

Review

Precision Fermentation of Low- and Non-Alcoholic Beer Using Non-*Saccharomyces* Yeast: A Framework for Process and Sensory Control

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Abstract

The production of low- and non-alcoholic beer remains a major technological challenge due to the need to restrict ethanol formation while maintaining acceptable sensory quality and fermentation-derived complexity. Conventional approaches, including physical dealcoholization and arrested fermentation, often result in flavor imbalance, reduced aroma intensity, diminished mouthfeel, and persistent wort-like off-flavors. In this context, non-*Saccharomyces* yeasts have emerged as promising biological tools due to their species- and strain-dependent carbohydrate utilization, aroma production potential, and intrinsic metabolic constraints. This review provides a structured and mechanistically informed synthesis of current knowledge regarding the application of non-*Saccharomyces* yeasts in low- and non-alcoholic beer production, with emphasis on metabolic regulation, fermentation process control, and sensory implications. Particular attention is given to sugar transport limitations, glycolytic regulation, carbon redistribution, redox balance, and the role of controllable process variables, including wort fermentability, pitching rate, oxygen availability, and temperature. The available evidence indicates that fermentation outcomes depend strongly on interactions between strain-specific metabolic traits and process design. Collectively, this review proposes a brewery-oriented precision fermentation framework in which strain-specific physiological constraints are deliberately aligned with controllable process variables to support rational strain selection, more predictable ethanol control, and targeted sensory optimization in low- and non-alcoholic beer production.

Keywords: non-*Saccharomyces* yeasts; low-alcohol beer; non-alcoholic beer; precision fermentation; sugar metabolism; fermentation process control; sensory optimization; brewing process design



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1. Introduction

The growing demand for low- and non-alcoholic beers (LNAB) has intensified the development of alternative production strategies aimed at reducing ethanol content while maintaining acceptable sensory quality and fermentation-derived complexity. Conventional technological approaches, including physical dealcoholization and restricted or arrested fermentation, are effective in limiting ethanol formation; however, they frequently result in reduced aroma intensity, flavor imbalance, diminished mouthfeel, and persistent wort-like off-flavors, thereby negatively affecting consumer acceptance and overall product quality [1–3].

These limitations have stimulated increasing interest in biological approaches based on the use of non-*Saccharomyces* yeasts, which enable intrinsic modulation of fermentation outcomes rather than post-process ethanol removal [4–6]. In contrast to conventional brewing with *Saccharomyces cerevisiae*, many brewing-relevant non-*Saccharomyces* species exhibit species- and strain-dependent limitations in the utilization of wort carbohydrates, particularly maltose and maltotriose, which constitute the dominant fermentable sugars in brewing systems. While some strains exhibit partial or substantial maltose utilization, fermentation in many brewing-relevant non-*Saccharomyces* systems remains preferentially associated with monosaccharide metabolism, thereby intrinsically constraining ethanol formation and making selected strains particularly attractive for LNAB production [7–9].

Beyond intrinsic ethanol limitation, restricted sugar utilization substantially influences the physicochemical and sensory properties of the final product. Incomplete carbohydrate metabolism contributes to elevated residual extract and enhanced mouthfeel, partially compensating for the reduced structural contribution of ethanol. At the same time, many non-*Saccharomyces* yeasts exhibit strain-dependent production of aroma-active metabolites, particularly esters, higher alcohols, and volatile compounds associated with fruity and floral sensory attributes, which may partially compensate for diminished ethanol-driven flavor perception in low- and non-alcoholic beer systems. Representative brewing-relevant species, including *Torulaspora delbrueckii*, *Metschnikowia pulcherrima*, *Pichia kluyveri*, and *Saccharomyces ludwigii*, have been associated with strain-dependent formation of esters, higher alcohols, terpenes, and volatile thiols contributing to fruity, floral, or complex sensory profiles [10–12].

However, the application of non-*Saccharomyces* yeasts in brewing is accompanied by significant technological challenges. Restricted fermentative metabolism frequently results in incomplete attenuation and insufficient reduction of wort-derived aldehydes, thereby contributing to undesirable wort-like off-flavors and increased variability in fermentation performance [1,13,14]. In addition, process imbalance may promote the formation of undesirable by-products, including volatile acidity, sulfur compounds, and diacetyl, which may negatively affect sensory quality if fermentation conditions are not appropriately controlled [11,15,16]. Furthermore, industrial implementation remains complicated by challenges related to fermentation reproducibility, microbiological stability, contamination risk, and process transferability from laboratory to pilot and industrial scales, thereby emphasizing the need for more robust and predictive fermentation strategies [14–16].

Beyond strain selection, fermentation process parameters critically influence fermentation outcomes. Variables such as wort composition, fermentability, pitching rate, temperature, oxygen availability, and fermentation duration directly affect sugar uptake, metabolic flux distribution, redox balance, aroma formation, and ethanol production [17–20]. Importantly, the influence of these variables is strongly strain-dependent and interacts with intrinsic physiological constraints, resulting in complex and often non-linear effects on fermentation performance and sensory outcomes [9,16].

Despite the growing body of literature addressing non-*Saccharomyces* yeasts in brewing, current research remains highly fragmented and frequently focuses on isolated aspects such as strain screening, sugar utilization, fermentation performance, aroma formation, or sensory characterization under specific experimental conditions [4,21,22].

Although these studies have significantly advanced understanding of individual strain performance and fermentation behavior, integrative and process-oriented frameworks specifically linking metabolic constraints, fermentation process control, and sensory outcomes in brewery-relevant low- and non-alcoholic beer systems remain comparatively limited [6,23]. Consequently, the present review moves beyond descriptive strain screening

by integrating strain physiology, process engineering, and sensory implications into a brewery-oriented precision fermentation framework.

In the context of this review, precision fermentation is conceptualized as the deliberate control of fermentation outcomes through coordinated manipulation of microbial physiology, substrate accessibility, and process conditions [5,24,25]. Rather than relying primarily on post-fermentation correction or empirical trial-and-error optimization, precision fermentation in low- and non-alcoholic brewing refers to the predictive alignment of strain-specific metabolic constraints with controllable technological variables to achieve targeted ethanol concentrations and desired sensory properties [18,23,26]. Within this framework, fermentation performance is treated as a design-driven and process-responsive system in which ethanol formation, aroma generation, mouthfeel, and sensory balance emerge from interactions between microbial metabolism and controllable operational conditions [27–30]. In contrast to conventional brewery optimization strategies, which frequently rely on empirical trial-and-error process adjustment or post-fermentation correction, this framework emphasizes predictive fermentation design based on deliberate coordination of strain physiology and controllable technological variables [24–26].

The objective of this review is therefore to provide a mechanistically informed and brewery-oriented synthesis of current knowledge regarding the use of non-*Saccharomyces* yeasts for low- and non-alcoholic beer production. By integrating strain-specific metabolic traits, fermentation process parameters, and sensory implications, this work aims to establish a precision fermentation framework supporting rational and brewery-relevant process development. Particular emphasis is placed on the interaction between metabolic constraints and controllable process variables as key determinants governing ethanol formation, fermentation performance, and product quality. The proposed framework should be interpreted as a conceptual synthesis of currently available evidence intended to support brewery-oriented decision making rather than as an experimentally validated predictive model.

2. Materials and Methods

This study was conducted as a structured narrative review informed by a transparent and reproducible literature search strategy, aimed at critically evaluating the application of non-*Saccharomyces* yeasts in low- and non-alcoholic beer (LNAB) production. Where alcohol categories are discussed throughout this review, terminology follows commonly applied brewery-oriented and European brewing conventions. Non-alcoholic beer generally refers to products containing up to approximately 0.5% alcohol by volume (v/v), whereas low-alcohol beer refers to products with reduced but higher ethanol concentrations. Because regulatory definitions may vary internationally, alcohol categories discussed in this review should be interpreted as brewery-relevant classifications rather than universally fixed legal thresholds. Particular emphasis was placed on metabolic constraints, sugar utilization, fermentation process control, sensory outcomes, and brewery-oriented implementation strategies. The review was designed to integrate microbiological, physiological, technological, and sensory perspectives into a mechanistically informed and application-oriented framework relevant to brewing practice and fermentation process optimization.

The literature search was performed using the Web of Science, Scopus, and Google Scholar databases. The search primarily focused on peer-reviewed publications published in English between January 2022 and February 2026 in order to capture recent advances in brewing-relevant non-*Saccharomyces* fermentation research, low- and non-alcoholic beer development, emerging precision fermentation approaches, and technological innovations in brewery process optimization. The selected timeframe was chosen to capture the rapid expansion of brewing-relevant non-*Saccharomyces* research, increasing industrial

interest in low- and non-alcoholic beer production, and recent developments in precision fermentation strategies. Earlier landmark studies and foundational brewing literature were selectively incorporated where necessary to provide mechanistic context regarding yeast physiology, sugar transport systems, carbohydrate metabolism, and brewery-relevant fermentation principles.

Representative search combinations included: (“non-*Saccharomyces* yeast” OR “non-*Saccharomyces* brewing”) AND (“low alcohol beer” OR “non-alcoholic beer” OR “low- and non-alcoholic beer”); (“maltose utilization” OR “maltotriose metabolism” OR “sugar transport”) AND (“brewing yeast” OR “non-*Saccharomyces*”); (“aroma formation” OR “esters” OR “higher alcohols” OR “sensory quality”) AND (“low alcohol beer” OR “non-alcoholic beer”); and (“precision fermentation” OR “fermentation control” OR “process optimization”) AND (“brewing” OR “non-*Saccharomyces* yeast”). Boolean operators (AND, OR) were applied to refine and combine search queries according to database-specific indexing and retrieval systems.

The literature identification process initially yielded 298 records across databases. Following duplicate removal, 237 records were screened based on titles and abstracts for thematic relevance to brewing applications of non-*Saccharomyces* yeasts in LNAB systems. Subsequently, 94 publications underwent full-text eligibility assessment. Studies were included if they addressed at least one of the following areas: (i) carbohydrate utilization and sugar transport in brewing-relevant non-*Saccharomyces* yeasts, particularly maltose and maltotriose metabolism; (ii) fermentation process parameters influencing ethanol formation; (iii) aroma-active metabolite formation and sensory implications; (iv) strain-dependent fermentation performance; or (v) brewery-relevant applications, including pilot-scale or industrially relevant fermentation systems. Studies focused exclusively on non-brewing fermentations without clear technological transferability to beer production, duplicate datasets, non-English publications, review-only articles without direct brewing applicability, or studies lacking sufficient physiological or process-related detail were excluded from the primary synthesis. Following eligibility assessment, 48 references were included in the final thematic synthesis. The literature identification and screening workflow is summarized in Figure 1.

The primary literature search and initial study screening were conducted by the first author based on thematic relevance, brewing applicability, and methodological suitability. Subsequently, thematic organization, interpretation of included studies, and manuscript structure were iteratively discussed with the co-authors to ensure conceptual consistency, scientific relevance, and alignment with the objectives of the review. Because this study was designed as a structured narrative review rather than a formal systematic review, independent dual-reviewer screening was not applied.

Given the heterogeneity of the available literature, this work was intentionally designed as a structured narrative review rather than a formal systematic review or meta-analysis. The objective was not to quantitatively aggregate findings across highly heterogeneous fermentation systems, but rather to critically evaluate recurring physiological patterns, strain-dependent metabolic constraints, and process-sensitive responses relevant to low- and non-alcoholic beer production.

Within the context of this review, the term mechanistic synthesis refers to the integrative interpretation of published evidence linking yeast physiology, carbohydrate metabolism, fermentation process variables, and sensory outcomes into a brewery-oriented conceptual framework. Rather than representing direct experimental mechanistic validation, this approach was intended to identify biologically plausible relationships, recurring physiological constraints, and process-sensitive interactions relevant to brewing practice. Recommendations and brewery-oriented guidance presented throughout the manuscript

should be interpreted as conceptual conclusions derived from synthesis of the available literature rather than as universally validated technological prescriptions. Where appropriate, proposed implementation strategies reflect the authors' interpretation of recurring trends and relationships reported across multiple studies.

