**TITLE: Chemical Variation Attributed to Storage and Shelf-life of Malt**

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**ABBREVIATIONS**

\*PCA, principal component analysis

\*ANOVA, analysis of variance

\*RP-UHPLC-MS, reverse-phase, ultra-high-performance liquid chromatography mass spectrometry

\*GC-MS, gas chromatography mass spectrometry

**ABSTRACT:**

This study presents a comprehensive analysis of the metabolomic changes in malt during storage,

utilizing advanced liquid chromatography mass spectrometry (LC-MS) and gas chromatography mass

spectrometry (GC-MS) platforms for non-targeted metabolomic profiling. We investigated malt samples

from two distinct barley varieties malted by two different malthouses. This research delves into the

dynamic metabolic transformations occurring during malt storage, revealing shifts in the

composition of saccharides, proteins, free amino nitrogen, and enzymes. We identified notable variations in key metabolomic profiles which provided new data on the influence of storage time on malt quality and offered a deeper understanding of the complex interplay of different metabolites. Our findings provide novel insights into how these changes in malt metabolites directly influence the sensory attributes of beer, bridging the gap in knowledge regarding malt storage chemistry and serving as a guide for optimizing malt quality in the brewing industry. This multifaceted analysis not only highlights the dynamic nature of malt aging but also elucidates the nuanced way these changes manifest in the brewing process, ultimately influencing the sensory experience of beer.

**INTRODUCTION:**

In the ever-evolving landscape of the brewing industry, a profound understanding of the intricate chemistry of malt is pivotal for crafting a diverse array of beer styles. Malted barley is an essential component in beer production, influencing both the brewing process and the ultimate flavor of the beer. Recent advances in metabolomics and sensory analysis have provided new insights into how malt's chemical attributes evolve during storage and their consequent impact on beer quality [1-4]. While traditional malting and brewing practices advocate for aging malt to improve its brewing performance and flavor consistency, emerging research suggests that fresh malt may offer superior flavor profiles, challenging long-held industry paradigms [5-8]. Our research draws upon extensive collaborations between academic institutions and industry leaders, including Colorado State University, University of California-Davis, Admiral Maltings, and Colorado Malting Company, to delve into the dynamics of malt chemistry during storage. The primary focus was to discern the chemical and sensory variations attributed to the aging process and to understand how these changes impact the shelf-life and flavor stability of malt used in brewing. Central to our investigation is the application of advanced analytical techniques such as UHPLC-MS, and GC-MS, to profile metabolites in malt that undergo significant changes during storage. These changes are hypothesized to affect key quality attributes such as flavor, color, and fermentability. By integrating metabolomic data with sensory analysis, we aim to provide a comprehensive understanding of how storage conditions—particularly the presence of oxygen and the occurrence of lipid oxidation and other non-enzymatic changes—affect malt quality.

Malt, a fundamental ingredient derived from the malting of barley grains, undergoes controlled hydration, germination and drying, resulting in a complex matrix including saccharides, proteins, free amino nitrogen (FAN), and enzymes fundamental for brewing [9-11]. Recent advancements in analytical techniques, particularly metabolomics, have opened doors to a deeper exploration of malt composition, shedding light on its multifaceted chemical makeup [8, 12-14]. However, an often neglected yet profoundly impactful phase is malt storage time. Despite extensive research into malt genetics, environmental factors, and malting techniques influencing flavor, the dynamics of malt chemistry during storage, specifically its metabolite composition, remain relatively uncharted territory [15]. During storage, malt is inevitably exposed to oxygen, triggering non-enzymatic processes such as lipid oxidation and staling reactions [15-18]. These chemical transformations have the potential to significantly alter the flavor profile of the resulting beer, marking the storage phase as a prime area for scientific investigation [19, 20]. This study embarks on a comprehensive exploration, aiming to unravel the chemical transformations occurring in malt during its storage period. The focus extends beyond the mere identification of metabolites; it delves into the nuanced interplay of various compounds, enzymes, and chemical reactions that shape malt chemistry over time. By elucidating the metabolic evolution of malt, we seek to bridge the existing knowledge gap regarding the influence of malt metabolites on beer flavor.

Despite the extensive body of knowledge documenting the impact of malt on beer flavor, the specific roles played by individual metabolites within malt remain enigmatic [21-23]. To unravel this mystery, our study employs advanced analytical tools, including non-targeted metabolomics. Malt samples sourced from two distinct barley varieties malted by two distinct malthouses are meticulously analyzed to discern metabolic disparities. This in-depth analysis extends to the beer brewed from these malt samples, enabling a comprehensive understanding of how these metabolic variances manifest in the final product. The subsequent sections of this research outline our meticulous methodology, explicit objectives, and the anticipated outcomes of our investigation. Through this scientific inquiry, we aim to not only unravel the complexities of malt chemistry during storage but also decipher the direct impact of specific malt metabolites on beer flavor. This knowledge, we believe, will not only enrich the scientific community's understanding of malt dynamics but also empower the brewing industry with valuable insights, aiding in the optimization of malt quality to meet the diverse demands of the modern brewing landscape.

The significance of this study is underscored by the potential to overturn conventional wisdom regarding malt aging, offering brewers and maltsters data-driven guidance on malt usage that could enhance both the efficiency of brewing operations and the flavor characteristics of beer [19, 20]. Furthermore, our research contributes to a broader understanding of malt's role in beer production, supporting the development of optimized malting techniques that align more closely with modern brewing requirements. This manuscript will present the findings from our systematic study on the impact of malt storage duration and conditions on its chemical makeup and sensory attributes, aiming to refine quality evaluation techniques for brewing and malting industries. Through our collaborative efforts, we strive to advance the scientific foundation necessary to drive innovation in malt production and utilization.

**Experimental**

**Plant materials, malting, and malt quality**

The malts used in this study were provided by three maltsters (herein labeled Maltster A, Maltster B, and Maltster C) as representatives of the pale malts within the specifications provided by the American Malting Barley Association [24]. Malt quality analyses were performed in-house at the Hartwick College Center for Craft Food & Beverage (Oneonta, New York, U.S.A.) using methods of analysis set forth by the American Society for Brewing Chemists for α -amylase, β-glucan, color, diastatic power (DP), extract, FAN, soluble protein, and total protein. Malting conditions and storage are outlined in Supplementary Methods 1.

*Malt Quality*

The malts utilized for this study all adhered to American Malting Barley Association malting specifications [16]. Malt quality analyses were performed either in-house or at the Hartwick College Center for Craft Food & Beverage (Oneonta, New York, U.S.A.) using methods of analysis set by the American Society for Brewing Chemists for α-amylase, β-glucan, color, diastatic power (DP), extract, FAN, soluble protein, total protein, and viscosity (Table 1).

**Mass spectrometry metabolomics of barley and malts**

Non-volatile metabolites in the barley and malts were detected using a non-targeted metabolomics approach. Analysis of non-volatiles was conducted using a methanol:water (95:5) monophasic extraction of the sample [25]. The aqueous layer was dried under a stream of nitrogen gas, derivatized via methoximation and silylation,[26] and then injected into a GC-MS using methods as previously described [27]. A portion of the aqueous fraction was also combined with the organic fraction and analyzed using reverse phase ultra-high performance liquid chromatography MS (UHPLC-MS) using methods as previously described [12]. Replicate injections (n = 3) of each sample were used as quality control to account for analytical variation. Coefficient of variation (CV) percentages were calculated for all metabolites between the duplicate samples to evaluate variation in the system and were reported as < 10%, considered acceptable variation for these metabolomics platforms [28]. Mass spectra from each of three metabolomics platforms were converted to the .cdf file format and processed and annotated using the workflows described in Bettenhausen et al.[21] (GC) and Bettenhausen et al. [12] (LC). Metabolite quantities were normalized to total ion current and relative abundance for each molecular feature was determined by the mean area of the chromatographic peaks among replicate injections (n = 2). Volatile metabolites were identified by spectral matching in RamSearch software [29] to an in-house database of over 1,500 compounds, MSFinder software (as a validation tool for LC annotations (v3.26, RIKEN Center for Sustainable Resource Science, Yokohama, Kanagawa, Japan),[30, 31] and to external and theoretical databases including NIST v14 (http://www.nist.gov), Metlin,[32, 33] Golm Metabolome Database,[34] Human Metabolome Database (HMDB),[35] and FooDB [36]. Chemical ontologies were established using HMDB and the ClassyFire package in R [37].

