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

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Abstract

Honey wine, also known as mead is made from honey, water and yeast. This beverage has experienced resurgence in popularity but to be competitive producers must consistently produce high quality products. This study focused on the effect of three fermentation temperatures and four different nutrient addition schedules on mead quality and fermentation parameters. Basic mead chemistry parameters were measured. Aroma composition was determined using HS-SPME-GCMS and descriptive analysis was used to determine the sensory differences of the meads. Significant differences were found for treatments receiving nutrients versus those receiving no nutrients. Aroma composition showed significant differences based on fermentation temperature and nutrient schedule. In particular, the coolest fermentation temperature resulted in meads with greater amounts of esters. However, despite the fact that a significant difference in aroma composition was found these do not result in any large sensory differences, particularly for those ferments with nutrient additions. Only the control was significantly different from the other treatments based on sensory data, although certain trends were found based on fermentation temperature and nutrient addition schedule. These results show that mead makers can use nutrient schedules and fermentation temperature to not only significantly reduce time-to-market but also to 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.

ABBREVIATIONS

HS-SPME: Head Space-Solid Phase Microextraction; GCMS: Gas Chromatography Mass Spectrometry; TOSNA: Tailored Organic SNA; YAN: Yeast Assimible Nitrogen; CDA: Canonical Discriminant Analysis

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 [1]. Improvements in agricultural practices made grapes and barley more available throughout temperate regions in Europe, contributing to the decline of mead's popularity [2]. Mead, like cider, has been riding on the coat tails of craft brewing's recent explosive growth in the United States, with at least 236 wineries making at least one mead in 2015 [3]. 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. All other meads produced domestically are classified as "other than standard" wines because they include alternate flavorings, colorings, or sources of fermentable sugars [4]. This range of additional ingredients is necessary to produce mead styles such as melomels and metheglins [5]. 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 [6]. However, fermenting at higher temperatures can negatively impact production of desirable aroma compounds [7]. Additionally, heat treatment and adverse storage conditions can have negative consequences for quality of both honey [8], and mead [9]. 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 their mainder composing of proteins, amino acids, vitamins and minerals among other compounds [8]. Additional nutrients, particularly nitrogen, are

often supplied to the must to minimize the risk of stuck and sluggish fermentations [10]. Added nutrients can also influence amino acid catabolism, which has an influence on the formation of esters and other aroma compounds [11].

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 [12], and any remaining nutrient not consumed by the intended microbe is available to be used by spoilage organisms [13]. 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, California), 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 [14], 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 [15,16]. 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 24h, 48h and 72h 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) which also accounts for the nitrogen needs of the particularly east 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.

MATERIALS AND METHODS

Materials

Honey was donated by Queen Bee Apiaries (Corvallis, OR) with an initial sugar content of 82°Bx. *S. cerevisiae* EC-1118 (Lallemand, Montreal, Canada) was used for fermentation mad Fermaid-O (Lallemand, Montreal, Canada) was the nutrient used for treatments.

Treatments

Stainless steel glycol jacketed 100L fermentation tanks (AAA, The Dalles, Oregon, USA) 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 three fermenters, one control, and one for each of the nutrient addition schedules. Treatments represent combinations of fermentation temperatures and nutrient addition schedules, and are listed in Table 1.

The style of mead chosen for this work was traditional semi-sweet style (final mead with 11% ethanol (v/v) and 6.3 °Bx), as this is the most popular style of mead available [5]. To achieve the semi-sweet style, honey was diluted using tap water to ~28.4°Bx. After the honey was diluted it was inoculated with yeast (EC118) and nutrient, following the below described nutrient schedule. The yeast was rehydrated in 40°C tap water at a rate of 100 g/L. A total volume of 1 L of rehydrated yeast was prepared and after 30 minutes at temperature, 10mL was added to each treatment.