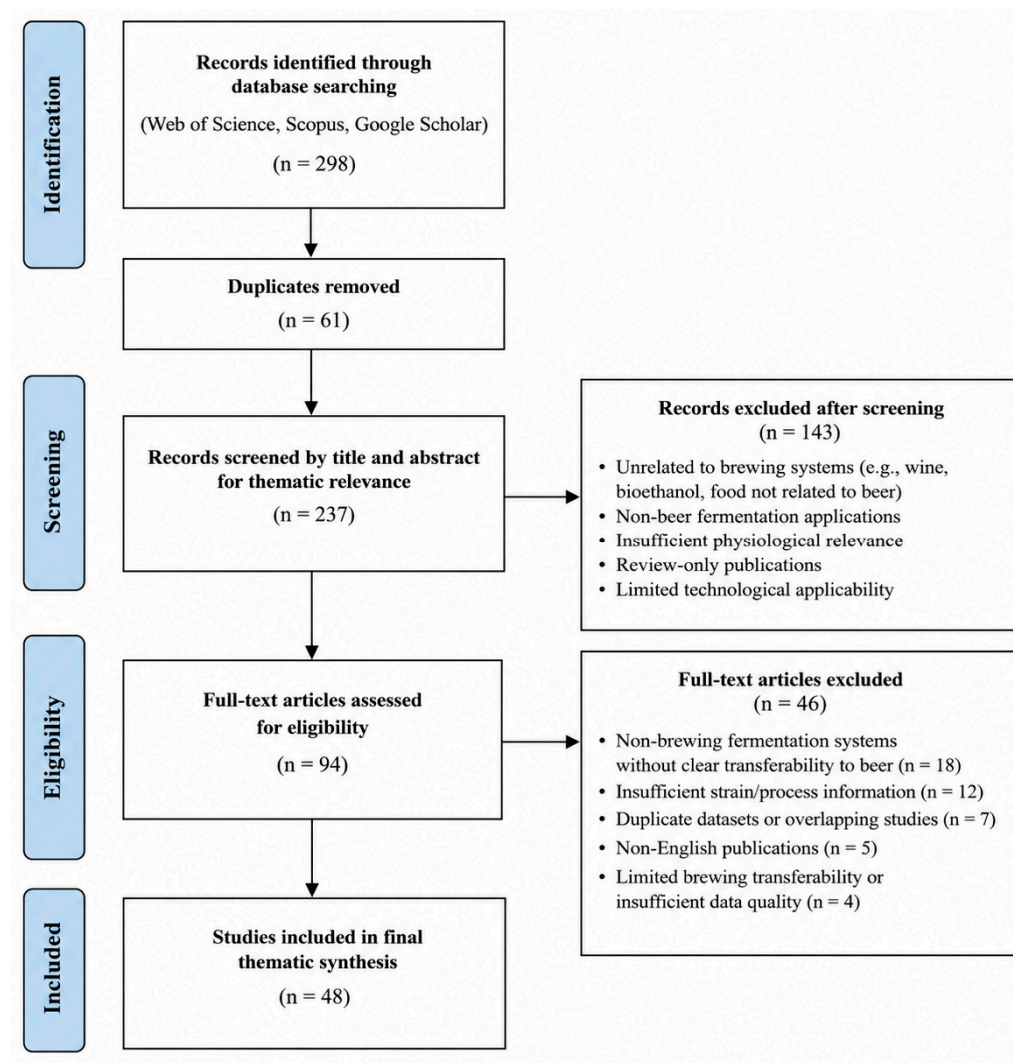


Figure 1. Literature identification and thematic screening workflow used in the structured narrative review. The literature search was conducted using Web of Science, Scopus, and Google Scholar databases. Records were screened for thematic relevance to brewing applications of non-*Saccharomyces* yeasts in low- and non-alcoholic beer systems. Studies lacking brewing applicability, sufficient physiological detail, or technological transferability were excluded from the primary synthesis.

The final review structure was organized into interconnected thematic sections covering wort carbohydrate composition, sugar transport limitations, glycolytic regulation, redox balance, strain functionality, fermentation process control, brewery implementation strategies, and sensory optimization. Interpretation of reported findings was additionally contextualized considering methodological heterogeneity among studies, including differences in wort composition, fermentation scale, oxygenation strategy, analytical methodology, strain selection, fermentation temperature, and propagation conditions.

3. Sugar Metabolism as a Central Constraint in Low-Alcohol Fermentation

3.1. Composition of Wort Carbohydrates

Wort carbohydrate composition represents a fundamental determinant of yeast metabolism and ethanol formation during beer fermentation because the availability and accessibility of fermentable carbon directly govern glycolytic activity and fermentation performance. In conventional brewing systems, fermentable carbohydrates are dominated by maltose, typically representing approximately 50–60% of total fermentable sugars, followed by maltotriose (15–20%), whereas glucose and fructose generally account for less than 10–15% of the fermentable extract [27,31,32].

The relative abundance and accessibility of these carbohydrates strongly influence fermentation behavior because ethanol production depends on the fraction of fermentable carbon entering glycolysis. In *Saccharomyces cerevisiae* fermentations, efficient sugar transport systems enable near-complete utilization of glucose and maltose together with substantial maltotriose consumption, resulting in apparent attenuation levels commonly ranging between approximately 75–85% under standard brewing conditions [7,8,33].

In contrast, fermentation performance among brewing-relevant non-*Saccharomyces* yeasts is considerably more variable and strongly species- and strain-dependent. While some strains exhibit partial or substantial maltose utilization, many brewing-relevant species remain preferentially associated with monosaccharide metabolism and limited access to maltose and maltotriose, thereby intrinsically constraining ethanol formation [5,9,21]. Consequently, attenuation in non-*Saccharomyces*-driven low- and non-alcoholic beer systems is frequently lower than in conventional brewing fermentations, although reported values vary substantially depending on strain selection, wort composition, oxygen availability, fermentation temperature, and process configuration [5,9,21].

Beyond its role as a nutrient source, wort carbohydrate composition therefore represents an important technological variable influencing ethanol formation, residual extract, mouthfeel, and overall sensory balance in low- and non-alcoholic beer systems. From a brewery perspective, deliberate manipulation of wort fermentability through grist composition, mashing profile adjustment, or carbohydrate availability may further support ethanol control and sensory optimization when combined with strain-specific physiological constraints [17,27,32].

3.2. Transport Limitations in Non-Saccharomyces Yeasts

A defining physiological characteristic of many brewing-relevant non-*Saccharomyces* yeasts used for low- and non-alcoholic beer production is species- and strain-dependent limitation in the uptake of maltose and maltotriose across the cell membrane. Because these carbohydrates constitute the dominant fermentable sugar fraction in brewing wort, variability in sugar transport capacity represents one of the principal determinants governing ethanol formation and fermentation performance in low- and non-alcoholic beer systems [7,8,34].

In brewing yeasts, maltose and maltotriose utilization depends on coordinated activity of membrane transport systems and intracellular carbohydrate metabolism. Efficient fermentative strains of *Saccharomyces cerevisiae* and *Saccharomyces pastorianus* possess specialized sugar transporters encoded by genes associated with the MAL loci and transporter families such as *AGT1* and *MTT1*, enabling active uptake of maltose and maltotriose into the cell, followed by intracellular hydrolysis and subsequent entry into glycolysis [8,35]. These transport systems substantially contribute to the high attenuation levels typically observed during conventional brewing fermentation.

In contrast, brewing-relevant non-*Saccharomyces* yeasts exhibit substantial interspecies and intraspecies variability regarding sugar uptake capacity. Species such as *Saccharomy-*

codes ludwigii frequently exhibit severely restricted maltose utilization, making them particularly attractive for low- and non-alcoholic beer production, whereas strains of *Torulaspora delbrueckii*, *Kluyveromyces marxianus*, or *Metschnikowia pulcherrima* may exhibit partial maltose utilization depending on strain identity, fermentation conditions, and wort composition [20,34,36,37]. In many cases, restricted fermentation performance has been associated with absence of homologous transport systems, lower transporter expression, reduced substrate affinity, or inefficient regulation of sugar transport pathways [29,34,36,38].

As a consequence, sugar utilization in many brewing-relevant non-*Saccharomyces* systems remains preferentially associated with glucose and fructose metabolism, although depletion kinetics vary substantially depending on strain phenotype, pitching rate, wort composition, oxygen availability, and fermentation temperature [5,9]. Following depletion of readily fermentable monosaccharides, fermentation activity frequently slows because the remaining carbohydrate fraction, dominated by maltose and maltotriose, remains only partially accessible for cellular metabolism. Although residual maltose concentrations vary considerably across species and process configurations, studies have reported substantial retention of unfermented maltose and maltotriose in low- and non-alcoholic beer fermentations involving maltose-negative or maltose-limited strains [9,20,25].

Importantly, restricted sugar transport should not be interpreted as the sole determinant of ethanol formation in non-*Saccharomyces* fermentations. Ethanol production is additionally influenced by factors including oxygen availability, redox balance, nutrient composition, fermentation temperature, and strain-specific physiological responses [36]. Nevertheless, carbohydrate accessibility frequently represents a central metabolic bottleneck because limited uptake of fermentable sugars constrains the amount of carbon available for downstream fermentative metabolism and thereby influences attenuation, carbon flux distribution, and fermentation outcomes in low- and non-alcoholic beer systems.

3.3. Glycolytic Flux and Ethanol Formation

Following sugar uptake, ethanol formation in brewing yeast is governed by glycolytic throughput and the subsequent allocation of carbon toward fermentative metabolism. Glycolysis represents the central metabolic pathway converting fermentable sugars into pyruvate, which is subsequently metabolized into ethanol under oxygen-limited brewing conditions through pyruvate decarboxylase and alcohol dehydrogenase activity [7–9]. In conventional *Saccharomyces cerevisiae* brewing systems, efficient uptake of wort carbohydrates enables sustained glycolytic activity and high fermentative flux, resulting in extensive attenuation and ethanol concentrations characteristic of conventional beer fermentation [31,32].

In brewing-relevant non-*Saccharomyces* yeasts, glycolytic activity frequently operates under physiologically constrained conditions due to species- and strain-dependent differences in carbohydrate accessibility, sugar transport efficiency, respiratory metabolism, and redox regulation [5,9,20]. Although glycolytic machinery itself generally remains functional, restricted access to fermentable sugars frequently reduces carbon availability for ethanol biosynthesis, thereby contributing to intrinsically lower ethanol production relative to conventional brewing systems. However, ethanol formation is not determined solely by sugar uptake capacity, as oxygen availability, nutrient composition, fermentation temperature, and strain-specific physiological responses may further modulate glycolytic activity and metabolic flux distribution [16,20,26].

As a consequence, ethanol concentrations achieved during non-*Saccharomyces*-driven low- and non-alcoholic beer fermentation vary substantially depending on strain phenotype and process configuration. Maltose-negative strains frequently produce beers below 0.5% *v/v* ethanol, whereas maltose-limited or metabolically flexible strains may generate

ethanol concentrations within broader low-alcohol ranges depending on fermentation conditions and wort fermentability [16,26,27]. These values should be interpreted as indicative rather than universally applicable because reported outcomes remain strongly strain- and process-dependent.

This physiological framework fundamentally distinguishes non-*Saccharomyces*-based low- and non-alcoholic beer production from conventional approaches such as arrested fermentation or post-fermentation dealcoholization. In conventional strategies, glycolytic activity is interrupted after substantial ethanol formation has already occurred or ethanol is removed following fermentation. In contrast, selected non-*Saccharomyces* fermentations may intrinsically constrain ethanol formation through strain-dependent metabolic limitations, restricted carbohydrate accessibility, and altered carbon allocation during fermentation itself [1,2,4].

Consequently, low- and non-alcoholic beer production using non-*Saccharomyces* yeasts should not be interpreted simply as reduced fermentation intensity but rather as a metabolically constrained and process-responsive fermentation system in which ethanol formation emerges from interactions between strain physiology, substrate accessibility, and controllable technological variables.

3.4. Redox Balance and Carbon Redistribution

Restriction of glycolytic flux has direct consequences for intracellular redox balance and carbon allocation during fermentation because maintenance of NAD^+ regeneration is essential for continued glycolytic activity under oxygen-limited brewing conditions. In conventional brewing fermentations, redox homeostasis is predominantly maintained through ethanol formation, in which pyruvate-derived acetaldehyde is reduced to ethanol via alcohol dehydrogenase, thereby regenerating NAD^+ required for sustained glycolytic flux [5,39]. However, when fermentative carbon flux is restricted, alternative metabolic pathways increasingly contribute to redox balancing and energy maintenance [27–29].

Under metabolically constrained conditions, many brewing-relevant non-*Saccharomyces* yeasts redistribute carbon toward alternative metabolic sinks, including glycerol formation, biomass synthesis, organic acid production, and, in some strains, respiratory metabolism depending on physiological characteristics and oxygen availability [16,19,22]. Carbon partitioning between ethanol, glycerol, biomass, and alternative metabolites remains highly species- and process-dependent; however, reduced ethanol formation is frequently accompanied by greater relative carbon allocation toward non-ethanolic metabolic products [18,19,39].

Among these pathways, glycerol formation represents a particularly important physiological response because it contributes to intracellular redox balancing through NAD^+ regeneration while additionally supporting osmotic adaptation and cellular stress tolerance [18,19,39]. From a technological perspective, glycerol may also influence perceived body and mouthfeel in low- and non-alcoholic beer systems. Several studies have reported elevated glycerol production in selected non-*Saccharomyces* fermentations relative to conventional *Saccharomyces* brewing systems, although reported concentrations remain strongly strain- and process-dependent and should not be interpreted as universally applicable [18,19,39].

At the same time, altered carbon redistribution may influence the accumulation of intermediate metabolites and undesirable fermentation by-products. Reduced aldehyde removal represents a particularly important challenge because insufficient metabolic reduction of wort-derived aldehydes, including compounds associated with green, wort-like sensory attributes, may contribute to persistent off-flavor perception in low- and non-alcoholic beer systems [1,13,14]. In addition, prolonged fermentation, nutrient limitation,

or physiological stress may promote accumulation of volatile acidity, sulfur compounds, or other sensory defects [11,15,16].

