing, and the ensuing quantitative-trait-locus analysis placed ethanol-tolerance loci genome-wide while a low-temperature locus intercepted *OSH6*, a gene of sterol metabolism and membrane fluidity [110]. That gene's presence in a stress-tolerance interval reinforces the membrane-centered reading of breeding, although the tolerance data themselves came from genetically modified strains (*HO* and *MAT* deletions, reciprocal hemizygosity) and therefore fall outside the non-GMO scope of this review. Hybridization thus stands out for tying its tolerance gains to the ergosterol-and-UFA axis, both transcriptionally and in cross-beverage lipidomics, making it a candidate substrate for a future measured signature.

6.3. Directed Evolution, Mutagenesis, Selection, and Consortia

The remaining non-GMO routes are well represented for wine non-*Saccharomyces*, but are lipid-silent. Non-transgenic mutagenesis works: UV mutagenesis of a wine *P. terricola* yielded mutants tolerating 10–11% *v*/*v* ethanol against the parental 8% *v*/*v* while modulating aroma; diethyl sulfate treatment gave only a marginal gain [100]. Spontaneous-mutant selection works under sequential stress: from a wine *T. delbrueckii* tolerant to 5% but not 10% *v*/*v* ethanol, isolating mutants resistant first to SO_2, then ethanol, then CO_2 overpressure produced derivatives that grow on 10% ethanol and stay industrially stable after 100 doublings [101]. The same group improved a sparkling-wine *T. delbrueckii* without genetic modification, mixing *T. delbrueckii* and *S. cerevisiae* cells, yielding clones with intermediate ethanol, SO_2, and copper resistance, and isolating spontaneous mutants resistant to high CO_2 pressure, approaching that of *S. cerevisiae* [102].

Consortium design exploits, rather than overrides, the non-*Saccharomyces* ethanol ceiling. Controlled multistarter co-cultures of *S. bacillaris*, *L. thermotolerans*, *T. delbrueckii*, and *M. pulcherrima* with *S. cerevisiae* achieved significant ethanol reduction, driven by *S. bacillaris* and *M. pulcherrima*, when the non-*Saccharomyces* dominated the inoculum at a 10,000:1 ratio [105]. Rational co-starter choice rests on the native tolerance ranking, in which *S. bacillaris* and *Lachancea* outrank the more sensitive *Metschnikowia* (most isolates fail to grow even at 8%) [2], while pairing *S. bacillaris* or *Z. bailii* with *S. cerevisiae* lowers wine ethanol in practice (an *S. bacillaris* sequential pairing reaching 11.34% *v*/*v*) [96]. Bioprospecting from extreme niches supplies the raw tolerance: isolates from high-sugar musts and high-ethanol flor biofilms yielded a *Hanseniaspora opuntiae* and a *Z. bailii* exceeding 10% *v*/*v* ethanol in synthetic medium [106].

6.4. The Lipid Signature of Improved Strains

Read across these non-GMO strategies, the improved wine non-*Saccharomyces* strains share a conspicuous gap: their characterization emphasized growth, fermentation, and aroma over the membrane lipidome. These strains span the evolved *T. delbrueckii* and *L. thermotolerans*, the ARTP *Hanseniaspora*, the mutagenized *P. terricola*, and the spontaneous *T. delbrueckii* mutants. The few improved non-*Saccharomyces* strains whose membrane lipid biology has been characterized at all are cross-beverage: an ALE *K. marxianus* whose tolerant derivative held higher total fatty-acid methyl esters and ergosterol than its parent [108], a bioethanol ALE strain whose lipid program was read only transcriptionally [107], and an ion-beam mutant of *Kazachstania* in which TFA rose by 105.48% with no significant shift in UFA proportions, a counterexample to the expectation that greater unsaturation confers greater tolerance, since ergosterol, trehalose, and ATPase activity rose instead [109]. Two of these exceptions show that a measured signature of an improved non-*Saccharomyces* strain is achievable; the wine counterparts lack it. The same evolved configurations, in *Saccharomyces* and cross-beverage hybrids, recall the qualitative remodeling already described in Sections 3.2 and 4.3: the reduced PE and more fluid membrane of an ALE-adapted hybrid [79], the ergosterol and UFA gains of a bioethanol-adapted strain [43] and the unsaturation gains of a rice-wine-adapted strain [44], and the saturated-chain reduction in a stepwise-adapted *S. cerevisiae* [39]. None of these has a wine non-*Saccharomyces* equivalent.

