THESIS

INFLUENCE OF BARLEY GENETICS ON BEER CHEMISTRY, FLAVOR, AND FLAVOR STABILITY

Submitted by

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In partial fulfillment for the requirements

For the Degree of Master of Science

Colorado State University

Fort Collins, Colorado

Fall 2017

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ABSTRACT

INFLUENCE OF BARLEY GENETICS ON BEER CHEMISTRY, FLAVOR, AND FLAVOR STABILITY

In the brewing industry, identifying superior ingredients that provide distinct flavors is an important area of research. While the contribution of raw ingredients such as yeast and hops to flavor is well understood, it is currently unclear if different genotypes of barley provide unique flavor to beer. In brewing, barley is malted to provide saccharides and enzymes for fermentation, however the malt also contains thousands of metabolites that may influence flavor. The goals of this study were to determine (*i*) if there would be metabolite differences among six commercial barley genotypes, (*ii*) if differences in barley chemistry are reflected in the chemistry of the beer, (*iii)* if the differences in the beer chemistry impact sensory attributes of beer, through flavor and flavor stability, and (*iv*) if there are barley and/or malt metabolites that can be markers for beer flavor and/or flavor stability. Six distinct malts were brewed into six beers using a recipe designed to evaluate differences in flavor. The malts were derived from the barley genotypes: Copeland, Expedition, Full Pint, Meredith, Metcalfe and PolarStar were grown and malted in either Canada or the U.S. Metabolomics was used to characterize chemical variation among the six malts and beers using RP-UHPLC-MS, HILIC-MS (non-volatile metabolites), HS/SPME-GC-MS (volatiles), and ICP-MS (metals). The metabolomics analysis detected 5,042 compounds in malt, and 217 were annotated as known metabolites and included amines (20 metabolites), amino acids (36), fatty acids/lipids (40), sugars (11), phenols (30), and others (80). A total of 4,568 compounds were detected in beer and included 246 annotated metabolites and included amines (9), amino acids (37), fatty acids/lipids/fatty acyls (28), sugars (10), phenols (20), esters (89), aldehydes (21), others (31). The chemical profiles of the six malts and beers were evaluated for metabolite variation using principal component analysis (PCA) and analysis of variance (ANOVA). Principal component analysis was conducted on the annotated metabolites and demonstrated that each of the six malts and beers contained unique chemical profiles. ANOVA characterized 150/217 malt metabolites (69.1%) and 150/246 beer

metabolites (60.9%) varied among genotype (ANOVA, FDR adjusted $p < 0.05$). The six beers were evaluated for flavor using a modified Quantitative Descriptive Analysis® (QDA) for 45 sensory traits at 0, 4, and 8 weeks of storage at 13 °C. PCA characterized flavor differences among the six beers at 8 weeks and Full Pint was described as fruity and Meredith as corn chip. The metabolite and sensory data were integrated using two approaches: Spearman's correlation and two-way orthogonal projection to latent structures (O2PLS). The analyses revealed associations between fruity or corn chip flavor in beer with beer purines/pyrimidines, volatile ketones, amines, and phenolics; and malt lipids, saccharides, phenols, amines, and alkaloids. Taken together, these data support a role of barley metabolites in beer flavor and flavor stability. As a raw ingredient, malted barley genotypes should be evaluated for a contribution to flavor, and this may be a future target for plant breeding efforts to selectively improve flavor and flavor stability quality in beer.

ACKNOWLEDGEMENTS

I want to begin this by thanking the person who was most responsible for my returning to graduate school to follow my dream of obtaining my M.S. and Ph.D. This person, my best friend in the whole world for almost 20 years, has since passed, but he is always in my thoughts and although this degree is for me and my future, it is very much in his memory that I persist and continue his wish for me.

There have been so many supportive people throughout this journey. My mother, an inspiration to all who meet her, has instilled me with a drive that never quits and always creates opportunities in the face of adversity, she has always encouraged my wild inclinations. My stepdad, the source of all that is logical and Vulcan talked me through so many of my darkest and my lightest moments. Big Jer has always provided us with a home and a safe place. My sister, who shares my sense of wonder, always craves adventure, and is always up for a good time.

I am incredibly grateful to have crossed paths with Dr. Adam Heuberger. Forever passionate about his work, I have definitely considered him "work dad" because he provides a balance of sage wisdom and the ability to let his fledglings fly with their ideas and creativity. He has been a true mentor to me, shared his vision and knowledge, and has been fatherly when it really mattered, for which I am forever thankful. I am also grateful for the committee members that have, in the past, been my instructors and colleagues: Drs. Marisa Bunning, Jack Avens, Corey Broeckling (whom no one will ever be as smart as, according to everyone in the Heuberger Lab), and Martha Stone. Each of them was in my corner, every step of the way.

Two very important and extraordinarily incredible people that have given me so much of their undivided time and wisdom to teach me – Drs. Jacqueline Chaparro and Amy Sheflin. I cannot imagine life without their invaluable insight, guidance, knowledge and their friendship. My lab mates in both the Heuberger and Jahn Labs have not only been family and drinking (therapy) buddies, but also have been indispensable resources and a wealth of knowledge. The entire staff of the Proteomics and Metabolomics

Facility, I owe many thanks and many drinks to for their tireless assistance, guidance, and figuring out "where it all went wrong" so that we could get it right.

I feel incredibly fortunate to have the support network for this endeavor that I do. I am constantly surrounded by such inspiring and talented women and men who strive to make this world a better place and encourage me to be more to the world, to do greater things, to not ever be satisfied with being average. Finally, I would like to thank the person in my life that has shown so much compassion, understanding, willingness, encouragement, and love during this whole tear-filled, emotional, very busy journey; the man who took on not only me and my drama, but furthering his own education at the same time, and remodeling an entire house to make us a home. Steven - thank you for all your support, love and tequila shots. I hope you read more than just the headline in this section.

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Background of the Brewing Industry

1.1.1. The brewing industry is important to the U.S. economy

The brewing industry is an important contributor to the U.S. and global economy and spans agricultural, food, and business sectors. The industry encompasses cereal grain growers, maltsters, brewers, engineers, and others who work together to produce, distribute, and sell beer to billions of consumers worldwide. A study released in 2014 demonstrated the brewing industry to have an estimated $101.5 billion impact to the U.S. economy and provided more than 424,000 jobs [1, 2].

Nationally, the U.S. now has as twice as many breweries as it did prior to prohibition, yet serving many more people. In 2017, there were an estimated 5,300 breweries in the U.S, experiencing a 16% yearly growth rate after 2010 [1, 2]. The brewing industry in Colorado is greatly expanding. There are over 330 breweries which currently require approximately 200,000,000 million pounds of barley to be malted and brewed into a beverage which not only contributes dollars to the economy, but also provides employment to thousands of people who work either directly or indirectly for the brewing industry [1, 2].

1.1.2. The brewing industry is divided between large-scale and craft production

The brewing industry is further defined by the number of barrels a company produces each year. Craft brewing is classified by annual production of less than 6 million barrels of beer per year. Large breweries such as MillerCoors or Anheuser-Busch produce at a significantly larger scale and for a global market. Approximately 99% of U.S. breweries are craft (5,234/5,301) and provide more than 125,000 jobs. There has been a 17% increase in craft breweries since 2015 with $19.6 billion being contributed to the national economy. This amounts to craft beer contributing 11% of the total volume produced and 19% of the dollar sales [3].

In addition to production volume, craft and large-scale breweries have developed different styles of brewing. Due to the need for increased volume and the demand for "light" beer, large-scale brewing typically involves brewing with adjunct cereal grains (*e.g.* rice, wheat, corn) in addition to barley. "Light" beer can be described as lower alcohol by volume (ABV) and less carbohydrates and calories due to reduced soluble mass (*e.g.* metabolites) per unit of product (*i.e.* water). The reduced requirement for carbohydrates or soluble material for yeast reduces the need for a protein- and saccharide-rich component to be used as 100% of the grain source. In contrast, when a protein increase is needed without the additional carbohydrate, corn is used as a portion of the grain source [4, 5]. This is referred to as "adjunct brewing," in contrast to 'all malt' brewing that is common in the craft portion of the industry. Therefore, large-scale and craft have slightly different supply chains and utilize different types of ingredients in their major products. In addition, the U.S. craft brewing industry has developed a focus on sustainable production [2, 6], diversity in flavor profiles, and seasonal brews with the caveat that this type of craft beer production is dependent upon barley crops that change with the season and are affected by yearly trends such as hail, drought or pathogens [7-9].

1.1.3. Beer brewing involves the fermentation of cereal grains to form a beverage

Brewing beer involves fermentation reactions that convert extracts from cereal grain sugars into alcohol. Metabolites from cereals such as barley and wheat (the 'mash') are extracted using hot water. This extract (the 'wort') is separated from the grains with help from a natural filtration method involving slowly draining the wort through the compact husks of the spent grains and then boiled with botanicals or other ingredients (*e.g.* hops, spices). This liquid is chilled and moved into a vessel where it awaits the addition of yeast, which utilizes the wort and its extracted metabolites (*e.g.* sugars and other nutrients) as a growth medium.

The yeast culture grows and ferments the liquid into beer. The final chemical makeup and flavor profile of beer is influenced by, for example, adjusting time and temperatures during different stages of the entire brewing process. An example of this influence is the difference between ale and lager yeasts

(*Saccharomyces cerevisiae* and *S. pastorianus*, respectively), which are two different species of brewing yeast. Ale yeasts ferment at warmer temperatures (~ 24 °C) and fermentation for a lager yeast occurs at much cooler temperatures (+/-10 °C) [5]. Lager yeasts, which are put through the fermentation process at higher temperatures can experience increased yeast cell death and lead to an off-flavor called "yeast bite" [10, 11], undesirable esters, and increased diacetyl (butterscotch) flavors. Yeast bite is a term that describes the different undesirable flavor and aroma characteristics that are directly related to lagering (the process of cold storing, which can effectively be done to ales and lagers) yeast at a temperature which is too high. The flavors and aromas associated with yeast bite have been described variously as smelling like rancid fats (cheese, soap, vomit) or beef or chicken soup or bullion, tasting of fatty acids, rotten ingredients, having a rubbery or sulfuric stench, and imparting a sour, bitter taste to beer [12-14].

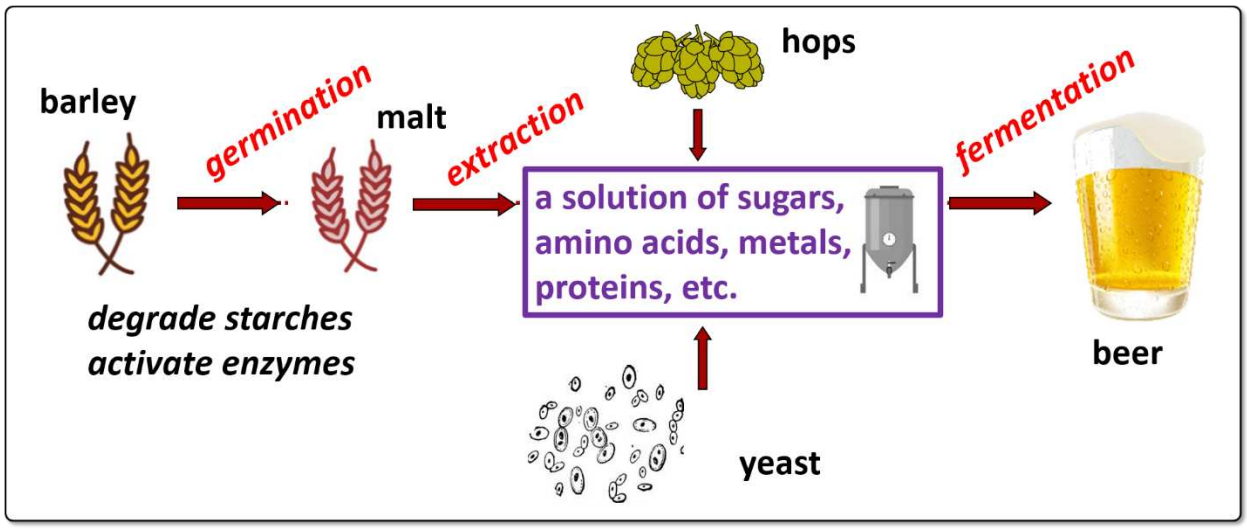


Figure 1a. Schematic of brewing process. This process involves four main ingredients – barley, yeast, water, and hops.

1.2. Raw Materials and Their Specifications Used in Brewing

Four main ingredients are required to produce beer: water, yeast, cereal grains, and hops. Beer usually contains 91-98% water. The amount of water used is upwards of 30 times the amount of beer produced. In the past, beer flavor was heavily influenced by the mineral content and purity of the local water [5, 15]. Now, much attention is given to water treatment and reuse for the brewing industry. The botanical ingredient hops (*Humulus lupulus L.)* is used in brewing to prolong shelf-life, provide

antimicrobial activity, and to provide specific flavors to the finished beer [16-18]. Hop flavors originate in the resins of the lupulin glands, which contain phenolic metabolites known as alpha-acids (α-acids). The α-acids are isomerized by heat, during the boil stage in brewing, to iso-α-acids, which are the main bittering metabolites in beer [12, 18-20]. In addition, hop glands contain oils with large amounts of terpenes that can provide aromatic attributes to beer flavor. Brewers can use different hop genotypes or brewing techniques to adjust the ratios of the α-acids and terpenes to achieve specific flavor.

Yeast (*S. cerevisiae*), has been used in food production for thousands of years to generate ethanol and carbon dioxide (in breads and brewing). It has always been present, but not recognized until 1680, when Antonie van Leeuwenhoek used a very primitive microscope to note the yeast flocs (groups) in fermenting wort [15, 21]. Two hundred years later (in 1883), the first yeast culture would be isolated by Emil Hansen and used in commercial brewing at the Carlsberg Brewery in Copenhagen [15, 21]. There are two main yeast species used for brewing: *S. cerevisiae* and *S. pastorianus* (*S. carlsbergensis*) [11, 22, 23] which provide the esters and higher alcohols that make beer. Cereal grains are one important ingredient in brewing. Grains are used to provide nutrients for yeast fermentation including carbohydrates and proteins. The contribution of grains to flavor, texture, and aroma will be the focus of this thesis.

1.2.1. Grain crops are an important component of the brewing industry

Cereal grains such as barley (*Hordeum vulgare L.*), wheat, oats, rice, or sorghum have seeds with high saccharide content in the form of starches. This is important to brewing because grain saccharides can provide nutrition for yeast, which perform fermentation reactions producing ethanol and carbon dioxide as byproducts. Further, when grains are milled and heated with water, they undergo biochemical changes which provide necessary nitrogen sources, amino acids, enzymes, and other compounds, such as lipids, for yeast nutrition.

Barley, in the Poaceae family, is a major grain used in brewing. Barley seed is the most "modifiable," because the endosperm is easily broken down to release saccharides, nitrogen, and enzymes as available to yeast. Enzymes, which are proteins that act as catalysts for biochemical reactions, such as

the conversion of complex carbohydrates into simple sugars, must be able to act upon their substrates and convert them into their intended products. The creation of an alcoholic beverage from a cereal grain is an intricate and complex example of the many functions of enzymes that are involved in the brewing process, as there are hundreds of enzymes and substrates that must interact concurrently and with specificity to achieve the appropriate result [24]. Wheat and sorghum are also widely used in brewing, although sorghum is less modifiable and has higher amounts of fats and oils which have the potential to lead to oxidation and early staling factors [11], however it has much less protein and must be supplemented with enzymes for an optimal amount of starch conversion to occur [25].

Oats, corn, rice, buckwheat, and quinoa are also used in brewing to provide saccharides and proteins for fermentation. These grains are brewed in conjunction with barley (they are added to the mash) and called "adjuncts." They were originally added by early American lager brewers to reduce cost, and they also add some desirable characteristics to the beer, such as nuttiness and texture from oats, commonly used in dark beer styles (*e.g.* a thick oatmeal stout). Nonetheless, using too much adjunct in the mash makes it difficult to extract wort due to increased viscosity, creating a ripple effect of slowed "run-off" or collection of the extract (which increases the chances of contamination and off-flavors) and reduced fermentability due to the lack of extract or sugar in the extract [15].

The use of only barley, as the main starch source, to brew beer, is referred to as "all-malt" brewing. Currently, this is mostly performed within the craft beer industry. Importantly, the shift to all-malt beer in the craft brewing industry requires unique properties within the malt. The all-malt brewing method requires malt to have higher extract (*i.e.* fermentable sugars) and reduced protein. There are two distinct varietal classes that are utilized in the brewing industry: "two-row" and "six-row." Two-row vs. six-row phenotypes are defined by the arrangement of kernels on the head of the barley. Six-row typically has higher protein and enzymes and reduced sugars. This is important in six-row because these attributes help to speed up the rate of conversion of sugars. The six-row also has thicker husks, which is important when creating a filter bed in the lautering portion of brewing, but can also contribute to polyphenol haze since the husks are naturally high in polyphenols (tannins) [4, 26-30]. However, the amount of excess

protein in 6-row makes it undesirable to use as the whole portion of carbohydrate. For a majority of the U.S., two-row, which contains more starch for conversion is used either as the total portion of carbohydrate or with adjuncts (*e.g.* corn, oats, rice) to increase the protein content. Two-row, however, lends much less body and flavor to the beer, which is a desirable attribute for most brewers who choose to impart their own flavor with more highly-kilned malts, yeast or hops.

Barley is normally malted for brewing [5]. Malting is the process by which the grain is 'modified' prior to extraction for fermentation (discussed in detail below). Wheat is also sometimes malted to create unique flavors and give different textures to beer. For example, a traditional German Hefeweisen is about 40-50% unmalted wheat and the remaining percentage is barley. Wheat has more protein, so it contributes to foam retention, haze, and a "thicker" mouthfeel. Other grains, such as rice or corn, are added to the mash after a pre-cook, which allows gelatinization of the starch (each grain varies as to the temperature required for gelatinization). Gelatinization and partial hemicellulose degradation must occur for enzymatic action to occur on any cereal grain [27].

1.2.2. Cereal grains provide "extract" in the brewing process

Malt extract refers to the amount of fermentable sugars available for yeast after mashing has ended and the wort is separated from the solids (spent grains that act as a filter bed). This wort is sent to the boil kettle to be boiled with botanicals, spices, or other ingredients (*e.g.* hops). The culmination of useful components (sugars, proteins, *etc.*) in the wort solution is referred to as the extract. One measure of the amount of sugars in the wort is 'specific gravity' (SG). If a wort has a SG of 1.040 at 20 °C (pure water being a SG of 1.000), the concentration of solids is 9.99% w/w (assuming the solids are all soluble monosaccharide in nature). The SG will change throughout fermentation as yeast consume and convert the sugars. There is a balance of solids/sugars and SG that is needed, but it varies among beer styles.

When the SG is too high, yeast do not consume all the sugars, resulting in an overly sweet flavor (although this can be addressed by adding more yeast to the culture). For some styles, that is desirable, as in German Doppelbock, which is traditionally a thick, dark, malty beer that is lightly hopped, not yeast-

forward, and moderately sweet. Monks in Bavaria often used it as nourishment in times of fasting [15]. Alternatively, if the SG is too low and there are not enough sugars for the yeast, more sugar (maltodextrin or honey, commonly) can be added to provide adequate yeast nutrition [5]. If no additional sugar is added, and SG remains low, the yeast will compete for the sugars available, the weaker yeast will die off prematurely and flocculate to the bottom, where they will begin to degrade, leading to unsavory savory off-flavors.

1.2.3. Malt-derived free amino nitrogen is essential for brewing

Free amino nitrogen (FAN) is an essential part of brewing, but can greatly influence the intended flavor of fresh beer and the flavor stability of the beer over time. During aging, remaining nitrogenous compounds tend to form undesirable flavors in the beer. For example, amino acids, such as L-lysine and L-proline, which are absorbed during fermentation at different rates, (L-lysine is absorbed rapidly and L-proline has little to no absorption) also influence the speed of fermentation. A normal fermentation time is about 72-100 hours. A wort that is supplemented with L-lysine can complete fermentation in approximately 48 hours with complete absorption of the L-lysine, but can lead to an increase in vicinal-diketone levels (VDK) in wort, leading to higher 2,3-butanedione and diacetyl (butterscotch, buttered popcorn) off-flavors over time. This is due to the rapid fermentation time that does not allow the VDK to be reabsorbed by yeast [31]. FAN is provided by the malt, and yeast utilize nitrogen-containing compounds to form enzyme and growth proteins. Wort with high FAN tends to produce excess higher alcohols (fusel) and esters that lead to undesirable flavor. After fermentation, excess nitrogen source can produce off-flavors in the beer due to increased esters, aldehydes, or fusel alcohol.

FAN levels are controlled by monitoring changes in grist (grain that has been milled in preparation for the mash step of brewing) composition and seasonal variations of raw materials (*i.e.* barley), to control yeast cultures and overall growth. Most brewing yeast requires approximately 100 mg of FAN per liter extract for adjunct brewing, and 200 mg/L for all-malt brewing to successfully ferment the wort [11, 32]. Traditionally, large-scale adjunct brewers have sought out barley that is higher in FAN.

FAN is therefore important for yeast nutrition – a wort that is lacking in amines can result in a "stuck fermentation" (fermentation that has halted) whereupon the yeast have run out of a nitrogen source and cannot continue to ferment. The resulting beer suffers, as stated earlier regarding raised VDK levels leading to increased production of diacetyl, and the unutilized amines that remain in the beer result in a chill haze (undesirable in most beers). Excess FAN can produce off-flavors such as meaty, hot-dog, umami flavors, as well as reduce the stability of the flavor over time [31].

Methionine has also been proposed to influence beer flavor. A study characterized the role of methionine and sulfur metabolites interacting in purine metabolite pathways [33]. Excess FAN levels (*i.e.* unabsorbed methionine) can lead to increased sulphur metabolites through Strecker Degradation of methionine. A study had proposed this pathway involved with the production of 5-methylthioadenosine (5-MTA), a metabolite marker of oxidation flavors in beer [13, 34]. Ethylene, a result of the breakdown of aldehyde and oxidative degradation of polyunsaturated fatty acids or amino acids (*e.g.* lysine or methionine), increases after storage, parallel with 5'-MTA, and both have been associated with staling flavor traits and decreased flavor stability in packaged beer [13, 35].

1.2.4. Malt enzymes are a critical component of the brewing process

Enzymes are critical in brewing and are carefully managed. Several barley enzymes catalyze chemical reactions during brewing. Approximately 40 endopeptidases have been identified in malt [24] which are broadly classified into "cysteine-, metallo-, aspartic-, and serine-proteinases." An example is cysteine-proteinases, also used as meat tenderizers due to the fact that they degrade protein, are the most important endo-proteinase involved in arranging protein during germination. However, although these are abundant in the malt, they have limited action on proteins due to the presence of inhibitor proteins. In the case of cysteine-proteinase, lipid transfer proteins block their access [36, 37]. Managing brewing enzymes is complex, as each enzyme can require a unique optimal temperature and pH in order to activate them. Many of these enzymes perform simultaneously, however, and do not reach their full potential due to the constraints of the temperature and pH. Enzymes are also inactivated at unique temperatures and pH,

resulting in a fluctuating substrate concentration, which leads to the degradation of starch and subsequent gelatinization [4, 24, 32] during the mash stage.

Alpha- and beta-amylase are two of the most widely studied enzymes in brewing. Alpha-amylase has optimal activity at 75 °C, where it solubilizes simple sugars to dextrin (dextrinization), and also limits beta-amylase saccharification. This results in a less fermentable wort, leaving a pool of un-fermentable sugars in the wort. This affects the quality of the wort and subsequent beer due to the abundance of unfermented residual sugars. During mashing, when most enzymes are activated/inactivated and act upon their substrates, measurements of temperature and pH are taken somewhat consistently, yet those are rarely constant. At any point, a particular set of enzymes is activated, forming a product until the substrate is reduced, then inactivated. The amounts of any given products formed relies on the catalysis rate and the rate at which the enzyme is inactivated, which is all temperature-dependent.

As stated earlier, the temperatures and pH constants that the enzymes require, are not always consistent with what occurs during mashing to achieve the optimum activity of the more well-known and necessary enzymes. The temperatures during mashing can affect the status of the wort and beer in the end. Temperatures that are too hot do not allow sufficient time for enzyme catalysis, leading to less of a product. Cooler temperatures do not allow some enzymes to activate, thereby leaving some products out of the mix. The final result is fewer fermentable sugars and a less desirable beer [4].

1.2.5. Malt β-glucans can be valuable during malting and brewing

The germination of the barley grain during malting results in the activation of many enzymes that convert the starch in barley into simple sugars. Beta-(β)-glucans are sugars that are found in the cell wall of the barley grain. They affect the extract yield, mashing and filtration efficiency, and excess beta-glucan can result in haze, which means a foggy, unclear beer (usually undesirable for most styles except those with wheat added, such as a Hefeweisen) and/or flavor defects in beer, such as a “Band-aid®” aroma and taste. Beta-glucans make it into the beer if β-glucanases are not activated (temperatures over 60 °C deactivate β-glucanase) or if the malt is poorly modified during the germination portion of malting [38].

Starch and protein degradation are essential for a clear wort and a resulting beer that is free of off-flavors or textures created by β-glucans.

1.2.6. Free fatty acids in malt are important components of flavor and foam

Although not a major component of beer, free fatty acids (FFA) are considered undesirable in the finished product. FFAs are a "foam-negative" compound, relating to their surface absorption tendencies upon interaction with foam-positive proteins [32, 39]. Medium chain fatty acids such as hexanoic, octanoic, and decanoic acid can result in off-flavors as "rancid, vomit, goat-like, cheese-like." These volatile off-flavors are formed by the yeast during fermentation [40-42]. However, long-chain unsaturated fatty acids, such as linoleic and linolenic acids, are more often derived from malt and lead to the formation of staling off-flavors (lipid oxidation) in beer. Saturated fatty acids (*e.g.* palmitic and stearic) from malt are also related to gushing (spontaneous foaming over when beer is opened). Trans-2-nonenal, a common staling compound in beer is formed from oxidized lipid components. Lipid oxidation in malt has also been reported to cause lautering (filtering) problems during the brewing process [43]. In recent years, an effort to create low-lipoxygenase malt (LOX-less) has been made. The kilning process affects the lipoxygenase in malt and its ability to oxidize lipids. Fatty acids in barley and malt also play a role in the amount of extract obtained from brewing and the attenuation (the percentage of sugars converted into alcohol and carbon dioxide by fermentation) [44].

1.3 Brewing Styles Require Different Ratios of Raw Materials

1.3.1. Craft brewing relies on all-malt brewing, but consistency and beer quality remain a challenge

All-malt (all-grain) brewing is the process of creating a beer using only barley, without the addition of adjuncts, sugars, or additional fermentable carbohydrates. Craft brewers have trended towards all-malt brewing for the creation of additional flavors, textures, colors, and aromas it provides. However, being all-malt, the desired organoleptic traits can easily turn to detriments if not controlled properly with

times and temperatures before, during, and after every process. The compounds that are featured (or minimized) during an all-malt brew are variable, depending on the barley genotype, malting process, and brewing procedure.

There are four main quality factors that encompass beer quality: appearance, mouthfeel, taste, and aroma. Together, taste and aroma contribute to the overall flavor of the beer. These organoleptic attributes are all variable and depend on style or consumer trends [5]. All-malt flavor is largely influenced by malt type (*e.g.* pale vs. dark malts) and creation of novel flavors is important to brewers and consumers, alike. The beer industry is driven partially by understanding how raw materials and brewing techniques create variation in quality and flavor. Variation in flavor can be achieved by using different strains of yeasts and by adding botanicals at various stages of brewing. For example, *S. cerevisiae* (brewing yeast) can be influenced in many ways by times and temperatures to give an ale or lager its desired style traits. For example, a Hefeweisen, is created using a *S. cerevisiae* strain that produces high levels of isoamyl acetate esters and 4-vinylguaiacol phenolic compounds. These compounds produce banana and clove like aromas, respectively.

In contrast, the India Pale Ale beer style is less defined by yeast and more due to the prevalence hops which produce highly floral, pine, or citrus aromas due to the extraction of essential oils from the lupulin glands of the female flower. Hop oils, volatile metabolites, are made up of a hydrocarbon fraction and an oxygenated fraction (and some sulfur-containing compounds which have been, to date, less studied). The hydrocarbon fraction contains the terpenes – myrcene and β-pinene (contribute pine, grapefruit, and grassy constituents) and sesquiterpenes – β-caryophyllene and α-humulene (contribute flowery, citrus, grassy and pine notes). Also included in the hydrocarbon fraction are alcohols such as linalool (contributes lemony citrus/fruity constituents typical of "Froot Loops" cereal) and geraniol (contributes a geranium, metallic constituents), as well as the esters geranyl isobutyrate (contributes sweet, floral, and fruity notes) and methyl-dec-4-enoate (contributes a fruity, plum-like, or floral note) [15, 18, 45].

The source of hop bitterness, α-acids, is concentrated in the resin glands of the hop flowers and considered the non-volatile metabolite portion of hops [45]. Isomerization of α-acids (*e.g.* humulones) to iso-α-acids (*e.g.* isohumulones) occurs when hops are boiled during brewing and serves to provide not only the bitter taste, but stabilization to beer foam and protection against microorganisms [45]. Hops were traditionally used as a preservation and flavoring method for beer. They have extraordinary bacteriostatic activity and inhibit the growth of Gram-positive bacteria [45]. Hops replaced other herbals (an herbal mixture called "gruit") which did not lend as much flavor or necessary anti-microbial properties. Today, there are over 100 genotypes of hops used in brewing, and their chemical profile can be influenced by the growing environment, the microbial flora, weather, and cultivation practices [12, 18-20]. For example, in England, lengths of string are used to trellis the hops on hillsides. Depending on the angle of the slope of the string, you may have a very different cone yield and much different concentrations of essential oils (lupulin) compared to someone on the other side of the hill [8, 10, 21, 46].

The type of malted barley used in brewing also affects the type of beer. Using a combination of paler (less roasted) malts will yield a lighter Pilsner-type beer, whereas using "dark chocolate" malts is preferred to give stouts their color and roasted, chocolate flavors.

Upon receiving a load of malt, the brewer receives a Certificate of Analysis (COA). This COA is the brewer's guarantee from a malthouse that the malted barley received meets the specifications required and that certain criteria are met for protein, diastatic power (which is the measure of the enzymatic (starch-converting) power of the malt), color, and many other traits. This COA, however, does not inform on future flavor or stability of the product, and malt is often stored for several months. It is highly variable how the COA can be interpreted, based on a brewer's experience with the malt and the malt's performance given a specific brewing method. For example, some brewers choose step-mashes based on the malt they are using and for which beer they are brewing, which consider various enzymatic activation times and temperatures, but this can also lead to astringency in the wort. Other brewers may not choose this method based on their own experience with that particular malt.

The COA informs on total extract and protein but it does not specify the composition of these chemical classes. This is importantbecause yeast can have a preference for certain sugars and proteins, and this can affect fermentation [31].

1.3.2. Malt type and flavor depend on barley chemistry during the malting process

Malt type and flavor vary by barley genotype, the location the barley was grown (*e.g.* Canada or Montana), the growing conditions (*e.g.* arid or temperate), the malting conditions (times and temperatures of each stage) and what specifications the barley is malted to for the brewery and purpose it is intended [28]. Examples of malt types include Pale Malt, which is the base malt for all-grain brewing. All-grain brewing refers to the percentage of whole, milled cereal grains used in the brewing process. All of the starch that is needed will come from the grain (barley, in this case), which is crushed in mill and malted. In contrast, extract brewing is the creation of wort by dissolving a malt extract (malt that has been already through the mashing stage and has been through the sugar-conversion process) in boiling water. All-grain brewing requires proper assessment of the barley prior to milling or brewing and special attention to the mashing and boiling process during brewing. No adjuncts are used in all-grain brewing; therefore one must make certain the base malt (pale malt) used contains enough nutrients, starch, and protein for the brew. For example, Vienna Malt, which is kiln-dried at higher temperatures, is slightly darker in color and more complex in flavor, but still maintains sufficient enzymatic power (diastatic power) to convert starch into sugar for the yeast. Darker malts, roasted chocolate malts intended for stouts and porters have much less sugar and are only meant to add flavor (coffee, roasted, chocolate) and aroma to the beer [4, 5, 11, 27, 47]. "Malt type" however, is usually created by pooling barley into a single sample, without discriminating based on barley genotype or its growing environment [27].

Barley has several grain chemistry requirements to be accepted by a malthouse. Moisture must be below 13.5% to prevent mold growth and mycotoxin accumulation in stored grain [32]. In addition, acceptable nitrogen levels and protein levels must be maintained (1.8-2.0% and 9.5-12.5%, respectively), kernels must be plump and uniform, damaged kernels must be below 5%, and it must be free of disease

(such as fusarium head blight, a destructive fungal disease of barley associated with mycotoxin contamination which causes the seed to become unusable). These specifications of initial quality are critical to the malting process. Each malting barley behaves differently during the malting process and in the future, it may be important to differentiate among genotypes, with regard to flavor attributes.

1.3.3. Protein content of barley affects malt and beer composition

Protein content of the barley grain affects the metabolomic composition and enzyme levels of malt, which is important for the brewer to be aware of, as high protein limits starch degradation, affects mouthfeel, foam stability, and decreases extract available to the brewer. Low protein limits enzymatic activity and modification is difficult. Hot and dry environments (such as in Colorado) tend to result in higher protein [35].

1.4. The Malting Process is Complex and Important to Brewing and Flavor

Malt is produced in three steps: steeping, germination, and kilning, a schematic which is shown in Figure 1b.

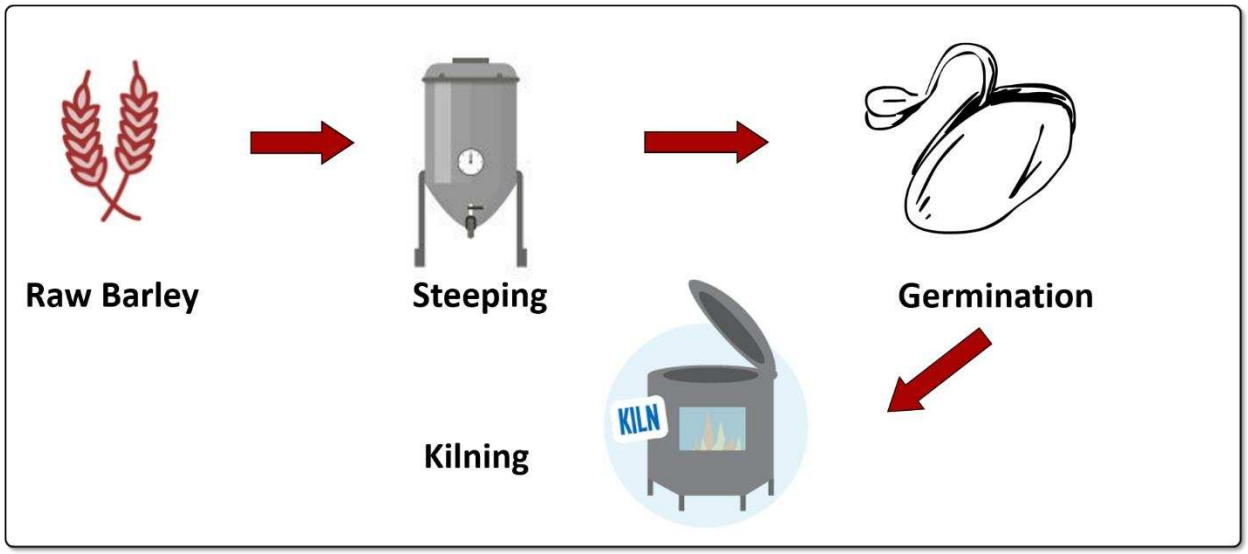


Figure 1b. Schematic of the malting process. Malting involves three main steps: steeping, germination, and kilning.

How these steps happen is influenced by the barley phenotype and has a major impact on flavor and flavor stability during brewing. The metabolites in malted barley (amines, sugars, amino acids, fatty acids) and the interactions they have with subsequent ingredients influence the flavor in beer. Steeping, the first step in malting barley, involves partially immersing dried barley grain in water (at 14-16°C) to increase moisture content (to a final moisture content of 42-48%) which stimulates germination. This stage takes 24-48 hours and requires turning and venting of the steeping grains (for oxygen replenishment and carbon dioxide release) [27].

Depending on the maltster, time of year, and barley genotype, these stages often vary. Once the "chit" (coleorhizae) has emerged from the grain, the barley is moved to germination beds. These beds maintain a specific temperature (between 16-20 °C) and aeration level (usually by auger) with the goal of stimulating enzymes that initiate endosperm modification, or protein degradation and hydrolyzation of starch. There are a number of enzymes released into action during germination. Enzymes to degrade β-glucan (Beta (β)-glucanase), for example. Excess β-glucan increases viscosity in wort and is an undesirable characteristic in beer. During the starch modification that occurs during germination, alpha- and beta-amylase are both initiated. These are essential enzymes which break up amylopectin and amylose. This break-up of amylopectin and amylose result in glucose chains of varying length, glucose, maltose, maltotriose and other saccharides for future consumption by yeast during fermentation.

Kilning is the last stage and is highly variable. Over the course of 24-30 hours, the now "green" malt is dried and cured. Kilning temperature is incrementally increased over the course of several hours to achieve they style of malt desired [32]. It is imperative to know the composition of barley when malting. Over-or under-modification can result in beer with too much or too little protein (resulting in underdeveloped yeast or off-flavors in aged beer) or too much or too little starch/sugar (which results in either sweet beer or hungry yeast) [4]. Kilning also influences the rate of lipid oxidation in malt. Lipid oxidation causes the formation of detrimental characteristics in barley and beer. For example, the development of trans-2-nonenal (cardboard off-flavor/aroma) is due to lipid oxidation in malt [43].

1.5. Previous Studies Support Significant Biochemical Variation among Barley Genotypes, within Malt Type

According to previous studies, further research would be required to determine the source of both volatile and non-volatile metabolites in beer, as several types of purines (which are nitrogenous bases which make up the DNA and RNA nucleobases; adenine and guanine are purines) were reported in beer, [13, 34, 35]. Barley and malt metabolites play an important biochemical role in the overall sensory qualities in beer. Metabolites can be defined as small molecules (<1200 Da) which include amino acids, lipids, fatty acids and carbohydrates, amongst others. Metabolites are the reactants and the products of reactions which are consumed and created during enzymatic actions. [35]. Metabolites can provide a residual fingerprint of metabolism at specific time points and this fingerprint can be used to establish the intricacies involved in the brewing process, fermentation of and eventual creation of a beverage from barley.