**Statistical Analyses**

***Metabolomics***

Volatile and non-volatile metabolite abundances were compared using analysis of variance (ANOVA) via the *aov* function in the R statistical environment v4.0 [38], with “Variety” and “Age” (month) as the major factors, and false discovery rate adjustment was performed on the ANOVA *p*-values using the Benjamini-Hochberg algorithm [39], for n=2 injections per malt source. Principal Component Analysis (PCA) was performed using the prcomp() function in R to assess systematic variation and identify grouping patterns across the 314 annotated metabolites [40]. Unit variance (UV) scaling was applied to the metabolite data prior to PCA. Additionally, Orthogonal Projection to Latent Structures (OPLS) was conducted using the ropls package in R to evaluate and separate the variation based on linearity and orthogonality [38] While PCA and OPLS provided a solid framework for visualizing metabolite patterns, increasing technical replication in future studies will help to improve the robustness of the statistical analyses. For heatmap visualization, metabolite abundances were normalized using z-score scaling (mean-centered and scaled to unit variance) across all timepoints for each maltster. This approach emphasizes relative changes in metabolite abundance over time.

**Results**

***Metabolomics Results***

Metabolite variation of the malted barley was determined using a non-targeted metabolomics workflow, and profiles were established using two MS platforms: non-volatile metabolites via UHPLC-MS and GC-MS. The UHPLC-MS and GC-MS platforms each detected over 5,000 features in the malt. From these, 314 non-volatile metabolites were confidently annotated (Supplementary Table 1). The metabolome of the malt was comprised of 8 different chemical classes, including organooxygen compounds (carbohydrates), organonitrogen compounds (amines, amides), organoheterocyclic compounds (MRPs, indoles), organic acids (amino acids, carboxylic acids), lipids (terpenoid lipids, fatty acid esters), alkaloids, alkanes/alkenes, and benzenoid compounds (phenolics, phenylpropanoids).

Analysis of variance (ANOVA) revealed that 92 of the 314 metabolites (29.3%) varied among malt sources for “Age”, 219 (69.7%) varied for “Variety”, and 87 (27.7%) varied due to an “Age × Variety” interaction (Supplementary Table 2; FDR-adjusted p ≤ 0.05). Principal Component Analysis (PCA) was used to assess overall metabolite variation. PCA of the 314 annotated hot steep metabolites generated four principal components that together explained 62.9% of the variation (Figure 1a), with PC1, PC2, and PC3 accounting for 28.8%, 11.7%, and 9.6%, respectively. Figure 1b complements this variation by looking at Maltster A and B. To further examine age-related trends within a single malt source, PCA was performed using only Maltster B samples (Figure 1c). In this analysis, PC1 and PC2 accounted for 36.1% and 26.7% of the variation, respectively. Samples separated along PC1 primarily by Month, with a general progression from Month 1 to Month 12, suggesting age-dependent metabolomic shifts within Maltster B. The tighter clustering of timepoints along PC2 further supports consistent patterns of change, despite underlying variability.

To explore which metabolites contributed to these age-related and maltster-driven trends, biplots were generated using GC-MS data for individual maltsters. For Maltster B, the biplot of PC1 (50.5%) and PC2 (12.5%) revealed clear separation of samples by age (Figure 2a). Month 1 and Month 12 samples loaded in opposite directions, indicating progressive shifts in metabolite profiles over time. Compounds including diacetyl, lactic acid, alanine, and GABA contributed strongly to these changes, supporting the idea of accelerated chemical reactivity or degradation with aging. In comparison, Maltster A samples were more tightly grouped overall, reflecting the PCA results. However, when plotted using PC1 (31.4%) and PC3 (12.4%), subtle time-dependent differences emerged (Figure 2b). Key contributors included maltose, L-proline, L-valine, and fructose, suggesting that even under stable conditions, certain sugars and amino acids shift slightly over time. These patterns help distinguish between broad maltster effects and more gradual, compound-specific changes due to age. To complement the PC1/PC3 biplot, a PC1 vs PC2 biplot was also constructed for Maltster A (Figure 2c). While timepoint separation was less pronounced, this view confirmed the overall metabolic consistency of these samples. Metabolites such as diacetyl, maltose, and L-valine contributed moderately to variation along PC1 and PC2, but without strong age-dependent clustering.

Boxplots were used to evaluate individual metabolite changes across maltsters and over time. Between Maltster A and B, several key compounds differed significantly, including L-proline, fructose, valine, and maltose, with p-values < 0.01 in most cases (Figure 3a). These differences reinforce the observed “maltster effect” and suggest metabolic contributions tied to production practices or variety.

Over time, Maltster B samples exhibited progressive changes in multiple amino acids and sugars, including increases in diacetyl, alanine, and lactic acid (Figure 3b). In contrast, Maltster A showed relatively minor shifts across the same metabolites, with tighter distributions across months (Figure 3c). These findings are consistent with PCA results, where Maltster A clustered more tightly regardless of age, while Maltster B showed more pronounced temporal separation.

To visualize broader metabolic patterns, heatmaps of GC-MS data were constructed. Maltster B’s heatmap revealed strong month-to-month shifts, especially in compounds like GABA, proline, and fructose, with clear separation between early and late samples (Figure 4a). Maltster A remained comparatively stable, with only subtle differences over time (Figure 4b). Together, these boxplots and heatmaps support the idea that Maltster B is undergoing more extensive biochemical change during storage, while Maltster A maintains a more consistent profile.

LC-MS analysis of the top 30 annotated non-volatile metabolites was conducted. Heatmaps for both maltsters revealed distinct chemical class clustering, particularly among fatty acids, ceramides, and glycosylated lipids (Supplementary Figures S1a- b). In Maltster B, notable shifts were observed across months, with temporal clustering driven largely by lipid-related metabolites. In contrast, Maltster A again showed relatively consistent profiles, with less distinct separation over time. While these findings support the broader aging trends observed by GC-MS, further targeted analysis would be required to validate the functional significance of these LC-MS compounds in the context of malt quality or shelf-life. Additional LC-MS heatmaps are provided in **Supplementary Figures S1a–b**, illustrating the top 30 lipid-associated metabolites by chemical class. While Maltster A showed relatively stable profiles across months, Maltster B (Supplementary Figure S1b) demonstrated more pronounced temporal shifts and clustering.

**DISCUSSION**

In the malting process of barley, a transformative alteration of the grain's metabolomic landscape occurs, characterized by the variation of a diverse array of organic compounds pivotal to the malt's properties and its subsequent applications in brewing and distilling. Initially, carbohydrates, predominantly polysaccharides in raw barley, are enzymatically broken down into simpler sugars such as maltose and other disaccharides [12, 41-43]. These sugars serve as fermentable substrates in beer production, directly influencing the efficiency and yield of fermentation [12].

***Month 1 – the beginning of the end***

In this section, we examine the metabolomic profiles of malts aged for one month from two distinct maltsters, as Maltster C was not examined further due to having only Months 1, 6, and 12 (Maltsters A and B, Tables 2 and 4). This analysis aims to elucidate the diversity and complexity of metabolites present, including specialized flavor-active compounds, lipids, and fatty acids, and their respective contributions to malt quality. By detailing the specific biochemical constituents and their transformations during the aging process, we seek to understand their roles in flavor, aroma, and color development. The comparative approach between Maltster A and Maltster B provides insights into how different malting processes influence these profiles, highlighting the impact of biochemical variations on the final malt characteristics. This discussion serves to advance the science of malt production and optimize the properties of malt for brewing and distilling applications. Comparative analysis between Maltster A and Maltster B reveals the extent to which processing variables influence these profiles (Figures 1a-c, 2a-c).

*Specialized and Flavor-Enacting Compounds*

For Maltster A, certain specialized metabolites such as 4-hydroxycinnamoylagmatine and indole-3-acrylic acid might play roles in the biosynthesis of complex molecules that contribute to flavor, aroma, and color. Ergothioneine, a thiol-containing compound, provides additional antioxidative benefits, which are crucial during the aging process to maintain malt quality. For Maltster B, indole, 3-methoxytyramine, diacetyl, and 3-hydroxyindolin-2-one are metabolites that directly influence the flavor profile of malt. Indole contributes to the formation of aromatic compounds during fermentation, enhancing the complexity of flavors. 3-methoxytyramine, related to plant physiological responses, can indirectly affect flavor through stress adaptation mechanisms in barley. Diacetyl is a key metabolite impacting malt flavor, known for imparting a buttery taste which, if not managed correctly during yeast metabolism, can lead to undesirable off-flavors. Similarly, 3-hydroxyindolin-2-one may participate in the formation of novel flavor and aroma compounds during fermentation, adding to the sensory profile of the final product [44].

