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Molecular Responses of *Saccharomyces cerevisiae* to Growth Under Conditions of Increasing Corn Syrup and Decreasing Molasses

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Abstract

Molasses, a by-product of raw sugar production, is widely used as a cost-effective carbon and nutrient source for industrial fermentations, including the production of baker's yeast (*Saccharomyces cerevisiae*). Due to the cost and limited availability of molasses, efforts have been made to replace molasses with cheaper and more readily available substrates such as corn syrup. However, the quality of dry yeast drops following the replacement of molasses with corn syrup, despite the same amount of total sugar being provided. Our understanding of how molasses replacement affects yeast physiology, especially during the dehydration step, is limited. Here, we examined changes in gene expression of a strain of baker's yeast during fermentation with increasing corn syrup to molasses ratios at the transcriptomic level. Our findings revealed that the limited availability of the key metal ions copper, iron, and zinc, as well as sulfur from corn syrup (i) reduced their intracellular storage, (ii) impaired the synthesis of unsaturated fatty acids and ergosterol, as evidenced by the decreasing proportions of these important membrane components with higher proportions of corn syrup, and (iii) inactivated oxidative stress response enzymes. Taken together, the molecular and metabolic changes observed suggest a potential reduction in nutrient reserves for fermentation and a possible compromise in cell viability during the drying process, which may ultimately impact the quality of the final dry yeast product. These findings emphasize the importance of precise nutrient supplementation when substituting molasses with cheaper substrates.

Keywords: molasses replacement; corn syrup; yeast; dehydration; metal ions; fatty acids; ergosterol; oxidative stress

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1. Introduction

The manufacturing process of baker's yeast (*Saccharomyces cerevisiae*) from inoculant to dry yeast involves two key stages: liquid fermentation and dehydration. Molasses, an industrial by-product of the raw sugar production process, makes a cost-effective culture medium for industrial-scale liquid fermentations because it contains high amounts of fermentable sugars, nitrogen compounds, metal ions, and vitamins [1,2]. According to the Republic of Turkey Ministry of Agriculture and Forestry, approximately three quarters (76%) of the world's molasses come from sugarcane grown in tropical climates of Asia

and South America, while the remaining quarter (24%) comes from sugar beet grown in the more temperate climates of Europe and North America. Depending on the cost and availability of molasses, it may be desirable to partially or fully replace molasses with other types of sugars, such as corn syrup. Corn syrup is made from the hydrolysis of corn starch. It contains a high amount of fermentable sugars; however, the availability of other nutrients from corn syrup is limited [3]. While the composition of molasses varies greatly from batch to batch, requiring frequent monitoring and adjustment of the growth media, corn syrup shows stable compositional characteristics [4].

The dehydration process of baker's yeast consists of centrifugation and washing, followed by filtration and drying [5]. During this process, the yeast undergoes significant osmotic and oxidative stress [6,7], which may affect the performance of the dried product, as evaluated by the measurement of gassing power during baking. Interestingly, such a decrease in the quality of the dried yeast product has been observed upon partial and complete substitution of molasses with corn syrup [8,9]. This suggests that the lack of nutrients in corn syrup affects stress resilience, as well as the viability or physiological condition of certain yeast strains after drying. Indeed, it has been hypothesized that strains that are fermented under sub-optimal conditions (e.g., missing vitamins or trace elements) may exhibit a suboptimal response to the drying stress (e.g., inadequate production of important osmo-protectants, impaired membrane functioning) and thus may be less well protected during the harsh drying process [10,11]. This, in turn, may have consequences for yeast performance during baking. The underlying molecular events resulting in decreased performance with increased corn syrup to molasses ratio remain to be elucidated.

Here, we used a combination of transcriptomics and analytical methods to decipher the molecular responses of a bakery strain grown under five different corn syrup to molasses ratios while providing a constant level of total fermentable sugars. Our results provide new insights into the nutrient requirements of baker's yeast for optimal resilience to stress and revival after drying and offer specific starting points for precision supplementation of micronutrients during the production of baker's yeast.

2. Materials and Methods

2.1. Strains and Growth

A commercial strain of *Saccharomyces cerevisiae*, which is routinely produced for bakery applications, was grown for investigation of changes in the yeast transcriptome. Yeast cream, derived from the penultimate step of industrial production, was used as an inoculum. The cream was inoculated into the growth media at 17 g dry weight/L for all fermentations.

2.2. Fermentation at Different Corn Syrup Inclusion Levels

A total of five fermentations were set up. Corn syrup (CS) inclusion levels ranged from 0 to 100% of total fermentable sugars in 25% increments, i.e., 0% CS, 25% CS, 50% CS, 75% CS, and 100% CS. Beet molasses was added up to 100% to keep the amount of total fermentable sugars consistent for all five fermentations. Fermentable sugar here was defined as the amount of sugar that can be metabolized by yeast during fermentation. Its concentration was determined by measuring the amount of carbon dioxide produced during yeast fermentation, which serves as an indirect measure of sugar utilization. Other than corn syrup and beet molasses, ammonia, phosphoric acid, ammonium sulphate, minerals (i.e., potassium, sodium, magnesium, zinc, copper), and B-group vitamins were added at constant concentrations across all five fermentations. A single biological replicate was conducted for each level of CS inclusion. Linear regression analysis was subsequently applied across five distinct CS inclusion levels to assess the relationship between CS level and gene expression pattern.