1.5.1. Barley chemistry is critical for brewing efficiency and beer quality

The chemistry of barley influences brewing parameters and the final beer quality. Recent studies have demonstrated that small molecules in barley grain are highly variable among barley genotypes, and that interactions between barley genetics and environment (GE) further influence the chemistry of the malt [4]. Malting quality is based on both differences in protein structures of barley (which tells us there is variation in enzymatic action) and the expression of the genes involved [48]. The metabolomics approach, which is essentially a study of the unique chemical fingerprint left behind by cellular processes, allows for deep study into these processes and the small-molecule profiles. Using this approach allows us to understand the mechanisms and indicators of certain biochemical processes, such as determining the compounds responsible for certain flavor traits in beer and the routes that these compounds take to arrive at the point where a staling/off-flavor is created [8, 34, 49-52].

1.5.2. Phenolic compounds in barley and malt affect beer flavor

Hordatines are a group of anti-fungal, phenolic compounds typically found in barley and wheat. They have been studied due to their antifungal activities against plant pathogens [53]. It has been determined that although the hordatine content does not differ according to style of beer, this secondary metabolite that is a defense system for the plant does contribute to flavor and influences human gut metabolism [54]. These particular compounds, hordatine β-glucosides, are also related to the astringent aftertaste in beer [36].

1.6. Beer Metabolomics

Metabolomics studies have recently been utilized in investigations to identify new markers of quality traits to breed superior plant lines. A study was performed on 72 lines of barley using non-targeted LC-MS to determine novel markers for breeding traits [35]. The results of this study suggest that metabolism and quality traits are co-influenced by barley GE factors and demonstrate the usefulness of metabolites as efficient markers of quality traits, suggesting the need for further research into the metabolites and biochemical processes which may contribute to beer flavor and flavor stability.

Given the breadth of variation in malt metabolites due to barley genotype described, there is the potential that malt genotype may also influence the flavor stability of beer [35, 55]. 'Flavor stability' is defined as the ability for beer to maintain its flavor profile over time. The flavor of beer changes with time and temperature. The quality and impact of raw materials is critical to the brewing process and beer flavor stability. Malt plays a key role as the keeper and transporter of precursors for many of the flavor compounds in beer [52]. Metabolomics is being used to determine the stability of beer under certain storage conditions using different hop genotypes [13, 18], to determine varietal differences among barley lines, to determine quality control methods and to track the metabolite changes that occur during the brewing process [56].

1.6.1. Beer quality is important

In the brewing industry, there has been a recent shift due to the advancements and push made by the American Society of Brewing Chemists (ASBC) to educate brewers, maltsters, producers, and consumers about beer science (www.asbcnet.org). Understanding the quality and science behind raw materials and the interactions in the process will increase the quality of beer flavor and flavor stability [26]. Beer possesses organoleptic traits which are discernable by consumers (aroma, taste, appearance, texture) and although metabolomics has been performed by many parties with improved methods, it is still the human sensory which is the most relevant, using the instrumentation as a secondary measurement to validate and correlate compounds with this qualitative process. It is important to understand the science behind the quality parameters involved in brewing and which metabolites and their interactions create flavors that consumers perceive. Otherwise, consistency, accuracy, and "true-to-brand" flavors are nonexistent [57].

1.6.2. Flavor stability in beer is complex

It is important to note that beer is a biologically active product [11]. The chemical composition of beer continuously changes during storage [50, 58]. The focus of recent research has been on the major chemical reactions that occur during storage, such as lipid oxidation, which forms the cardboard-flavor component, trans-2-nonenal. Lipid oxidation is also responsible for the increase in n-hexenal and acetaldehyde and contribute a sweet-solvent or green-apple off-flavor to aged beer.

Strecker degradation (which converts α-amino acids into aldehydes) forms Strecker aldehydes, which are volatile staling compounds such as benzaldehyde (which contributes an almond-like aroma). Heterocyclic compounds are formed by way of manipulation of an oxidation reaction, originating out of the Strecker degradation (Paal Knorr Synthesis), resulting in furans, furanones and nitrogen-heterocyclic pyrazines and pyrroles, which cause formation of harsh, smoke-flavored, phenolic off-flavors during storage [59].

Maillard reactions (chemical reaction between amino acids and reducing sugars) play an important role in flavor and flavor stability. The formation of α-dicarbonyls derived from carbohydrate degradation were discovered to be highly correlated with beer flavor deterioration and contributed to increased bread-like, caramel, burnt notes in the beer [60]. Furfural (2-furfural ethyl ether), another Maillard intermediate, the result of etherification of ethanol and Maillard compounds, is correlated as being higher in beers with high alcohol levels brewed with dark malts (stouts, porters) and results in a typical staling flavor of solvent, harsh, and very bitter [58].

Control and modification to increase the stability of packaged beer is necessary to maintain quality over time. Prolonging shelf-life and promoting flavor stability is a challenge for the industry. There are many places that biochemical reactions happen to induce off-flavors, including the introduction of oxygen, light, or heat. These are factors that are increasingly controlled by the breweries that package beer. Examples of controlling oxygen include oxygen-scavenging liners inside of the caps on bottles and even changing bottling conditions to introduce less oxygen while packaging beer. Control of light and heat is sometimes a challenge after the beer leaves the brewery, as some beer is transported long distances in non-cooled trucks and/or stored/sold where it can be exposed to light.

1.6.3. "True-to-Brand" concept for beer

"True-to-brand" (TTB) is the concept that a product tastes the same every time it is consumed. TTB is important for breweries with flagship beers that are exported or consumed after long periods of time, given beer is not flavor stable. Many times, beer that a brewery creates is meant to be shipped cool, and stored in a dark, cool place. However, circumstances do not always allow for those conditions to be met and beer ends up sitting in a warm place, or in a window-front. This accelerates the aging process, contributes to off flavors (such as cardboard or paper-like tastes and aromas), and decreases the TTB flavor, increasing consumer dissatisfaction [6].

The determination of the aforementioned off-flavor characteristics (cardboard, staling) in beer is a product of sensory analysis by trained panelists and scored according to the appropriate method. For

example, in qualitative sensory analysis, beer is sampled for sensory evaluation at certain time points (*e.g.* at 0, 4, 8 weeks) and served blind to a trained sensory panel. Standardization of samples (time, temperature, volume) is employed to reduce noise and bias. Panelists analyze the samples for taste, aroma, mouthfeel, and appearance and either give a quantitative score for each descriptor (*e.g.* nutty, fruity) or generate a qualitative descriptor for the product [13]. This method relates directly to the determination of TTB for specific brands or styles. Panelists are trained on the control beer and, with the use of flavor standards, trained on what the control beer would taste like with specific off-flavors at different levels, such as diacetyl (buttery) or isoamyl acetate (apple, fruity) at specific time points. This can help a brewery determine what the expiration date should be (or the date that the beer no longer tastes/smells like the beer it was brewed to be).

1.7 Several malt metabolites are known to influence flavor and flavor stability

Malt quality and barley genotype both have the potential to influence beer flavor and flavor stability. Malted barley contributes thiols, purines, amines, fatty acids and phenolics that are known to influence flavor [4, 61]. The amounts of these compounds that are contained within each genotype of malt varies by barley genotype (GE), malting times and temperatures and storage conditions of both barley and malt.

Sulfur-compounds which react with ketones create a cat/goat flavor in beer [57]. Sulfur flavor is desirable in some styles of beer (*e.g.* Saison Farmhouse style), but is generally associated with aging and poor flavor stability. For example, 3-methyl-2-butene-1-thiol (associated with ‘lightstruck’ flavor) is often found in beer which has been exposed to excessive light or aging, caused by a reaction between hop alpha-acids and riboflavin in beer, is easily controlled by proper quality control and monitoring of the compounds.

Many phenolic compounds are created by malts, as well as the malty, sweet, roasty flavors that are perceived [11, 32, 45]. Isobutyraldehyde in malt, an aldehyde, is considered an off flavor of harsh,

raw grain. Although the influence of this aldehyde on flavor mellows with age, it is caused by water that is too hot during the sparging phase (or sprinkling of temperature-controlled water onto the mash to extract the wort for boiling) of brewing, crushing malt too fine, or holding the mash phase for too long causes an abundance of isobutyraldehyde [61]. Acetaldehyde is another malt-derived compound that influences flavor, and is associated with "green apple" or "latex paint." Acetaldehyde is detectable in most beers and generally increases with age. It is a precursor of ethanol produced by yeast during fermentation and should be reabsorbed by yeast later in the process, but if too much oxygen is present in packaging, the ethanol will change back into acetaldehyde [11, 32, 45].

1.7.1. Barley genetics and influence on grain chemistry.

The influence of genotype and environment on barley is important. The chemical components of malted barley contribute in many ways, directly and indirectly, to the energy and nutrients required for brewing and fermentation. As mentioned previously, the two major barley varietals are 2-row and 6-row, each with their own set of traits. Row type represents a defining unit of genetic diversity in barley, as their breeding pools are kept separate. Malting quality is determined by genetic traits and GE interactions [27, 28]. These quality factors can include extract yield, enzyme content, diastatic power, and protein content, *etc.* Phenotypic traits such as protein content (which includes FAN, total malt protein, wort soluble protein, and the Kolbach Index % of soluble/malt protein) is influenced by the environment where it is grown and expressed accordingly.

The metabolites that display co-variation with genetic factors can play a role in the future of barley breeding and identifying biomarkers for certain agronomic and quality traits. For example, quality traits that are important to the brewing industry, such as Beta-glucans, diastatic power, α-amylase, and fine extract all have been correlated with metabolites [4, 11, 27, 30, 44, 62]. However, the full extent of biochemical processes that occur and how these metabolites correlate with the traits are still largely unknown [35].

1.7.2. The difference between barley genotypes and association to beer quality requires further study

Malt houses often do not denote or separate barley genotypes prior to malting. Several genotypes can be pooled and conditions adjusted to produce a final malt with specifications expected by the brewery. Further, the COA does not distinguish which barley genotype has been malted at that time and if it was pooled with other genotypes or not. Further, there are many different microbes that affect the display or retention of certain features in barley, depending on the environment where the barley was grown (*i.e.* Canada or the U.S.) [63]. These microbes affect the expression or suppression of phenotypic traits and are either suppressed or expressed. These factors are all players in the downstream result of how malting and the subsequent malted barley have an effect on beer flavor and flavor stability. The brewing industry may benefit by applying genotype differentiation as a component of malting. Brewers can track barley growing locations, malt houses, the malting quality specifications, how it was ground into grist for the brewing process, the brewing parameters, and how the malt worked within those parameters.

One of the first barley genotypes to claim a genetic contribution to beer flavor is Full Pint. Full Pint, a doubled haploid from parents "Orca" and "Harrington," was developed at Oregon State University to have enhanced agronomic and disease resistance properties [29, 64]. Full Pint contains higher levels of α-amylase, increased diastatic power, and lower protein. According to Briess Malting and American Malting Barley Association (AMBA), the results of flavor trials described Full Pint as having a clean sweetness, very little astringency, tart, bread-like, and salted popcorn-like with above-average foam quality in beer [55].

Meredith, a Canadian barley genotype, has been utilized in large-scale brewing since 1997, but it has only moderate agronomic qualities, poor yield compared to newer varieties, and less-than-desirable disease resistance compared to newer varieties, according to AMBA [55]. Meredith has higher protein content than Full Pint, but lower than much older AC Metcalfe and Copeland, and have lower alpha-amylase and diastatic power than Full Pint, leaving it slow to convert during brewing except in the case of

high-adjunct brews, for which it has been optimized [29, 64].The results of flavor trials describe Meredith as having a light bitter, earthy, slightly sulfur flavor [65].

1.8. Hypothesis and Goals

Previous research supports that barley genetics may influence beer flavor and flavor stability through variation in metabolites. The purpose of this study was to investigate six genotypes of malted barley and the corresponding beers using four different metabolomics platforms (UPLC-MS, ZIC-HILIC, ICP-MS, c) and sensory evaluation. The experimental design was intended to elucidate the influence of GE on the barley and malt chemical composition, and how it contributes to beer flavor and flavor stability. This research investigated the claim that had, until recently been stated by many barley producers and maltsters, that barley is "all the same," in regard to its contribution to the base pale malt created for brewing [13, 34, 35].

Hypotheses:

1. There will be metabolomic differences among barley genotypes;
2. The differences in barley chemistry will be reflected in the chemistry of the final product (beer);
3. Differences in the beer chemistry will impact sensory attributes of beer through flavor and flavor stability;
4. Certain barley and/or malt metabolites can be markers for beer flavor and/or flavor stability.

Goals/objectives:

1. To use metabolomics platforms to evaluate flavor in malt and beer;
2. To evaluate flavor and flavor stability based on sensory and metabolite analysis;
3. To determine beer volatile and/or non-volatile metabolite markers for flavor and flavor stability;
4. To evaluate possible beer markers for aging;
5. To determine the co-varying metabolites regarding how malt genotype affects beer flavor and the associations among metabolites that influence beer flavor and flavor stability.

Chapter 2 – Methodology

2.1. Plant Materials

A total of six malts were selected for brewing in this study that were generated from the 2-row barley genotypes: Copeland, Expedition, Full Pint, Meredith, Metcalfe, and PolarStar (Table 1). All genotypes are widely used for both domestic and craft brewing and are considered interchangeable to produce major beer styles. Further, the malt genotypes were chosen to include four growing locations (Montana and Oregon, U.S.A.; Alberta and Saskatchewan, Canada), and four regional malt-houses Rahr, Malteurop, Briess, and Cargill [66].

Barley, a cultivated cereal grain from the Poaceae family, is a diploid species with 14 chromosomes dating back to about 3000 B.C. Two-row and six-row barley, used for modern brewing, possess spikes which contain flowers and mature seeds and consist of spikelets attached to the central rachis (stem) with the number of florets per rachis (axes) node used to define the row type [67]. In the two-row variety, only the central spikelet develops a fertile flower and seed and in the six-row variety, all three spikelets at each node develop seeds. There are over 150 cultivated varieties in the U.S. of which 50% is used for livestock feed and 25% is malted and used for beer and distilled spirits [4, 55].

2.2. Brewing Method

The beer for this study was brewed at Haas Innovations' "Innovation Brewery" in Washington on a 2.5 hectoliter system. This recipe was developed by Christian Holbrook at New Belgium Brewing Company (Fort Collins, CO) and was designed to be malt-forward for the purposes of this study. Hop addition was limited to 8 international bittering units (IBU) strictly as a bittering component for the beer; ale yeast (*S. cerevisiae*) was provided by New Belgium Brewing; water was minimally treated with Calcium (100 ppm), SO_4 (65 ppm), and Cl (95 ppm) to achieve an acceptable pH for mash-in. Yeast pitch-rate was 10^6/ml/°P + 10^6 and fermentation temperature was held at 20 °C until completion, after

which the beers were bottled and pressurized under CO2. The specifications set for the purposes of this study were intended to reduce variation and provide normalization of quality among the resulting beers (*e.g.* taste, aroma, appearance, mouthfeel). Brewing specifications for this study are provided in Table 2. Due to space limitations at the brewery, the beers were produced in three rounds of two beers. It takes up to 6 hours to create a wort that is ready for fermentation and up to 7 days for fermentation to complete. Hence, the beer could not all be produced at one time.

2.3. Malt and Beer Metabolite Extraction

The malt samples, after they arrived in the Heuberger Lab at Colorado State University (Fort Collins, CO) were visually assessed for chaff or extraneous matter prior to milling. Grain was milled using a Thomas® Wiley Mini-Mill (Thomas Scientific, Swedesboro, NJ), using a #40 mesh sieve. Before the first sample, and between subsequent genotypes, the mill was thoroughly cleaned using 70% ethanol (v:v) (J.T. Baker, UltraPure reagent grade) and dried with compressed air for removal of residual dust. The ground malt was stored in -80 °C until removal for metabolomics analysis to avoid lipid oxidation of ground whole barley samples, which can contribute to rancid off-flavors [33, 68, 69]. Malt samples were ground in a Wiley Mill, measured to 100 mg, and placed in -80 until further analysis [70].

Beer samples were measured to 1 ml and dried down in Speedvac centrifugal dryer at room temperature. After drying, 1ml of MTBE:MeOH solution (3:2 for malt; 2:1 for beer) was added to the sample vials. The samples were vortexed for 60 minutes at -20 °C and centrifuged for 20 min at 2000 rcf at 4°C. The sample vials were removed, placed on ice, and 50 µL aliquots were taken for RP/LC-MS and dispersed into separate, labeled auto-sampler vials with 100 µL inserts. After the aliquots were taken, 750 µL of cold HPLC grade water was dispensed into sample remaining in the original sample vials. These samples were then centrifuged for 10 mins at 2000 rcf and at 4 °C. The organic layer was placed in clean, labeled glass tubes and the aqueous layer was aliquoted into labeled Eppendorf tubes. The Eppendorf tubes were centrifuged for 15 mins at 3500 rcf and at 4 °C. All samples were stored in -20 °C until further analysis.

2.4. Reversed-Phase Ultra High Performance-Liquid Chromatography-Mass Spectrometery (RP-UHPLC-MS) Metabolite Detection of Non-volatiles

This is a commonly-used technique to detect and quantify moderately polar to non-polar non-volatile compounds such as alkaloids, purines, amino acids, lipids and terpenes. Autosampler vials containing the 50 µL of aqueous layer from extraction were removed from the freezer and placed into trays after randomization of samples was performed and noted. After samples were randomized and placed into trays, 2 µL of extract was injected twice (n=2 replicates) onto a Waters Acquity UPLC system in discrete, randomized blocks, and separated using a Waters Acquity UPLC CSH Phenyl Hexyl column (1.7 µM, 1.0 x 100 mm) and a gradient from solvent A (2mM ammonium hydroxide, 0.1% formic acid) to solvent B (Acetonitrile, 0.1% formic acid).

Injections were made in 100% A, held at 100% A for 1 min, ramped to 98% B over 12 minutes, held at 98% B for 3 minutes, and then returned to starting conditions over 0.05 minutes and allowed to re-equilibrate for 3.95 minutes, with a 200 µL/min constant flow rate. The column and samples were held at 65 °C and 6 °C, respectively. The column eluent was infused into a Waters Xevo G2 Q-TOF-MS with an electrospray source in positive mode, scanning 50-2000 m/z at 0.2 seconds per scan, alternating between MS (6 V collision energy) and MSE mode (15-30 V ramp). Calibration was performed using sodium iodide with 1 ppm mass accuracy. The capillary voltage was held at 2200 V, source temp at 150 °C, and nitrogen desolvation temp at 350 °C with a flow rate of 800 L/hr [34, 71, 72].

2.5. Hydrophilic Interaction Liquid Chromatography- Mass Spectrometry (HILIC-MS) Analysis of Non-volatile Metabolites

This is a technique to detect and quantify moderately polar to polar non-volatile compounds such as peptides, carbohydrates, organic acids, free fatty acids, amino acids, and saccharides. Autosampler vials containing 50 µL of aqueous layer from extraction were removed from the freezer and placed into trays after randomization of samples was performed and noted. Analysis of these non-volatile metabolites was completed using a ZIC-HILIC (Zwitterionic Hydrophilic Interaction Chromatography)

column. For analysis of non-volatiles using the ZIC-HILIC column, 3 µL of extract was injected twice (n=2 replicates) onto a Waters Acquity UPLC system in discrete, randomized blocks, and separated using a EMD Millipore ZIC-pHilic (5 µM, 2.0 x 150 mm), using a gradient from solvent B (Acetonitrile) to solvent B (Water, 10 mM ammonium bicarbonate, pH 9.6). Flow rate was 0.27 mL / minute and the column was held at 50 °C. The mobile phase A was water with 10 mM ammonium bicarbonate, adjusted to pH 9.6 with a 50% ammonium hydroxide solution, and mobile phase B was acetonitrile. The gradient was as follows: time (t) = 0 min, 10% A; t = 1.5 min, 10% A; t = 8.5 min, 38% A; t = 11 min, 60% A; t = 11.5 min, 100% A, 0.2 mL/min flow; t = 16.5 min, 100% A; t = 17 min, 10% A; t = 18 min, 10% A, 0.6 mL/min flow; t = 22 min 10% A; t = 22.5 min, 10% A, 0.27 mL/min flow; t = 23 min, 10% A, end of equilibration. The column eluent was infused into a Waters Xevo G2 Q-TOF-MS with an electrospray source in negative ionization mode, scanning 50-1200 m/z at 0.2 seconds per scan, alternating between MS (6 V collision energy) and MSE mode (15-30 V ramp). Calibration was performed using sodium formate with 1 ppm mass accuracy. The capillary voltage was held at 2200 V, source temp at 150 °C, and nitrogen desolvation temp at 350 °C with a flow rate of 800 L/hr [69].

2.6. Solid-Phase Micro-Extraction Gas Chromatography Mass Spectrometry (SPME/GC-MS) Metabolite Detection of Volatiles

SPME/GC-MS identifies volatile molecules [14, 73] in an extremely complex matrix of metabolites from water, yeast, malt, salts, hops, and other flavorings which, through all stages of malting through packaging, are interacting and reacting to each other and environmental influences. Volatiles in beer (organoleptic compounds) contribute to the aroma and flavor such as esters, aromatic alcohols, terpenes, and aldehydes. These volatiles are the result, not only of fermentation by the yeast, but of the interactions of all the raw materials. This includes the byproducts created by unused or un-retained amino acids.

Volatiles analysis was completed using SPME/GC-MS. Bottles of beer sample were opened and degassed in an ultrasonic bath for 15 minutes at 5 °C. NaCl was added at 1.8mg/6mL of beer into 20mL

vials. Three replicates of each beer sample made from the six genotypes were randomized into a single block (18 total). The beer headspace was analyzed using a 50/30 µm DVB/PDMS/Carboxen SPME fiber (Sigma Aldrich, St. Louis, MO) installed and conditioned per manufacturer recommendations before use. The fiber was exposed to headspace above the sample, which was heated to 60°C for 60 minutes. After extraction, the fiber was immediately inserted into the GC port for desorption. The fiber was conditioned between each desorption. SPME/GC-MS analysis was performed on a Thermo Scientific® ISQ and operated in splitless mode for trace analysis, desorbed at 260°C for 5 minutes. GC ramping was carried out at 40°C for 10 min, to 210 at 4C/min, then to 220°C for 5 minutes, at 30°C/min for a total run time of approximately 60 minutes. A DB-WAX column (0.25mm i.d. x 30m x 0.25 µm film thickness) was used with He gas flow at 1 mL/min, with MS operated at 70 eV in EI mode. Transfer line, source, and quadrupoles temperatures were set at 220, 230, and 150°C, respectively. Detection was performed in full scan mode from 30-200 amu.

2.7. Metabolomics Data Processing

For each sample, a matrix of molecular features (defined by retention time and mass (m/z)) was generated using XCMS software in R v.3.2.4 [74]. Samples were normalized to the total ion current (TIC) and the relative abundance (quantity) of each molecular feature was determined by the mean area of the chromatographic peak among replicate injections (n = 2) for samples analyzed via UPLC-MS and ZIC-HILIC-MS. Mass spectra were generated using an algorithm in RamClust, an R package that clusters masses into spectra (‘spectral clusters’) based on co-variation and co- elution in the data set [75]. Compounds were annotated based on retention time and spectral matching to in-house libraries using RamSearch software [75]) that included in-house libraries of authentic standards, as well as to external libraries NIST v.14 (http://www.nist.gov), Metlin [76, 77], HMDB [78], and the Golm Metabolome Database [79]. Analytes detected via ICP-MS were referenced to standard solutions to ensure proper quantification. Identification of metabolites via c was performed based on information provided in the NIST v.14.

2.8. Inductively Coupled Plasma Mass Spectrometry (ICP-MS) Elemental Analysis

ICP-MS is a method identifying elementals/metals such as copper, lead, arsenic, *etc.* Calibration curves for each element of interest (Li, Be, B, Cd, Se, As, Na, P, S, Mg, K, Ca, Al, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Sr, Mo, Ba, W, and Pb) were made by adding requisite materials to make solutions ranging from 0.1 ppb to 1000 ppb. Samples were weighed in triplicate for each genotype from previously milled grain stored at -80C. Each sample of 100 mg for was placed in individual borosilicate tubes (B, Si not analyzed). To each sample were added: 66.7 uL of internal standard solution at 10 ppm of Y, Ga, Bi, and In to produce a final concentration of 20 ppb of each, and 1.5 mL nitric acid (trace metal grade, BDH) at 70% (w:v) with a molarity of 15.9 M, and left covered overnight to digest. All experimental and calibration samples were treated identically and during the same time period. After 24 hours had passed, samples were randomized and placed in a sand bath heated to 120 °C for 2.5 hours until all orange vapor had subsided. After samples had cooled to room temperature, 750 µL 30% hydrogen peroxide (v:v) was added to each, and then further digested in sand bath for 1 hour at 120 °C.

Once at room temperature, 2 mL of digested samples were transferred to individual 15 mL polypropylene falcon tubes. Sample volume was raised to 10 mL using 18 MΩ water, then 4.5 mL of the above solution was diluted with 18MΩ to a final volume of 15 mL for a final internal standard concentration of 20 ppb and 3% HNO3 into new falcon tubes, then analyzed.

Elemental concentrations of Li, Be, B, Cd, Se, As, Na, P, S, Mg, K, Ca, Al, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Sr, Mo, Ba, W, and Pb were measured using an Elan DRC (dynamic reaction cell) II mass spectrometer (PerkinElmer) connected to a Seaspray™ MEINHARD nebulizer and a quartz cyclonic spray chamber. Samples were introduced using an ASX-520 autosampler (CETAC Technologies). Li, Be, B, Na, P, S, Mg, K, Ca, W, As, and Pb were measured in standard mode. Cd, Se, and As were measured in DRC mode using oxygen as the reactive gas. Al, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Sr, Mo, and Ba were measured in DRC mode using ammonia as the reactive gas. Before analysis the nebulizer gas flow and lens voltage were optimized for maximum Indium signal intensity (45753 counts per second), 0.82 and

9.0 respectively. A daily performance check was also run which ensured that the instrument was operating properly and obtained a CeO+:Ce+ of 0.029 and a Ba++:Ba of 0.018. A calibration curve was obtained by analyzing 7 dilutions of a multi-element stock solution made from a mixture of single-element stock standards (Inorganic Ventures). To correct for instrument drift, a quality control (QC) solution (pooled sample, prepared by mixing 2mL of each digested individual sample) was run every 10th sample.

Data were processed using Excel. Each element was subjected to internal standard corrections and subsequently drift corrected [1]. Corrections were chosen based on minimizing the coefficient of variance (CV) for the QC samples. After drift correction, samples were corrected for the dilution factor. Limits of detection (LOD) and limits of quantification (LOQ) were calculated as 3 times or 10 times the standard deviation of the blank divided by the slope of the calibration curve respectively [2, 3]. Final concentrations are given in ppb (µg/L). Measured calculations below the LOQ were assigned to LOQ/2 [4]. Elements with concentrations below the limit of detection (Li, Be, V, Co, W) or that may be introduced from materials used (B) were eliminated from downstream analysis [51].

2.9. Sensory Analysis

Sensory analysis of the six beers was performed by a trained panel at New Belgium Brewing Company (Fort Collins, CO). Beer sub-samples were stored at room temperature during weeks zero through 4 and stored at 4 °C during weeks 4 through 8. Beer was evaluated for quality at 0, 4, and 8 weeks after bottling. Beer was evaluated using a modified Quantitative Descriptive Analysis® (QDA) for 45 sensory traits. QDA is a sensory evaluation technique based on the notion that humans are good at judging relative differences in sensory, but lack in the ability to evaluate absolute differences [80, 81].

QDA was modified at New Belgium Brewing Company to fit the parameters for quantification of beer sensory. For each evaluation event, ten or more expert panelists were instructed to establish robust

qualitative sensory descriptors for each sample and determine a perceived quantity for each trait that encompasses taste, aroma, mouthfeel and appearance [82].

2.10. Statistical Analysis

Principal Component Analysis (PCA) was conducted on metabolite and sensory data after mean-centering and UV-scaling in SIMCA v.14.1 (Sartorius Stedim Biotech, Sweden) [83, 84]. Metabolite abundances were compared using two-way ANOVA, via the "aov" function in the R statistical environment v. 3.2.4 for malt and beer genotypes (each of six) and growing location (U.S. or Canada) with a p (probability) threshold of 0.05. Benjamini-Hochberg (BH) correction was applied to ANOVA results when conducting multiple comparisons to account for falsely rejected statistical hypotheses, otherwise known as "false discovery rate" (FDR) [85]. O2PLS models were conducted in SIMCA v. 14.1 with R^2 and Q^2 scores for both malt and beer models. The Q^2 score is an estimate of the predictive ability of the model [84, 86, 87]. SIMCA uses the "leave one out" cross-validation method, by which the data is divided into seven parts and 1/7th of the data are removed and the model is built on the remaining 6/7th of data remaining and the removed 1/7th of data are predicted from a new model. This is repeated until all the data have been predicted. These new predicted data are compared with the original data and the predicted residual sum of squares is predicted for the whole dataset [86, 87]. In the case of this study with a low n (n=6), this type of cross-validation is considered valid [86]. "Good" predictions will have high Q^2 scores ($Q^2 > 0.5$). Low Q^2 scores ($Q^2<0.5$) indicate a lack of predictability. Heatmaps were prepared in R v.3.2.4 using "gplots" package [88] with "heatmap.2" function, "ggplots2" package [89], "Reshape2" package [90] with "melt" function, and "stats" package [91] with hclust function for hierarchical clustering. z-scores were calculated using the mean and standard deviation of the metabolite abundances where X is the abundance of a metabolite, μ is the mean content for the metabolite across all samples and σ is the standard deviation across all samples or $z=(X-\mu)/\sigma$. Data were z-transformed and resulting z-scores were used to create heatmaps utilizing hierarchical clustering and Spearman's rank correlation

methods. Only annotated compounds were included in statistical models, including both univariate data (ANOVA, PCA, Spearman's correlation heatmaps) and multivariate data (O2PLS), created to improve reproducibility for future work.

Chapter 3 – Results and Discussion

3.1. Results

3.1.1. The six malts had similar malting quality indicating validity of flavor evaluation using a single brewing recipe.

It is difficult to compare malts for an influence on beer flavor if the genotypes differ for major malt quality traits (*e.g.* extract, protein). The six genotypes evaluated in this study were malted among four commercial maltsters (from four growing locations), and resulted in very similar malting quality (Table 1). The malt extract ranged between 79.6 to 83.3% and protein was between 10.86-13.62%. One malt, Full Pint, was bred to be significantly higher in α-amylase [28, 29, 55] and contained approximately 22 DU more than the other five genotypes. Full Pint was also approximately 116.4 more ppm β-glucan than the other five genotypes. In the mash stage, α-amylase hydrolyzes large α-linked polysaccharides into monosaccharides; its concentration decreases slowly at lower mash temperatures (below 68 °C).

Emphasizing α-amylase at higher temperatures (68-69 °C) will result in more unfermentable sugars (increased sweetness) and a much more full-bodied (thicker mouthfeel and texture) and lower alcohol by volume (ABV) beer, lower mash temperatures (below 68 °C) will result in more of a medium-bodied and higher-ABV beer. One possible effect high α-amylase could have, as in Full Pint, is an increased perceived sweetness due to the unfermented sugars, as yeast at an average pitch-rate may not ferment the sugars to full attenuation [4, 24]. It is important to note flavor was not influenced by these malting quality factors alone and that Full Pint was not perceived as "sweet," but as "fruity." Given that the recipe was standardized for the purposes of this study, this "extra sweetness" could have had an effect on results. The high abundance of β-glucans in Full Pint may also have had an effect upon sensory results. Excess β-glucans, which are the result of undermodified malt (they retain intact cell walls and undegraded proteins), play a role in creating the body (texture, mouthfeel) of a beer, but also contribute to low extract during brewing. These low extracts are due to increased wort viscosity and resulting lautering problems. Low friability (the ability of the malt grain to be optimally crushed and exposed for enzymatic action and

conversion during brewing) in Full Pint may also have contributed to low extract and incomplete conversion of sugars. The average of all other genotypes was 91% (80% is industry minimum for malt), whereas Full Pint was 55.5%, a 35.5% difference. With low friability, wort filterability decreases, again creating lautering difficulties and decreasing the amount and quality of extract from the mash stage. All other malt quality traits were very similar among the six genotypes such as moisture, diastatic power (DP) and pH, indicating that these malts can be brewed into beer using identical recipes.

3.1.2 Metabolomics analysis of malt extracts revealed variation in small molecules among the six genotypes

Malt was evaluated for metabolite variation using a non-targeted metabolomics workflow. Malt was ground to a powder, metabolites were extracted, and profiles were established using three different mass spectrometry (MS) platforms: non-volatile metabolites via RP/LC-MS and HILIC-MS, and ICP-MS for metals. The RP/LC-MS and HILIC-MS platforms detected 2492 and 2550 metabolites, respectively. Of 5,042 detected compounds, 217 were annotated as known metabolites and included amines (20), amino acids (36), fatty acids/lipids (40), sugars (11), phenols/benzenoids (30), and others (80). (Table 3). ICP-MS detected 20 metals including copper, iron, calcium, and sulfur that are known to be important for yeast nutrition and brewing.

Principal component analysis (PCA) was performed to evaluate variation among the malt metabolite profiles, and was independently performed for metabolites and metals. The data set was reduced to include only the 217 annotated malt metabolites or 20 metals. For metabolites, a total of 5 PCs were generated that explained 47.8% of the variation. The PCA demonstrated chemical variation among the six malt genotypes (Figure 2). PC1 and PC2 explained 31.4% and 16.4% of the variation (respectively) and separated three genotypes Full Pint, Copeland, and Expedition from a cluster of the other three genotypes (Figure 2a, left).

The PC1 and PC2 loadings (Figure 2a, right) indicated trends in metabolite classes that drive the variation among the six malts. Specifically, lipids (green) were generally higher in Full Pint compared to the other five genotypes

Other classes, such as amino acids were generally equally distributed in the loadings plot, and this indicated that none of the six genotypes has a major trend in being higher or lower in any one chemical class. An analysis of additional components revealed separation among the cluster of three genotypes, specifically PC5 (6.8% of the variation) revealed separation among Meredith, Metcalfe, and Polarstar (Figure 2b). Further, the PCA was evaluated to understand if metabolite variation could be attributed to maltster. The PC1 and PC2 scores plot was colored according to each of the four maltsters (Figure 2c). The data revealed that for the two maltsters that were replicated (Rahr and Malteurop), the respective genotypes were on different places on the PC scores plot. This indicates that maltster was not the major influence of metabolite variation among the six malts. PCA of the 20 metals resulted in 5 principal components that explained 57% of the variation. The PC scores plot of PC1 and PC2 (42.7% and 14.3% of the variation) separated most of the genotypes (Figure 2d, left). Of the six malts, Full Pint and PolarStar had the most distinct metals profiles. The PC loadings plot (Figure 2d, right) revealed that Full Pint was higher in trace metals such as copper (Cu), zinc (Zn), and manganese (Mn).

Taken together, PCA indicated each of the six malts had a distinct profile of metabolites and metals that was largely attributed to genotype. Of the six malts, Full Pint appeared to be the most unique, partially due to lipid content (Figures 2a, right), and partially due to other classes of metabolites that were different (*i.e.* amino acids, purines and amines), however there were no major trends in chemical classes.

The six malts were further evaluated for metabolite variation using analysis of variance (ANOVA). The analysis revealed 150 of the 217 annotated compounds (69.1%) varied among genotype (ANOVA, FDR adjusted $p < 0.05$). Metabolites within all chemical classes varied, specifically amines (20/20) and amino acids (30/36), lipids (30/47), phenols/benzenoids (15/24), and others. This significance

included non-volatile metabolites (fatty acids/lipids/nitrogenous compounds/sugars) according to the factors of genotype and the location grown (*i.e.* Canada or U.S.). The factor of location in the initial

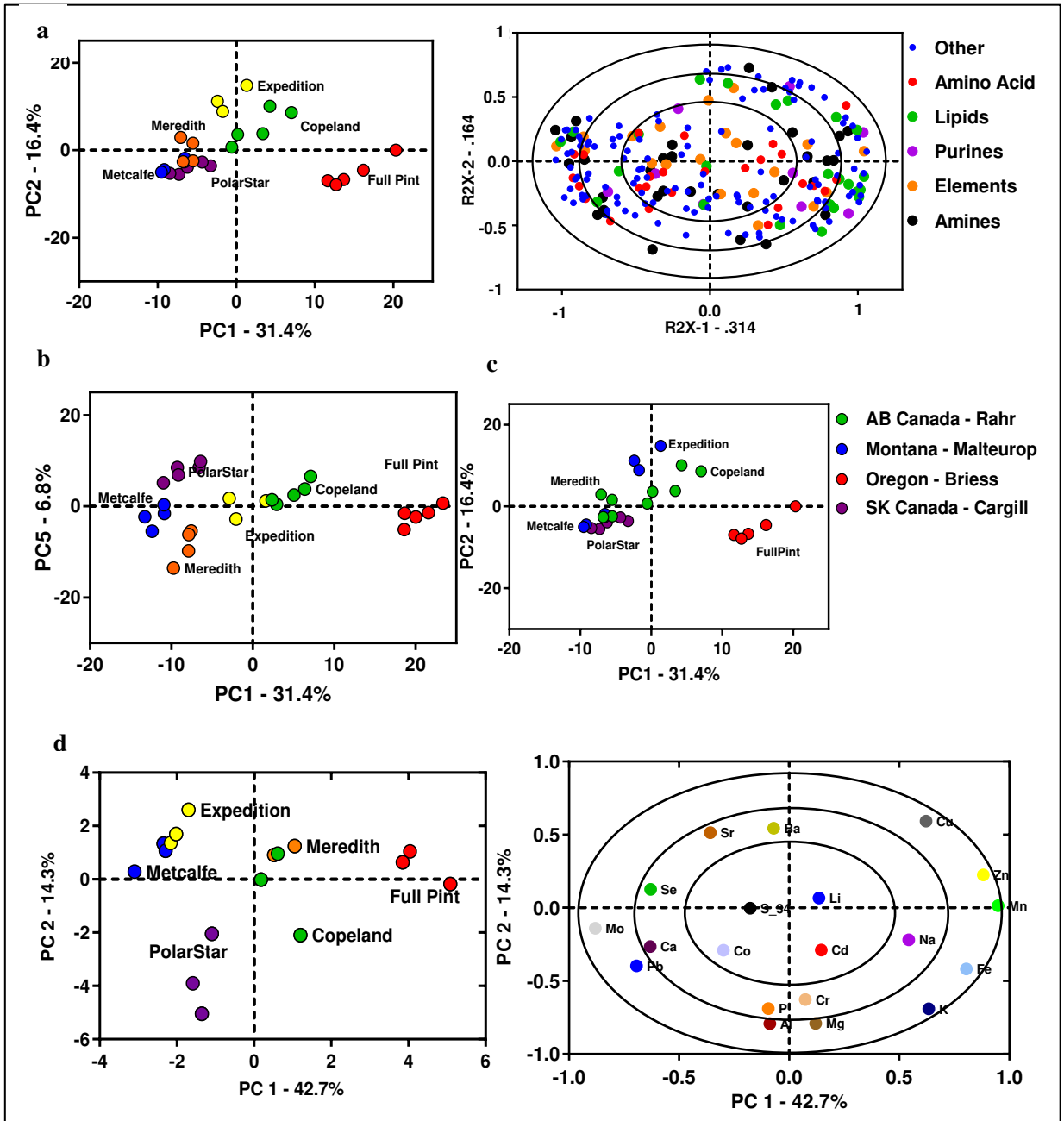


Figure 2. Principal component analysis (PCA) of malt metabolites of the six genotypes.
PCA was performed on 217 metabolites detected among the six malts. **(a)** PC scores (left) and correlated scaled loadings (right) plot for PC1 and PC2 of six malts, and loadings were colored according to chemical class. **(b)** PC scores plot for PC1 and PC5 provides additional separation among the malt genotypes. **(c)** PC scores plot (PC1, PC2) colored according to each of the four maltsters, indicated metabolite variation was more driven by genotype than maltster. **(d)** PCA conducted on 20 metals for the six malts with PC scores (left) and loadings (right). All analyses were conducted on n = 3-5 extraction replicates per genotype.