*Lipids and Fatty Acids*

Lipids contribute to beer flavor and aroma by releasing volatile compounds during malting and mashing, adding complexity to the beer’s aroma. However, excessive lipid content can cause off-flavors and stability issues. Lipids also affect foam stability – too much can harm head retention – and can contribute to haze formation, which brewers mitigate via cold conditioning and fining. Key lipid classes include phosphatidylethanolamines (PE-NMe(16:0/18:1(9Z)) and PE-NMe(14:1(9Z)/14:1(9Z))), which are crucial for maintaining cellular membrane integrity and functionality. Sphingolipids such as phytoceramide and various ceramides (e.g., N-(11Z)-icosenoylphytosphingosine and Cer(d18:0/16:0)) contribute to cellular signaling and membrane stability. Glycerolipids like DG(18:1(9Z)/18:2(9Z,12Z)/0:0) and complex glycosylated lipids such as glucosylceramide (d18:1/26:1(17Z)) are involved in lipid metabolism that influences the flavor and aroma development through oxidative processes. These lipid constituents are necessary not only for the structural aspects of the grains but also influence the sensory attributes of the malt through their roles in oxidative stability and flavor compound formation, contributing to both the physical properties and the sensory quality of the aged malt (Supplementary Figures S1a-b).

For Maltster B, Metabolites like DG(16:0/18:3(9Z,12Z,15Z)/0:0), PIP(20:2(11Z,14Z)/18:0), and various phospholipids (e.g., PE-NMe(14:1(9Z)/14:1(9Z)) and PE-NMe(16:0/18:1(9Z))) play crucial roles in the germination process by impacting the lipid metabolism and membrane dynamics in barley grains. These lipids are necessary for maintaining cellular integrity and facilitating water uptake during malting, which are critical for enzymatic activity and efficient sugar release—factors directly affecting the malt’s fermentability and flavor [45, 46]. Lipids in malted barley serve as an energy source during the germination process. Germination is a pivotal step in malting, where the barley grain begins to sprout and convert starches into sugars. Lipids provide the energy needed for the enzymatic reactions that break down starches into fermentable sugars. Lipids can contribute to the flavor and aroma profile of the finished beer. During the malting and mashing processes, lipids can release volatile compounds that add complexity to the beer's aroma and flavor. However, excessive lipid content can also lead to off-flavors and stability issues in the final product. Lipids can impact the foam stability of beer. Too much lipid content can lead to poor head retention in the beer, which is undesirable for many beer styles. Brewers often take measures to reduce lipid levels during brewing to ensure better foam quality. Lipids are essential for yeast health and fermentation. Yeast requires lipids as a source of nutrients and energy during fermentation. Adequate lipid levels are important to support yeast growth and ensure complete and healthy fermentation. High levels of lipids can contribute to haze formation in beer. Haze can be problematic for some beer styles, particularly those that should be clear and bright. Brewers often take steps to minimize lipid-related haze, such as cold conditioning and fining agents [5, 47].

*Carbohydrates and Sugars*

Hexose, fructose, and complex oligosaccharides like 3-fucosyllactose influence the fermentability of malt. Simple sugars such as glucose and fructose are pivotal for yeast fermentation, directly correlating with the efficiency and yield of the brewing process. They provide the necessary substrates for alcohol production and contribute to the sweetness and body of the beer. Additionally, complex carbohydrates may affect the viscosity and mouthfeel of the malt extract, influencing the sensory attributes of the beer.

*Nitrogenous Compounds*

Citric acid, a key component in the tricarboxylic acid cycle, impacts the acid-base balance during mashing and affects enzymatic activity. N-oleoyl phenylalanine and other amino acid derivatives like Tyrosyl-Leucine contribute to protein synthesis and potentially influence flavor development through Maillard reactions and other pathways. Amino acids like L-proline, L-phenylalanine, and peptides such as histidinyl-proline contribute to protein solubility and enzymatic reactions during malting and brewing. L-proline and L-phenylalanine are precursors to aromatic compounds that enhance the flavor complexity of the beer. Peptides, through their role in protein structure and metabolism, can affect the stability of foam and the overall clarity of the beer [48-50] (Figures 3a-c).

*Phenolic and Antioxidant Compounds*

In the case of Maltster A, stilbenes and β-carotene are notable for their antioxidant properties, which help protect the malt against oxidative damage, thereby enhancing the stability and shelf life of the malt. These compounds are important for preventing off-flavors and maintaining the quality of the malt during storage. Metabolites such as (all-E,3R,3'R)-zeaxanthin alpha-L-rhamnopyranoside and 1-O-sinapoylglucose, present in the one-month aged samples of Maltster B, impact the antioxidant properties of the malt. These compounds can contribute to the stability and color of the beer, as well as provide health benefits associated with antioxidants. The presence of phenolic compounds is also crucial for the development of a desirable bitterness and astringency in beer, balancing the overall flavor profile [51-54].

***Month 12- The end of the beginning***

The metabolites observed in aged malt (Maltsters A and B) at month 12, indicate a complex transformation of biochemical constituents during the aging process (Tables 3 and 5). These transformations are necessary for developing the flavor, aroma, and stability of the malt over time (Figures 3b–c, 4a–b).

*Lipids and Fatty Acids*

For Maltsters A and B, several fatty acids and their derivatives, such as oleic acid, linoleic acid, palmitic Acid, and various forms of FAHFA (fatty acid esters of hydroxy fatty acids), are prominent. Oleic and linoleic acids are unsaturated fats that contribute to the development of flavor and oxidative stability in aged malt. Palmitic acid, a saturated fatty acid, plays a role in the consistency and mouthfeel of the final brew. The presence of unique lipid structures like MG(18:3(9Z,12Z,15Z)/0:0/0:0) and various FAHFAs indicates lipid oxidation and enzymatic transformations that can affect the malt's organoleptic properties. Metabolites such as 9(10)-EpODE, 12,13-EpOME, and 12,13-DHOME, derivatives from the oxidation of fatty acids, are involved in the aging process, potentially contributing to the flavor and stability of the malt. These compounds are part of the plant's response mechanism to stress and play a role in signaling that can influence the aging characteristics of malt. Compounds like N-(11Z)-icosenoylphytosphingosine and 1-palmitoyl-sn-glycero-3-phospho-(1'-sn-glycerol) maintain the structural integrity of cell membranes in the grains, even as they age [17, 42, 46, 55].

*Nitrogenous Compounds and Amino Acids*

Ergothioneine, a sulfur-containing amino acid, and phenylalanyl-asparagine, an amino acid linkage, are important for protecting the malt against oxidative damage and for contributing to flavor formation, respectively. N(6),N(6)-dimethyladenosine, a modified nucleoside, indicates changes in the nucleic acid metabolism during aging.

*Phenolics and Vitamins*

β-carotene and Vitamin A are significant for their antioxidant properties, protecting lipids and other sensitive molecules from oxidation during storage. These compounds are crucial for maintaining the quality and longevity of aged malt. Molecules like 3,3',4'5-tetrahydroxystilbene represent the phenolic transformations occurring in malt, contributing to the antioxidant capacity and color of the malt. These changes are beneficial for the stability and sensory profiles of beers and whiskies derived from aged malt [11, 56].

The presence of specialized metabolites such as various glycosylated and acylated derivatives reflects ongoing metabolic activity and transformation in the stored malt, influencing its functional properties and the complexity of flavors in the final product. The diversity and complexity of these metabolites underscore the dynamic biochemical processes occurring during the aging of malt, each contributing uniquely to the development of a rich flavor profile, enhanced stability, and overall quality of the malt used in brewing and distilling.

***Metabolites over time display variability***

Upon examining the metabolites in malts over the 12-month period (Figures 4a-b, Supplementary Figures S1a-b), we observed several notable differences and similarities in the metabolite profiles after one month of aging. These observations provide insights into the biochemical dynamics that occur during the aging process of malt, which are key for understanding the development of flavor, aroma, and overall malt quality.