Three different nutrient addition schedules were tested. For each nutrient schedule a total of 100 mg/L of yeast assimilable nitrogen (YAN) was added to each ferment, as the starting diluted honey was lacking in nitrogen with only 32 mg/L YAN. Schedule A was the control and did not receive any nutrients. Schedule B was time-based, with additions occurring at 24h, 48h and 72h after pitch with each addition of 33.3 mg/L YAN. Treatment C had additions occurring at 24h, 48h and 72 h and a final addition at either 168h after pitching the yeast or 1/3 through fermentation, whichever came first [17], with each addition consisting of 25 mg/L YAN. For each addition, slurry of Fermaid-O and water was made so that after all additions the same amount of nitrogen and volume has been added to the ferments.

Fermentation and storage

Temperature and Brix (°Bx) for each fermenter were measured every 24h with a DMA 35 N density meter (Anton Paar, Graz, Austria). Fermenters 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 the above equation divided by the number of doses for each treatment. For addition purposes, slurry was formed in advance at a ratio of 10mL water to 1g of nutrient. This slurry was manually agitated before each addition in order to return the nutrients to suspension. Fermentations were moved to cold storage at 3 ± 1°C when they reached 6.3°Bx (the target for the semi-sweet mead style). Sulfur dioxide (5% SO_2 solution, $K_2S_2O_5$, Brew craft, Vancouver, Washington) was added to fermenters until 30 ppm free SO_2 was reached.

Chemical analysis

pH was measured using anion selective electrode (Mettler Toledo, Greifensee, Switzerland), and residual sugar was determined using the Rebele in process [17]. Ethanol content was calculated using the ASBC Beer – 4 methods [18]. Yeast amino nitrogen is a combination of both primary amino nitrogen and ammonia. Primary amino nitrogen was measured with an o-phthaldialdehyde/N-acetyl-L-cysteine (OPA/NAC) assay [19], and ammonia was measured using an enzymatic test kit (R-Biopharm AG, Darmstadt, Germany). Free SO_2 was measured by the aspiration method [17].

Aroma chemical analysis

Chemicals: Purity and manufacturer for standards used in gas chromatograph-mass spectrometry (GCMS) calibration curves and internal standards are found in Table 2. Other chemicals used include ethanol (HPLC grade, Pharmco-AAPER), sodium hydroxide (NaOH, 99%, Macron) and Milli-Q water from Millipore Continental water system.

Sample preparation: Mead samples were defrosted at room temperature prior to analysis. Samples were diluted with a model solution (saturated NaOH, 10 % ethanol and 1g/L citric acid) prior

to analysis. Each mead (0.9mL) was added to 7.8mL of model solution. In 20mL amber glass, screw cap vials (22.5x75.5mm, Sigma-Aldrich), followed by 150µL of both isotopically-labeled internal standard solutions (Table 2). Vials were capped tightly with headspace screw caps (Restek, Bellefonte, Pennsylvania). Samples were held in a stack cooler at 7°C until analyzed.

Head space-Solid phase Microextraction Gas Chromatography Mass Spectrometry (HS-SPME GCMS) – Method was adapted from method one found in [20]. A three-phase Stable flex fiber (50/30µm DVB/CAR/PDMS, 2cm, 24Ga, Sigma-Aldrich) was used for HS-SPME. Prior to analysis, the fiber was conditioned at 250°C for 1h. 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 (5son, 2soff). 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 10min 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 min length, 0.25mm ID, and 0.25µm of film thickness connected in sequence to an Rxi-1ms, 15min length, 0.25mmID, and 0.25µm 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^{O}C for 10min which then increase data 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 32.2k Paanda linear velocity of 21.5cm/s. Total run time was 73-75min. GCMS transfer line temperature was 250°C and ion source was 200°C. Spectra were acquired using electron impact ionization (EI,70eV) in a full scan mode from 3.8min to 65min with a scan range of 50 to 303m/z and an event time of 0.20s.