Nowadays, baker's yeast is typically propagated in yeast production plants using multistage fermentations, ending with fed-batch steps [12]. To enhance the industrial relevance of our findings, fed-batch fermentation was employed in this study. The fermentation process was conducted in a 30 L fermenter using a fed-batch approach, with culture volumes starting at 9 L and increasing to 16 L. The fermentation temperature was kept at 30 °C throughout. The fermentation process was stopped at 20 h. Throughout the fermentation, agitation, aeration, and pH were tightly regulated by controlling stirring speed, airflow rate, and H_2SO_4 addition. A dissolved oxygen cascade was implemented to control oxygen levels. All settings were consistently applied across all five fermentations.

The 4 h time point corresponded to the mid-exponential growth phase of the strain, as determined by OD600 measurements. During this growth phase, gene expression is at its peak and reflects the yeast's immediate physiological responses to the fermentation environment. Therefore, at time point 4 h, 100 mL of yeast culture was collected from each fermentation and centrifuged at 1957× *g*. The supernatant was discarded and the pellet snap-frozen in liquid nitrogen and stored at −80 °C before RNA extraction. The 20 h time point was chosen for fatty acid and sterol composition analysis as it corresponds to the end of the fermentation process, providing a snapshot of yeast health and membrane composition just prior to downstream processing, including drying. Therefore, at time point 20 h, 100 mL of yeast culture was collected for measurement of cellular fatty acid and sterol composition. The cell pellets were obtained as described above, stored at −80 °C, freeze-dried, and ground prior to metabolite extraction.

2.3. RNA Sequencing and Data Analysis

Total RNA was extracted from the cell pellet using the RNAprep Pure kit (Tiangen Biotech, Beijing, China). The RNA library was prepared with the NEBNext® Ultra™ RNA Library Prep Kit for Illumina® (New England Biolabs, Ipswich, MA, USA), and paired-end sequencing was performed on the Illumina NovaSeq 6000 system (2 × 150 bp; Novogene, Beijing, China). In total, ~6 Gbp of raw data were generated for each sample. After removal of the low-quality reads and adaptors by fastp (version 0.23.2) [13], RNA-seq reads were aligned to the *Saccharomyces cerevisiae* S288C reference genome (Accession: GCA_000146045.2) using STAR (version 2.7.11a) [14]. Between 1.8×10^7 and 2.0×10^7 reads were uniquely aligned for each sample. Subsequently, mapped reads were sorted by Samtools (version 1.17) [15] and quantified by featureCounts (version 2.0.6) [16]. The resulting counts matrix was converted into a DESeq dataset and normalized for differential expression analysis using DESeq2 (version 1.42.0) [17]. The percentage of corn syrup inclusion was treated as a continuous variable. A linear regression model was used to analyze the effect of the gradually increased corn syrup. Adjusted *p*-values were calculated using the Benjamini–Hochberg FDR method. Differentially expressed genes (DEGs) were called if the following two criteria were met: (i) log2 fold change (LFC) > 1 or <−1, and (ii) adjusted $p < 0.05$. DEGs were selected for further analysis.

Sequencing data were deposited in the SRA database under BioProject identifier PRJNA1291913.

2.4. Functional Analysis of DEGs

The list of DEGs was uploaded into the Database for Annotation, Visualization, and Integrated Discovery (DAVID) platform (http://david.ncifcrf.gov (accessed on 1 March 2025)) to search for enriched gene sets in both up and downregulated genes [18]. The Functional Annotation Tool in the online version of DAVID was used with default settings, and the top twenty Gene Ontology (GO) terms from the Biological Process category were reported. Additionally, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway

analysis (https://www.genome.jp/kegg/ (accessed on 11 April 2025)) was conducted using the same gene list to identify significantly enriched pathways [19]. ShinyGO 0.77 (https://bioinformatics.sdstate.edu/go/ (accessed on 11 April 2025)) was used to plot the chart and network of KEGG enriched pathways [20].

2.5. Extraction and Measurement of Cellular Fatty Acid and Sterol Composition

Extraction and derivatization of cellular lipids were carried out according to Williams et al. [21], with some modifications. Briefly, a total of 50 mg of freeze-dried yeast powder was saponified with 2 mL of 1.3 M KOH in a methanol/H_2O (9:1) solution at 95 °C for 30 min. The mixture was neutralized by adding 0.5 mL of 6 M HCl. Lipid extraction was initiated by adding 2 mL of a chloroform/methanol (1:1) mixture, followed by the addition of 1 mL of saturated brine and 1 mL of water. The mixture was vortexed and centrifuged at 900× *g* for 10 min. The lower organic layer was collected. To remove excess water, anhydrous Na_2SO_4 was added until the solution reached saturation, resulting in observable salt precipitation. A total of 20 µL of sample was derivatized with 180 µL of N,O-bis (trimethylsilyl) trifluoroacetamide (BSTFA) with 1% trimethylchlorosilane (TMCS) (Sigma-Aldrich). The derivatization mixture was incubated at 95 °C for 1 h before analysis by GC-MS. GC-MS analysis was carried out using an Agilent 8890 gas chromatograph equipped with a 5977B single quadrupole mass detector. Chromatographic separation was performed on an Agilent HP-5MS column (30 m × 250 µm × 0.25 µm). Fatty acids and sterols were identified through mass spectral matching against the National Institute of Standards and Technology (NIST) library, using a matching score threshold of >95% for compound confirmation. Relative proportions of the identified fatty acids or sterols from the total were subsequently calculated.