There was a wide range of metabolites such as amino acids (e.g., L-threonine, L-valine, L-isoleucine), organic acids (e.g., citric acid, malic acid), and other key compounds (e.g., acetaldehyde, diacetyl, phenol). This similarity across the data indicates that both types of malt undergo comparable biochemical transformations during aging, suggesting a consistency in the production of key flavor and aroma compounds across different malting processes. However, differences in the intensity and distribution of specific metabolites such as lactic acid and acetic acid were noted, reflecting distinct aging characteristics between the malts over time and between maltsters. These variations could be attributed to differences in the malting conditions, the origin of the grain, or microbial activities during the malting process, which might influence the metabolic pathways activated.

Maltster A malts, for instance, show a more pronounced presence of metabolites such as lactic acid and methanol, possibly indicating higher concentrations or a greater analytical focus on these compounds. Conversely, the data from Maltster B displays a broader distribution of amino acids and compounds related to glycolysis, suggesting a different enzymatic activity profile that could impact the enzymatic breakdown and synthesis of flavor precursors during malting.

The observed differences in metabolite concentration and emphasis between the malts over time, per maltster, suggest that while the overall metabolic footprint is similar, the specifics of the malting process or conditions employed by each maltster lead to unique modifications in the malt's metabolomic profile. These modifications likely influence the sensory properties and functional quality of the malt, thus affecting the characteristics of the beer when these malts are utilized in brewing. This data can inform on tailoring malting processes to enhance desired malt attributes, ultimately contributing to the production of superior quality beers with distinct flavors and aromas. While the current study focuses on the metabolic changes observed in aged malt, the impact of these changes on sensory attributes will be evaluated in a subsequent study examining the resultant beer. This two-phase approach ensures a comprehensive understanding of how malt aging influences both chemical composition and flavor outcomes.

**Conclusion**

This study provides a comprehensive analysis of the chemical variations in malt during storage, utilizing advanced metabolomic profiling techniques such as LC-MS and GC-MS. Significant shifts in saccharides, proteins, and lipids were observed, highlighting the dynamic nature of malt's chemical composition over time. These changes may directly influence the sensory attributes of beer, affecting its flavor and aroma. The findings emphasize the role of storage time in determining malt quality, with longer storage periods resulting in both beneficial and detrimental chemical transformations. Additionally, the study underscores the impact of lipid oxidation during storage, which plays a pivotal role in flavor stability and overall malt quality. These insights bridge the knowledge gap regarding malt storage chemistry and offer valuable guidance for optimizing malt quality in the brewing industry.

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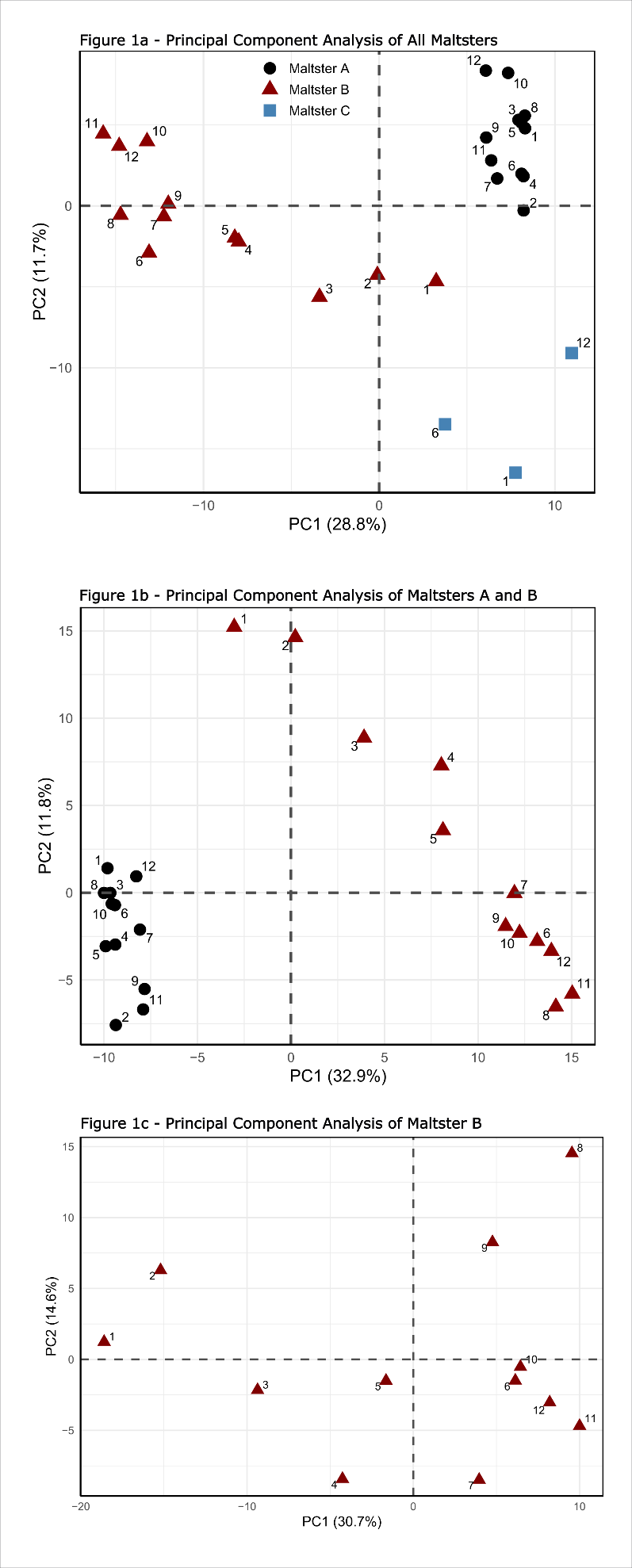
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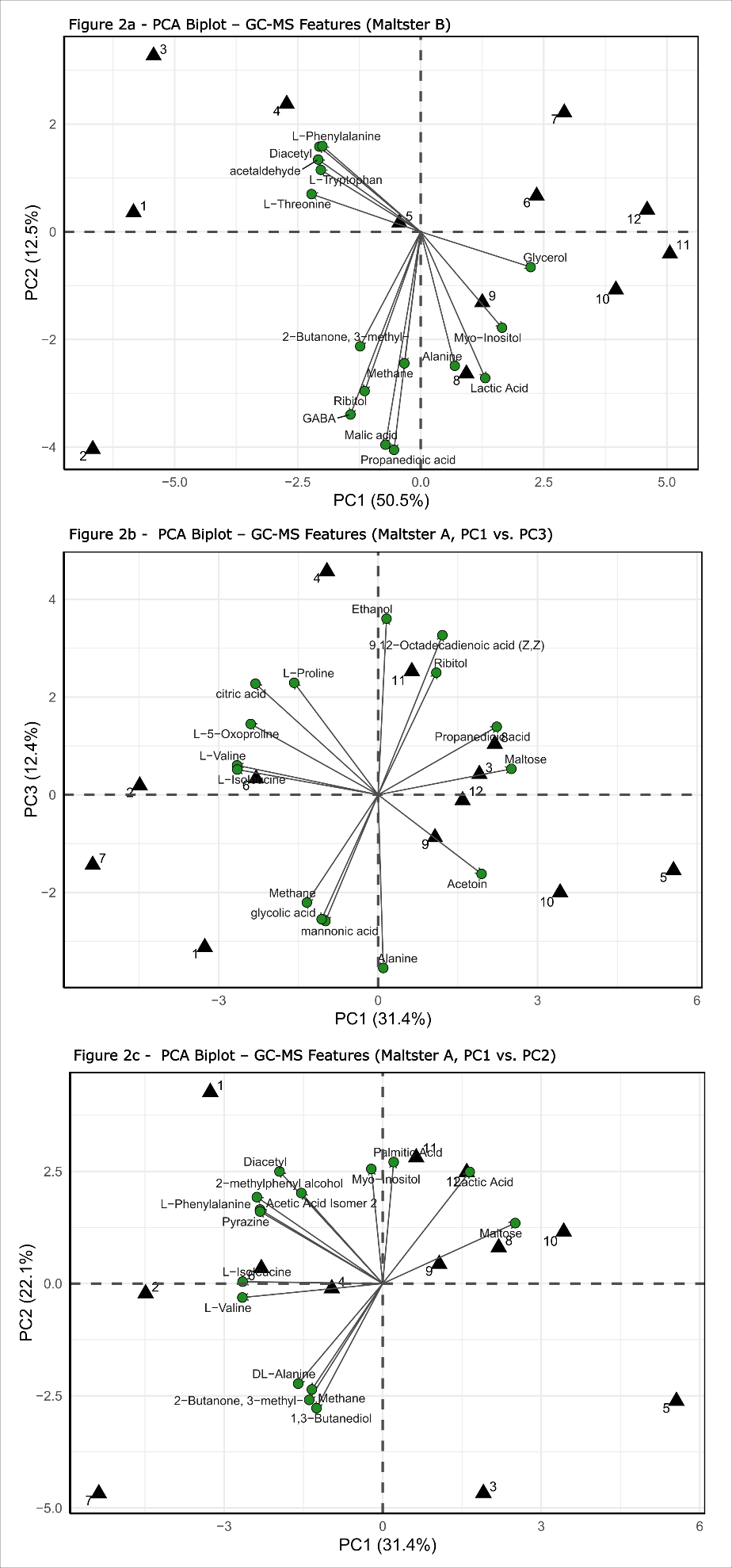
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**Figures**

