AN ABSTRACT OF THE THESIS OF

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Title: Influence of Fermentation Temperature and Nutrient Addition Schedules on Sensory and Chemical Characteristics of Traditional Honey Wine

Abstract approved: ______________________________

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Honey wine, also known as mead, is one of the oldest alcoholic beverages known to man. Made primarily from honey, water, and yeast, this beverage has recently been experiencing a resurgence in popularity. It is imperative that commercial producers focus on consistently creating quality products to differentiate themselves from competitors in the growing market. This study focused on the effect of three fermentation temperatures (12.8 °C, 18.3 °C and 23.9 °C) and four different nutrient addition schedules (no added nutrients; at 24 h, 48 h and 72 h after pitch; $\frac{1}{4}$, $\frac{1}{2}$, and $\frac{3}{4}$ through fermentation; and a combination of both) on mead quality and fermentation parameters. Quantitative analysis of aroma compounds in the finished meads were determined using headspace solid-phase microextraction coupled with gas chromatography-mass spectrometry (HS-SPME-GCMS). Sensory differences in the meads were determined

using a trained panel evaluating the intensity of five aroma and five taste attributes.

Significant differences were found for treatments receiving nutrients versus those receiving no nutrients. Additional fermentation rates were higher with those treatments that received nutrients and those fermented at warmer temperatures. Significant differences between treatments were found for pH, residual sugar, and yeast assimilable nitrogen (YAN), but no significant differences were found between the treatments that all received nutrients.

According to sensory analysis, only the treatment which received no nutrients and was fermented at the lowest temperature was significantly different from the other treatments, although certain trends were found based on fermentation temperature and nutrient addition schedule. Treatments at 12.8 °C were characterized by "warm" and "fruity" attributes, while treatments at 18.3 °C were characterized by the "floral", "lemon", and "sour" attributes and treatments at 23.9 °C were characterized by the "sweet" and "fresh" attributes. Treatments without nutrients were characterized by the "warm", "fruity", and "fresh" attributes while treatments receiving nutrients were characterized by the "lemon", "floral", and "honey aroma" attributes.

Twenty-two aroma compounds were quantified. Ten compounds of these measured were significantly impacted by temperature, schedule, or an interaction between temperature and schedule. Six compounds were detected at levels exceeding their aroma thresholds: ethyl butyrate, ethyl hexanoate, ethyl octanoate, ethyl decanoate, linalool, and 3-methyl-1-pentanol. Differences for the meads were found for aroma analysis. Treatments exhibited significant separation based on fermentation temperature and nutrient schedule. In particular, the coolest fermentation tempera-

ture resulted in meads with greater amounts of esters. However, despite the fact that significant differences in aroma composition were found, these do not result in any large sensory differences, particularly for those ferments with nutrient additions.

These results show that mead makers can use nutrient schedules and fermentation temperature to not only significantly reduce time-to-market but also potentially achieve sensory goals. Additional work is required to determine whether nutrient blends tailored to particular implementations can be applied using commonly-accepted nutrient schedules.

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Influence of Fermentation Temperature and Nutrient Addition Schedules on Sensory and Chemical Characteristics of Traditional Honey Wine

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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

John Twilley, Author

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Chapter 1

1.1 History

Honey wine, also known as mead, is an ancient alcoholic beverage produced from diluted honey which has been fermented with yeast to a typical strength of 8 % to 18 % (v/v) alcohol. Mead has a long and extensive history. Pot shards containing trace evidence of fermented honey, rice, and grapes have been found at Neolithic sites in China and have been dated as old as 7000 BC (McGovern et al., 2004). Bronze mixing and serving vessels found in the likely tomb of King Midas circa 700 BC contained a mixture of grape wine, barley beer, and honey mead (McGovern et al., 1999). Contemporaneous with this find was the discovery of a bronze bowl from a grave in Bavaria which also contained mead as verified by the presence of pollen (Rösch, 2005). Plutarch, a famous ancient Greek essayist, identified mead as the libation to the gods before wine (Harrison, 1908), and mead was the original intoxicant of choice in Greek mythology for men and gods alike (López-Ruiz, 2012).

Mead, once acclaimed as Odin's gift to humanity, began to decline in popularity as a result of the development of Roman viticulture and the increasing use of land for agriculture in Europe, which made grapes and barley considerably less expensive than honey (Alves, 2006). By the late Renaissance, mead was the province of the wealthy and the sick (Unger, 2004). Sir Kenelm Digby, an English courtier and diplomat, was

a renowned mead maker. First published in 1669, Digby's collection of mead recipes represents perhaps the most complete records of their kind. A number of his recipes are documented in sufficient detail as to be reproduced by modern mead makers with only slight modifications to ingredients and processes (Digby, 1669/1910).

Mead is currently undergoing a cultural renaissance in the United States, alongside other alcoholic beverages. Members of the American Mead Makers Association reported mead sales increased by 84 % from 2012 to 2014, with 236 domestic wineries making at least one mead in 2015 – an increase of 42 wineries over the previous year (Herbert & Herbert, 2015). The largest commercial mead competition in the world, Mazer Cup International, was capped at 425 entries in 2018, with 400 entries in 2017 and 350 in 2016.

1.2 Styles

Traditional mead, also known as "show" mead, consists of only three ingredients: honey, water, and yeast. In addition to traditional mead, there exists an extensive array of mead styles focused primarily on adjuncts and other ingredients (Beer Judge Certification Program [BJCP], 2015). Broad categories include braggots, metheglins, and melomels, which are made with malted grain, spices, and fruits, respectively (BJCP, 2015). Some of these styles are rooted in history: a typical recipe for a metheglin includes herbs such as rosemary, thyme, and mint, as well as spices like ginger and cinnamon, and was often crafted as a health tonic (Digby, 1669/1910). Other styles arose through cultural precedent. Pyments, a type of melomel

made with grapes or grape juices, are the modern equivalent of the honeyed wine enjoyed by Greeks and Romans (Alves, 2006). Still other styles have their origins rooted in particular regions of the world. In Poland, traditional meads are differentiated by the ratio of honey to water used to make them: półtorak, dwójniak, and trójniak each use a mixture consisting of one part honey and one-half, one, and two parts water, respectively (Tasting Poland, n.d.). In Ethiopia, portions of the gesho (*Rhamnus prinoides*) plant are added to the must prior to fermentation when making tej, a local honey wine often used as a medicine (Debebe, Chandravanshi, & Redi-Abshiro, 2016). In Uganda, the mead enturire, is made by first fermenting malted sorghum flour before adding honey for secondary fermentation, resulting in a braggot (Mukisa et al., 2010). These examples are among many which span the globe (Steinkraus, 1995).

1.3 Honey

Honey is one of the oldest sweeteners known to man, with depictions of honey hunters harvesting honey from wild beehives in cave paintings dating to at least 2000 BC (Pager, 1976). Bees will incidentally collect pollen while gathering nectar from flowers for honey production. This pollen can be used to not only identify the variety of honey (Kasprzyk, Depciuch, Grabek-Lejko, & Parlinska-Wojtan, 2018), but also detect potential adulteration (Ohe, Oddo, Piana, Morlot, & Martin, 2004).

Honey is effectively a sugar suspension in water, with fructose and glucose representing approximately three-quarters of its solid composition by mass (Hermosín,

Chicón, & Cabezudo, 2003). In most cases, honey contains more fructose than glucose (da Silva, Gauche, Gonzaga, Costa, & Fett, 2016). Most of the remaining mass of honey—typically seventeen to twenty percent—consists of water (Kamal & Klein, 2011). The sugars contribute to honey's hygroscopic nature (Martin, 1958). They also determine how rapidly honey crystallizes. Honey with more glucose than fructose crystallizes more quickly (da Silva et al., 2016). This crystallization can make honey more difficult to process on an industrial scale due to its non-Newtonian behavior. One common way to reverse the crystallization process involves the application of heat. Unfortunately, treating honey with heat can have a negative impact on product quality (Moreira, Maria, Pietroluongo, & Trugo, 2010), due to the potential loss of volatile organic compounds which are important for aroma.

The remaining components of honey include other sugars such as disaccharides, organic acids, proteins and minerals as well as a wide variety of volatile organic compounds (da Silva et al., 2016). Many of these components are primarily responsible for honey's distinctive aromas, flavors and colors: for example, dark honeys usually have more minerals than light honeys (Abu-Jdayil, Ghzawi, Al-Malah, & Zaitoun, 2002).

1.4 Yeast

Saccharomyces cerevisiae is one of the most commonly used organisms for winemaking (Jackson, 2000), and thus in making mead. *S. cerevisiae* ferments more completely and produces ethanol more efficiently while generating less acetic acid when compared

with enologically-useful microbes (Magyar & Tóth, 2011). This organism preferentially consumes monosaccharides like fructose and glucose, which are the primary constituents of honey (Fraenkel, 1982).

A wide variety of strains of *S. cerevisiae* are commercially available today. These different strains can be used to produce various styles of meads and other fermented beverages. For successful fermentation, *S. cerevisiae* requires adequate sources of sugar and nutrients. Some strains require more nutritional support in the form of nitrogen (Scott Laboratories, 2017). Additionally, nutritional requirements vary over the life cycle of the yeast – for example, nitrogen requirements are highest during the exponential phase as yeast cells multiply (Jackson, 2000), while specific amino acids such as glycine have been observed to improve stationary phase fermentation in ways that ammonia does not (Bisson, 1999). Yeast nutrients have been developed to support these diverse requirements. They typically consist of diammonium phosphate (DAP), vitamins, yeast hulls and other compounds such as organic acids which are alleged to assist yeast as they grow and develop.

The traditional mead making process can be distilled into a small number of steps essential to a successful fermentation (Figure 1.1). Honey is first diluted with water to provide an environment in which the yeast can grow. The dilution must be sufficient to prevent the yeast from experiencing excessive osmotic pressures (Pratt, Bryce, & Stewart, 2003). The must is then inoculated with yeast before being stored in a temperature-controlled vessel. After fermentation is complete, the mead is racked off the yeast lees before being either aged or packaged for consumption. However, these steps alone are insufficient for many ferments. As an example, the non-sugar

components in honey provide inadequate support for yeast nutrition as compared to those found in grapes. Therefore, the yeast will require additional nutrients in order to effectively ferment.

1.5 Nutrients

Nitrogen is a key yeast nutrient of which honey does not have enough. Typical yeast assimilable nitrogen (YAN) levels in honey musts are on the order of 30 mg/L (Mendes-Ferreira et al., 2010). Grape wine must requires a minimum of 120 mg/L to 140 mg/L of YAN to avoid incomplete fermentations (Alexandre & Charpentier, 1998). This requirement suggests that additional nitrogen is needed for a healthy mead fermentation.

The timing of nutrient addition is also thought to be important to a successful fermentation. Nutrients can be added all at once at the beginning of the fermentation, or staggered over the course of fermentation (Seguinot et al., 2018). Schedules range from the simple to the complex. Some commercial mead makers will add nutrient once a day for several days after pitch, while others will add nutrient based on "sugar break" or fermentation progress (data not shown). One popular schedule for nutrient addition is called tailored organic staggered nutrient addition (TOSNA). This schedule involves first calculating a target YAN level based on the starting gravity of the must, the nitrogen requirements of the particular yeast strain, and the presence of fruit or fruit juices. This amount is then divided into four individual additions to be added separately: at 24 h, 48 h and 72 h, and finally 7 d after pitch or at $\frac{1}{3}$ sugar

break, whichever comes first (Moutela, n.d.). Variations on this theme are endless.

Nitrogen availability is known to have a significant positive influence on the production of some aroma compounds, including isobutanol, ethyl butanoate, ethyl hexanoate, and ethyl octanoate (Rollero et al., 2015). Esters in particular are known to benefit from higher YAN levels due to increased availability of precursors (Ugliano, Travis, Francis, & Henschke, 2010). Higher alcohols, isobutanoic acid, and isovaleric acid all exhibited very similar non-linear trends with regard to YAN supplementation, peaking at approximately 250 mg/L total nitrogen (Carrau et al., 2008). The addition of nitrogen can extend the growth phase experienced by the yeast, which can increase the amount of compounds such as propanol which are predominantly created during the growth phase (Mouret et al., 2014). This effect may be enhanced when ammonium ions are used as supplements, as this is the preferential source of nitrogen during the growth phase (Bell & Henschke, 2005).

Not all nitrogen sources are equal: different nitrogen sources have been observed to create different aroma profiles (Barbosa, Mendes-Faia, & Mendes-Ferreira, 2012). A combination of amino acids and inorganic nitrogen in the form of ammonium chloride (NH_4Cl) produced increased amounts of acetate esters and decreased amounts of fusel oils than NH_4Cl alone (Torrea et al., 2011). Combinations of individual amino acids can directly influence the production of aroma compounds beyond their availability as precursors (Fairbairn, McKinnion, Musarurwa, Ferreira, & Bauer, 2017). Removing methionine from a blend of amino acids resulted in a significant increase in higher alcohols and some ethyl esters, including ethyl octanoate and ethyl decanoate (Barbosa et al., 2012).

Nitrogen levels can also influence the production of hydrogen sulfide (H_2S) and other sulfur-containing compounds. While it is known that supplementation can decrease H_2S production in wines (Vos & Gray, 1979), the relationship between the two is more complex: moderate (260 mg/L) supplementation with DAP resulted in significantly higher H_2S production than high (410 mg/L) supplementation (Ugliano, Kolouchova, & Henschke, 2010). Similar results were seen in an earlier study with higher concentrations of sulfides and disulfides in the high-nitrogen wines (Ugliano et al., 2009), but those same wines were reported to have lower sulfide-related aromas than other wines in the same study.

1.6 Temperature

Besides nutrition, the temperature of fermentation is also important for mead styles. Honey itself is affected by temperature during storage and handling, experiencing changes in antioxidant and phenolic content when exposed to elevated temperatures before fermentation (da Silva et al., 2016). Fermentation temperature has been shown to affect the time required to complete fermentation and aroma composition. It is known that increasing fermentation temperature reduces the time required to complete fermentation, but it has also been shown to increase the production of H_2S in nutrient-poor ferments (Bohlscheid, Osborne, Ross, & Edwards, 2011). Finally, concerns about temperature control do not end once fermentation is complete. Storage of mead at elevated temperatures has been shown to significantly increase levels of 5-hydroxymethylfurfural, a spoilage indicator in honey (Kahoun, Rezkova, & Kralovsky,

2017).

Fermentation temperature can affect the production of aroma compounds. When comparing fermentations at 15 °C and 28 °C, Molina, Swiegers, Varela, Pretorius, and Agosin (2007) reported significantly higher levels of ethyl hexanoate, ethyl octanoate, and ethyl decanoate in the cooler fermentations. Some of these differences may be due to evaporative losses. Mouret et al. (2014) measured equal production of ethyl hexanoate and ethyl octanoate at 18 °C and 24 °C total using online gas chromatography, but final liquid concentrations were higher in the 18 °C fermentations. Another source of differences may be due to changes in yeast metabolism to acclimate to lower temperatures. Significant changes in lipid composition of *S. cerevisiae* have been observed when fermenting at 13 °C versus 25 °C (Torija et al., 2003). Effective temperature control is therefore essential to maximizing tank utilization while producing consistent, high-quality results from fermenting mead.

1.7 Purpose

The purpose of this research is to examine the impact of fermentation temperature and nutrient addition schedule on mead quality and fermentation performance as measured by sensory and chemical analyses. This research will immediately assist the mead industry by providing guidance towards producing large volumes of high-quality mead.

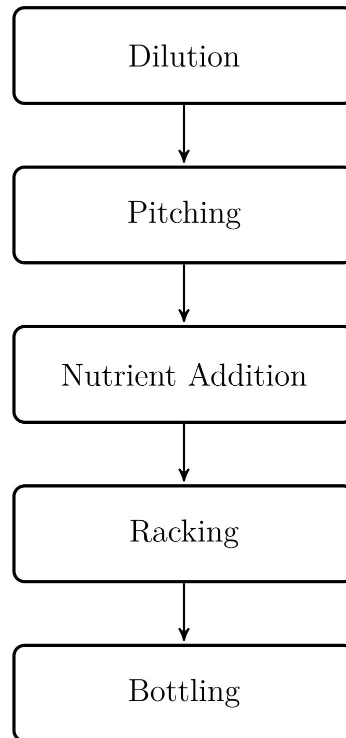

Figure 1.1: Flow diagram for mead making.

Chapter 2

2.1 Introduction

Honey wine, also called mead, is an alcoholic beverage made by fermenting diluted honey with yeast (typically *S. cerevisiae*). This type of fermented beverage has an extensive history with evidence of fermented honey found at Neolithic sites dating as far back as 7000 BC (McGovern et al., 2004). Improvements in agricultural practices made grapes and barley more available throughout temperate regions in Europe, contributing to the decline of mead's popularity (Alves, 2006). Mead, like cider, has been riding on the coattails of craft brewing's recent explosive growth in the United States, with at least 236 wineries making at least one mead in 2015 (Herbert & Herbert, 2015). However, the craft brewing industry has recently experienced some consolidation and contraction, partly due to quality issues. Mead makers must be vigilant in order to avoid these pitfalls.

"Traditional" mead is made from honey, water, and yeast. This style of mead is classified in the United States as an "agricultural wine" under the Internal Revenue Code which limits commercial producers to using honey as the only fermentable sugar source and hops as the only optional flavoring (27 C.F.R §24.200, 2018; 27 C.F.R §24.203, 2018). All other meads produced domestically are classified as "other than standard" wines because they include alternate flavorings, colorings, or sources

of fermentable (27 C.F.R §24.218, 2018). This range of additional ingredients is necessary to produce mead styles such as melomels and metheglins (BJCP, 2015). Like all wines, meads can be fermented to varying levels of dryness and ethanol content.

An important parameter for mead quality is fermentation temperature. Chemical reaction rates, including those of enzymes inside *S. cerevisiae*, are known to increase proportionally with temperature (Arrhenius, 1889). However, fermenting at higher temperatures can negatively impact production of desirable aroma compounds (Peng, Li, Cui, & Guo, 2015). Additionally, heat treatment and adverse storage conditions can have negative consequences for quality of both honey (da Silva, Gauche, Gonzaga, Costa, & Fett, 2016) and mead (Kahoun, Rezkova, & Kralovsky, 2017). Consequently, temperature is an important parameter to control to produce high quality mead.

Another important factor for mead quality is yeast health during fermentation. Honey does not provide a complete nutrient source for *S. cerevisiae* as its composition is roughly 60 % to 80 % sugars and 15 % to 21 % water with the remainder composing of proteins, amino acids, vitamins and minerals among other compounds (da Silva et al., 2016). Additional nutrients, particularly nitrogen, are often supplied to the must to minimize the risk of stuck and sluggish fermentations (Bisson, 1999). Added nutrients can also influence amino acid catabolism, which has an influence on the formation of esters and other aroma compounds (Bisson, 2004).

It is important to note that too much nutrient can be as problematic as too little. The addition of excessive nitrogen has been seen to trigger cell death in yeast (Tesnière, Delobel, Pradal, & Blondin, 2013), and any remaining nutrient not consumed

by the intended microbe is available to be used by spoilage organisms such as *Brettanomyces bruxellensis* (Aranda, Matallana, & del Olmo, 2011). A wide variety of yeast nutrient formulations are commercially available to ensure good yeast nutrition. Some examples include Go-Ferm and Fermaid-O (Scott Laboratories, Petaluma, CA), designed for use during yeast rehydration and during fermentation, respectively.

The timing of yeast addition has a significant impact on its influence on mead quality. The exponential phase of yeast growth requires more nitrogen than later phases (Jackson, 2000), while additions during the stationary phase has been observed to increase fermentation rates significantly as well as influence the production of some aroma compounds in synthetic grape must (Barbosa, Falco, Mendes-Faia, & Mendes-Ferreira, 2009; Seguinot et al., 2018). An informal survey of commercial mead makers (data unpublished) described a variety of nutrient addition schedules. One popular approach is to divide the total amount of nutrient to be added into three equal doses, with those doses added 24 h, 48 h and 72 h after pitch. Another example divides the nutrient into two equal doses, administered at one-quarter and one-half sugar depletion. More complex nutrient regimens exist as well: for example, tailored organic staggered nutrient addition (TOSNA) also accounts for the nitrogen needs of the particular yeast as well as the amount of honey in the must.

The purpose of this study was to evaluate the effects of different fermentation temperatures and nutrient addition schedules on fermentation parameters and mead quality. This information will be directly applicable to commercial producers who must balance high quality requirements with a need to maximize efficiency.

2.2 Materials and Methods

2.2.1 Materials

Honey was donated by Queen Bee Apiaries (Corvallis, OR) with an initial sugar content of 82 °Bx. Honey was diluted to 25.1 °Bx with tap water prior to fermentation. This concentration was appropriate to achieve a traditional semi-sweet mead at completion of fermentation (11 % (v/v) ethanol; 6.3 °Bx) (BJCP, 2015).

S. cerevisiae EC-1118 (Lallemand, Montreal, Canada) was used for fermentation. The yeast was rehydrated at a ratio of 1 g yeast per 10 mL tap water. Incubation was done in a water bath at 40 °C for 20 min. Each carboy received 10 mL of yeast solution.

Fermaid-O (Lallemand, Montreal, Canada) was used as the yeast nutrient, as it has a high content of organic nitrogen in the form of amino acids instead of diammonium phosphate (DAP). For addition purposes, a slurry was formed in advance at a ratio of 1 g of nutrient to 10 mL tap water. This slurry was manually agitated before each addition to ensure the nutrients were in suspension. The total mass of nutrient added to each non-control treatment was calculated from Moutela, n.d.:

$$m = \frac{f_y * f_B}{50} * V_m \tag{2.1}$$

where f_y is a factor based on the particular yeast being used, f_B is grams of sugar per liter of must, and V_m is the volume of the must in gallons. An approximation for f_B is the must's original °Bx times 10. The value of 0.75 for f_y was chosen in

accordance with the classification of EC-1118 by Scott Laboratories (2017) as having "low" relative nitrogen needs.

2.2.2 Treatments

Stainless steel glycol-jacketed 100 L fermentation tanks (AAA, The Dalles, OR) were used as water baths. These tanks were each set to the following temperatures: 12.8 °C, 18.3 °C and 23.9 °C. Each tank contained four 3.7 L carboys, one for each of the nutrient addition schedules. Each treatment was performed in triplicate. Treatments represent combinations of fermentation temperatures and nutrient addition schedules.