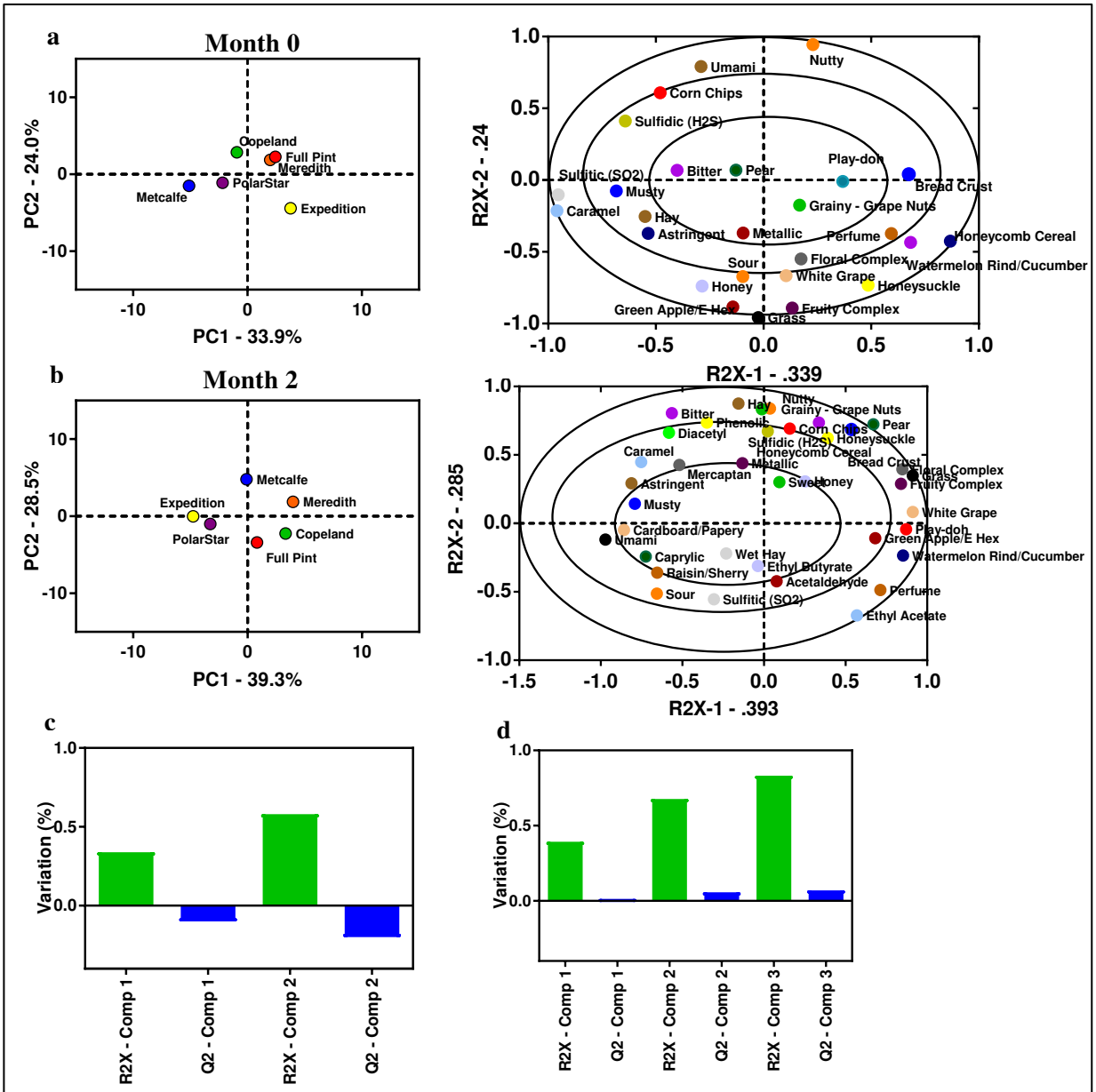


Figure 3. Principal component analysis (PCA) of sensory traits of the six genotypes. PCA was performed on 45 sensory traits detected among the six beers. (a) PC scores (left) and correlation scaled loadings (right) plot for PC1 and PC2 of sensory of six beers at 0 months, and loadings were colored according to sensory trait. (b) PC scores plot (PC1, PC2) colored according sensory traits detected in the six beers at 2 months, indicated sensory is more highly detectible in all beers at 2 months as shown by the separation between genotypes. The R^2 X score is the percent of variation that was explained by the model (how well the model fits the data), where an R^2 X score is close to 1 (or 100%) is considered a "good model." The Q^2 score is the percent of variation of the X (the sensory traits) variables with PCA and predicted by the model according to cross-validation. A $Q^2 > 0.5$ indicates good predictability. (c) At month 0, the model is explained by two principal components that indicate 60% of variation can be explained by the month 0 sensory traits. (d) At month 2, the model was explained by three principal components that indicate 80% of variation was explained by the month 2 sensory traits.

ANOVA analyzing all metabolites revealed that 46% of all metabolites were significant (ANOVA, FDR adjusted $p < 0.05$, 2,311 out of the 5,042 metabolites).

Taken together, these data indicated variation in the malt metabolite profiles of the six genotypes. Full Pint, Expedition and Copeland exhibited the most unique profiles within the chemical classes that varied. The amino acid and lipid classes are important to fermentation due to their indispensable nutrients for yeast health. Nitrogen sources are imperative for yeast growth, reproduction, and production of enzymes yielding ethanol and carbon dioxide. Sugars (monosaccharides, oligosaccharides) did not vary significantly across genotype or location grown.

Copper, in barley malt, averages about 5ppm and is an important element in the fermentation stage of brewing. Fermentation is negatively affected by Cu at levels from 5 to 10 ppm causing abnormal yeast growth, development, and reproduction which lead to sluggish fermentations [92]. At levels below 5 ppm, Cu reacts with sulfides to reduce sulfur flavors and aromas in beer. Zinc is an important enzymatic co-factor and a requirement for healthy yeast development, protein synthesis and phospholipid membrane composition and stability; it also increases fermentation rate and ester production, but is found at trace levels in most barley malt [93]. Manganese is an important enzyme regulator in the mash stage of brewing, but is found at trace levels in barley malt [93].

3.2. Sensory Differences Observed in Beer after Two Months of Storage

The brewing recipe used for the six malts is described in Table 2. The study was designed to utilize an industrial scale system based on the concern that brewing on pico- or micro-levels would be difficult to replicate and compare malts for an influence on flavor. The recipe is relatively malt-forward with low hop levels and yeast with low flavor-producing esters, designed to evaluate malt flavor.

Sensory analysis of the six beers was conducted using modified quantitative descriptive analysis® (QDA) of 45 traits that encompass taste, aroma, and mouthfeel. Principal component analysis was conducted on the scores generated by the QDA panel for the sensory traits on beer at 0 months

(Figure 3a, left) and 2 months (Figure 3b, left) of storage. PCA was performed to evaluate variation among the sensory trait profiles. The data set included 45 sensory traits with no replicates. For Month 0, a total of 2 principal components (Figure 3a, left) were generated that explained 57.9% of the variation. The PC scores plot demonstrated variation among the six malts. PC1 explained 33.9% of the variation and separated three genotypes: Full Pint, Meredith, and Expedition from a cluster of the other genotypes.

The PC loadings plot for Month 0 (Figure 3a, right) demonstrated the sensory traits, on the correlation scale, that 10 of the 45 sensory traits were attributed to beer flavor at Month 0. The summary of fit for this model (Figure 3d) displayed two components which explained the variation and was elucidated with R^2 and Q^2 scores. The R^2 score is the percent of variation that was explained by the model (how well the model fits the data), where an R^2 X score is close to 1 (or 100%) is considered a "good model." The Q^2 score is the percent of variation of the X (the sensory traits) variables with PCA and predicted by the model according to cross-validation. Although the R^2 X scores in this model were below 0.6, (R^2 X score is 0.339 in component 1 and 0.579 in component 2) which indicates an acceptable biological model, the Q^2 scores were negative. A negative Q^2 indicates that there may have been noise, outliers, or a small n, but in this case (Q^2 score is -0.1 in component 1 and -0.2 in component 2) indicated it is not a good indicator of predictability at Month 0.

The PC loadings plot for Month 2 (Figure 3b, right) demonstrated the sensory traits, on the correlation scale, that at least 17 of the 45 sensory traits were able to be attributed to beer flavor at Month 2. The summary of fit for this model (Figure 3e) displayed three components which explained the variation and is elucidated by R^2 X and Q^2 scores. The R^2 X and Q^2 scores in this model at Month 2 were higher. R^2 X scores for components 1, 2, and 3 were 0.39, 0.67, and 0.83, respectively. The Q^2 scores for components 1, 2, and 3 were -0.01, 0.05, and 0.07, respectively. These higher Q^2 scores were attributed to better predictability of these traits at Month 2 (Table 8). The sensory traits in the Month 2 model were better matched to the QDA panel's analysis of sensory traits at Month 2. The sensory traits that were analyzed in Month 2 revealed higher predictability than the traits analyzed at Month 0, according to the agreement between the QDA panel's reports and to the predictability of the model (Figure 3e). The model

at Month 2 indicated 20% more reliability in prediction (80% in component 3) as opposed to only 60% in component 2 of the Month 0 model (Figure 3d). This indicated that sensory traits at Month 2 were more apparent and could be predicted based on these analyses.

3.3. Metabolomics of the six beers revealed variation in metabolites attributed to the six malt genotypes

Beer metabolite variation was evaluated using four MS platforms: RP/LC-MS, HILIC-MS, GC-MS, and ICP-MS for metals which detected approximately 1,659, 2,057, 852, and 20 metabolites and elements, respectively. Of the metabolites detected through LCMS, 70 were annotated and remained in the final analysis. Of the metabolites detected through ZIC HILIC, 38 were annotated and remained in the final analysis. Of the metabolites detected through SPME, 138 were annotated and remained in the final analysis. Of 3,716 detected non-volatile compounds in beer, 108 were annotated as known metabolites and included amines (9), amino acids (37), fatty acids/lipids/fatty acyls (28), sugars (10), phenols/benzenoids (20), and others (3). (Table 3). Of the 852 volatile compounds detected in beer 138 were annotated as known metabolites and included esters (89), aldehydes (21), and others (28) (Table 3).

Principal component analysis (PCA) was performed to evaluate variation among the beer metabolite profiles. The data set included the 246 annotated beer metabolites and 20 elements. A total of 6 principal components were generated that explained 47.8% of the variation (Figure 4a). The PC scores plot demonstrated variation among several of the beers (Figure 4a). PC1 explained 23.4% of the variation and PC2 explains 16.6% of the variation in this model and separated three genotypes: Full Pint, Copeland, and Expedition from a cluster of the other genotypes. PC5 explained 7.9% of the variation (Figure 4b) that separates Meredith, PolarStar, and Metcalfe.

A PC scores plot of 138 volatile metabolites only (PC1 – 24.2% of variation and PC2 – 17.2% of variation) separates Full Pint, Copeland, and Expedition from a tighter cluster of the remaining three genotypes (Figure 4c). The PC scores plot of 108 non-volatile compounds only (PC1 – 27.5% of variation and PC2 – 17.6% of variation) separates Meredith from PolarStar and Meredith in PC2 and Full Pint,

Copeland, and Expedition in PC1. The separation generated by these plots suggested that the non-volatile compounds were more influential on the variance in the PCA (Figure 4d). These initial unbiased PCA analyses of malt metabolite data focused the attention of this study towards the metabolites which had a relationship with flavor traits associated with each genotype.

ICP-MS revealed 20 elements in the beer. PCA was performed to evaluate variation among the beer elemental profiles. A total of 5 principal components were generated that explained 57.2% of the variation. The PC scores plot demonstrated variation among several of the six malts (Figure 4e, left). PC1 explained 42.1% of the variation and PC2 explained 15.1% of the variation. Full Pint was separated from the other genotypes. Of these six beers, Full Pint and Expedition are the most unique. Manganese (Mn), Zinc (Zn), Sulfur (S), and Iron (Fe) revealed variation in Full Pint (Figure 4e, right). Section 3.3 discusses Mn and Zn. Sulfur and Iron are also important elements in the brewing process and affect beer flavor and flavor stability.

The six beers were further evaluated for metabolite variation using ANOVA. The analysis revealed 150 of the 246 annotated compounds (60.9%) varied among genotype (ANOVA, FDR adjusted $p < 0.05$). Metabolites within all chemical classes varied, specifically amines (10/10), purines (10/12), amino acids (30/33), phenols/benzenoids (15/24), esters/aldehydes (70/96), and others (71). This significance included non-volatile metabolites (nitrogenous compounds/sugars) and volatile metabolites (esters/aldehydes/ketones) according to the factors of genotype and the location grown (*i.e.* Canada or U.S.). The factor of location in the initial ANOVA analyzing all metabolites revealed that 27% of all metabolites were significant (ANOVA, FDR adjusted $p < 0.05$, 1,267 out of the 4,568 metabolites).

3.3.1. Beer metabolite influence on beer flavor

The metabolites separating the genotypes are displayed in the PC loadings plots of principal components (Figures 4e-i). These metabolites were attributed to specific flavor traits (Tables 3, 4-6) and illustrated associations between sensory characteristics and genotype. Metabolites were colored according to their known contribution to beer flavor (Tables 4 through 6). In the “umami” (savory) loadings

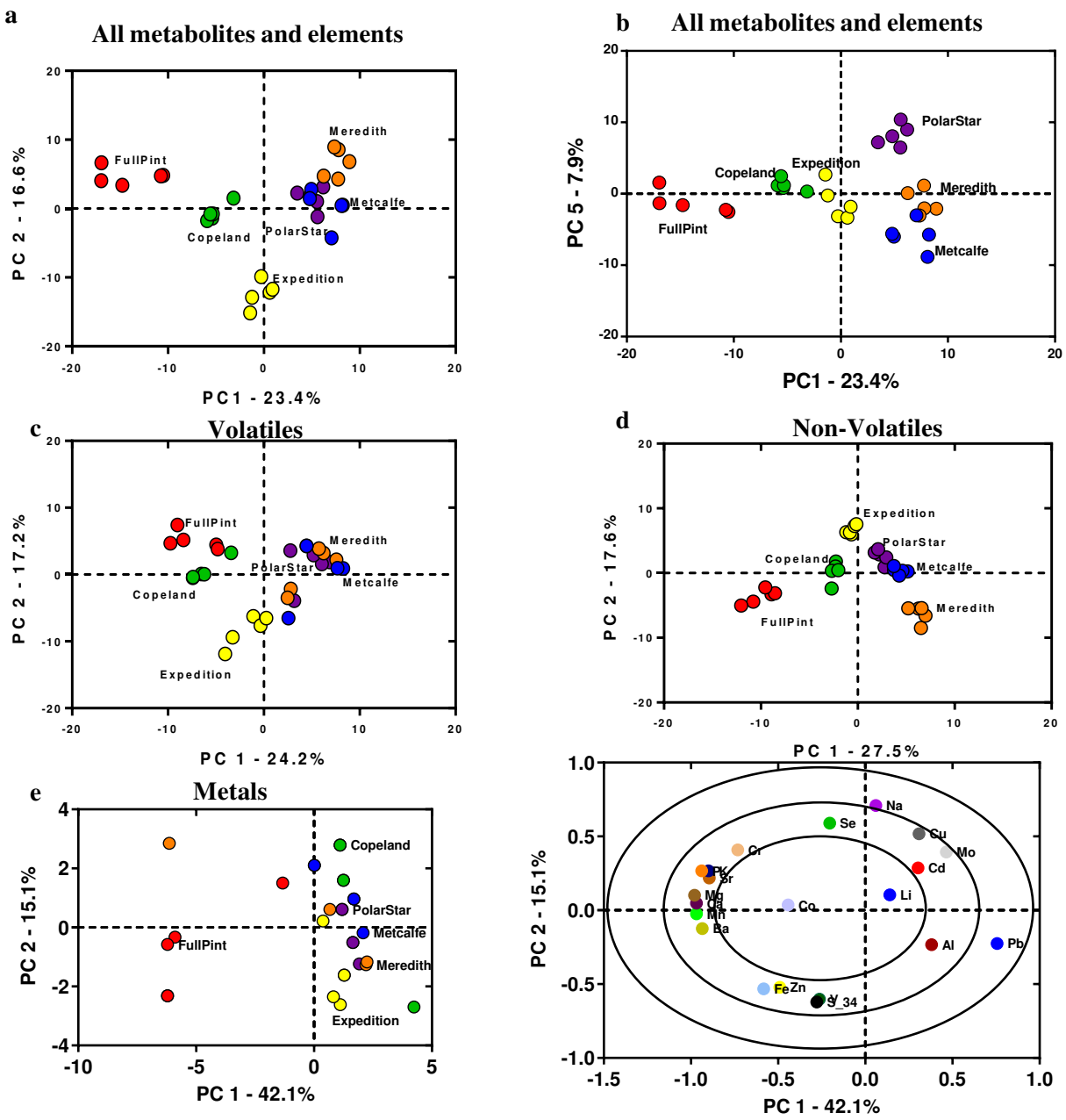
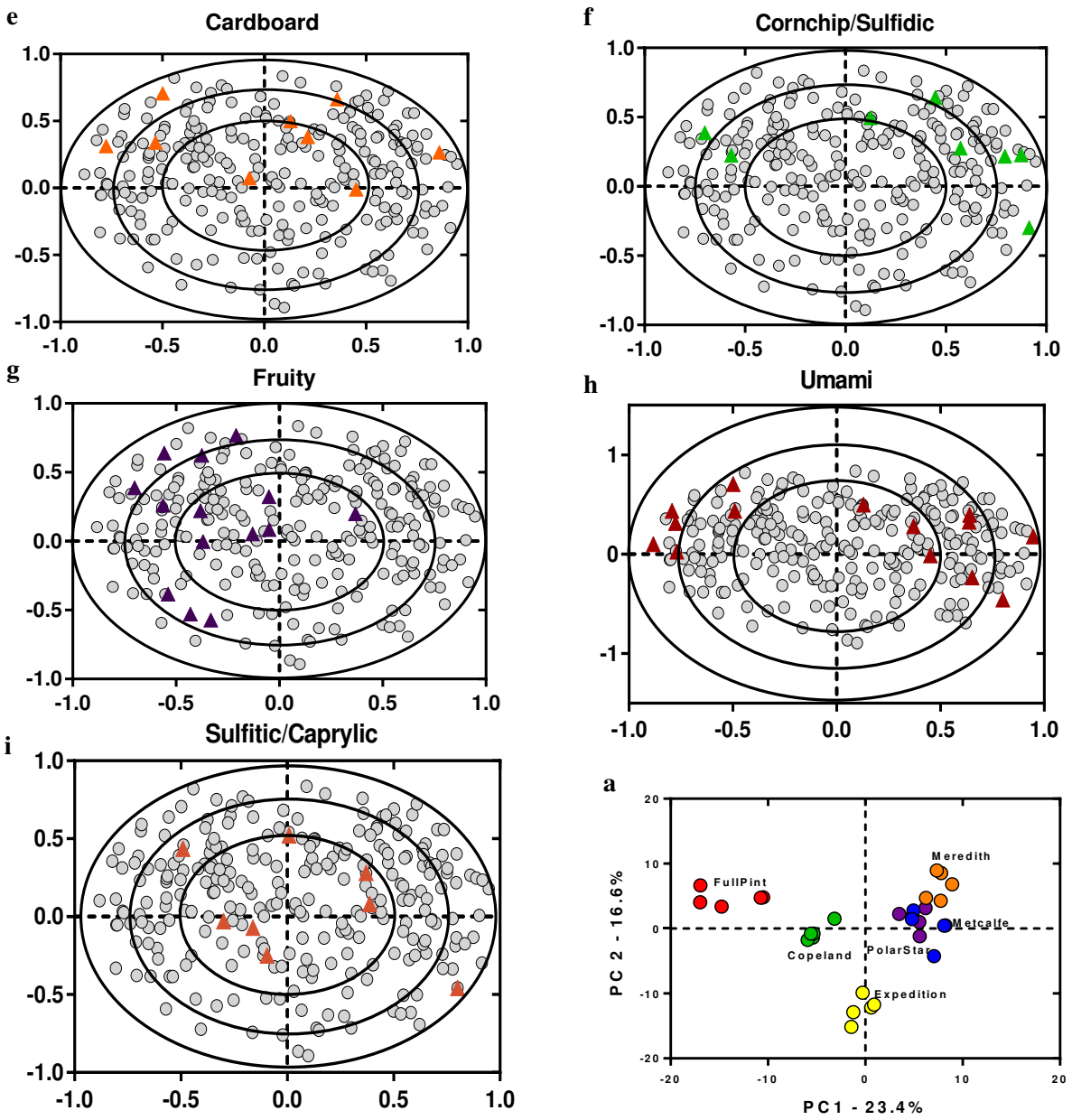


Figure 4a-d. Principal component analysis (PCA) of beer metabolites of the six genotypes.

PCA was performed on 246 metabolites (volatiles, non-volatiles, and metals) detected among the six beers. **(a)** PC scores for all metabolites and elements. PC1 and PC2 of six beers indicated separation of Full Pint, Copeland, and Expedition from the other three genotypes. **(b)** PC scores plot for PC1 and PC5 provided additional separation among the malt genotypes. **(c)** PC scores plot for PC1 and PC2 with 138 volatile metabolites provided less separation among the beer genotypes. **(d)** PC scores plot (PC1, PC2) with 108 non-volatile metabolites indicated additional separation between genotypes. **(e)** PCA conducted on 20 metals for the six malts with PC scores (left) and loadings (right). All analyses were conducted on n = 3-5 extraction replicates per genotype.



Figures 4e-i. PC loadings plots of beer metabolites of the six genotypes.
PCA was performed on 217 volatile and non-volatile metabolites and 20 metals detected among the six beers. PC correlated scaled loadings plots for PC1 and PC2 of six beer genotypes were colored according to chemical class. **(e)** Orange triangles denote metabolites associated with "cardboard" sensory traits. Separation was seen (Figure 4a included for reference) amongst Full Pint, Meredith and Metcalfe and the other three genotypes for "cardboard." **(f)** Green triangles denote "cornchip" or sulfidic sensory traits. Separation was seen between a cluster of Meredith, Metcalfe, and PolarStar and the other three genontypes for "cornchip." **(g)** Purple triangles denote "fruity" sensory traits. Full Pint was separated in this plot from the other 5 genotypes. (**h**) Dark red triangles denote denote "umami" sensory traits. Separation was seen between Full Pint, then Meredith and Metcalfe from the other three genotypes. **(i)** Dark orange triangles denote "sulfitic/caprylic" sensory traits. These were distributed amongst Copeland, Polarstar, and Metcalfe. Grey circles are other metabolites. All analyses were conducted on n = 3-5 extraction replicates per genotype.

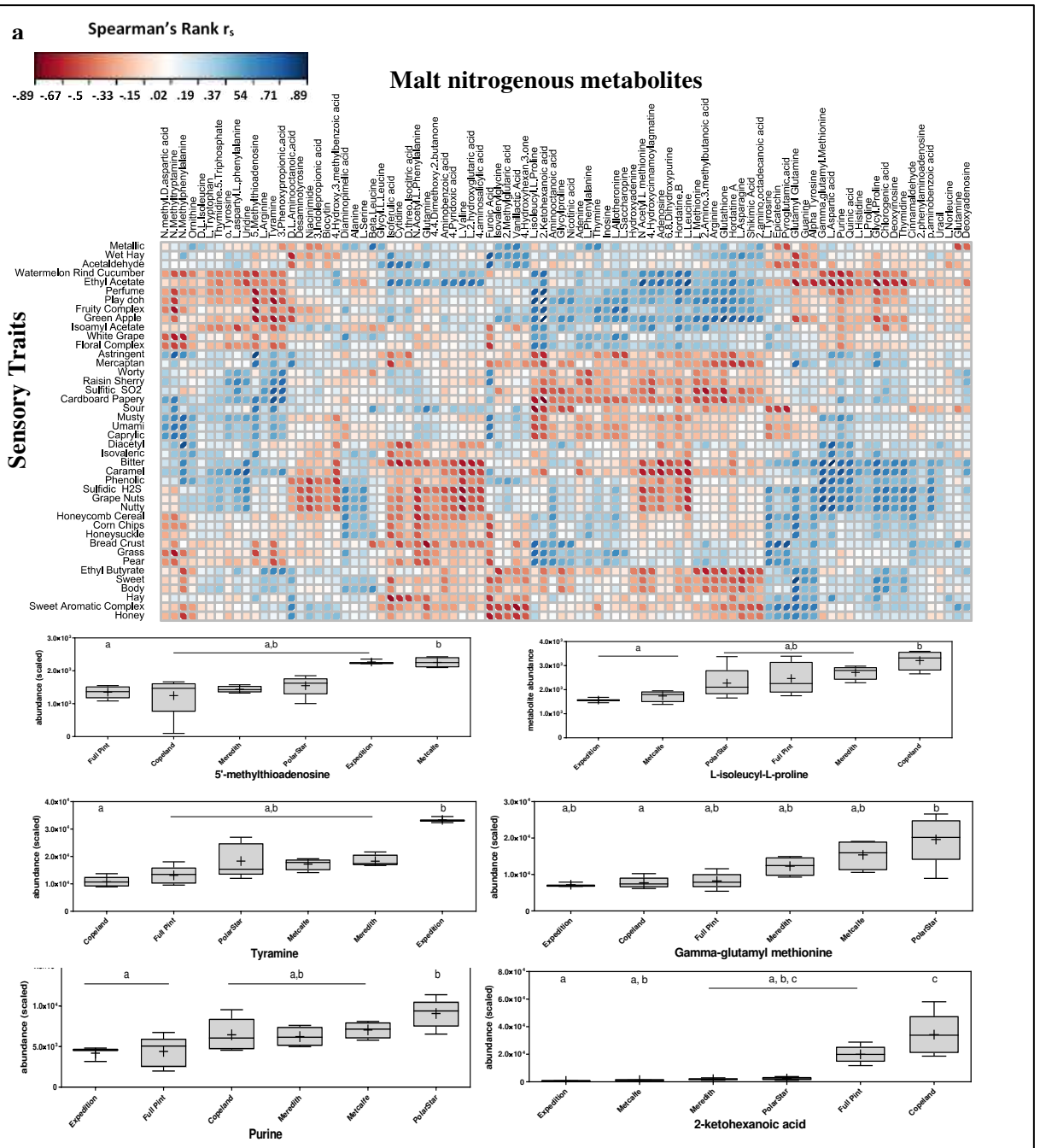


Figure 5. Univariate association of malt nitrogenous metabolites with beer sensory traits after 2 months of storage.

The association between malt metabolites and beer sensory was evaluated with Spearman's rank correlation **(a)** Correlation plot (heat map) of r_s values for malt metabolites (x-axis) and sensory traits (y-axis). Colors and the eccentricity of the ellipse indicated strength and direction of the r_s correlation, and the data were arranged using hierarchical clustering. **(b)** Box plots were used to indicate example metabolite variation in the system. Data are presented as the mean metabolite abundance ± standard error of the mean (S.E.M.) for n =3-5 extraction replicates per genotype. ANOVA with a Tukey post-hoc ($p < 0.05$) was performed on the data in the box plots, and differences are denoted with letters (e.g. a, b, c).

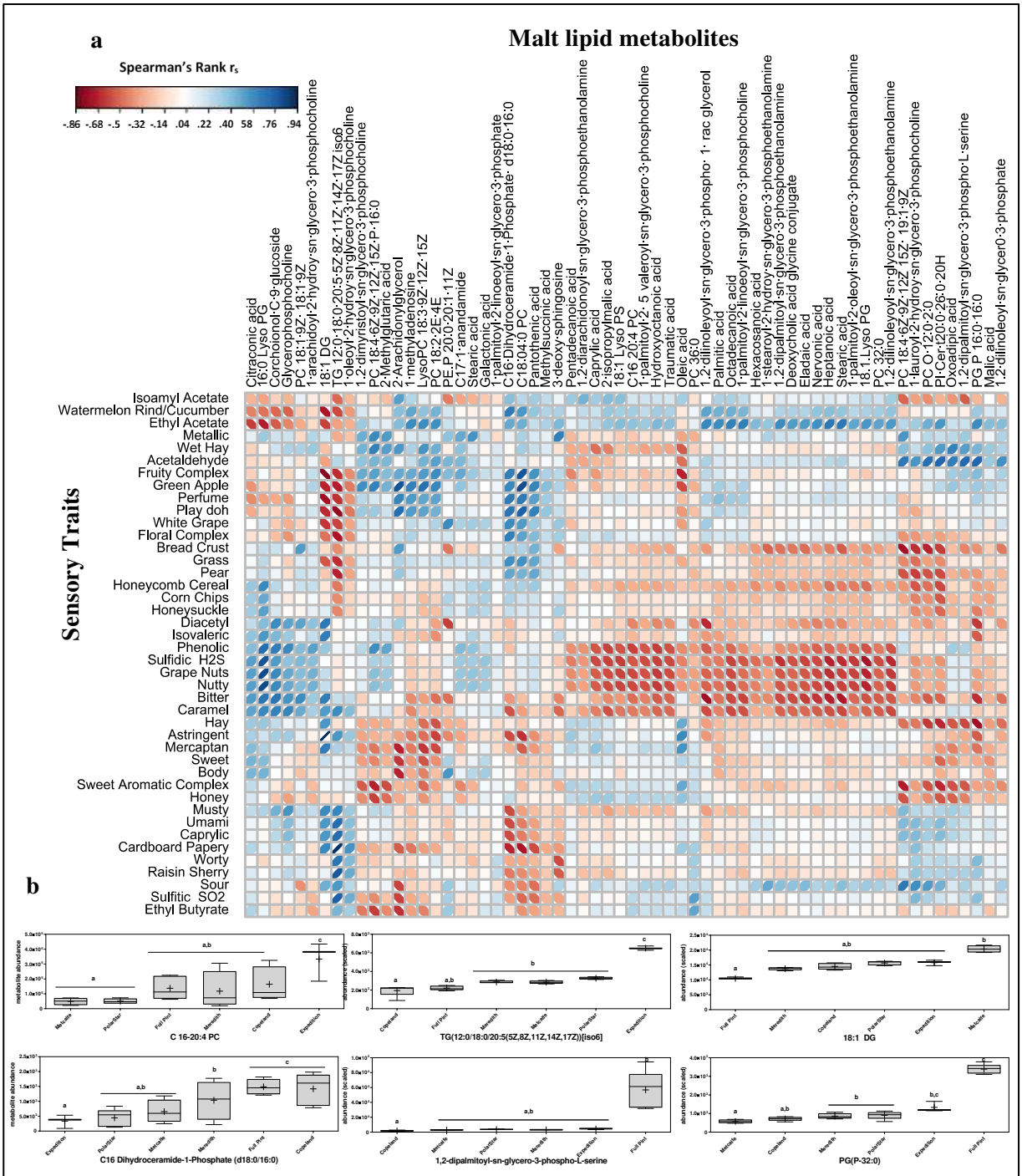


Figure 6. Univariate association of malt lipid metabolites with beer sensory traits after 2 months of storage.

The association between malt metabolites and beer sensory was evaluated with Spearman's rank correlation **(a)** Correlation plot of r_s values for malt metabolites (x-axis) and sensory traits (y-axis). Colors and the eccentricity of the ellipse indicates strength and direction of the r_s correlation, and the data was arranged using hierarchical clustering. (b) Box plots were used to indicate example metabolite variation in the system. Data are presented as the mean metabolite abundance ± S.E.M. for n =3-5 extraction replicates per genotype. ANOVA with a Tukey post-hoc ($p < 0.05$) was performed on the data in the box plots, and differences are denoted with letters (e.g. a, b, c).

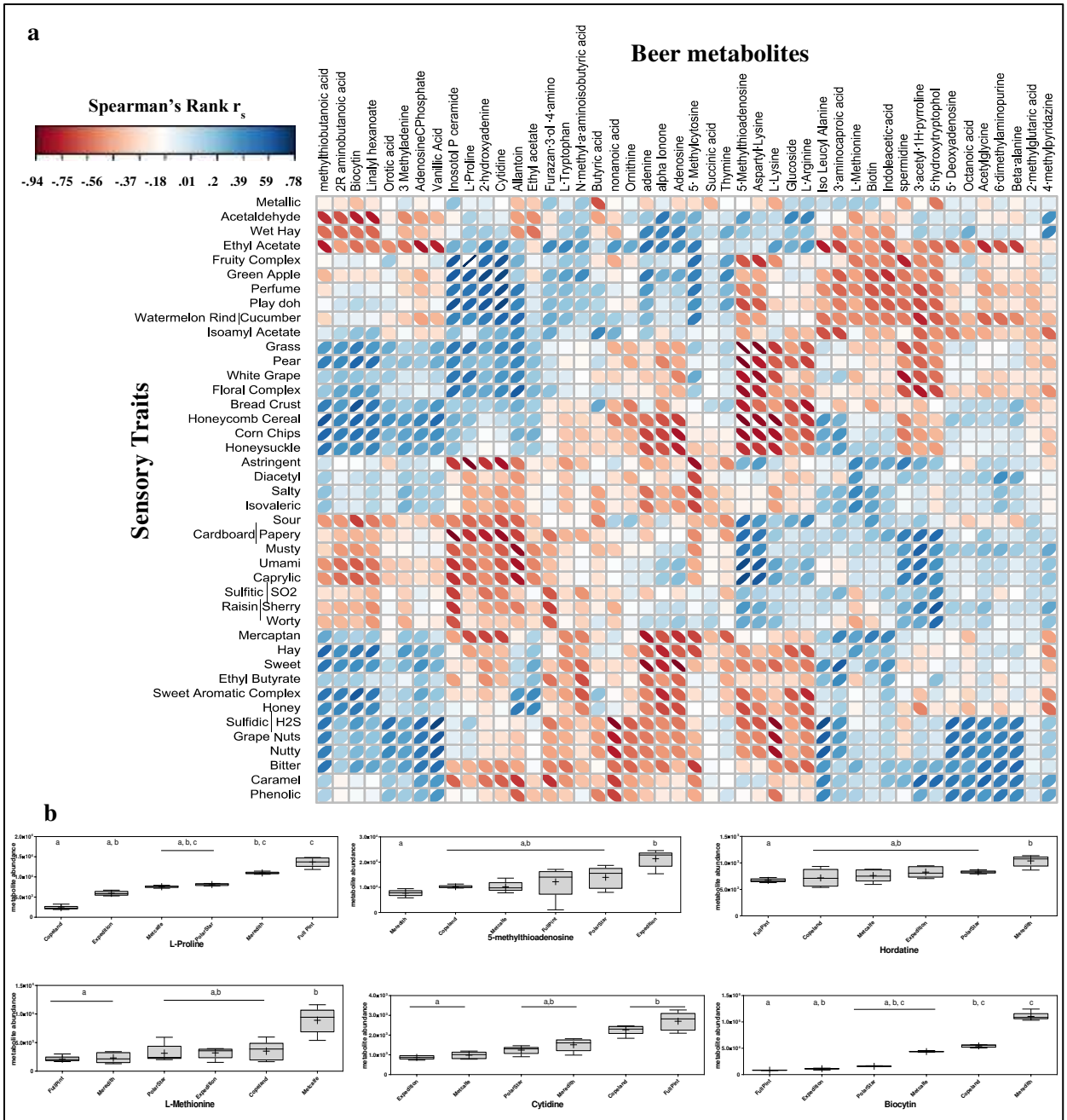


Figure 7. Univariate association of beer metabolites with beer sensory traits after 2 months of storage.

The association between nitrogenous beer metabolites and beer sensory was evaluated with Spearman's rank correlation **(a)** Correlation plot of r_s values for malt metabolites (x-axis) and sensory traits (y-axis). Colors and the eccentricity of the ellipse indicates strength and direction of the r_s correlation, and the data was arranged using hierarchical clustering. (b) Box plots were used to indicate example metabolite variation in the system. Data are presented as the mean metabolite abundance ± S.E.M. for n =3-5 extraction replicates per genotype. ANOVA with a Tukey post-hoc ($p < 0.05$) was performed on the data in the box plots, and differences are denoted with letters (e.g. a, b, c).

(Figure 4h), there was separation between umami characteristics and non-umami characteristics fell amongst Full Pint, Meredith, Metcalfe and PolarStar (Figure 4a, also shown in this panel for reference). There were distinct metabolites shown to be related to the sensory trait "umami" and associated with those genotypes (Figure 4h).

The metabolites associated with "fruity" sensory traits (Figure 4g) were detected in the direct path of the Full Pint and Copeland genotypes. The metabolites associated with "corn chip or sulfitic" (sulfitic refers to the aroma of burnt rubber or a lit match) (Figure 4f) were predominantly near Meredith. "Cardboard," a common off-flavor property related to staling, (Figure 4e) and "sulfidic or caprylic" (sulfidic refers to rotten egg aroma and caprylic refers to "vomit or barnyard" organoleptic properties) (Figure 4i) metabolite loadings were distributed equally amongst Full Pint, Metcalfe and Meredith. These loadings plots (Figures 4e-i) provided a false impression of flavor prediction based on metabolites that were associated with the six genotypes and their flavor attributes at 2 months. Brewing and fermentation are integrative processes and it is the combination of these metabolites from which flavor is derived, not simply one or two innocuous metabolites.