3. Results

3.1. Gene Expression in Yeast Fermentations

Analysis of gene expression trends across a broad gradient of corn syrup (CS) concentrations can provide insight into the underlying molecular mechanisms of the impact of CS inclusion on yeast physiology and stress resilience. Therefore, we analyzed the gene expression patterns of a strain of baker's yeast grown at five different corn syrup concentrations—0%, 25%, 50%, 75%, and 100%—while maintaining a consistent total amount of fermentable sugars across all fermentations through the addition of molasses.

Analysis of the RNA-seq data revealed the expression profiles of a total of 6425 protein-coding genes. Differentially expressed genes (DEGs) were identified through linear regression analysis, which assessed the relationship between increasing syrup concentrations and mRNA levels. A total of 213 genes exhibited a slope coefficient greater than 1 (significantly upregulated) or less than −1 (significantly downregulated), signifying at least a two-fold change in expression between 0% and 100% corn syrup inclusion (Figure 1A). Among these 213 genes, 92 were upregulated while 121 were downregulated as the proportion of corn syrup increased (Figure 1B, Table S1).

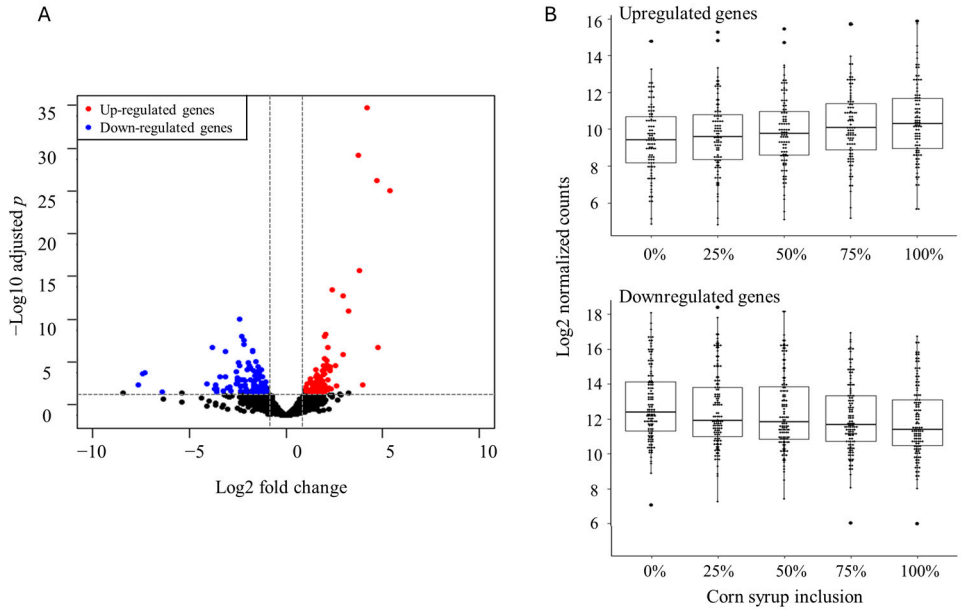

Figure 1. RNA-seq analysis of yeast grown with increased corn syrup inclusion. (**A**) Volcano plot of DEGs. Genes with log2 (fold change) > 1 or <−1 (vertical lines) and adjusted $p < 0.05$ (horizontal line) were colored. Red points: upregulated genes; Blue points: downregulated genes. Black points: genes with no significant differential expression. (**B**) The 92 upregulated and 121 downregulated genes identified upon increasing the syrup inclusion.

3.2. Functional Enrichment Analysis

To gain a deeper understanding of the transcriptomic response of yeast cells to the varying media, we employed Gene Ontology (GO) classification to assess the enrichment of functionally related gene groups. Enriched GO terms were derived from identified DEGs. Through a comprehensive analysis of the GO terms and the functional characteristics of the DEGs, we identified the top twenty most enriched GO terms for both upregulated and downregulated genes, ranked by enrichment score [−log10 (*p*)].

GO enrichment analysis revealed that processes associated with the uptake of iron, copper, and zinc were most significantly enriched among the upregulated genes in response to increased corn syrup (Figure 2A).

GO enrichment analysis identified significant downregulation of genes involved in cellular respiration, particularly those associated with the tricarboxylic acid (TCA) cycle and the electron transport chain (ETC) (Figure 2B). Furthermore, genes critical for the biosynthesis of iron cofactors, including heme and iron–sulfur cluster, as well as those involved in oxidative stress responses, were also significantly downregulated (Figure 2B).

To further investigate the regulatory pathways, KEGG pathway analysis was conducted for the upregulated (Table 1) and downregulated genes (Figure 3). For upregulated genes, no significantly enriched pathways were identified at a false discovery rate (FDR) threshold of 0.01, which is consistent with the observation that genes involved in metal ion uptake are individual genes not included within the KEGG pathway database. To obtain additional insights, a less stringent criterion was applied by relaxing the *p*-value threshold to 0.3, and pathways were ranked based on fold enrichment. This analysis revealed that sterol biosynthesis, nucleotide metabolism, and cysteine and methionine metabolism were the top three pathways, each exhibiting a fold enrichment greater than five (Table 1).