**Figure 1a -** Principal Component Analysis of All Maltsters  
  
PCA of all malt samples (n = 36) derived from three maltsters (A, B, and C), showing sample separation based on metabolite variation across PC1 (28.8%) and PC2 (11.7%). Maltster A clustered tightly regardless of age, while Maltsters B and C exhibited broader dispersion. These patterns support a maltster-specific effect on the metabolomic profile.

**Figure 1b -**  PCA of Maltsters A and B Only  
  
Subset PCA of Maltsters A and B (Maltster C removed) showing distinct clustering by maltster across PC1 (32.9%) and PC2 (11.8%). Maltster A remained tightly grouped, while Maltster B samples were more widely distributed. No consistent separation by age was observed, indicating metabolite variation is more strongly associated with production source than storage duration.

**Figure 1c -**  PCA of Maltster B Samples Colored by Age  
  
PCA of Maltster B samples only, separated across PC1 (30.7%) and PC2 (14.6%). Coloring by month of storage shows progression from Month 1 to Month 12, suggesting age-dependent shifts in metabolite composition within this maltster’s samples.

**Figure 2a -** PCA Biplot – GC-MS Features (Maltster B)  
  
Biplot of Maltster B samples using GC-MS metabolite data, showing PC1 (50.5%) and PC2 (12.5%) axes. Storage duration (Month 1–12) is labeled. Key metabolites contributing to temporal variation include diacetyl, GABA, lactic acid, alanine, and acetaldehyde. Samples show progressive separation along PC1, consistent with aging-related metabolic changes.

**Figure 2b -**  PCA Biplot – GC-MS Features (Maltster A, PC1 vs. PC3)  
  
Biplot of Maltster A samples showing PC1 (31.4%) and PC3 (12.4%). While clustering remains relatively tight, slight progression over time is observed. Metabolites contributing to PC3 include L-proline, maltose, and mannonic acid. This figure complements the PC1/PC2 comparison by capturing additional variance associated with age.

**Figure 2c -**  PCA Biplot – GC-MS Features (Maltster A, PC1 vs. PC2)  
  
Biplot of Maltster A samples showing PC1 (31.4%) and PC2 (22.1%), with samples labeled by month. Limited temporal separation suggests that Maltster A maintains a more consistent metabolomic profile during aging. Metabolites like diacetyl, maltose, and valine contribute to variation but do not drive strong clustering.

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**Figure 3a -**  Boxplots of Amino Acids and Key Metabolites Between Maltsters  
  
Boxplots comparing metabolite abundances between Maltster A and Maltster B at Month 1. Compounds include L-proline, fructose, maltose, L-valine, L-isoleucine, and others. P-values are shown for each compound (e.g., L-proline p = 0.000). These differences highlight distinct chemical signatures in early-stage malt between producers.

**Figure 3b** - Boxplots of Metabolite Trends Over Time (Maltster B)  
  
Temporal trends for key amino acids and small molecules in Maltster B samples over 12 months. Compounds include L-threonine, L-tryptophan, maltose, and diacetyl. Increasing or decreasing trends reflect aging effects and contribute to broader variation seen in PCA and biplot analyses.

**Figure 3c** - Boxplots of Metabolite Trends Over Time (Maltster A)  
  
Metabolite abundances for Maltster A samples across months 1–12. Compared to Maltster B, these values remain relatively stable, with minor fluctuations in compounds such as L-proline, acetaldehyde, and maltose. Supports the interpretation that Maltster A undergoes less dramatic chemical change during storage.

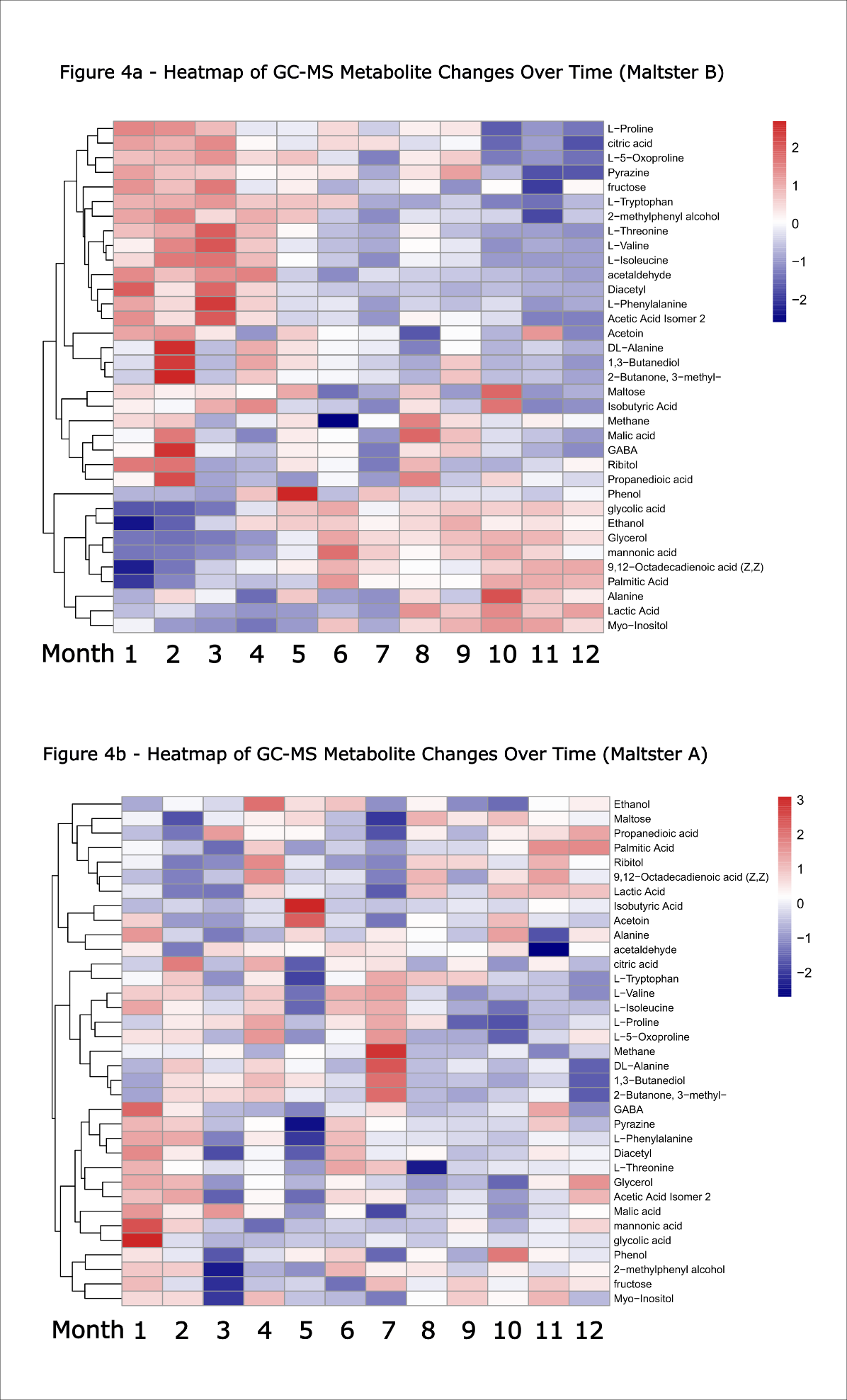


Figure 4a - Heatmap of GC-MS Metabolite Changes Over Time (Maltster B)  
  
Heatmap shows z-score–normalized metabolite abundance. Red indicates higher-than-average abundance; blue indicates lower-than-average abundance across timepoints.