3.4. Univariate Analysis Revealed Malt and Beer Metabolites that were Associated with Beer Flavor

The malt and beer metabolite data were integrated with beer flavor data using Spearman's rank correlation analysis combined with hierarchical clustering. The flavor data were z-transformed prior to analysis. The analysis of malt was performed independently for two chemical classes: nitrogenous compounds and lipids, due to the known contribution of these classes to brewing and beer flavor (reviewed in Section 1.2.3 and 1.2.6). Several nitrogenous malt metabolites were found to be associated with flavor in beer (Figure 5a and b). For example, the clustering of the flavors "green apple," ethyl acetate, isoamyl acetate, and "fruity" were positively correlated ($r > 0.71$, $p < 0.05$) with L-isoleucyl-L-proline (amino acid), and 2-ketohexanoic acid (an oxo-keto-acid which is metabolized by yeast during fermentation and is involved in the formation of fusel alcohols from aldehydes) [94]. These malt

metabolites are associated with the sensory at Month 2 of the Full Pint and Copeland genotypes. The boxplots (Figure 5b) revealed the relative abundances of the malt metabolites for the genotypes.

The "cornchip," "caramel," and "grapenuts," flavor traits were associated with gamma-glutamyl-methionine (dipeptide), ornithine (an amine, the result of arginine catabolism by lactic acid bacteria in the mashing stage of brewing) and purines. Relative abundances (Figure 5b) illustrated Meredith, Metcalfe, and Polarstar as being highly abundant in these metabolites. Tyramine (amino acid) and 5'-methylthioadenosine (purine intermediate) were associated with "cardboard," "umami," and "astringent" flavor traits. Relative abundances of these metabolites (Figure 5b) were higher in Meredith and Expedition. Several malt metabolites were associated with beer sensory traits at Month 2, however integration of these metabolites with sugars, lipids, phenolics, and other metabolites is key to the production of flavors.

Lipids in malt that were associated with flavor traits are shown in Figure 6a. Lipids are important traits of malt chemistry that contribute directly to the beer. They not only contribute to flavor, but also to viscosity, head retention (foam), and flavor stability. The lipid content and composition varied in each genotype (Figure 6b) in the study. Abundances of lipids in malt also varied depending on lipid class. The triglyceride class, including saturated fat metabolites such as palmitic and stearic acid, was associated with the "fruity" and "watermelon rind" sensory traits. Relative abundance boxplots (Figure 6b) revealed that Full Pint is higher in the saturated fat class of lipids. Phosphatidylcholines and glycerophosphocholines were associated with the "corn chip," "grape nuts," and "sulfidic" sensory traits. The relative abundance boxplots displaed higher content in Meredith and Expedition. Malt lipid abundances generally vary by genotype and are important to brewing. These classes of lipid also appears to have an effect on beer flavor, foam retention, and mouthfeel.

Nitrogenous compounds (amines, amino acids, purines) in beer were associated with flavor traits. For example, 5'-methylthioadenosine (a purine nucleoside) was shown to be negatively correlated with most "fruity" traits, but was positively correlated with typical staling traits of "cardboard," "umami," and sulfitic (SO_2) (Figure 7). The sulfur compounds were also positively correlated with other amino acids

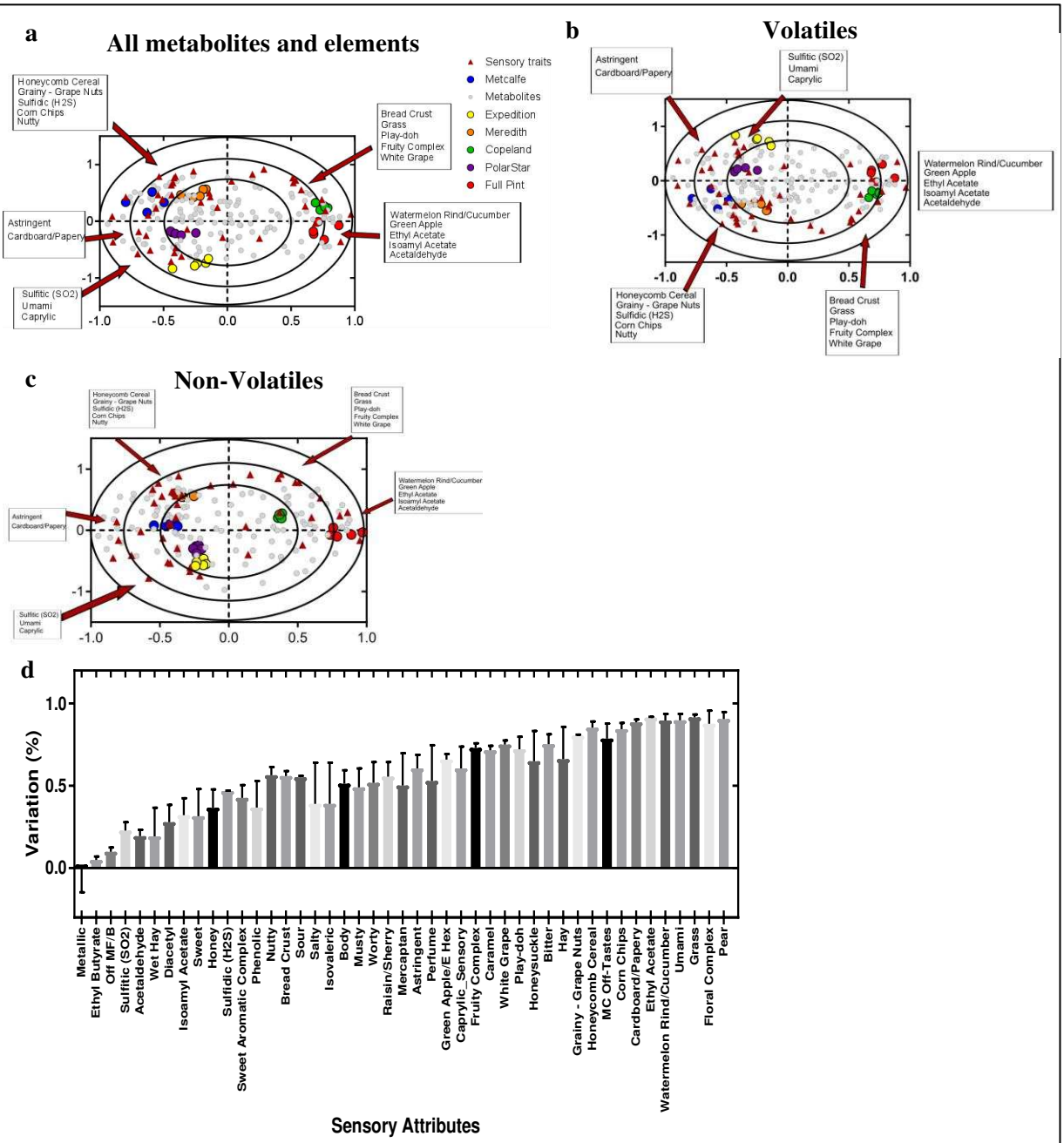


Figure 8. Multivariate association of beer metabolites with beer sensory traits after 2 months of storage.

The association between beer metabolites and beer sensory was evaluated with two-way orthogonal partial least squares (O2PLS) and performed on 246 metabolites, 20 metals, and 45 sensory traits **(a)** O2PLS overview biplot of all 246 metabolites and 20 metals, genotype, and sensory trait associations showed separation amongst the six genotypes and co-variation with sensory traits and metabolites. **(b)** Biplot of 138 volatile metabolites, genotype, and 45 sensory trait associations. **(c)** Biplot of 108 non-volatile metabolites, genotype, and 45 sensory trait associations provided additional separation among the beer genotypes **(d)** Cumulative prediction plot (Q^2Y) of sensory traits by beer metabolites. This plot represents, based on the O2PLS beer model, the predictability of these sensory traits. The higher the %, the more reliably it is predicted, based on metabolite composition (derived from barley genetics) and abundance in the beer.

and dipeptides in beer which were dominant in Meredith, such as biocytin and the flavor traits associated with it, “corn chip,” “bread crust,” “sulfitic,” and “sulfidic.” As stated earlier (Sec. 1.2.3), an excess of any FAN in the beer is related to off-flavors, flavor instability, and production of fusel alcohols [31]. These compounds created in beer also displayed positive correlation to the savory flavor that we see in Meredith. Thymine (a DNA nucleobase) and cytidine (a nucleoside), co-varying metabolites, were associated with the fruitier flavors also connected with Full Pint. L-proline, an amino acid which is not readily absorbed by yeast during fermentation, was noted as the most abundant in Meredith and Full Pint. Integration of these metabolites during brewing and fermentation is how flavor is created in beer. Abundances of these metabolites vary generally by genotype. This interaction between genotype and metabolites was confirmed using univariate analysis (ANOVA and PCA, which were in agreement that genotype is a driving factor in separation seen in the beer PCAs (Figure 4).

3.5. Integration of Chemical Data Revealed Significant Variation among Genotypes

3.5.1. Two-way Orthogonal Projection to Latent Structures (O2PLS)

In the univariate analysis of malt and beer (unbiased PCA and biased ANOVA), it was determined that the malt and beer metabolite data sets explained separation among genotypes. Each PC was explained by the variation within the metabolite data set or the sensory traits. Given that the limitation of univariate analysis is that it can explain only one dependent y variable for each x variable, a more robust method of analysis (O2PLS) was conducted to incorporate multiple y variables.

The metabolite and sensory data were integrated using O2PLS analysis. O2PLS is an extension of multivariate regression which builds on orthogonal projection to latent structure by adding multiple y-variables [87, 95-97]. It is a data integration technique, and in this study, provided a multivariate-level integration of sensory and metabolite data. O2PLS extends from the standard partial least squares (PLS) model by assuming the x variables (metabolites) and y variables (sensory traits) are weighted similarly.

One block is created to model the associations between x and y and another block is created to model the remaining parts of x and y separately and then as residuals. In other words, this states that there are many components in each data set that are unique to the dataset and may or may not be linked to components of the other data set(s). O2PLS provides information about the interrelated features among the datasets without regression against one class variable (as in O2PLS with discriminant analysis or O2PLS-DA) [35, 87, 95-98]. This method was developed to identify covariation across two multivariate data sets [98]. Covariation between the sensory data set and the beer metabolite data set was determined by setting the sensory traits as O2PLS "y" variables and the metabolites as "x" variables. This O2PLS malt model identified 5 components in the beer metabolite data set. Cross-validation of this O2PLS model was conducted by SIMCA with the "leave one out" method (Section 2.10, Table 10).

In both the malt and beer model, there were n=6 genotypes. With a limited sample size, power (the probability of statistically significant evidence of differences among groups) is reduced. Cross validation ("leave one out" explained in Section 2.10) was performed in SIMCA. The Q^2 score is an estimate of the predictive ability of this model. It provides a qualitative measure of consistency between the original data and the predicted data. Q^2 scores do not imply significance, however. The acceptable values for biological data is $Q^2 > 0.4$ (on a scale from 0 to 1) [35, 87, 95, 96, 98]. Table 11 displays the cross-validation scores for beer [99].

Multiple trends were seen based on the distribution of metabolites, metals, and sensory data in an O2PLS overview plot of the beer genotypes (Figure 8a). Full Pint and Copeland were separated from the other four genotypes and are associated with the "fruity," "watermelon rind," ethyl acetate, and acetaldehyde sensory traits. Meredith and Metcalfe were associated with the "corn chip," sulfidic (H2S), and "honeycomb cereal" sensory traits. PolarStar and Expedition were associated with the "umami" and "cardboard" flavors. According to the O2PLS model, 25 sensory traits associated with Full Pint and Meredith beer were over 50% predictable, given the metabolite data (Table 9, Figure 8d). Given this indicator of predictability, sensory traits could be estimated based on the beer metabolite data.

The O2PLS model plotting the beer sensory data set with the volatile metabolite data set displayed similar trends as in the overview plot (Figure 8b). There was separation seen among genotypes, but with Full Pint and Copeland clustered closer together, PolarStar and Expedition clustered together, and Meredith and Metcalfe clustered more closely together. These same trends were seen in the O2PLS biplot of non-volatile metabolites plotted against sensory traits (Figure 8c). These overview plots indicated that each genotype possessed a unique volatile chemical profile, but some profiles are more similar to each other. In these overview biplots, the sensory traits that were seen in previous PCAs of the beer (Figure 2), as well as the correlation plot (Figure 7) were displayed in this more robust multivariate analysis.

3.5.2. Variable line plot data were used to identify sets of metabolites that co-vary with sets of sensory traits

Variable line plots using the multivariate O2PLS model for beer were created using sets of metabolites that correlated with sets of sensory traits, which was consistent with the sensory experience that a sensory panel may have. Variable line plots of metabolite data were created for Full Pint and Meredith, based on the O2PLS model, and confirmed the specific sensory traits attributed to Full Pint and Meredith genotypes. The metabolite contribution to Full Pint sensory traits (*e.g.* "fruity," "ethyl acetate," and "watermelon rind") demonstrated that many metabolites were being integrated to create flavors in beer (Figure 9a). In Full Pint, the nitrogenous compounds were consistent with those identified in the correlation plot of beer metabolites interrelated to sensory traits and the boxplots of abundances of these metabolites (Figure 7).

For example, cytidine, a nucleotide excreted by yeast early in fermentation and under storage conditions was seen in the correlation plot (Figure 7a) and abundant in Full Pint (Figure 7b) [5]. L-tryptophan and L-arginine are both abundant in Full Pint and may have had an effect on flavor as they interact with other volatile compounds, purines, or alkaloids. Purines, as in Full Pint, 2-hydroxyadenine

3-methyladenine, are taken up by yeast, but are not held by them, so they diffuse back into the beer. The 5' nucleotide class is recognized to have an influence on flavor in beer when concentration levels are high [5, 62, 100].

Aromatic monoterpenes contributed to the unique flavor traits attributed to beer made with Full Pint. The impact of monoterpenes on the finished beer is hard to quantify because of additional chemical changes during transesterification (the process of exchanging the organic group R″ of an ester with the organic group R′ of an alcohol) by yeast cells during fermentation. Important terpenes include linalool and geraniol, which contribute floral characteristics to beer flavor, as well as limonene and α-terpineol, which contribute citrus characteristics [12, 16, 18]. Little is known about the sulfur-containing hydrocarbons and their aromatic contribution. Another metabolite seen in Full Pint is alpha-Ionone, which is a volatile ketone associated with floral, pear, and melon rind attributes [12]. This compound is synthesized from citral (terpene) and acetone (which is synthesized from ketosis of Free Fatty Acids) and may contribute to the unique flavor profile of "fruity," "watermelon rind," and ethyl acetate in Full Pint (Figure 7, Figure 9a).

Variable line plots of sensory traits attributed to Meredith and the metabolites that contribute to those traits display that the traits are consistent those identified in the Spearman's correlation heatmap of beer metabolites and sensory traits along with boxplots of abundances of these metabolites (Figure 5, Figure 7, Figure 9). The metabolites that contributed to the unique flavor profile of Meredith remained amino acids and terpenes, but there were more sulfur-containing compounds associated with this genotype (*i.e.* 2-methylthiobutanoic acid and tryptophyl-cysteine). This was evidence that the unique flavor profiles of each genotype are the combination of many metabolites found in beer. Purines and pyrimidines found in malt and beer which are subject to Maillard reactions have been associated with the "corn chip" flavor in beer (as well as the flavor of corn tortillas)[101].

3.5.3. O2PLS and variable line plot data for malt genotypes displays trends

In malt, trends were observed among genotypes. Separation among genotypes was explained by sensory traits and the metabolites associated with them in the O2PLS biplot overview (Figure 10a). The metabolites are varied in their contribution as was indicated in the heatmaps (Figures 5a and 6a) which correlated two chemical classes of metabolites in malt – lipids and those with a nitrogenous base (amino acids, pyrimidines, *etc.*). In the O2PLS Biplot (Figure 10a) of malt, a more distinct separation among genotypes indicated distinct flavor profiles are attributed to each genotype based on the malt composition Full Pint was associated with the "fruity," "sweet," acetaldehyde, and "watermelon rind" sensory traits and Meredith was associated with the "corn chip," sulfidic (H_2S), and "honeycomb cereal" sensory traits. According to the O2PLS model, the traits associated with Full Pint and Meredith were over 50% predictable, given the metabolite data. Sixteen out of 45 sensory traits in the malt model had cumulative prediction rates (Q^2Y) of greater than 50% (Figure 10b, supplementary Table 8), indicating which sensory traits could be reliably predicted based on malt metabolite data.

In the Full Pint malt variable line plot, (Figure 10c), more lipids that possibly play a role in the flavor traits at Month 2 were seen. In Meredith malt (Figure 10d), sulfur-containing amines, amino acids and sugars played a role in contribution to the "honeycomb cereal," and "corn chip" flavors (Figure 6e). Taken together, the malt data indicated that each genotype had a unique flavor profile derived from the integration of many metabolites.

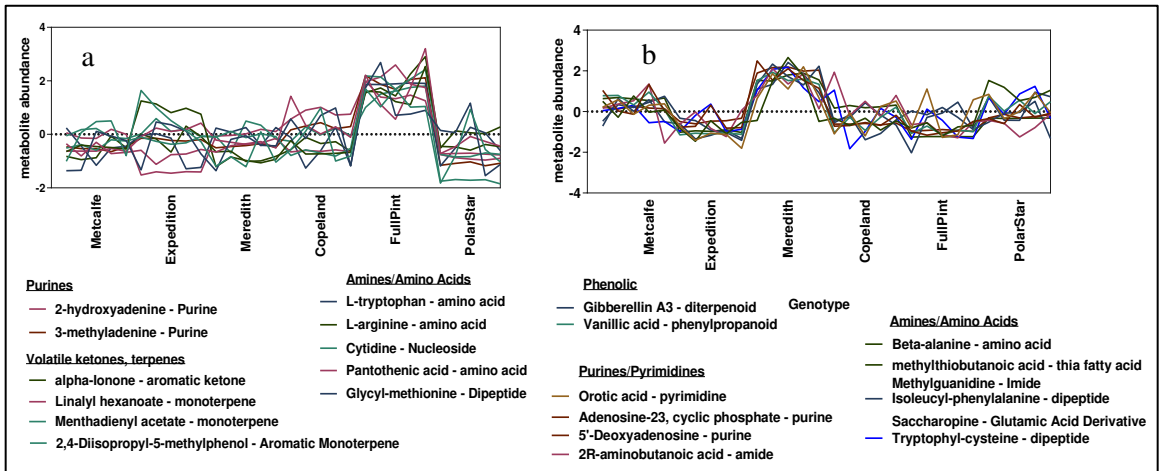


Figure 9. Multivariate association of beer metabolites with beer sensory traits after 2 months of storage.

The association between beer metabolites and beer sensory was evaluated with O2PLS and performed on 246 metabolites, 20 metals, and 45 sensory traits. Variable line plots graphed the contribution of more highly abundant metabolites contributing to sensory traits associated with each beer genotype. **(a)** Variable line plot of beer metabolites most abundant in Full Pint, as explained by the O2PLS model that includes all metabolites and metals. **(b)** Variable line plot of beer metabolites most abundant in Meredith, as explained by the O2PLS model that includes all metabolites and metals.

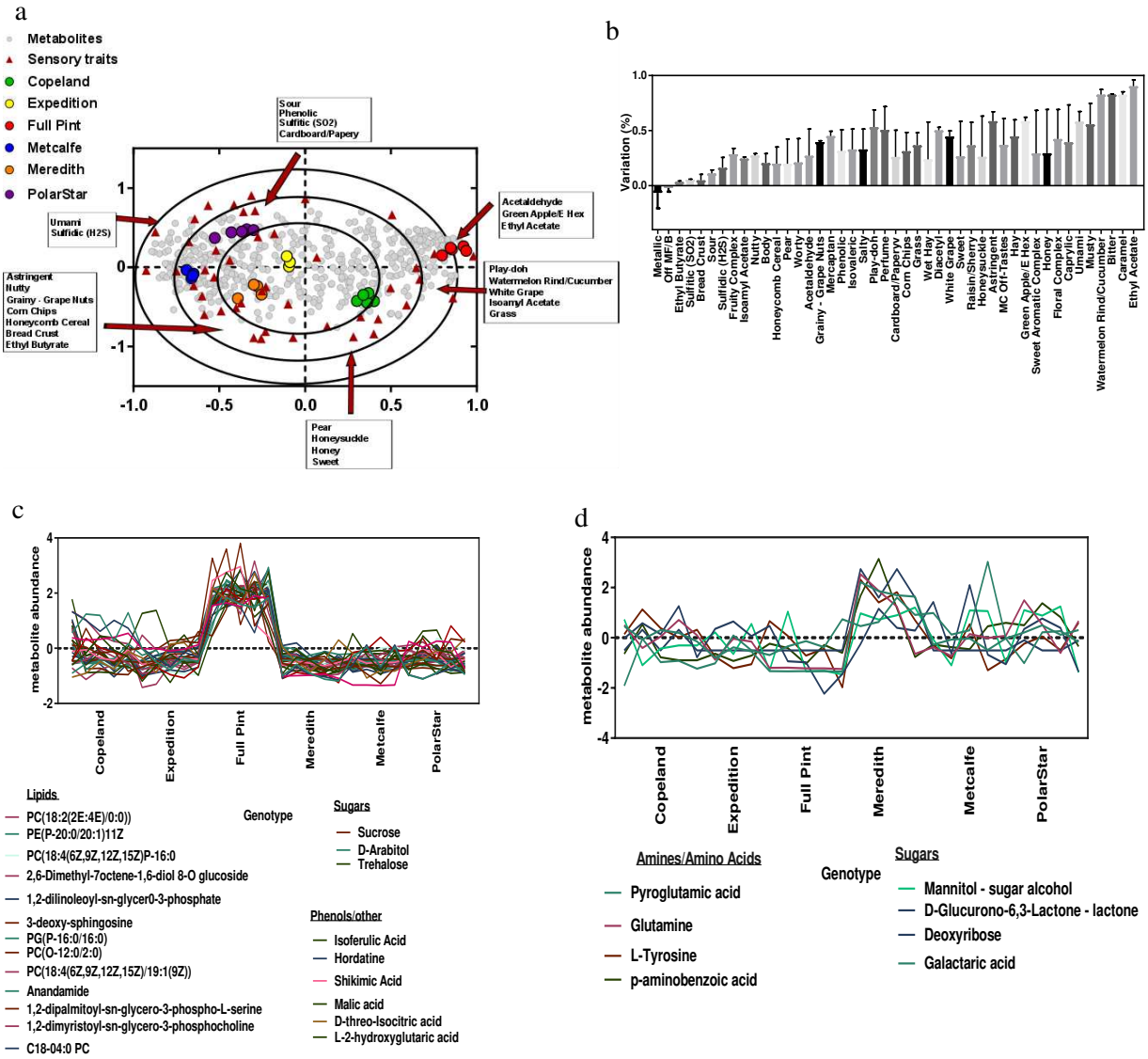


Figure 10. Multivariate association of malt metabolites with beer sensory traits after 2 months of storage.

The association between malt metabolites and beer sensory was evaluated with two-way orthogonal partial least squares (O2PLS) and performed on 217 metabolites, 20 metals, and 45 sensory traits **(a)** O2PLS overview biplot of all 246 metabolites and 20 metals, genotype, and sensory trait associations showed separation amongst the six genotypes. **(b)** Cumulative prediction plot (Q^2Y) of sensory traits by beer metabolites. This plot represents, based on the O2PLS model, the predictability of these sensory traits. **(c)** Variable line plot of malt metabolites most abundant in Full Pint, as explained by the O2PLS model that includes all metabolites and metals. **(b)** Variable line plot of malt metabolites most abundant in Meredith, as explained by the O2PLS model that includes all metabolites and metals. All analyses were conducted on n = 3-5 extraction replicates per genotype

Table 4. Malt metabolites associated with Full Pint

Chemical Class	Metabolite Name	Structure	Platform***	Reported Sensory	Beer Sensory[d]	Malt Sensory[e]
5'-deoxy-5'-thionucleosides	MTA	C11H15N5O3S	b/a	Umami-like, sulfurous	Nutty/Corn chip/Grainy/Sulfitic/Sulfidic	Phenolic/Sulfidic
6-aminopurine	Adenine	C5H5N5	b /a		Corn chip	Play-doh/Nutty/Grainy Corn Chips/Honeycomb Cereal
Acylaminosugar	N-acetylmannosamine	C8H15NO6	b			
Alkaloid	N-methyltryptamine	C11H14N2	a	Glutanimous	Umami/Astringent/Cardboard	Sulfitic/Cardboard/Sulfidic
Alpha-Amino Acid	Glutamine	C5H10N2O3	b	Fruity, Vegetal, Umami, Savory		Bread Crust/Corn Chip
Alpha-amino acid	Ornithine	C5H12N2O2	b		Corn chip/HoneySuckle/HoneyComb	Sulfitic/Cardboard/Sulfidic
Amine	Methylamine	C6H13NO	c	Vegetable, grape, carrot, cabbage	Sulfidic	Wet Hay
Amino Acid	Alanine	C3H7NO2	b	Sweet		Phenolic/Sulfidic
Amino Acid	Aminooctanoic acid	C8H17NO2	b			Bread Crust
Amino Acid	Biocytin	C16H28N4O4S	a		HoneyComb/Nutty/Corn chip	Pear/Isoamyl Acetate
Amino Acid	DAPA	C7H14N2O4	b			Bread Crust
Amino Acid	L-Arginine	C6H14N4O2	a	Sweet, bitter, astringent	Bread Crust	Grainy-Grape nut cereal
Amino Acid	L-Asparagine	C4H8N2O3	b /a	Savory	Bread Crust	Bread Crust
Amino Acid	L-Methionine	C5H11NO2S	b/a		Isovaleric/Corn chip/Umami/	Umami
Amino Acid	L-Tryptophan	C11H12N2O2	b/ a	bitter-sweet, methanol-like	Sulfitic/Sulfidic/Corn chip/Ethyl Butyrate	Grainy/Astringent/Mercaptan
Amino Acid	L-Tyrosine	C9H11NO3	b	Vegetal, Savory		Bread Crust/Honeycomb Cereal
Amino Acid	Vitamin B5	C9H17NO5	b/a	Astringent, Salty	Bitter/Astringent/Sour/Isovaleric/Ethyl Butyrate	Sulfitic/Sulfidic
Benzenoid	3-Phenoxypropionic acid	C9H10O3	b			Phenolic/Sulfidic
Benzenoid	PABA	C7H7NO2	b	aminobenzoic acid, hay-like	Wet Hay	Sulfidic/Sulfitic/Phenolic

Carboxylic Acid Derivative	3-O-methyldopa	C10H13NO4	b	Bitter, lit match		Corn chip/Grainy/Sulfidic/Phenolic
Carboxylic Acid Derivative	Ethyl Dimethylcarbamate	C5H11NO2	c	carboximidic acids	Corn chip/HoneyComb/Nutty/Sulfidic	Sulfidic
Carboxylic Acid Derivative	Maleic acid	C4H4O4	b			Bread Crust
Carboxylic Acid Derivative	N-(1-Deoxy-1-fructosyl)leucine	C12H23NO7	a			Sweet/Bread Crust/Honeycomb Cereal
Carboxylic Acid Derivative	N-1(1-Deoxy-1-fructosyl)isoleucine	C12H23NO7	a			Sulfitic/Sulfidic/Sour
Carboxylic Acid Derivative	N-acetylglutamic acid	C7H11NO5	b	Sulfrous, glutaminous, Umami		Sulfitic/Sulfidic/Phenolic/Cardboard
Carboxylic Acid Derivative	o-Tyrosine	C9H11NO3	b			Corn Chips/Honeycomb Cereal
Carboxylic Acid Derivative	Pyroglutamic acid	C5H7NO3	b	soapy, astringent, less intense sour	Umami	Mercaptan/Corn Chips
Carboxylic Acid Ester	Isoamyl formate	C6H12O2	c	Plum, vinous, ethereal		Sulfitic/Sulfidic
Dialkyldisulfide	Methyl propyl disulfide	C4H10S2	c	Garlic, burnt rubber,	HoneyComb	Corn Chips/Honey Comb
Dicarboxylic Acid	Fumaric acid	C4H4O4	c	Sour	Sour/Sulfitic/Phenolic	Sulfitic/Sulfidic/Sour
Dicarboxylic Acid	TXIB	C16H30O4	c	Butyric	Ethyl Butyrate/Sulfitic/Phenolic	Sulfitic/Sulfidic/Phenolic
Dipeptide	Glutamyl Methonine	C10H16N3O6	a			Corn Chips/Hay/Mercaptan
Dipeptide	Glycylproline	C7H12N2O3	ZIC-HILIC-MS/a	Toasty, roasty, malty		Sweet/Bread Crust
Disaccharide	Isomaltose	C12H22O11	a		Watermelon Rind/Cucumber/Fruity/White Grape/Isoamyl Acetate	Hay/Corn Chips
Ester	N-allyl-L-alanine	C6H11NO3S	c		Ethyl Butyrate/Corn chip/Isovaleric	Mercaptan
Fatty Acid Ester	Oct-3-enoic acid, oct-3-en-2-yl ester	C16H28O2	c		Corn chip/HoneyComb/Nutty/Sulfidic	Phenolic/Umami/Caramel/Bitter/Astringent

Fatty Acid Ester	Diethyl decanedioate	C14H26O4	c	Fruity, Melon, Quince, Wine, Mild		Sweet/Honey/Sweet Aroma
Fatty Acid Ester	Isobutyl 3-methyl-2-butenoate;	C9H16O2	c	Green, spicy, mint	Nutty/Sulfitic/Sulfidic/Phenolic	Nutty/Sulfitic/Sulfidic/Phenolic
Fatty Acid Ester	Isobutyl caprylate	C12H24O2	c	Swiss cheesy, winey, fatty, sweet	Ethyl Butyrate/Caprylic/Isovaleric/Corn chip	Mercaptan
Fatty Acid Ester	Methyl 2-(methylthio)Butyrate	C6H12O2S	c	Musty, onion, sulfurous	Isovaleric/Mercaptan/Sulfitic/Sulfidic	Sulfitic/Sulfidic
Fatty Acid Ester	Pelargonic Acid	C9H18O2	c	Unpleasant, rancid, old oil		Grain/Mercaptan/Corn Chips/Nutty/Astringent
fatty acid methyl esters - Fatty Acyl	Methyl hexanoate	C7H14O2	c	goaty, fatty acid, vegetable oil, sweaty, caprylic		Bread Crust
Fatty Acyl	Isopentyl Hexanoate	C11H22O2	c	Milky, fruity		Honeycomb cereal/Corn Chips
Fatty Acyl	Pentadecanoic acid	C15H30O2	b			Bread Crust
Flavanol glycoside	Rutin	C27H30O16	b			Sulfidic/EthylButyrate/Sulfitic
Furan	Furan, 2-nonadecanoyl	C23H40O2	c	Aromatic, roasty, nutty	Corn chip/HoneyComb/Nutty	Metallic
Glycerophospholipid	PA(16:0/18:2)	C37H69NO8P	b			Nutty/Sulfidic
Hydroxy Acid	Galactonate	C6H12O7	b			Sulfidic/Nutty
Hydroxy Acid	L-Lactic acid	C3H6O3	b	Acidic		Sulfidic/Nutty
Hydroxy Acid	Malic acid	C4H6O5	b	sour-like, sweettart		Green Apple/Ethyl Acetate
Hydroxy Fatty Acid	Hydroxyisocaproic acid	C6H12O3	b	caproic, goaty, sulfurous		Sulfitic/Sulfidic/Phenolic/Cardboard
Hydroxyindole	5-hydroxytryptophol	C10H11NO2	a	Old almonds, unpleasant	Caprylic/Musty/Sulfidic/Sulfitic	Pear/Grass/Fruity
Monosaccharide	Deoxyribose	C5H10O4	a	Sweet		Honeycomb Cereal/Corn Chip/Grainy
Monosaccharide phosphate	Glucose-1-phosphate	C6H13O9P	b			Sulfitic/Sulfidic
Phenethylamine	Tyramine	C8H11NO	a	Cheddar cheesey		Sulfitic/Sulfidic
Phenol	Vanillic acid	C8H8O4	b		Nutty/Sulfitic/Sulfidic/HoneyComb/Grainy	Acetaldehyde

Purine	Purine	C5H4N4	b			Phenolic/Nutty/Sulfidic/Bitter
Purine nucleoside	Adenosine	C10H13N5O4	a	Bitter, glutamate-like, Corn chippy	Nutty/Sulfitic/Grainy/Corn chip	Play-doh/Watermelon Rind
Purine nucleoside	Guanosine	C10H13N5O5	b	Glutamate-like, grain		Astringent/Sulfidic/Nutty/Phenolic
Purine nucleoside	Inosine	C10H12N4O5	b	Meaty, Savory	Bread Crust	Bread Crust
Pyrazine	Dithiouracil	C4H4N2S2	c	Toasted, roasted, Corn, toasted bread	Bread Crust	Sulfitic/Sulfidic/Wet Hay
Pyridine	4-methylpyridine	C6H7N	c	roasted, nutty, cocoa, peanut	Phenolic/Sulfidic	Wet Hay
Pyridinecarboxylic acid	Also Vitamin B3	C6H6N2O	b			Bread Crust
Pyridinecarboxylic acid	Vitamin B3	C6H5NO2	b/a	Sour, metallic	Corn chip	Metallic
Pyrimidone	Uracil	C4H4N2O2	b			Sulfitic/Sulfidic
Saccharide	Alpha-Sophorose	C12H22O11	b			Corn Chips/mercaptan
Secondary alcohol	Furazan-3-ol, 4-amino	C2H3N3O2	c	Aromatic, roasty, nutty	Corn chip/HoneyComb/Nutty	Play-doh
Sulfur Compound	4-(Methylthio)-1-butanamine	C5H13NS	c	Cabbage, garlic, potato, sulfury, vegetable	Sulfidic/Sulfitic/Mercaptan	Sulfidic/Phenolic
Sulfur Compound	Benzothiazole	C7H5NS	c	Rubbery, sulfury, cooked, gasoline	Mercaptan/Astringent	Wet Hay/Metallic/Sulfidic
Triglyceride	TG(50:5)iso6	C52H98O6	a			Sulfitic/Sulfidic

***Platform - a denotes RP/LC-MS; b denotes HILIC/LC-MS; c denotes SPME/GC-MS; d denotes if this metabolite was found in malt, e denotes if this metabolite was found in beer, sensory associated with beer at Month 2, according to O2PLS beer model.