Identification of all compounds was based on comparison of retention time and spectra with pure standards and NIST11 database [20]. Quantification for all compounds and validation of method was the same as described in [21].

Sensory analysis

Sensory analysis was performed at the Oregon State University Arbuthnot Dairy Center (Corvallis, Oregon) on Monday, Wednesday, and Friday during the last two each day weeks of May in 2018. The panelists participated in six 1h 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 atleast 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 3). All standards were placed in black INAO tasting lasses [22], 20min 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 descript or for sessions one and two. At the end of these condsession, panelists evaluated the intensities of the standards on a 100mm visual analogue 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 warm-up mead.

Mead bottles were opened and poured into black ISO tasting glasses [22], 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 100mm 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, Utah).

Statistical analysis

Wine chemistry parameters and aroma chemistry results were examined with analysis of variance and Tukey's HSD using R version 3.4.4 [23]. Canonical discriminant analysis (CDA) was performed on the sensory and aroma chemistry data using the XLSTAT (Adding soft, New York, NY, USA).

RESULTS AND DISCUSSION

Fermentation

The time to complete fermentation was significantly different (α=0.05) based on temperature and nutrient schedule (Figure 1, Table 1). A significant interaction was also observed between these two parameters. As anticipated fermentations that were warmer and that had nutrient additions completed fermentation significantly faster than fermentations at cooler temperatures. This has been shown previously in mead work with mead [24], and for beer [25], and cider [26] fermentations.

Significant differences were found for several basic chemical parameters including pH, residual sugar and YAN (Table 1). 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. The ethanol content for all treatments was within the accepted range [5], for standard meads and was 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.

Aroma chemistry

The aroma composition of the meads was found to differ based on temperature and nutrient schedule. Three of the compounds, isoamylacetate, 2-nonanone, and nonanoic acid, were not detected in any treatment (Table 4). Eleven compounds were detected in amounts which were not statistically significant across treatments. Eight compounds were impacted due only

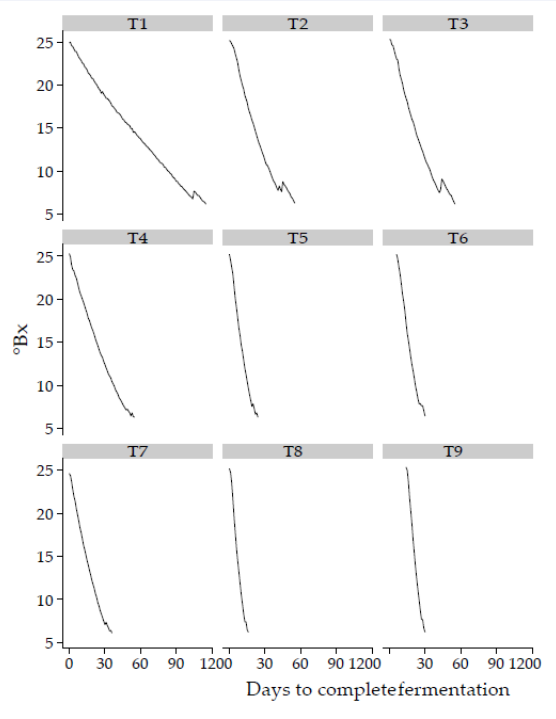

Figure 1 Fermentation curves for each treatment.

Table 1: Wine chemistry by treatment.

	Fermentation Temp (°C)	Nutrient Schedule[1]	Days to complete fermentation (d)	Ethanol%(v/v)	YAN (mg/L)	Residual sugar(g/L)	pH
Diluted honey					32	318.6	3.57
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}
T3	12.8	C	44 ± 7^{a}	11.14 ± 0.36	13.4 ± 1.1^{a}	64.4 ± 8.7^{ab}	3.59 ± 0.03^{a}
T4	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}
T5	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}
T6	18.3	C	20 ± 2^{bc}	11.16 ± 0.04	12.5 ± 1.9^{a}	63.7 ± 6.3^{ab}	3.49 ± 0.01^{cd}
T7	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}
T8	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}
T9	23.9	C	14 ± 1^{c}	11.54 ± 0.02	10.7 ± 2.0^{a}	61.6 ± 3.7^{a}	3.46 ± 0.02^{d}

to nutrient schedule, five compounds were impacted due to temperature and one compound was impacted due to the interaction of both parameters (Table 5). Only six compounds were detected in levels higher than their known aroma thresholds (Table 4).