The boundary the non-GMO frame imposes is itself illuminating. Transgenic work shows the membrane can be reshaped at a single regulatory node: reverting one lipid transcription-factor allele (*INO2*) lowers ethanol tolerance together with ergosterol, PE-synthesis, and *OLE1* expression [111], and a gain-of-function Pdr1p raises the UFA:SFA ratio and ergosterol while cutting reactive oxygen species under fusel-alcohol stress [112]. Sharp as the mechanism is, engineering a lipid transcription factor is transgenic, outside the non-GMO scope of winemaking. The legitimate routes instead improve, select, or

combine wine non-*Saccharomyces* strains for ethanol tolerance or ethanol-yield reduction without measuring their membrane lipidomes. That gap makes a predictive lipid signature necessary, but measured signatures are documented so far mainly in *Saccharomyces* and cross-beverage analogs, preventing direct validation from these wine studies.

7. Prediction of Ethanol Tolerance from Lipid Profiles

Across mechanisms, comparisons, interventions, and improvements, one thread recurs: the membrane lipidome is a readout of what the cell can withstand. This section sets out the deficits that keep the field descriptive rather than predictive and formalizes the recurring membrane descriptors as a candidate predictive lipid signature.

Three structural deficits keep the field descriptive rather than predictive. The first is methodological fragmentation: lipid data on the wine non-*Saccharomyces* are generated by diverse, complementary techniques (GC-FAME; ultra-high-performance liquid chromatography–tandem mass spectrometry, UHPLC-MS/MS; FT-NIR; Laurdan-GP), each capturing a different facet of the lipidome, and individual studies apply these techniques to examine endpoints as different as growth-based tolerance, acute survival, fermentation performance, and deliberate ethanol-yield reduction, yet the resulting data are seldom integrated into the combined compositional and biophysical view that a predictive reading requires. The second is the near-absence of dose-resolved data in these yeasts; that is, lipidome profiles measured across increasing ethanol concentrations rather than at a single point. Even before ethanol enters, the lipidome already shows high inter- and intraspecific variability, as the broadest 2026 survey of thirteen species documents [17], and for ecologically central taxa such as *S. bacillaris* and *Hanseniaspora*, to our knowledge, no comprehensive quantitative membrane profile has yet been reported. How that lipidome then shifts as ethanol rises has rarely been resolved. The third is interpretive: the compositional indices in common use, chiefly the unsaturation index and the sterol:phospholipid ratio, were developed largely on *S. cerevisiae* and have yet to be tested against direct membrane measurements in the non-*Saccharomyces* (Section 4.3). Where untargeted lipidomics has been pushed to phenotype, the couplings can even run counter to expectation: across industrial strains, phosphatidylinositol correlated negatively with ethanol tolerance, against the canonical oleic-acid paradigm [113]. Relevant data are therefore accumulating, the multispecies UHPLC-MS/MS profiling of indigenous non-*Saccharomyces* fermentations among them [12]; to our knowledge, however, these accumulating data have not yet been assembled into a predictive model.

That assembly is the thesis of this review: a small set of membrane descriptors (ergosterol and the ergosterol:phospholipid ratio, UFA:SFA, oleic acid, the low-PE signature, the PC:PI balance, and membrane fluidity) can serve as a candidate predictive lipid signature, a lipidome read as a biomarker of ethanol tolerance and of fermentation performance. The precedent is strong but uneven. Formal models that infer tolerance from lipid composition exist almost exclusively for *S. cerevisiae* and cross-beverage systems (Table 6). Two lipid-based classifiers are methodological precedents rather than models of an ethanol endpoint: the closest non-*Saccharomyces* precedent, a PCA/LDA of fatty-acid profiles, sets the less tolerant *Issatchenkia* apart from *S. cerevisiae* but stops short of quantitative prediction [16], and a PLS-DA of lipid composition on *Saccharomyces* hybrids and parents classifies the culture condition, not the strain's tolerance [82]. The only quantitative wine model, in turn, is a mixed-panel regression of oleic acid, palmitoleic acid and ergosterol that pools the non-*Saccharomyces* with *S. cerevisiae*; it explains ethanol tolerance well across five strains but has not yet been validated as a predictor. That is, its predictive error has not been estimated outside the data used to fit it, by cross-validation or on strains withheld from it [15] (Table 6). This is the central gap: to our knowledge no validated, multivariate model yet

predicts either ethanol tolerance or fermentation performance for wine non-*Saccharomyces* specifically. Genome-scale metabolic models of several wine non-*Saccharomyces* already predict their respiratory and fermentative behavior from genome content [114], yet none infers ethanol tolerance or performance from the membrane lipidome.