Table 5. Malt metabolites associated with malt flavor in Meredith

Chemical Class	Metabolite Name	Structure	Platform	Reported Sensory	Beer Sensory[d]	Malt Sensory[e]
Acyl glycine	Deoxygcholylglycine	C26H43NO5	b			Perfume/Play-doh/Fruity
Alkene	1-pentadecene	C15H30	a			Perfume/Acetaldehyde/Green Apple
Alkylamine	Dimethylethanolamine	C4H11NO	c			Green Apple/Ethyl Acetate
Alpha Amino Acid	N-methyl-a-aminoisobutyric acid	C5H11NO2	a		Perfume/Fruity/GreenApple/PlayDoh/Ethyl Acetate	Ethyl Butyrate/Bread Crust
Amino Acid	L-Allothreonine	C4H9NO3	b	Sweet, bitter, astringent		Play-doh/Watermelon Rind
Amino Acid	L-Valine	C5H11NO2	a	Sweet, bitter, astringent		Fruity/Play-doh
Amino Acid	L-Valine	C5H11NO2	b	bitter, sweet, astringent		Perfume/Play-doh
Benzenoid	2,4-Di-tert-butylphenol	C14H20O	c	Bitter, astringent, Phenolic		Acetaldehyde
Benzenoid	2-phenylbutyric acid	C10H12O2	b			Acetaldehyde
Benzenoid	4-aminosalicylic acid	C7H7NO3	b	Solventy, fruity-astringent		Green Apple/Ethyl Acetate/Perfume
Benzenoid	Creosotinic Acid	C8H8O3	b			Green Apple/Ethyl Acetate
Benzenoid/Aldehyde	Benzaldehyde	C7H6O	c	bitter almond, cherry stone, almond	Floral/Grass	Floral/Grass/Ethyl Acetate/Watermelon Rind
Carboximidic acid	PEA	C18H37NO2	a			Green Apple/Ethyl Acetate
Carboxylic Acid Derivative	N-(1-Deoxy-1-fructosyl)methionine	C11H21NO7S	a			Acetaldehyde/Perfume
Carboxylic Acid Derivative	N-Acetyl-L-Phenylalanine	C11H13NO3	b			Acetaldehyde
Carboxylic Acid Derivative	NMDA	C5H9NO4	b	Sour, glutamate-like		Acetaldehyde
Carboxylic Acid Derivative	N-oleoyl-alanine	C21H39NO3	a			Perfume/Acetaldehyde/Green Apple
Carboxylic Acid Derivative	pyrrolidonecarboxylic acid	C5H7NO3	b /a			Acetaldehyde/Green Apple
Ceramide Phosphate	CerP(d18:0/16:0)	C34H70NO6P	b			Play-doh/Watermelon Rind/Fruity
Disaccharide	Trehalose	C12H22O11	b/a	o-glycosyl compounds	PlayDoh/GreenApple/Fruity/Ethyl Acetate	Green Apple/Ethyl Acetate

Endocannabinoid	C17:1 anandamide	C19H37NO2	b			Perfume/Fruity/Play-doh
Fatty Acid Ester	Methyl caprylate	C9H18O2	c	Fruity, cinnamony		Green Apple/Ethyl Acetate
Fatty Acid Ester	Methyl decanoate/Methyl caprate	C11H22O2	c	Sweet, coconut, fruity	Bread Crust/Fruity	Ethyl Acetate/Green Apple
Fatty Acid Ester	Methyl dodecanoate	C13H26O2	c	grape, fruity, apple		Green Apple/Ethyl Acetate
Fatty Acid Ester	Methyl tetradecanoate	C15H30O2	c	Perfumey, herbal, petals	Perfume/EthylAcetate/Fruity/Watermelon/PlayDoh	Perfume/Ethyl Acetate/Green Apple
Fatty Acid Ester	Nonyl Phenylacetate	C17H26O2	c	Fruity, fruit, soapy, tropical, tea-like	Fruity/Ethyl Acetate	Metallic
Fatty Acyl	Alchornoic Acid	C20H36O3	a			Perfume/Acetaldehyde/Green Apple
Fatty Acyl	Caprylic Acid	C8H16O2	c	caprylic, goaty, fatty acid, vegetable oil, wet dog	Acetaldehyde	Green Apple/Ethyl Acetate
Fatty Acyl	Citraconic acid	C5H6O4	b	Citric		Green Apple/Ethyl Acetate
Fatty Acyl	Elaidic acid	C18H34O2	b			Perfume/Green Apple/Ethyl Acetate
Fatty Acyl	Heptanoic acid	C7H14O2	b	Rancid		Green Apple/Perfume/Acetaldehyde
Fatty Acyl	Nervonic acid	C24H46O2	b			Perfume
Fatty Acyl	Turanose	C12H22O11	b/a	reducing disaccharide	Ethyl Acetate/Perfume/Fruity	Ethyl Acetate/Perfume/Fruity
Fatty Acyl Glycoside	2,6-Dimethyl-7octene-1,6-diol 8-O glucoside	C16H30O7	a	anise-like, fennel		Green Apple/Ethyl Acetate
Fatty Acyl	2-amino-octadecanoic acid	C18H37NO2	a			Acetaldehyde
Glycerophosphocholine	PC(16:0-18:1)	C42H82NO8P	b			Green Apple/Ethyl Acetate
Glycerophosphocholine	PC(18:4(6Z,9Z,12Z,15Z)/19:1(9Z))	C40H80NO8P	a			Green Apple/Acetaldehyde
Glycerophosphoethanolamine	GPE(P-18:0/20:4)	C5H14NO6P	b			Green Apple/Ethyl Acetate
Glycerophospholipid	PE-NMe(32:0)	C38H76NO8P	b			Green Apple/Ethyl Acetate
Hydroxy Acid	L-2-hydroxyglutaric acid	C5H8O5	b			Ethyl Acetate/Green Apple
Hydroxy Acid	Malic acid	C4H6O5	b	sour-like, sweettart		Green Apple/Ethyl Acetate

Hydroxycinnamic Acid	Isoferulic Acid	C10H10O4	ZIC-HILIC_LC-MS/a	Clovey, spicy, fruity		Perfume/Play-doh/Acetaldehyde/Green Apple
Inositolphosphorylceramide	PI-Cer(t20:0/26:0)	C52H104NO12P	a			Green Apple/Ethyl Acetate
Intermediate	Shikimate	C7H10O5	b			Green Apple
Keto Acid	ketoisocaproate	C6H10O3	b	Sweet, fruity		Metallic/Acetaldehyde
Keto Acid	Oxoadipic acid	C6H8O5	b			Acetaldehyde/Perfume
Lysophospholipid	Lyso PC(18:2)	C26H50NO7P	a			Green Apple/Ethyl Acetate
monoglycerophospholipid	LysoPC(18:3(9Z,12Z,15Z))	C26H48NO7P	a			Green Apple/Ethyl Acetate
Monosaccharide	D-fructose	C6H12O6	b	sweet		Green Apple/Ethyl Acetate
Monosaccharide	D-Tagatose	C6H12O6	b/a	Sweet	Fruity/Perfume/EthylAcetate/Green Apple/IsoamylAcetate	Bitter/Astringent, Caramel
Monosaccharide	Sucrose	C12H22O11	b	nonreducing sugar	Sweet/Honey/Ethyl Butyrate	Green Apple/Ethyl Acetate
Nucleoside	Cytidine	C9H13N3O5	b	glutamate-like, bitter	Watermelon/Ethyl Acetate/PlayDoh	Acetaldehyde
Oligosaccharide	Stachyose	C24H42O21	b		Green Apple/Fruity	Astringent/Grainy
Organooxygen Compound	NeuAc	C11H19NO9	b			Perfume/Acetaldehyde/Green Apple
Peptide	Glutathione	C10H17N3O6S	b			Green Apple/Play-doh
Phenol	3-Ethylphenol	C8H10O	c			Perfume/Acetaldehyde/Green Apple
Phenol	N-[4-(cyanomethyl)phenyl]-5-(phenoxymethyl)furan-2-carboxamide	C20H16N2O2	c			Green Apple/Ethyl Acetate
Phosphatidylcholine	PC(18:4)	C26H46NO7P	a			Green Apple/Ethyl Acetate
Phosphatidylcholine	PC(28:0)	C36H72NO8P	b			Green Apple/Ethyl Acetate
Phosphatidylcholine	PC(32:0)	C40H80NO7P	b			Green Apple/Ethyl Acetate
Phosphatidylethanolamine	PE(40:1)	C48H94NO8P	a			Green Apple/Ethyl Acetate
phosphatidylglycero	PG(P-32:0)	C38H75O10P	a			Green Apple/Ethyl Acetate
Phosphatidylglycerolphosphate	PGP(36:4)	C42H76O13P2	a			Green Apple/Ethyl Acetate

Phospholipid	PC(O-14:0)		a		Green Apple/Ethyl Acetate
Purine nucleoside	1-methyladenosine	C11H15N5O4	b	Milk-like, salty	Play-doh/Perfume
Purine nucleoside	2-Phenylamino adenosine	C16H18N6O4	b		Green Apple/Ethyl Acetate
Pyrimidine nucleoside	Ribothymidine	C10H14N2O6	b		Play-doh
S-Containing Amino Acid Derivative	N-Acetyl-L-methionine	C7H13NO3S	ZIC-HILIC-MS/a	Sulfrous, glutaminous, Umami	Play-doh
Sugar Alcohol	D-Arabitol	C5H12O5	b		Green Apple/Fruity
Sugar Alcohol	Galactitol	C6H14O6	b	sweet	Green Apple/Ethyl Acetate
UFA	Oleic acid	C18H34O2	b		Fruity

***Platform - a denotes RP/LC-MS; b denotes HILIC/LC-MS; c denotes SPME/GC-MS; d denotes if this metabolite was found in malt, e denotes if this metabolite was found in beer, sensory associated with beer at Month 2, according to O2PLS beer model.

Table 6. Beer metabolites associated with flavor in Full Pint

Chemical Class	Metabolite Name	Structure	Platform	Reported Sensory	Beer Sensory[d]	Malt Sensory[e]
Aldehyde	Methyl Benzoate	C8H8O2	c	Fruity, herbal, floral	Acetaldehyde	
Alpha Amino Acid	N-methyl-a-aminoisobutyric acid	C5H11NO2	a		Perfume/Fruity/GreenApple/PlayDoh/Ethyl Acetate	Ethyl Butyrate/Bread Crust
Amino Acid	Acetylglycine	C4H7NO3	b	Fruity	Fruity/Perfume/EthylAcetate/Green Apple	
Amino Acid	Beta-alanine	C3H7NO2	b		Wet Hay/Acetaldehyde	
Benzenoid	Beta-Ionone (2,4-Di-tert-butylphenol)	C14H22O	c	raspberry, citrus, woodlands, violet, kettle hop aroma, artificial raspberry, strawberry, floral, berry	Wet Hay/Acetaldehyde	
Benzenoid	Phenethyl alcohol	C8H10O	c	alcohol, flowery, honey-like, roses, swee	Wet Hay/Acetaldehyde	
Benzenoid/Aldehyde	Benzaldehyde	C7H6O	c	bitter almond, cherry stone, almond	Floral/Grass	Floral/Grass/Ethyl Acetate/Watermelon Rind
Disaccharide	Trehalose	C12H22O11	b/a	o-glycosyl compounds	PlayDoh/GreenApple/Fruity/Ethyl Acetate	Green Apple/Ethyl Acetate
Fatty Acid Ester	Isopentyl hexanoate/Isoamyl caprylate	C11H22O2	c	fruity, solvent, perfumed, tropical fruits	Fruity/Ethyl Acetate/White Grape/Watermelon/Green Apple	
Fatty Acid Ester	Methyl decanoate/Methyl caprate	C11H22O2	c	Sweet, coconut, fruity	Bread Crust/Fruity	Ethyl Acetate/Green Apple
Fatty Acid Ester	Methyl tetradecanoate	C15H30O2	c	Perfumey, herbal, petals	Perfume/EthylAcetate/Fruity/Watermelon/PlayDoh	Perfume/Ethyl Acetate/Green Apple
Fatty Acid Ester	Nonyl Phenylacetate	C17H26O2	c	Fruity, fruit, soapy, tropical, tea-like	Fruity/Ethyl Acetate	Metallic
Fatty Acyl	Butanoic Acid	C5H10O2	b	buttery, rancid, cheesy	PlayDoh	
Fatty Acyl	Butyric Acid	C5H10O2	c	Fatty acid methyl ester	Watermelon Rind/Cucumber/Fruity/White Grape	
Fatty Acyl	Caprylic Acid	C8H16O2	c	caprylic, goaty, fatty acid, vegetable oil, wet dog	Acetaldehyde	Green Apple/Ethyl Acetate
Fatty Acyl	Citraconic acid	C5H6O4	b	Citric		Green Apple/Ethyl Acetate
Fatty Acyl	Turanose	C12H22O11	b/a	reducing disaccharide	Ethyl Acetate/Perfume/Fruity	Ethyl Acetate/Perfume/Fruity
Glycosylamines	Nicotinomide riboside	C11H15N2O5	a		Fruity/Perfume/PlayDoh/GreenApple	
Hydroxypyrimidine	5-Methylcytosine	C5H7N3O	a		Perfume/Fruity/GreenApple/PlayDoh	
Ketone	2,2-Dimethyl-1,3-cyclohexanedione	C8H12O	c	sweet, caramel, maple	acetaldehyde	
Monosaccharide	D-Tagatose	C6H12O6	b/a	Sweet	Fruity/Perfume/EthylAcetate/Green Apple/IsoamylAcetate	Bitter/Astringent, Caramel

Monosaccharide	Sucrose	C12H22O11	b	nonreducing sugar	Sweet/Honey/Ethyl Butyrate	Green Apple/Ethyl Acetate
N-alkylpyrrolidine	1-Acetonylpyrrolidine	C6H13NO	a	Proline-derived Maillard product	Play-doh	
Nucleoside	Cytidine	C9H13N3O5	b	glutamate-like, bitter	Watermelon/Ethyl Acetate/PlayDoh	Acetaldehyde
Oligosaccharide	Maltopentaose	C36H52O26	a	sweet	Perfume/EthylAcetate/Fruity/Watermelon/PlayDoh	
Oligosaccharide	Stachyose	C24H42O21	b		Green Apple/Fruity	Astringent/Grainy
Phenol	Apigenin-6-C-glucoside	C21H19O10-	a	Grassy, hoppy	Grassy/WhiteGrape/Floral/Ethyl Acetate	
Polysaccharide	Oligosaccharide		a		Perfume/EthylAcetate/Fruity/Watermelon/PlayDoh	
Pyrimidine	Vitamin B1	C12H17ClN4OS	b		Green Apple	
Pyrimidne Nucleoside	Thymidine	C10H14N2O5	b	Sweet, nutty	Fruity/Green Apple	Caramel/Nutty/Umami/Bitter
S-Containing Amino Acid Derivative	N-Acetyl-L-methionine	C7H13NO3S	b/a	Sulfrous, glutaminous, Umami		Play-doh

***Platform - a denotes RP/LC-MS; b denotes HILIC/LC-MS; c denotes SPME/GC-MS; d denotes if this metabolite was found in malt, e denotes if this metabolite was found in beer, sensory associated with beer at Month 2, according to O2PLS beer model.

Table 7. Beer metabolites associated with Meredith

Chemical Class	Metabolite Name	Structure	Platform ***	Sensory Detected	Beer Sensory[d]	Malt Sensory[e]
Imidazopyrimidines	DMAP	C7H9N5	b		Umami/Sulfitic/Sulfidic	
5'-deoxy-5'-thionucleosides	5-MTA	C11H15N5O3S	b/a	Umami-like, sulfurous	Nutty/Corn chip/Grainy/Sulfitic/Sulfidic	Phenolic/Sulfidic
5'-deoxyribonucleoside	5'-Deoxyadenosine	C10H13N5O3	a	Glutamate-like	Sulfidic/Sulfitic/Caprylic/Umami/Caramel/Phenolic/Acetaldehyde	
6-aminopurine	Adenine	C5H5N5	b /a		Corn chip	Play-doh/Nutty/Grainy
Aldehyde	hydroxymethylfurfural	C6H6O3	a	stale, vegetable oil, Paper-like, vegetables, bready, caramel	Corn chip/HoneyComb/Nutty	
Aldehyde	Cinnamaldehyde	C10H10O	a	Spicy, bitter, Phenolic	Honeysuckle/Sweet/Sweet-Aromatic/Honey/HoneyComb	
Aldehyde	Methional	C4H10OS	c	Umami	Umami/Sulfitic/Isovaleric/Grainy/Sulfidic	
alkaloid	N-methyltryptamine	C11H14N2	a	Glutanimous	Umami/Astringent/Cardboard	Sulfitic/Cardboard/Sulfidic
Alpha Amino Acid	Methionine sulfoxide	C5H11NO3S	a	Biomarker of oxidative stress	Sulfidic/Sulfitic/Wet Hay	
Alpha Amino Acid	Sarcosine, N-(3-phenylpropionyl)-, pentyl ester	C17H25NO3	c		Corn chip/HoneyComb/Metallic	
Alpha-amino acid	Ornithine	C5H12N2O2	b		Corn chip/HoneySuckle/HoneyComb	Sulfitic/Cardboard/Sulfidic
Amine	Methylamine	C6H13NO	c	Vegetable, grape, carrot, cabbage	Sulfidic	Wet Hay
Amino Acid	α-aminoButyrate	C4H9	a	sweet-bitter, sour, glutamate-like	Corn chip/HoneyComb/HoneySuckle	
Amino Acid	Biocytin	C16H28N4O4S	a		HoneyComb/Nutty/Corn chip	Pear/Isoamyl Acetate

Amino Acid	Isoleucine	C6H13NO2	a	bitter-sweet, astringent	Sulfitic/Sulfidic/Grainy/ Musty/Isovaleric/Umami	
Amino Acid	L-Arginine	C6H14N4O2	a	Sweet, bitter, astringent	Bread Crust	Grainy-Grape nut cereal
Amino Acid	L-Asparagine	C4H8N2O3	b /a	Savory	Bread Crust	Bread Crust
Amino Acid	L-Lysine	C6H14N2O2	a	lemony	Honey Comb/Nutty/Corn chip/Grainy	
Amino Acid	L-Methionine	C5H11NO2S	b/a		Isovaleric/Corn chip/Umami/	Umami
Amino Acid	L-Tryptophan	C11H12N2O2	b/ a	bitter-sweet, methanol-like	Sulfitic/Sulfidic/Corn chip/Ethyl Butyrate	Grainy/Astringent/ Mercaptan
Amino Acid	Vitamin B5	C9H17NO5	b/a	Astringent, Salty	Bitter/Astringent/Sour/Isovaleric/Ethyl Butyrate	Sulfitic/Sulfidic
Benzenoid	PABA	C7H7NO2	b	aminobenzoic acid, hay-like	Wet Hay	Sulfidic/Sulfitic/Phenolic
Carboxylic Acid Derivative	Ethyl Dimethylcarbamate	C5H11NO2	c	carboximidic acids	Corn chip/HoneyComb/Nutty/Sulfidic	Sulfidic
Carboxylic Acid Derivative	Pyroglutamic acid	C5H7NO3	b	soapy, astringent, less intense sour than other org. acids	Umami	Mercaptan/Corn Chips
Chalcone	Xanthohumol	C21H22O5	b	Bitter		Phenolic
Dialkyldisulfide	Methyl propyl disulfide	C4H10S2	c	Garlic, burnt rubber,	HoneyComb	Corn Chips/Honey Comb
Dicarboxylic Acid	Fumaric acid	C4H4O4	c	Sour	Sour/Sulfitic/Phenolic	Sulfitic/Sulfidic/Sour
Dipeptide	Glycyl-methionine	C7H14N2O3S	a		Bread Crust	
Dipeptide	Isoleucyl-phenylalanine	C15H52N2O3	a		Sulfitic/Corn chip	
Dipeptide	Prolyl-arginine	C11H21N5O3	a		Corn chip/Nutty/Sulfitic	
Dipeptide	Prolyl-cysteine	C8H14N2O3S	a		Sulfitic	
Dipeptide	Tryptophyl-cysteine	C14H17N3O3S	a		Sulfitic/Ethyl Butyrate/Corn chip/Grainy	
Disaccharide	Isomaltose	C12H22O11	a		Watermelon Rind/Cucumber/Fruity/White Grape/Isoamyl Acetate	Hay/Corn Chips

Ester	N-allyl-L-alanine	C6H11NO3S	c		Ethyl Butyrate/Corn chip/Isovaleric	Mercaptan
Fatty Acid Ester	Isobutyl 3-methyl-2-butenoate;	C9H16O2	c	Green, spicy, mint	Nutty/Sulfitic/Sulfidic/Phenolic	Nutty/Sulfitic/Sulfidic/Phenolic
Fatty Acid Ester	Methyl 2-(methylthio) Butyrate	C6H12O2S	c	Musty, onion, sulfurous	Isovaleric/Mercaptan/Sulfitic/Sulfidic	Sulfitic/Sulfidic
Fatty Acid Ester	Isobutyl caprylate	C12H24O2	c	Swiss cheesy, winey, fatty, sweet	Ethyl Butyrate/Caprylic/Isovaleric/Corn chip	Mercaptan
Fatty Acid Methyl Ester	S-Methyl hexanoate	C7H14O2 S	c	cooked vegetable, sulfury, soapy	Bread Crust	
fatty acid methyl esters - Fatty Acyl	Methyl-4-(methylthio) Butyrate	C6H12O2S	a		OffFlavor/Sulfidic/Sulfitic	
Furan	DMPF	C6H8O4	c	Aromatic, roasty, nutty, cooling	Corn chip/HoneyComb/Nutty	
Furan	Furan, 2-nonadecanoyl	C23H40O2	c	Aromatic, roasty, nutty	Corn chip/HoneyComb/Nutty	Metallic
Hydroxyindole	5-hydroxytryptophol	C10H11NO2	a	Old almonds, unpleasant	Caprylic/Musty/Sulfidic/Sulfitic	Pear/Grass/Fruity
Ketone/Furan	2-propionylfuran	C7H8O2	c	Aromatic, roasty, nutty (common in aged beer)	Corn chip/HoneyComb/Nutty/Sulfidic	
Monoterpenoid	Linalyl hexanoate	C16H28O2	a	Barnyardy	Honey Comb/Nutty/Corn chip/Grainy	
Phenol	Vanillic acid	C8H8O4	b		Nutty/Sulfitic/Sulfidic/HoneyComb/Grainy	Acetaldehyde
Purine	Purine	C5H4N4	b			Phenolic/Nutty/Sulfidic/Bitter
Purine nucleoside	Adenosine	C10H13N5O4	a	Bitter, glutamate-like, Corn chippy	Nutty/Sulfitic/Grainy/Corn chip	Play-doh/Watermelon Rind
Purine nucleoside	Inosine	C10H12N4O5	b	Meaty, Savory	Bread Crust	Bread Crust
Pyrazine	Dithiouracil	C4H4N2S2	c	Toasted, roasted, Corn, toasted bread	Bread Crust	Sulfitic/Sulfidic/Wet Hay

Pyridine	4-methylpyridine	C6H7N	c	roasted, nutty, cocoa, peanut	Phenolic/Sulfidic	Wet Hay
Pyridinecarboxylic acid	Vitamin B3	C6H5NO2	b/a	Sour, metallic	Corn chip	Metallic
Pyrimidinecarboxylic Acid	Vitamin B13	C5H4N2O4	b	matches, sulfurous	Nutty/Sulfitic/Sulfidic/HoneyComb/Grainy	
Pyrrol	2-Methylpyrrole	C5H7N	c	Sulfury, bitter	Corn chip/Honeycomb	
Secondary alcohol	Furazan-3-ol, 4-amino	C2H3N3O2	c	Aromatic, roasty, nutty	Corn chip/HoneyComb/Nutty	Play-doh
Sulfur Compound	4-(Methylthio)-1-butanamine	C5H13NS	c	Cabbage, garlic, potato, sulfury, vegetable	Sulfidic/Sulfitic/Mercaptan	Sulfidic/Phenolic
Sulfur Compound	Benzothiazole	C7H5NS	c	Rubbery, sulfury, cooked, gasoline	Mercaptan/Astringent	Wet Hay/Metallic/Sulfidic
Terpene	alpha-Ionone	C13H20O	a	raspberry, cedarwood	Bread Crust	
Thia Fatty Acid	2-Hydroxy-4-(methylthio) butyric acid	C5H10O3S	a	precursor to methianol	Corn chip/Grainy/Sulfitic/Nutty/Umami	
Thioester	Methylthio-2-(propanoyloxy)propanoate	C7H12O3S	c	sulfuric ester	Honey Comb/Nutty/Corn chip/Grainy/Sulfitic/Sulfidic	

***Platform - a denotes RP/LC-MS; b denotes HILIC/LC-MS; c denotes SPME/GC-MS; d denotes if this metabolite was found in malt, e denotes if this metabolite was found in beer, sensory associated with beer at Month 2, according to O2PLS beer model.

3.6 Discussion

Six malts and their finished beers were evaluated using a metabolomics approach. The malts and beers were determined to have distinct metabolomic profiles according to the genotype of barley used to create them. Recent research has demonstrated that utilization of non-targeted metabolomics to characterize phenotypic variation among barley genotypes is on the rise. This characterization of variation is important as an approach to understand the complex metabolic pathways of the brewing process and to begin to note biomarkers that are indicative of traits in crops that are of importance to the brewing industry [35].

It is known that the concentrations of metabolites in a given system can regulate gene expression, which further regulates metabolic activity [102]. Changes in the expressions of any gene can result in a ripple effect, increasing or decreasing enzyme and regulatory protein concentrations and having a great effect on resulting metabolites [24]. To begin to understand this type of variation, such as the effect drought conditions may have on barley which is being grown for beer production, we must first begin to identify the metabolites which have an effect on the end product and then look to the interactions of these metabolites in the process, the creation of beer.

3.6.1. The main findings in the study

The main findings of this study include: relationships among non-volatile and volatile metabolites that contribute to beer flavor, possibly to beer flavor stability and trends that suggest that the metabolomic makeup of barley genotypes (GE) is a factor in determining the flavor in beer. PCA and PC loadings plots, as well as O2PLS analysis showed many compounds that were significantly ($p < 0.05$) associated with flavor in fresh beer (Month 0) and flavor in beer at Month 2. Beer genotype was shown to have statistically ($p < 0.05$) significant influence on which flavors were associated a Month 0 and Month 2 (Figure 3).

3.6.2. Beer flavor in the Full Pint genotype is influenced by non-volatile and volatile metabolites

Interesting trends were observed among beer metabolites and flavor traits. In this study, the metabolites in the Full Pint genotype of malt and subsequent beer made with this genotype were found to have a relationship with specific flavor traits, namely "fruity," ethyl acetate, "pear" at Month 2. These nitrogenous compounds in beer, such as cytidine, 5-methylcytosine, adenine, and thymine (all forms of DNA nucleobases) are important factors to consider, as they are the building blocks of amino acids. Beer, being a pyrimidine-rich food, contains cytidine, a nucleotide excreted by yeast early in fermentation and under storage conditions (Figure 7, Figure 9a) [4, 11, 27]. Cytidine is dependent upon the amount of sulfites, a natural product of fermentation, in the beer. Another compound contributing to these sensory traits in Full Pint is alpha-Ionone, which is a volatile ketone associated with floral, pear, and melon rind attributes [12]. This compound is abundant in Full Pint and contributes to the unique flavor profile (Figure 7, Figure 9a).

Oxidation of sulfites to free radicals can cause a reaction whereupon bitterness is increased in aged beer. Sulfur dioxide (SO_2) and sulfites resulting from fermentation can function as mild oxidizing agents, but also importantly, as reducing agents. The production of reactive oxygen species is initiated by enzymes and exposure to light or heat. Iron and copper (Figure 2d, Figure 4e) stimulate the formation and interconversions of free radicals from oxygen into compounds which have deleterious effects on the flavor and flavor stability. Cytidine and alpha-Ionone are two products which are affected in this process and found to form adducts when exposed to free radicals, sulfites, and SO_2 [4, 103]. Beer flavor and flavor stability are impacted by oxygen in packaged beer and the resulting reactions that occur due to its presence.

3.6.3 Sulfur-containing compounds influence beer flavor traits and flavor stability

5'-methylthioadenosine, a purine intermediate in the methionine and S-adenosylmethionine (SAM) pathways, has been investigated as a biomarker for aging in beer [13, 34]. The metabolism of 5'-MTA has previously been investigated in protein-rich foods (*i.e.* beer) mostly with the intent of reducing

purines for gout-related illnesses, as it is involved in uric acid synthesis and other polyamine synthesis [104]. Non-volatile biomarkers, specifically purines, for beer flavor stability have not been previously connected or ascribed to beer flavor or flavor stability. 5'-MTA has been described as an indirect marker of beer flavor stability [13] due to the increase over time after an accelerated aging regime correlated with sensory traits that are undesirable (*e.g.* corn chip, stale). Baseline levels of 5'-MTA were variable among genotypes in this study, but the trend over time was consistent among beer types in the development of off-flavors. Although not highly abundant, it was noted as positively correlated (Figure 7) with undesirable flavors over time, disrupting the "TTB" flavor and flavor stability.

Biocytin, another sulfur-containing compound, is an amide formed from biotin (a vitamin) and the amino acid L-lysine. Meredith was abundant in biocytin, which is associated with the flavors "corn chip," "honeycomb cereal," and sulfidic/sulfitic (Figure 7, Figure 9b). L-lysine (Sec. 1.2.3) is readily and quickly absorbed by yeast, however biocytin is not a readily available form of the amino acid, so yeast would not utilize it as readily. Since biocytin is acted upon by enzymes to make biotin available for metabolism, this may leave excess L-lysine to break down into components (aldehydes and ketones) that contribute to flavor instability and savory flavors (Figure 7) [23, 105].

Methylthiobutanoic acid, a thia fatty acid compound found in beer, generally as a result of yeast desatuase activity, is part of the enzyme complex responsible for fatty acid biosynthesis. This compound is found to increase when malt, hop, or yeast quality is poor. It is transformed from amino acids, such as L-methionine or L-cysteine, hydrogen sulfide (H_2S) and reactive oxygen into a free sulfur species which is highly reactive and forms other compounds in beer over time with the remaining FAN [52].

3.6.4. Antioxidant activity in malt and beer

Antioxidant activity decreases during the germination phase of malting, but then increases considerably during steeping and kilning. Phenolic compounds are bound until enzymatic activity is increased enough to release them. Chlorogenic acid, ferulic acid, vanillic acid, and p-coumaric acid all have strong antioxidant activity in barley and malt. Maillard reaction products during kilning are possible

due to the thermal breakdown of carbohydrates during germination, when reducing sugars and amino acids are released. It has been shown that lipoxygenase activity was decreased during kilning due to the increase in phenolic compounds [106]. In the selection of barley genotypes for malting and brewing, it is important to know not only abundance, but composition of the phenolic compounds. In this study, phenolic compounds are important in their role in flavor and flavor stability. Phenolic compounds, such as the flavan-3-ols (catechins and epicatechins) and hydroxycinnamic acids (ferulic acid and p-coumaric acid) have a strong impact on the colloidal stability (foam and haze), flavor (astringent, clove), and antioxidant activity (increased shelf-life) of beer.

Hordatines (and their precursors, hydroxycinnamoylagmatines) were detected in malt and beer. Hordatines (Figures 5-7, Figures 9-10) are present in malt, and beer as phenolic secondary metabolites. Polyamides such as putrescine, spermidine, and spermine (all found in the samples in this study) that are conjugated with hydroxycinnamic acids (*e.g.* p-coumaric and ferulic acids) form phenolamides (hydroxycinnamic acid amides) which are a stress response against biotic or abiotic factors [107, 108]. Hordatines have exceptional antifungal capacity and act as defense compounds in both barley seedlings and in older plants post-pathogen [109]. Hordatines or their glycosides (glycosides are compounds containing a carbohydrate and a non-carbohydrate residue in the same molecule, wherein the carbohydrate residue is attached by an acetal linkage at carbon atom 1 to a non-carbohydrate residue or aglycone. The sugar component is called the glycone. If the carbohydrate portion is glucose, the resulting compound is a glucoside [110]) are able to withstand high temperatures and amounts of processing from barley into beer and present themselves in beer as very astringent and medicinal, affecting flavor negatively. The total hordatine content, in regards to composition, has not been fully studied, but it has been discovered that hordatine content varies among barley genotype and is positively correlated with the alcohol by volume (ABV) of beer [53]. In Figure 3a, hordatines are shown to have a positive correlation with the “fruity”, “sweet,” and astringent sensory traits, which have also been shown to be positively correlated with other phenolic non-volatile metabolites. This is also seen in Figure 6d, the variable line plot of contributing metabolites with respect to Full Pint.

3.6.5. Free amino nitrogen in beer influences flavor and flavor stability

Nitrogenous compounds vary in their chemical composition and their influence on beer flavor and flavor stability. The main source of amino acids is found in malt and yeast, malt contributing FAN, peptides, and polypeptides. Deamination and transamination are reactions responsible for the creation of organic acids, aldehydes, esters, and alcohols in the beer [111-113].

According to this study, more research needs to be performed to determine composition of FAN, not only abundance in the wort and beer. FAN is a general term, and is comprised of all amino acids. The liberation of FAN in the mash is highly dependent upon proteinase activity in the malt. Proteinases in germinating barley are responsible for the breakdown of storage proteins into soluble proteins (peptides and amino acids). Proteinase classes do not all show a relationship or correlation to the content of soluble nitrogen that will be available in malt for subsequent mashing[114]. It is important to consider the ability of barley (from a breeding perspective) to efficiently degrade grain storage proteins that result in appropriate levels of FAN for brewing.

In The Spearman's correlation heat maps of nitrogenous compounds in malt and beer (Figures 5 and 7) display the effects of high abundances of amino acids. The higher abundances of amino acids such as L-tryptophan and L-arginine are associated with the "fruity" flavors in Full Pint (Figure 9), however L-lysine abundance is associated with the "corn chip" flavor in Meredith. FAN measurements are currently a "blunt instrument" for the determination of wort quality in regards to yeast growth and fermentation efficiency [31]. Levels of FAN (Table 2) do not take into consideration the total composition. FAN is absorbed at different rates by ale yeast, but not much is known about the absorption rate or utilization given anything other than controlled brewing situations. L-Proline (abundant in Full Pint) has been observed to not be absorbed well by yeast, however the reason is unclear. L-methionine and L-valine are absorbed at an intermediate rate, but that says nothing of "how much" is absorbed by yeast and what is left over. L-tryptophan, L-tyrosine, L-alanine, L-glycine, and L-phenylalanine are all absorbed at an exponentially slow rate [105]. These are all compounds that are seen in all genotypes, but vary in abundances and attribution to sensory traits. When fermentation is supplemented with amino

acids, lysine, for example, yeast cell concentration is affected and the supplement is utilized very quickly (as lysine is very rapidly absorbed) and then cell growth rapidly drops off after a large spike in activity. The increase or decrease in specific amino acids will affect the fermentation efficiency and the uptake of other nitrogenous compounds. With an increase in L-methionine, amino acids that are usually absorbed equally as rapidly are not absorbed at all. This underutilization and remainder of amino acids may possibly have a detrimental effect on the fermentation and stability of the beer [27, 31, 105, 115].

3.6.6. Free fatty acids in malt affect beer flavor and flavor stability

Lipids and fatty acids represent a small fraction of barley grain weight (about 2%), but they play an important role in malting and brewing, leading to significant changes in flavor and flavor over time. Varying lipid compounds (Figure 6) are associated with the "sweet" and "fruity" types of sensory traits, but also to the "cardboard" staling traits. Many studies have shown that the content of specific fatty acids in malt have an adverse effect on beer quality by negatively influencing beer flavor (*e.g.* foam instability, hazy appearance) and flavor stability [44].

Lipids and fatty acids are all essential in yeast activation and cell growth under anaerobic conditions. Increased or decreased amounts lead to fermentation issues. Certain fatty acids (unsaturated fatty acids such as linolenic and linoleic) have a high tendency to result in oxidative degradation leading to staling flavors over time in beer [116]. In this study, the correlation of fatty acids to specific sensory traits is an important factor to consider when determining beer flavor and flavor stability. The total content, as well as the composition of free fatty acids in barley, malt, and beer may play a role and indicate that they should be considered together with other metabolites when considering genotype.

3.7 Conclusions

The purpose of this study was to investigate the effect of barley genetics (GE) on beer flavor and flavor stability utilizing a metabolomics approach. Research methods utilized RP/UPLC-MS, HILIC-MS, ICP-MS, and SPME/GC-MS in combination with QDA to profile the changes between malt and beer, the metabolites involved, and the predicted co-varying metabolites that could be used to predict flavor and flavor stability in beer. There is increasing interest in the study of barley genetic, the influence of GE interactions, and how they affect the malting of barley and the creation of beer. The studies conducted for this thesis identified volatile and non-volatile possible biomarkers for identifying flavor and flavor stability through barley type chosen. These results suggest that, after confirmative study, barley genome identifiers could be used in agriculture to increase certain flavor and flavor stability characteristics when breeding barley for beer production.

The analysis of malt in this study revealed 217 compounds that changed quantitatively from malt into beer. Of these changing compounds, there are many that show promise in further investigation studying the flavor stability of beer, including lipids, purines, and amines. The analysis of beer in this study revealed 246 compounds that changed quantitatively from malt into beer. Of these significantly changing compounds, there are many that show promise in further investigation studying the flavor stability of beer, including purines, amines, phenolics, and alkaloids. The results confirm the hypothesis that (*i*) there are metabolite differences among six commercial barley genotypes (*ii*) differences in barley chemistry are reflected in the chemistry of the beer (*iii)* the differences in the beer chemistry impact sensory attributes of beer, through flavor and flavor stability and (*iv*) there are potentially barley and/or malt metabolites that can be markers for beer flavor and/or flavor stability. Metabolites in malt and beer are found to influence flavor and flavor stability of beer and the co-variance of these metabolites (volatiles and non-volatiles). Univariate (ANOVA, PCA, Spearman's correlation) and multivariate (O2PLS) analyses depict variation observed among malt and beer genotypes. These metabolites may be attributed to differences in genetics, environmental conditions, malting or brewing parameter differences,

or other influences outside of the control of study parameters. Future design studies with more control could help to normalize for variation. For example, malting and brewing of the genotypes were not done in replicate and this could have assisted in comparison amongst genotypes. The assumption that brewing was controlled was made, nonetheless no two brews/fermentations are ever exactly alike. There were several confounding factors that could not be separated, therefore given the small "n" in the study (n=6), it can be stated that barley GE does have an effect on beer flavor and flavor stability.

More study needs to be done in the area of accelerated aging and with a more targeted approach now that we have identified potential biomarkers for aging. This includes more targeted studies to determine the composition, not just the quantity of FAN in malt and beer [27, 115] and the effect of the increase or decrease in these metabolites at pivotal points in the brewing process on beer flavor and flavor over time. The co-variation of metabolites, including the interactions of non-volatiles with non-volatiles and volatiles, requires further study to determine the depth of impact upon flavor and flavor over time since each barley genotype is affected by gene and environmental (GE) conditions (including seasonal, yearly changes) and each malting is the result of those GE interactions in the raw barley.

3.8 Broader Impacts

This research could provide novel methods to predict sensory traits based on volatile and non-volatile metabolite abundances, of use to maltsters and brewers seeking greater understanding of the chemistry and interactions of raw ingredients at a molecular level. These raw ingredients are essential to beer flavor and flavor stability. Modern malting and brewing processes should involve a deeper look into barley and malt through metabolomics, proteomics, lipidomics, and ionomics to understand the associate of amino acids, lipids, alkaloids, volatile compounds, and other unknowns to flavor. This should involve understanding of the composition, as well as the abundance, of the compounds and how they affect beer flavor and flavor stability. Knowledge and understanding of metabolites which are related to genes and environmental circumstances could provide insight to producers of barley for crop improvements or

experimental barley lines. This research suggests that designing effective brewing schemes based on a deeper understanding of malt and the finished beer will require moving beyond the acceptance of blunt instruments for precise measurements.

Brewers should pay particular attention to malt and malt quality, as it is the interactions between high quality malt and other ingredients (hops, yeast) that create substantial flavor. In brewing, it is common to think that only one strong raw ingredient is making a major contribution. For example, in creating an imperial IPA with high IBU, the brewer will commonly choose a cheaper, lower-quality malt because s/he does not think that malt plays a large role in the flavor. This is incorrect. There are specific malt-hop interactions that contribute to flavor development and flavor stability. Brewers should see the need to connect all of the ingredients and to choose only high-quality ingredients of which they know the interactions and results. For example, paying attention to the protein quantity and composition in malt will help the brewer make decisions regarding amounts and composition of hops to add. For example, using a low-quality malt with higher protein will not improve the flavor or character of a heavily-hopped beer, but using a lower-protein, higher-quality flavor-forward malt will improve aroma, mouthfeel, foam retention, and overall flavor.

Tables

Table 1. Barley malt genotypes used in this study

Malt Cultivar	Maltster	Malthouse Location	Moisture (%)	FGDB (%)	Color (°)	DP (L)	AA (DU)	TP (%)	SP (%)	S/T	FAN (ppm)	Viscosity (CPU)	β-glucan	Friability (%)	pH
Metcalfe	Malt-europ	Great Falls, MT	4.9	82.4	1.55	127	62.7	10.86	4.1	37.8	177	1.5	96	93	5.9
Expedition	Malt-europ	Great Falls, MT	5	81.1	1.74	144	58.7	12.24	4.82	39.4	204	1.48	105	89.5	5.9
Meredith	Rahr	Alix, AB	5.5	83.3	1.83	156	59.8	10.91	4.65	42.6	189	1.49	115	90.5	6.0
Full Pint	Briess	Chilton, WI	9	79.6	1.78	181	82	13.62	4.78	35.1	173	1.59	220	55.5	5.9
Polar Star	Cargill	Biggar, SK	4.9	82.3	2.04	156	65.7	10.98	4.64	42.2	173	1.48	72	93	6.1
Copeland	Rahr	Alix, AB	5.2	82.4	1.49	126	52.1	11.14	4.25	38.1	161	1.5	130	89.2	6.1

*data provided by New Belgium Brewing, Briess Malting, Rahr Malting, Malt-europ Malting, and Cargill Malting.