Temperature effects

CDA resolved 100% of the variance in both factors when using fermentation temperature as the grouping factor while considering all detected compounds (Figure 2). Significant separation for all three temperatures was observed. Treatments fermented at 23.9°C were characterized by ethylisobutyrate, linalool, phenethylacetate, and phenethylalcohol, while treatments fermented at 18.3°C were characterized by ethyldodecanoate. Treatments fermented at 12.8°C were characterized by arrange of compounds including isobutylacetate, ethylpropanoate, hexanoicacid, ethyloctanoate, ethyl hexanoate, and 3-methyl-1-pentanol.

Lower fermentation temperature was related to greater amounts of esters in mead, including ethyloctanoate and ethyldecanoate. 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 [27]. Ethylesters were also founding greater amounts in those meads with nutrient additions versus those without, which was anticipated as the added nutrient provides more substrate for ester synthesis [28].

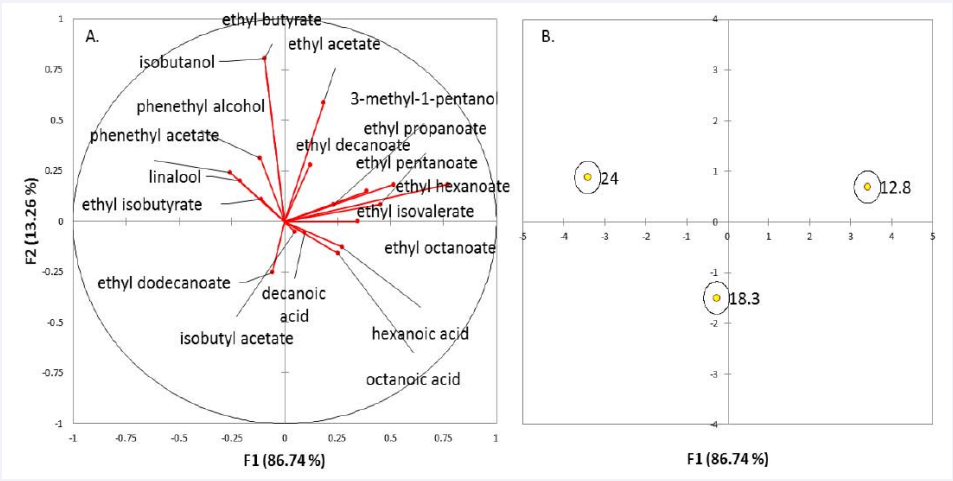

Figure 2 Separation of fermentation temperatures using CDA. Loadings for aroma compounds are in sub figure A, and scores are plotted on subfigure B. Circles represent 95% confidence intervals surrounding the treatment means.