Four different nutrient addition schedules were tested. Schedule A was the control and did not receive any nutrients. Schedule B was time-based, with additions occurring at 24 h, 48 h and 72 h after pitch. Schedule C was based on fermentation progress or "sugar break", with additions occurring at $\frac{1}{4}$, $\frac{1}{2}$, and $\frac{3}{4}$ through fermentation, corresponding to 20.4 °Bx, 15.7 °Bx and 11.0 °Bx, respectively. Schedule D had additions occurring at 24 h, 48 h and 72 h and a final addition at either 168 h after pitching the yeast or $\frac{1}{3}$ through fermentation (18.8 °Bx), whichever came first (Moutela, n.d.).

2.2.3 Fermentation and storage

Fermentation progress was measured every 24 h with a DMA 35N density meter (Anton Paar, Graz, Austria). Ferments which reached addition targets, either time-based or progress-based, received doses of nutrient. These doses were equal fractions of the

total dose calculated in accordance with Equation 2.1 divided by the number of doses for each treatment. When carboys reached 6.3 °Bx, samples were taken for chemical analysis before the carboys were moved to cold storage at 3 ± 1 °C. Samples were stored in a freezer at -80 °C. Sulfur dioxide (5 % SO_2 solution, $K_2S_2O_5$, Brewcraft, Vancouver, WA) was added to carboys until 30 ppm free SO_2 was reached. When all fermentations were complete, treatments were combined for sensory analysis and additional SO_2 was added to maintain 30 ppm free SO_2. Treatments were bottled in 375 mL green glass bottles with aluminum cap closures (Amcor, Paris, France) and stored in a wine cellar at 15 °C.

2.2.4 Chemical analysis

pH was measured using an ion-selective electrode (Mettler Toledo, Greifensee, Switzerland), and residual sugar was determined using the Rebelein process (Iland, Bruer, Edwards, Caloghiris, & Wilkes, 2013). Ethanol content was calculated using the ASBC (1958/1975) Beer-4 method. Free amino nitrogen was measured with an *o*-phthaldialdehyde/N-acetyl-L-cysteine (OPA/NAC) assay (Dukes & Butzke, 1998), and ammonia was measured using an enymatic test kit (R-Biopharm AG, Darmstadt, Germany). Free SO_2 was measured by the aspiration method (Iland et al., 2013).

2.2.5 Sensory analysis

Sensory analysis was performed at the Oregon State University Arbuthnot Dairy Center (Corvallis, OR) on Monday, Wednesday, and Friday during the last two weeks of May in 2018. The panelists participated in six 1 h sessions (12-1pm, 2:30-3:30pm, and 5-6pm), one on each day. Panelists had to be non-smokers, free of any oral diseases and piercings, drank mead or white wine at least once a week, and be over 21 years old. 20 panelists (7 male and 13 female) participated in the sensory analysis. The facilities had a mixture of natural and artificial light. Any background odors were eliminated with air purifiers (WINIX5500, Winix Inc., East Dundee, IL). The room temperature was maintained at 24 ± 2 °C and portable sensory booths (Flipside Products, Inc., Cinncinati, IL) were used to separate the panelists.

For the first two sessions, panelists underwent training sessions on aroma, flavor, and taste descriptors determined from preliminary tastings (data not shown) (Table 2.1). All standards were placed in black INAO tasting glasses (ISO, 1977) 20 min prior to tasting so any aromas could equalize and were presented to panelists in random order, labeled with three-digit identifier codes. Panelists were asked to identify the descriptor for sessions one and two. At the end of the second session, panelists evaluated the intensities of the standards on a 100 mm visual analog scale with word anchors of none or extreme. For the three evaluation sessions, the panelists evaluated the meads. In each session, they evaluated all treatments in addition to a warm-up mead.

Mead bottles were opened and poured into black INAO tasting glasses (ISO,

1977) approximately 30 minutes prior to each session. Meads were presented in random order following a balanced incomplete block design to reduce any possible order effects and labeled with three-digit identifier codes. Each mead was analyzed in triplicate, one replicate per day. Panelists evaluated the intensity of the different descriptors using 100 mm visual analog scales with word anchors of none and extreme. All observations for training sessions and evaluation sessions were recorded using online surveys (Qualtrics, Provo, UT).

2.2.6 Gas chromatograph-mass spectrometry (GCMS)

2.2.6.1 Chemicals

Purity and manufacturer for standards used in gas chromatograph-mass spectrometry (GCMS) calibration curves and internal standards are found in Table 2.4. Other chemicals used include ethanol (HPLC grade, Pharmco-AAPER), sodium hydroxide (NaOH, 99 %, Macron) and Milli-Q water from Millipore Continental water system.

2.2.6.2 Sample preparation

Mead samples were defrosted at room temperature prior to analysis. Samples were diluted with a model solution (saturated NaOH, 10 % ethanol and 1 g/L citric acid) prior to analysis. Each mead (0.9 mL) was added to 7.8 mL of model solution. In 20 mL amber glass, screw cap vials (22.5x75.5 mm, Sigma-Aldrich), followed by 150 µL of both isotopically-labeled internal standard solutions (Table 2.4). Vials were capped

tightly with head space screwcaps (Restek, Bellefonte, PA). Samples were held in a stack cooler at 7 °C until analyzed.

2.2.6.3 Headspace solid-phase microextraction (HS-SPME)

A three-phase Stableflex fiber (50/30 µm DVB/CAR/PDMS, 2 cm, 24 Ga, Sigma-Aldrich) was used for HS-SPME. Prior to analysis, the fiber was conditioned at 250 °C for 1 h. HS-SPME occurred using a Shimadzu AOC-5000xt auto-sampler (Shimadzu, Kyoto, Japan) fitted with a stack cooler set. Samples were incubated for 10 min at 60 °C, during which time the incubator was agitated at 500 rpm (5 s on, 2 s off). The sample was extracted for 60 min with no further agitation. The fiber was then injected into the GCMS for 10 min at 250 °C followed by fiber conditioning for 10 min at 250 °C in an NDL heater.

GCMS analysis was performed using a Shimadzu QP-2010 Ultra mass spectrometer chromatograph with a split/splitless injector. The GC column was a Stabilwax, 30 m in length, 0.25 mm ID, and 0.25 µm of film thickness connected in sequence to a Rxi-1ms, 15 m in length, 0.25 mm ID, and 0.25 µm of film thickness (Restek Corporation, Bellefonte, PA). Method parameters for the GC oven are as follows: injector temperature was 250 °C and a split ratio of 0.5. The initial column oven temperature was held at 35 °C for 10 min which then increased at a rate of 4.0 °C/min to 250 °C and held for 10 min. Flow control was set using linear velocity at a starting pressure of 32.2 kPa and a linear velocity of 21.5 cm/s. Total run time was 73.75 min. GCMS transfer line temperature was 250 °C and ion source was 200 °C. Spectra were ac-

quired using electron impact ionization (EI, 70 eV) in a full scan mode from 3.8 min to 65 min with a scan range of 50 to 303 m/z and an event time of 0.20 s.

Identification of all compounds was based on comparison of retention time and spectra with pure standards and NIST 11 database (US Secretary of Commerce, 2011). Quantification for all compounds and validation of method was the same as described in Song, Xia, and Tomasino, 2015.

2.2.7 Statistical analysis

Wine chemistry parameters and aroma chemistry results were examined with analysis of variance and Tukey's HSD using R version 3.5.1 (R Core Team, 2018). Canonical discriminant analysis (CDA) was performed on the sensory and aroma chemistry data using the `candisc` package (Friendly & Fox, 2017).

2.3 Results

Treatments in nutrient schedule C demonstrated unexpected results in wine chemistry, sensory, and aroma chemistry which indicated that they were contaminated. This will be discussed further in Section 2.4. As a result, these treatments were not included in these results. The complete set of data is described in Appendix A.

2.3.1 Fermentation

The time to complete fermentation was significantly different ($\alpha = 0.05$) based on temperature and nutrient schedule (Table 2.2). A significant interaction was also observed between these two parameters (Table 2.3). Fermentations that were warmer completed fermentation significantly faster than fermentations at cooler temperatures. The significant difference for nutrient schedule was due to differences between treatments that received no nutrients and those that received nutrients. Fermentations that received nutrients completed fermentation significantly faster than treatments that received no nutrients. No significant difference was found between the treatments that received nutrients (Table 2.2). The significant interaction shows that treatments fermented at cooler temperatures with no nutrients took much longer to complete than the others.

Significant differences were found for several basic chemical parameters including pH, residual sugar and yeast assimilable nitrogen (YAN) (Table 2.2). No significant difference was found between the treatments that received nutrients. Fermentations that received nutrients had higher levels of YAN, lower levels of residual sugar, and higher pH values than treatments that received no nutrients. YAN levels decreased as temperature increased in fermentations that received nutrients. Residual sugar levels decreased as temperature increased for nutrient schedule D, in contrast to schedule B where residual sugar levels increased as temperature increased. The ethanol content for all treatments was within the accepted range (BJCP, 2015) for standard meads and were not significantly different. As anticipated, treatments that had nutrient

additions had significantly greater amounts of YAN after fermentation compared to those that did not have any added nutrient, but no significant interaction was observed between fermentation temperature and nutrient schedule.

2.3.2 Aroma chemistry

The aroma composition of the meads were found to differ based on temperature and nutrient schedule. Three of the compounds, isoamyl acetate, 2-nonanone, and nonanoic acid, were not detected in any treatment (Tables 2.5). Eleven compounds were detected in amounts which were not statistically significant across treatments. Eight compounds were impacted due only to nutrient schedule, five compounds were impacted due to temperature and one compound was impacted due to the interaction of both parameters (Table 2.6). Only six compounds were detected in levels higher than the aroma thresholds described in Table 2.7.

CDA resolved 100 % of the variance in both factors when using fermentation temperature as the grouping factor while considering all detected compounds (Figure 2.1). Significant separation for all three temperatures was observed. Treatments fermented at 23.9 °C were characterized by ethyl isobutyrate, phenethyl acetate, and phenethyl alcohol, while treatments fermented at 18.3 °C were characterized by ethyl dodecanoate. Treatments fermented at 12.8 °C were characterized by a range of compounds including isobutyl acetate, ethyl propanoate, hexanoic acid, ethyl octanoate, ethyl hexanoate, and 3-methyl-1-pentanol.

100 % of the differences between nutrient schedules for all detected compounds

were expressed in both factors with CDA (Figure 2.2). Nutrient schedules showed significant separation. Schedules B and D were separated from schedule A along the F1 axis. The F2 axis then separated schedules B and D from each other. Schedule B was characterized by ethyl isovalerate, linalool, and octanoic acid, at lower concentrations of all compounds in comparison to other schedules. Schedule D was characterized by a range of compounds, including ethyl hexanoate, ethyl octanoate, ethyl decanoate, and ethyl dodecanoate, while schedule A was characterized by hexanoic acid.

2.3.3 Sensory analysis

CDA explained 60.7 % of the difference in the first two factors when using treatment as the grouping factor (Figure 2.3). With the exception of Treatment 1, all treatments were not significantly different from each other. Treatment 1 was correlated with the attributes “warm” and “fresh”, while the remaining treatments were clustered along an axis consisting of the attributes “lemon” and “honey aroma”. The differences among the remaining treatments along this axis were not statistically significant.

Using fermentation temperature as the grouping factor allowed CDA to explain 100 % of the difference in two factors (Figure 2.4). While no significant differences were found, there are certainly trends based on temperature and some sensory characterizations can be found. Treatments at 12.8 °C were characterized by “warm” and “fruity” attributes, while treatments at 18.3 °C were characterized by the “floral”, “lemon”, and “sour” attributes and treatments at 23.9 °C were characterized by the

"sweet" and "fresh" attributes.

CDA was used to determine any differences based solely by nutrient schedule. 100 % of the differences were found in two factors when using nutrient schedule as the grouping factor (Figure 2.5). No significant differences were observed between schedules, however unlike separation based on temperature much greater overlaps were found for nutrient schedule, specifically with schedules B and D. Schedule A was characterized by the "warm", "fruity", and "fresh" attributes while schedules B and D were characterized by the "lemon", "floral", and "honey aroma" attributes.

2.4 Discussion

Treatments in nutrient schedule C exhibited multiple characteristics indicative of microbial spoilage. The levels of ethyl isovalerate were highest in schedule C with the single highest level in the coldest treatment in schedule C. Ethyl isovalerate is a product of esterification of isovaleric acid and ethanol under acidic conditions. Isovaleric acid has a rancid cheesy aroma and can be produced by *B. bruxellensis* (Kosel et al., 2017), a common wine contaminant. The levels of cheesy flavor were highest in schedule C, again with the single highest level in the coldest treatment in schedule C. Spoilage organisms like *B. bruxellensis* will metabolize any remaining sugars resulting in a rise in pH (Fleet, 2011). Treatments from schedule C had the lowest levels of residual sugars and highest pH levels of all treatments. Therefore, schedule C has been removed from the analysis and from further discussion. Unfortunately, no screening for microorganisms was performed due to time constraints.

2.4.1 Fermentation

Time to complete fermentation was significantly affected by both temperature and nutrient addition, which was anticipated based on previous work with mead (Steinkraus & Morse, 1966). Similar behavior has been observed in beer (D'Amore, 1992) and cider (Kelkar & Dolan, 2012). The reduction in time to complete fermentation was identical for nutrient schedules B and D, which received the same amount of nutrients but in three and four doses, respectively. This result is in agreement with Bely, Sablayrolles, and Barre (1990) where nutrient additions in synthetic grape must were reported to be equally effective regardless of the number of additions as long as the additions were before the halfway point of fermentation. This is in contrast to Gobbi, Comitini, D'Ignazi, and Ciani (2013) where a reduction of fermentation time for Verdiccio grape must was reported when the same amount of YAN was added in three doses as compared to four doses. However, this study's first dose was added no earlier than 24 h after pitch whereas the initial dose used by Gobbi et al. (2013) was added at the start of fermentation. It is interesting to note that the reduction of fermentation times observed between the treatments at 12.8 °C and 18.3 °C for nutrient schedule A which received no nutrients was coincidentally comparable to that observed between schedule A and schedules B and D at 12.8 °C. It is known that nutrient additions result in faster fermentations and that fermentations lacking in necessary nutrients can result in stuck fermentations (Beltran, Esteve-Zarzoso, Rozès, Mas, & Guillamón, 2005). In addition to shorter fermentation times, Specht (2000) reported that fermentations with higher levels of added YAN also finished with

higher amounts of YAN after fermentation was complete, which is in agreement with this study's results. No trends in ethanol or residual sugar were found or expected for either fermentation temperature or nutrient schedule due to the design of the experiment which focused on a uniform production of ethanol across all treatments.

2.4.2 Aroma chemistry

Both fermentation temperature and nutrient additions are known to influence aroma composition of fermented beverages (Deed, Fedrizzi, & Gardner, 2017; Ugliano, Travis, Francis, & Henschke, 2010). The majority of compounds measured in this study are those related to fermentation, specifically ethyl and acetate esters, but several higher alcohols and volatile fatty acids were also detected. Esters are considered important as they are known to influence fruity aromas (Saerens et al., 2008). While the amount of ethyl and acetate esters produced varies based on yeast strain, the production pathways and precursors are completely different. Acetate esters are formed quickly and are derived from the amino acid content of the starting material (Saerens et al., 2008). Ethyl esters are formed from medium-chain fatty acids within the yeast cell and are then transferred to the surrounding medium, with longer chain esters having more difficulty with this transfer (Saerens et al., 2008). Therefore ester synthesis is due to concentrations of substrates, activity of enzymes and hydrolysis of esters (Saerens et al., 2008).

Of the four acetate esters measured in this study, only ethyl acetate was significantly different across treatments and it was observed at levels roughly an order of

magnitude below the reported threshold (Guth, 1997). This suggests that it is not responsible for any spoilage-like aromas in the mead (Kosel et al., 2017). The lower fermentation temperature was related to greater amounts of esters in mead, including ethyl octanoate and ethyl decanoate. This is most likely due to the fact that lower fermentation temperatures are known to maintain esters, while the warmer ferments are known to drive off and volatilize esters (Killian & Ough, 1979). Ethyl esters were also found in greater amounts in those meads with nutrient additions versus those without, which was anticipated as the added nutrient provides more substrate for ester synthesis (Saerens et al., 2008).

Fermentation temperature was also found to greatly impact the amount of phenethyl alcohol and phenethyl acetate in the finished meads. Phenethyl alcohol is known to impart “floral” aroma and phenethyl acetate, particularly at high concentrations, has a “honey-like” aroma (Swiegers, Bartowsky, Henschke, & Pretorius, 2005). The presence of these compounds is thought to be due to the amount of phenylalanine in the starting material and synthesis depends on the yeast used (Etschmann, Bluemke, Sell, & Schrader, 2002). Higher temperatures are known to favor higher alcohols, such as phenethyl alcohol (Molina, Swiegers, Varela, Pretorius, & Agosin, 2007), and phenethyl acetate is formed from yeast enzymatic reactions (Albertazzi, Cardillo, Servi, & Zucchi, 1994). While the higher fermentation temperature treatment was characterized by these 2 compounds (Figure 2.1), there was actually no significant difference between the temperature treatments (Table 2.6) which suggests that it is the lack of esters in the higher fermentation temperature that brings out this characterization.

Nutrient additions also showed differences in many fermentation compounds. Nutrient schedules B and D are significantly different from schedule A along the F1 axis which is separated clearly by longer chain esters, acetate esters, and alcohols corresponding to schedules B and D, with the shorter chain compounds associated with no nutrient addition. As stated previously all of these compounds are related to substrate composition and yeast fermentation. However during fermentation many of the synthesis pathways may be inhibited due to production of specific compounds or a lack of resources, known as feedback inhibition (Suástegui & Shao, 2016). Therefore the aroma compositional differences seen that differentiate schedules B and D may be due to the complex production pathways that may be altered based on nutrient availability at different times of fermentation.

It would be of interest to investigate the specific nutrient needs for mead making. Most nutrient products currently available are tailored for the brewing and winemaking industries. Honey has a very different original composition in comparison, therefore a different nutrient addition may be more effective. Additionally different mead styles may have various nutrient needs, due to the addition of other ingredients beyond honey (BJCP, 2015) and various processing steps. Beyond fermentation impacts the type of nutrient added could greatly impact the final aroma and flavor of the product.

2.4.3 Sensory analysis

Treatment 1 was the only treatment which was significantly different than the rest of the treatments, with characteristics of “warm” and “fresh”. This treatment was fermented at the coldest temperature with no nutrient addition. It was anticipated that the cooler fermentation temperature would produce “fresher” aromas, as cooler temperatures are known to retain more aroma compounds such as isoamyl acetate which result in fruity and fresh aromatics (Killian & Ough, 1979).

There is little published work on the effects of fermentation temperature on mead quality and sensory data, but there is an extensive body of literature on other types of fermented beverages (Pajović, Popović, & Krstić, 2011; Peng et al., 2015). All show similar trends with this study’s results, that cooler temperatures result in “fresh” and “fruity” aromatics (Figure 2.1). The lack of sensory differentiation in the two warmer temperatures suggests that the temperature difference was not large enough to impact sensory perception. Reynolds, Cliff, Girard, and Koop (2001) showed no significant differences in Semillon wines fermented at 15 °C and 20 °C. Much work shows inconsistent chemical and sensory results based on fermentation temperature. This is most likely due to the choice of yeast and fermentable sugar source (Deed et al., 2017; Garruti, de Abreu, Franco, & da Silva, 2006). To determine the fermentation temperatures that have a significant impact on mead quality it is suggested to select more extreme temperatures in the future in order to discover the quality differences (Cottrell & McLellan, 1986). It should also be noted that the honey used in this fermentation did not have a high temperature process point, as honey is often

subjected to heat treatments during extraction (da Silva et al., 2016). Investigating the interaction of honey extraction temperature and mead fermentation temperature may show some interesting impacts to quality and provide useful information to mead makers.

There is a large amount of work previously conducted with regard to the effects of nutrient addition on sensory of fermented beverages. Several studies reported no significant differences in finished products as a result of adding nutrients, which is in agreement with this study's results (Jin, Chen, Li, Li, & Li, 2015; Joshi, Bhutani, & Sharma, 1990). Multiple studies have found an increase in fruitiness after adding nutrients (Hernández-Orte, Ibarz, Cacho, & Ferreira, 2005; Ugliano et al., 2010), which is most likely influenced by the composition of the starting material. These studies also showed an increase in other characteristics (floral and citrus aromas) presumably due to nutrient addition. Treatments which received nutrients were characterized by "lemon" and "floral" aromas, which suggests that the nutrients included precursors to aroma compounds linked to these aromas or the nutrients help facilitate the formation of aroma compounds by avoiding the diversion of microbial resources towards amino acid synthesis (Jackson, 2000).

It is interesting to note that there were few differences in final mead sensory analysis despite the fact that the aroma composition of the meads did show significant differences. Aroma perception is complex. While the odor thresholds of all the tested compounds are known, once these compounds are in a complex mixture, such as mead, their odor activity is greatly altered (Ferreira, 2010). It can be seen that the many aroma compound differences do not show any causal relationship with

sensory perception of meads, or alternatively the differences shown are too small to result in differences of sensory perception (Sáenz-Navajas, Campo, Fernández-Zurbano, Valentin, & Ferreira, 2010).

2.5 Conclusion

The differences in fermentation parameters, aroma composition, and sensory perception of meads fermented at different temperatures and nutrient addition schedules show how the choice of production processes can impact final mead quality. The lack of sensory differentiation compared to aroma composition based on nutrient schedule suggest that it is not when the nutrient addition occurs but potentially how much and what type of nutrient is used. Additionally, fermenting at lower temperatures result in a greater retention of esters and other fermentation-derived aroma compounds, as seen in other fermented beverages. Mead makers desiring quicker ferments can choose to either increase fermentation temperature or use nutrients based on their individual requirements without sacrificing quality. More research is needed to develop nutrient blends optimized for mead quality and to identify temperature ranges suitable for reliably consistent fermentation.

Table 2.1: Aroma (A) and flavor (F) standards for the chosen descriptors.