FGDB – fine-grind, dry basis; Color – based on Standard Reference Method (SRM); DP – diastatic power, based on Lintner units; AA – alpha amylase, based on diastatic units (DU), 30 or above is required for proper conversion; TP – total protein, should be <14%; SP – soluble protein, based on dry basis; S/T – soluble/total ratio, a minimum of 30 is required to prevent lautering issues; FAN – free amino nitrogen, standard value is 180ppm and above; Viscosity – typically 1.45 – 160 centipoise units (CPU); β-glucan – <180 indicates good lauterability, but this test only indicates the number of molecules found, not the molecular weight; Friability – indicator of lautering performance, >90% indicates good friability, <90% indicates undermodification (high viscosity polysaccharides such as Beta glucan, leading to lautering difficulties).

Table 2. Brewing specifications for this study

Area	Specification	Notes	Results
Malt & Grist			
Malt	Pale/Pilsen	Single genotype per batch as supplied by New Belgium Brewing	
Grist Specification	Standard/No Spec	Consistent from batch to batch; per Haas specifications; analyzed per ASBC Malt-15 or comparable method	
Wort Production			
Mash Salts	Ca= 100ppm; SO4 = 65-70ppm; Cl = 95-100ppm	No water information provided	
Mash pH Target	5.4		
Grist:Water Ratio	2.8:1		
Wort Original Gravity	12.5°P		11.7°P
Post Primary FV EA	2.85°P		2.92°P
ABV Target	5%		4.8%
RDF Target	63.40%		62.43%
Mash Strike Temp	40°C		
Saccharafication Temp	65°C		
Mash Off Temp	76°C		
Boil Time - Minutes	120		
Kettle Salts	Ca = 55ppm; SO4 = 42ppm; Cl = 59ppm; Lactic = 25ppm	Need Haas/Yakima water information	
Beer IBU Target	8	Use Crop 2014 T90 Nugget; Bittering addition only	7.9
Wort Knockout Temp	18°C		
Cold Wort Sample	Yes	One cold wort sample to be collected per batch for wort analyses	
Air	30g/hr		
Area	Specification	Notes	Results
Fermentation & Finishing			
Yeast	NBB Ale/WLP001 CA Ale		
Pitch count	10^6/ml/°P + 10^6	Per Nexcelom Cellometer count from slurry	
Primary FV Temp	20°C		
Area	Specification	Notes	Results
Malt & Grist			
VDK Spec.	≤30 ppb		

Post Fermentation FV Temp	minus 1°C	
Post Fermentation Sample	Yes	One post fermentation sample to be collected per batch for wort analyses
Maturation/Stabilization Time	3-5 Days	
Filtration Medium	DE	
Filtration D.O.	≤50 ppb	

Packaging		
Bottling	22oz glass w/NBB Crown	2x12 22oz bottles per batch - supplied by NBB
Keg/Draft	1/6 bbl. (19.5L) NBB Cooperage	Remainder of beer to be kegged after completion of bottling
Package Beer T.P.O.	≤50 ppb	

*This table was provided by Haas Innovations, Inc.

FV EA – Fermentation Vessel Apparent Extract; ABV – alcohol by volume; RDF – real degree of fermentation; IBU – international bittering units; T.P.O – total packaged oxygen; DE – diatomaceous earth; VDK – vicinal diketones

Table 3. Annotated metabolites detected in malt and beer

Chemical Class	Metabolite	Structure	Platform***	Tissue Detected**	Reported Sensory	Beer Sensory[d]	Malt Sensory[e]	HMDB or PubChem ID*	ANOVA
5'-deoxy-5'-thionucleosides	5-MTA	C11H15N5O3S	b/a	M/B	Umami-like, sulfurous	Nutty/Cornchip/Grainy/Sulfitic/Sulfidic	Phenolic/Sulfidic	HMDB01173	$p < 0.05$
5'-deoxyribonucleoside	5'-Deoxyadenosine	C10H13N5O3	A	B	Glutamate-like	Sulfidic/Sulfitic/Caprylic/Umami/Caramel/Phenolic/Acetaldehyde		HMDB01983	$p < 0.05$
6-aminopurine	3-methyladenine	C6H7N5	b/a	B	6-aminopurine class	Umami/Astringent/ Cardboard/Ethyl Butyrate/Mercaptan		HMDB11600	$p < 0.05$
6-aminopurine	Adenine	C5H5N5	b /a	M/B		Cornchip	Play-doh/Nutty/Grainy	HMDB00034	$p < 0.05$
6-aminopurine	Isoguanine	C5H5N5O	a	M/B	6-aminopurine class	PlayDoh	Play-doh/Perfume	HMDB00403	$p = 0.12$
Acid	Methyl heptyl carbonate	C9H18O3	c	B		Grainy/Diacetyl			$p = 0.22$
Acid	Gluconic acid	C6H12O7	b	M	Fruity, honey, wine		Umami/Phenolic/Raisin/Sherry/Cardboard	HMDB00625	$p = 0.06$
Acid	Carbonic acid, monoamide, N-butyl, hexyl ester	C11H23NO2	c	M/B		Mercaptan	Mercaptan		$p = 0.23$
Acid	Ethyl 2-methylpropyl carbonate	C7H14O3	c	M/B	Agave, blueberry, rhubarb	Fruity Complex	Ethyl Butyrate	6420652	$p < 0.05$
Acrylic Acid Ester	Ethyl acrylate	C5H8O2	c	M/B	Bitter, pineapple, fruity, metallic	Raisin/Sherry/Musty/Phenolic	Metallic	HMDB33978	$p < 0.05$
Acyl glycine	Deoxygcholylglycine	C26H43NO5	b	M			Perfume/Play-doh/Fruity	HMDB00631	$p = 0.06$
Acyl glycine	Isovalerylglycine	C7H13NO3	b	M			Acetaldehyde/Phenolic	HMDB00678	$p = 0.10$
Acylaminosugar	N-acetylmannosamine	C8H15NO6	b	M			Corn Chips/Honeycomb Cereal	HMDB01129	$p < 0.05$
Alcohol	Isopropyl methyl carbinol	C5H12O	c	B	.malty, cherry, almond, chocolate, apple, cheese, unripe banana	Astringent/Bitter		HMDB33777	$p < 0.05$
Alcohol	4-methylphenyl ethanol	C9H12O	c	M/B		Astringent	Metallic	10817	$p = .31$

Aldehyde	Cinnamaldehyde	C10H10O	a	B	Spicy, bitter, Phenolic, cinnamony, sweet	Honeysuckle/Sweet/Sweet-Aromatic/Honey/HoneyComb		HMDB03441	$p < 0.05$
Aldehyde	hydroxymethylfurfural	C6H6O3	a	B	stale, vegetable oil, Paper-like, vegetables, bready, caramel	Cornchip/HoneyComb/Nutty		HMDB34355	$p < 0.05$
Aldehyde	Isobutanol	C4H10O	c	B	malty, grainy, husk-like, varnish, fruity, banana, melon, green malt, green leaves, bitter, alcoholic	Astringent/Bitter		HMDB06006	$p < 0.05$
Aldehyde	Methional	C4H10OS	c	B	Umami	Umami/Sulfitic/Isovaleric/Grainy/Sulfidic		HMDB31857	$p < 0.05$
Aldehyde	Methyl Benzoate	C8H8O2	c	B	Fruity, herbal, floral	Acetaldehyde		HMDB33968	$p < 0.05$
Aldehyde	Nonanal	C9H18O	c	B	bitter, astringent, Cardboard, aldehydic	Ethyl Butyrate		HMDB59835	$p < 0.05$
Aldehyde	Methanetricarbaldehyde	C4H4O3	c	M/B	Sulfrous, methane gas	Isovaleric/EthylButyrate/Sulfitic/Bitter	Suflitic/Sufidic/Ethyl Butyrate	551778	$p < 0.05$
Aliphatic Alcohol	Isobutanol	C4H10O	c	M	malty, grainy, husk-like, varnish, fruity, banana, melon, green malt, green leaves, bitter, alcoholic		Phenolic	HMDB06006	$p < 0.05$
Alkaloid	N-Methoxy-1-vinyl-beta-carboline	C14H12N2O	a	M			Ethyl Butyrate/Bread Crust	HMDB30379	$p = 0.11$
Alkaloid	Trigonelline	C7H7NO2	b	M	Light bitterness, bell pepper, melon		Pear/Bread Crust/Metallic	HMDB00875	$p < 0.05$
alkaloid	N-methyltryptamine	C11H14N2	a	M/B	Glutanimous	Umami/Astringent/Cardboard	Sulfitic/Cardboard/Sulfidic	HMDB04370	$p < 0.05$
Alkane	Methylmethane	C6H14O2	c	M/B	Fruity	Sweet/Ethyl Butyrate/Sweet-Aromatic	Metallic/Ethyl Butyrate	6324	$p < 0.05$
Alkene	1-pentadecene	C15H30	a	M			Perfume/Acetaldehyde/Green Apple	HMDB31082	$p < 0.05$

Alkylamine	Dimethylethanolamine	C4H11NO	c	M			Green Apple/Ethyl Acetate	HMDB32231	$p < 0.05$
Alpha Amino Acid	5-hydroxy norvaline	C5H11NO3	a	B		Bread Crust/IsoamylAcetate		HMDB31658	$p < 0.05$
Alpha Amino Acid	Methionine sulfoxide	C5H11NO3S	a	B	Biomarker of oxidative stress	Sulfidic/Sulfitic/Wet Hay		HMDB02005	$p < 0.05$
Alpha Amino Acid	Sarcosine, N-(3-phenylpropionyl)-, pentyl ester	C17H25NO3	c	B		Cornchip/HoneyComb/Metallic		91741223	$p < 0.05$
Alpha Amino Acid	N-methyl-a-aminoisobutyric acid	C5H11NO2	a	M/B		Perfume/Fruity/GreenApple/PlayDoh/Ethyl Acetate	Ethyl Butyrate/Bread Crust	HMDB02141	$p < 0.05$
Alpha Amino Acid	Sarcosine	C3H7NO2	c	M/B		Wet Hay	Acetaldehyde	HMDB00271	$p < 0.05$
Alpha-Amino Acid	Glutamine	C5H10N2O3	b	M	Fruity, Vegetal, Umami, Savory		Bread Crust/Corn Chip	HMDB00641	$p < 0.05$
Alpha-amino acid	Ornithine	C5H12N2O2	b	M/B		Cornchip/HoneySuckle/HoneyComb	Sulfitic/Cardboard/Sulfidic	HMDB00214	$p < 0.05$
Amine	Spermine	C10H26N4	a	B				HMDB01256	$p < 0.05$
Amine	2-(Methylthio)ethylamine	C3H9NS	c	M/B	Sulfurous, rancid oily nutty	Cardboard/Astringent	Cardboard/Astringent/Caramel/Musty/Umami	87697	$p < 0.05$
Amine	Methylamine	C6H13NO	c	M/B	Vegetable, grape, carrot, cabbage	Sulfidic	Wet Hay	HMDB00164	$p = 0.30$
Amino Acid	Acetylglycine	C4H7NO3	b	B	Fruity	Fruity/Perfume/EthylAcetate/Green Apple		HMDB00532	$p < 0.05$
Amino Acid	Beta-alanine	C3H7NO2	b	B		Wet Hay/Acetaldehyde		HMDB00056	$p < 0.05$
Amino Acid	Betonicine	C7H13NO3	a	B		Umami/Astringent/Cardboard		HMDB29412	$p < 0.05$
Amino Acid	Isoleucine	C6H13NO2	a	B	bitter-sweet, astringent	Sulfitic/Sulfidic/Grainy/Musty/Isovaleric/Umami		HMDB00172	$p < 0.05$
Amino Acid	L-Lysine	C6H14N2O2	a	B	lemony	Honey Comb/Nutty/Cornchip/Grainy		HMDB00182	$p < 0.05$
Amino Acid	α-aminoButyrate	C4H9	a	B	sweet-bitter, sour, glutamate-like	Cornchip/HoneyComb/HoneySuckle		HMDB00650	$p < 0.05$
Amino Acid	Alanine	C3H7NO2	b	M	Sweet		Phenolic/Sulfidic	HMDB00161	$p < 0.05$

Amino Acid	Aminoadipic acid	C6H11NO4	b	M			Astringent/Phenolic/Nutty	HMDB00510	$p < 0.05$
Amino Acid	Aminooctanoic acid	C8H17NO2	b	M			Bread Crust	HMDB00991	$p < 0.05$
Amino Acid	Betaine	C5H11NO2	b	M			Grainy	HMDB00043	$p < 0.05$
Amino Acid	Beta-Leucine	C6H13NO2	a	M	Sour, astringent		Metallic	HMDB03640	$p < 0.05$
Amino Acid	DAPA	C7H14N2O4	b	M			Bread Crust	HMDB01370	$p < 0.05$
Amino Acid	L-Allothreonine	C4H9NO3	b	M	Sweet, bitter, astringent		Play-doh/Watermelon Rind	HMDB04041	$p < 0.05$
Amino Acid	L-Aspartic acid	C4H7NO4	b	M	methanol-like, sour, glutamate-like		Bitter/Astringent/Caramel	HMDB00191	$p < 0.05$
Amino Acid	L-Histidine	C6H9N3O2	b	M	light sweetness, bitter, astringent		Astringent/Phenolic	HMDB00177	$p < 0.05$
Amino Acid	L-Leucine	C6H13NO2	b/a	M	sour, bitter, astringent		Mercaptan/Play-doh	HMDB00687	$p < 0.05$
Amino Acid	L-Norleucine	C6H13NO2	b	M	Sour vomity, goaty		Ethyl Butyrate	HMDB01645	$p < 0.05$
Amino Acid	L-Phenylalanine	C9H11NO2	ZIC-HILIC-MS/a	M	sweet-sour, bitter, astringent		Astringent/Grainy/Bitter	HMDB00159	$p < 0.05$
Amino Acid	L-Serine	C3H7NO3	b	M	Astringent, sweet, bitter		Umami/Caramel/Astringent/Bitter/Grainy/Mercaptan	HMDB00187	$p < 0.05$
Amino Acid	L-Threonine	C4H9NO3	b	M	Sweet, bitter, astringent		Metallic	HMDB00167	$p < 0.05$
Amino Acid	L-Tyrosine	C9H11NO3	b	M	Vegetal, Savory		Bread Crust/Honeycomb Cereal	HMDB00158	$p < 0.05$
Amino Acid	L-Valine	C5H11NO2	a	M	Sweet, bitter, astringent		Fruity/Play-doh	HMDB00883	$p < 0.05$
Amino Acid	L-Valine	C5H11NO2	b	M	bitter, sweet, astringent		Perfume/Play-doh	HMDB00883	$p < 0.05$
Amino Acid	Biocytin	C16H28N4O4S	a	M/B		HoneyComb/Nutty/Cornchip	Pear/Isoamyl Acetate	HMDB03134	$p < 0.05$
Amino Acid	L-Arginine	C6H14N4O2	a	M/B	Sweet, bitter, astringent	Bread Crust	Grainy-Grape nut cereal	HMDB00517	$p < 0.05$
Amino Acid	L-Asparagine	C4H8N2O3	b /a	M/B	Savory	Bread Crust	Bread Crust	HMDB00168	$p < 0.05$

Amino Acid	L-Methionine	C5H11NO2S	b/a	M/B		Isovaleric/Cornchip /Umami/	Umami	HMDB00696	$p < 0.05$
Amino Acid	L-Proline	C5H9NO2	b/a	M/B		Perfume/Fruity/GreenApple/PlayDoh	Umami/Phenolic/Grainy/ Astringent	HMDB00162	$p < 0.05$
Amino Acid	L-Tryptophan	C11H12N2O2	b/ a	M/B	bitter-sweet, methanol-like	Sulfitic/Sulfidic/Cornchip/Ethyl Butyrate	Grainy/Astringent/Mercaptan	HMDB00929	$p < 0.05$
Amino Acid	Vitamin B5	C9H17NO5	b/a	M/B	Astringent, Salty	Bitter/Astringent/Sour/Isovaleric/Ethyl Butyrate	Sulfitic/Sulfidic	HMDB00210	$p < 0.05$
Benzene/Toluene	M-ethyltoluene	C9H12	c	M	Solventy		Fruity	HMDB59848	$p = 0.55$
Benzenetriol/Phenol	phloroglucinol	C6H6O3	b	M	Phenolic		Wet hay/Metallic/ Sour	HMDB13675	$p = 0.12$
Benzenoid	Beta-Ionone (2,4-Di-tert-butylphenol)	C14H22O	c	B	raspberry, citrus, woodlands, violet, kettle hop aroma, artificial raspberry, strawberry, floral, berry	Wet Hay/Acetaldehyde		HMDB13816	$p = 0.70$
Benzenoid	Isobutyl benzoate	C11H14O2	c	B	Present in banana, sweet cherry, papaya, beer, cider and cocoa, Musty ,fruity	White Grape/Pear/Grass/Floral		HMDB40583	$p = 0.42$
Benzenoid	Phenethyl alcohol	C8H10O	c	B	alcohol, flowery, honey-like, roses, swee	Wet Hay/Acetaldehyde		HMDB33944	$p = 0.07$
Benzenoid	2,4-Di-tert-butylphenol	C14H20O	c	M	Bitter, astringent, Phenolic		Acetaldehyde	HMDB13816	$p = 0.43$
Benzenoid	2-phenylbutyric acid	C10H12O2	b	M			Acetaldehyde	HMDB00329	$p = 0.32$
Benzenoid	3-Phenoxypropionic acid	C9H10O3	b	M			Phenolic/Sulfidic	HMDB02229	$p = 0.25$
Benzenoid	4-aminosalicylic acid	C7H7NO3	b	M	Solventy, fruity-astringent		Green Apple/Ethyl Acetate/Perfume	HMDB14378	$p < 0.05$
Benzenoid	4-aminosalicylic acid	C7H7NO3	b	M	Solventy, fruity-astringent		Green Apple/Ethyl Acetate/Perfume	HMDB14378	$p < 0.05$
Benzenoid	4-Hydroxy-3-methylbenzoic acid	C8H8O3	b	M			Fruity	HMDB04815	$p < 0.05$

Benzenoid	5-Methoxysalicylic acid	C8H8O4	b	M	Tea-like, bitter, solventy, Phenolic		Acetadehyde/Phenolic	HMDB01868	$p = 0.21$
Benzenoid	Creosotinic Acid	C8H8O3	b	M			Green Apple/Ethyl Acetate	HMDB02390	$p = 0.07$
Benzenoid	Ethyl Benzoate	C9H10O2	c	M	apple, banana, sweet cherry. Also present in milk, butter, wines, black tea, bourbon vanilla and fruit brandies.		Musty/Phenolic	HMDB33967	$p = 0.22$
Benzenoid	PABA	C7H7NO2	b	M	Astringent		Grainy/Astringent	HMDB01392	$p = 0.08$
Benzenoid	Pyrogallol	C6H6O3	b	M	Bitter, metallic		Metallic/Fruity	HMDB13674	$p = 0.06$
Benzenoid	Ethanone	C10H13NO	c	M/ B	Floral, citrusy, herbal, green vegetable, fruity	Pear/White Grape/Watermelon/ Grass/Floral/Fruity	Fruity/Perfume		$p = 0.06$
Benzenoid	4-Methoxyphenylacetone	C10H12O2	c	M/B	Fruity, sweet, spicy, anisic	Isoamyl Acetate/Floral/Watermelon	Isoamyl Acetate/Floral	HMDB32891	$p = 0.15$
Benzenoid	PABA	C7H7NO2	b	M/B	aminobenzoic acid, hay-like	Wet Hay	Sulfidic/Sulfitic/Phenolic	HMDB01392	$p = 0.45$
Benzenoid	p-Methoxybenzoic acid, tridecyl ester	C21H34O3	c	M/B	Perfumey	Pear/Grass/Floral/Isoamyl Acetate	Fruity/Play-doh/Perfume	522365	$p = 0.26$
Benzenoid/Aldehyde	Benzaldehyde	C7H6O	c	M/B	bitter almond, cherry stone, almond	Floral/Grass	Floral/Grass/ Ethyl Acetate/Watermelon Rind	HMDB06115	$p < 0.05$
Benzenoid/Phenol	Phenol	C6H6O	c	M	Phenolic, metallic, bitter		Metallic	HMDB00228	$p < 0.05$
Benzofuran	Hordatine A/B	C28H38N8O5	a	M/B		Isovaleric/EthylButyrate/Sulfitic/Bitter	Cardboard/Musty/Phenolic/Bitter	HMDB30461 HMDB30459	$p < 0.05$
Benzoic Acid Ester		C9H10O2	c	B	Phenolic, clove-like, bitter,	Sour/Raisin/Sherry/ Musty		HMDB33967	$p < 0.05$
Benzyloxycarbonyl	Phenylmethyl butanoate	C11H14O2	b	M	passion fruit, mountain papaya, cherimoya, black tea, Bourbon vanilla and hog plum.		Astringent/Bitter	HMDB33376	$p < 0.05$

Carboximidic acid	PEA	C18H37NO2	a	M			Green Apple/Ethyl Acetate	HMDB02100	$p < 0.05$
Carboxyclic Acid Derivative	Cyclopentanecarboxylic acid	C6H10O2	c	M			Sour/Metallic		$p < 0.05$
Carboxylic Acid	Formic acid	CH2O2	c	B	Cucumber, rose, fruity	Astringent/Mercaptan/Isovaleric/Ethyl Butyrate		HMDB00142	$p < 0.05$
Carboxylic Acid	D-Pantethine	C22H42N4O8S2	b	M	Sulfurous, metallic		Metallic	HMDB03828	$p < 0.05$
Carboxylic Acid	Furoic Acid	C5H4O3	a	M			Sour/Wet Hay	6919	$p < 0.05$
Carboxylic Acid	Acetic Acid	C2H4O2	c	M/B	Sour	Phenolic/Sour	Sour	HMDB00042	$p < 0.05$
Carboxylic Acid Derivative	3-O-methyldopa	C10H13NO4	b	M	Bitter, lit match		Cornchip/Grainy/Sulfidic/Phenolic	HMDB01434	$p < 0.05$
Carboxylic Acid Derivative	5-ALA	C5H9NO3	b	M			Umami/Caramel/Musty/Bitter/Astringent	HMDB01149	$p < 0.05$
Carboxylic Acid Derivative	Maleic acid	C4H4O4	b	M			Bread Crust	HMDB00176	$p < 0.05$
Carboxylic Acid Derivative	N-(1-Deoxy-1-fructosyl)leucine	C12H23NO7	a	M			Sweet/Bread Crust/Honeycomb Cereal	HMDB37840	$p < 0.05$
Carboxylic Acid Derivative	N-(1-Deoxy-1-fructosyl)methionine	C11H21NO7S	a	M			Acetaldehyde/Perfume	HMDB37841	$p < 0.05$
Carboxylic Acid Derivative	N-1(1-Deoxy-1-fructosyl)isoleucine	C12H23NO7	a	M			Sulfitic/Sulfidic/Sour	HMDB39780	$p < 0.05$
Carboxylic Acid Derivative	N-acetylglutamic acid	C7H11NO5	b	M	Sulfrous, glutaminous, Umami		Sulfitic/Sulfidic/Phenolic/Cardboard	HMDB01138	$p < 0.05$
Carboxylic Acid Derivative	N-Acetyl-L-Phenylalanine	C11H13NO3	b	M			Acetaldehyde	HMDB00512	$p < 0.05$
Carboxylic Acid Derivative	N-alpha-acetyllysine	C8H16N2O3	b	M			Metallic	HMDB00446	$p < 0.05$
Carboxylic Acid Derivative	NMDA	C5H9NO4	b	M	Sour, glutamate-like		Acetaldehyde	HMDB02393	$p < 0.05$
Carboxylic Acid Derivative	N-oleoyl-alanine	C21H39NO3	a	M			Perfume/Acetaldehyde/Green Apple	44423663	$p < 0.05$
Carboxylic Acid Derivative	o-Tyrosine	C9H11NO3	b	M			Corn Chips/Honeycomb Cereal	HMDB06050	$p < 0.05$

Carboxylic Acid Derivative	pyrrolidonecarboxylic acid	C5H7NO3	b /a	M			Acetaldehyde/Green Apple	HMDB00805	$p < 0.05$
Carboxylic Acid Derivative	trans-aconitic acid	C6H6O6	b	M			Isoamyl Acetate/Fruity	HMDB00958	$p < 0.05$
Carboxylic Acid Derivative	Ethyl Dimethylcarbamate	C5H11NO2	c	M/B	carboximidic acids	Cornchip/HoneyComb/Nutty/Sulfidic	Sulfidic	12709	$p < 0.05$
Carboxylic Acid Derivative	Pyroglutamic acid	C5H7NO3	b	M/B	soapy, astringent, less intense sour than other org. acids	Umami	Mercaptan/Corn Chips	HMDB00267	$p < 0.05$
Carboxylic Acid Derviative	N-(1-Deoxy-1-fructosyl)phenyl alanine	C15H21NO7	a	M	Cooked. Canned vegetable-like		Metallic	HMDB37846	$p < 0.05$
Carboxylic Acid Ester	2-methylpropyl formate (Isobutyl formate)	C5H10O2	c	B	fruity, solvent	Fruity Complex		HMDB31247	$p < 0.05$
Carboxylic Acid Ester	Ethyl propionate	C5H10O2	c	B	fruity, rum	Astringent/Mercaptan		HMDB30058	$p < 0.05$
Carboxylic Acid Ester	Isobutyl formate	C5H10O2	c	B	chemical, ethereal, sweet	Mercaptan/Hay/Astringent/Ethyl Butyrate		HMDB31247	$p < 0.05$
Carboxylic Acid Ester	Methionol acetate	C6H12O2S	a	B	sulfurous, herbal mushroom cabbage, asparagus, potato, cheesy - contributor to mercaptan	Umami/Mercaptan/Isovaleric		HMDB31717	$p < 0.05$
Carboxylic Acid Ester	Decyl formate	C11H22O2	c	M	Fruity, waxy		Caramel/Bitter/Astringent	79541	$p < 0.05$
Carboxylic Acid Ester	Isoamyl formate	C6H12O2	c	M	Plum, vinous, ethereal		Sulfitic/Sulfidic	HMDB34163	$p < 0.05$
Carboxylic Acid Ester	Methionol acetate	C6H12O2S	c	M	Apple, melon, pineapple		Sour/Metallic	HMDB31717	$p < 0.05$
Carboxylic Acid Ester	2-methylbutyl formate	C6H12O2	c	M/B	Pungent, vinegar, Dry, Earthy, Vinous, Green	Acetaldehyde/Wet Hay	Ethyl Butyrate/Nutty/Grainy/Mercaptan	118210	$p < 0.05$
Carboxylic Acid Ester	Ethyl acetate	C4H8O2	c	M/B	Solvent, fruity, sweet	Honeysuckle/Honey/Sweet/Sweet-aromatic/Pear/Ethyl Acetate	Honeysuckle/Wet Hay/Sour	HMDB31217	$p < 0.05$

Ceramide Phosphate	CerP(d18:0/16:0)	C34H70NO6P	b	M			Play-doh/Watermelon Rind/Fruity	5283582	$p = 0.07$
Chalcone	Xanthohumol	C21H22O5	b	B	Bitter		Phenolic	HMDB37479	$p = 0.10$
Cinnamic Acid	3,4,5-Trimethoxycinnamic acid	C12H14O5	b	M			Sour/Sulfitic	HMDB02511	$p < 0.05$
Cinnamic Acid Derivative	4-Hydroxycinnamoylagmatine	C14H20N4O2	a	M	Astringent		Fruity	HMDB33460	$p < 0.05$
Cinnamic Acid Derivative/Aldehyde	Cinnamaldehyde	C9H8O	b	M	Phenolic, astringent and cinnamon, clovey		Astringent	HMDB03441	$p < 0.05$
Cinnamic Acid Ester	Ethyl cinnamate	C11H12O2	c	M/B	spicy, fruity, sweet	Isovaleric/Ethyl Butyrate/Astringent/Bitter	Astringent	HMDB33834	$p < 0.05$
Cyclic ester/lactone	Gluconolactone	C6H10O6	b	M	Odorless, acidy		Astringent/Nutty	HMDB00150	$p < 0.05$
Dialkyl ethers/Benzyl Alcohol	Phenylethyl Alcohol	C8H10O	c	M/B	cool, fresh, leafy, metallic, green, hyacinth	Hay	Ethyl Butyrate/Sour	HMDB33944	$p < 0.05$
Dialkylamine	Spermidine	C7H19N3	a	B	morning mouth	Umami/Astringent/Cardboard/Ethyl Butyrate/Mercaptan		HMDB01257	$p < 0.05$
Dialkyldisulfide	Methyl propyl disulfide	C4H10S2	c	M/B	Garlic, burnt rubber,	HoneyComb	Corn Chips/Honey Comb	HMDB31872	$p < 0.05$
Dicarboxylic acid	Diethyl malonate	C7H12O4	c	M/B	guava fruit, melon, concord grape, pineapple, blackberry and many wines and spirits	Sulfitic	Metallic	HMDB29573	$p < 0.05$
Dicarboxylic Acid	Fumaric acid	C4H4O4	c	M/B	Sour	Sour/Sulfitic/Phenolic	Sulfitic/Sulfidic/Sour	HMDB00134	$p < 0.05$
Dicarboxylic Acid	Succinic acid	C4H6O4	c	M/B	Musty, cooked, apple, fruity	Nutty/Sulfitic/Acetaldehyde	Sour/Sulfitic	HMDB00254	$p < 0.05$
Dicarboxylic Acid	TXIB	C16H30O4	c	M/B	Butyric	Ethyl Butyrate/Sulfitic/Phenolic	Sulfitic/Sulfidic/Phenolic	HMDB59777	$p < 0.05$
Dicarboxylic Acid Derivative	Butyl oxalate	C10H18O4	c	B	Nutty, bitter tea	Honeysuckle		HMDB40196	$p < 0.05$
Dicarboxylic Acid Derivative	Oxalic acid, monoamide, N-(2-ethylhexyl), ethyl ester	C12H23NO3	c	B		Umami/Caramel/Sulfitic/Grainy		2914761	$p < 0.05$

Dicarboxylic Acid Derivative	Oxalic acid, dicyclobutyl ester	C10H14O4	c	M/B	Apple, fruity, grape, Musty	Pear/Grass/Floral/Isoamyl Acetate	Sweet/Bread Crust	6420604	$p < 0.05$
dicarboxylic sugar acid	Galactaric acid	C6H10O8	b	M			Bitter/Astringent/Mercaptan	HMDB00639	$p < 0.05$
Dipeptide	Aspartyl-Lysine	C10H19N3O5	a	B	Sour, glutamate-like	Sulfitic/Ethyl Butyrate		HMDB28758	$p < 0.05$
Dipeptide	Glycyl-methionine	C7H14N2O3S	a	B		Bread Crust		HMDB28847	$p < 0.05$
Dipeptide	Isoleucyl-phenylalanine	C15H52N2O3	a	B		Sulfitic/Cornchip		HMDB28914	$p < 0.05$
Dipeptide	Prolyl-arginine	C11H21N5O3	a	B		CornChip/Nutty/Sulfitic			$p < 0.05$
Dipeptide	Prolyl-cysteine	C8H14N2O3S	a	B		Sulfitic		HMDB29014	$p < 0.05$
Dipeptide	Tryptophyl-cysteine	C14H17N3O3S	a	B		Sulfitic/Ethyl Butyrate/Cornchip/Grainy		HMDB29080	$p < 0.05$
Dipeptide	GLN-Met	C10H19N3O4S	a	M			Umami/Musty/Nutty/Astringent/Bitter	HMDB29155	$p < 0.05$
Dipeptide	Glycylproline	C7H12N2O3	ZIC-HILIC-MS/a	M	Toasty, roasty, malty		Sweet/Bread Crust	HMDB00721	$p < 0.05$
Dipeptide	L-aspartyl-L-phenylalanine	C13H16N2O5	b	M			Metallic/Wet Hay/Sour	HMDB00706	$p < 0.05$
Dipeptide	L-isoleucyl-L-Proline	C11H20N2O3	a	M			Isoamyl Acetate	HMDB11174	$p < 0.05$
Dipeptide	Glycyl-L-leucine	C8H16N2O3	b /a	M/B	Substrate for glycyl-leucine dipeptidase	Honeysuckle/Pear	Fruity Complex	HMDB00759	$p < 0.05$
Disaccharide	Cellobiose	C12H22O11	b	M			Ethyl Butyrate/Phenolic	HMDB00055	$p < 0.05$
Disaccharide	Melibiose	C12H22O11	b	M	Astringent		Nutty/Phenolic	HMDB00048	$p < 0.05$
Disaccharide	Isomaltose	C12H22O11	a	M/B		Watermelon Rind/Cucumber/Fruity/White Grape/Isoamyl Acetate	Hay/Corn Chips	HMDB02923	$p < 0.05$
Disaccharide	Trehalose	C12H22O11	b/a	M/B	o-glycosyl compounds	PlayDoh/GreenApple/Fruity/Ethyl Acetate	Green Apple/Ethyl Acetate	HMDB00975	$p < 0.05$

Disaccharide/Phenolic Glycoside	5-(3',5')-Dihydroxylphenyl-gamma valerolactone	C18H22O10	a	M			Sour/Metallic	HMDB60030	$p < 0.05$
Dissacharide	D-Maltose	C12H22O11	b	M	Sweetening agent		Ethyl Butyrate	HMDB00163	$p < 0.05$
Dissacharide	Lactulose	C12H22O11	b	M			Wet Hay/Metallic/Sour	HMDB00740	$p < 0.05$
Diterpene Alcohol	Geranylgeraniol	C20H34O	c	M/B	peach, raspberry, grapefruit, red apple, plum, lime, orange, lemon, watermelon, pineapple and blueberry.	White Grape/Pear/Grass/Floral/Perfume	White Grape/Pear/Grass/Floral/Perfume		$p < 0.05$
Diterpenoid	Gibberellic Acid	C19H22O6	a	M/B		Metallic/Nutty/Acetaldehyde/HoneyComb	Metallic	HMDB03559	$p = 0.15$
Diureide	Allantoin	C4H6N4O3	b	M/B	oxidation of uric acid	IsoamylAcetate/Floral	Metallic	HMDB00462	$p = 0.09$
Endocannabinoid	C17:1 anandamide	C19H37NO2	b	M			Perfume/Fruity/Play-doh		$p = 0.22$
Endocannabinoid	MAG(0:0/20:4n6)	C23H38O4	a	M			Metallic/Fruity	HMDB04666	$p = 0.39$
Enone	4-Hexene-3-one	C6H10O	a	M	Ethereal, green, pungent, tropical, metallic		Metallic/Wet Hay	HMDB35239	$p = 0.14$
Ester	Isoamyl Acetate/Isopentyl Acetate	C7H14O2	c	B	fruity, banana, Pear, solvent, estery, apple, sweet	Pear/HoneyComb/Honeysuckle/Sweet-Aromatic		HMDB31528	$p < 0.05$
Ester	N-allyl-L-alanine	C6H11NO3S	c	M/B		Ethyl Butyrate/Cornchip/Isovaleric	Mercaptan	15558642	$p < 0.05$
Ester/Phenol	Chlorogenic acid	C16H18O9	b	M/B	Phenolic, astringent		Astringent	HMDB03164	$p < 0.05$
Fatty Acid	Methylacetoin	C5H10O2	a	M	Fruity, berry		Fruity/White Grape/Play-doh	8261	$p < 0.05$
Fatty Acid	Monogalactosyl diacylglycerol (MGDG)		b	M			Umami/Phenolic		$p < 0.05$
Fatty Acid	Octanoic anhydride	C16H30O3	c	M	Fecal or vomity		Isovaleric/salty	69340	$p < 0.05$
Fatty Acid Ester	Ethyl Pentadecanoate	C17H34O2	c	M/B		Acetaldehyde/Phenolic	Umami/Paper/Cardboard/Musty	38762	*c*

Fatty Acid Ester	Oct-3-enoic acid, oct-3-en-2-yl ester	C16H28O2	c	M/B		Cornchip/HoneyComb/Nutty/Sulfidic	Phenolic/Umami/Caramel/Bitter/Astringent		$p < 0.05$
Fatty Acid Ester	Ethyl octanoate	C10H20O2	c	B	Sour appley	Sour		HMDB40195	$p < 0.05$
Fatty Acid Ester	Isopentyl hexanoate/Isoamyl caprylate	C11H22O2	c	B	fruity, solvent, perfumed, tropical fruits	Fruity/Ethyl Acetate/White Grape/Watermelon/Green Apple		HMDB33618	$p < 0.05$
Fatty Acid Ester	Linalyl Butyrate	C14H24O2	c	B	Floral, fruity	Ethyl Butyrate		HMDB30427	$p < 0.05$
Fatty Acid Ester	Diethyl decanedioate	C14H26O4	c	M	Fruity, Melon, Quince, Wine, Mild		Sweet/Honey/Sweet Aroma	HMDB40429	$p < 0.05$
Fatty Acid Ester	Ethyl nonanoate	C11H22O2	c	M	Fruity, pineapple, banana		Fruity complex	HMDB40193	$p < 0.05$
Fatty Acid Ester	Methyl 4-octenoate	C9H16O2	c	M	Fruity, sweet, astringent, pineapple		Nutty/Bitter/Grainy/Umami/Caramel	HMDB39794	$p < 0.05$
Fatty Acid Ester	Methyl caprylate	C9H18O2	c	M	Fruity, cinnamony		Green Apple/Ethyl Acetate	HMDB31291	$p < 0.05$
Fatty Acid Ester	Methyl dodecanoate	C13H26O2	c	M	grape, fruity, apple		Green Apple/Ethyl Acetate	HMDB31018	$p < 0.05$
Fatty Acid Ester	Pelargonic Acid	C9H18O2	c	M	Unpleasant, rancid, old oil		Grain/Mercaptan/Corn Chips/Nutty/Astringent	HMDB00847	$p < 0.05$
Fatty Acid Ester	2-Methylacetophenone	C20H38O7S	c	M/B	Nutty, Phenolic, honey	Phenolic/Worty	Phenolic	HMDB32386	$p < 0.05$
Fatty Acid Ester	Amyl laurate	C17H34O2	c	M/B	Goaty, vomity	Astringent/Mercaptan/Ethyl Butyrate/Isovaleric	Mercaptan	62571	$p < 0.05$
Fatty Acid Ester	Diethyl decanedioate	C12H22O4	c	M/B	Fruity, melon, winey, quince, apple, Pear	Honeysuckle/Honey/Sweet/Sweet-aromatic/Pear	Honeycomb Cereal/Sweet	HMDB40429	$p < 0.05$
Fatty Acid Ester	Ethyl 2-methylpentanoate	C5H10O2	c	M/B	Apple, fresh, fruity, melon, pineapple	Pear/White Grape/Watermelon/Grass/Floral/Fruity	Pear	HMDB31579	$p < 0.05$
Fatty Acid Ester	Ethyl dodecanoate	C12H24O	c	M/B	apple, apricot, guava, melon, *etc.* crispbread, ginger, whisky, fruit brandies and wine. flavouring agent.	OffMouthFeel/Bread Crust/Grass	Grainy	HMDB33788	$p < 0.05$