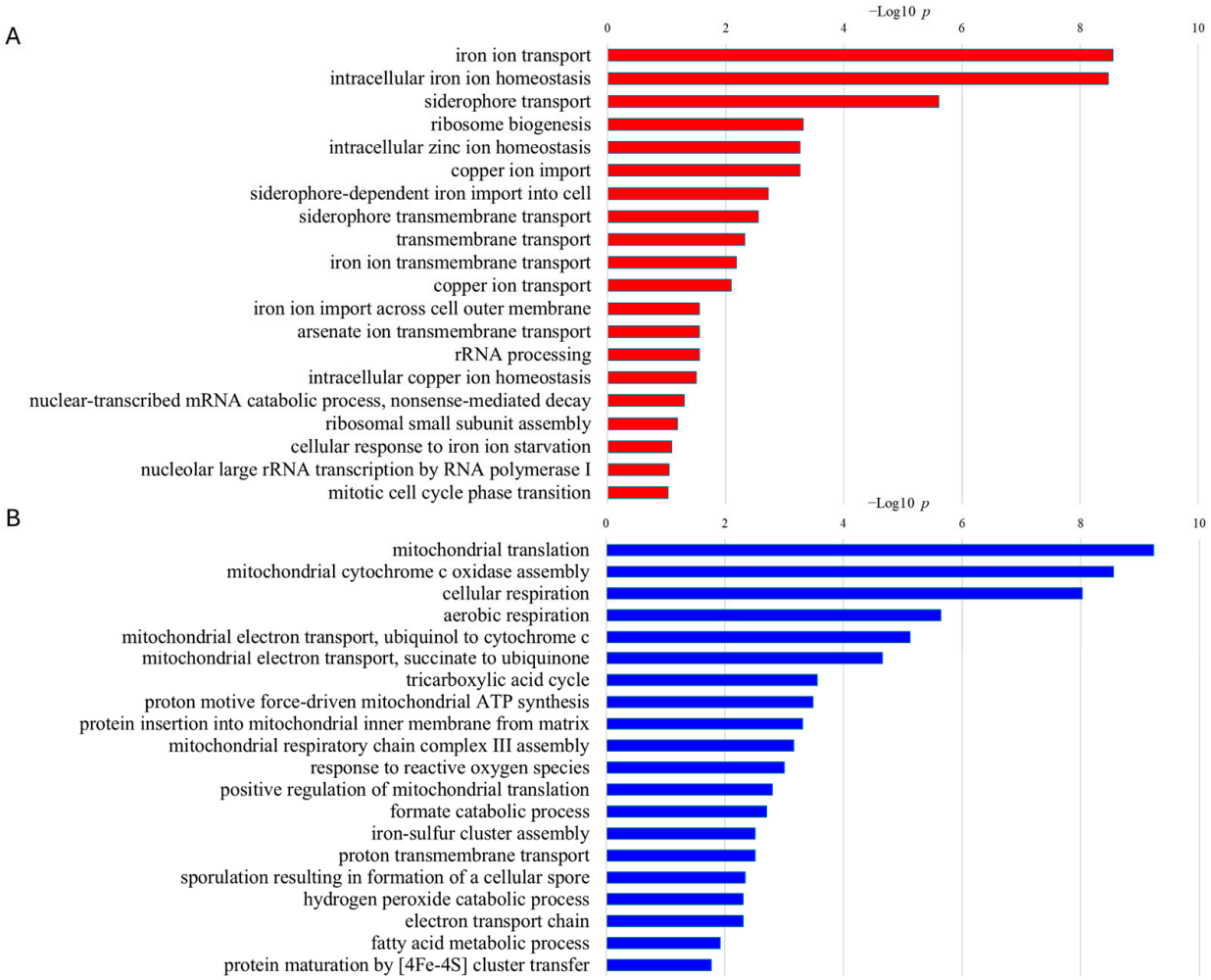

Figure 2. Bar chart of the twenty most significant GO biological processes that are associated with either upregulated genes (**A**) or downregulated genes (**B**). *X*-axis represents the statistical significance of the enrichment (−log10 (*p*)).

Table 1. KEGG pathway enrichment analysis of upregulated genes.

Pathway	Fold Enrichment	*p*-Value
Steroid biosynthesis	8.6	0.20
Nucleotide metabolism	5.5	0.10
Cysteine and methionine metabolism	5.4	0.10
Ribosome	2.5	0.08
Biosynthesis of amino acids	2.5	0.20
Cell cycle—yeast	2.4	0.22
Metabolic pathways	1.4	0.16

For downregulated genes, KEGG pathway analysis identified several significantly enriched pathways (FDR < 0.01; Figure 3A). Notable pathway terms included oxidative phosphorylation, carbon metabolism, peroxisome, TCA cycle, glyoxylate and dicarboxylate metabolism, and metabolic pathways. Both oxidative phosphorylation and the TCA cycle are involved in cellular respiration, while the general term peroxisome pathway, in this case, contains enzymes involved in oxidative stress responses. Enzymes involved in the term metabolic pathways here cover a wide range, with a primary focus on those involved in cellular respiration. The glyoxylate and dicarboxylate metabolism and carbon metabolism primarily include enzymes that either produce precursors for the TCA cycle or are directly

involved in the TCA cycle itself (Figure 3B). These findings are consistent with the results of the GO enrichment analysis, further supporting the observed functional patterns.