Attribute	Standard	Preparation Notes	Supplier
Cheesy (F)	Parmesan cheese	Grated	Market of Choice (Corvallis, OR)
Floral (A)	Essential oils (gardenia, jasmine) diluted in mineral oil	83 mL/L of each essential oil	Barnhouse Blue (San Clemente, CA)
Fresh (A)	Yogurt, lettuce, and cucumber	Blended, frozen, and defrosted 30 min before serving	Market of Choice (Corvallis, OR)
Fruity (A)	Fruit cocktail in pear juice	Chunks and liquid	Market of Choice (Corvallis, OR)
Honey (A, F)	Honey	None	Queen Bee Apiaries (Corvallis, OR)
Lemon (A)	Lemon, rind and meat	Chopped into small pieces	Market of Choice (Corvallis, OR)
Sour (F)	Tartaric acid	3 g/L	Davison Winery Supply (McMinnville, OR)
Sweet (F)	Sucrose	100 g/L	Domino (Yonkers, NY)
Warm (F)	190 proof grain ethanol	20 %	Luxco (St. Louis, MO)

Table 2.2: Fermentation temperature, nutrient schedule, and basic wine chemistry parameters for finished meads.

	Fermentation temperature (°C)	Nutrient schedule[1]	Days to complete fermentation (d)[2]	Ethanol % (v/v)	YAN (mg/L)[2]	Residual sugar (g/L)[2]	pH[2]
T1	12.8	A	105 ± 7^{d}	11.40 ± 0.02	6.2 ± 2.0^{b}	72.5 ± 4.6^{bc}	3.48 ± 0.01^{cd}
T2	12.8	B	44 ± 7^{a}	10.78 ± 0.18	13.6 ± 2.1^{a}	61.2 ± 7.8^{a}	3.58 ± 0.03^{a}
T4	12.8	D	44 ± 7^{a}	11.14 ± 0.36	13.4 ± 1.1^{a}	64.4 ± 8.7^{ab}	3.59 ± 0.03^{a}
T5	18.3	A	48 ± 4^{a}	11.56 ± 0.02	5.5 ± 1.3^{b}	72.4 ± 3.5^{bc}	3.41 ± 0.03^{b}
T6	18.3	B	20 ± 2^{bc}	11.54 ± 0.00	12.3 ± 1.9^{a}	64.1 ± 3.0^{ab}	3.52 ± 0.02^{c}
T8	18.3	D	20 ± 2^{bc}	11.16 ± 0.04	12.5 ± 1.9^{a}	63.7 ± 6.3^{ab}	3.49 ± 0.01^{cd}
T9	23.9	A	31 ± 2^{b}	10.18 ± 1.26	6.2 ± 2.5^{b}	76.8 ± 4.9^{c}	3.32 ± 0.01^{e}
T10	23.9	B	14 ± 2^{c}	11.68 ± 0.04	10.8 ± 1.4^{a}	69.3 ± 1.7^{abc}	3.45 ± 0.01^{d}
T12	23.9	D	14 ± 1^{c}	11.54 ± 0.02	10.7 ± 2.0^{a}	61.6 ± 3.7^{a}	3.46 ± 0.02^{d}

[1] A: control, B: 24 h, 48 h and 72 h, D: 24 h, 48 h and 72 h and either 168 h or $\frac{1}{3}$ through fermentation, whichever came first (Moutela, n.d.).
[2] For each attribute, values described by the same letters do not differ significantly (Tukey's HSD; $\alpha = 0.05$).

Table 2.3: p-Values ($\alpha = 0.05$) for effects of temperature, schedule, and interaction between temperature and schedule on wine chemistry results.

	Temperature	Schedule	Interaction
Days to complete fermentation	0.00	0.00	0.00
YAN	0.02	0.00	0.29
Residual sugar	0.18	0.00	0.15
pH	0.00	0.00	0.02

Table 2.4: Quantification parameters.

Compound	ISTD	Ret. Time (min)	Target Ion m/z	Confirming Ions m/z	Calibration Range (µg/L)	Purity	CAS No.	Source
d3-ethyl acetate	(1)	5.04	64	76, 133		99	90691-33-1	CDN
d3-ethyl butyrate	(2)	12.49	74	89, 61		99.8	113435-99-7	CDN
2-methyl-d3-propyl-d4 alcohol	(3)	13.78	50	81, 61		99.8	1219804-53-1	CDN
d9-ethyl-2-methylbutyrate	(4)	14.09	66	107, 94		99	7452-79-1	CDN
d11-ethyl hexanoate	(5)	21.89	91	110, 63		98	2159-19-5	CDN
d5-2-nonanone	(6)	27.83	63	64, 75		99	1398065-76-3	CDN
d15-ethyl octanoate	(7)	30.15	91	105, 66		98	1219798-38-5	CDN
d4-diethyl succinate	(9)	35.98	105	77, 122		99	52089-62-0	CDN
d11-hexanoic acid	(10)	39.72	63	77, 93		98.5	95348-44-0	CDN
d17-nonanoic acid	(11)	47.82	77	63, 125		99	130348-94-6	CDN
ethyl acetate	1	5.08	61	70, 88	0 – 2233.32	99.5	141-78-6	BDH
ethyl propanoate	2	7.93	57	74, 102	0 – 260	99	105-37-3	Aldrich
ethyl isobutyrate	4	9.52	71	88, 116	0 – 262.22	99	97-62-1	Aldrich
isobutyl acetate	1	11.19	56	73, 86	0 – 47.02	99	110-19-0	Aldrich
ethyl butyrate	2	12.61	71	88, 60	0 – 226.22	99+	105-54-4	Fluka
isobutanol	3	14.16	56	57, 77	0 – 1162.22	99+	78-83-1	Sigma-Aldrich
ethyl isovalerate	4	14.92	88	57, 60	0 – 229.33	98+	108-64-5	Sigma-Aldrich
isoamyl acetate	1	16.98	70	55, 61	0 – 222.89	99+	123-92-2	Sigma-Aldrich
ethyl pentanoate	5	17.73	85	57, 101	0 – 46.84	98+	539-82-2	Sigma-Aldrich
3-methyl-1-butanol	3	20.07	73	70, 42	0 – 4657.78	99+	123-51-3	Sigma-Aldrich
ethyl hexanoate	5	22.25	88	99, 70	0 – 222.67	99+	123-66-0	Aldrich
3-methyl-1-pentanol	3	24.51	56	69, 84	0 – 224.67	99+	589-35-5	Sigma-Aldrich
2-nonanone	6	27.97	58	57, 71	0 – 244.22	99+	821-55-6	Sigma-Aldrich
ethyl octanoate	7	30.63	88	101, 127	0 – 1151.11	98+	106-32-1	Sigma-Aldrich
linalool	8	32.47	93	71, 121	0 – 223.78	97+	78-70-6	Aldrich
diethyl succinate	9	36.16	101	129, 128	0 – 566.11	99+	123-25-1	Sigma-Aldrich
ethyl decanoate	7	37.30	88	101, 155	0 – 230.22	99+	110-38-3	Aldrich
phenethyl acetate	3	40.04	104	91, 78	0 – 566.11	98+	110-45-7	Sigma-Aldrich
hexanoic acid	10	40.10	60	73, 87	0 – 581.11	99	142-62-1	Aldrich
phenethyl alcohol	3	42.02	91	92, 122	0 – 4533.33	99+	60-12-8	Aldrich
ethyl dodecanoate	7	43.56	101	88, 70	0 – 264	98+	106-33-2	Aldrich
octanoic acid	10	45.59	60	73, 101	0 – 1146.67	98+	124-07-2	Sigma-Aldrich
nonanoic acid	11	48.19	73	60, 115	0 – 232.89	98	112-05-0	Sigma-Aldrich
decanoic acid	11	50.66	60	73, 129	0 – 226	98	334-48-5	Sigma

Table 2.5: Mean concentration ± standard deviation (µg/L) of aroma compounds from different treatments using ANOVA with Tukeyś HSD ($\alpha = 0.05$)

Compound	1	2	4	5	6
Ethyl acetate	817.4 ± 103.7^{abc}	398.8 ± 213.4^{ab}	938.7 ± 99.4^{c}	484.1 ± 328.3^{abc}	303.4 ± 69.4^{a}
Ethyl propanoate	24.6 ± 2.3^{a}	23.0 ± 2.4^{a}	23.6 ± 6.4^{a}	20.9 ± 3.5^{a}	20.5 ± 2.6^{a}
Ethyl isobutyrate	n.d.	3.3 ± 5.8^{a}	n.d.	3.3 ± 5.8^{a}	6.6 ± 5.7^{a}
Isobutyl acetate	2.0 ± 1.7^{a}	1.9 ± 1.7^{a}	n.d.	0.9 ± 1.5^{a}	1.0 ± 1.8^{a}
Ethyl butyrate	579.5 ± 25.7^{ab}	515.4 ± 91.2^{abc}	626.6 ± 37.5^{ab}	475.5 ± 35.1^{bc}	398.9 ± 31.2^{c}
Isobutanol	2318.1 ± 102.9^{ab}	2061.7 ± 364.9^{abc}	2506.5 ± 150.2^{ab}	1902.1 ± 140.6^{bc}	1595.5 ± 124.7^{c}
Ethyl isovalerate	5.5 ± 4.8^{a}	5.6 ± 4.8^{a}	5.8 ± 5.0^{a}	n.d.	2.7 ± 4.7^{a}
Isoamyl acetate	n.d.	n.d.	n.d.	n.d.	n.d.
Ethyl pentanoate	0.8 ± 0.2^{a}	0.8 ± 0.0^{a}	0.7 ± 0.1^{a}	0.6 ± 0.2^{ab}	0.3 ± 0.0^{b}
Ethyl hexanoate	26.0 ± 0.9^{ef}	30.8 ± 0.9^{a}	36.9 ± 1.2^{b}	20.6 ± 0.7^{cd}	19.4 ± 1.2^{c}
3-methyl-1-pentanol	n.d.	2.5 ± 1.0^{a}	4.7 ± 5.1^{a}	n.d.	0.9 ± 1.6^{a}
2-nonanone	n.d.	n.d.	n.d.	n.d.	n.d.
Ethyl octanoate	32.4 ± 2.1^{c}	65.9 ± 3.2^{a}	89.0 ± 6.2^{b}	37.0 ± 1.5^{c}	45.3 ± 3.6^{cd}
Linalool	0.9 ± 0.8^{a}	0.5 ± 0.2^{a}	0.7 ± 0.4^{a}	0.1 ± 0.1^{a}	0.0 ± 0.1^{a}
Ethyl decanoate	3.4 ± 1.1^{a}	3.9 ± 3.4^{a}	6.7 ± 4.2^{a}	1.5 ± 1.3^{a}	2.1 ± 1.8^{a}
Phenethyl acetate	20.8 ± 3.2^{a}	18.0 ± 5.4^{a}	19.3 ± 1.8^{a}	15.7 ± 1.8^{a}	16.2 ± 3.2^{a}
Hexanoic acid	373.1 ± 494.6^{a}	51.3 ± 2.2^{a}	73.7 ± 49.6^{a}	53.8 ± 54.8^{a}	74.4 ± 40.2^{a}
Phenethyl alcohol	1141.9 ± 294.5^{a}	1033.7 ± 181.5^{a}	1411.6 ± 609.8^{a}	941.2 ± 170.6^{a}	813.5 ± 168.3^{a}
Ethyl dodecanoate	8.0 ± 2.9^{a}	13.6 ± 2.1^{ab}	17.1 ± 9.3^{ab}	4.1 ± 0.5^{a}	13.7 ± 2.2^{ab}
Octanoic acid	414.2 ± 534.6^{a}	51.7 ± 12.0^{a}	439.0 ± 700.4^{a}	65.5 ± 74.5^{a}	714.3 ± 715.7^{a}
Nonanoic acid	n.d.	n.d.	n.d.	n.d.	n.d.
Decanoic acid	15.3 ± 1.7^{c}	32.5 ± 4.0^{a}	33.9 ± 5.1^{a}	17.5 ± 2.0^{bc}	26.1 ± 3.5^{ab}

* For each compound, values described by the same letters do not differ significantly.

Table 2.5: Mean concentration ± standard deviation (µg/L) of aroma compounds from different treatments using ANOVA with Tukeyś HSD ($\alpha = 0.05$) (continued)

Compound	8	9	10	12
Ethyl acetate	495.0 ± 51.9^{abc}	859.0 ± 61.0^{bc}	508.1 ± 11.5^{abc}	482.0 ± 311.4^{abc}
Ethyl propanoate	13.0 ± 11.5^{a}	21.0 ± 1.7^{a}	16.2 ± 1.8^{a}	11.6 ± 10.0^{a}
Ethyl isobutyrate	n.d.	6.9 ± 6.0^{a}	n.d.	7.3 ± 6.4^{a}
Isobutyl acetate	0.9 ± 1.5^{a}	0.8 ± 1.4^{a}	n.d.	0.9 ± 1.5^{a}
Ethyl butyrate	518.0 ± 78.8^{abc}	641.1 ± 86.7^{a}	530.1 ± 42.6^{abc}	665.2 ± 28.4^{a}
Isobutanol	2072.0 ± 315.1^{abc}	2564.3 ± 347.0^{a}	2120.3 ± 170.2^{abc}	2660.9 ± 113.5^{a}
Ethyl isovalerate	n.d.	n.d.	0.5 ± 0.7^{a}	3.0 ± 5.1^{a}
Isoamyl acetate	n.d.	n.d.	n.d.	n.d.
Ethyl pentanoate	0.8 ± 0.1^{a}	0.7 ± 0.2^{a}	0.4 ± 0.1^{ab}	0.6 ± 0.2^{ab}
Ethyl hexanoate	29.2 ± 1.2^{ae}	17.0 ± 1.2^{c}	26.5 ± 2.1^{ef}	23.6 ± 1.9^{df}
3-methyl-1-pentanol	2.3 ± 2.9^{a}	0.7 ± 0.6^{a}	1.9 ± 0.3^{a}	0.7 ± 0.7^{a}
2-nonanone	n.d.	n.d.	n.d.	n.d.
Ethyl octanoate	71.2 ± 8.3^{a}	34.2 ± 4.1^{c}	60.3 ± 3.3^{ad}	58.7 ± 5.2^{ad}
Linalool	0.4 ± 0.4^{a}	0.1 ± 0.1^{a}	1.1 ± 0.0^{a}	n.d.
Ethyl decanoate	4.7 ± 4.5^{a}	3.1 ± 1.7^{a}	4.7 ± 0.3^{a}	4.9 ± 2.3^{a}
Phenethyl acetate	21.8 ± 4.5^{a}	18.2 ± 1.8^{a}	19.2 ± 0.5^{a}	23.3 ± 3.1^{a}
Hexanoic acid	17.1 ± 3.4^{a}	31.1 ± 10.9^{a}	16.2 ± 0.5^{a}	21.5 ± 8.9^{a}
Phenethyl alcohol	1377.9 ± 124.0^{a}	1460.4 ± 445.3^{a}	1139.8 ± 20.1^{a}	1390.0 ± 161.4^{a}
Ethyl dodecanoate	29.9 ± 14.7^{b}	6.3 ± 1.8^{a}	14.1 ± 1.3^{ab}	15.3 ± 4.6^{ab}
Octanoic acid	12.1 ± 2.3^{a}	25.7 ± 8.5^{a}	12.9 ± 0.4^{a}	16.6 ± 7.6^{a}
Nonanoic acid	n.d.	n.d.	n.d.	n.d.
Decanoic acid	32.5 ± 2.7^{a}	16.6 ± 3.4^{bc}	32.1 ± 2.5^{a}	31.4 ± 3.4^{a}

* For each compound, values described by the same letters do not differ significantly.

Table 2.6: p-Values ($\alpha = 0.05$) for effects of temperature, schedule, and interaction between temperature and schedule on aroma compounds.

	Temperature	Schedule	Interaction
Ethyl acetate	0.01	0.01	0.09
Ethyl propanoate	0.05	0.12	0.69
Ethyl isobutyrate	0.19	0.78	0.16
Isobutyl acetate	0.66	0.63	0.55
Ethyl butyrate	0.00	0.00	0.97
Isobutanol	0.00	0.00	0.97
Ethyl isovalerate	0.03	0.76	0.82
Ethyl pentanoate	0.01	0.00	0.02
Ethyl hexanoate	0.00	0.00	0.00
3-methyl-1-pentanol	0.32	0.09	0.43
Ethyl octanoate	0.00	0.00	0.00
Linalool	0.01	0.80	0.01
Ethyl decanoate	0.32	0.11	0.94
Phenethyl acetate	0.31	0.06	0.34
Hexanoic acid	0.21	0.31	0.41
Phenethyl alcohol	0.14	0.05	0.69
Ethyl dodecanoate	0.38	0.00	0.15
Octanoic acid	0.30	0.81	0.16
Decanoic acid	0.52	0.00	0.20

Table 2.7: Aroma descriptors and detection thresholds (µg/L) for compounds measured in finished meads.

	Description	Threshold
Ethyl acetate	Solvent-like, fruity	7500[a]
Ethyl propanoate	Pineapple-like	1840[b]
Ethyl isobutyrate	Fruity, aromatic	15[a]
Isobutyl acetate	Banana-like, sweet fruity	1600[c]
Ethyl butyrate	Fruity	20[a]
Isobutanol	Alcoholic	40000[a]
Ethyl isovalerate	Fruity, grape-like	18[c]
Isoamyl acetate	Banana, pear	30[a]
Ethyl pentanoate	Apple-like	10[b]
Ethyl hexanoate	Apple-like, fruity, aniseed-like	5[a]
3-methyl-1-pentanol	–	–
2-nonanone	Ketonic, varnishy	200[c]
Ethyl octanoate	Apple-like, sweet, fruity	2[a]
Linalool	Citrus-like, floral	15[a]
Ethyl decanoate	Caprylic, fruity	2[a]
Phenethyl acetate	Rose-like, honey-like	250[a]
Hexanoic acid	Caprylic, sweaty	3000[a]
Phenethyl alcohol	Rose-like, perfumy	10000[a]
Ethyl dodecanoate	Caprylic, estery	2000[c]
Octanoic acid	Caprylic	4500[c]
Nonanoic acid	–	–
Decanoic acid	Tallowy, caprylic	15000[a]

[a] 10 % ethanol (Guth, 1997),
[b] wine (Etiévant, 1991),
[c] beer (Angelino, 1991)

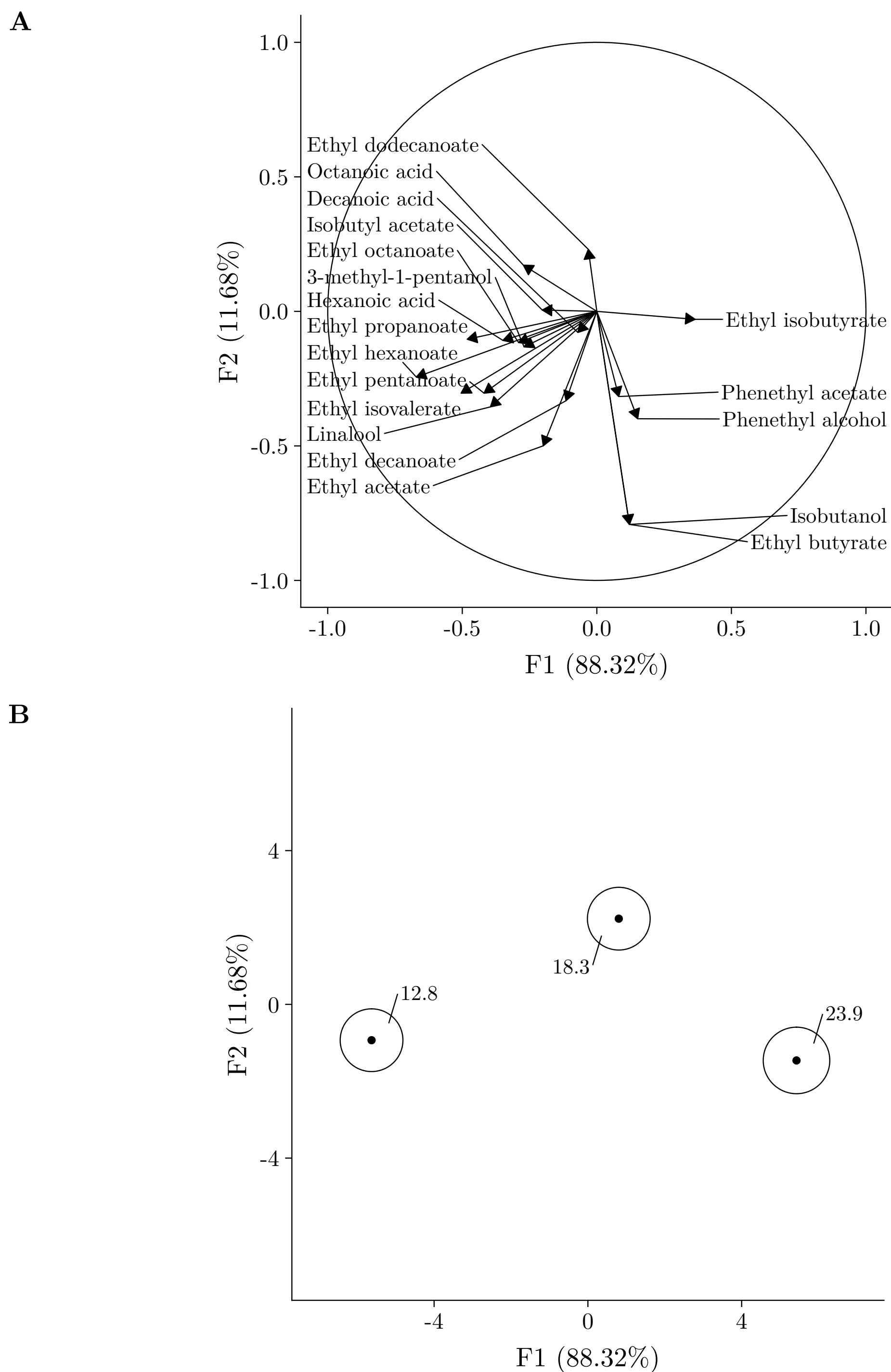

Figure 2.1: Separation of fermentation temperatures using CDA. Loadings for aroma compounds are in subfigure A, and scores are plotted on subfigure B. Circles represent 95 % confidence intervals surrounding the treatment means.