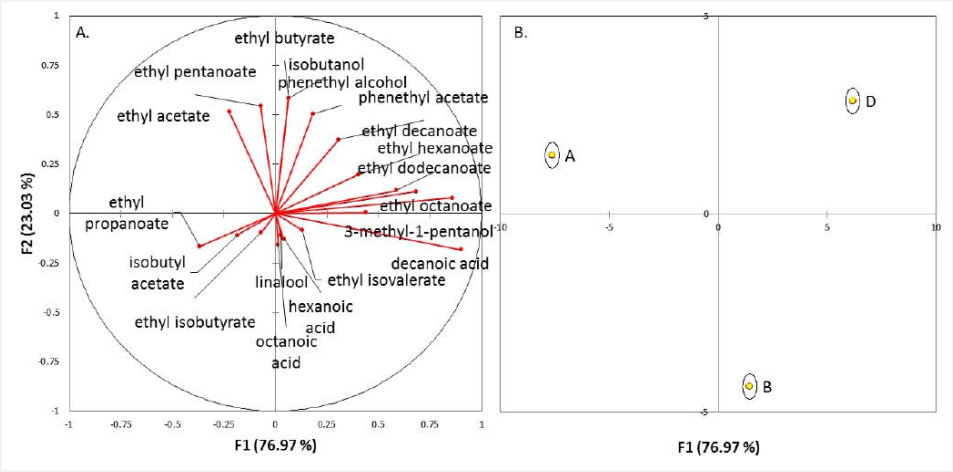

Figure 3 Separation of nutrient schedules using CDA. Loadings for aroma com-pounds are in sub figure A, and scores are plotted on subfigure B. Circles represent 95% confidence intervals surrounding the treatment means.

Fermentation temperature was also found to greatly impact the amount of phenethylalcohol and phenethylacetate in the finished meads. Phenethylalcohol is known to impart "floral" aroma and phenethylacetate, particularly at high concentrations, has a "honey-like" aroma [29]. 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 [30]. Higher temperatures are known to favor higher alcohols, such as phenethyl alcohol [31], and phenethyl acetate is formed from yeast enzymatic reactions [32]. While the higher fermentation temperature treatment was characterized by these 2 compounds (Figure 2), there was actually no significant difference between the temperature treatments (Table 5), which suggests that it is the lack of esters in the higher fermentation temperature that brings out this characterization.

Nutrient effects

100% of the differences between nutrient schedules for all detected compounds were expressed in both factors with CDA (Figure 3). Nutrient schedules showed significant separation. Schedules B and C were separated from schedule A along the F1 axis, with separation based on longer chain esters, acetate esters, and alcohols. The F2 axis then separated schedules B and C from each other based on shorter chain compounds. Schedule B was characterized by linalool, hexanoic and octanoic acid. Schedule C was characterized by a range of compounds, including ethyl hexanoate, ethyl octanoate, ethyl decanoate, and ethyldodecanoate, while schedule A was characterized by ethyl propanoate and ethyl acetate.

As stated previously, 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 [32]. Therefore the aroma compositional differences seen that differentiate schedules B and C may be due to the complex production pathways that may be altered based on nutrient availability at different times of fermentation.

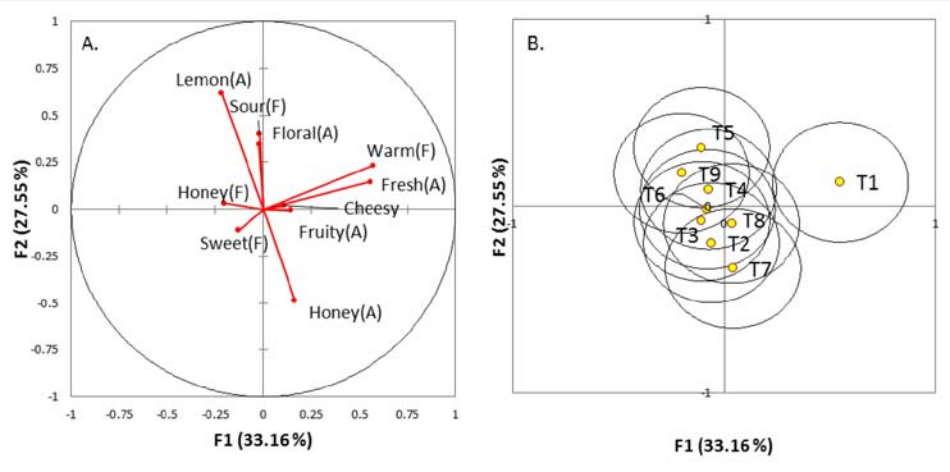

Figure 4 Separation of treatments using CDA. Loadings for sensory attributes are in sub figure A, and scores are plotted on subfigure B. Circles represent 95% confidence intervals surrounding the treatment means.
Abbreviations: CDA: Canonical Discriminant Analysis

Table 2: Quantification parameters.