Oxygen availability may further modulate carbon allocation patterns because some brewing-relevant non-*Saccharomyces* species exhibit partial respiratory metabolism, enabling redirection of carbon away from ethanol formation under moderate oxygen conditions. However, the magnitude and direction of these effects remain highly species- and strain-dependent and are additionally influenced by fermentation conditions and wort composition [19,24].

Recent brewing-focused studies further support the view that fermentation behavior in non-*Saccharomyces* yeasts is better understood as a dynamic interaction among carbohydrate accessibility, redox balancing, respiratory activity, aroma-active metabolite formation, and physiological stress adaptation rather than as a single linear fermentative pathway [21,40,41]. This broader physiological perspective reinforces the importance of considering fermentation outcomes as emergent properties of interacting metabolic and process-related variables rather than isolated physiological traits.

Consequently, low- and non-alcoholic beer fermentation using non-*Saccharomyces* yeasts should be understood not merely as ethanol-restricted fermentation but rather as a system of metabolic redistribution in which carbon allocation, redox balance, and process conditions collectively determine fermentation performance and sensory outcomes.

3.5. Metabolic Implications for Low-Alcohol Fermentation Systems

The metabolic features described above collectively distinguish many brewing-relevant non-*Saccharomyces* fermentations from conventional brewing systems and establish the physiological basis for controlled ethanol limitation in low- and non-alcoholic beer production. Species- and strain-dependent differences in carbohydrate accessibility, glycolytic throughput, redox balancing, and carbon redistribution collectively influence attenuation, ethanol formation, aroma development, and fermentation performance [5,7,8].

Importantly, fermentation outcomes emerge not solely from intrinsic strain physiology but from interactions among microbial metabolism, substrate accessibility, and controllable process conditions. Depending on strain phenotype and fermentation management, constrained carbon flux may be redistributed toward biomass formation, glycerol production, respiratory metabolism, aroma-active metabolite synthesis, or incomplete reduction of wort-derived intermediates, thereby generating distinct fermentation and sensory outcomes [12,16,19].

These physiological interactions help explain characteristic features frequently associated with low- and non-alcoholic beers produced using selected non-*Saccharomyces* yeasts, including intrinsically reduced ethanol formation, elevated residual extract, altered aroma profiles, enhanced mouthfeel, and increased susceptibility to aldehyde-associated wort-like off-flavors under suboptimal fermentation conditions [1,10,11]. However, the magnitude and direction of these effects remain strongly species-, strain-, and process-dependent and should not be interpreted as universally applicable across all non-*Saccharomyces* brewing systems.

Successful fermentation design therefore depends on understanding strain-specific physiological constraints and their interaction with controllable technological variables governing ethanol formation and sensory performance. Since brewing-relevant non-*Saccharomyces* yeasts differ substantially in carbohydrate utilization, metabolic flexibility, oxygen responsiveness, and aroma formation potential, effective implementation requires aligning strain functionality with the targeted alcohol category and desired sensory profile. The principal functional differences among brewing-relevant strains are discussed in the following section.

4. Strain Selection and Functional Design of Non-*Saccharomyces* Yeasts

4.1. Strain Selection as a Functional Decision, Not a Taxonomic Choice

Strain selection represents one of the most critical determinants of low- and non-alcoholic beer fermentation because it defines the intrinsic limits of sugar uptake, ethanol formation, aroma generation, stress tolerance, and process robustness. In contrast to conventional brewing with *Saccharomyces cerevisiae*, where fermentation performance is relatively predictable, brewing-relevant non-*Saccharomyces* yeasts exhibit pronounced interspecies and intraspecies variability, making strain-level characterization essential for reproducible fermentation outcomes [22,42,43].

From a brewery-oriented perspective, species identity alone is insufficient to guide fermentation design. Even strains belonging to the same species may differ substantially in sugar utilization efficiency, fermentation kinetics, aroma production, volatile acidity, redox behavior, and tolerance toward osmotic or oxidative stress [12,44]. For example, strains of *Saccharomyces ludwigii* are frequently associated with severely restricted maltose utilization and low ethanol production, whereas *Torulaspora delbrueckii*, *Pichia kluyveri*, *Metschnikowia pulcherrima*, and *Cyberlindnera saturnus* may exhibit markedly different fermentation kinetics, aroma formation patterns, and alcohol yields depending on strain identity and fermentation conditions [17,26,36]. Recent comparative studies further illustrate the magnitude of strain-level variability within brewing-relevant species. For example, commercial *Saccharomyces ludwigii* strains have been reported to differ substantially in attenuation, residual sugar retention, aroma formation, and overall sensory performance despite belonging to the same species [20,45]. Similarly, distinct *Torulaspora delbrueckii* strains have demonstrated considerable variation in fermentation kinetics, alcohol production, and flavor development, emphasizing the limitations of species-level generalizations when designing low- and non-alcoholic beer fermentations [42,46,47].

Consequently, strain selection should be approached as a functional process decision based on physiological performance rather than taxonomic classification alone. Within the framework of precision fermentation, yeast selection becomes a predictive design step in which microbial physiology is aligned with targeted ethanol concentration, desired sensory attributes, and brewery-relevant process conditions. Thus, successful implementation depends not only on selecting a strain capable of limiting ethanol formation but also on selecting one whose metabolic behavior remains sufficiently stable under brewery-relevant process conditions, including wort composition, oxygen availability, pitching strategy, and temperature management [19,31,47].

4.2. Functional Classification Based on Sugar Utilization Phenotype

For practical fermentation design, brewing-relevant non-*Saccharomyces* yeasts may be functionally classified according to their sugar utilization phenotype, which strongly influences ethanol formation and determines the required intensity of process control. From a brewery-oriented perspective, three broad functional categories may be distinguished: maltose-negative strains, maltose-limited strains, and metabolically flexible strains. These categories should be interpreted as functional and process-oriented groupings rather than rigid taxonomic classifications because fermentation performance remains strongly species-, strain-, and process-dependent.

Maltose-negative strains primarily utilize glucose and fructose while leaving most maltose and maltotriose unfermented. Representative examples include selected strains of *Saccharomyces ludwigii*, which are frequently associated with intrinsically restricted ethanol formation and are therefore commonly considered attractive candidates for non-alcoholic beer production [9,36]. However, fermentation outcomes remain strongly influenced by wort composition and process configuration, and restricted attenuation may

contribute to elevated residual sweetness, reduced fermentation-derived complexity, and increased sensitivity to wort carbohydrate composition [25,26].

Maltose-limited strains exhibit partial maltose utilization and may therefore support production within broader low-alcohol ranges depending on strain physiology, wort fermentability, and fermentation management. Selected strains of *Torulaspora delbrueckii*, *Metschnikowia pulcherrima*, and *Cyberlindnera saturnus* have been reported to provide improved aroma complexity and a more pronounced fermentation character relative to strongly maltose-negative strains, although tighter process control is frequently required to prevent unintended ethanol formation [16,25,42].

Metabolically flexible strains exhibit broader carbohydrate utilization, including partial metabolism of maltose and, in some cases, limited maltotriose utilization. While these yeasts may support more beer-like sensory profiles and improved attenuation, ethanol concentrations may increase substantially depending on fermentation conditions, pitching strategy, and wort fermentability [16,22]. Consequently, metabolically flexible strains are often considered more suitable for low-alcohol beer production, co-fermentation strategies, or brewery systems in which fermentation variables can be more tightly controlled [33,45,46].

Importantly, the functional categories presented above should be interpreted as indicative brewing frameworks rather than universally predictive classifications, as considerable variability exists even among strains belonging to the same species. Recent comparative studies of commercial maltose-negative and maltotriose-negative strains further highlight pronounced variability in attenuation, sensory performance, and process robustness among yeasts selected for similar low- and non-alcoholic brewing applications. The proposed categories are intended as brewery-oriented decision-support groupings rather than discrete biological classes, as individual strains may simultaneously exhibit characteristics associated with multiple functional categories depending on strain identity and fermentation conditions.

4.3. Linking Strain Phenotype to Process Design Requirements

The functional classification of brewing-relevant non-*Saccharomyces* yeasts influences not only expected ethanol formation but also process sensitivity and the operational flexibility of fermentation systems. Because metabolic limitations differ substantially among strains, the required intensity of process control varies according to the physiological characteristics of the selected yeast. Maltose-negative strains frequently operate under relatively strong intrinsic metabolic constraints and therefore often require less intensive process intervention to maintain ethanol concentrations within the non-alcoholic range. Since fermentable carbon accessibility is already substantially restricted, moderate fluctuations in fermentation parameters may exert comparatively smaller effects on final alcohol concentration relative to metabolically flexible strains [1,13,43,47].

In contrast, maltose-limited and metabolically flexible strains frequently exhibit greater sensitivity to process variables such as wort fermentability, oxygen availability, temperature, pitching rate, and fermentation duration. Under brewery-relevant conditions, relatively small variations in fermentation management may alter sugar utilization efficiency, glycolytic activity, aroma formation, redox balance, and ethanol production depending on strain phenotype and process configuration [14,36,48–51]. Recent brewery-oriented studies further indicate that fermentation performance in low- and non-alcoholic beer systems is best interpreted as an interaction between strain functionality and controllable process variables rather than a fixed physiological outcome.

Consequently, strain phenotype may strongly influence the breadth of the operational fermentation window and the predictability of fermentation outcomes. From a precision fermentation perspective, strain selection therefore establishes the physiological framework within which subsequent process variables, including wort composition, oxygen management, and temperature, must be optimized to achieve targeted ethanol concentrations and sensory outcomes.

4.4. Aroma Potential as a Design Trade-Off

The application of brewing-relevant non-*Saccharomyces* yeasts extends beyond ethanol reduction and increasingly represents an opportunity for targeted aroma modulation in low- and non-alcoholic beer systems. Numerous strains exhibit enhanced production of aroma-active metabolites, including esters, thiols, higher alcohols, terpenes, and other volatile compounds associated with fruity and floral sensory characteristics that may partially compensate for diminished ethanol-driven flavor perception [10–12,52]. Representative examples include *Pichia kluyveri*, frequently associated with enhanced thiol release and fruity aroma intensity, *Torulaspora delbrueckii*, linked to ester production and improved fermentation-derived complexity, and *Cyberlindnera saturnus*, known for characteristic fruity and phenolic aroma contributions depending on fermentation conditions [26,36,52].

However, aroma enhancement is intrinsically linked to metabolic activity and therefore frequently involves trade-offs between sensory complexity and ethanol control. Conditions promoting increased aroma formation, including elevated metabolic throughput, increased oxygen availability, or intensified fermentation activity, may simultaneously increase ethanol formation or stimulate production of undesirable metabolites, including volatile acidity, sulfur compounds, or sensory imbalance depending on strain physiology and process configuration [5,38,44,53]. Consequently, aroma formation should not be interpreted as universally beneficial, as highly aroma-active strains may also exhibit increased process sensitivity or less predictable fermentation behavior under brewery-relevant conditions.

As a result, strain selection should not be viewed solely as a strategy for ethanol limitation but rather as a multidimensional design decision balancing alcohol control, aroma intensity, fermentation robustness, and target sensory objectives. Strains optimized for strict ethanol limitation may exhibit reduced fermentation-derived complexity, whereas highly aroma-active strains often require tighter operational control to maintain targeted alcohol concentrations and desirable sensory outcomes.

4.5. Process Robustness and Industrial Applicability

Beyond metabolic phenotype and aroma potential, industrial implementation depends strongly on process robustness and strain stability under brewery-relevant conditions. Compared with *Saccharomyces cerevisiae*, many brewing-relevant non-*Saccharomyces* yeasts may exhibit reduced tolerance toward osmotic stress, oxygen fluctuations, pH variation, and fermentation-related environmental stressors depending on strain identity and fermentation conditions, potentially resulting in prolonged lag phases, inconsistent fermentation performance, or increased variability in aroma production [4,22,26,54].

Furthermore, industrial implementation requires consideration of microbiological stability, contamination risk, filtration behavior, and process reproducibility, particularly in low- and non-alcoholic beer systems where reduced ethanol concentrations may increase microbial susceptibility and shorten flavor stability [1,13,54]. Recent studies additionally emphasize that strain-dependent differences in process robustness may strongly influence scalability and reproducibility between laboratory, pilot, and industrial brewing systems.

Consequently, laboratory-scale strain screening alone is frequently insufficient for brewery implementation. Pilot-scale validation should additionally assess fermentation kinetics, attenuation, ethanol formation, glycerol production, volatile acidity, process reproducibility, microbiological stability, and sensory performance under brewery-relevant production conditions [47,55]. Depending on strain functionality, practical evaluation may further include filtration performance, fermentation consistency across repeated batches, and tolerance toward industrial process fluctuations.

From a precision fermentation perspective, industrially relevant strains should not only demonstrate the capacity to limit ethanol formation but also maintain sufficiently reproducible metabolic behavior across different fermentation batches and process conditions. Process robustness therefore represents an essential criterion for selecting strains suitable for scalable low- and non-alcoholic beer production.