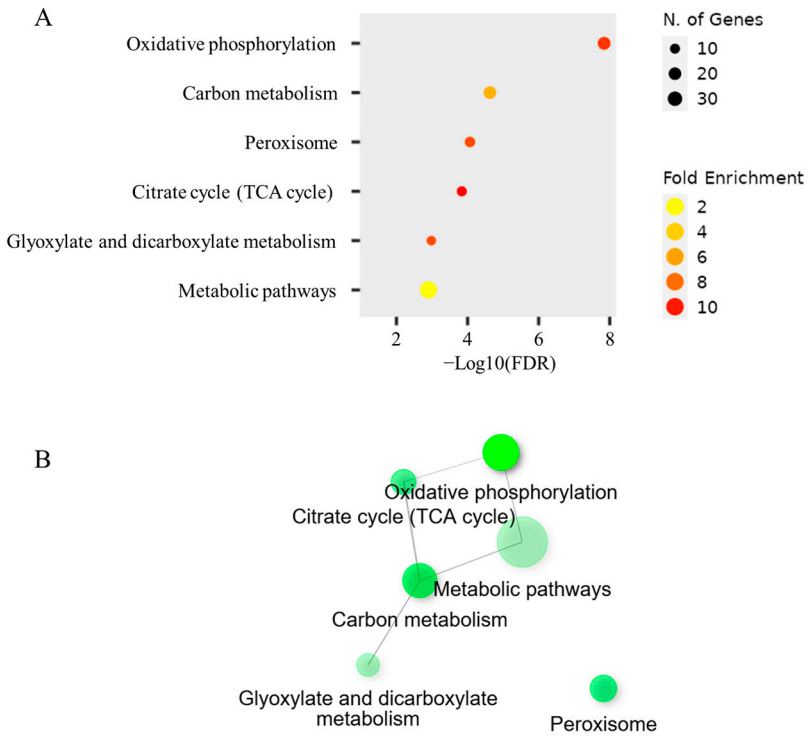

Figure 3. KEGG pathway enrichment analysis of downregulated genes. (**A**) Significantly enriched pathways (FDR < 0.01) in the KEGG pathway analysis. Larger nodes represent a higher number of genes detected in the pathway, and the different colors represent different fold enrichment. (**B**) Network of significantly enriched pathways (FDR < 0.01). Each node represents a term, and edges indicate functional similarity based on shared genes. Two pathways (nodes) are connected if they share 20% or more genes. Node size reflects gene counts, and darker nodes are more significantly enriched gene sets.

3.3. Expression of Genes Involved in Respiration

The GO enrichment analysis showed a noticeable downregulation of respiration, including both the TCA cycle and the ETC, in samples with increased corn syrup inclusion.

The genes associated with the TCA cycle and ETC are shown in Figure 4A,B. Three genes involved in the TCA cycle and sixteen genes in the ETC were found to be downregulated with increased levels of corn syrup. The downregulated genes are mainly those that require heme (e.g., *CYT1*, *SDH3*), iron–sulfur clusters (e.g., *RIP1*, *ACO1*), or copper (e.g., *SCO1*) as cofactors, suggesting that the lack of these cofactors may be responsible for the observed downregulation.

3.4. Expression of Genes Involved in Oxidative Stress Response

The GO enrichment analysis identified two Gene Ontology (GO) terms: response to reactive oxygen species [GO:0000302] and hydrogen peroxide catabolic process [GO:0042744], both of which are associated with the oxidative stress response and are downregulated with increased corn syrup inclusion. A common set of genes, including cytochrome c peroxidase (*CCP1*), catalase T (*CTT1*), and catalase A (*CTA1*), were enriched in both terms, emphasizing their role in oxidative stress mitigation.

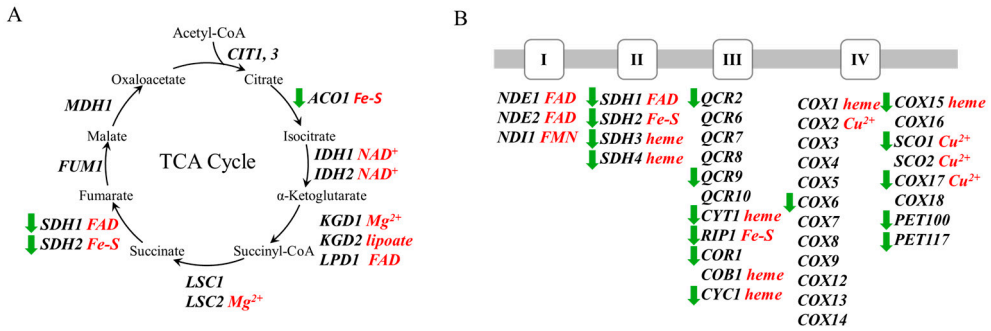

Figure 4. Expression response of cellular respiration to corn syrup inclusion. Schematic representation of cellular respiration, including the TCA cycle (**A**) and the electron transport chain (**B**) [22]. Solid arrows indicate known enzymatic steps. Cofactors required for each enzymatic step are highlighted in red. Upregulated and downregulated enzymes are marked with green upward and downward arrows, respectively. Subunits of ETC complexes, along with proteins required for their assembly, are shown beneath their respective complexes.