Analyte	ISTD	Ret. Time(min)	Target Ion m/z	Confirming Ions m/z	Calibration Range (µg/L)	Purity	CASNo.	Source
d3-ethylacetate	(1)	5.04	64	76,133		99	90691-33-1	CDN
d3-ethylbutyrate	(2)	12.49	74	89,61		99.8	113435-99-7	CDN
2-methyl-d3-propyl-d4alcohol	(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-ethylhexanoate	(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-ethyloctanoate	(7)	30.15	91	105,66		98	1219798-38-5	CDN
d4-diethylsuccinate	(9)	35.98	105	77,122		99	52089-62-0	CDN
d11-hexanoicacid	(10)	39.72	63	77,93		98.5	95348-44-0	CDN
d17-nonanoicacid	(11)	47.82	77	63,125		99	130348-94-6	CDN
ethylacetate	1	5.08	61	70,88	0–2233.32	99.5	141-78-6	BDH
ethylpropanoate	2	7.93	57	74,102	0–260	99	105-37-3	Aldrich
ethylisobutyrate	4	9.52	71	88,116	0–262.22	99	97-62-1	Aldrich
isobutylacetate	1	11.19	56	73,86	0–47.02	99	110-19-0	Aldrich
ethylbutyrate	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
ethylisovalerate	4	14.92	88	57,60	0–229.33	98+	108-64-5	Sigma-Aldrich
isoamylacetate	1	16.98	70	55,61	0–222.89	99+	123-92-2	Sigma-Aldrich
ethylpentanoate	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
ethylhexanoate	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
ethyloctanoate	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
diethylsuccinate	9	36.16	101	129,128	0–566.11	99+	123-25-1	Sigma-Aldrich
ethyldecanoate	7	37.30	88	101,155	0–230.22	99+	110-38-3	Aldrich

phenethylacetate	3	40.04	104	91,78	0–566.11	98+	110-45-7	Sigma-Aldrich
hexanoicacid	10	40.10	60	73,87	0–581.11	99	142-62-1	Aldrich
phenethylalcohol	3	42.02	91	92,122	0–4533.33	99+	60-12-8	Aldrich
ethyldodecanoate	7	43.56	101	88,70	0–264	98+	106-33-2	Aldrich
octanoicacid	10	45.59	60	73,101	0–1146.67	98+	124-07-2	Sigma-Aldrich
nonanoicacid	11	48.19	73	60,115	0–232.89	98	112-05-0	Sigma-Aldrich
decanoicacid	11	50.66	60	73,129	0–226	98	334-48-5	Sigma

Table 3: Aroma (A) and flavor (F) standards for the chosen descriptors.

Attribute	Standard	Preparation Notes
Cheesy (F)	parmesan cheese	grated
Floral (A)	gardenia, jasmine essential oils	83 mL/L of each oil in mineral oil
Fresh (A)	plain yogurt, lettuce and cucumber	blended together prior to serving
Fruity (A)	fruit cocktail in pear juice	use both chunks and juice
Honey (A, F)	honey	(same honey from meads)
Lemon (A)	lemon, rind and pulp	chopped into small pieces
Sour (F)	tartaric Acid	3/g L in distilled water
Sweet (F)	sucrose	100 g/L in distilled water
Warm (F)	190 proof alcohol	20% solution diluted with distilled water

Table 4: Concentration (µg/L) of aroma compounds in each treatment.