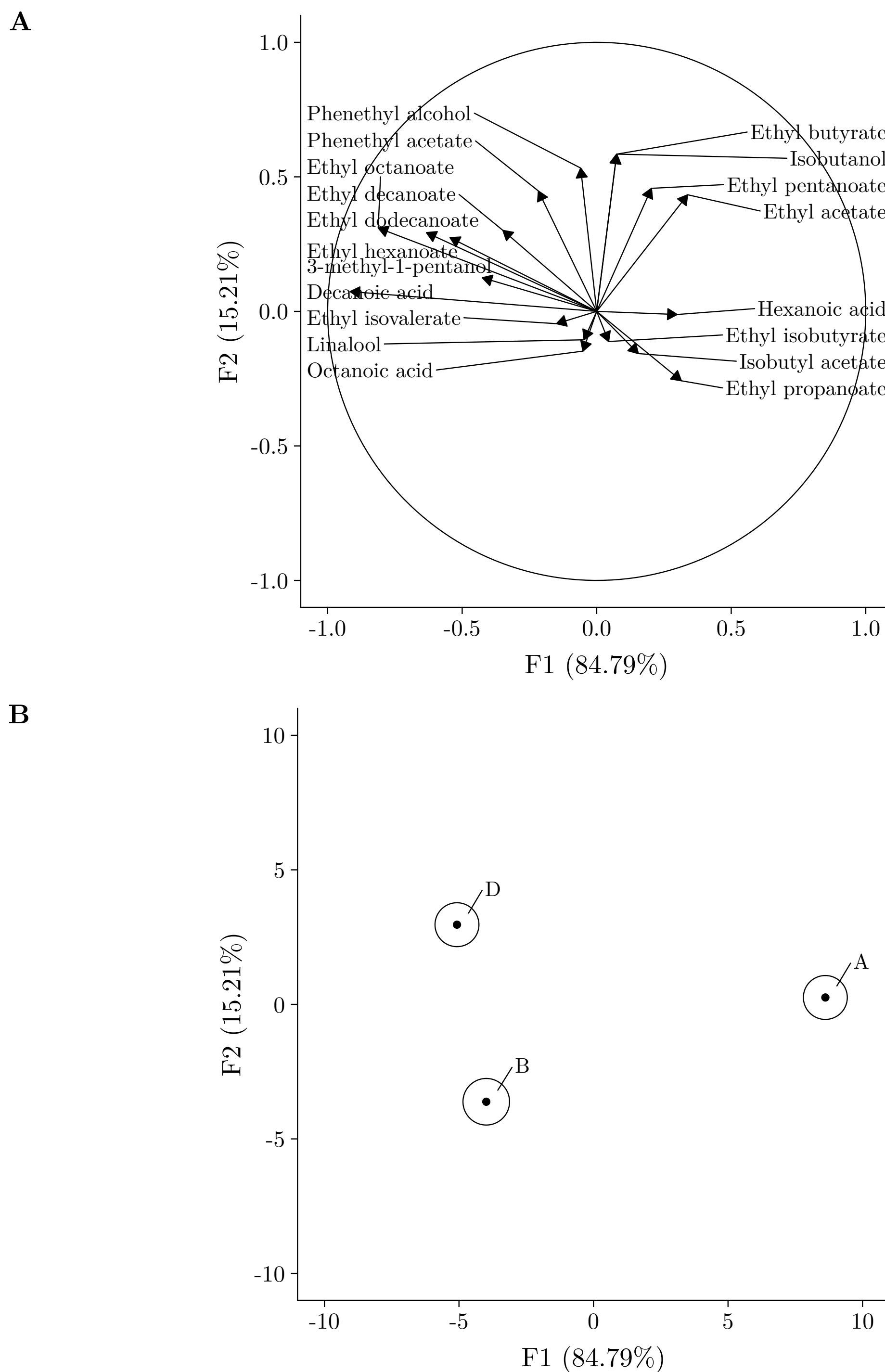

Figure 2.2: Separation of nutrient schedules using CDA. Loadings for aroma compounds are in subfigure A, and scores are plotted on subfigure B. Circles represent 95 % confidence intervals surrounding the treatment means.

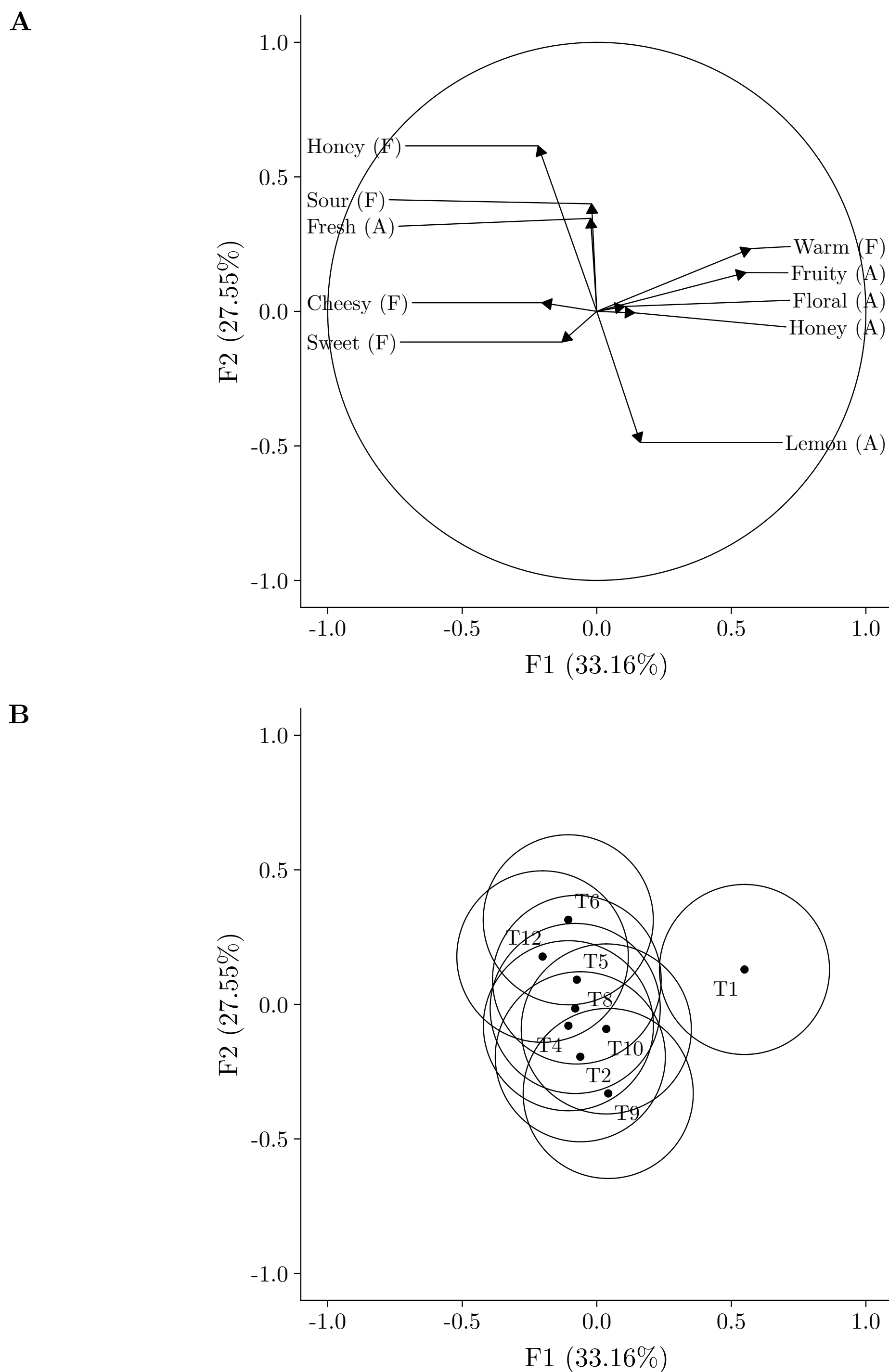

Figure 2.3: Separation of treatments using CDA, after removing outliers. Loadings for sensory attributes are in subfigure A, and scores are plotted on subfigure B. Circles represent 95 % confidence intervals surrounding the treatment means.

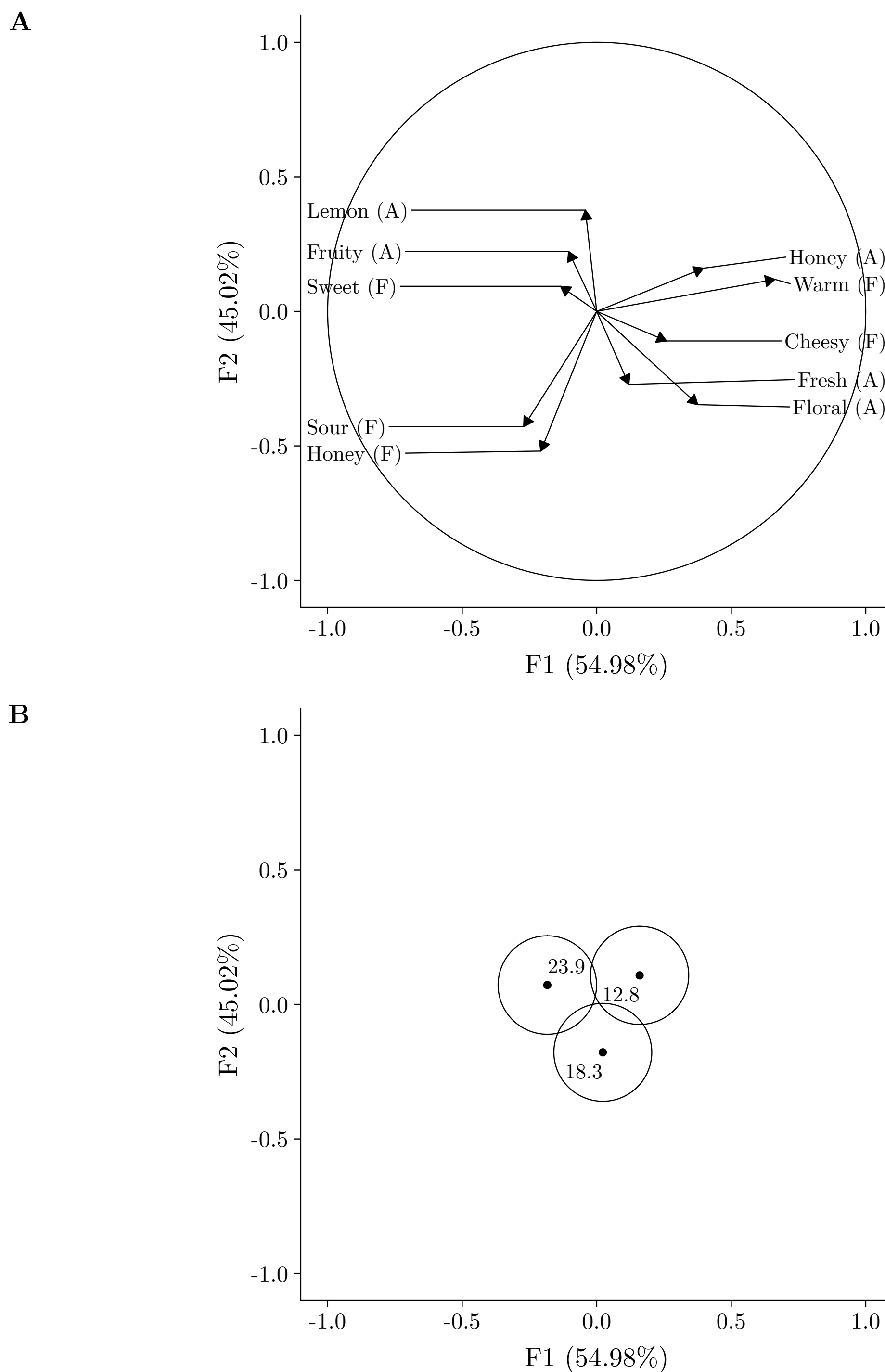

Figure 2.4: Separation of fermentation temperatures using CDA, after removing outliers. Loadings for sensory attributes are in subfigure A, and scores are plotted on subfigure B. Circles represent 95 % confidence intervals surrounding the treatment means.

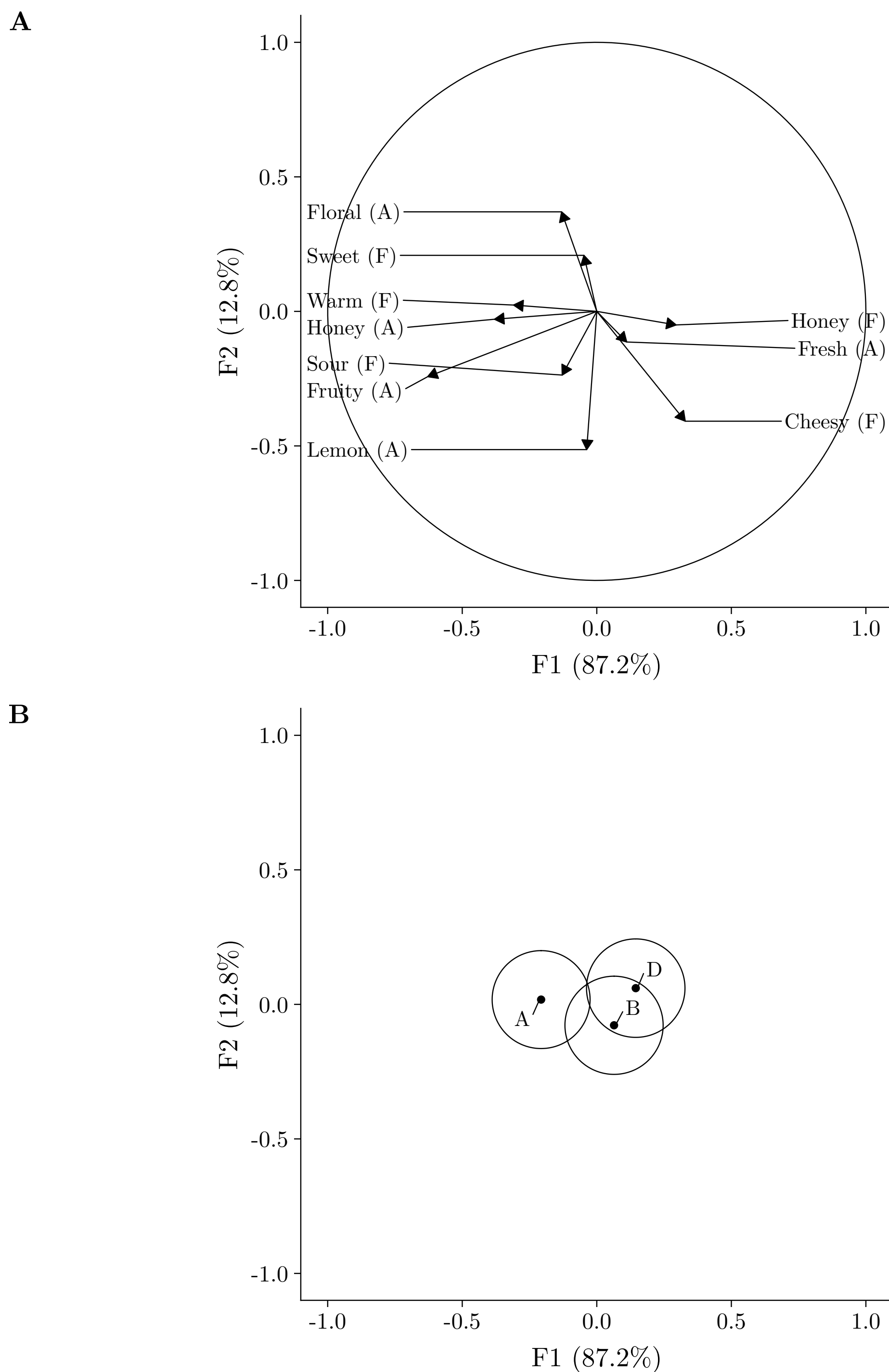

Figure 2.5: Separation of nutrient schedules using CDA, after removing outliers. Loadings for sensory attributes are in subfigure A, and scores are plotted on subfigure B. Circles represent 95 % confidence intervals surrounding the treatment means.

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Chapter 3

Mead has a compelling argument for being the oldest alcoholic beverage, yet mead research has only just recently come into its own. Much of the work has been done in the past fifty years, with many researchers focusing on regional styles. The increase in commercial mead making has brought with it a corresponding increase in studies aiming to improve quality and efficiency. Increasing fermentation temperature increases the rate of mead fermentation much as with many other fermented beverages, as does adding nutrients under all schedules included in this study. This efficiency gain came at no cost to quality, as most treatments were indistinguishable with regard to sensory analysis. Still more gains could be made with improvements to nutrient supplements tailored to complement honey's unique composition and identifying higher fermentation temperatures that decrease fermentation time without compromising on aroma and flavor. Each of these avenues of exploration could be complemented by the additional dimension of yeast strain selection. Some known strains may be more suitable for mead fermentation than others, and new strains could potentially be isolated from honey samples. Genetically modified strains of *S. cerevisiae* or other organisms could be also customized to better accommodate the needs of the mead industry.

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APPENDIX

Chapter A

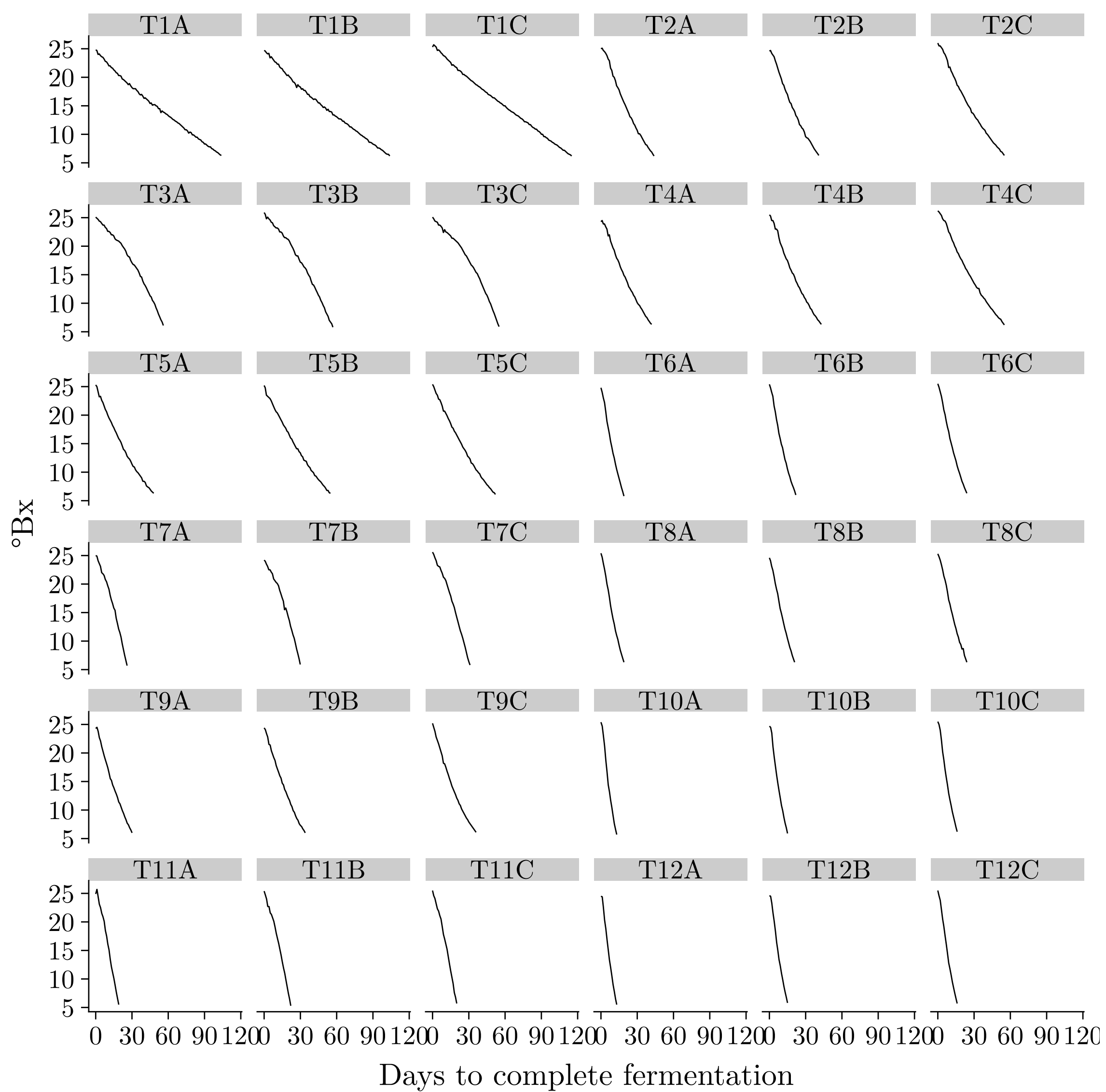

Figure A.1: Individual fermentation profiles for all replicates.