4.6. Strain Selection as the Starting Point of Process Design

From a brewery-oriented perspective, strain selection should begin with defining the target product category because the metabolic phenotype of the selected yeast strongly influences the achievable range of ethanol formation, fermentation performance, and sensory characteristics [15,56,57]. For strictly non-alcoholic beers targeting ethanol concentrations below approximately 0.5% *v/v*, maltose-negative strains, including selected *Saccharomyces ludwigii* isolates, are frequently considered attractive candidates due to their intrinsically restricted carbohydrate utilization and comparatively predictable ethanol limitation [36,42,52]. However, fermentation outcomes remain dependent on wort composition and process conditions and should not be interpreted as universally applicable across all strains.

In contrast, production of low-alcohol beers within the approximate range of 0.5–1.5% *v/v* may benefit from maltose-limited strains capable of generating greater fermentation complexity and more developed aroma profiles while still maintaining reduced ethanol formation relative to conventional brewing systems [16,25]. Representative examples include selected strains of *Torulaspora delbrueckii*, *Metschnikowia pulcherrima*, and *Cyberlindnera saturnus*, although reported alcohol concentrations and sensory outcomes remain strongly influenced by fermentation management and wort fermentability [17,26,36].

Where stronger fermentation character and improved attenuation are desired, metabolically flexible strains or controlled co-fermentation systems may support greater sensory complexity, although these approaches typically require substantially tighter process regulation to maintain targeted alcohol concentrations [43,46,58]. Depending on strain functionality, practical control may involve adjustment of wort fermentability, oxygen availability, pitching strategy, fermentation temperature, and fermentation duration.

From a brewery-oriented perspective, strain selection therefore represents not merely a microbiological decision but the starting point of process engineering, strongly influencing fermentation behavior and operational flexibility [57]. Within the framework of precision fermentation, the selected strain establishes the physiological boundaries within which controllable technological variables can be manipulated to achieve targeted ethanol concentrations and desired sensory outcomes [25,56]. The functional classification of brewing-relevant non-*Saccharomyces* yeasts and their practical relevance for low- and non-alcoholic beer production are summarized in Table 1.

Table 1. Functional classification of brewing-relevant non-*Saccharomyces* yeasts according to sugar utilization phenotype, indicative ethanol formation, sensory functionality, and brewery-oriented application in low- and non-alcoholic beer production.

Functional Strain Group	Representative Yeast Species	Typical Sugar Utilization Phenotype	Indicative Ethanol Range *	Main Advantage	Main Limitation	Practical Brewing Application	Key References
Maltose-negative strains	<i>Saccharomyces ludwigii</i> , selected <i>Pichia</i> spp.	Glucose/fructose utilization; maltose and maltotriose largely unfermented	Typically <0.5% v/v	Strong intrinsic ethanol limitation; relatively predictable alcohol control	High residual sweetness; reduced fermentation-derived complexity	Non-alcoholic beer production	[20,36,59]
Maltose-limited strains	<i>Torulaspora delbrueckii</i> , <i>Metschnikowia pulcherrima</i> , <i>Wickerhamomyces anomalus</i>	Partial maltose utilization; limited maltotriose metabolism	Typically 0.5–1.5% v/v	Improved fermentation character and aroma complexity	Greater sensitivity of ethanol formation to process conditions	Low-alcohol beer production	[9,29,44,59]
Metabolically flexible strains	Selected <i>Torulaspora delbrueckii</i> , <i>Metschnikowia</i> spp., co-fermentation-compatible strains	Broader carbohydrate utilization including partial maltose metabolism and limited maltotriose use	Frequently >1.0% v/v depending on process conditions	More beer-like fermentation profile and improved attenuation	Requires tighter process control to avoid excessive ethanol formation	Low-alcohol beer, hybrid fermentation, and controlled co-fermentation strategies	[16,59,60]
Aroma-active strains **	<i>Pichia kluyveri</i> , <i>Cyberlindnera saturnus</i> , selected ester-producing non- <i>Saccharomyces</i> strains	Limited sugar fermentation combined with elevated ester, thiol, or volatile aroma production	Variable; typically low under controlled conditions	Fruity/floral aroma enhancement; sensory compensation for reduced ethanol	Aroma inconsistency and increased process sensitivity	Aroma-focused low- and non-alcoholic beer production	[10,12,41,49]

* ethanol ranges are intended only as indicative brewery-oriented outcomes commonly reported in the literature and should not be interpreted as fixed thresholds for a given species or strain. ** Aroma-active strains are presented as a sensory-functional category rather than a distinct metabolic phenotype and may overlap with maltose-negative, maltose-limited, or metabolically flexible strains. Consequently, the categories presented in Table 1 should not be interpreted as mutually exclusive classifications.

5. Process Parameters as Regulators of Constrained Metabolism

Once strain-specific metabolic constraints are established, fermentation performance becomes increasingly dependent on process conditions. In low- and non-alcoholic beer production using brewing-relevant non-*Saccharomyces* yeasts, variables such as wort fermentability, temperature, pitching rate, oxygen availability, and fermentation duration regulate the extent to which constrained metabolic systems convert accessible substrates into ethanol, aroma-active metabolites, biomass, and residual extract [17–19]. Because many brewing-relevant non-*Saccharomyces* strains operate under species- and strain-dependent limitations in sugar accessibility, glycolytic throughput, and redox balancing, fermentation performance frequently exhibits heightened sensitivity to process conditions relative to conventional brewing systems [4,50,51].

Under brewery-relevant conditions, operational variables may substantially influence sugar utilization efficiency, carbon allocation, aroma formation, redox homeostasis, and final ethanol concentration depending on strain physiology and fermentation configuration. Consequently, successful fermentation management requires coordinated adjustment of process conditions according to strain-specific physiological constraints rather than isolated optimization of individual variables [15,43].

From a precision fermentation perspective, process control in low- and non-alcoholic beer systems should therefore be understood as deliberate regulation of constrained metabolism, in which fermentation outcomes emerge from interactions among microbial physiology, substrate accessibility, and controllable technological variables. Rather than relying on post-fermentation correction or empirical trial-and-error optimization, this approach emphasizes predictive alignment of process parameters with strain-specific metabolic behavior to achieve targeted ethanol concentrations and desired sensory outcomes [25,57].

5.1. Process Parameters as Modulators of Carbon Flux Distribution

In brewing-relevant non-*Saccharomyces* fermentations, process parameters do not determine whether ethanol is formed but rather regulate the extent to which metabolically constrained systems redistribute available carbon between fermentation, respiration, biomass formation, glycerol production, and aroma-active metabolite synthesis [4,5,7,9]. Because sugar accessibility is already physiologically restricted in many strains, fermentation outcomes frequently exhibit heightened sensitivity to process conditions, with operational variables modulating glycolytic throughput, redox balancing, and carbon allocation patterns [47,51].

In contrast to *Saccharomyces cerevisiae*, which exhibits strong fermentative metabolism even under partially aerobic conditions due to the Crabtree effect, several brewing-relevant non-*Saccharomyces* yeasts exhibit reduced or absent Crabtree behavior depending on species and strain identity. This physiological characteristic may permit partial redirection of carbon toward respiratory metabolism when oxygen becomes available, thereby reducing ethanol formation while maintaining metabolic activity under selected fermentation conditions [4,19,24].

Consequently, changes in fermentation temperature, oxygenation strategy, pitching rate, or substrate accessibility may alter fermentation kinetics, ethanol formation, aroma development, and redox homeostasis depending on strain physiology and process configuration. From a brewery-oriented perspective, fermentation outcomes in low- and non-alcoholic beer systems should therefore be interpreted as the result of coordinated interactions among microbial physiology and controllable technological variables rather than isolated process effects.

5.2. Wort Fermentability as a Primary Control Lever

Among process variables, wort fermentability represents one of the most influential upstream determinants governing ethanol formations because it directly defines the proportion and accessibility of carbohydrates available for yeast metabolism. In low- and non-alcoholic beer production, deliberate reduction of wort fermentability may substantially limit glycolytic substrate availability and thereby constrain carbon flux toward ethanol formation [17,18,20].

Technological interventions commonly applied to reduce wort fermentability include elevated mash temperatures, shortened saccharification regimes, suppression of β -amylase activity, and incorporation of adjuncts exhibiting limited enzymatic accessibility. These strategies reduce the generation of readily fermentable carbohydrates, particularly maltose, thereby contributing to more predictable ethanol limitation during fermentation [48,57,61].

However, the effect of wort fermentability remains strongly strain-dependent. In maltose-negative strains, reductions in fermentability may exert comparatively smaller effects because carbohydrate accessibility is already intrinsically constrained [7,47]. In contrast, maltose-limited and metabolically flexible strains frequently exhibit substantially greater sensitivity to wort composition and increases in fermentable sugar availability may

contribute to elevated ethanol formation depending on strain phenotype, mash profile, and fermentation management [42,49,51].

From a brewery-oriented perspective, wort fermentability should therefore be interpreted not as a fixed technological parameter but as a controllable upstream regulator of carbon accessibility that interacts closely with strain physiology to influence ethanol formation, attenuation, and sensory outcomes.

5.3. Temperature Control and Metabolic Activation

Fermentation temperature represents a major determinant of metabolic activity because it directly affects enzymatic reaction rates, membrane transport efficiency, and overall fermentation kinetics. In non-*Saccharomyces* fermentations, temperature influences not only sugar uptake and ethanol formation but also the production of aroma-active metabolites and the balance between fermentative and respiratory metabolism [17,24,41].

Elevated fermentation temperatures generally increase glycolytic activity and metabolic throughput, frequently leading to enhanced sugar consumption and moderately increased ethanol formation. Under brewery-relevant conditions, increasing fermentation temperature from approximately 15 °C to 22–25 °C has in some studies been associated with moderate increases in ethanol concentration, often within several tenths of a percent *v/v* depending on strain phenotype and wort composition [51,60,62].

At the same time, elevated temperatures may enhance ester biosynthesis and aroma intensity, particularly in aroma-active strains such as *Cyberlindnera saturnus* and selected ester-producing non-*Saccharomyces* yeasts, potentially improving sensory complexity in low- and non-alcoholic beer systems [10,26,52]. However, excessive thermal activation may simultaneously increase fermentation variability, stimulate undesirable by-product formation, or compromise ethanol control.

Conversely, lower fermentation temperatures (<15 °C) generally reduce metabolic throughput and ethanol formation while improving process stability. Nevertheless, excessively low temperatures may prolong fermentation time, impair aroma formation, and reduce overall fermentation-derived complexity [1,62,63]. Beyond its influence on fermentation kinetics, temperature acts as an important metabolic regulator shaping ethanol formation, aroma development, and overall fermentation robustness.

5.4. Pitching Rate and Biomass–Fermentation Trade-Off

Pitching rate substantially influences fermentation performance because it affects the balance between biomass formation and fermentative metabolism during early fermentation stages. In brewing-relevant non-*Saccharomyces* systems, this relationship becomes particularly important because constrained sugar accessibility may amplify the impact of yeast growth dynamics on carbon allocation, fermentation kinetics, and ethanol formation [4,11,14].

Lower pitching rates may favor biomass formation over fermentative metabolism, potentially reducing ethanol formation through greater diversion of available carbon toward cellular growth and maintenance. However, insufficient inoculation may prolong lag phase duration, increase susceptibility to microbial instability, and contribute to less predictable fermentation performance or elevated formation of undesirable metabolites under brewery-relevant conditions [14,60,64].

In contrast, higher pitching rates frequently shorten lag phase duration, improve fermentation robustness, and support more reproducible metabolic performance. Nevertheless, in strains exhibiting partial maltose utilization or broader metabolic flexibility, increased pitching rates may simultaneously promote greater sugar utilization and ethanol

formation depending on strain physiology, wort fermentability, and fermentation management [4,16,64].

Consequently, pitching rate represents a practical trade-off between process robustness, fermentation reproducibility, aroma development, and strict ethanol limitation. From a brewery-oriented perspective, optimal pitching strategies therefore depend strongly on strain phenotype, fermentation objectives, and the targeted alcohol category.

5.5. Oxygen Availability and Metabolic Switching

Oxygen availability plays a central role in regulating fermentation performance because it influences sterol biosynthesis, membrane integrity, cellular viability, and the balance between respiratory and fermentative metabolism. In contrast to *Saccharomyces cerevisiae*, which exhibits strong fermentative metabolism even under partially aerobic conditions, several brewing-relevant non-*Saccharomyces* yeasts may exhibit partially respiratory behavior depending on species, strain identity, and fermentation conditions, making oxygen management particularly relevant for controlling ethanol formation and metabolic performance [4,5,24,39].

Controlled oxygen exposure during pitching or early fermentation stages may support membrane synthesis, biomass formation, and fermentation robustness while, in selected strains, enabling partial redirection of carbon away from ethanol production through respiratory metabolism [17,65]. Under brewery-relevant conditions, carefully managed oxygen availability may therefore contribute to improved process reproducibility and influence aroma-active metabolite formation.