Fatty Acid Ester	Ethyl Oleate	C12H22O2	c	M/B		White Grape/Pear/Grass/Floral	Watermelon Rind/White Grape	522255	$p < 0.05$
Fatty Acid Ester	Ethyl tridecanoate	C15H30O2	c	M/B	Fatty, fruity	Caramel/Sour/Raisin/Sherry	Caramel/Phenolic	HMDB59833	$p < 0.05$
Fatty Acid Ester	Ethyl undecanoate	C13H26O2	c	M/B	coconut, pineapple, sweet, fruity, green, soapy, Pear	Ethyl Butyrate	Isovaleric/Salty	HMDB29552	$p < 0.05$
Fatty Acid Ester	Ethyl-5-methylhexanoate	C9H18O2	c	M/B	Apple, fruity, sweet	Pear/Grass/Floral/Isoamyl Acetate	Pear/Honeysuckle	HMDB59822	$p < 0.05$
Fatty Acid ester	Heptyl decanoate	C17H34O2	c	M/B		Caprylic/Sulfitic/Isovaleric/Umami	Astringent	108902	$p < 0.05$
Fatty Acid Ester	Isobutyl 3-methyl-2-butenoate;	C9H16O2	c	M/B	Green, spicy, mint	Nutty/Sulfitic/Sulfidic/Phenolic	Nutty/Sulfitic/Sulfidic/Phenolic	121709	$p < 0.05$
Fatty Acid Ester	Isobutyl Butyrate	C8H16O2	c	M/B	Soapy, waxy, rancid, vomity	Caprylic/Sour/Bitter/Isovaleric	Astringent	HMDB34161	$p < 0.05$
Fatty Acid Ester	Isobutyl caprylate	C12H24O2	c	M/B	Swiss cheesy, winey, fatty, sweet	Ethyl Butyrate/Caprylic/Isovaleric/Cornchip	Mercaptan	HMDB59868	$p < 0.05$
Fatty acid ester	Isopentyl 8-methylnon-6-enoate	C15H28O2	c	M/B	Cinnamic acid ester - cocoa, floral, Musty, orchid	White Grape/Pear/Grass/Floral	White Grape/Pear/Grass/Floral		$p < 0.05$
Fatty Acid Ester	Methyl 2-(methylthio)Butyrate	C6H12O2S	c	M/B	Musty, onion, sulfurous	Isovaleric/Mercaptan/Sulfitic/Sulfidic	Sulfitic/Sulfidic	HMDB41306	$p < 0.05$
Fatty Acid Ester	Methyl decanoate/Methyl caprate	C11H22O2	c	M/B	Sweet, coconut, fruity	Bread Crust/Fruity	Ethyl Acetate/Green Apple	HMDB33848	$p < 0.05$
Fatty Acid Ester	Methyl tetradecanoate	C15H30O2	c	M/B	Perfumey, herbal, petals	Perfume/EthylAcetate/Fruity/Watermelon/PlayDoh	Perfume/Ethyl Acetate/Green Apple	HMDB30469	$p < 0.05$
Fatty Acid ester	Methyl tetradecanoate	C16H32O2	c	M/B	fatty acids, caprylic, vegetable oil	Mercaptan/Astringent/Isovaleric	Mercaptan	HMDB30469	$p < 0.05$
Fatty Acid Ester	Nonyl Phenylacetate	C17H26O2	c	M/B	Fruity, fruit, soapy, tropical, tea-like	Fruity/Ethyl Acetate	Metallic	562667	$p < 0.05$
Fatty Acid Methyl Ester	Methyl caprylate	C9H18O2	c	B	perfumey, aldehydic, herbal, orange, sweet	Sour/Bitter/Isovaleric		HMDB31291	$p < 0.05$
Fatty Acid Methyl Ester	Methyl pentanoate	C6H12O2	c	B	Apple, fruity, green, pineapple, sweet	Pear		HMDB31207	$p < 0.05$
Fatty Acid Methyl Ester	S-Methyl hexanoate	C7H14O2 S	c	B	cooked vegetable, sulfury, soapy	Bread Crust		HMDB35238	$p < 0.05$

fatty acid methyl esters - Fatty Acyl	Methyl-4-(methylthio)Butyrate	C6H12O2S	a	B		OffFlavor/Sulfidic/Sulfitic		HMDB37619	$p < 0.05$
fatty acid methyl esters - Fatty Acyl	Methyl hexanoate	C7H14O2	c	M	goaty, fatty acid, vegetable oil, sweaty, caprylic		Bread Crust	HMDB35238	$p < 0.05$
Fatty Acyl	2-ethylbutanoic acid	C15H22O3	c	B	found in cereals and cereal products, green, ethereal, fruity, cocoa	Fruity		HMDB31221	$p < 0.05$
Fatty Acyl	Butanoic Acid	C5H10O2	b	B	buttery, rancid, cheesy	PlayDoh		HMDB00039	$p < 0.05$
Fatty Acyl	Butyric Acid	C5H10O2	c	B	Fatty acid methyl ester	Watermelon Rind/Cucumber/Fruity/White Grape		HMDB00039	$p < 0.05$
Fatty Acyl	Cyclopentylacetic acid	C7H12O2	c	B	Jasmonic acid ester	White Grape/Pear/Grass/Floral		71606	$p < 0.05$
Fatty Acyl	Hexanoic Acid	C6H12O2	a	B	goaty, fatty acid, vegetable oil, sweaty, caprylic	Caprylic/Isovaleric/Bitter/Sour		HMDB00535	$p < 0.05$
Fatty Acyl	1-(3-Methylbutanoyl)-6-apiosylglucose	C16H28O11	a	M	Bitter, Phenolic		Phenolic	HMDB39953	$p < 0.05$
Fatty Acyl	9,10-epoxy-11-hydroxy-12-octadecenoic acid	C18H34O4	a	M	Herbal, Chrysanthemum, Cereal-like		Pear/Isoamyl Acetate/Grass	5283015	$p < 0.05$
Fatty Acyl	Alchornoic Acid	C20H36O3	a	M			Perfume/Acetaldehyde/Green Apple	44256507	$p < 0.05$
Fatty Acyl	Cerotic acid	CH3(CH2)24COOH	b	M			Metallic	HMDB02356	$p < 0.05$
Fatty Acyl	Citraconic acid	C5H6O4	b	M	Citric		Green Apple/Ethyl Acetate	HMDB00634	$p < 0.05$
Fatty Acyl	Corchoionol C-9-glucoside	C19H30O8	a	M	Bitter		Nutty/Phenolic	HMDB29772	$p < 0.05$
Fatty Acyl	Elaidic acid	C18H34O2	b	M			Perfume/Green Apple/Ethyl Acetate	HMDB00573	$p < 0.05$
Fatty Acyl	Heptanoic acid	C7H14O2	b	M	Rancid		Green Apple/Perfume/Acetaldehyde	HMDB00666	$p < 0.05$

Fatty Acyl	Hexadecanoic acid	C16H32O2	b	M	Bitter		Bitter/Astringent/Grainy	HMDB00220	$p < 0.05$
Fatty Acyl	Isopentyl Hexanoate	C11H22O2	c	M	Milky, fruity		Honeycomb cereal/Corn Chips	16617	$p < 0.05$
Fatty Acyl	megultol	C6H10O5	b	M	Oily, Papery, flaxseed-like, bitter		Caramel/Bitter/Astringent	HMDB00355	$p < 0.05$
Fatty Acyl	Methylsuccinic acid	C5H8O4	b	M	Bitter, glutamate, sour		Bread Crust/Ethyl Butyrate	HMDB01844	$p < 0.05$
Fatty Acyl	Nervonic acid	C24H46O2	b	M			Perfume	HMDB02368	$p < 0.05$
Fatty Acyl	N-tert-butyl arachidonoyl amine	C24H41NO	a	M			Phenolic/Nutty/Umami	5283397	$p < 0.05$
Fatty Acyl	Pentadecanoic acid	C15H30O2	b	M			Bread Crust	HMDB00826	$p < 0.05$
Fatty Acyl	Stearic acid	C18H36O2	b	M	Waxy		Metallic	HMDB00827	$p < 0.05$
Fatty Acyl	Traumatic acid	C12H20O4	b	M			Fruity	HMDB00933	$p < 0.05$
Fatty Acyl	2-isopropylmalic acid	C7H12O5	b	M/B	Cereal, Fatty, Fruity	Pear/Bread Crust	Grass/Pear	HMDB00402	$p < 0.05$
Fatty Acyl	2-Methylglutarate	C6H10O4	b	M/B	Glutamate-like, bitter	Bitter/Astringent/Umami/Cardboard	Metallic/Acetaldehyde	HMDB00422	$p < 0.05$
Fatty Acyl	9-Decenoic acid	C10H18O2	c	M/B		White Grape/Pear/Grass/Floral	Watermelon Rind	HMDB31003	$p < 0.05$
Fatty Acyl	Capric Acid	C10H20O2	c	M/B	Goaty, unplesant, oily, old meat	Caprylic/Sulfitic/Isovaleric/Umami	Caramel/Bitter/Nutty/Grainy/Umami	HMDB00511	$p < 0.05$
Fatty Acyl	Caprylic Acid	C8H16O2	c	M/B	caprylic, goaty, fatty acid, vegetable oil, wet dog	Acetaldehyde	Green Apple/Ethyl Acetate	HMDB00482	$p < 0.05$
Fatty Acyl	Turanose	C12H22O11	b/a	M/B	reducing disaccharide	Ethyl Acetate/Perfume/Fruity	Ethyl Acetate/Perfume/Fruity	HMDB11740	$p < 0.05$
Fatty Acyl Glycoside	2,6-Dimethyl-7octene-1,6-diol 8-O glucoside	C16H30O7	a	M	anise-like, fennel		Green Apple/Ethyl Acetate	HMDB33218	$p < 0.05$
Faty Acid Ester	Ethyl heptadecanoate	C9H18O2	c	M/B	Fruit punch, fatty, perfumey	Isovaleric/Ethyl Butyrate/Astringent/Bitter	Mercaptan/Grainy/Nutty/Ethyl Butyrate	26397	$p < 0.05$

Faty Acyl	2-amino-octadecanoic acid	C18H37NO2	a	M			Acetaldehyde	409323	$p < 0.05$
Flavanoid	delphin	C27H31O17	a	B		Phenolic		HMDB30693	$p < 0.05$
Flavanol	Hydroxyflavone		a	B		Sour/Raisin/Sherry			$p < 0.05$
Flavanol glycoside	Rutin	C27H30O16	b	M			Sulfidic/EthylButyrate/Sulfitic	HMDB03249	$p < 0.05$
Furan	DMPF	C6H8O4	c	B	Aromatic, roasty, nutty, cooling	Cornchip/HoneyComb/Nutty		HMDB39784	$p < 0.05$
Furan	Furfural	C5H4O2	c	B	Caramel, bready, Papery, husky	Aromatic, roasty, nutty		HMDB32914	$p < 0.05$
Furan	2,5-Dimethyl-2,5-dihydrofuran	C6H10O	c	M			Caramel	557796	$p < 0.05$
Furan	Furan, 2-nonadecanoyl	C23H40O2	c	M/B	Aromatic, roasty, nutty	Cornchip/HoneyComb/Nutty	Metallic	573623	$p < 0.05$
Glucoside	Indoxyl Beta-D-Glucoside	C8H7NO	b	M			Metallic	258533	$p < 0.05$
Glucoside	Terpene glycoside		a	M/B		Isovaleric/Bitter/Ethyl Butyrate	Phenolic/Ethyl Butyrate		$p < 0.05$
Glutamic acid derivative	Saccharopine	C11H20N2O6	b/ a	M/B	glutamate-like	Fruity/Bread Crust/Umami/Grainy/Nutty	Play-doh/Fruity	HMDB00279	$p < 0.05$
Glycerophosphocholine	PC(16:0-18:1)	C42H82NO8P	b	M			Green Apple/Ethyl Acetate	6506401	$p < 0.05$
Glycerophosphocholine	PC(18:4(6Z,9Z,12Z,15Z)/19:1(9Z))	C40H80NO8P	a	M			Green Apple/Acetaldehyde	52922915	$p < 0.05$
Glycerophosphoethanolamine	GPE(P-18:0/20:4)	C5H14NO6P	b	M			Green Apple/Ethyl Acetate	86289532	$p < 0.05$
Glycerophospholipid	PA(16:0/18:2)	C37H69NO8P	b	M			Nutty/Sulfidic	9547167	$p < 0.05$
Glycerophospholipid	PE-NMe(32:0)	C38H76NO8P	b	M			Green Apple/Ethyl Acetate	445468	$p < 0.05$
Glycosylamines	Nicotinomide riboside	C11H15N2O5	a	B		Fruity/Perfume/PlayDoh/GreenApple		HMDB00855	$p < 0.05$
Hexose	Allose	C6H12O6	b	M	Spicy, bitter, Phenolic		Astringent	HMDB01151	$p < 0.05$
Hydroxy Acid	Galactonate	C6H12O7	b	M			Sulfidic/Nutty	HMDB00565	$p < 0.05$

Hydroxy Acid	L-2-hydroxyglutaric acid	C5H8O5	b	M			Ethyl Acetate/Green Apple	HMDB00694	$p < 0.05$
Hydroxy Acid	L-Lactic acid	C3H6O3	b	M	Acidic		Sulfidic/Nutty	HMDB00190	$p < 0.05$
Hydroxy Acid	Malic acid	C4H6O5	b	M	sour-like, sweettart		Green Apple/Ethyl Acetate	HMDB00744	$p < 0.05$
Hydroxy Acid Derivative	2-acetylphenanthrene	C16H12O	c	M	Earthy		Pear	80060	$p < 0.05$
Hydroxy Fatty Acid	Hydroxyisocaproic acid	C6H12O3	b	M	caproic, goaty, sulfurous		Sulfitic/Sulfidic/Phenolic/Cardboard	HMDB00746	$p < 0.05$
Hydroxycinnamic Acid	Isoferulic Acid	C10H10O4	ZIC-HILIC_LC-MS/a	M	Clovey, spicy, fruity		Perfume/Play-doh/Acetaldehyde/Green Apple	HMDB00955	$p < 0.05$
Hydroxyindole	5-hydroxytryptophol	C10H11NO2	a	M/B	Old almonds, unpleasant	Caprylic/Musty/Sulfidic/Sulfitic	Pear/Grass/Fruity	HMDB01855	$p < 0.05$
Hydroxypyrimidine	5-Methylcytosine	C5H7N3O	a	B		Perfume/Fruity/GreenApple/PlayDoh		HMDB02894	$p < 0.05$
Hypoxanthine	6,8-Dihydroxypurine	C5H4N4O2	a	M	eggy		Play-doh	HMDB01182	$p < 0.05$
Imidazopyrimidine	Xanthine	C5H4N4O2	b	M			Sulfidic/Nutty	HMDB00292	$p < 0.05$
Imidazopyrimidines	DMAP	C7H9N5	b	B		Umami/Sulfitic/Sulfidic		HMDB00473	$p < 0.05$
Indole	N,N-dimethylindoliumolate	C10H11NO2	a	B		Isovaleric/Sulfitic/Sour		HMDB60063	$p < 0.05$
Indole	N-Methylpropionamide	C4H9NO	c	M/B	Mushroomy, earthy, metallic	Grainy/Nutty/Metallic	Grainy/Nutty	14470	$p < 0.05$
Indolyl Carboxylic Acid Derivative	Indoleacetic acid	C10H9NO2	b	B	indole	Umami/Astringent/Cardboard/Ethyl Butyrate/Mercaptan		HMDB00197	$p < 0.05$
Indolyl Carboxylic Acid Derivative	Indolepropionate	C11H11NO2	b	M	NA		Bread Crust/Metallic	HMDB02302	$p < 0.05$
Inorganic Non-Metal Compound	Pyrophosphate	O7P2	b	M			Sulfitic	HMDB00250	$p < 0.05$

Inositolphosphorylceramide	PI-Cer(t20:0/26:0)	C52H104NO12P	a	M			Green Apple/Ethyl Acetate	70699095	$p < 0.05$
Intermediate	Shikimate	C7H10O5	b	M			Green Apple	HMDB03070	$p < 0.05$
Intermediate. Substrate for Succinate-CoA	Itaconic acid	C5H6O4	b	M			Honeycomb cereal/Ethyl Butyrate	HMDB02092	$p < 0.05$
Isothiocyanate	Methane, isothiocyanate	C2H3NO	c	M/B	Pungent, mustardy, astringent	Umami/Astringent/ Cardboard/Ethyl Butyrate/Mercaptan	Suflitic/Sufidic	HMDB34106	$p < 0.05$
Keto Acid	2-Ketocaproic acid	C6H10O3	b	M	Fruity		White Grape/Pear/ Grass/Floral/ Watermelon Rind	HMDB01864	$p < 0.05$
Keto Acid	2-oxoglutarate	C5H4O5	b	M	Fruity, metallic		Metallic	HMDB62781	$p < 0.05$
Keto Acid	ketoisocaproate	C6H10O3	b	M	Sweet, fruity		Metallic/Acetaldehyde	HMDB00695	$p < 0.05$
Keto Acid	Oxoadipic acid	C6H8O5	b	M			Acetaldehyde/Perfume	HMDB00225	$p < 0.05$
Ketone	2,2-Dimethyl-1,3-cyclohexanedione	C8H12O	c	B	sweet, caramel, maple	acetaldehyde		550967	$p < 0.05$
Ketone	Cyclopentanone	C5H8O	c	B	Ethyl ether, bitter	Bitter		8452	$p < 0.05$
Ketone	Diacetyl	C4H6O2	c	B	Butterscotch, butter popCorn, caramel	Ethyl Butyrate/Astringent /Isovaleric/Bitter		HMDB03407	$p < 0.05$
Ketone	Ethanone-2-acetylphenanthrene	C16H12O	c	B		Isoamyl Acetate/Floral/Grass		80060	$p < 0.05$
Ketone	1,3-cyclohexanedione	C6H8O2	c	M	Bitter, burnt, coffee-like		Umami/Cardboard/Musty/ Raisin/Caprylic	10434	$p < 0.05$
Ketone	Acetylacetaldehyddimethylacetal	C6H12O3	a	M	Bitter, ethereal, Musty, nutty		Metallic	HMDB33851	$p < 0.05$
Ketone/Furan	2-propionylfuran	C7H8O2	c	B	Aromatic, roasty, nutty (common in aged beer)	Cornchip/HoneyComb/Nutty/Sulfidic		HMDB40280	$p < 0.05$

Lactone	Nonalactone	C9H16O2	c	B	perfumey, aldehydic, herbal, orange, sweet•concentration increases in aged beer	Raisin/Sherry/Caramel		HMDB31514	$p < 0.05$
Lactone	DHAA	C6H6O6	b	M			Astringent	HMDB01264	$p < 0.05$
lysophospholipid	Lyso PC(18:2)	C26H50NO7P	a	M			Green Apple/Ethyl Acetate	11005824	$p < 0.05$
Modified Amino Acid (post-translational)	Hydroxyproline	C5H9NO3	b	M			Phenolic/Astringent	HMDB00725	$p < 0.05$
Monoglycerophospholipid	20:0 LYSO PC	C28H58NO7P	b	M			Metallic/Sour/Wet Hay	24779473	$p < 0.05$
monoglycerophospholipid	LysoPC(18:3(9Z,12Z,15Z))	C26H48NO7P	a	M			Green Apple/Ethyl Acetate	HMDB10388	$p < 0.05$
Monosaccharide	Deoxyribose	C5H10O4	a	M	Sweet		Honeycomb Cereal/Corn Chip/Grainy	HMDB03224	$p < 0.05$
Monosaccharide	D-fructose	C6H12O6	b	M	sweet		Green Apple/Ethyl Acetate	HMDB00660	$p < 0.05$
Monosaccharide	D-Galactose	C6H12O6	b	M	Less sweet than other sugars		Nutty/Astringent	HMDB00143	$p < 0.05$
Monosaccharide	Glucose	C6H12O6	b	M	Sweet		Fruity Complex	HMDB00122	$p < 0.05$
Monosaccharide	D-Tagatose	C6H12O6	b/a	M/B	Sweet	Fruity/Perfume/EthylAcetate/Green Apple/IsoamylAcetate	Bitter/Astringent, Caramel	HMDB03418	$p < 0.05$
Monosaccharide	Sucrose	C12H22O11	b	M/B	nonreducing sugar	Sweet/Honey/Ethyl Butyrate	Green Apple/Ethyl Acetate	HMDB00258	$p < 0.05$
Monosaccharide phosphate	Glucose-1-phosphate	C6H13O9P	b	M			Sulfitic/Sulfidic	HMDB01586	$p < 0.05$
Monoterpenoid	2,4,5-Trimethyl-phenol	C13H20O	a	B	Aromatic monoterpene, herbal, Phenolic	Grass/Pear		HMDB29823	$p < 0.05$
Monoterpenoid	Linalyl hexanoate	C16H28O2	a	B	Barnyardy	Honey Comb/Nutty/Cornchip/Grainy		HMDB30429	$p < 0.05$
Monoterpenoid	Menthadienyl acetate	C12H18O2	a	B	Spearminty	Floral/Grass		HMDB38292	$p < 0.05$

N-alkylpyrrolidine	1-Acetonylpyrrolidine	C6H13NO	a	B	Proline-derived Maillard product	Play-doh		HMDB40030	$p < 0.05$
Nucleoside	Cytidine	C9H13N3O5	b	M/B	glutamate-like, bitter	Watermelon/Ethyl Acetate/PlayDoh	Acetaldehyde	HMDB00089	$p < 0.05$
Oligosaccharide	Maltopentaose	C36H52O26	a	B	sweet	Perfume/EthylAcetate/Fruity/Watermelon/PlayDoh		HMDB12254	$p < 0.05$
Oligosaccharide	Maltotetraose	C24H42O21	ZIC-HILIC-MS/a	M	Sweet Starchy -		Pear/Bread Crust	HMDB01296	$p < 0.05$
Oligosaccharide	Maltotriose	C18H32O16	b	M			Nutty/Astringent/Grainy	HMDB01262	$p < 0.05$
Oligosaccharide	Stachyose	C24H42O21	b	M/B		Green Apple/Fruity	Astringent/Grainy	HMDB03553	$p < 0.05$
Organonitrogen Compound	Methylguanidine	C2H7N3	b	B	Guanidine class	Sulfitic		HMDB01522	$p < 0.05$
Organonitrogen Compound	N6-methylagmatine	C6H14N4	a	B	Beany	Grass/Floral/PlayDoh/Bread Crust		HMDB39252	$p < 0.05$
Organooxygen compound		C6H8O6	b	M	Mentholic, mild		Phenolic	HMDB06355	$p < 0.05$
Organooxygen Compound	NeuAc	C11H19NO9	b	M			Perfume/Acetaldehyde/Green Apple	HMDB00230	$p < 0.05$
Peptide	Glutathione	C10H17N3O6S	b	M			Green Apple/Play-doh	HMDB00125	$p < 0.05$
Phenethylamine	Tyramine	C8H11NO	a	M	Cheddar cheesey		Sulfitic/Sulfidic	HMDB00306	$p < 0.05$
Phenol	Apigenin-6-C-glucoside	C21H19O10-	a	B	Grassy, hoppy	Grassy/WhiteGrape/Floral/Ethyl Acetate		HMDB29260	$p < 0.05$
Phenol	Stilbene	C14H12	c	B		Wet Hay/Acetaldehyde		638088	$p < 0.05$
Phenol	3-Ethylphenol	C8H10O	c	M			Perfume/Acetaldehyde/Green Apple	HMDB59873	$p < 0.05$
Phenol	Catechin (+)	C15H14O6	b	M	Tea-like, sour cherry		Grass/Floral/Fruity Comple	HMDB02780	$p < 0.05$
Phenol	N-[4-(cyanomethyl)phenyl]-5-(phenoxymethyl) furan-2-carboxamide	C20H16N2O2	c	M			Green Apple/Ethyl Acetate	9260786	$p < 0.05$

Phenol	5-methoxy-3,7-dihydroxyflavanone	C16H14O5	c	M/B		Wet Hay/Acetaldehyde	Acetaldehyde	597405	$p < 0.05$
Phenol	Generic terpene		c	M/B		Wet Hay/Acetaldehyde	Phenolic		$p < 0.05$
Phenol	Vanillic acid	C8H8O4	b	M/B		Nutty/Sulfitic/Sulfidic/HoneyComb/Grainy	Acetaldehyde	HMDB00484	$p < 0.05$
Phenol/Benzenoid	Tyrosol	C8H10O2	c	B	bitter, chemical, sour aftertaste, old fruit, pepper, unpleasant	Astringent		HMDB04284	$p < 0.05$
Phenol/Benzenoid	Tyrosol	C8H10O2	b	M	bitter, chemical, sour aftertaste, old fruit, pepper, unpleasant		Fruity	HMDB04284	$p < 0.05$
Phenylacetaldehyde	Hydratropaldehyde	C9H10O	c	B	Floral, citrusy, herbal, green vegetable, fruity	Grass/White Grape		HMDB31626	$p < 0.05$
Phenylpropanoic Acid	P-Aminophenylalanine	C9H12N2O2	a	B	phenylpropanoic acids	Astringent/Cardboard/Bitter		HMDB30397	$p < 0.05$
Phenylpropanoic Acid	Hydroxyphenyllactic acid	C9H10O4	b	M/B	vomity, sour	Isovaleric/Ethyl Butyrate/Astringent/Bitter	Ethyl Butyrate	HMDB00755	$p < 0.05$
Phenylpropanoic Acid	Phenyllactic acid	C9H10O3	a	M/B	Aromatic, cyclic	Umami/Astringent/Cardboard/Ethyl Butyrate/Mercaptan	Metallic	HMDB00779	$p < 0.05$
Phenylpropanoid	Desaminotyrosine	C9H10O3	b	M			Pear/Isoamyl Acetate	HMDB02199	$p < 0.05$
Phenylpropanoid	Epicatechin	C15H14O6	b/a	M			Ethyl Butyrate/Bread Crust	HMDB01871	$p < 0.05$
Phosphatidylcholine	PC(18:1(9Z)/18:1(9Z))	C44H84NO8P	b	M			Phenolic/Nutty	10350317	$p < 0.05$
Phosphatidylcholine	PC(18:4)	C26H46NO7P	a	M			Green Apple/Ethyl Acetate	452110	$p < 0.05$
Phosphatidylcholine	PC(28:0)	C36H72NO8P	b	M			Green Apple/Ethyl Acetate	5459377	$p < 0.05$
Phosphatidylcholine	PC(32:0)	C40H80NO7P	b	M			Green Apple/Ethyl Acetate	24779471	$p < 0.05$
Phosphatidylcholine	PC(36:4)	C44H81NO8P+	b	M			Fruity/Metallic	5283486	$p < 0.05$

Phosphatidylethanolamine	Lyso PE(16:0/18:2)	C39H76NO8P	b	M			Phenolic/Caramel/Astringent	5283496	$p < 0.05$
Phosphatidylethanolamine	PE(40:1)	C48H94NO8P	a	M			Green Apple/Ethyl Acetate		$p < 0.05$
phosphatidylglycero	PG(P-32:0)	C38H75O10P	a	M			Green Apple/Ethyl Acetate	446440	$p < 0.05$
Phosphatidylglycerolphosphate	PGP(36:4)	C42H76O13P2	a	M			Green Apple/Ethyl Acetate	HMDB13495	$p < 0.05$
Phosphatidylserine	PSer(36:1)	C42H80NO10P	b	M/B		Pear	Isoamyl Acetate/Green/Fruity	9547087	$p < 0.05$
Phosphocholine	PC(12:0/0:0)	C20H42NO7P	b	M			Metallic	460605	$p < 0.05$
Phosphocholine	PC(18:1)	C44H84NO8P	b	M			Umami/Musty/Cardboard/Carprylic	16081932	$p < 0.05$
Phosphoethanolamine	C12 Sphingosyl PE (d17:1/12:0)	C31H63N2O6P	a	B		Pear/Floral/Grass/White Grape			$p < 0.05$
Phosphoethanolamine	Lyso PE (18:0)	C23H48NO7P	b	M			Play-doh/Isoamyl Acetate/Green/Fruity	46891690	$p < 0.05$
Phospholipid	PC(O-14:0)		a	M			Green Apple/Ethyl Acetate		$p < 0.05$
Polysaccharide	Oligosaccharide		a	B		Perfume/EthylAcetate/Fruity/Watermelon/PlayDoh			$p < 0.05$
Primary Alcohol	Butyl Alcohol	C4H10O	c	B	ether	Sulfitic/Sulfidic/Grainy/Musty/Isovaleric		HMDB04327	$p < 0.05$
Primary Alcohol	Spritus vini	C2H6O	c	M/B	Phenolic, alcoholic	Phenolic/Metallic	Metallic	HMDB00108	$p < 0.05$
Purine	Guanine	C5H5N5O	b	M			Mercaptan	HMDB00132	$p < 0.05$
Purine	Purine	C5H4N4	b	M/B			Phenolic/Nutty/Sulfidic/Bitter	HMDB01366	$p < 0.05$
Purine nucleoside	1-methyladenosine	C11H15N5O4	b	M	Milk-like, salty		Play-doh/Perfume	HMDB03331	$p < 0.05$
Purine nucleoside	2-Phenylaminoadenosine	C16H18N6O4	b	M			Green Apple/Ethyl Acetate	HMDB01069	$p < 0.05$

Purine nucleoside	Deoxyadenosine	C10H13N5O3	a	M	Glutamate-like, bitter		Nutty/Grainy /Astringent	HMDB00101	$p < 0.05$
Purine nucleoside	Deoxyinosine	C10H12N4O4	b	M	Waxy		Isovaleric/Salty	HMDB00071	$p < 0.05$
Purine nucleoside	Guanosine	C10H13N5O5	b	M	Glutamate-like, grain		Astringent/Sulfidic/Nutty /Phenolic	HMDB00133	$p < 0.05$
Purine nucleoside	Isocitric acid	C6H8O7	b	M	Vegetal, sour vomit		Isovaleric/Mercaptan	HMDB00195	$p < 0.05$
Purine nucleoside	Adenosine	C10H13N5O4	a	M/B	Bitter, glutamate-like, Cornchippy	Nutty/Sulfitic/Grainy/Cornchip	Play-doh/Watermelon Rind	HMDB00050	$p < 0.05$
Purine nucleoside	Inosine	C10H12N4O5	b	M/B	Meaty, Savory	Bread Crust	Bread Crust	HMDB00195	$p < 0.05$
Purine nucleotide	AMP	C10H14N5O7P	b	B	Bitter, chalky, glutamate-like	Isovaleric/EthylButyrate/Sulfitic/Bitter		HMDB00045	$p < 0.05$
Pyrazine	Maltol	C6H6O3	b	M	caramel, malty, sweet, toasted, roasted		Sulfitic/PhenolicNutty	HMDB30776	$p < 0.05$
Pyrazine	Dithiouracil	C4H4N2S2	c	M/B	Toasted, roasted, Corn, toasted bread	Bread Crust	Sulfitic/Sulfidic/Wet Hay	1712448	$p < 0.05$
Pyridine	2-oxo-4-phenylbut-3-enoic acid	C10H8O3	c	M			Grainy	5356206	$p < 0.05$
Pyridine	4-(2-Phenylethyl)pyridine	C13H13N	c	M/B	Putrid, rancid, sour, sickening	Hay/Mercaptan/Ethyl Butyrate/Cornchip	Ethyl Butyrate	220846	$p < 0.05$
Pyridine	4-methylpyridine	C6H7N	c	M/B	roasted, nutty, cocoa, peanut	Phenolic/Sulfidic	Wet Hay	7963	$p < 0.05$
Pyridine	Isoquinoline	C9H7N	c	M/B	anise, herbal, sweet, benzaldehyde	Perfume/Fruity/GreenApple/PlayDoh	Perfume/Play-doh//Metallic	HMDB34244	$p < 0.05$
Pyridine	Pyridine-3-carbonitrile, 1,2-dihydro-6-amino-4-methyl-2-thioxo	C7H7N3S	c	M/B		Pear/Grass/Floral/Isoamyl Acetate	Pear/Grass/Floral/Isoamyl Acetate	5373995	$p < 0.05$
Pyridinecarboxylic acid	Vitamin B3	C6H6N2O	b	M			Bread Crust	HMDB01406	$p < 0.05$
Pyridinecarboxylic acid	Vitamin B3	C6H5NO2	b/a	M/B	Sour, metallic	Cornchip	Metallic	HMDB01488	$p < 0.05$
Pyrimidine	Vitamin B1	C12H17ClN4OS	b	B		Green Apple		HMDB00235	$p < 0.05$

Pyrimidine	2-amino-6methylpyrimidin-4-one	C5H7N3O	c	M/B		Honey/Sweet/Sweet-Aromatic/HoneySuckle	Sweet	1532	$p < 0.05$
Pyrimidine nucleoside	Ribothymidine	C10H14N2O6	b	M			Play-doh	HMDB00884	$p < 0.05$
Pyrimidine nucleoside	Uridine	C9H12N2O6	b	M	Contributes to many flavors		Umami/Musty/Nutty/Astringent/Bitter	HMDB00296	$p < 0.05$
Pyrimidine Nucleotide	Thymidine 5'-Triphosphate	C10H17N2O14P3	b	M			Bread Crust/Metallic/Nutty/Phenolic	HMDB01342	$p < 0.05$
Pyrimidinecarboxylic Acid	Vitamin B13	C5H4N2O4	b	B	matches, sulfurous	Nutty/Sulfitic/Sulfidic/HoneyComb/Grainy		HMDB00226	$p < 0.05$
Pyrimidne Nucleoside	Thymidine	C10H14N2O5	b	M/B	Sweet, nutty	Fruity/Green Apple	Caramel/Nutty/Umami/Bitter	HMDB00273	$p < 0.05$
Pyrimidone	Uracil	C4H4N2O2	b	M			Sulfitic/Sulfidic	HMDB00300	$p < 0.05$
Pyrrol	2-Methylpyrrole	C5H7N	c	B	Sulfury, bitter	Cornchip/Honeycomb		HMDB33114	$p < 0.05$
Pyrrol	3-acetamidopyrrolidine	C6H13NO2	c	M/B		Bread Crust	White Grape/Pear/Grass/Floral/Isoamyl Acetate	522715	$p < 0.05$
Pyrrol	3-Acetylpyrrole	C6H7NO	c	M/B	Found in cereals and cereal products.	Sour	Umami	2737793	$p < 0.05$
Quinolone Carboxylic Acid	Xanthurenic acid	C10H7NO4	b	M	Bitter		Pear/Bread Crust/Metallic	HMDB00881	$p = 0.22$
Saccharide	Alpha-Sophorose	C12H22O11	b	M			Corn Chips/mercaptan		$p < 0.05$
S-Containing Amino Acid Derivative	N-Acetyl-L-methionine	C7H13NO3S	ZIC-HILIC-MS/a	M/B	Sulfrous, glutaminous, Umami		Play-doh	HMDB11745	$p < 0.05$
Secondary alcohol	Furazan-3-ol, 4-amino	C2H3N3O2	c	M/B	Aromatic, roasty, nutty	Cornchip/HoneyComb/Nutty	Play-doh		$p < 0.05$

Sesquiterpenoid	Nerolidol	C15H26O	c	M/B	Nerolidol belongs to the family of Sesquiterpenes. These are terpenes with three consecutive isoprene units. Bitter	Cardboard/Umami/Sour/Bitter	Astringent/Nutty/Phenolic	HMDB35662	$p = 0.77$
Sesquiterpenoid	trans-Farnesol	C15H26O	c	M/B	Anise, floral, grapefruit, waxy, lily	Umami/Astringent/Cardboard/Ethyl Butyrate	Ethyl Butyrate	HMDB59849	$p = 0.55$
Sphingolipid	Inosotol-P-ceramide	C50H100NO13P	a	B		Grassy/WhiteGrape/Floral/Ethyl Acetate		HMDB12237	$p < 0.05$
Sugar Acid	D-Galacturonic acid	C6H10O7	b	B	medium chain fatty acid	Pear/Bread Crust/Floral		HMDB02545	$p < 0.05$
Sugar Acid	Quinic acid	C7H12O6	b	M	Bitter or fruity		Astringent/Bitter	HMDB03072	$p < 0.05$
Sugar Acid	Threonic acid	C4H8O5	b	M			Nutty/Grainy	HMDB00943	$p < 0.05$
Sugar Acid Derivative	Muramic acid	C9H17NO7	a	M			Astringent	HMDB03254	$p < 0.05$
Sugar Alcohol	D-Arabitol	C5H12O5	b	M			Green Apple/Fruity	HMDB00568	$p < 0.05$
Sugar Alcohol	D-Threitol	C4H10O4	a	M	Bitter		Metallic	HMDB04136	$p < 0.05$
Sugar Alcohol	Galactitol	C6H14O6	b	M	sweet		Green Apple/Ethyl Acetate	HMDB00107	$p < 0.05$
Sugar Alcohol	Mannitol	C6H14O6	b	M			Ethyl Butyrate	HMDB00765	$p < 0.05$
Sulfur Compound	3,5-dithiahexanol 5,5-dioxide	C4H10O3S2	c	M	Sulfurous		Nutty/Isovaleric/Salty/Grainy	548382	$p < 0.05$
Sulfur Compound	2-Mercapto-4-phenylthiazole	C9H7NS2	c	M/B	Garbagy	Caprylic/Musty/Caramel/Cardboard/Sulfitic	Sour/Sulfitic	3000729	$p < 0.05$
Sulfur Compound	4-(Methylthio)-1-butanamine	C5H13NS	c	M/B	Cabbage, garlic, potato, sulfury, vegetable	Sulfidic/Sulfitic/Mercaptan	Sulfidic/Phenolic	533935	$p < 0.05$
Sulfur Compound	Benzothiazole	C7H5NS	c	M/B	Rubbery, sulfury, cooked, gasoline	Mercaptan/Astringent	Wet Hay/Metallic/Sulfidic	HMDB32930	$p < 0.05$
Terpene	alpha-Ionone	C13H20O	a	B	raspberry, cedarwood	Bread Crust		HMDB59883	$p = 0.35$

Thia Fatty Acid	2-Hydroxy-4-(methylthio)butyric acid	C5H10O3S	a	B	Fatty acid derivative obtained by insertion of S, precursor to methianol	Cornchip/Grainy/Sulfitic/Nutty/Umami		11427	$p < 0.05$
Thioester	Methylthio-2-(propanoyloxy)propanoate	C7H12O3S	c	B	sulfuric ester	Honey Comb/Nutty/Cornchip/Grainy/Sulfitic/Sulfidic		HMDB40003	$p < 0.05$
Tricarboxylic Acid	Citric acid	C6H8O7	b	M	Acid-like, sweet, lemon		Fruity	HMDB00094	$p < 0.05$
Triglyceride	TG(50:5)iso6	C52H98O6	a	M			Sulfitic/Sulfidic	9543990	$p < 0.05$
Trisaccharide	D-Raffinose	C18H32O16	b	M	Sweet, bitter		Phenolic	HMDB03213	$p < 0.05$
UFA	Oleic acid	C18H34O2	b	M			Fruity	HMDB00207	$p < 0.05$
Vitamin	Biotin	C10H16N2O3S	b	B		Astringent		HMDB00030	$p < 0.05$
Xanthine	1,3-dimethyluric acid	C7H8N4O3	b	M			Metallic	HMDB01857	$p = 0.21$
Xanthine	3-Methylxanthine	C6H6N4O2	b	M	NA		Fruity/Isoamyl Acetate	HMDB01886	$p = 0.15$

***Platform - a denotes RP/LC-MS; b denotes HILIC/LC-MS; c denotes SPME/GC-MS; **Tissue - M if this metabolite was found in malt, B if this metabolite was found in beer; *HMDB database was used (denoted with prefix HMDB) to identify metabolites, PubChem was used in cases where metabolites were not found on HMBD (no prefix before ID); d denotes if this metabolite was found in malt, e denotes if this metabolite was found in beer, sensory associated with beer at Month 2, according to O2PLS beer model.