Z-scored heatmap showing metabolite changes over time (Months 1–12) for Maltster B. Compounds include L-proline, diacetyl, GABA, citric acid, and L-valine. Distinct clustering patterns illustrate dynamic biochemical shifts associated with storage and aging.

Figure 4b - Heatmap of GC-MS Metabolite Changes Over Time (Maltster A)  
  
Heatmap shows z-score–normalized metabolite abundance. Red indicates higher-than-average abundance; blue indicates lower-than-average abundance across timepoints. Z-scored heatmap of Maltster A samples across aging timepoints. Compared to Maltster B, patterns are more consistent, with less dramatic shifts across metabolites. Provides visual confirmation of PCA and boxplot findings highlighting maltster-specific storage effects.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Table 1 – Malting Quality Analysis** | | | | | | | | | | | | | | | | | |
| **Sample ID** | **Date** | **Friability (%)** | **Glassy (WUG) %** | **Half glassy (PUG) %** | **Moisture (%)** | **Extract (FGDB) %** | **Total Protein (%)** | **Soluble Protein (%)** | **S/T (%)** | **Alpha Amylase (DU)** | **Diastatic Power (°L)** | **FAN (mg/L)** | **β-glucan (mg/L)** | **Color (°SRM)** | **pH** | **Clarity** | **Filtration Time** |
| Maltster A | 1 month | 92.3 | 0.2 | 0.0 | 3.2 | 78.1 | 10.58 | 4.57 | 43.2 | 52.5 | 122 | 162 | 71 | 2.25 | 5.94 | slightly hazy | Normal |
| Maltster B | 1 month | 78.3 | 2.2 | 0.5 | 6.7 | 80.7 | 11.69 | 4.95 | 42.3 | 44.2 | 141 | 168 | 63 | 3.06 | 5.97 | clear | Normal |
| Maltster C | 1 month | 92.3 | 1.3 | 0.6 | 3.6 | 80.3 | 10.10 | 4.29 | 42.5 | 57.4 | 102 | 157 | 85 | 3.35 | 5.88 | hazy | Normal |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Maltster A | 12 months | 91.5 | 0.3 | 0.0 | 4.1 | 77.9 | 10.50 | 4.23 | 40.3 | 50.5 | 125 | 159 | 72 | 2.2 | 5.96 | hazy | Normal |
| Maltster B | 12 months | 77.6 | 2.3 | 0.5 | 7.0 | 80.2 | 11.35 | 4.90 | 43.2 | 40.1 | 140 | 166 | 60 | 3 | 5.96 | clear | Normal |
| Maltster C | 12 months | 92.0 | 1.5 | 0.5 | 4.2 | 80.1 | 10.21 | 4.10 | 40.2 | 56.2 | 110 | 160 | 83 | 3.01 | 5.90 | hazy | Normal |

| **Table 2 – Metabolites contributing to variation in Month 1, Maltster B** | | | |
| --- | --- | --- | --- |
| **Primary ID** | **Chemical Class** | **Metabolite Name** | **Contribution Score** |
| LCPOS-C4579 | Tryptamines and derivatives | Tryptophanamide | 3.37641 |
| LCPOS-C6688 | Medium-chain fatty acids | 9-mercaptodethiobiotin | 3.26073 |
| LCPOS-C6474 | Dipeptides | Histidinyl-Proline | 3.24626 |
| LCPOS-C3274 | Indoles | Indole | 3.21591 |
| LCPOS-C2979 | Methoxyphenols | 3-Methoxytyramine | 2.9805 |
| GC-C230 | Ketones | Diacetyl | 2.90998 |
| LCPOS-C1626 | Linoleic acids and derivatives | DG(16:0/18:3(9Z,12Z,15Z)/0:0) | 2.88762 |
| LCPOS-C2122 | Glycerophosphoinositol phosphates | PIP(20:2(11Z,14Z)/18:0) | 2.73118 |
| LCPOS-C1716 | Hexoses | Hexose | 2.59742 |
| LCPOS-C1274 | Monomethylphosphatidylethanolamines | PE-NMe(14:1(9Z)/14:1(9Z)) | 2.49914 |
| LCPOS-C1629 | Xanthophylls | (all-E,3R,3'R)-Zeaxanthin alpha-L-rhamnopyranoside | 2.22488 |
| LCMS-LCNEG\_C03375 | Fatty acid esters | O-oleoylanandamide | 2.19978 |
| GC-C198 | Aldehydes | acetaldehyde | 2.11683 |
| LCPOS-C1818 | Indolines | 3-hydroxyindolin-2-one | 2.10004 |
| LCPOS-C0407 | Hydroxycinnamic acid glycosides | 1-O-Sinapoylglucose | 1.97046 |
| LCPOS-C1604 | Glycosyldiacylglycerols | 1-18:2-2-18:3-monogalactosyldiacylglycerol | 1.8757 |
| LCMS-LCNEG\_C02166 | Monomethylphosphatidylethanolamines | PE-NMe(16:0/18:1(9Z)) | 1.84673 |
| GC-C021 | Amino acids | L-Proline | 1.83274 |
| GC-C110 | Organic acids | Acetic acid Isomer 2 | 1.80636 |
| GC-C037 | Amino acids | L-Phenylalanine | 1.68338 |
| LCPOS-C6021 | Phosphosphingolipids | IPC 18:0;3/18:0;1 | 1.63483 |
| LCPOS-C1117 | Alpha amino acids and derivatives | L-Hexahydro-3-imino-1,2,4-oxadiazepine-3-carboxylic acid | 1.56217 |
| LCMS-LCNEG\_C01191 | Ceramides | N-(1,3-dihydroxytricos-4-en-2-yl)-2-hydroxyhenicos-3-enamide | 1.48271 |
| GC-C022 | Saccharides | fructose | 1.46461 |
| LCPOS-C1329 | Pyrimidine nucleotide sugars | dTDP-4-acetamido-4,6-dideoxy-D-galactose | 1.4426 |
| LCPOS-C2655 | Phosphosphingolipids | N-nonadecanoylsphinganine-1-phosphocholine | 1.42199 |
| GC-C047 | Polyols | Ribitol | 1.40375 |
| LCPOS-C3245 | Oligosaccharides | 3-Fucosyllactose | 1.38708 |
| LCPOS-C2100 | Secondary alcohols | 5,8,11,14,17,20,23,26-octamethyl-4,7,10,13,16,19,22,25,28-nonaoxadotriacontan-2-ol | 1.38678 |
| LCPOS-C1857 | Cinnamaldehydes | 2-Pentyl-3-phenyl-2-propenal | 1.368 |
| GC-C062 | Amino acids | L-Tryptophan | 1.35145 |
| GC-C031 | Carboxylic acids | citric acid | 1.3477 |
| GC-C029 | Amino acids | L-Threonine | 1.26198 |
| LCPOS-C5654 | Phosphoethanolamines | N-(octadecanoyl)-tetradecasphing-4-enine-1-phosphoethanolamine | 1.24237 |
| GC-C180 | Organoheterocyclic compounds | Pyrazine | 1.17168 |
| LCPOS-C3296 | Pyridines and derivatives | 5-(3-pyridinylmethyl)-1,3,5-triazinane-2-thione | 1.15579 |
| GC-C288 | Phenols | 2-methylphenyl alcohol | 1.09379 |
| LCPOS-C6241 | Glycosyl-N-acylsphingosines | Lactosylceramide (d18:1/12:0) | 1.09165 |
| LCMS-LCNEG\_C03128 | Oligosaccharides | Oligosaccharide 2 | 1.07156 |
| LCPOS-C1506 | Phosphosphingolipids | SM(d18:1/24:1(15Z)) | 1.02572 |
| LCPOS-C2142 | Linoleic acids and derivatives | DG(18:4(6Z,9Z,12Z,15Z)/16:0/0:0) | 1.01186 |