However, oxygen management requires careful balancing because excessive oxygen exposure may stimulate metabolic activity, alter sugar utilization efficiency, or reduce the predictability of ethanol control depending on strain physiology and wort composition [5,7,16,39]. Conversely, insufficient oxygen availability may impair membrane functionality, reduce fermentation robustness, and increase susceptibility to physiological stress or stress-associated off-flavor formation [14,60].

From a precision fermentation perspective, oxygen management should therefore be interpreted not merely as a fermentation support variable but as a controllable metabolic regulator capable of influencing carbon allocation among respiration, biomass formation, and alcoholic fermentation. The practical effect of oxygenation nevertheless remains strongly strain- and process-dependent.

5.6. Fermentation Time and Metabolic Endpoints

Fermentation duration defines the cumulative extent of metabolic activity and therefore represents an important determinant of ethanol formation, attenuation, aroma development, and sensory stability in low- and non-alcoholic beer systems. In brewing-relevant non-*Saccharomyces* fermentations, the period of active metabolic activity frequently corresponds to depletion of accessible monosaccharides and subsequent stabilization of fermentation behavior, although the duration of this phase remains strongly strain- and process-dependent [1,16,19].

In contrast to conventional brewing systems, extending fermentation beyond the period of active sugar utilization does not necessarily result in substantially improved attenuation because residual wort carbohydrates, particularly maltose and maltotriose, may remain partially or entirely metabolically inaccessible depending on strain physiology [9,42,44,59]. Consequently, prolonged fermentation does not always translate into improved fermentation performance and may instead increase susceptibility to metabolic imbalance or undesirable sensory outcomes.

Several studies have reported that extended fermentation periods may alter aroma composition, increase volatile acidity, or promote accumulation of stress-associated metabolites, particularly under oxygen-limited or nutritionally constrained conditions [1,11,15]. From a brewery-oriented perspective, fermentation endpoints in non-*Saccharomyces*-based low- and non-alcoholic beer production should therefore be defined according to metabolic stabilization and strain-specific fermentation behavior rather than fermentation duration alone.

Within a precision fermentation framework, clearly defined fermentation endpoints represent an important process control strategy supporting predictable ethanol concentrations while minimizing the risk of sensory instability, metabolic imbalance, and off-flavor development.

5.7. Integrated Process Windows and System Sensitivity

The process variables discussed above interact to define the operational conditions under which ethanol formation, fermentation performance, and sensory quality may be predictably managed in low- and non-alcoholic beer systems. Because brewing-relevant non-*Saccharomyces* fermentations frequently operate under metabolically constrained conditions, changes in wort fermentability, temperature, oxygen availability, pitching rate, or fermentation duration may substantially influence carbon allocation, fermentation kinetics, aroma formation, and final product characteristics depending on strain physiology and process configuration [16,20,50].

Compared with conventional brewing systems, in which fermentation behavior is often more robust across broader operational conditions, many non-*Saccharomyces* fermentations may exhibit comparatively greater process sensitivity [56]. Consequently, successful low- and non-alcoholic beer production cannot rely solely on isolated parameter optimization but instead requires coordinated process design integrating strain physiology with controllable fermentation variables [33]. From a precision fermentation perspective, predictable product quality emerges from alignment between metabolic constraints and operational management rather than from yeast selection or technological intervention alone [4].

Collectively, the interactions discussed above define the practical process space within which ethanol formation, fermentation performance, and sensory outcomes may be managed during low- and non-alcoholic beer production (Figure 2). The brewery-relevant operational implications of the principal process variables are summarized in Table 2.

Table 2. Core process variables regulating constrained metabolism during precision fermentation of low- and non-alcoholic beer using non-*Saccharomyces* yeasts. The table summarizes brewery-relevant process implications, principal metabolic mechanisms, and expected fermentation effects. Reported outcomes should be interpreted as strain- and process-dependent tendencies rather than fixed technological limits.

Process Variable	Principal Metabolic Effect	Expected Fermentation Outcome	Sensory Implication	Risk If Uncontrolled	Key References
Wort fermentability	Regulates accessibility of fermentable carbohydrates, particularly maltose and maltotriose	Alters glycolytic substrate availability and ethanol formation potential	Residual sweetness, body, and mouthfeel modulation	Excessive sweetness or insufficient attenuation	[17,18,20,48,57,61]
Pitching rate	Influences balance between biomass formation and fermentative metabolism	Affects fermentation robustness, lag phase duration, and reproducibility	More or less consistent aroma formation	Under-pitching: microbial instability; over-pitching: altered ethanol formation	[4,11,14,16,60,64]

Table 2. Cont.

Process Variable	Principal Metabolic Effect	Expected Fermentation Outcome	Sensory Implication	Risk If Uncontrolled	Key References
Fermentation temperature	Regulates enzymatic activity, membrane transport, and metabolic throughput	Modulates fermentation kinetics, aroma formation, and ethanol formation	Altered ester production and sensory balance	Increased fermentation variability or reduced aroma complexity	[1,17,24,26,41,52]
Oxygen availability	Influences sterol synthesis, membrane integrity, and respiratory metabolism	Affects viability, metabolic performance, and carbon allocation	Reduced or increased formation of stress-associated off-flavors	Excessive oxygen: altered metabolism; insufficient oxygen: reduced robustness	[4,5,7,17,24,39,59]
Fermentation duration	Defines cumulative extent of metabolic activity	Influences stabilization of fermentation endpoints and attenuation	Aroma maturation and sensory stability	Prolonged fermentation may promote metabolic imbalance or off-flavors	[1,9,11,15,16,19,39,44,59]

Fermentation outcomes remain strongly strain-, wort-, and process-dependent and should be interpreted as brewery-oriented tendencies rather than universally predictive relationships.

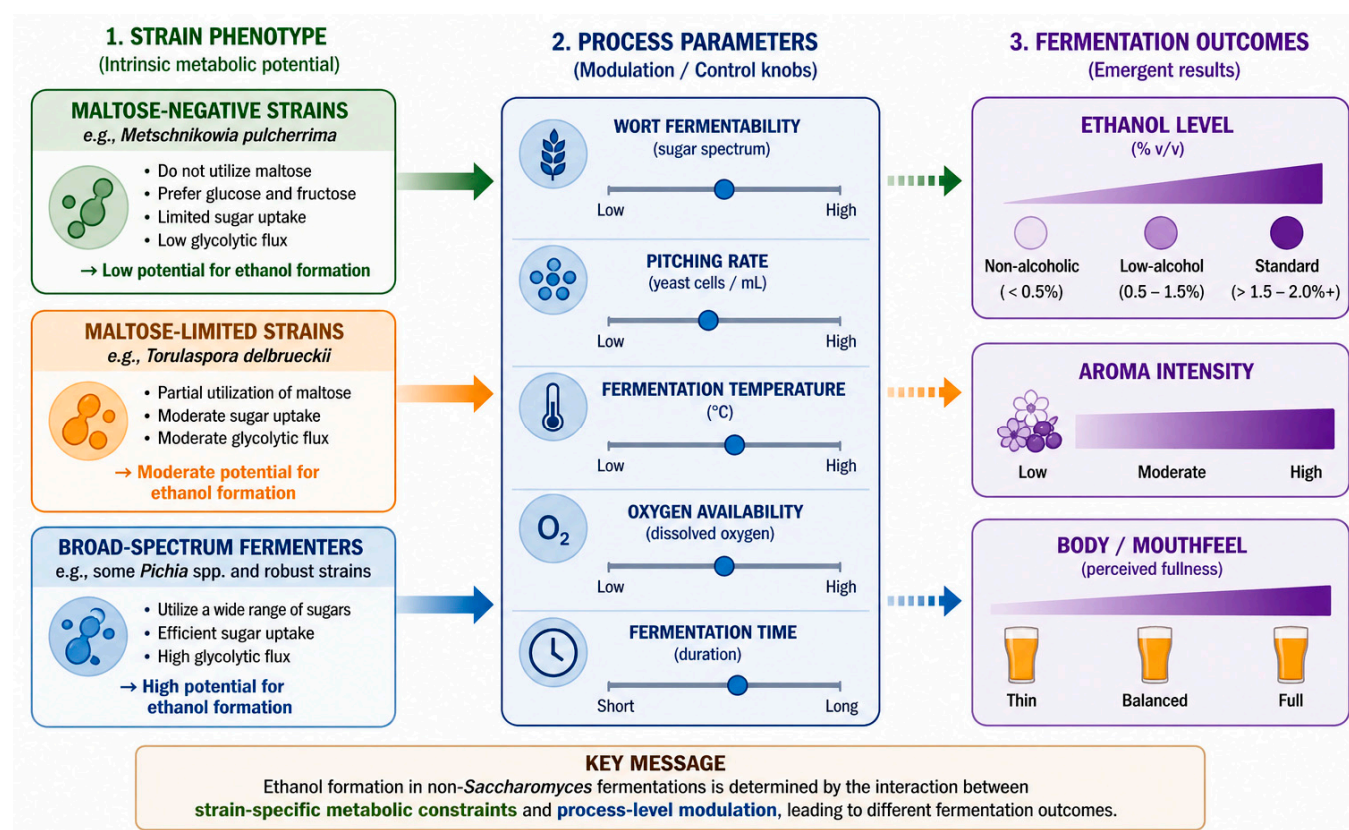


Figure 2. Conceptual framework illustrating interactions between strain-specific metabolic traits and controllable process variables during precision fermentation of low- and non-alcoholic beer using non-*Saccharomyces* yeasts. Strain phenotype influences sugar uptake capacity, glycolytic throughput, and metabolic flexibility, while wort fermentability, pitching rate, temperature, oxygen availability, and fermentation duration modulate carbon allocation between respiration, biomass formation, aroma-active metabolite synthesis, and alcoholic fermentation. Fermentation outcomes, including ethanol concentration, aroma intensity, mouthfeel, and sensory balance, emerge from coordinated interactions among microbial physiology and controllable process conditions. Relationships shown represent a conceptual brewery-oriented framework and should not be interpreted as universally predictive across all strains or fermentation systems.

6. Process Design Strategies for Low- and Non-Alcoholic Beer Production

Although strain selection and process parameter optimization establish the physiological basis for ethanol control, successful implementation additionally depends on the overall fermentation strategy applied during production. Because brewing-relevant non-*Saccharomyces* fermentations frequently operate under metabolically constrained and process-sensitive conditions, brewery-oriented process design must balance ethanol limitation with sensory complexity, fermentation robustness, microbiological stability, and process reproducibility [1,2,4].

Rather than representing a single technological solution, low- and non-alcoholic beer production using non-*Saccharomyces* yeasts increasingly relies on integrated process configurations in which strain phenotype, wort composition, fermentation conditions, and post-fermentation management are jointly optimized to achieve targeted product characteristics [25,43].

6.1. Single-Strain Fermentation Strategies

Single-strain fermentation represents one of the most straightforward implementation strategies for low- and non-alcoholic beer production using non-*Saccharomyces* yeasts because fermentation outcomes remain more directly associated with the physiological behavior of a selected strain. This approach may provide comparatively simplified operational control, reduced microbiological complexity, and greater process predictability relative to multi-strain systems, making it attractive for brewery implementation [6,25,36].

In practice, single-strain systems are commonly applied using maltose-negative or maltose-limited yeasts capable of intrinsically restricting ethanol formation while simultaneously contributing aroma-active metabolites. Representative examples include selected strains of *Saccharomyces ludwigii*, *Torulaspora delbrueckii*, and *Metschnikowia pulcherrima*, although reported fermentation outcomes remain strongly strain- and process-dependent [10,63–65]. Depending on strain physiology, wort fermentability, and fermentation management, such systems may support production of beers within non-alcoholic or low-alcohol categories without requiring physical dealcoholization.

However, single-strain systems frequently face limitations associated with incomplete attenuation, elevated residual sweetness, restricted fermentation-derived complexity, or insufficient aldehyde reduction. Consequently, achieving acceptable product quality often requires careful alignment among strain phenotype, wort composition, and fermentation conditions [1,13].

6.2. Sequential and Co-Fermentation Strategies

To overcome sensory limitations associated with restricted carbohydrate metabolism, increasing attention has been directed toward sequential and co-fermentation systems combining non-*Saccharomyces* and *Saccharomyces* strains. These strategies are generally designed to balance the ethanol-limiting characteristics of selected non-*Saccharomyces* yeasts with improved fermentation completeness, aroma complexity, and sensory balance provided by *Saccharomyces cerevisiae* under brewery-relevant conditions [3,44,58].

In sequential fermentation systems, brewing-relevant non-*Saccharomyces* yeasts are commonly applied during early fermentation stages to modulate aroma formation and influence ethanol accumulation prior to inoculation with *Saccharomyces cerevisiae*, which may subsequently contribute to attenuation and reduction of residual wort character. In contrast, co-fermentation systems involve simultaneous inoculation of both yeast groups, thereby creating competitive and complementary metabolic interactions throughout fermentation [12,25].

Representative combinations involving *Torulaspora delbrueckii*, *Metschnikowia pulcherrima*, or *Pichia kluyveri* together with *Saccharomyces cerevisiae* have been associated with enhanced sensory complexity and modified aroma profiles in selected brewing studies, although fermentation performance remains strongly dependent on inoculation timing, strain compatibility, and fermentation management [10,16,44].