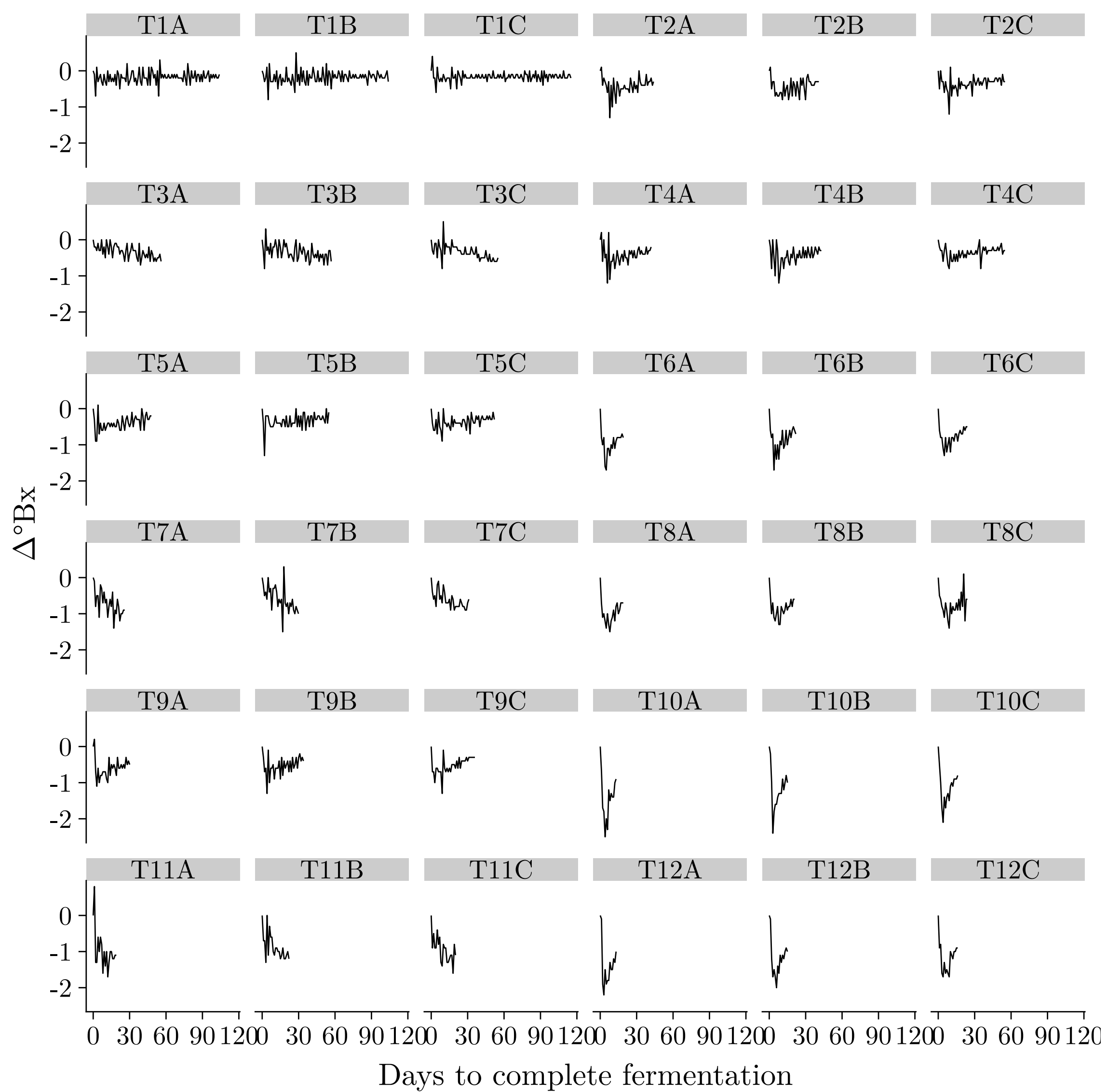

Figure A.2: Individual fermentation profiles for all replicates, in Δ°Bx/d.

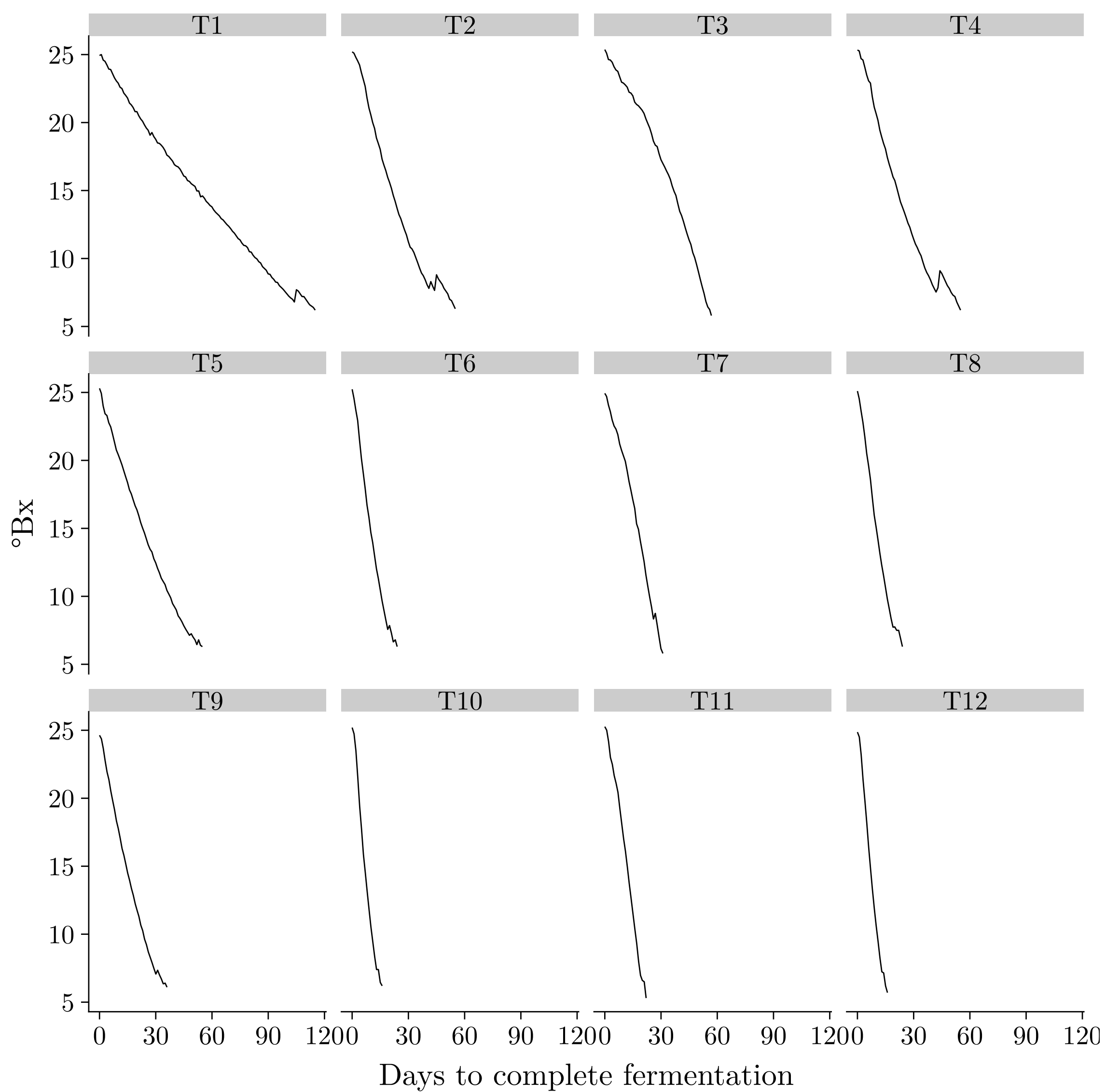

Figure A.3: Individual fermentation profiles for all treatments.

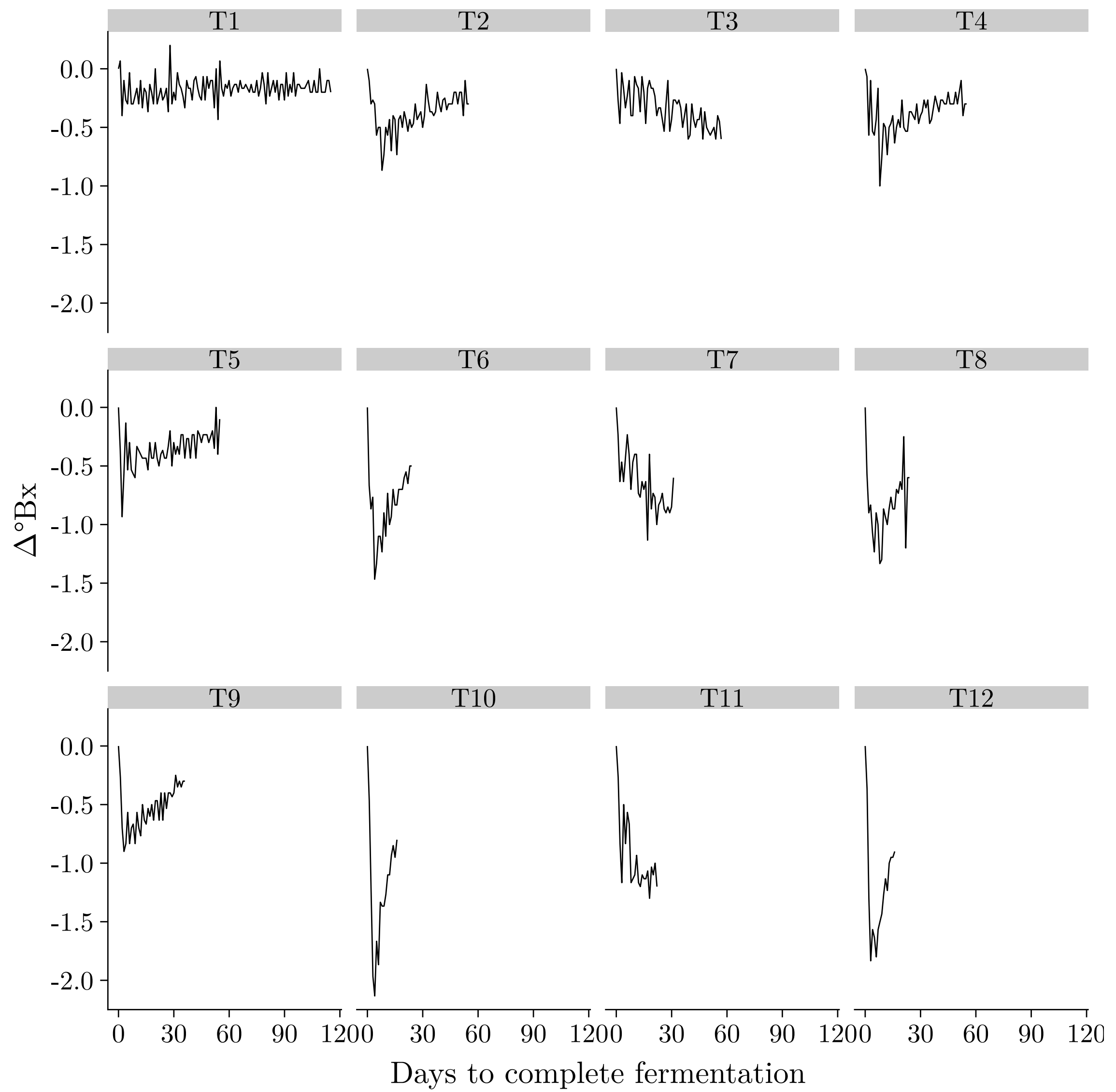

Figure A.4: Individual fermentation profiles for all treatments, in Δ°Bx/d.

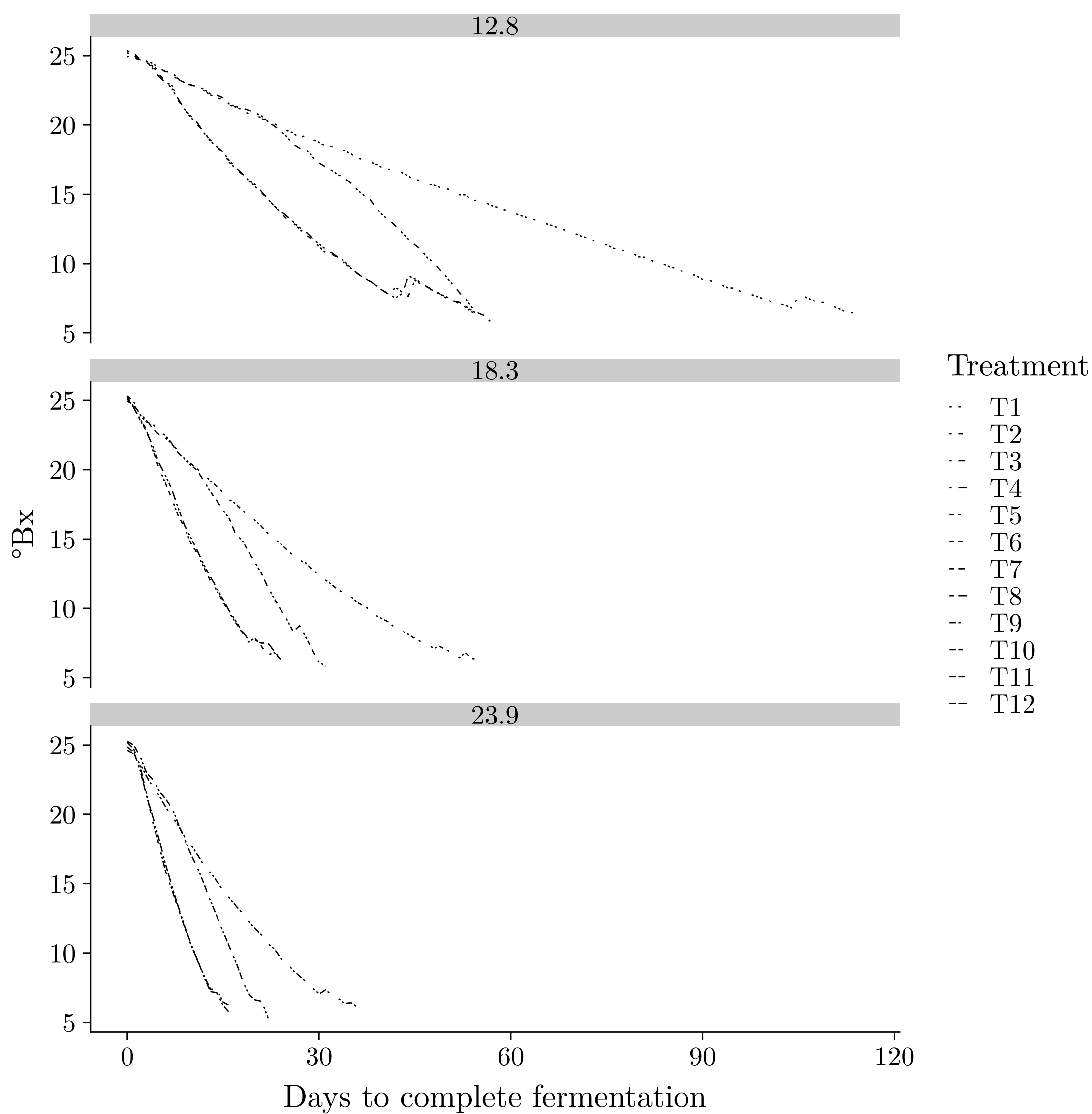

Figure A.5: Fermentation profiles for all treatments, grouped by temperature.

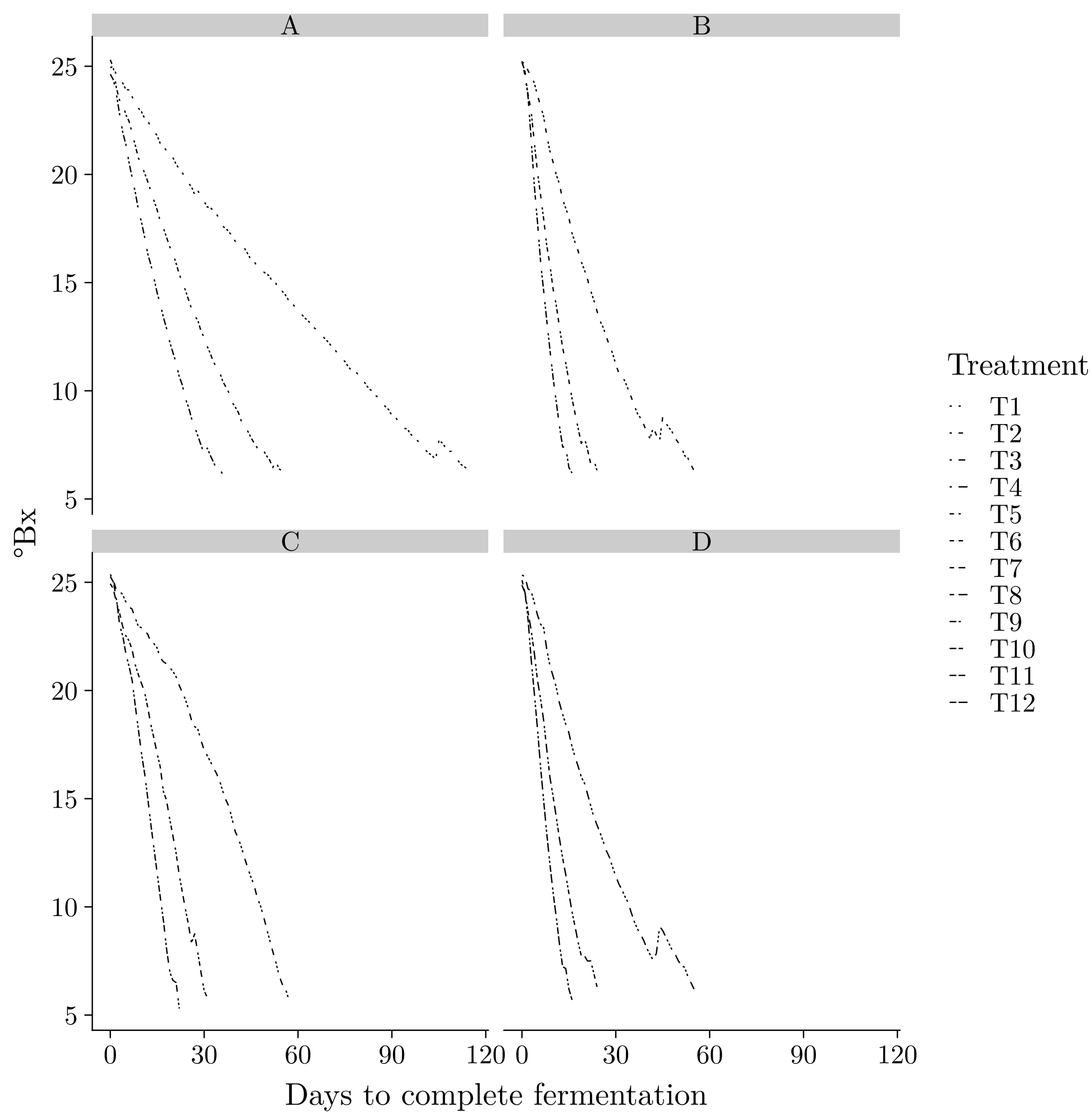

Figure A.6: Fermentation profiles for all treatments, grouped by nutrient addition schedule.

Table A.1: Fermentation temperature, nutrient schedule, and basic wine chemistry parameters for all replicates.

	Fermentation temperature (°C)	Nutrient schedule[1]	Days to complete fermentation (d)	YAN (mg/L)	Residual sugar (g/L)	pH
T1A	12.8	A	101.3	7.4 ± 0.2	69.1 ± 2.1	3.46 ± 0.00
T1B	12.8	A	100.4	7.5 ± 0.2	70.2 ± 2.3	3.49 ± 0.00
T1C	12.8	A	112.6	3.7 ± 0.4	78.0 ± 0.7	3.48 ± 0.01
T2A	12.8	B	40.8	14.4 ± 0.9	51.5 ± 1.3	3.61 ± 0.00
T2B	12.8	B	38.3	15.3 ± 0.5	65.5 ± 4.4	3.54 ± 0.01
T2C	12.8	B	51.5	11.1 ± 0.6	66.5 ± 1.4	3.58 ± 0.00
T3A	12.8	C	60.5	16.6 ± 0.3	48.4 ± 0.4	3.63 ± 0.00
T3B	12.8	C	59.6	16.8 ± 0.0	42.7 ± 0.1	3.63 ± 0.00
T3C	12.8	C	60.4	18.2 ± 0.0	59.5 ± 1.5	3.62 ± 0.01
T4A	12.8	D	39.3	14.6 ± 0.8	55.5 ± 1.3	3.62 ± 0.00
T4B	12.8	D	40.1	13.3 ± 0.2	64.7 ± 8.3	3.57 ± 0.00
T4C	12.8	D	51.4	12.3 ± 0.3	73.0 ± 2.1	3.57 ± 0.00
T5A	18.3	A	44.6	6.7 ± 0.1	71.5 ± 2.1	3.43 ± 0.00
T5B	18.3	A	51.8	5.8 ± 0.3	70.1 ± 4.9	3.42 ± 0.01
T5C	18.3	A	48.3	3.9 ± 0.4	75.5 ± 1.4	3.36 ± 0.01
T6A	18.3	B	17.5	14.3 ± 0.4	64.6 ± 0.8	3.51 ± 0.01
T6B	18.3	B	20.2	12.3 ± 0.1	62.2 ± 5.8	3.54 ± 0.00
T6C	18.3	B	22.5	10.1 ± 0.0	65.5 ± 0.0	3.50 ± 0.01
T7A	18.3	C	26.7	13.8 ± 0.3	58.1 ± 0.7	3.52 ± 0.01
T7B	18.3	C	32.0	11.6 ± 1.0	53.1 ± 0.7	3.52 ± 0.01
T7C	18.3	C	31.6	17.3 ± 0.2	62.5 ± 1.4	3.51 ± 0.01
T8A	18.3	D	17.9	15.0 ± 0.0	64.6 ± 5.7	3.51 ± 0.00
T8B	18.3	D	19.8	11.2 ± 0.1	57.6 ± 5.7	3.48 ± 0.00
T8C	18.3	D	22.8	11.4 ± 0.0	69.0 ± 2.1	3.49 ± 0.01
T9A	23.9	A	28.0	7.1 ± 0.2	80.2 ± 5.7	3.31 ± 0.01
T9B	23.9	A	31.3	8.4 ± 0.1	78.1 ± 4.0	3.31 ± 0.00
T9C	23.9	A	32.6	3.1 ± 0.1	72.0 ± 0.7	3.33 ± 0.00
T10A	23.9	B	11.8	11.7 ± 0.4	71.0 ± 0.0	3.46 ± 0.01
T10B	23.9	B	13.9	11.7 ± 1.0	69.5 ± 1.4	3.45 ± 0.00
T10C	23.9	B	14.9	9.1 ± 0.2	67.5 ± 0.0	3.44 ± 0.01
T11A	23.9	C	18.6	14.3 ± 0.6	67.4 ± 1.1	3.47 ± 0.01
T11B	23.9	C	22.1	12.0 ± 0.0	42.4 ± 11.5	3.50 ± 0.00
T11C	23.9	C	20.1	15.4 ± 0.3	61.5 ± 2.8	3.46 ± 0.01
T12A	23.9	D	12.0	12.1 ± 0.3	63.9 ± 0.6	3.45 ± 0.01
T12B	23.9	D	13.9	12.0 ± 0.0	59.4 ± 6.2	3.49 ± 0.01
T12C	23.9	D	14.7	8.1 ± 0.1	61.5 ± 2.8	3.46 ± 0.01

[1] A: control, B: 24 h, 48 h and 72 h, D: 24 h, 48 h and 72 h and either 168 h or $\frac{1}{3}$ through fermentation, whichever came first (Moutela, n.d.).