Table 8. Prediction-set for O2PLS malt model

Sensory Attribute	R^2Y score	Q^2Y Score
Acetaldehyde	0.0991737	0.443486
Astringent	0.646448	0.524312
Bitter	0.831459	0.824104
Body	0.142795	0.265809
Bread Crust	0.0100212	0.0869789
Caprylic	0.153457	0.634507
Caramel	0.807876	0.844767
Cardboard/Papery	0.0893195	0.432384
Corn Chips	0.193194	0.433486
Diacetyl	0.522189	0.483081
Ethyl Acetate	0.863048	0.944201
Ethyl Butyrate	0.0266071	0.0399551
Floral Complex	0.234808	0.613313
Fruity Complex	0.32364	0.248534
Grainy - Grape Nuts	0.388888	0.405056
Grass	0.285679	0.447727
Green Apple/E Hex	0.611167	0.560251
Hay	0.341168	0.554151
Honey	0.0142063	0.575409
Honeycomb Cereal	0.0937831	0.306999
Honeysuckle	0.00136269	0.524186
Isoamyl Acetate	0.23669	0.255833
Isovaleric	0.460432	0.198852
MC Off-Tastes	0.202386	0.54017
Mercaptan	0.481869	0.420232
Metallic	0.0333305	-0.164452
Musty	0.421569	0.692083
Nutty	0.286268	0.26258
Off MF/B	0.00318802	-0.0450587
Pear	0.0401927	0.35817
Perfume	0.656006	0.355448
Phenolic	0.450976	0.179066
Play-doh	0.643303	0.421705
Raisin/Sherry	0.218963	0.514719
Salty	0.460432	0.198852
Sour	0.133403	0.0893256
Sulfidic (H2S)	0.229074	0.0937773
Sulfitic (SO2)	0.0506304	0.0575267
Sweet/Aromatic Complex	0.0154256	0.569746

Sweet	0.0407514	0.49093
Umami	0.516042	0.646783
Watermelon Rind/Cucumber	0.860183	0.794029
Wet Hay	0.000163345	0.479047
White Grape	0.409741	0.483346
Worty	0.0588562	0.364109

*This table is displayed as Figure 10b. Cumulative prediction plot (Q^2Y) of sensory traits by malt metabolites. This table represents, based on the O2PLS malt model, the predictability of these 45 sensory traits based on malt metabolite content. The higher the Q^2Y, the more reliably it is predicted, based on metabolite composition (derived from barley genetics) and abundance in the beer (Section 3.5.1). $Q^2Y > 0.5$ is a good model of predictability. 16 of these 45 traits has a $Q^2Y > 0.5$, indicating high predictability in these sensory traits. The R^2Y score indicates the validity of the data provided for this model. $R^2Y > 0.4$ is considered reliable data for the prediction.

Table 9. Prediction-set for O2PLS beer model

Sensory Attribute	R^2Y score	Q^2Y Score
Bread Crust	0.538143	0.580881
Corn Chips	0.871138	0.816944
Grainy - Grape Nuts	0.801074	0.808842
Honeycomb Cereal	0.828093	0.879393
Nutty	0.529274	0.600022
Grass	0.927872	0.903398
Hay	0.80053	0.522656
Watermelon Rind/Cucumber	0.925345	0.868882
Fruity Complex	0.71014	0.750282
Green Apple/E Hex	0.683466	0.63413
Pear	0.935761	0.874907
White Grape	0.768547	0.73362
Sweet /Aromatic Complex	0.481227	0.371976
Caramel	0.734875	0.695474
Honey	0.445761	0.288874
Floral Complex	0.933526	0.820339
Honeysuckle	0.779484	0.518719
Perfume	0.683273	0.374428
Sulfitic (SO2)	0.264354	0.19087
Sulfidic (H2S)	0.465546	0.469936
Musty	0.572292	0.409531
Play-doh	0.775553	0.66941
Sweet	0.432409	0.199103
Bitter	0.795513	0.709862
MC Off-Tastes	0.851328	0.722459
Sour	0.558114	0.546154
Salty	0.566906	0.213424
Umami	0.925619	0.870144
Astringent	0.663878	0.546757
Body	0.570435	0.456327
Off MF/B	0.118121	0.081133
Metallic	0.102961	-0.10411
Acetaldehyde	0.222242	0.16748
Caprylic	0.699152	0.511201
Cardboard/Papery	0.873029	0.898003
Diacetyl	0.353147	0.205061

Ethyl Acetate	0.911091	0.918176
Ethyl Butyrate	0.063946	0.033927
Isoamyl Acetate	0.394895	0.250061
Isovaleric	0.566906	0.213424
Mercaptan	0.640876	0.361921
Phenolic	0.482181	0.253246
Raisin/Sherry	0.618171	0.492297
Wet Hay	0.316384	0.070148
Worty	0.607689	0.428036

*This table is displayed as Figure 8d. Cumulative prediction plot (Q^2Y) of sensory traits by beer metabolites. This table represents, based on the O2PLS beer model, the predictability of these 45 sensory traits based on malt metabolite content. The higher the Q^2Y, the more reliably it is predicted, based on metabolite composition (derived from barley genetics) and abundance in the beer (Section 3.5.1). $Q^2Y > 0.5$ is a good model of predictability. 25 of these 45 traits has a $Q^2Y > 0.5$, indicating high predictability in these sensory traits. The R^2Y score indicates the validity of the data provided for this model. $R^2Y > 0.4$ is considered reliable data for the prediction.

Table 10. O2PLS cross-validation (leave one out) for malt model

Component	R2X	R2X(cum)	R2	Limit	R2(cum)	Q2	Q2(cum)	R2Y	R2Y(cum)
Model		0.745			0.977		0.913		1
Predictive		0.642			0.977		0.913		1
P1	0.279	0.279	0.302	0.01	0.302	0.199	0.199	0.307	0.307
P2	0.0963	0.375	0.313	0.01	0.615	0.211	0.41	0.325	0.632
P3	0.12	0.495	0.142	0.01	0.757	0.139	0.549	0.145	0.777
P4	0.0858	0.581	0.112	0.01	0.869	0.19	0.739	0.114	0.891
P5	0.061	0.642	0.108	0.01	0.977	0.174	0.913	0.109	1
Orthogonal in X(OPLS)		0.103			0				
O1	0.0621	0.0621	0		0				
O2	0.0409	0.103	0		0				

*The cross-validation for the beer O2LS model separates the components into three groups: 1. Components that express information found in both X and Y, called predictive. 2. Components that express information found only in X, called orthogonal in X. 3. Components that express information found only in Y, called orthogonal in Y. The row labeled "Model" provides overall performance statistics of the O2PLS model. For this model, there were 5 predictive components. These are the X and Y data found in the model (*i.e.* information in X, the malt metabolites, which are predictive to Y, the flavor traits). The orthogonal components in this model are listed (Orthogonal in X(OPLS)) and contain the information from the data which is unique to X (*i.e.* information in the metabolite data (X) that is orthogonal to Y (sensory traits). O1 and O2 are the orthogonal Y components (2 in this model). These contain the information in the data that is unique to Y (sensory traits) and that is orthogonal to X (metabolites). R^2X is the amount of X (metabolite) variation modeled in the component. $R^2X(cum)$ is the cumulative R^2X up to the specified component. R^2 is the amount of Y variation modeled by X in each component, using the X model. $R^2(cum)$ is the cumulative R^2 up to the specified component. Q^2 is the cross-validated R^2 for the component. Limit is the critical value of Q^2 under which the component is insignificant. $Q^2(cum)$ is the cumulative Q^2 up to the specified component. Note that unlike $R^2X(cum)$, $Q^2(cum)$ is not additive. R^2Y is the amount of Y variation modeled by Y in the component, using the Y model. $R^2Y(cum)$ is the cumulative R^2Y up to the specified component [84, 87].

Table 11. O2PLS cross-validation (leave one out) for beer model

Component	R2X	R2X(cum)	R2	R2(cum)	Q2	Limit	Q2(cum)	R2Y	R2Y(cum)
Model		0.763		0.983			0.943		1
Predictive		0.643		0.983			0.943		1
P1	0.219	0.219	0.309	0.309	0.214	0.01	0.214	0.315	0.315
P2	0.143	0.362	0.309	0.618	0.293	0.01	0.507	0.313	0.627
P3	0.116	0.478	0.158	0.775	0.16	0.01	0.667	0.161	0.788
P4	0.0972	0.575	0.0933	0.869	0.0993	0.01	0.767	0.0948	0.883
P5	0.0682	0.643	0.114	0.983	0.176	0.01	0.943	0.117	1
Orthogonal in X(OPLS)		0.119		0					
O1	0.0659	0.0659	0	0					
O2	0.0535	0.119	0	0					

*The cross-validation for the beer O2LS model separates the components into three groups: 1. Components that express information found in both X and Y, called predictive. 2. Components that express information found only in X, called orthogonal in X. 3. Components that express information found only in Y, called orthogonal in Y. The row labeled "Model" provides overall performance statistics of the O2PLS model. For this model, there were 5 predictive components. These are the X and Y data found in the model (*i.e.* information in X, the beer metabolites, which are predictive to Y, the flavor traits). The orthogonal components in this model are listed (Orthogonal in X(OPLS)) and contain the information from the data which is unique to X (*i.e.* information in the metabolite data (X) that is orthogonal to Y (sensory traits). O1 and O2 are the orthogonal Y components (2 in this model). These contain the information in the data that is unique to Y (sensory traits) and that is orthogonal to X (metabolites). R^2X is the amount of X (metabolite) variation modeled in the component. $R^2X(cum)$ is the cumulative R^2X up to the specified component. R^2 is the amount of Y variation modeled by X in each component, using the X model. $R^2(cum)$ is the cumulative R^2 up to the specified component. Q^2 is the cross-validated R^2 for the component. Limit is the critical value of Q^2 under which the component is insignificant. $Q^2(cum)$ is the cumulative Q^2 up to the specified component. Note that unlike $R^2X(cum)$, $Q^2(cum)$ is not additive. R^2Y is the amount of Y variation modeled by Y in the component, using the Y model. $R^2Y(cum)$ is the cumulative R^2Y up to the specified component [84, 87].

References

1. Brewers Association. Craft Beer Sales by State [Web article]. Brewers Association; 2016. Available from: https://www.brewersassociation.org/statistics/by-state/.

2. Brewers Association. Craft Beer Sales Statistics [Web article]. Brewers Association; 2017. Available from: https://www.brewersassociation.org/statistics/by-state/.

3. Sizemore, C. Why Big Beer Is Struggling in the Age of Craft Beer: Forbes 2015. Available from: https://www.forbes.com/sites/moneybuilder/2015/06/09/why-big-beer-is-struggling-in-the-age-of-craft-beer/#16a6e52d47a4.

4. Gupta, M, Abu-Ghannam, N, Gallaghar, E. Barley for Brewing: Characteristic Changes During Malting, Brewing and Applications of Its by-Products. Comprehensive Reviews in Food Science and Food Safety. 2010;9(3):318-28. doi: 10.1111/j.1541-4337.2010.00112.x.

5. Briggs. Brewing Science and Practice. 2004.

6. Brewers Association. Brewers Association Export Development Program. 2016.

7. Donadini, G, Porretta, S. Uncovering Patterns of Consumers' Interest for Beer: A Case Study with Craft Beers. Food Research International. 2017;91:183-98. doi: http://dx.doi.org/10.1016/j.foodres.2016.11.043.

8. Bokulich, NA, Thorngate, JH, Richardson, PM, Mills, DA. Microbial Biogeography of Wine Grapes Is Conditioned by Cultivar, Vintage, and Climate. Proc Natl Acad Sci U S A. 2014;111(1):E139-48. doi: 10.1073/pnas.1317377110. PubMed PMID: 24277822; PubMed Central PMCID: PMCPMC3890796.

9. Costantini, EAC, Lorenzetti, R, Malorgio, G. A Multivariate Approach for the Study of Environmental Drivers of Wine Economic Structure. Land Use Policy. 2016;57:53-63. doi: http://dx.doi.org/10.1016/j.landusepol.2016.05.015.

10. Tang, K, Li, Q. Biochemistry of Wine and Beer Fermentation. 2017:281-304. doi: 10.1016/b978-0-444-63666-9.00011-x.

11. Bokulich, NA, Bamforth, CW. The Microbiology of Malting and Brewing. Microbiol Mol Biol Rev. 2013;77(2):157-72. doi: 10.1128/MMBR.00060-12. PubMed PMID: 23699253; PubMed Central PMCID: PMCPMC3668669.

12. Dong, L, Hou, Y, Li, F, Piao, Y, Zhang, X, Zhang, X*et al.* Characterization of Volatile Aroma Compounds in Different Brewing Barley Cultivars. J Sci Food Agric. 2015;95(5):915-21. doi: 10.1002/jsfa.6759. PubMed PMID: 24862930.

13. Heuberger, AL, Broeckling, CD, Sedin, D, Holbrook, C, Barr, L, Kirkpatrick, K*et al.* Evaluation of Non-Volatile Metabolites in Beer Stored at High Temperature and Utility as an Accelerated Method to Predict Flavour Stability. Food Chem. 2016;200:301-7. doi: 10.1016/j.foodchem.2016.01.022. PubMed PMID: 26830592.

14. Silva, GAd, Augusto, F, Poppi, RJ. Exploratory Analysis of the Volatile Profile of Beers by Hs–Spme–Gc. Food Chemistry. 2008;111(4):1057-63. doi: http://dx.doi.org/10.1016/j.foodchem.2008.05.022.

15. Hornsey, IS. Beer: History and Types. Encyclopedia of Food and Health. Oxford: Academic Press; 2016. p. 345-54.

16. Schönberger, C, Kostelecky, T. 125th Anniversary Review: The Role of Hops in Brewing. Journal of the Institute of Brewing. 2011;117(3):259-67. doi: 10.1002/j.2050-0416.2011.tb00471.x.

17. Johnson, EA, Haas, GJ. Antimicrobial Activity of Hops Extract against Clostridium Botulinum, Clostridium Difficile and Helicobacter Pylori. Google Patents; 2001.

18. Inui, T, Tsuchiya, F, Ishimaru, M, Oka, K, Komura, H. Different Beers with Different Hops. Relevant Compounds for Their Aroma Characteristics. Journal of Agricultural and Food Chemistry. 2013;61(20):4758-64. doi: 10.1021/jf3053737.

19. Ceslova, L, Holcapek, M, Fidler, M, Drstickova, J, Lisa, M. Characterization of Prenylflavonoids and Hop Bitter Acids in Various Classes of Czech Beers and Hop Extracts Using High-Performance Liquid Chromatography-Mass Spectrometry. J Chromatogr A. 2009;1216(43):7249-57. doi: 10.1016/j.chroma.2009.09.022. PubMed PMID: 19786280.

20. Quifer-Rada, P, Vallverdu-Queralt, A, Martinez-Huelamo, M, Chiva-Blanch, G, Jauregui, O, Estruch, R*et al.* A Comprehensive Characterisation of Beer Polyphenols by High Resolution Mass Spectrometry (Lc-Esi-Ltq-Orbitrap-Ms). Food Chem. 2015;169:336-43. doi: 10.1016/j.foodchem.2014.07.154. PubMed PMID: 25236235.

21. Hartmeier, W, Reiss, M. Production of Beer and Wine. In: Osiewacz HD, editor. Industrial Applications. Berlin, Heidelberg: Springer Berlin Heidelberg; 2002. p. 49-65.

22. Suomalainen, H, Lehtonen, M. The Production of Aroma Compounds by Yeast. Journal of the Institute of Brewing. 1979;85(3):149-56. doi: 10.1002/j.2050-0416.1979.tb06846.x.

23. Pires, EJ, Teixeira, JA, Branyik, T, Vicente, AA. Yeast: The Soul of Beer's Aroma--a Review of Flavour-Active Esters and Higher Alcohols Produced by the Brewing Yeast. Appl Microbiol Biotechnol. 2014;98(5):1937-49. doi: 10.1007/s00253-013-5470-0. PubMed PMID: 24384752.

24. Bamforth, CW. Current Perspectives on the Role of Enzymes in Brewing. Journal of Cereal Science. 2009;50(3):353-7. doi: http://dx.doi.org/10.1016/j.jcs.2009.03.001.

25. EtokAkpan, OU. Preliminary Study of Fat Oxidation in Sorghum and Maize Brewing. World Journal of Microbiology and Biotechnology. 2004;20(6):569-73. doi: 10.1023/B:WIBI.0000043169.65135.b4.

26. Iimure, T, Sato, K. Beer Proteomics Analysis for Beer Quality Control and Malting Barley Breeding. Food Research International. 2013;54(1):1013-20. doi: 10.1016/j.foodres.2012.11.028.

27. Schwarz, P, Li, Y. Malting and Brewing Uses of Barley. Barley: Wiley-Blackwell; 2010. p. 478-521.

28. Brewers Association. Malting Barley Characteristics for Craft Brewers.2016.

29. American Malting Barley Association. Malting Barley Quality Requirenments; 2017. Available from: www.ambainc.org

30. Barley Malting Institute, BaMBR. Quality Factors in Malting Barley; 2017. Available from: http://bmbri.ca/variety-development/quality-factors-in-malting-barley/.

31. Lekkas, S, Hill, Taidi, and Hodgson. The Importance of Free Amino Nitrogen in Wort and Beer. MBAA TQ. 2005;Vol. 42(2):113-6. doi: 10.1094/TQ-42-0113.

32. Bamforth, CW. Malting Technology and the Uses of Malt. Bhatty Ma, editor: American Association of Cereal Chemists; 1993.

33. Hughey, CA, McMinn, CM, Phung, J. Beeromics: From Quality Control to Identification of Differentially Expressed Compounds in Beer. Metabolomics. 2015;12(1):11. doi: 10.1007/s11306-015-0885-5.

34. Heuberger, AL, Broeckling, CD, Lewis, MR, Salazar, L, Bouckaert, P, Prenni, JE. Metabolomic Profiling of Beer Reveals Effect of Temperature on Non-Volatile Small Molecules During Short-Term Storage. Food Chem. 2012;135(3):1284-9. doi: 10.1016/j.foodchem.2012.05.048. PubMed PMID: 22953855.

35. Heuberger, AL, Broeckling, CD, Kirkpatrick, KR, Prenni, JE. Application of Nontargeted Metabolite Profiling to Discover Novel Markers of Quality Traits in an Advanced Population of Malting Barley. Plant Biotechnol J. 2014;12(2):147-60. doi: 10.1111/pbi.12122. PubMed PMID: 24119106.

36. Kageyama, N, Inui, T, Fukami, H, Komura, H. Elucidation of Chemical Structures of Components Responsible for Beer Aftertaste. J Am Soc Brew Chem. 2011;69:255.

37. Further Elucidation of Beer Flavor Instability: The Potential Role of Cysteine-Bound Aldehydes. Journal of the American Society of Brewing Chemists. 2015. doi: 10.1094/asbcj-2015-0531-01.

38. Cimini, A, De Francesco, G, Perretti, G. Effect of Crossflow Microfiltration on the Clarification and Stability of Beer from 100% Low-B-Glucan Barley or Malt. LWT - Food Science and Technology. 2017;86:55-61. doi: http://dx.doi.org/10.1016/j.lwt.2017.07.033.

39. Spevacek, AR, Benson, KH, Bamforth, CW, Slupsky, CM. Beer Metabolomics: Molecular Details of the Brewing Process and the Differential Effects of Late and Dry Hopping on Yeast Purine Metabolism. Journal of the Institute of Brewing. 2016;122(1):21-8. doi: 10.1002/jib.291.

40. Horak, T, Culik, J, Cejka, P, Jurkova, M, Kellner, V, Dvorak, J*et al.* Analysis of Free Fatty Acids in Beer: Comparison of Solid-Phase Extraction, Solid-Phase Microextraction, and Stir Bar Sorptive Extraction. J Agric Food Chem. 2009;57(23):11081-5. doi: 10.1021/jf9028305. PubMed PMID: 19904941.

41. Castellari. Determination of Carboxylic Acids, Carbohydrates, Glycerol, Ethanol, and 5-Hmf in Beer by High-Performance Liquid Chromatography and Uv–Refractive Index Double Detection. Journal of Chromatographic Science. 2001;39.

42. Nardini, M. Determination of Free and Bound Phenolic Acids in Beer. Food Chemistry. 2004;84(1):137-43. doi: 10.1016/s0308-8146(03)00257-7.

43. Kaukovirta-Norja, A, Laakso, S, Reinikainen, P, Olkku, J. The Effect of Kilning on the Capability of Malt to Oxidise Lipids. Journal of the Institute of Brewing. 1998;104(6):327-32. doi: 10.1002/j.2050-0416.1998.tb01004.x.

44. Cozzolino, D, Roumeliotis, S, Eglinton, J. Relationships between Fatty Acid Contents of Barley Grain, Malt, and Wort with Malt Quality Measurements. Cereal Chemistry. 2015;92(1):93-7. doi: 10.1094/cchem-04-14-0071-r. PubMed PMID: WOS:000348239600015.

45. De Keukeleire, D. Fundamentals of Beer and Hop Chemistry. Química Nova. 2000;23:108-12.

46. Cordero-Bueso, G, Arroyo, T, Serrano, A, Tello, J, Aporta, I, Velez, MD*et al.* Influence of the Farming System and Vine Variety on Yeast Communities Associated with Grape Berries. Int J Food Microbiol. 2011;145(1):132-9. doi: 10.1016/j.ijfoodmicro.2010.11.040. PubMed PMID: 21185102.

47. O'Sullivan, TF, Walsh, Y, O'Mahony, A, Fitzgerald, GF, van Sinderen, D. A Comparative Study of Malthouse and Brewhouse Microflora. Journal of the Institute of Brewing. 1999;105(1):55-61. doi: 10.1002/j.2050-0416.1999.tb00006.x.

48. Shewry, U. Barley: Chemistry and Technology, Second Edition: American Association of Cereal Chemists International; 2014.

49. Jeandet, P, Heinzmann, SS, Roullier-Gall, C, Cilindre, C, Aron, A, Deville, MA*et al.* Chemical Messages in 170-Year-Old Champagne Bottles from the Baltic Sea: Revealing Tastes from the Past. Proc Natl Acad Sci U S A. 2015;112(19):5893-8. doi: 10.1073/pnas.1500783112. PubMed PMID: 25897020; PubMed Central PMCID: PMCPMC4434772.

50. Vanderhaegen, B, Neven, H, Verachtert, H, Derdelinckx, G. The Chemistry of Beer Aging – a Critical Review. Food Chemistry. 2006;95(3):357-81. doi: 10.1016/j.foodchem.2005.01.006.

51. Mahmood, N, Petraco, N, He, Y. Elemental Fingerprint Profile of Beer Samples Constructed Using 14 Elements Determined by Inductively Coupled Plasma-Mass Spectrometry (Icp-Ms): Multivariation Analysis and Potential Application to Forensic Sample Comparison. Anal Bioanal Chem. 2012;402(2):861-9. doi: 10.1007/s00216-011-5452-y. PubMed PMID: 21983983.

52. Yin. Impact of Malt on Beer Flavor Stability References. 2013.

53. Pihlava, J-M, Kurtelius, T, Hurme, T. Total Hordatine Content in Different Types of Beers. Journal of the Institute of Brewing. 2016;122(2):212-7. doi: 10.1002/jib.311.

54. Kohyama, N, Ono, H. Hordatine a B-D-Glucopyranoside from Ungerminated Barley Grains. Journal of Agricultural and Food Chemistry. 2013;61(5):1112-6. doi: 10.1021/jf304453c.

55. Association, AMB. Barley for Beer. 2015.

56. Spevacek, AR. Beer Metabolomics Molecular Details of the Brewing Process and the Differential Effects of Late and Dry Hopping. 2016.

57. Bennett, SJE. Off-Flavours in Alcoholic Beverages. In: Saxby MJ, editor. Food Taints and Off-Flavours. Boston, MA: Springer US; 1996. p. 290-320.

58. Vanderhaegen, BD, Filip; Daenen, Luk. Aging Characteristics of Different Beer Types. Food Chemistry. 2007;103(2):404-12. doi: 10.1016/j.foodchem.2006.07.062.

59. Coghe. Sensory and Instrumental Flavour Analysis of Wort Brewed with Dark Specialty Malts. J Inst Brew. 2004;110(2):94-103.

60. Bravo, A, Herrera, JC, Scherer, E, Ju-Nam, Y, Rübsam, H, Madrid, J*et al.* Formation of A-Dicarbonyl Compounds in Beer During Storage of Pilsner. Journal of Agricultural and Food Chemistry. 2008;56(11):4134-44. doi: 10.1021/jf703696p.

61. Steiner, E, Auer, A, Becker, T, Gastl, M. Comparison of Beer Quality Attributes between Beers Brewed with 100% Barley Malt and 100% Barley Raw Material. Journal of the Science of Food and Agriculture. 2012;92(4):803-13. doi: 10.1002/jsfa.4651.

62. Briggs, D. Malts and Malting. 1st ed: Blackie Academic and Professional; 1998.

63. Bulgarelli, D, Garrido-Oter, R, Munch, PC, Weiman, A, Droge, J, Pan, Y*et al.* Structure and Function of the Bacterial Root Microbiota in Wild and Domesticated Barley. Cell Host Microbe. 2015;17(3):392-403. doi: 10.1016/j.chom.2015.01.011. PubMed PMID: 25732064; PubMed Central PMCID: PMCPMC4362959.

64. American Malting Barley Association. Economic Significance of Barley; 2016. Available from: www.ambainc.org.

65. Centre for Malting Barley Technology. Meredith Specifications. MBAA, editor. 2013.

66. Schooley, C. Personal Communication regarding malt. 2017.

67. Ullrich, Sa. Barley Chemistry and Technology. American Association of Cereal Chemists International 2014.

68. Sargent. Guide to Achieving Reliable Quantitative Lc-Ms Measurements. 2013.

69. Di Palma, S, Boersema, PJ, Heck, AJ, Mohammed, S. Zwitterionic Hydrophilic Interaction Liquid Chromatography (Zic-Hilic and Zic-Chilic) Provide High Resolution Separation and Increase Sensitivity in Proteome Analysis. Anal Chem. 2011;83(9):3440-7. doi: 10.1021/ac103312e. PubMed PMID: 21443167.

70. American Society of Brewing Chemists. Barley Milling. 1977.

71. Broeckling, CD, Heuberger, AL, Prenni, JE. Large Scale Non-Targeted Metabolomic Profiling of Serum by Ultra Performance Liquid Chromatography-Mass Spectrometry (Uplc-Ms). J Vis Exp. 2013;(73):e50242. doi: 10.3791/50242. PubMed PMID: 23524330; PubMed Central PMCID: PMCPMC3639512.

72. Turner, MF, Heuberger, AL, Kirkwood, JS, Collins, CC, Wolfrum, EJ, Broeckling, CD*et al.* Non-Targeted Metabolomics in Diverse Sorghum Breeding Lines Indicates Primary and Secondary Metabolite Profiles Are Associated with Plant Biomass Accumulation and Photosynthesis. Front Plant Sci. 2016;7:953. doi: 10.3389/fpls.2016.00953. PubMed PMID: 27462319; PubMed Central PMCID: PMCPMC4939745.

73. da Silva, GC, da Silva, AA, da Silva, LS, Godoy, RL, Nogueira, LC, Quiterio, SL *et al.* Method Development by Gc-Ecd and Hs-Spme-Gc-Ms for Beer Volatile Analysis. Food Chem. 2015;167:71-7. doi: 10.1016/j.foodchem.2014.06.033. PubMed PMID: 25148961.

74. Smith, CA, *et al.* Xcms: Processing Mass Spectrometry Data for Metabolite Profiling Using Nonlinear Peak Alignment,Matching,and Identification. Anal Chem. 2006;73(3):779-87.

75. Broeckling, CD, Afsar, FA, Neumann, S, Ben-Hur, A, Prenni, JE. Ramclust: A Novel Feature Clustering Method Enables Spectral-Matching-Based Annotation for Metabolomics Data. Anal Chem. 2014;86(14):6812-7. doi: 10.1021/ac501530d. PubMed PMID: 24927477.

76. Tautenhahn, R, Cho, K, Uritboonthai, W, Zhu, Z, Patti, GJ, Siuzdak, G. An Accelerated Workflow for Untargeted Metabolomics Using the Metlin Database. Nat Biotech. 2012;30(9):826-8. doi: 10.1038/nbt.2348

77. Zhu, Z-J, Schultz, AW, Wang, J, Johnson, CH, Yannone, SM, Patti, GJ*et al.* Liquid Chromatography Quadrupole Time-of-Flight Mass Spectrometry Characterization of Metabolites Guided by the Metlin Database. Nat Protocols. 2013;8(3):451-60. doi: http://www.nature.com/nprot/journal/v8/n3/abs/nprot.2013.004.html#supplementary-information.

78. Wishart, DS, Jewison, T, Guo, AC, Wilson, M, Knox, C, Liu, Y*et al.* Hmdb 3.0--the Human Metabolome Database in 2013. Nucleic Acids Res. 2013;41(Database issue):D801-7. Epub 2012/11/20. doi: 10.1093/nar/gks1065. PubMed PMID: 23161693; PubMed Central PMCID: PMCPMC3531200.

79. Hummel, J, Strehmel, N, Bölling, C, Schmidt, S, Walther, D, Kopka, J. Mass Spectral Search and Analysis Using the Golm Metabolome Database. The Handbook of Plant Metabolomics: Wiley-VCH Verlag GmbH & Co. KGaA; 2013. p. 321-43.

80. Francois, N. Beer Astringency Assessed by Time–Intensity and Quantitative Descriptive Analysis: Influence of Ph and Accelerated Aging. Food Quality and Preference. 2006;17(6):Pages 445-52. doi: https://doi.org/10.1016/j.foodqual.2005.05.008.

81. Stone, H, Sidel, JL. 1 - Introduction to Sensory Evaluation. Sensory Evaluation Practices (Third Edition). San Diego: Academic Press; 2004. p. 1-19.

82. Stone, H, Sidel, JL. 6 - Descriptive Analysis. Sensory Evaluation Practices (Third Edition). San Diego: Academic Press; 2004. p. 201-45.

83. Umetrics. Multivariate Data Analysis for Omics. 2008.

84. Umetrics. User Guide to Simca 13. 2012.

85. Benjamini, Y, Hochberg, Y. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. Journal of the Royal Statistical Society Series B (Methodological). 1995;57(1):289-300.

86. Umetrics. Multivariate Simca – P and Multivariate Analysis Frequently Asked Questions. 2008.

87. Umetrics. Simca O2PLS multivariate Notes. 2008.

88. Warnes, GR, Bolker, B, Bonebakker, L, Gentleman, R, Liaw, WHA, Lumley, T*et al.* Gplots: Various R Programming Tools for Plotting Data. R Package Version 2.17. 0. Computer software] Available online at: http://CRAN R-project org/package= gplots. 2015.

89. Wickham, H. Ggplot2: Elegant Graphics for Data Analysis (Use R!): Springer; 2010.

90. Wickham, H. R Reshape2 Package: Flexibly Reshape Data: A Reboot of the Reshape Package. 2014.

91. Team, RC. R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing; 2014.

92. Webber, HFP, Taylor, L, Marsh, AS. Observations on Traces of Copper in Brewing, Especially in Relation to Yeast. Journal of the Institute of Brewing. 1955;61(3):231-7. doi: 10.1002/j.2050-0416.1955.tb02792.x.

93. Passaghe, P, Bertoli, S, Tubaro, F, Buiatti, S. Monitoring of Some Selected Heavy Metals Throughout the Brewing Process of Craft Beers by Inductively Coupled Plasma Mass Spectrometry. European Food Research and Technology. 2015;241(2):199-215. doi: 10.1007/s00217-015-2445-7.

94. Pires, EJ. Biochemistry of Beer Fermentation.2015.

95. Umetrics. Application Note Integrating Information from Multiple Datasets Using O2PLS Integrating Information from Multiple Datas. 2015.

96. Bouhaddani, SE, Houwing-Duistermaat, J, Salo, P, Perola, M, Jongbloed, G, Uh, HW. Evaluation of O2pls in Omics Data Integration. BMC Bioinformatics. 2016;17 Suppl 2:11. doi: 10.1186/s12859-015-0854-z. PubMed PMID: 26822911; PubMed Central PMCID: PMCPMC4959391.

97. Umetrics. O2PLS Multivariate graphic.2016.

98. Bylesjo, M, Eriksson, D, Kusano, M, Moritz, T, Trygg, J. Data Integration in Plant Biology: The O2PLS Method for Combined Modeling of Transcript and Metabolite Data. Plant J. 2007;52(6):1181-91. doi: 10.1111/j.1365-313X.2007.03293.x. PubMed PMID: 17931352.

99. Worley, B, Powers, R. Multivariate Analysis in Metabolomics. Current Metabolomics. 2013;1(1):92-107.

100. Liu, C. Determination of Purines in Beer by Hplc Using a Simple and Rapid Sample Pretreatment. Journal of the American Society of Brewing Chemists. 2015. doi: 10.1094/asbcj-2015-0409-01.

101. Harding, RJ, Nursten, HE, Wren, JJ. Basic Compounds Contributing to Beer Flavour. Journal of the Science of Food and Agriculture. 1977;28(2):225-32. doi: 10.1002/jsfa.2740280218.

102. Bradley, PH, Brauer, MJ, Rabinowitz, JD, Troyanskaya, OG. Coordinated Concentration Changes of Transcripts and Metabolites in Saccharomyces Cerevisiae. PLOS Computational Biology. 2009;5(1):e1000270. doi: 10.1371/journal.pcbi.1000270.

103. Guido, LF. Sulfites in Beer: Reviewing Regulation, Analysis and Role. Scientia Agricola. 2016;73:189-97.

104. Johnson, RJ, Nakagawa, T, Sanchez-Lozada, LG, Lanaspa, MA, Tamura, Y, Tanabe, K*et al.* Umami: The Taste That Drives Purine Intake. J Rheumatol. 2013;40(11):1794-6. doi: 10.3899/jrheum.130531. PubMed PMID: 24187156.

105. Jones, M, Pierce, JS. Absorption of Amino Acids from Wort by Yeasts. Journal of the Institute of Brewing. 1964;70(4):307-15. doi: 10.1002/j.2050-0416.1964.tb01996.x.

106. Maillard, MN, Soum, MH, Boivin, P, Berset, C. Antioxidant Activity of Barley and Malt: Relationship with Phenolic Content. LWT - Food Science and Technology. 1996;29(3):238-44. doi: https://doi.org/10.1006/fstl.1996.0035.

107. Burhenne, K, Kristensen, BK, Rasmussen, SK. A New Class of N-Hydroxycinnamoyltransferases. Purification, Cloning, and Expression of a Barley Agmatine Coumaroyltransferase (Ec 2.3.1.64). J Biol Chem. 2003;278:13919.

108. Kristensen, BK, Burhenne, K, Rasmussen, SK. Peroxidases and the Metabolism of Hydroxycinnamic Acid Amides in Poaceae. Phytochemistry Reviews. 2004;3(1):127-40. doi: 10.1023/B:PHYT.0000047800.59980.6e.

109. Pihlava, J-M. Identification of Hordatines and Other Phenolamides in Barley (Hordeum Vulgare) and Beer by Uplc-Qtof-Ms. Journal of Cereal Science. 2014;60(3):645-52. doi: 10.1016/j.jcs.2014.07.002.

110. Friedrich, W, Galensa, R. Identification of a New Flavanol Glucoside from Barley (Hordeum Vulgare L.) and Malt. Eur Food Res Technol. 2002;214:388.

111. Gresser, A. Properties and Quality. Handbook of Brewing: Wiley-VCH Verlag GmbH & Co. KGaA; 2009. p. 359-97.

112. Taylor, B, Organ, G. Sensory Evaluation. Handbook of Brewing: Wiley-VCH Verlag GmbH & Co. KGaA; 2009. p. 675-701.

113. Gresser, A. Stability of Beer. Handbook of Brewing: Wiley-VCH Verlag GmbH & Co. KGaA; 2009. p. 399-435.

114. Kihara, M, Saito, W, Okada, Y, Kaneko, T, Asakura, T, Ito, K. Relationship between Proteinase Activity During Malting and Malt Quality. Journal of the Institute of Brewing. 2002;108(3):371-6. doi: 10.1002/j.2050-0416.2002.tb00563.x.

115. Yu, J, Huang, S, Dong, J, Fan, W, Huang, S, Liu, J*et al.* The Influence of Lox-Less Barley Malt on the Flavour Stability of Wort and Beer. Journal of the Institute of Brewing. 2014;120(2):93-8. doi: 10.1002/jib.122.

116. Bravi, E, Marconi, O, Sileoni, V, Perretti, G. Determination of Free Fatty Acids in Beer. Food Chem. 2017;215:341-6. Epub 2016/08/21. doi: 10.1016/j.foodchem.2016.07.153. PubMed PMID: 27542484.