Both mitochondrial respiration and peroxisomal β-oxidation contribute to the generation of superoxide anion and hydrogen peroxide H_2O_2. Superoxide anions are first converted into H_2O_2 before being further degraded. The breakdown of H_2O_2 into water is facilitated by mitochondrial cytochrome c peroxidase (CCP1), cytosolic catalase T (CTT1), and peroxisomal catalase A (CTA1) [23] (Figure 5).

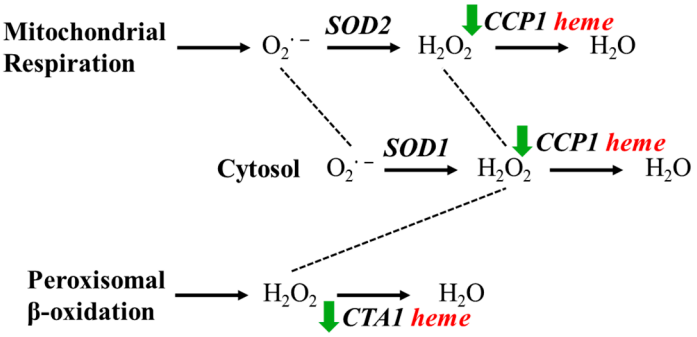

Figure 5. Oxidative stress response to corn syrup inclusion. Oxidative stress response, including both superoxide anion and H_2O_2 degradation. Solid arrows indicate known enzymatic steps, and dashed lines denote chemical transport between organelles. Cofactors required for each enzymatic step are highlighted in red. Upregulated and downregulated enzymes are marked with green upward and downward arrows, respectively.

3.5. Expression of Genes Involved in Synthesis of Membrane Lipids

The transcriptomic analysis revealed an 8.6-fold enrichment in steroid biosynthesis with increased syrup inclusion (Table 1). The specific enzymes identified in steroid biosynthesis here are specifically involved in sterol biosynthesis, a subset of the broader steroid biosynthesis (Figure 6A). The biosynthesis of ergosterol can be divided into three modules: mevalonate, farnesyl pyrophosphate, and ergosterol biosynthesis [24]. The latter pathway includes enzymes that rely on iron as an essential redox cofactor, such as the ERG5 and ERG11, which utilize heme, and ERG3 and ERG25, which employ oxo-diiron as a cofactor [25]. In this study, *ERG3* and *ERG11* were significantly upregulated (Figure 6A).

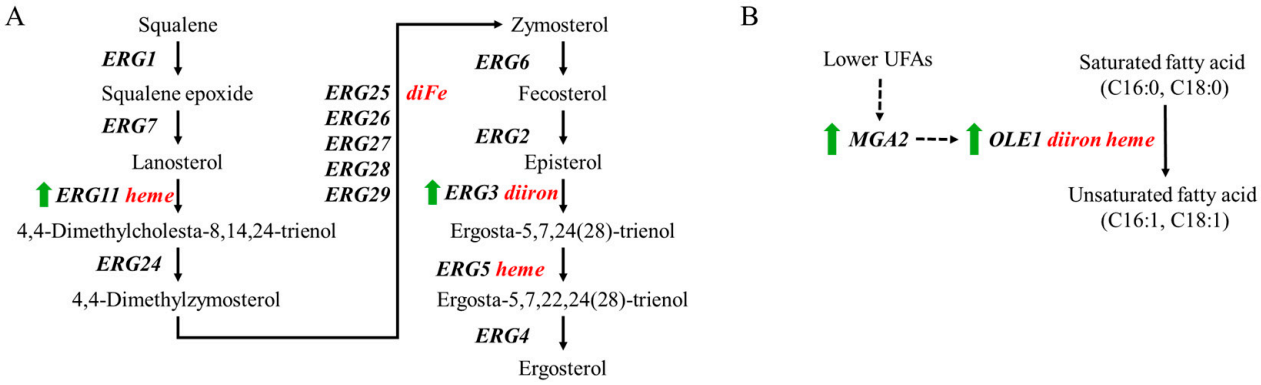

Figure 6. Expression response of membrane lipid biosynthesis pathways to corn syrup inclusion. Schematic representation of metabolic pathways involved in membrane lipid synthesis, highlighting the ergosterol biosynthesis pathway (**A**) and the unsaturated fatty acid biosynthesis pathway (**B**) [22]. Solid arrows indicate known enzymatic steps, while dashed arrows represent expression regulation. Cofactors required for each enzymatic step are highlighted in red. Upregulated and downregulated enzymes are marked with green upward and downward arrows, respectively.