Compound	T1	T2	T4	T5	T6	T8	T9	T10	T12	aroma threshold (µg/L)	reference
Acetate esters											
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}	495.0 ± 51.9^{abc}	859.0 ± 61.0^{abc}	508.1 ± 11.5^{abc}	482.0 ± 311.4^{abc}	7,500	[40]
Isobutyl acetate	2.0 ± 1.7	1.9 ± 1.7	n.d.	0.9 ± 1.5	1.0 ± 1.8	0.9 ± 1.5	0.8 ± 1.4	n.d.	0.9 ± 1.5	1,600	[41]
Isoamyl acetate	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1,100	[42]
Phenethyl acetate	20.8 ± 3.2	18.0 ± 5.4	19.3 ± 1.8	15.7 ± 1.8	16.2 ± 3.2	21.8 ± 4.5	18.2 ± 1.8	19.2 ± 0.2	23.3 ± 3.1	250	[40]
Ethyl esters											
Ethyl propanoate	24.6 ± 2.3	23.0 ± 2.4	23.6 ± 6.4	20.9 ± 3.5	20.5 ± 2.6	13.0 ± 11.5	21.0 ± 1.7	16.2 ± 1.8	11.6 ± 10.0	1,840	[42]
Ethyl isoburyate	n.d.	3.3 ± 5.8	n.d.	3.3 ± 5.8	6.6 ± 5.7	n.d.	6.9 ± 6.0	n.d.	7.3 ± 6.4	15	[40]
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}	518.0 ± 78.8^{abc}	641.1 ± 86.7^{a}	530.1 ± 42.6^{abc}	665.2 ± 28.4^{a}	20	[40]
Ethyl isovalerate	5.5 ± 4.8	5.6 ± 4.8	5.8 ± 5.0	n.d.	2.7 ± 4.7	n.d.	n.d.	0.5 ± 0.7	3.0 ± 5.1	18	[41]
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}	0.8 ± 0.1^{a}	0.7 ± 0.2^{a}	0.4 ± 0.1^{ab}	0.6 ± 0.2^{ab}	10	[42]
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}	29.2 ± 1.2^{ae}	17.0 ± 1.2^{c}	26.5 ± 2.1^{ef}	23.6 ± 1.9^{df}	5	[40]
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}	71.2 ± 8.3^{a}	34.2 ± 4.1^{c}	60.3 ± 3.3^{ad}	58.7 ± 5.2^{ad}	2	[40]
Ethyl decanoate	3.4 ± 1.1	3.9 ± 3.4	6.7 ± 4.2	1.5 ± 1.3	2.1 ± 1.8	4.7 ± 4.5	3.1 ± 1.7	4.7 ± 0.3	4.9 ± 2.3	2	[40]
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}	29.9 ± 14.7^{b}	6.3. ± 1.8^{a}	14.1 ± 1.3^{ab}	15.3 ± 4.6^{ab}	2,000	[41]
Alcohols											