Table 6. Lipid-based models and correlates linking membrane composition to the ethanol or fermentation endpoint named in each row.

Model	Organism	System	Endpoint and Metric	Cross-Domain	Ref.
PLS regression, cross-validated	*S. cerevisiae* (22 industrial, primarily wine)	Wine	Final ethanol: R^2 = 0.91, Q^2 = 0.83; biomass: R^2 = 0.97, Q^2 = 0.94	—	[41]
Multiple regression (oleic + palmitoleic + ergosterol), in-sample fit	mixed panel (3 non-*Saccharomyces* + 2 *S. cerevisiae*)	Wine	Ethanol tolerance as EC_{50} for growth: R^2 = 95.30%, not cross-validated	—	[15]
Multivariate association (low-PE + high fluidity), qualitative	*S. cerevisiae* (5 representatives from a 61-strain screen)	Wine and other origins	Growth-based tolerance: lipid biomarker, no fitted model	—	[8]
Laurdan-GP correlation, single variable	*S. cerevisiae* (wine)	Wine	Viability loss after an 18% *v*/*v* ethanol shock: Pearson r = −0.654 with GP	—	[22]
Measured signature, descriptive (evolved strain versus parent)	*Kazachstania* sp.	Cross-beverage	Tolerance at a fixed dose: TFA-driven, UFA:SFA-independent	[M]	[109]

Symbols: R^2, coefficient of determination; Q^2, cross-validated predictive ability; [M] cross-beverage/domain.

8. Conclusions and Future Perspectives

The membrane lipidome of the wine non-*Saccharomyces* co-varies with ethanol tolerance, and separately with fermentation performance, but has not yet been assembled into a formal predictive model. The route to closing that gap runs from *S. cerevisiae* and the better-studied genera outward to the wine non-*Saccharomyces*. ALE provides a transferable mechanism: in a non-*Saccharomyces* yeast, evolved tolerance arose through transcriptional reprogramming of unsaturated fatty acid and, at high ethanol contents, sterol biosynthesis rather than through ploidy change [107], and a measured signature is achievable outside *Saccharomyces*: an evolved non-*Saccharomyces* derivative carries higher total fatty-acid methyl esters and ergosterol than its parent [108]. The desaturase axis correlates quantitatively with tolerance in this frame: *OLE1* expression tracks growth at 10% *v*/*v* ethanol at r = 0.8054 across fifteen strains [34]. One qualification applies to any model, however: tolerance does not always follow unsaturation. In an improved *Kazachstania* the gain was driven by absolute TFA, ergosterol, trehalose, and ATPase activity with no shift in UFA:SFA at all [109]. In addition, apiculate wine yeasts such as *Hanseniaspora* remain a particular unknown, their fermentation behavior still only partly mapped [115]. A predictive signature must therefore be multivariate, not a single index.

Four priorities follow. First, build a harmonized lipidomic dataset for the wine non-*Saccharomyces*, applying an integrated, consistent methodology across species and ethanol doses so that values become comparable and modelable [17]. Second, move beyond the predominantly linear models in Table 6 (chiefly PLS-family and regression models) toward non-linear learners, following the Random Forest template already applied to the lipidome of a marine non-*Saccharomyces* yeast [116], a shift justified by benchmarks in which gradient boosting outperformed linear models and stress resistance proved more predictable than nutrient-limited growth [117]. Third, prioritize the least-measured species, with *S. bacillaris*, ethanol-persistent yet lipidomically among the least characterized (Section 4.2), the most conspicuous gap. Fourth, deploy inexpensive biophysical proxies for rapid screening:

Laurdan-GP already correlates fluidity with tolerance in a wine *S. cerevisiae* strain [22], and synchrotron Fourier-transform infrared (synchrotron-FTIR) offers a label-free biophysical readout of membrane lipid state under stress [32]. Pursuing these priorities would turn the membrane lipidome of the wine non-*Saccharomyces* from a descriptive record into a predictive lipid signature, making strain selection, intervention design, and non-GMO improvement lipid-informed.

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