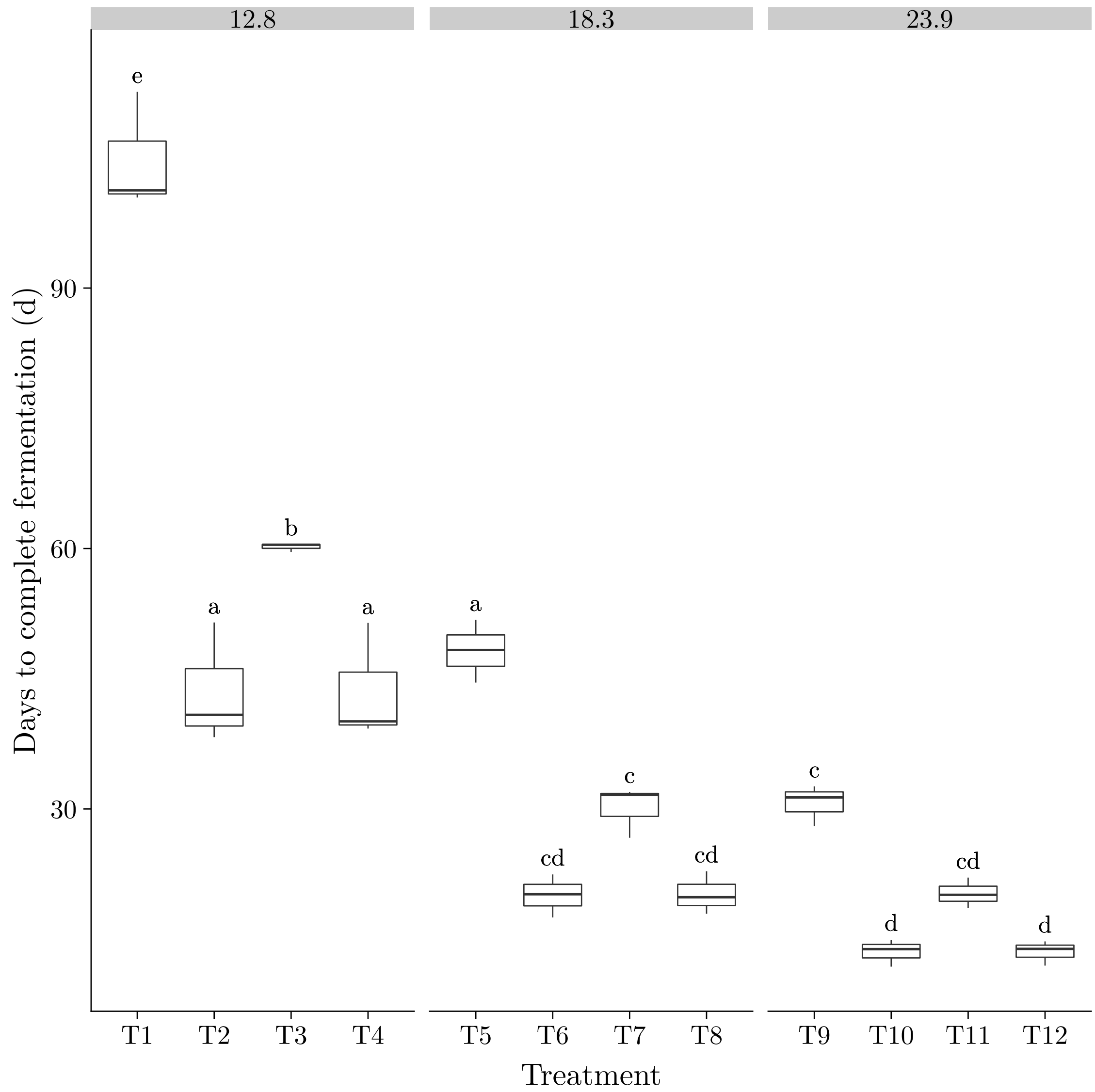

Figure A.7: Days to complete fermentation for all treatments, grouped by temperature, using ANOVA with Tukey's HSD ($\alpha = 0.05$). Values described by the same letters do not differ significantly.

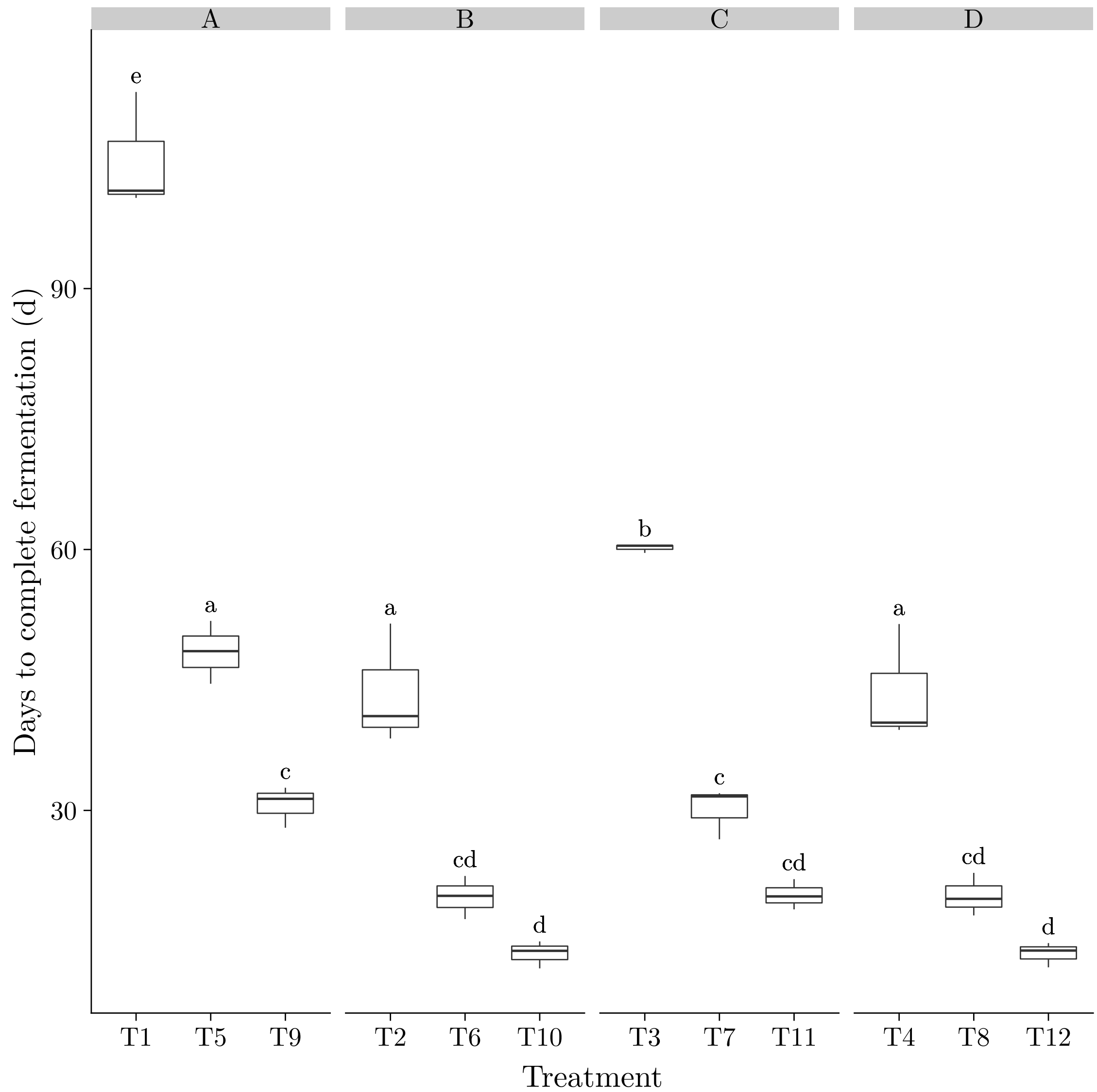

Figure A.8: Days to complete fermentation for all treatments, grouped by schedule, using ANOVA with Tukey's HSD ($\alpha = 0.05$). Values described by the same letters do not differ significantly.

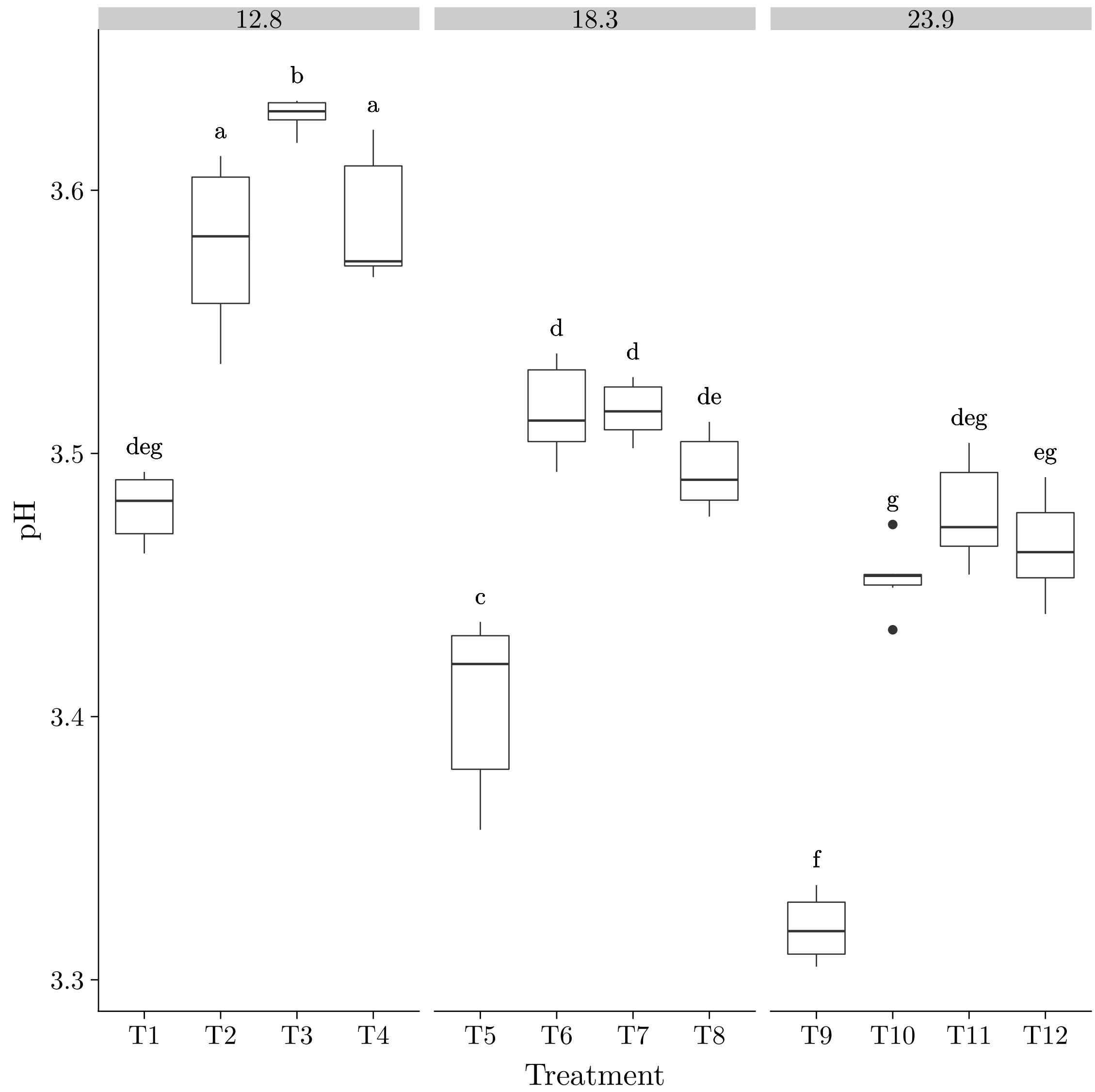

Figure A.9: pH values for all treatments, grouped by temperature, using ANOVA with Tukey's HSD ($\alpha = 0.05$). Values described by the same letters do not differ significantly.

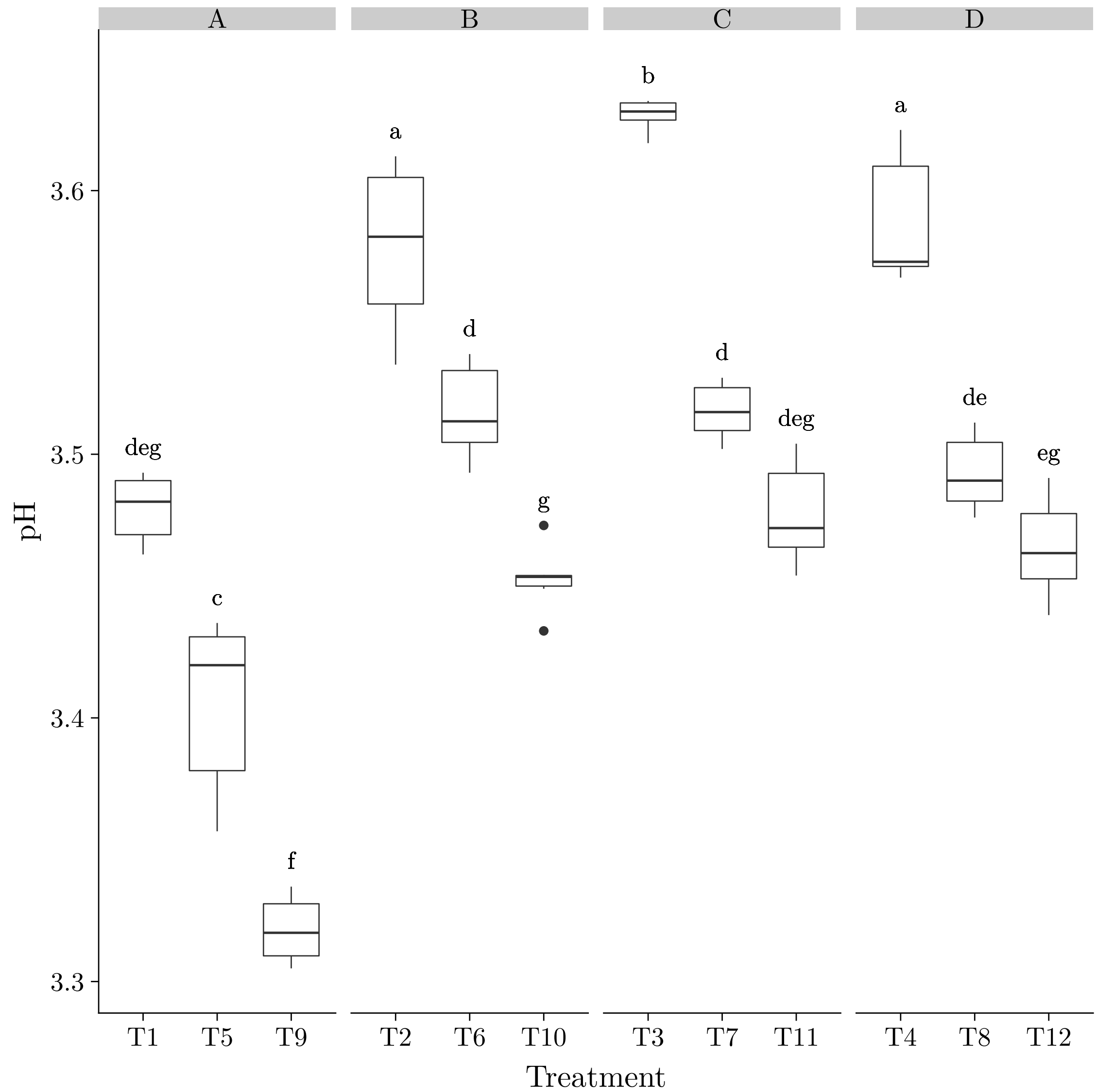

Figure A.10: pH values for all treatments, grouped by schedule, using ANOVA with Tukey's HSD ($\alpha = 0.05$). Values described by the same letters do not differ significantly.

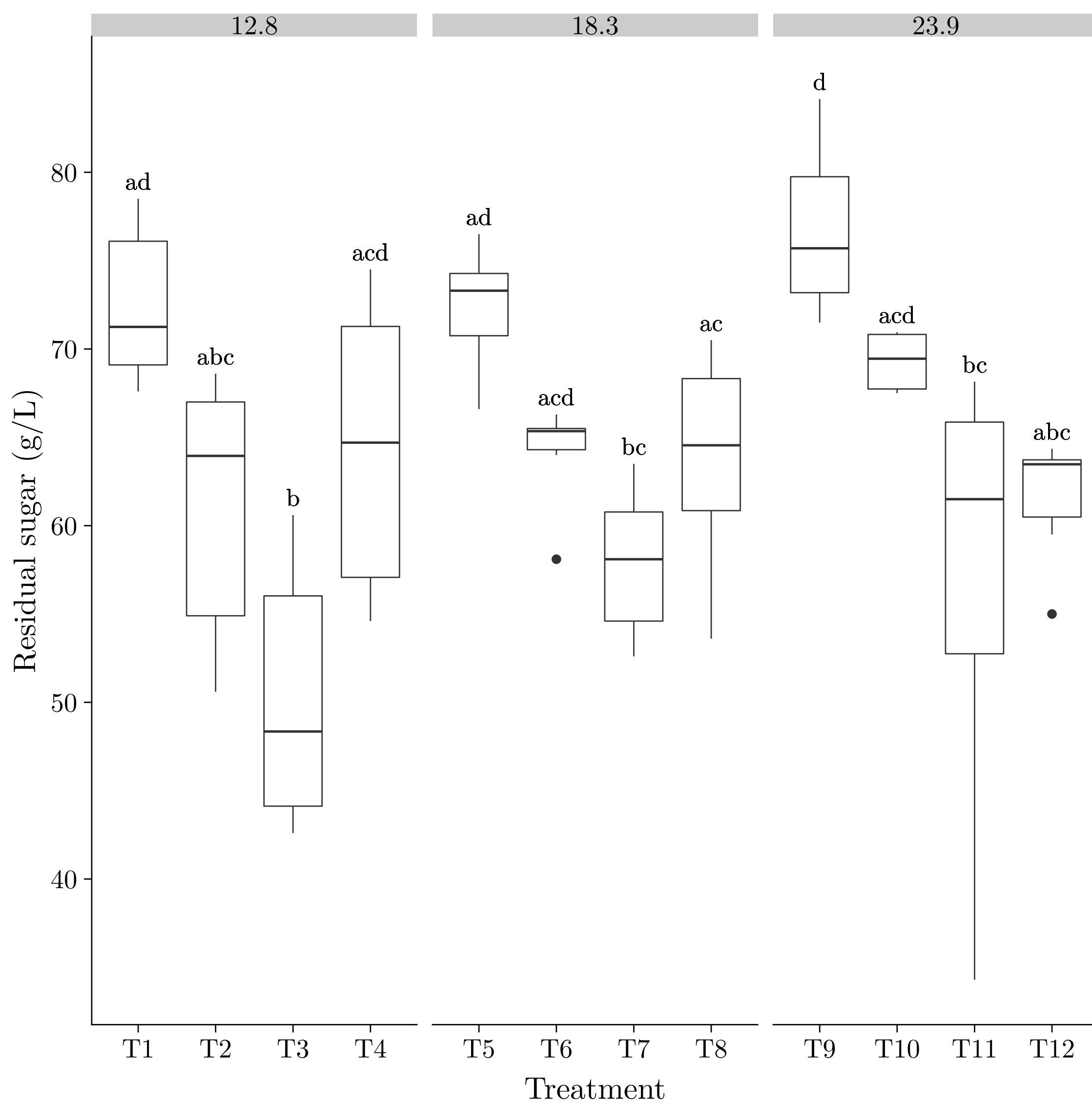

Figure A.11: Residual sugar for all treatments, grouped by temperature, using ANOVA with Tukey's HSD ($\alpha = 0.05$). Values described by the same letters do not differ significantly.