| **Table 3 – Metabolites contributing to variation in Month 12, Maltster B** | | | |
| --- | --- | --- | --- |
| **Primary ID** | **Chemical Class** | **Metabolite Name** | **Contribution Score** |
| LCMS-NEG\_C01759 | Long-chain fatty acids | Oleic acid | 1.90089 |
| LCPOS-C0038 | Linoleic acids and derivatives | MG(18:3(9Z,12Z,15Z)/0:0/0:0) | 1.89402 |
| LCPOS-C0704 | Dipeptides | Phenylalanyl-Asparagine | 1.80155 |
| LCMS-NEG\_C00951 | Linoleic acids and derivatives | Linoleic acid | 1.75575 |
| LCMS-NEG\_C02267 | Long-chain fatty acids | FAHFA(16:1(9Z)/13-O-18:0) | 1.58905 |
| LCMS-NEG\_C01106 | Medium-chain fatty acids | 9(10)-EpODE | 1.56781 |
| LCMS-NEG\_C03302 | Long-chain fatty acids | 12,13-EpOME | 1.52377 |
| LCMS-NEG\_C01638 | Long-chain fatty acids | FAHFA(16:0/9-O-18:0) | 1.50566 |
| LCPOS-C1966 | Phytoceramides | N-(11Z)-icosenoylphytosphingosine | 1.46419 |
| LCPOS-C6509 | Indoles | 1H-Indole-3-carboxaldehyde | 1.45849 |
| LCMS-NEG\_C02255 | Carotenes | β-Carotene | 1.39039 |
| LCPOS-C4619 | Histidine and derivatives | Ergothioneine | 1.36988 |
| GC-C016 | Fatty acids | Palmitic Acid | 1.36434 |
| LCMS-NEG\_C01800 | Stilbenes | 3,3',4'5-Tetrahydroxystilbene | 1.36262 |
| LCPOS-C0042 | Indolyl carboxylic acids and derivatives | 2'-alpha-mannosyl-L-tryptophan | 1.29043 |
| LCMS-NEG\_C01714 | Long-chain fatty acids | Ricinoleic acid | 1.272 |
| LCMS-NEG\_C02085 | Long-chain fatty acids | (R)-3-Hydroxy-tetradecanoic acid | 1.26016 |
|  |  |  |  |
| **Primary ID** | **Chemical Class** | **Metabolite Name** | **Contribution Score** |
| LCMS-NEG\_C02075 | Long-chain fatty acids | A-12(13)-EpODE | 1.24989 |
| GC-C013 | Fatty acids | 9,12-Octadecadienoic acid (Z,Z) | 1.24383 |
| LCPOS-C5218 | Long-chain ceramides | N-octadecanoyl-15-methylhexadecasphinganine | 1.24292 |
| LCPOS-C4029 | Purine nucleosides | N(6),N(6)-dimethyladenosine | 1.17004 |
| LCMS-NEG\_C09383 | Long-chain fatty acids | 12,13-DHOME | 1.15876 |
| LCPOS-C2074 | Lysophosphatidylglycerols | 1-palmitoyl-sn-glycero-3-phospho-(1'-sn-glycerol) | 1.14473 |
| LCPOS-C1892 | Retinoids | Retinal | 1.14193 |
| LCMS-NEG\_C02469 | Linoleic acids and derivatives | 13(S)-Hydroperoxylinolenic acid;(13S)-Hydroperoxy-cis-9,15-trans-11-octadecatrienoic acid;(13S)-HPLA | 1.09869 |
| LCPOS-C4020 | Pyridinecarboxylic acids | 1-(3-methoxypropyl)-4,6-dimethyl-2-oxo-1,2-dihydropyridine-3-carboxylic acid | 1.09661 |
| LCPOS-C1554 | Stigmastanes and derivatives | 22:0-Glc-Stigmasterol | 1.07103 |
| GC-C001 | Alcohols | Glycerol | 1.05309 |
| LCMS-NEG\_C09239 | Long-chain fatty acids | (R)-3-Hydroxy-hexadecanoic acid | 1.03984 |
| LCPOS-C0953 | Glycosyldiacylglycerols | 1-18:1-2-16:0-digalactosyldiacylglycerol | 1.03367 |
| LCPOS-C2162 | Retinoids | Vitamin A | 1.03057 |
| LCPOS-C0735 | Long-chain ceramides | N-arachidonoylsphinganine | 1.02197 |
| LCPOS-C0838 | Isoflavonoid O-glycosides | Genistin | 1.02189 |
| LCPOS-C0242 | Ceramides | Cer(d18:2/18:1) | 1.0001 |