Isobutanol	2318 ± 102.9^{ab}	2061.7 ± 364.9^{abc}	2506.5 ± 150.2^{ab}	1902.1 ± 140.6^{bc}	1595.5 ± 124.7^{c}	2072.0 ± 315.1^{abc}	2564.3 ± 347.0a	2120.3 ± 170.2^{abc}	2660.9 ± 113.5^{a}	40,000	[40]
3-Methyl-1-pentanol	n.d.	2.5 ± 1.0	4.7 ± 5.1	n.d.	0.9 ± 1.6	2.3 ± 2.9	0.7 ± 0.6	1.9 ± 0.3	0.7 ± 0.7	n/a	
Phenethyl alcohol	1141.9 ± 294.5	1033.7 ± 181.5	1411.6 ± 609.8	941.2 ± 170.6	813.5 ± 168.3	1377.9 ± 124.0	1460.4 ± 445.3	1139.8 ± 20.1	1390.0 ± 161.4	10,000	[40]
Fatty acids											
Hexanoic acid	373.1 ± 49.6	51.3 ± 2.2	73.7 ± 49.6	53.8 ± 54.8	74.4 ± 40.2	17.1 ± 3.4	31.1 ± 10.9	16.2 ± 0.5	21.5 ± 8.9	3,000	[40]
Octanoic acid	414.2 ± 53.4^{a}	51.7 ± 12.0^{b}	439.0 ± 70.0^{a}	65.5 ± 74.5^{b}	714.3 ± 71.6^{a}	12.1 ± 2.3^{b}	25.7 ± 8.5^{b}	12.9 ± 0.4^{b}	16.6 ± 7.6^{b}	4,500	[41]
Nonanoic acid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	3,000	[40]
Decanoic acid	15.3 ± 1.7^{c}	32.5 ± 4.0^{a}	33.9 ± 4.0^{a}	17.5 ± 2.0^{bc}	26.1 ± 3.5^{ab}	32.5 ± 2.7^{a}	16.6 ± 3.4^{bc}	32.1 ± 2.5^{a}	31.4 ± 3.4^{a}	15,000	[40]
Misc.											
2-nonanone	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	200	[40]
Linalool	0.9 ± 0.8	0.5 ± 0.2	0.7 ± 0.4	0.1 ± 0.1	0.0 ± 0.1	0.4 ± 0.4	0.1 ± 0.1	1.1 ± 0.0	n.d.	15	[40]

*Superscript letters denote significant differences calculated by tukey multiple comparison. n.d. = not detected

Table 5: *p*-values (α=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
Isobutylacetate	0.66	0.63	0.55
Phenethylacetate	0.31	0.06	0.34
Ethyl propanoate	0.05	0.12	0.69
Ethylisobutyrate	0.19	0.78	0.16
Ethy lbutyrate	0.00	0.00	0.97
Ethylisovalerate	0.03	0.76	0.82
Ethylpentanoate	0.01	0.00	0.02
Ethylhexanoate	0.00	0.00	0.00
Ethyloctanoate	0.00	0.00	0.00
Ethyldecanoate	0.32	0.11	0.94
Ethyldodecanoate	0.38	0.00	0.15
Isobutanol	0.00	0.00	0.97
3-Methyl-1-pentanol	0.32	0.09	0.43
Phenethyl alcohol	0.14	0.05	0.69
Hexanoic acid	0.21	0.31	0.41
Octanoic acid	0.30	0.81	0.16
Decanoic acid	0.52	0.00	0.20
Linalool	0.01	0.80	0.01

Sensory analysis

CDA explained 60.7% of the difference in the first two factors when using treatment as the grouping factor (Figure 4). With the exception of Treatment 1, all treatments were not significantly different from each other. Treatment1 was correlated with the attributes "warm" and "fresh", while there maining treatments were clustered along an axis consisting of the attributes "lemon" and "honey aroma". The differences among there maining treatments along this axis were not statistically significant.

Using fermentation temperature as the grouping factor allowed CDA to explain100% of the difference in two factors (data not shown). 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 (data not shown).No significant differences were found based on fermentation temperature or nutrient schedule, as all confidence intervals were overlapping.

Treatment 1 was the only one which was significantly different than the rest of the treatments, with characteristics of "warm'' and "fresh''. This treatment was the colder fermentation 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, which result in fruity and fresh aromatics [27].

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 [7,33]. All show similar trends with this study's results, that cooler temperatures result in "fresh" and "fruity" aromatics (Figure 3). The lack of sensory differentiation for the two

Warmer temperature suggests that the temperature difference was not large enough to impact sensory perception. Much work shows in consistent chemical and sensory results based on fermentation temperature. This is most likely due to the choice of yeast and fermentable sugar source [34,35].

There is a large amount of work previously conducted with regard to the effects of nutrient addition on sensory of fermented beverages. Multiple studies have found an increase in fruitiness after adding nutrients [36,37], 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 [14].

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 [38]. 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 [39-41].

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.

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