Although unsaturated fatty acids (UFAs) were not significantly enriched in the GO and KEGG pathway enrichment analyses, they play a vital role—similar to ergosterol—in maintaining the physicochemical integrity of the plasma membrane [21]. Therefore, we also investigated the regulation of UFA biosynthesis. A comparable analytical approach was applied to the UFA biosynthetic pathway as was used for the sterol pathway. The synthesis of UFAs is mediated by an iron-dependent enzyme, OLE1. Furthermore, the expression of *OLE1* and *MGA2*, two genes involved in the biosynthesis of unsaturated fatty acids, was significantly upregulated with increasing levels of syrup inclusion in the media (Figure 6B).

3.6. Sterol and Fatty Acids Profiling of Dried Yeast Grown at Different Corn Syrup Inclusion Levels

To verify the finding of the transcriptomics analysis that corn syrup inclusion affects the expression of genes related to the biosynthesis of membrane lipids, we analyzed the sterol and fatty acid composition of cells harvested from media with different levels of corn syrup inclusion (Figure 7).

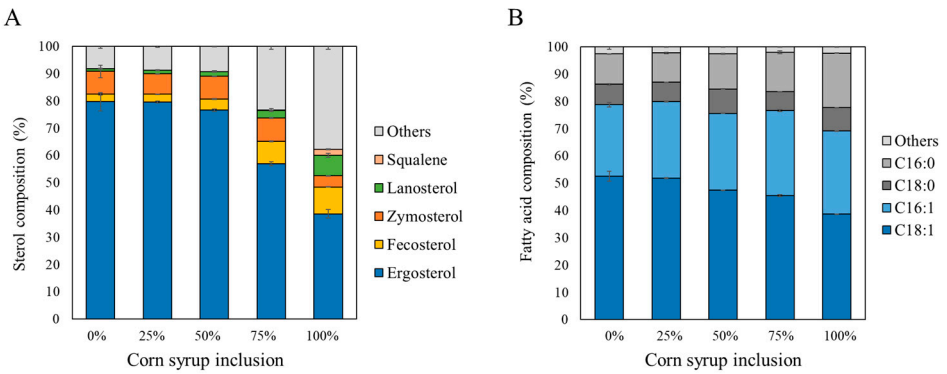

Figure 7. Impact of corn syrup inclusion on ergosterol and unsaturated fatty acid biosynthesis. Relative abundances of (**A**) sterols and (**B**) cellular fatty acids in yeast cultured at varying levels of syrup inclusion. Sterol profiles include several highly similar intermediates that could not be conclusively identified using the current MS database; these intermediates are collectively labelled as "Others" in (**A**). Fatty acid components contributing less than 5% in all samples are grouped as "Others" in (**B**).

The relative abundance of sterols in yeast was quantified and analyzed. Among the detected sterols, ergosterol was the most abundant, comprising 38–80% of the total sterol content (Figure 7A). Higher levels of corn syrup inclusion (>25%) were associated with a significant reduction in ergosterol abundance ($p < 0.05$), accompanied by larger proportions of sterol pathway intermediates, particularly fecosterol and lanosterol (Figure 7A).

Similarly, the relative abundance of fatty acids was assessed across different corn syrup inclusion levels. Palmitic acid (C16:0) and stearic acid (C18:0) were the predominant saturated fatty acids (SFAs), while palmitoleic acid (C16:1) and oleic acid (C18:1) were the major unsaturated fatty acids (UFAs). Compared to SFAs (C16:0 and C18:0), UFAs (C16:1 and C18:1) were more abundant, accounting for 69–80% of the total fatty acid content. A significant reduction in UFA relative abundance was observed for fermentations with corn syrup inclusion levels of >25% compared to those with 0% and 25% ($p < 0.05$). Notably, oleic acid (C18:1) showed a gradual and significant decline ($p < 0.05$) when corn syrup inclusion exceeded 25% compared to 0% corn syrup inclusion (Figure 7B).

4. Discussion

Strains of *Saccharomyces cerevisiae* show sensitivity to the replacement of molasses with corn syrup during fermentation for the production of dried yeast. This sensitivity is attributed to the reduced nutrient content in corn syrup and often manifests in decreased yeast quality, i.e., gassing power [8,9]. Here, we monitored the molecular responses of a strain of baker's yeast to a gradient of increasing levels of corn syrup through RNA sequencing and showed that several factors contribute to the deleterious effects of corn syrup inclusion on yeast fermentation and dry yeast quality.

In our study, strikingly, genes associated with the uptake of iron (e.g., *ARN1*, *FTR1*, *SIT1*), copper (e.g., *CCC2*, *CTR2*), and zinc (e.g., *IZH1*, *IZH2*, *COT1*) were significantly upregulated in response to the increase in corn syrup. It appeared that the lower levels of iron, copper, and zinc in corn syrup compared to molasses led to reduced intracellular levels, thereby activating genes responsible for the uptake of these nutrients. Additionally, the upregulation of genes related to the uptake and synthesis of sulfur-containing amino acids, such as cysteine and methionine (e.g., *MUP1*, *MET2*, *MET6*), suggests a potential sulfur deficiency in the media when higher corn syrup inclusion levels were applied. Although gene expression patterns suggest ion limitation, we did not directly quantify intracellular concentrations in this study, preventing definitive confirmation of this hypothesis. Future studies could include quantification of intracellular levels of these ions to validate the findings.