However, mixed fermentation systems frequently introduce greater process complexity because fermentation outcomes may depend strongly on inoculation timing, inoculum ratio, oxygen availability, substrate competition, and strain compatibility. Consequently, sequential and co-fermentation approaches generally require tighter operational control to maintain predictable ethanol concentrations, reproducible sensory outcomes, and fermentation robustness [16,51].

6.3. Wort Design and Matrix Optimization

Because ethanol formation is fundamentally constrained by substrate accessibility, wort composition itself represents an important upstream design variable in low- and non-alcoholic beer production. Beyond conventional process adjustments targeting fermentability, optimization of carbohydrate composition, nitrogen availability, dextrin content, and adjunct incorporation may substantially influence metabolic activity, fermentation robustness, aroma formation, and sensory perception [17,20,66].

In particular, adjustment of wort fermentability may partially compensate for strain-specific metabolic limitations by modulating the proportion and accessibility of carbohydrates available for glycolysis. Depending on strain physiology and carbohydrate utilization phenotype, modifications of mash profile and wort composition may influence ethanol formation, attenuation, and fermentation kinetics [17,20,66,67]. Likewise, dextrin-rich wort formulations may contribute to enhanced body and mouthfeel, partially compensating for the structural contribution typically provided by ethanol in conventional beer systems [10,11].

Beyond carbohydrate composition, matrix properties such as amino nitrogen availability, osmotic balance, and adjunct-derived substrate accessibility may further influence fermentation robustness and metabolic behavior in brewing-relevant non-*Saccharomyces* systems. Consequently, wort optimization should not be interpreted merely as a preparatory technological step but as an important determinant of the metabolic environment within which constrained fermentation systems operate.

From a precision fermentation perspective, wort design therefore represents deliberate engineering of substrate accessibility and metabolic context aimed at balancing ethanol control, fermentation performance, and target sensory outcomes.

6.4. Process Robustness, Reproducibility, and Scale-Up Considerations

Despite promising laboratory-scale results, successful industrial implementation of non-*Saccharomyces*-based low- and non-alcoholic beer production remains strongly dependent on process robustness and reproducibility under brewery-relevant conditions. Fermentation systems performing consistently at laboratory scale may exhibit altered metabolic behavior during pilot- or industrial-scale production due to differences in oxygen transfer, thermal gradients, mixing conditions, fermentation geometry, microbial competition, and physiological stress responses [1,4,30,64,68].

Compared with conventional brewing systems, many brewing-relevant non-*Saccharomyces* fermentations may exhibit comparatively greater sensitivity to process variation depending on strain identity and fermentation configuration. Variations in pitching strategy, fermentation temperature, oxygenation regime, or wort composition may influence sugar utilization

efficiency, aroma formation, attenuation, and ethanol production, thereby affecting process reproducibility and product consistency [16,20,30,69].

Consequently, industrial implementation should extend beyond laboratory-scale strain screening and include pilot-scale validation focused on fermentation kinetics, ethanol reproducibility, attenuation stability, aroma development, aldehyde reduction, microbiological stability, and sensory consistency across repeated fermentation batches [58,60]. Depending on strain functionality, additional evaluation of contamination risk, filtration behavior, and fermentation robustness under realistic brewery conditions may further improve process transferability.

Several recent brewery-oriented studies further illustrate the importance of pilot-scale validation during implementation of non-*Saccharomyces* yeasts. Myncke et al. [20] demonstrated substantial differences in attenuation, residual sugar retention, and sensory performance among commercial maltose-negative strains evaluated under brewing-relevant conditions, highlighting the limitations of species-level assumptions. Similarly, Kelanne et al. [59] reported distinct fermentation performance and sensory outcomes when applying *Saccharomyces ludwigii* and *Torulaspora delbrueckii* for ale-type low-alcohol beer production. Additional industrially relevant investigations have identified oxygen management, filtration performance, and batch-to-batch reproducibility as critical factors influencing successful scale-up and commercial implementation of low- and non-alcoholic beer fermentation systems [55,60].

In this context, process robustness should be considered equally important as intrinsic ethanol-limiting capacity when evaluating strains suitable for commercial application [56]. From a brewery-oriented precision fermentation perspective, reproducibility across different fermentation scales ultimately represents a prerequisite for predictable product quality and industrial feasibility.

6.5. Strategic Selection of Fermentation Configurations

The diversity of available fermentation strategies highlights that no universal technological solution exists for low- and non-alcoholic beer production using non-*Saccharomyces* yeasts. Instead, the suitability of a given fermentation configuration depends on the targeted alcohol category, desired sensory profile, acceptable process complexity, and degree of operational control available within the brewery environment [22,25].

Single-strain fermentations frequently provide comparatively simplified operational control and reduced microbiological complexity, making them attractive options for low- and non-alcoholic beer production under brewery-relevant conditions. In contrast, sequential and co-fermentation systems may support greater sensory complexity and enhanced fermentation-derived character but often require tighter operational control due to increased metabolic interactions, strain compatibility considerations, and greater process sensitivity [12,16,42].

Similarly, wort optimization strategies may complement or partially compensate for strain-associated metabolic limitations by reshaping substrate accessibility, attenuation potential, mouthfeel, and sensory perception [48,61]. Consequently, successful brewery implementation increasingly relies on integrated process design in which strain phenotype, fermentation strategy, and wort composition are jointly aligned to achieve targeted ethanol concentrations and desired product quality.

To facilitate practical translation of the physiological and process-related principles discussed above into brewery-oriented fermentation planning, Figure 3 presents a conceptual brewery-oriented framework integrating strain phenotype, fermentation strategy, process sensitivity, and target alcohol category in low- and non-alcoholic beer production. Rather than prescribing a universal technological pathway, the framework is intended

to illustrate how fermentation configurations may be aligned with targeted ethanol and sensory objectives under different brewing scenarios.

The practical advantages, limitations, and brewery-relevant implementation characteristics of the principal fermentation strategies discussed above are summarized in Table 3. Because different fermentation strategies present distinct trade-offs between ethanol control, sensory complexity, operational robustness, and implementation complexity, direct comparison becomes essential for rational process selection. Table 3 therefore summarizes the principal characteristics, strengths, limitations, and brewery-relevant considerations associated with single-strain, sequential, co-fermentation, and wort optimization strategies for low- and non-alcoholic beer production.

Table 3. Brewery-oriented implementation strategies for precision fermentation of low- and non-alcoholic beer using non-*Saccharomyces* yeasts. The table summarizes key technological interventions linking wort design, fermentation strategy, process stabilization, and monitoring with their mechanistic rationale and expected technological outcomes during brewery-scale production.

Brewery Implementation Stage	Strategy	Mechanistic Rationale	Expected Technological Outcome	Sensory Implication	Key References
Wort design	High-temperature mashing (dextrinization)	Reduced β -amylase activity limits maltose formation and glycolytic substrate availability	Reduced ethanol formation potential; increased residual extract	Fuller body and mouthfeel; increased sweetness	[2,17,18,20]
Wort design	Adjustment of fermentable sugar composition	Reduced glucose and maltose accessibility limits fermentative carbon flux	Greater control of ethanol formation within target alcohol categories	Reduced risk of excessive attenuation	[19,20,29]
Raw material strategy	Use of low-fermentability adjuncts (e.g., unmalted cereals, β -glucan-rich substrates)	Reduced enzymatic accessibility lowers fermentable sugar release	Greater flexibility in ethanol and attenuation control	Enhanced body and texture	[19,20,46]
Fermentation strategy	Primary fermentation with non- <i>Saccharomyces</i> yeasts	Restricted sugar utilization intrinsically limits ethanol production	Intrinsically constrained ethanol formation depending on strain physiology	Mild fermentation character; elevated residual sweetness	[4,29,59]
Fermentation strategy	Co-fermentation with <i>Saccharomyces cerevisiae</i>	Partial utilization of residual sugars improves attenuation	Enhanced fermentation completeness depending on strain compatibility	Greater aroma complexity; altered ethanol formation	[9,16,60]
Fermentation strategy	Sequential fermentation	Temporal separation of aroma formation and attenuation	Greater flexibility in balancing ethanol and sensory outcomes	Improved fermentation-derived complexity	[16,19,70]
Process stabilization	Controlled oxygenation and pitching strategy	Supports membrane integrity, yeast vitality, and metabolic stability	Greater fermentation reproducibility and robustness	Reduced risk of stress-associated off-flavors	[4,18,24,46]
Process monitoring	Monitoring of ethanol, extract, glycerol, and volatile acidity	Enables earlier identification of metabolic imbalance	Improved process consistency and quality control	Reduced sensory instability	[15,29,55]
Scale-up implementation	Standardization of fermentation conditions across batches	Reduces variability associated with oxygen transfer, mixing, and wort composition	Improved batch-to-batch reproducibility	More stable sensory profile	[4,29,59]

Reported outcomes should be interpreted as brewery-oriented tendencies influenced by strain physiology, wort composition, and fermentation management rather than universally predictive technological outcomes.

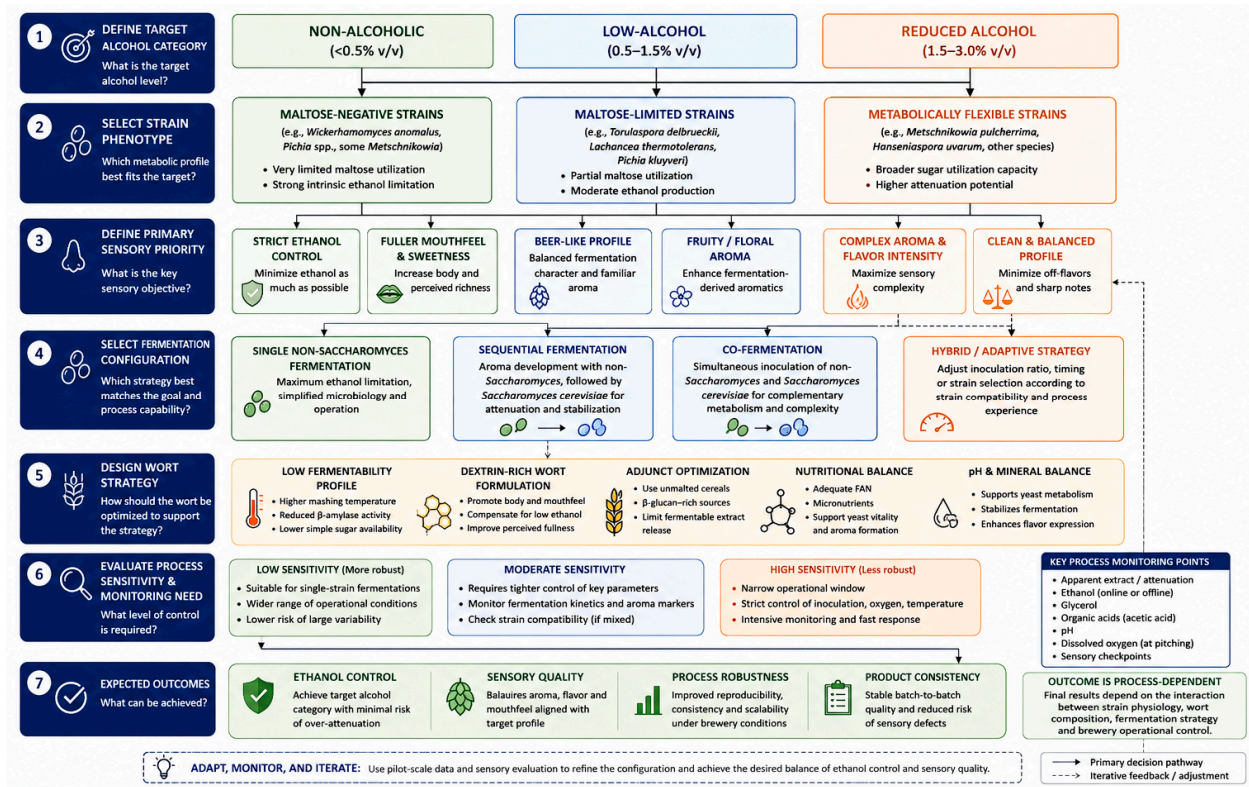


Figure 3. Decision-support framework for precision fermentation design of low- and non-alcoholic beer using non-*Saccharomyces* yeasts. The framework integrates target alcohol category, wort fermentability, strain phenotype, inoculation strategy, fermentation conditions, process monitoring, and sensory evaluation to support brewery-oriented optimization of ethanol control and product quality. Fermentation outcomes are conceptualized as process-dependent interactions between metabolic constraints and controllable operational variables rather than as strain-driven effects alone. The framework is intended as a conceptual decision-support tool based on currently available evidence and requires further experimental validation under brewery-relevant conditions.