|  |  |  |  |
| --- | --- | --- | --- |
| **Table 4– Metabolites contributing to variation in Month 1 – Maltster A** | | | |
| **Primary ID** | **Chemical Class** | **Metabolite Name** | **Contribution Score** |
| LCMS-NEG\_C01800 | Stilbenes | 3,3',4'5-Tetrahydroxystilbene | 3.73764 |
| LCMS-NEG\_C02255 | Carotenes | β-Carotene | 3.25929 |
| LCMS-NEG\_C02166 | Monomethylphosphatidylethanolamines | PE-NMe(16:0/18:1(9Z)) | 3.25418 |
| LCMS-NEG\_C03875 | Linoleic acids and derivatives | Corchorifatty acid F | 3.15927 |
| LCPOS-C0387 | Phytoceramides | Phytoceramide | 3.03434 |
| LCPOS-C3447 | Secondary alcohols | N-[(15Z)-2-hydroxytetracosenoyl]sphingosine | 3.00482 |
| LCMS-NEG\_C00727 | Linoleic acids and derivatives | DG(18:1(9Z)/18:2(9Z,12Z)/0:0) | 2.98094 |
| LCPOS-C0246 | Coumaric acids and derivatives | 4-Hydroxycinnamoylagmatine | 2.80407 |
| LCMS-NEG\_C01683 | Phenylalanine and derivatives | N-Oleoyl phenylalanine | 2.73206 |
| GC-C031 | Carboxylic Acids | citric acid | 2.65363 |
| LCPOS-C1274 | Monomethylphosphatidylethanolamines | PE-NMe(14:1(9Z)/14:1(9Z)) | 2.64845 |
| LCMS-NEG\_C01613 | Glycosyl-N-acylsphingosines | Glucosylceramide (d18:1/26:1(17Z)) | 2.61076 |
| LCMS-NEG\_C03448 | Secondary alcohols | N-2-hydroxypalmitoylsphingosine | 2.60197 |
| LCMS-NEG\_C09383 | Long-chain fatty acids | 12,13-DHOME | 2.56827 |
| LCPOS-C0038 | Linoleic acids and derivatives | MG(18:3(9Z,12Z,15Z)/0:0/0:0) | 2.52014 |
| LCPOS-C0129 | Glycosyl-N-acylsphingosines | N-acetylneuraminyl-Galactosylceramide | 2.45115 |
| LCPOS-C0335 | Long-chain fatty acids | 8,11,14-Eicosatrienoic acid | 2.42835 |
| LCPOS-C1010 | Phosphoglycosphingolipids | PI-Cer(d20:1/16:0) | 2.42592 |
| **Primary ID** | **Chemical Class** | **Metabolite Name** | **Contribution Score** |
| LCMS-NEG\_C00546 | Ceramides | N-Palmitoylsphingosine | 2.41226 |
| LCMS-NEG\_C02518 | Medium-chain keto acids and derivatives | ferulic acid | 2.39676 |
| LCPOS-C3624 | Stigmastanes and derivatives | 20:0-Glc-Sitosterol | 2.34983 |
| LCPOS-C6241 | Glycosyl-N-acylsphingosines | Lactosylceramide (d18:1/12:0) | 2.33023 |
| LCPOS-C6509 | Indoles | 1H-Indole-3-carboxaldehyde | 2.26352 |
| LCPOS-C2695 | Phytoceramides | Cer(t18:0/16:0) | 2.25276 |
| LCMS-NEG\_C02063 | Ceramides | N-acetylsphinganine | 2.21767 |
| LCPOS-C1930 | Phytoceramides | N-(2-hydroxytetracosanoyl)phytosphingosine | 2.20481 |
| LCMS-NEG\_C01242 | Long-chain fatty acids | 21-hydroxyhenicosanoic acid | 2.16187 |
| LCPOS-C6430 | Dipeptides | Tyrosyl-Leucine | 2.14652 |
| LCPOS-C0668 | Indoles | Indole-3-acrylic acid | 2.09673 |
| LCPOS-C3758 | Secondary alcohols | N-(1,3-dihydroxyoctadeca-4,8-dien-2-yl)-2-hydroxyoctadecanamide | 2.09105 |
| LCPOS-C0429 | Phosphosphingolipids | SM(d18:0/26:1(17Z)) | 2.05555 |
| LCPOS-C2636 | Fatty acid esters | 1-O-stearoyl-Cer(d18:1/16:0) | 2.03242 |
| LCMS-NEG\_C00399 | Long-chain fatty acids | (9S,10S)-9,10-dihydroxyoctadecanoate | 2.00613 |
| LCPOS-C5037 | Phytoceramides | N-docosanoyl-4-hydroxysphinganine | 1.98409 |
| LCMS-NEG\_C09663 | Alpha amino acids and derivatives | Imidazolone | 1.97003 |
| LCPOS-C1253 | Monomethylphosphatidylethanolamines | PE-NMe(16:0/16:0) | 1.9627 |
| LCPOS-C0505 | Ceramides | N-2-hydroxylignoceroylsphingosine | 1.90736 |
| **Primary ID** | **Chemical Class** | **Metabolite Name** | **Contribution Score** |
| LCPOS-C4029 | Purine nucleosides | N(6),N(6)-dimethyladenosine | 1.84748 |
| LCPOS-C1754 | C20-gibberellin 6-carboxylic acids | Gibberellin A12;GA12 | 1.82265 |
| LCPOS-C0145 | Stigmastanes and derivatives | 20:1-Glc-Stigmasterol | 1.81647 |
| LCPOS-C0512 | Glycosyl-N-acylsphingosines | 1-(beta-D-galactosyl)-N-[(2R)-2-hydroxybehenoyl]sphingosine | 1.73269 |
| LCMS-NEG\_C03446 | Glycosyl-N-acylsphingosines | Galactosylceramide (d18:1/14:0) | 1.71445 |
| LCPOS-C0333 | Phosphosphingolipids | SM(d18:0/16:1(9Z)(OH)) | 1.68126 |
| LCPOS-C6536 | Dipeptides | Phenylalanylphenylalanine | 1.67674 |
| LCPOS-C3819 | Tocotrienols | epsilon-Tocopherol | 1.65567 |
| LCPOS-C3022 | Medium-chain fatty acids | 12-aminododecanoic acid | 1.60612 |
| LCMS-NEG\_C02469 | Linoleic acids and derivatives | 13(S)-Hydroperoxylinolenic acid;(13S)-Hydroperoxy-cis-9,15-trans-11-octadecatrienoic acid;(13S)-HPLA | 1.59762 |
| LCPOS-C0399 | Oligosaccharides | Oligosaccharide 3 | 1.57226 |
| LCPOS-C0284 | Fatty acid esters | 2-Octenoylcarnitine | 1.56618 |
| LCPOS-C2707 | Glycosyldiacylglycerols | 1-18:2-2-16:0-monogalactosyldiacylglycerol | 1.56598 |
| LCMS-NEG\_C00967 | Long-chain fatty acids | 2-hydroxynonadecanoic acid | 1.50202 |
| LCPOS-C3043 | Alkylglucosinolates | Glucocamelinin | 1.48649 |
| LCMS-NEG\_C00456 | Long-chain fatty acids | 9,12,13-TriHOME | 1.48124 |
| LCMS-NEG\_C01640 | Phytoceramides | N-(2R-Hydroxydocosanoyl)-2S-amino-1,3S,4R-octadecanetriol | 1.44073 |
| LCMS-NEG\_C00705 | Phytoceramides | (4S)-N-(2,3-dihydroxytetracosanoyl)-4-hydroxysphinganine | 1.43688 |
| LCPOS-C1188 | Glycosyldiacylglycerols | 1-18:3-2-18:3-monogalactosyldiacylglycerol | 1.43513 |
| **Primary ID** | **Chemical Class** | **Metabolite Name** | **Contribution Score** |
| LCMS-NEG\_C01094 | Long-chain fatty acids | 15(16)-EpODE | 1.43368 |
| GC-C185 | Amino acid compounds | Alanine | 1.41711 |
| LCPOS-C3510 | Long-chain ceramides | N-(2-hydroxyoctadecanoyl)eicosasphinganine | 1.39094 |
| LCPOS-C2979 | Methoxyphenols | 3-Methoxytyramine | 1.38285 |
| LCMS-NEG\_C01191 | Ceramides | N-(1,3-dihydroxytricos-4-en-2-yl)-2-hydroxyhenicos-3-enamide | 1.38103 |
| LCPOS-C4762 | Isoflavonoid O-glycosides | Isoflavonoid O-glycosides | 1.35819 |
| LCMS-NEG\_C03135 | Short-chain keto acids and derivatives | Alpha-ketoisovaleric acid | 1.34756 |
| LCPOS-C0407 | Hydroxycinnamic acid glycosides | 1-O-Sinapoylglucose | 1.30957 |
| LCPOS-C2100 | Secondary alcohols | 5,8,11,14,17,20,23,26-octamethyl-4,7,10,13,16,19,22,25,28-nonaoxadotriacontan-2-ol | 1.28535 |
| LCPOS-C4636 | Quinoline carboxylic acids | 3-(carboxymethyl)-2-quinolinecarboxylic acid | 1.27247 |
| LCMS-NEG\_C02436 | Tocopherols | Alpha-Tocopherol | 1.26741 |
| LCPOS-C1926 | Phosphoethanolamines | PE-Cer(d15:2(4E,6E)/24:0(2OH)) | 1.26153 |
| LCPOS-C0744 | Phytoceramides | N-eicosanoyl-4-hydroxysphinganine | 1.24138 |
| LCPOS-C1370 | Methoxypyrazines | Methoxypyrazine | 1.20577 |
| LCPOS-C0953 | Glycosyldiacylglycerols | 1-18:1-2-16:0-digalactosyldiacylglycerol | 1.18967 |
| LCPOS-C0843 | Epigallocatechins | 4',3''-di-O-methylapocynin-D | 1.16556 |
| LCPOS-C5137 | Phytoceramides | N-octadecanoyl-4-hydroxysphinganine | 1.15179 |
| LCPOS-C2482 | Fatty aldehydes | Docosanal | 1.14087 |
| LCPOS-C1173 | Long-chain ceramides | Cer(d18:0/18:0) | 1.13764 |
|  |  |  |  |
| **Primary ID** | **Chemical Class** | **Metabolite Name** | **Contribution Score** |
| LCMS-NEG\_C11218 | Hexoses | D-Glucose | 1.11974 |
| LCPOS-C1818 | Indolines | 3-hydroxyindolin-2-one | 1.11782 |
| LCMS-NEG\_C11180 | Hydroxybenzoic acid derivatives | 4-Hydroxybenzoic acid | 1.08993 |
| LCPOS-C2655 | Phosphosphingolipids | N-nonadecanoylsphinganine-1-phosphocholine | 1.06915 |
| LCPOS-C5534 | Pyrimidinecarboxylic acids | ethyl 2-amino-4-hydroxypyrimidine-5-carboxylate | 1.06407 |
| LCPOS-C1966 | Phytoceramides | N-(11Z)-icosenoylphytosphingosine | 1.04337 |
| LCPOS-C0266 | Stigmastanes and derivatives | 22:2-Glc-Sitosterol | 1.03404 |
| LCMS-NEG\_C00979 | Oligosaccharides | Maltotriose | 1.02148 |
| LCPOS-C2662 | Linoleic acids and derivatives | DG(18:3(6Z,9Z,12Z)/18:2(9Z,12Z)/0:0) | 1.00793 |

| **Table 5– Metabolites contributing to variation in Month 12 – Maltster B** | | | |
| --- | --- | --- | --- |
| **Primary ID** | **Chemical Class** | **Metabolite Name** | **Contribution Score** |
| LCPOS-C1554 | Stigmastanes and derivatives | 22:0-Glc-Stigmasterol | 2.88348 |
| LCPOS-C4020 | Pyridinecarboxylic acids | 1-(3-methoxypropyl)-4,6-dimethyl-2-oxo-1,2-dihydropyridine-3-carboxylic acid | 2.31752 |
| LCPOS-C0250 | Medium-chain fatty acids | decanedioic acid | 1.73011 |
| LCMS-NEG\_C02484 | Methylpyridines | (S)-Actinidine | 1.72945 |
| LCMS-NEG\_C01547 | Ceramides | Cer(d18:0/26:1(17Z)) | 1.70056 |
| GC-C064 | Dicarboxyclic Acids | Propanedioic acid | 1.31639 |
| GC-C016 | Fatty Acids | Palmitic Acid | 1.00597 |
| LCPOS-C1978 | Linoleic Acids | DG(16:0/18:2(9Z,12Z)/0:0) | 1.00096 |