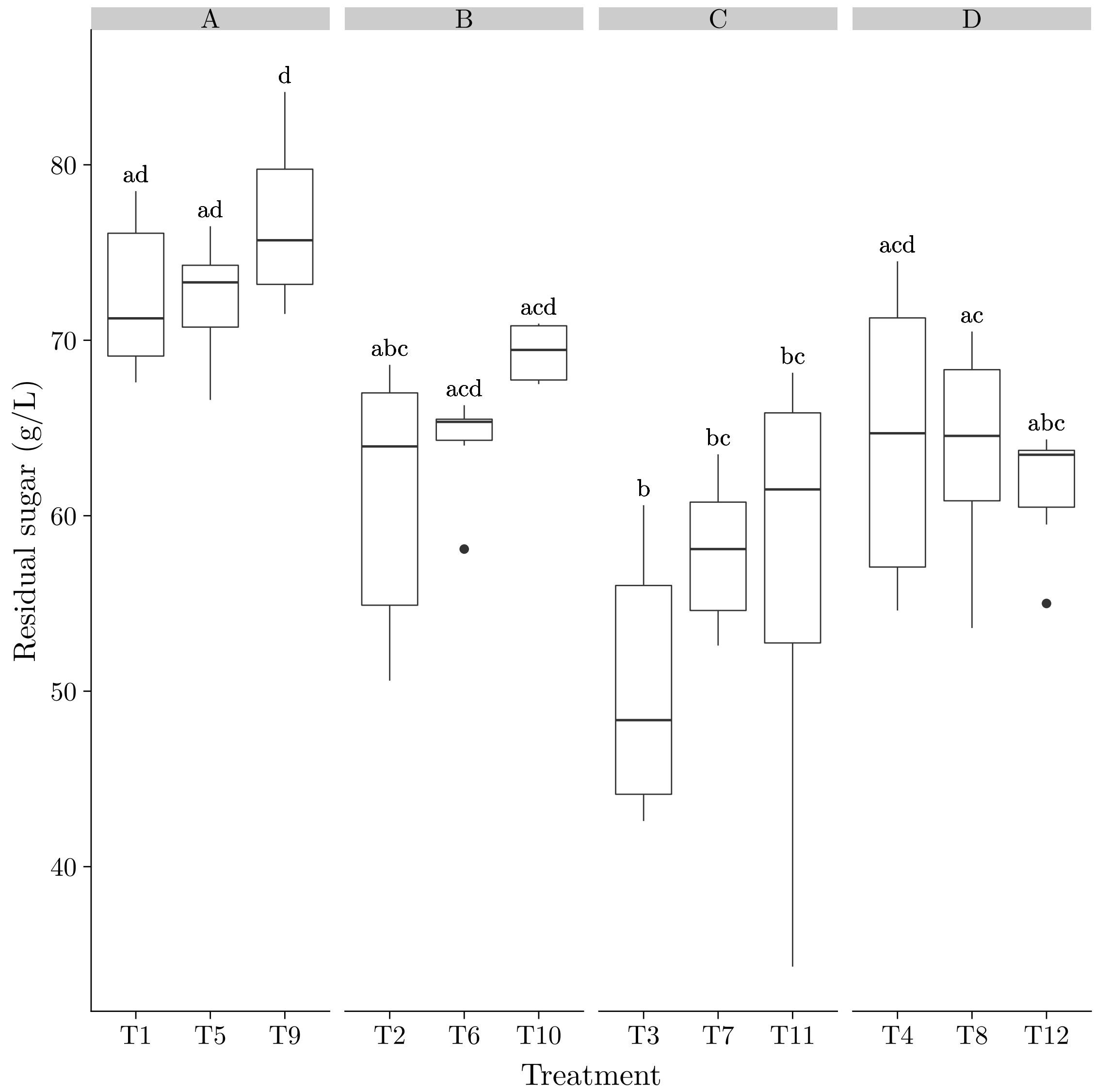

Figure A.12: Residual sugar for all treatments, grouped by schedule, using ANOVA with Tukey's HSD ($\alpha = 0.05$). Values described by the same letters do not differ significantly.

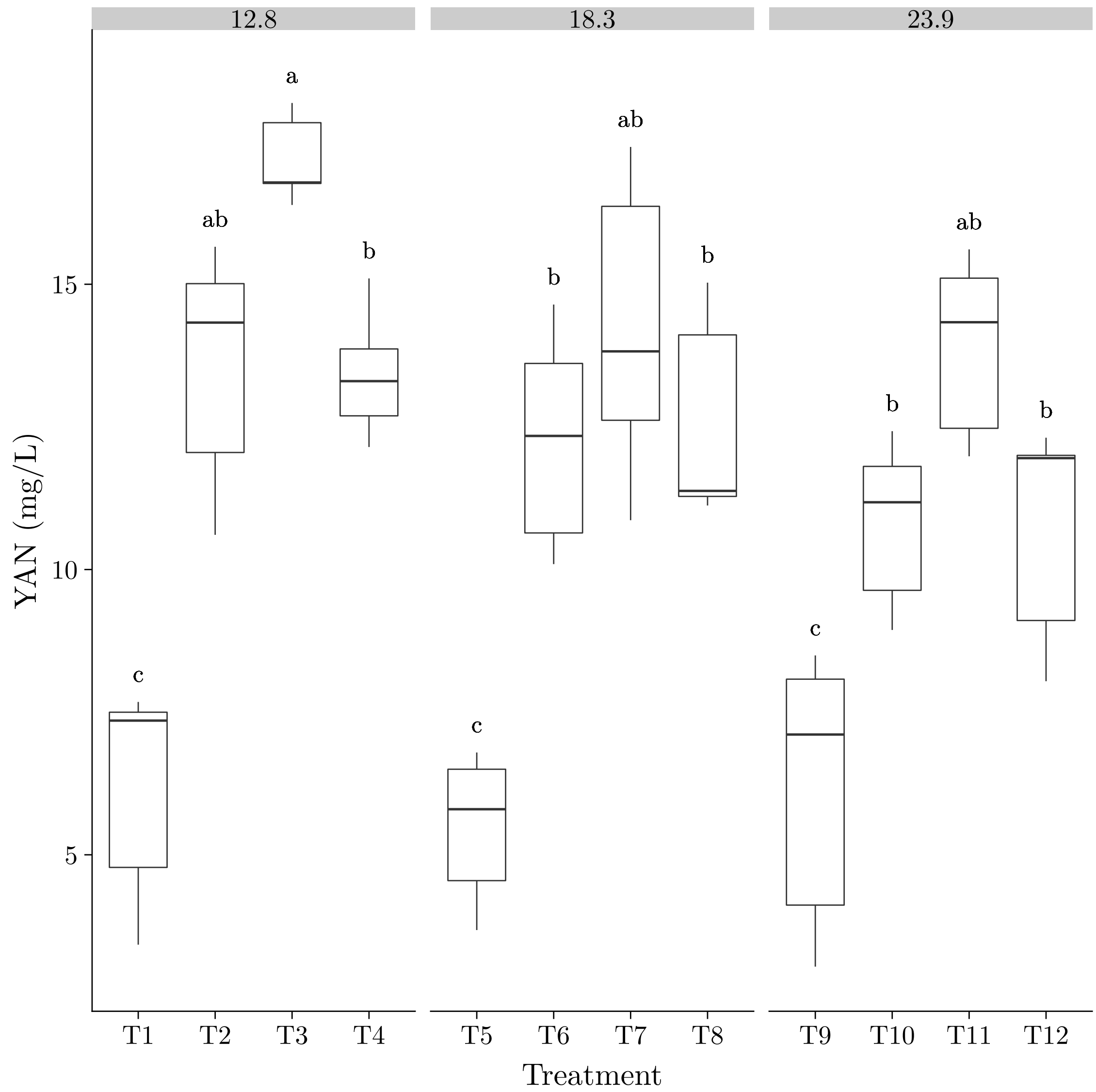

Figure A.13: Yeast assimilable nitrogen for all treatments, grouped by temperature, using ANOVA with Tukey's HSD ($\alpha = 0.05$). Values described by the same letters do not differ significantly.

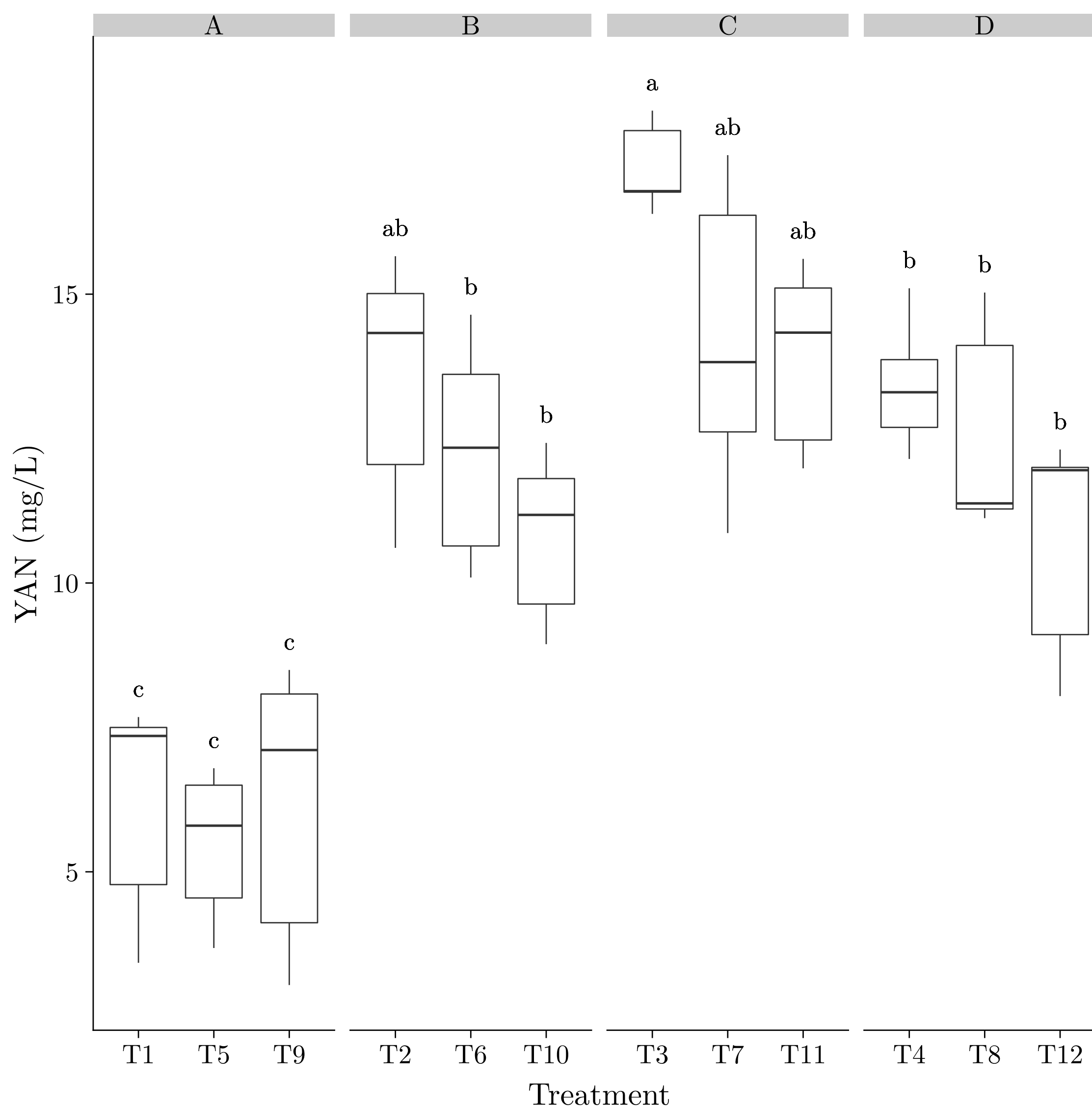

Figure A.14: Yeast assimilable nitrogen for all treatments, grouped by schedule, using ANOVA with Tukey's HSD ($\alpha = 0.05$). Values described by the same letters do not differ significantly.

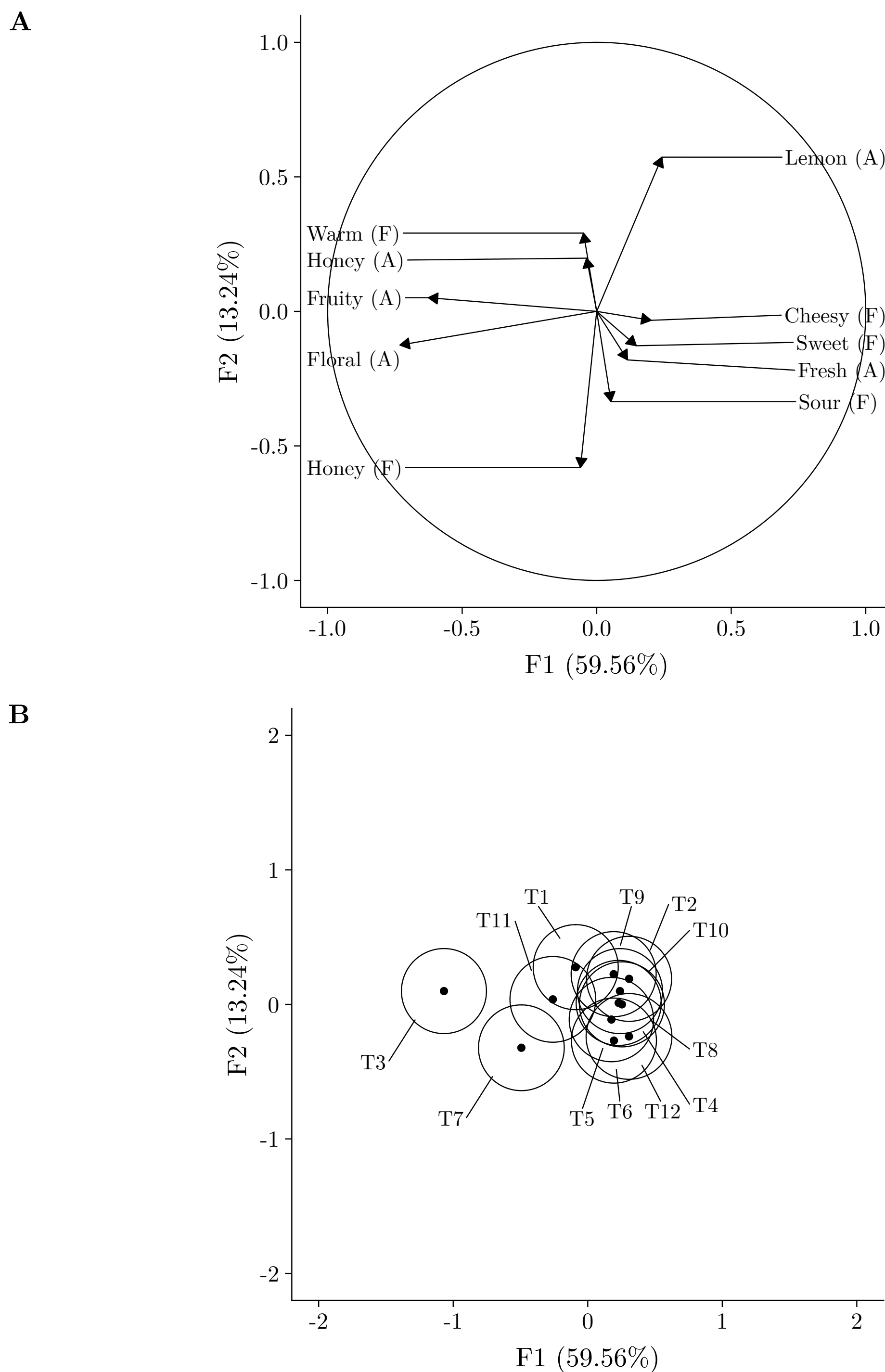

Figure A.15: Separation of treatments using canonical discriminant analysis (CDA). Loadings for sensory attributes are in subfigure A, and scores are plotted on subfigure B. Circles represent 95 % confidence intervals surrounding the treatment means.

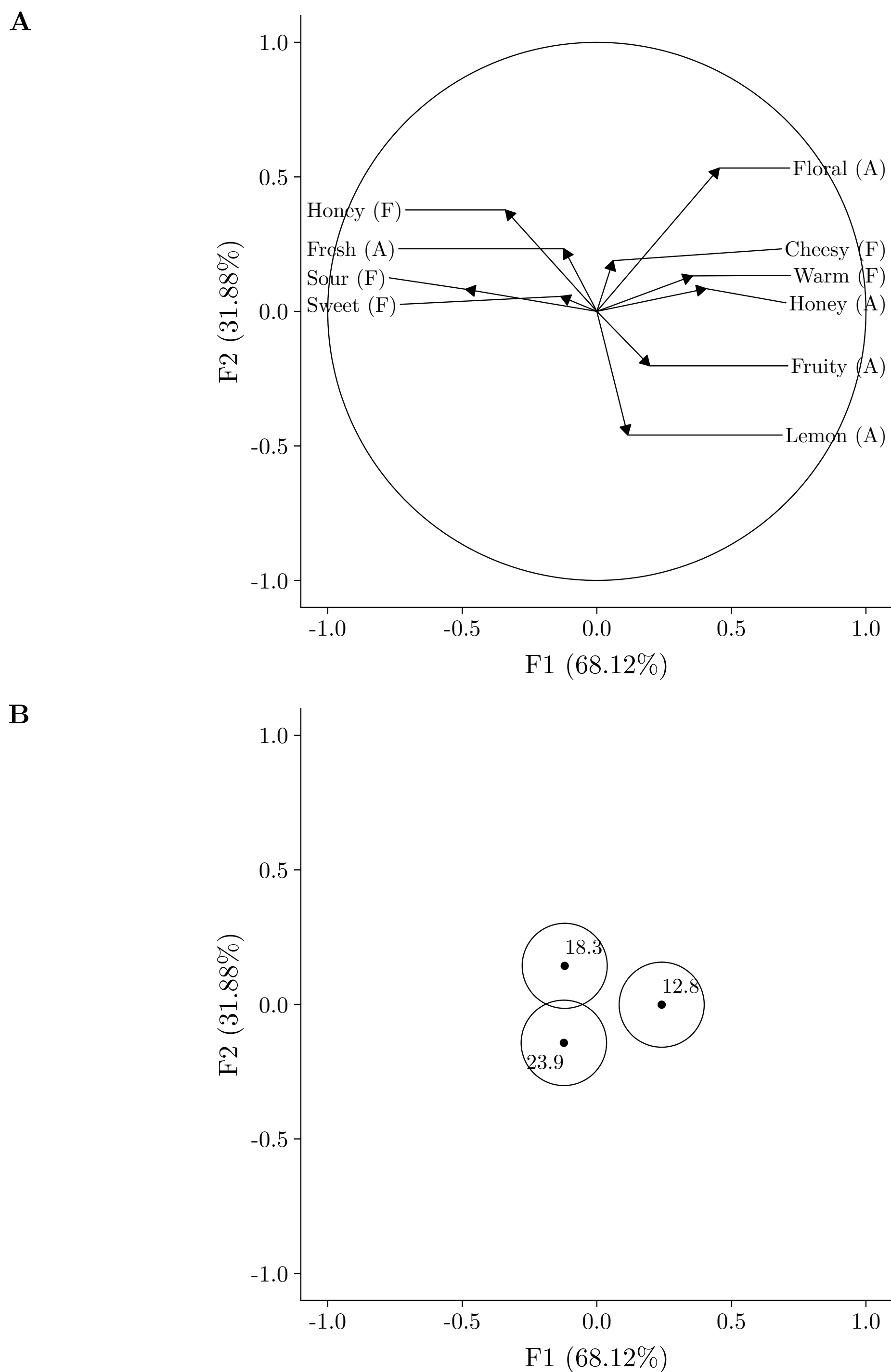

Figure A.16: Separation of fermentation temperatures using canonical discriminant analysis (CDA). Loadings for sensory attributes are in subfigure A, and scores are plotted on subfigure B. Circles represent 95 % confidence intervals surrounding the treatment means.

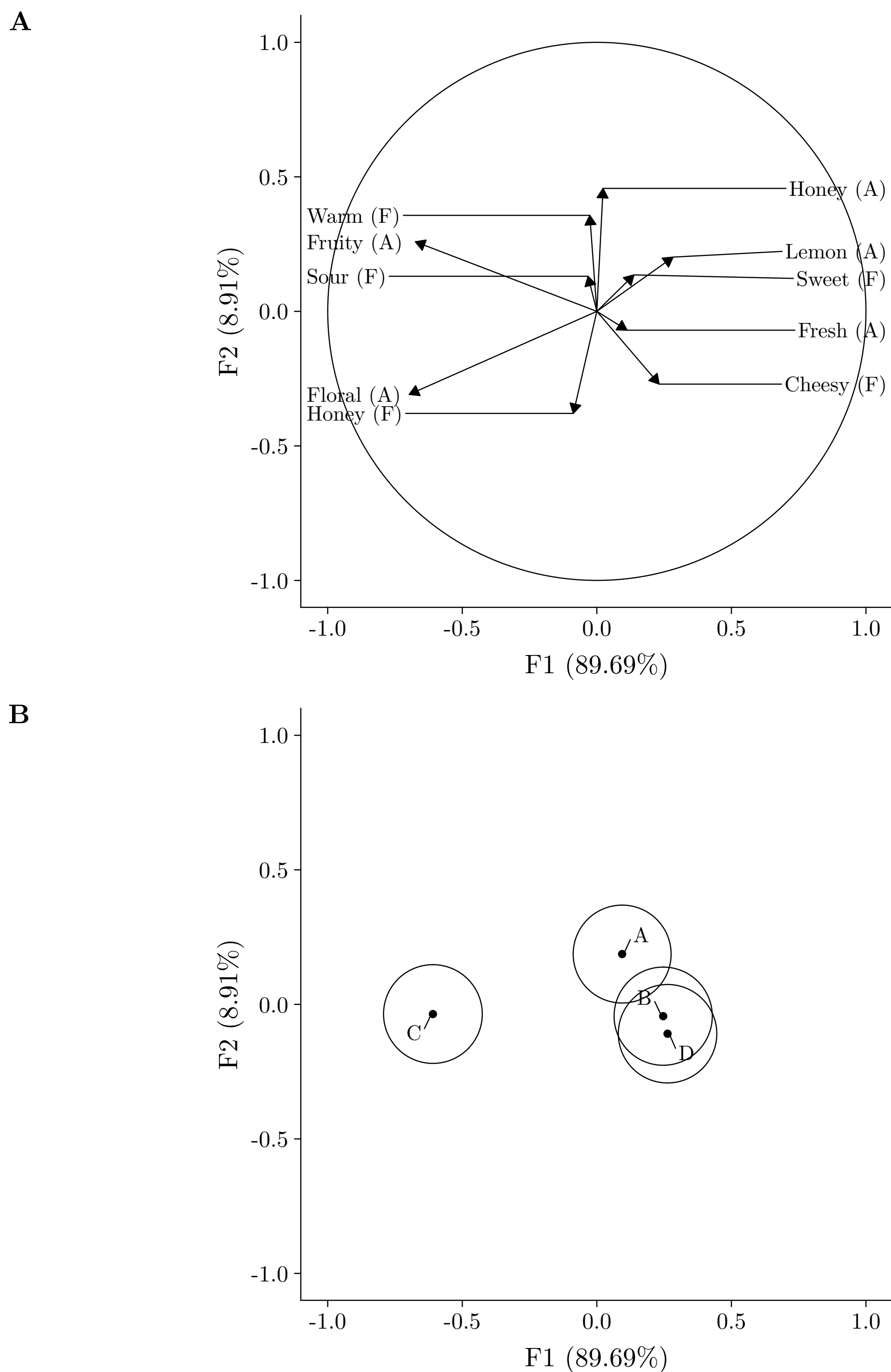

Figure A.17: Separation of nutrient schedules using canonical discriminant analysis (CDA). Loadings for sensory attributes are in subfigure A, and scores are plotted on subfigure B. Circles represent 95 % confidence intervals surrounding the treatment means.