7. Sensory Optimization and Product Design in Low- and Non-Alcoholic Beer

Low- and non-alcoholic beer production presents a distinctive sensory challenge because ethanol reduction fundamentally alters aroma perception, flavor intensity, mouthfeel, sweetness balance, and overall product complexity. In conventional beer, ethanol acts not only as an intoxicating component but also as an important sensory modulator affecting viscosity, volatile release, flavor persistence, and integration of aroma-active compounds. Consequently, reduced ethanol concentrations are frequently associated with beers perceived as thinner, less complex, or exhibiting more pronounced wort-like sensory characteristics relative to conventional products [1,2,14].

In brewing-relevant non-*Saccharomyces* systems, sensory properties emerge from coordinated interactions among strain-specific metabolism, wort composition, and fermentation process conditions rather than ethanol concentration alone [44]. Because these fermentations operate under metabolically constrained conditions, factors such as sugar accessibility, carbon redistribution, oxygen availability, and fermentation intensity may influence aroma formation, residual extract, mouthfeel, and sensory stability depending on strain physiology and process configuration. Representative brewing-relevant species, including *Torulaspora delbrueckii*, *Metschnikowia pulcherrima*, *Pichia kluyveri*, and *Saccharomycodes ludwigii*, have been associated with distinct sensory profiles ranging from fruity and floral

characteristics to fuller mouthfeel or residual wort-like notes under different fermentation conditions [10,12,44].

Consequently, sensory quality in low- and non-alcoholic beer systems should be interpreted as an integrated outcome of metabolic activity, strain functionality, and process control rather than as a secondary consequence of ethanol limitation alone [5,15]. Within the context of this review, a beer-like profile refers to sensory characteristics resembling conventional beer, including balanced fermentation-derived aroma, reduced wort-like character, appropriate bitterness perception, and acceptable integration of flavor, body, and overall drinkability. Fermentation-derived complexity refers to the contribution of yeast-generated metabolites, including esters, higher alcohols, thiols, terpenes, and related volatile compounds, to overall sensory perception. Sensory integration describes the extent to which aroma, flavor, mouthfeel, sweetness, bitterness, and ethanol perception form a coherent and balanced sensory experience.

7.1. Residual Extract and Mouthfeel Enhancement

One of the most characteristic sensory consequences of non-*Saccharomyces*-based fermentation is elevated residual extract resulting from incomplete utilization of maltose and maltotriose. Because many brewing-relevant strains exhibit restricted access to these carbohydrates, portions of wort sugars may remain partially or entirely unfermented, thereby contributing to increased viscosity, enhanced sweetness perception, and greater structural fullness of the final product depending on strain physiology and wort composition [1,9,11].

In low- and non-alcoholic beer systems, elevated residual extract may serve as an important compensatory mechanism partially replacing the sensory contribution of ethanol. Since ethanol substantially contributes to body, flavor persistence, and mouthfeel in conventional beer, dextrin retention and residual carbohydrate content may improve perceived fullness and reduce sensory thinness frequently associated with ethanol-reduced products [2,10]. In addition to residual carbohydrates, altered redox balance may further influence mouthfeel through changes in glycerol production. Selected brewing-relevant non-*Saccharomyces* strains have been associated with elevated glycerol formation relative to conventional brewing systems, potentially contributing to viscosity and structural perception depending on strain functionality and fermentation conditions [19,39,71].

However, elevated residual extract does not universally improve sensory quality. Excessive sweetness, incomplete attenuation, or disproportionate carbohydrate retention may negatively affect flavor balance, particularly in products lacking sufficient bitterness, carbonation, or fermentation-derived aroma complexity. Consequently, successful sensory optimization depends on balancing residual extract with aroma formation, bitterness perception, and overall flavor integration rather than maximizing sweetness or body independently.

7.2. Aroma Formation and Metabolic Trade-Offs

Aroma modulation represents one of the most extensively explored functional traits of brewing-relevant non-*Saccharomyces* yeasts because it contributes directly to sensory diversification and product differentiation in low- and non-alcoholic beer systems. Unlike ethanol reduction strategies relying on physical dealcoholization, biological fermentation using non-*Saccharomyces* yeasts enables simultaneous formation of aroma-active metabolites that may partially compensate for diminished ethanol-associated flavor intensity [10,12,43].

Depending on strain physiology and fermentation conditions, brewing-relevant non-*Saccharomyces* yeasts may exhibit enhanced formation of esters, higher alcohols, thiols, terpenes, and volatile compounds associated with fruity, floral, spicy, tropical, or citrus sensory characteristics. Species including *Cyberlindnera saturnus*, *Torulaspora*

delbrueckii, *Pichia kluyveri*, and selected strains of *Saccharomyces ludwigii* have been associated with distinctive aroma profiles and increased sensory complexity under selected fermentation conditions, although reported outcomes remain strongly strain- and process-dependent [16,25,52].

Nevertheless, aroma enhancement remains intrinsically linked to metabolic activity and therefore frequently involves trade-offs between sensory complexity and ethanol control. Conditions promoting greater fermentation activity, including elevated temperature, improved sugar accessibility, or intensified metabolic throughput, may simultaneously increase ethanol formation or alter the formation of undesirable by-products depending on strain phenotype and process configuration [18,21,41].

Consequently, aroma optimization should not be interpreted simply as maximizing volatile production but rather as balancing metabolic activity to achieve desirable sensory complexity while maintaining targeted alcohol concentrations, fermentation robustness, and product stability.

7.3. Ethanol–Aroma Interactions and Sensory Compensation Mechanisms

Ethanol reduction influences sensory quality not only through decreased alcohol concentration itself but also through altered interactions between volatile compounds and the beer matrix. In conventional beer systems, ethanol contributes to aroma retention, volatility modulation, flavor persistence, and integration of sensory attributes. Consequently, reduced ethanol concentrations may alter aroma perception even when measurable concentrations of aroma-active metabolites remain comparable [2,14].

Because ethanol affects both partitioning behavior and sensory perception of volatile compounds, low- and non-alcoholic beers may exhibit reduced aroma persistence, altered retronasal perception, or changes in flavor integration relative to conventional products depending on matrix composition and sensory profile. In some cases, fruity esters and volatile aroma compounds may become less effectively integrated into the sensory matrix, contributing to beers perceived as less complex or less balanced despite measurable volatile production [10,23].

In non-*Saccharomyces*-based fermentations, partial sensory compensation may arise through increased formation of aroma-active metabolites capable of offsetting reduced ethanol-mediated flavor delivery. Enhanced ester biosynthesis, terpene release, thiol liberation, or strain-specific volatile signatures may contribute to perceived aroma complexity depending on strain physiology, wort composition, and fermentation conditions [52,72,73].

However, successful sensory compensation depends on balanced sensory integration rather than maximization of volatile intensity alone. Excessive aroma formation without sufficient structural support from mouthfeel or residual body may generate imbalanced sensory profiles, whereas insufficient aroma compensation may contribute to products perceived as wort-like, thin, or sensorially incomplete. Consequently, ethanol reduction and aroma optimization should be regarded as interdependent rather than independent sensory objectives in low- and non-alcoholic beer design.

7.4. Off-Flavor Formation and Process Imbalance

Despite their technological potential, brewing-relevant non-*Saccharomyces* fermentations may present several sensory risks associated with metabolically constrained fermentation. One of the most persistent quality challenges in low- and non-alcoholic beer production involves incomplete reduction of wort-derived aldehydes, which may contribute cereal-like, green, wort-like, or insufficiently fermented sensory characteristics negatively affecting product acceptance [1,13,14].

Because many brewing-relevant non-*Saccharomyces* strains exhibit reduced fermentative intensity or shorter periods of active fermentation relative to conventional brewing yeasts, metabolic reduction of aldehydes may remain less complete depending on strain physiology and fermentation conditions. Consequently, compounds including methional, 3-methylbutanal, and trans-2-nonenal may contribute to wort-like sensory perception depending on fermentation duration, oxygen availability, wort composition, and process management [14,36,71].

Additional sensory risks may include volatile acidity, sulfur-associated off-notes, excessive sweetness, insufficient bitterness integration, and fermentation instability associated with prolonged lag phases or process imbalance. In mixed fermentation systems, microbial competition and altered metabolic interactions may further influence variability of sensory outcomes depending on inoculation strategy, fermentation timing, and strain compatibility [15,42,51].

Importantly, these sensory risks should not be interpreted solely as intrinsic strain limitations but rather as consequences of interactions between microbial physiology and process management. In many cases, optimization of fermentation temperature, oxygenation strategy, pitching rate, wort composition, and fermentation timing may contribute to improved sensory stability and reduced risk of undesirable sensory deviations depending on strain functionality and brewery conditions [69].

7.5. Sensory Design Through Process Control

Because sensory outcomes emerge from interactions between metabolism and fermentation conditions, process parameters represent important tools for targeted sensory modulation in low- and non-alcoholic beer production [17]. Variables such as wort fermentability, fermentation temperature, oxygen availability, pitching rate, and fermentation duration not only influence ethanol formation but may simultaneously affect aroma biosynthesis, residual extract, glycerol production, attenuation, and sensory balance [18,26].

For example, elevated fermentation temperatures may promote ester formation and aroma intensity while simultaneously influencing ethanol formation or fermentation variability depending on strain physiology and process conditions. Likewise, reduced wort fermentability may support ethanol limitation while also affecting body and sweetness perception through altered carbohydrate accessibility. Oxygen availability may further influence aroma outcomes through modulation of respiratory metabolism and redox balance, whereas pitching strategy may shape fermentation robustness and volatile formation dynamics [16,19,24].

Consequently, sensory optimization should not be approached through isolated modification of single variables but rather through coordinated adjustment of interacting process parameters according to strain-specific metabolic behavior [33]. In this context, fermentation design may be interpreted as a form of sensory engineering in which targeted product characteristics emerge from deliberate modulation of constrained metabolism [44].

To facilitate integration of the mechanistic relationships discussed throughout this review, Figure 4 presents a systems-oriented conceptual framework linking strain metabolism, process control, and sensory outcomes in low- and non-alcoholic beer production. The framework illustrates how strain-specific physiological constraints may interact with controllable process variables to influence carbon allocation, aroma formation, mouthfeel, ethanol concentration, and resulting sensory profiles. Rather than representing independent technological variables or universally predictive relationships, these factors collectively define a brewery-oriented sensory process space within which targeted product design may be achieved.

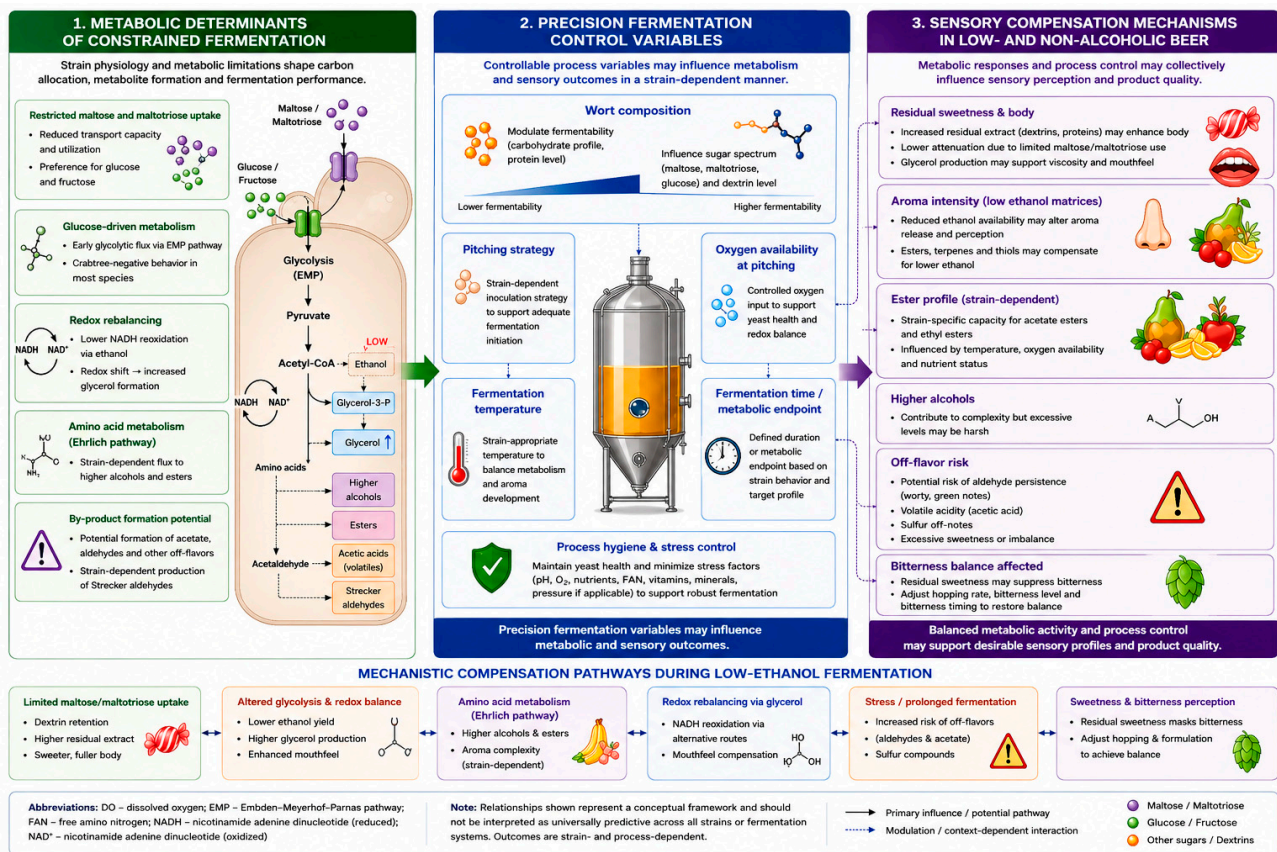


Figure 4. Conceptual framework linking metabolic determinants, controllable fermentation variables, and sensory compensation mechanisms during low- and non-alcoholic beer production using non-*Saccharomyces* yeasts. Restricted sugar utilization, altered glycolytic activity, redox balancing, and amino acid metabolism interact with fermentation variables, including wort composition, pitching strategy, oxygen availability, temperature, and fermentation duration, thereby influencing ethanol formation, aroma development, mouthfeel, residual sweetness, and sensory stability. Relationships shown represent a conceptual brewery-oriented framework and may vary depending on strain physiology, wort composition, and fermentation conditions.