Iron serves as an essential cofactor (in the form of heme, iron–sulfur, or diiron) for enzymes engaged in various cellular processes [26], and its deficiency prompts yeast cells to prioritize iron utilization in vital pathways. Copper and zinc also function as cofactors for cellular enzymes [27], while sulfur is an essential element for various biological processes, including the synthesis of iron–sulfur clusters and sulfur-containing amino acids [28,29]. As a result of the reduced intracellular levels of iron and sulfur, the yeast strain appeared to suppress the synthesis of iron cofactors, including heme (e.g., *COX15*, *YAH1*) and iron–sulfur clusters (e.g., *YFH1*, *GRX5*, *IBA57*, *BOL3*) observed from our transcriptomic data.

4.1. Decrease in Respiratory Activity

Cellular respiration, encompassing both the TCA cycle and the ETC, is characterized by high consumption of iron, copper, and sulfur [30–32]. This is attributed to the fact that many of the enzymes involved in respiration require specific cofactors such as heme (e.g., CYT1, SDH3), copper (e.g., SCO1), and iron–sulfur clusters (e.g., RIP1, ACO1). Since this non-essential pathway can be substituted with anaerobic fermentation for energy

production, it seems logical that downregulation of respiration-related genes was observed upon an increase in corn syrup. This is consistent with other studies that showed that cellular respiration was compromised under iron-limiting conditions, as evidenced by the changes in gene expression [33–35].

During yeast production, respiration is preferred over anaerobic fermentation, as the pathway is metabolically more efficient and leads to both higher energy generation and biomass production [12,36]. Moreover, the respiratory capacity serves as a critical indicator of yeast quality, as it directly influences carbon dioxide production, which enhances the gassing power of the final yeast product, both wet and dry [37]. Studies have demonstrated that nutrient supplementation can enhance yeast respiration, thereby improving overall yeast quality [38]. In line with these findings, our study demonstrates that respiration is downregulated because of suboptimal fermentation when molasses is replaced with increasing proportions of corn syrup.

4.2. Decrease in Oxidative Stress Tolerance

Oxidative stress defense mechanisms are essential for defense against harmful reactive oxygen byproducts. All of the downregulated enzymes (CCP1, CTT1, CTA1) involving oxidative stress responses require heme as a cofactor [39]. The increased expression of these antioxidant enzymes is often associated with cellular protection against oxidative stress. In this study, the limitation of iron and sulfur ions resulted in the downregulation of antioxidant enzymes that would otherwise mitigate oxidative damage within cells. It is not clear from the results whether downregulation of genes involved in antioxidative stress response is the cause or effect of the downregulation of respiration.

4.3. Decrease in Osmotic Stress Tolerance

Ergosterol is the most abundant sterol in yeast [40]. It is a critical component of the yeast cell membrane, affecting membrane fluidity, permeability, and the cell's ability to adapt to changing environments due to temperature or pressure, as well as to mechanical, osmotic, or oxidative stress [20]. Interestingly, a previous study demonstrated that supplementation of fermentation media with ergosterol can improve cell viability during and after dehydration [41]. Like ergosterol, disruptions in UFA synthesis can dramatically alter membrane fluidity and morphology, potentially leading to cell death under severe stress conditions, e.g., drying [42,43].

Increased inclusion of corn syrup exacerbated iron deficiency, consequently restricting iron availability for various iron-dependent processes, including ergosterol and UFA biosynthesis. This reduction in ergosterol and UFA levels likely triggered compensatory mechanisms to restore ergosterol and UFA synthesis, as indicated by the upregulation of *ERG3*, *ERG11*, *MGA2*, and *OLE1*. However, at syrup inclusion levels exceeding 25%, severe iron starvation prevented adequate restoration of both ergosterol and UFA levels, as evidenced by the analytical data. This hypothesis aligns with previous studies on the response of ergosterol and UFA biosynthesis to iron deficiency [33,44]. Other than iron, zinc has also been shown to enhance the biosynthesis of ergosterol and trehalose [45]. Therefore, a lack of zinc may also contribute to the reduced synthesis and observed lower proportions of ergosterol in the cell membranes. While iron limitation may contribute to the reduction in ergosterol and UFA biosynthesis, other factors such as altered energy metabolism or stress-induced membrane remodeling may also play a role in modulating membrane lipid composition [46–48]. The reduced ergosterol and UFAs content in yeast grown in syrup inclusion media may contribute to the decreased survival rate during dehydration.

5. Conclusions

In summary, deficiencies in iron, copper, zinc, and sulfur in the fermentation media with high levels of corn syrup inclusion (>25%) led to decreased intracellular iron storage, which impaired cellular respiration. Furthermore, the iron deficiency compromised yeast viability during dehydration, the final step in manufacturing, during which the cells are subjected to considerable oxidative and osmotic stress. This appeared to result from two distinct mechanisms. Firstly, the lack of iron and sulfur hindered the activity of stress response enzymes, and secondly, the lack of iron impaired the synthesis of ergosterol and unsaturated fatty acids and their incorporation into the cell membranes. Taken together, these effects lead to higher rates of cell death during the dehydration process, and in consequence, a reduction in dry yeast quality. We propose that higher proportions of corn syrup inclusion require careful tuning of supplementation, specifically with regard to the quantity of iron, copper, zinc, and sulfur.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/fermentation11080432/s1, Table S1: Significantly up or downregulated differentially expressed genes.

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