Table A.2: Mean concentration ± standard deviation (µg/L) of aroma compounds from different treatments using ANOVA with Tukeyś HSD ($\alpha = 0.05$)

Compound	1	2	3	4	5	6
Ethyl acetate	817.4 ± 103.7^{abc}	398.8 ± 213.4^{ab}	722.0 ± 85.7	938.7 ± 99.4^{c}	484.1 ± 328.3^{abc}	303.4 ± 69.4^{a}
Ethyl propanoate	24.6 ± 2.3^{a}	23.0 ± 2.4^{a}	26.0 ± 6.2	23.6 ± 6.4^{a}	20.9 ± 3.5^{a}	20.5 ± 2.6^{a}
Ethyl isobutyrate	n.d.	3.3 ± 5.8^{a}	7.1 ± 6.1	n.d.	3.3 ± 5.8^{a}	6.6 ± 5.7^{a}
Isobutyl acetate	2.0 ± 1.7^{a}	1.9 ± 1.7^{a}	n.d.	n.d.	0.9 ± 1.5^{a}	1.0 ± 1.8^{a}
Ethyl butyrate	579.5 ± 25.7^{ab}	515.4 ± 91.2^{abc}	651.7 ± 61.9	626.6 ± 37.5^{ab}	475.5 ± 35.1^{bc}	398.9 ± 31.2^{c}
Isobutanol	2318.1 ± 102.9^{ab}	2061.7 ± 364.9^{abc}	2606.9 ± 247.6	2506.5 ± 150.2^{ab}	1902.1 ± 140.6^{bc}	1595.5 ± 124.7^{c}
Ethyl isovalerate	5.5 ± 4.8^{a}	5.6 ± 4.8^{a}	11.2 ± 0.6	5.8 ± 5.0^{a}	n.d.	2.7 ± 4.7^{a}
Isoamyl acetate	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Ethyl pentanoate	0.8 ± 0.2^{a}	0.8 ± 0.0^{a}	13.1 ± 2.6	0.7 ± 0.1^{a}	0.6 ± 0.2^{ab}	0.3 ± 0.0^{b}
Ethyl hexanoate	26.0 ± 0.9^{ef}	30.8 ± 0.9^{a}	34.2 ± 0.8	36.9 ± 1.2^{b}	20.6 ± 0.7^{cd}	19.4 ± 1.2^{c}
3-methyl-1-pentanol	n.d.	2.5 ± 1.0^{a}	0.7 ± 1.3	4.7 ± 5.1^{a}	n.d.	0.9 ± 1.6^{a}
2-nonanone	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Ethyl octanoate	32.4 ± 2.1^{c}	65.9 ± 3.2^{a}	56.3 ± 2.1	89.0 ± 6.2^{b}	37.0 ± 1.5^{c}	45.3 ± 3.6^{cd}
Linalool	0.9 ± 0.8^{a}	0.5 ± 0.2^{a}	3.5 ± 2.5	0.7 ± 0.4^{a}	0.1 ± 0.1^{a}	0.0 ± 0.1^{a}
Ethyl decanoate	3.4 ± 1.1^{a}	3.9 ± 3.4^{a}	4.6 ± 1.3	6.7 ± 4.2^{a}	1.5 ± 1.3^{a}	2.1 ± 1.8^{a}
Phenethyl acetate	20.8 ± 3.2^{a}	18.0 ± 5.4^{a}	21.7 ± 5.3	19.3 ± 1.8^{a}	15.7 ± 1.8^{a}	16.2 ± 3.2^{a}
Hexanoic acid	373.1 ± 494.6^{a}	51.3 ± 2.2^{a}	44.1 ± 22.7	73.7 ± 49.6^{a}	53.8 ± 54.8^{a}	74.4 ± 40.2^{a}
Phenethyl alcohol	1141.9 ± 294.5^{a}	1033.7 ± 181.5^{a}	1345.2 ± 522.4	1411.6 ± 609.8^{a}	941.2 ± 170.6^{a}	813.5 ± 168.3^{a}
Ethyl dodecanoate	8.0 ± 2.9^{a}	13.6 ± 2.1^{ab}	6.1 ± 1.2	17.1 ± 9.3^{ab}	4.1 ± 0.5^{a}	13.7 ± 2.2^{ab}
Octanoic acid	414.2 ± 534.6^{a}	51.7 ± 12.0^{a}	27.9 ± 14.2	439.0 ± 700.4^{a}	65.5 ± 74.5^{a}	714.3 ± 715.7^{a}
Nonanoic acid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Decanoic acid	15.3 ± 1.7^{c}	32.5 ± 4.0^{a}	21.6 ± 1.4	33.9 ± 5.1^{a}	17.5 ± 2.0^{bc}	26.1 ± 3.5^{ab}

[*] For each compound, values described by the same letters do not differ significantly.

Table A.2: Mean concentration ± standard deviation (µg/L) of aroma compounds from different treatments using ANOVA with Tukeyś HSD ($\alpha = 0.05$) (continued)

Compound	7	8	9	10	11	12
Ethyl acetate	507.5 ± 279.2	495.0 ± 51.9^{abc}	859.0 ± 61.0^{bc}	508.1 ± 11.5^{abc}	643.8 ± 132.1	482.0 ± 311.4^{abc}
Ethyl propanoate	25.2 ± 1.6	13.0 ± 11.5^{a}	21.0 ± 1.7^{a}	16.2 ± 1.8^{a}	21.5 ± 1.9	11.6 ± 10.0^{a}
Ethyl isobutyrate	n.d.	n.d.	6.9 ± 6.0^{a}	n.d.	n.d.	7.3 ± 6.4^{a}
Isobutyl acetate	1.2 ± 2.1	0.9 ± 1.5^{a}	0.8 ± 1.4^{a}	n.d.	1.3 ± 2.2	0.9 ± 1.5^{a}
Ethyl butyrate	566.7 ± 92.9	518.0 ± 78.8^{abc}	641.1 ± 86.7^{a}	530.1 ± 42.6^{abc}	627.8 ± 80.4	665.2 ± 28.4^{a}
Isobutanol	2266.9 ± 371.7	2072.0 ± 315.1^{abc}	2564.3 ± 347.0^{a}	2120.3 ± 170.2^{abc}	2511.1 ± 321.7	2660.9 ± 113.5^{a}
Ethyl isovalerate	8.7 ± 0.3	n.d.	n.d.	0.5 ± 0.7^{a}	2.9 ± 5.0	3.0 ± 5.1^{a}
Isoamyl acetate	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Ethyl pentanoate	3.4 ± 0.3	0.8 ± 0.1^{a}	0.7 ± 0.2^{a}	0.4 ± 0.1^{ab}	0.9 ± 0.2	0.6 ± 0.2^{ab}
Ethyl hexanoate	28.1 ± 0.9	29.2 ± 1.2^{ae}	17.0 ± 1.2^{c}	26.5 ± 2.1^{ef}	19.0 ± 1.0	23.6 ± 1.9^{df}
3-methyl-1-pentanol	1.3 ± 2.2	2.3 ± 2.9^{a}	0.7 ± 0.6^{a}	1.9 ± 0.3^{a}	0.5 ± 0.6	0.7 ± 0.7^{a}
2-nonanone	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Ethyl octanoate	60.9 ± 4.7	71.2 ± 8.3^{a}	34.2 ± 4.1^{c}	60.3 ± 3.3^{ad}	40.2 ± 4.1	58.7 ± 5.2^{ad}
Linalool	0.6 ± 0.2	0.4 ± 0.4^{a}	0.1 ± 0.1^{a}	1.1 ± 0.0^{a}	4.8 ± 3.9	n.d.
Ethyl decanoate	4.4 ± 3.3	4.7 ± 4.5^{a}	3.1 ± 1.7^{a}	4.7 ± 0.3^{a}	3.6 ± 2.9	4.9 ± 2.3^{a}
Phenethyl acetate	25.2 ± 7.6	21.8 ± 4.5^{a}	18.2 ± 1.8^{a}	19.2 ± 0.5^{a}	32.2 ± 8.3	23.3 ± 3.1^{a}
Hexanoic acid	31.9 ± 12.8	17.1 ± 3.4^{a}	31.1 ± 10.9^{a}	16.2 ± 0.5^{a}	17.3 ± 1.9	21.5 ± 8.9^{a}
Phenethyl alcohol	1313.0 ± 494.3	1377.9 ± 124.0^{a}	1460.4 ± 445.3^{a}	1139.8 ± 20.1^{a}	1335.7 ± 620.0	1390.0 ± 161.4^{a}
Ethyl dodecanoate	11.8 ± 1.1	29.9 ± 14.7^{b}	6.3 ± 1.8^{a}	14.1 ± 1.3^{ab}	13.0 ± 6.2	15.3 ± 4.6^{ab}
Octanoic acid	30.8 ± 17.9	12.1 ± 2.3^{a}	25.7 ± 8.5^{a}	12.9 ± 0.4^{a}	14.5 ± 3.9	16.6 ± 7.6^{a}
Nonanoic acid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Decanoic acid	26.1 ± 3.0	32.5 ± 2.7^{a}	16.6 ± 3.4^{bc}	32.1 ± 2.5^{a}	19.3 ± 1.0	31.4 ± 3.4^{a}

* For each compound, values described by the same letters do not differ significantly.

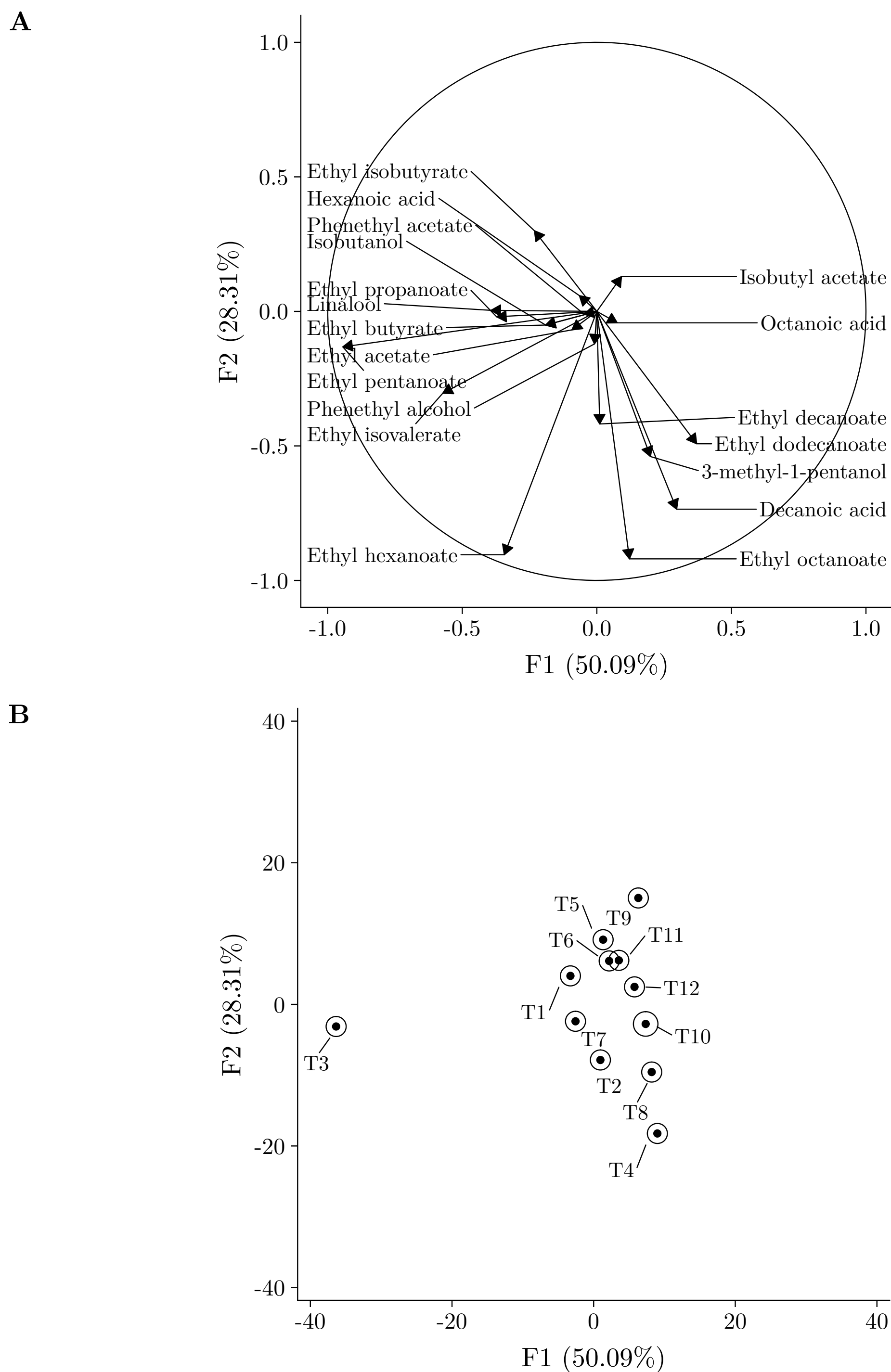

Figure A.18: Separation of treatments using canonical discriminant analysis (CDA). Loadings for aroma compounds are in subfigure A, and scores are plotted on subfigure B. Circles represent 95 % confidence intervals surrounding the treatment means.

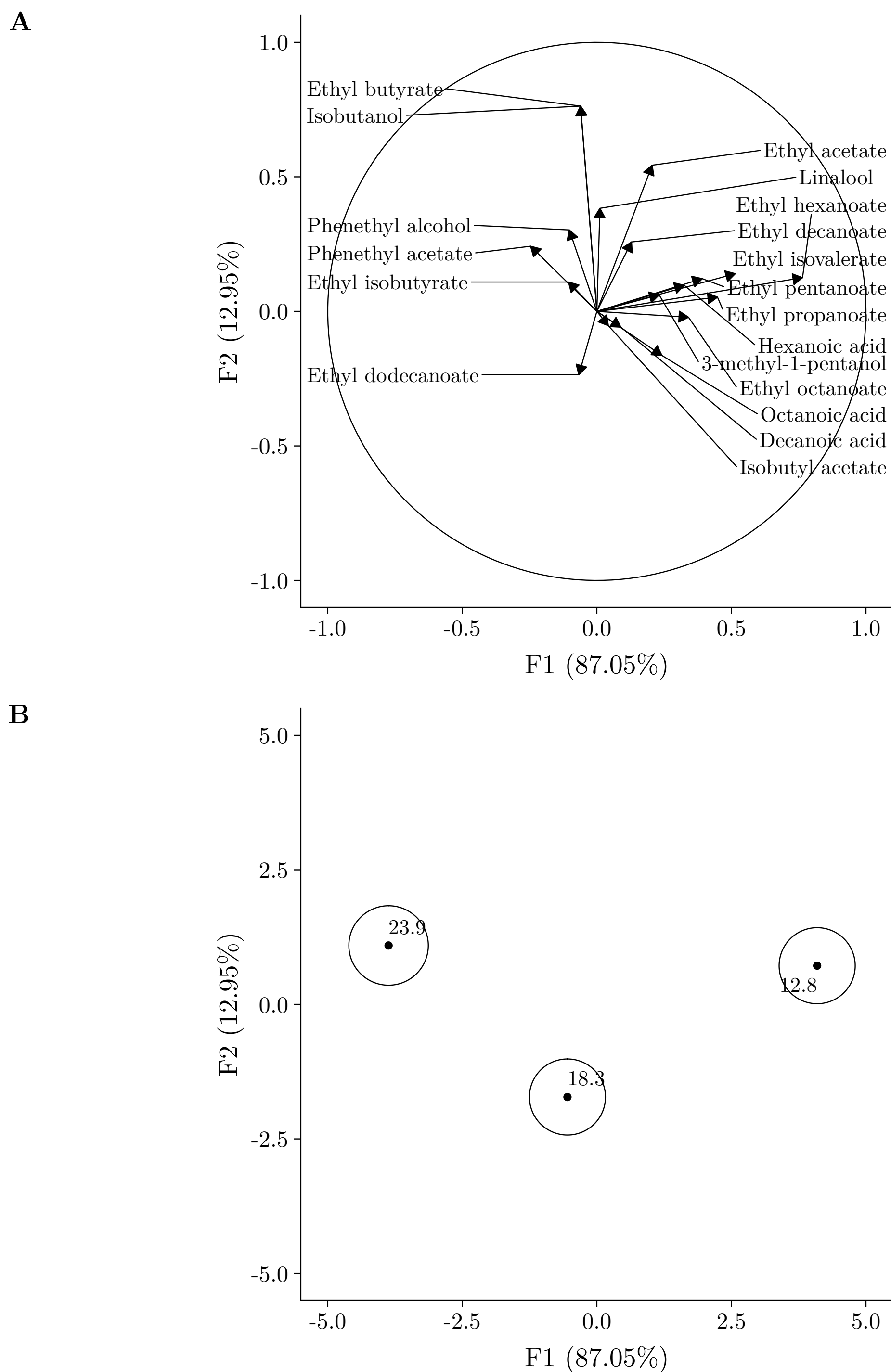

Figure A.19: Separation of fermentation temperatures using canonical discriminant analysis (CDA). Loadings for aroma compounds are in subfigure A, and scores are plotted on subfigure B. Circles represent 95 % confidence intervals surrounding the treatment means.

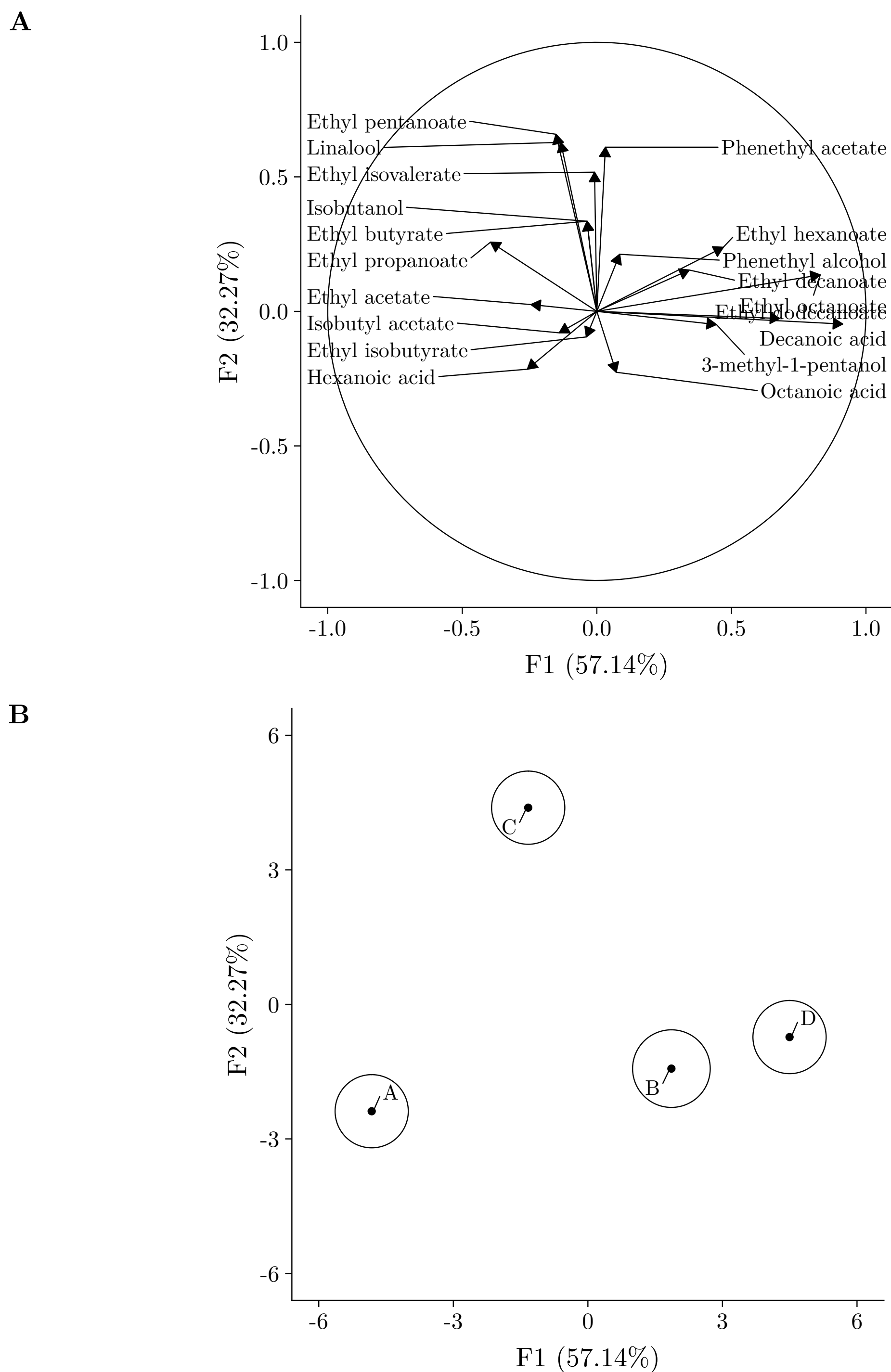

Figure A.20: Separation of nutrient schedules using canonical discriminant analysis (CDA). Loadings for aroma compounds are in subfigure A, and scores are plotted on subfigure B. Circles represent 95 % confidence intervals surrounding the treatment means.

Table A.3: p-Values ($\alpha = 0.05$) for effects of temperature, schedule, and interaction between temperature and schedule on aroma compounds.

	Temperature	Schedule	Interaction
Ethyl acetate	0.00	0.01	0.18
Ethyl propanoate	0.03	0.03	0.74
Ethyl isobutyrate	0.70	0.86	0.03
Isobutyl acetate	0.95	0.82	0.58
Ethyl butyrate	0.00	0.00	0.84
Isobutanol	0.00	0.00	0.84
Ethyl isovalerate	0.00	0.01	0.54
Ethyl pentanoate	0.00	0.00	0.00
Ethyl hexanoate	0.00	0.00	0.00
3-methyl-1-pentanol	0.39	0.10	0.47
Ethyl octanoate	0.00	0.00	0.00
Linalool	0.08	0.00	0.16
Ethyl decanoate	0.42	0.19	0.95
Phenethyl acetate	0.10	0.00	0.33
Hexanoic acid	0.17	0.28	0.40
Phenethyl alcohol	0.35	0.17	0.92
Ethyl dodecanoate	0.28	0.00	0.11
Octanoic acid	0.27	0.51	0.14
Decanoic acid	0.41	0.00	0.04