7.6. Target-Oriented Sensory Profiles

Because consumer expectations differ substantially across low- and non-alcoholic beer categories, successful fermentation design increasingly depends on defining target-oriented sensory objectives rather than pursuing universal optimization strategies. Sensory preferences may vary according to intended beer style, alcohol category, consumer expectations, and desired balance among freshness, complexity, drinkability, sweetness, bitterness, and fermentation character [2,14].

For strictly non-alcoholic beer production, fermentation strategies frequently prioritize clean sensory profiles, moderate sweetness, controlled body, and suppression of wort-derived off-flavors while maintaining acceptable aroma intensity. In contrast, low-alcohol products may tolerate slightly greater ethanol formation in exchange for enhanced fermentation complexity, ester production, and more beer-like sensory characteristics depending on product objectives and brewery conditions [16,25].

Likewise, aroma-forward products targeting fruity or craft-oriented sensory profiles may benefit from metabolically flexible strains or mixed fermentation systems despite greater process complexity, whereas mainstream-oriented products may prioritize reproducibility, fermentation robustness, and sensory consistency over maximal aroma diversification [42,43].

From a precision fermentation perspective, successful low- and non-alcoholic beer production therefore depends on coordinated alignment of strain phenotype, process conditions, and sensory objectives into a coherent product design strategy rather than independent optimization of individual fermentation variables [70,74].

Because fermentation outcomes frequently involve trade-offs among ethanol limitation, aroma complexity, mouthfeel, process robustness, and sensory stability, direct comparison becomes important for brewery-oriented process selection. Table 4 summarizes representative fermentation design tendencies and sensory trade-offs associated with non-*Saccharomyces*-based low- and non-alcoholic beer production [44,59].

Table 4. Target-oriented fermentation design strategies for low- and non-alcoholic beer production using non-*Saccharomyces* yeasts. The table integrates target product objective, strain phenotype, wort design, and fermentation strategy to support brewery-oriented process selection balancing ethanol control and sensory quality. Reported approaches represent brewery-relevant tendencies and may vary depending on strain physiology, wort composition, and production objectives.

Target Product Objective	Representative Strain Phenotype	Recommended Wort Strategy	Recommended Fermentation Strategy	Typical Alcohol Category	Expected Sensory Profile	Operational Control Requirement	Key References
Strict non-alcoholic beer production	Maltose-negative strains (<i>Saccharomyces ludwigii</i> , selected <i>Pichia</i> spp.)	Reduced fermentability wort; dextrin-supportive mash profile	Primary fermentation using non- <i>Saccharomyces</i> yeast	Non-alcoholic beer	Mild fermentation character; fuller body; increased residual sweetness	Moderate	[20,36,59]
Aroma-enhanced non-alcoholic beer	Aroma-active strains (<i>Cyberlindnera saturnus</i> , <i>Pichia kluyveri</i>)	Moderate fermentability; aroma-supportive wort composition	Controlled oxygenation and aroma-oriented fermentation management	Non-alcoholic to low-alcohol beer	Fruity/floral aroma enhancement; greater sensory complexity	Greater	[10,12,41,49]
Balanced low-alcohol beer production	Maltose-limited strains (<i>Torulaspora delbrueckii</i> , <i>Wickerhamomyces anomalus</i>)	Moderate fermentability with controlled carbohydrate accessibility	Primary fermentation or controlled co-fermentation	Low-alcohol beer	Improved fermentation character and balanced aroma profile	Greater	[9,29,44,59]
Beer-like low-alcohol profile with improved attenuation	Metabolically flexible strains or sequential fermentation systems	Controlled fermentability combined with carbohydrate restriction	Sequential fermentation or controlled co-fermentation with <i>Saccharomyces cerevisiae</i>	Low-alcohol to reduced-alcohol beer	More beer-like flavor profile; lower residual sweetness	High	[16,59,60,70]

Fermentation outcomes should be interpreted as strain- and process-dependent tendencies rather than universally predictive outcomes. The strain phenotypes shown in Table 4 represent brewery-oriented design tendencies rather than mutually exclusive categories. Individual strains may simultaneously exhibit metabolic and sensory-functional characteristics associated with multiple categories.

8. Limitations and Future Perspectives

Despite substantial advances in the application of non-*Saccharomyces* yeasts for low- and non-alcoholic beer production, several scientific and technological limitations continue to constrain broader implementation and reproducibility across brewing systems. One of the principal challenges remains the pronounced strain dependency of fermentation performance, because even closely related strains may differ substantially in sugar utilization, fermentation kinetics, aroma formation, redox balance, stress tolerance, and resulting sensory outcomes [1,4,22].

An additional limitation concerns the considerable methodological heterogeneity among existing studies. Differences in wort composition, oxygenation strategy, fermen-

tation temperature, pitching rate, inoculation timing, fermentation duration, analytical methodologies, and sensory evaluation approaches frequently complicate direct comparison across studies and limit transferability of findings to brewery-relevant conditions [16,25]. Consequently, reported strain performance often reflects specific experimental configurations rather than universally transferable physiological behavior.

Furthermore, despite growing mechanistic understanding at laboratory scale, comparatively limited information remains available regarding pilot-scale reproducibility, industrial process robustness, and long-term sensory stability under commercial production conditions. Because fermentation performance depends strongly on interactions among strain physiology, wort matrix, and process management, scale-dependent variation may influence ethanol formation, aroma expression, and product consistency [51,73,75,76].

Future progress in low- and non-alcoholic beer production will likely depend on transitioning from descriptive strain screening toward more predictive and systems-oriented fermentation strategies integrating microbial physiology, process engineering, sensory science, and brewery-scale validation. Although many of the physiological relationships discussed throughout this review are supported by published evidence, the integrated precision fermentation framework proposed herein should be regarded as a conceptual brewery-oriented synthesis rather than an experimentally validated predictive model. Future studies combining strain characterization, process monitoring, sensory evaluation, and pilot-scale validation will be required to assess its predictive capability and practical applicability across different brewing scenarios. Greater emphasis on strain-level characterization, standardized experimental reporting, and pilot-scale validation may substantially improve reproducibility and industrial transferability of non-*Saccharomyces*-based fermentation systems.

In addition, emerging approaches integrating metabolomics, volatile profiling, real-time fermentation monitoring, and predictive process modeling may further support brewery-oriented precision fermentation frameworks capable of improving ethanol control, sensory consistency, and process robustness under industrial conditions [25,44].

8.1. Toward Predictive and Precision Fermentation Systems

Future development of low- and non-alcoholic beer production using non-*Saccharomyces* yeasts will likely increasingly depend on predictive and systems-oriented fermentation strategies rather than empirical optimization alone. Because fermentation outcomes emerge from coordinated interactions among strain physiology, substrate accessibility, and process conditions, improved mechanistic understanding may support more rational, reproducible, and brewery-relevant fermentation design [30,34,46].

In particular, integration of metabolic phenotyping, transcriptomics, metabolic flux analysis, fermentation kinetics, volatile compound profiling, and strain-specific physiological characterization may facilitate development of predictive fermentation frameworks capable of estimating ethanol formation and sensory tendencies under different process configurations. Beyond phenotypic characterization, transcriptomic and metabolic flux analyses may provide deeper mechanistic insight into regulatory pathways governing sugar utilization, carbon redistribution, redox balancing, and aroma formation in non-*Saccharomyces* fermentations. Such approaches may help identify strain-specific metabolic bottlenecks and improve prediction of fermentation outcomes under different brewing conditions [16,20].

Likewise, advances in metabolomics, real-time fermentation monitoring, inline analytical technologies, and data-assisted process modelling may increasingly support adaptive fermentation management during production, thereby improving process reproducibility and enabling more targeted sensory optimization [30].

Rather than replacing brewer expertise, such approaches may increasingly function as decision-support tools facilitating more precise control of constrained fermentation systems and supporting targeted product development under brewery-relevant conditions.

8.2. Digital Brewing and Adaptive Fermentation Control

Beyond predictive modelling, future brewery implementation may increasingly benefit from digital fermentation approaches integrating process monitoring with adaptive fermentation control. In this context, digital twins, process simulation, and real-time fermentation monitoring may support brewery-oriented estimation of how fermentation variables influence ethanol formation, aroma development, and sensory outcomes before undesirable deviations occur [30,55,59]. Artificial intelligence and machine-learning approaches may further enhance these systems by integrating multi-omics datasets, fermentation kinetics, and process variables into data-driven decision-support tools. Such approaches may support prediction of fermentation trajectories, early identification of process deviations, and optimization of fermentation conditions for targeted ethanol and sensory outcomes.

Such approaches may be particularly relevant in brewing-relevant non-*Saccharomyces* systems because relatively small changes in oxygen availability, fermentation temperature, wort fermentability, or pitching strategy may influence fermentation performance depending on strain physiology and process configuration. Consequently, adaptive fermentation systems capable of responding dynamically to metabolic changes may contribute to improved process robustness and reduced batch-to-batch variability during industrial production [22,64,69].

Rather than replacing brewer expertise, digital process tools may increasingly function as decision-support systems facilitating more precise management of metabolically constrained fermentation systems and supporting targeted product development under brewery-relevant conditions [62,70].

8.3. Future Research Priorities

Future research should increasingly prioritize strain-level characterization rather than broad species-level generalization, particularly regarding sugar transporter functionality, carbohydrate accessibility, redox regulation, aroma metabolism, and fermentation robustness under brewery-relevant conditions [7,8,49]. Greater attention should additionally be directed toward systematic comparison of strain-specific responses under standardized fermentation conditions to improve reproducibility and comparability across studies.

Further progress will also require closer integration between laboratory-scale experimentation and industrial validation, including systematic assessment of fermentation reproducibility, sensory stability, microbiological robustness, and process consistency across repeated production cycles [1,51].

In addition, broader integration of metabolomics, transcriptomics, metabolic flux analysis, volatile compound profiling, fermentation monitoring, and brewery-scale validation may support improved mechanistic understanding of how strain physiology interacts with controllable process variables during low- and non-alcoholic beer production. Combining these multi-omics approaches with predictive modelling may further facilitate development of more robust and transferable precision fermentation strategies for industrial brewing applications [30,44].

Ultimately, successful implementation of non-*Saccharomyces* yeasts in low- and non-alcoholic beer production will likely depend on transitioning from descriptive strain screening toward brewery-oriented precision fermentation frameworks integrating microbial physiology, process engineering, and sensory science into reproducible fermentation design [4,70].

9. Conclusions

Non-*Saccharomyces* yeasts represent a promising biological strategy for low- and non-alcoholic beer production because ethanol limitation emerges intrinsically from strain-dependent metabolic constraints rather than post-fermentation alcohol removal. However, successful implementation depends not solely on strain selection but on coordinated interactions among microbial physiology, wort composition, and fermentation management governing ethanol formation, aroma development, and sensory quality.

The evidence reviewed highlights that fermentation performance in brewing-relevant non-*Saccharomyces* systems is highly strain- and process-dependent, requiring deliberate alignment of strain phenotype with controllable variables such as wort fermentability, temperature, oxygen availability, pitching strategy, and fermentation duration. Consequently, successful low- and non-alcoholic beer production should be approached as precision management of constrained fermentation systems. Future progress will likely depend on brewery-oriented precision fermentation frameworks integrating physiology, process control, and sensory design to support more reproducible and targeted product development.

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Abbreviations

The following abbreviations are used in this manuscript:

NAB	Non-Alcoholic Beer
LAB	Low-Alcohol Beer
LNAB	Low- and Non-Alcoholic Beer
NSY	Non- <i>Saccharomyces</i> Yeasts
DO	Dissolved Oxygen
NAD ⁺	Nicotinamide Adenine Dinucleotide (oxidized form)
ATP	Adenosine Triphosphate
<i>v/v</i>	Volume per Volume
°C	Degrees Celsius
mg·L ^{−1}	Milligrams per Liter
MAL	Maltose Transporter Gene Family
AGT1	Alpha-Glucoside Transporter 1
MTT1	Maltotriose Transporter 1

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