International Journal of Food Science and Biotechnology
2026, Vol. 11, No. 2, pp. 85–96
https://doi.org/10.11648/j.ijfsb.20261102.11

Research Article

Effect of Honey-to-Water Ratio and Yeast Inoculum Size on the Physicochemical and Sensory Properties of Honey Wine '*Mies*'

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Abstract

Mies is a traditional Tigreayan honey wine produced typically from honey, gesho, and water, prepared with varying ingredient proportions and requiring prolonged fermentation. To shorten the fermentation duration, producers for retail are increasingly using commercial yeasts. This study aimed to determine the optimal yeast inoculum size and proportion of honey-to-water ratio, and subsequently evaluate their effects on the physicochemical and sensory properties of mies, as well as on fermentation time. Honey-to-water ratios of 1: 2.45, 1: 4.25, and 1: 6.0 (w/v) were used based on survey data (Mean ± SD), and treatments were inoculated with 3%, 4%, and 5% (w/w) yeast relative to honey weight. The honey used contained 18.8% moisture, 81.2% total soluble solids, pH of 3.93, 3.57 g/kg hydroxymethylfurfural, and 0.17% ash. Must samples showed pH, titratable acidity, and °Brix values ranging from 3.81-3.91, 3.17-4.43 g/L, and 15.14-32.58%, respectively. Final mies samples exhibited TSS of 3.95-18.45%, pH of 3.51-3.74, titratable acidity of 3.56-5.58 g/L, and ethanol levels of 6.2-8.75%. All physicochemical and sensory parameters showed significant difference ($p<0.05$). Fermentation time ranged from 24 to 120 hours, strongly influenced by the proportion of honey used. Honey-to-water ratios significantly affected the physicochemical properties of the product, whereas sensory quality was influenced by both the substrate ratio and yeast inoculum size. Overall, mies produced with a moderate 1: 4.25 honey-to-water ratio exhibited superior sensory performance and was most preferred by panelists followed to the spontaneously fermented control.

Keywords

Mies, Physicochemical Properties, Sensory Attributes, *S. cerevisiae*, Fermentation

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Received: 14 May 2026; **Accepted:** 27 May 2026; **Published:** 10 June 2026

1. Introduction

Fermentation, a low-input process, has been used to provide inexpensive, safe, and nutritious foods for low-income people [35]. Among fermented products, alcoholic beverages are widely consumed and deeply embedded in human dietary traditions. In Ethiopia, they are predominantly produced at the household level and consumed in considerable quantities [23]. Beyond their dietary role, these beverages enhance food security, generate income, and sustain socio-cultural practices. Globally, their production contributes substantially to the world economy [38]. Consequently, communities across the world continue to produce and consume traditional fermented beverages prepared from locally available materials using indigenous knowledge systems [1, 38]. *Mies* is an indigenous honey wine originating from Tigray, produced primarily from honey, water, and the leaves and twigs of *gesho* (*Rhamnus prinoides L.*). It is common in religious and secular social events, including weddings, birthdays, festivals, and funerals. In recent years, *mies* has increasingly appeared in resorts, grocery stores, hotels, and restaurants in urban and semi-urban areas, expanding its contribution to household and small-scale enterprise income. Traditional alcoholic beverages in Ethiopia reportedly hold an almost equal market share with modern industrial beverages [19, 23]. Ethiopia is one of Africa's largest honey producers and ranks tenth globally [37]. About 80% of the country's honey production is utilized for *mies* production [25]. Honey is a complex natural product containing nearly 200 constituents [22]. Owing to this composition, honey provide nutritional and therapeutic properties and is often regarded as a medicinal food [31]. As a honey wine, *mies* may provide health benefits derived from honey antioxidants, particularly polyphenols and flavonoids [30, 36], as well as additional metabolites generated during microbial fermentation and bioactive compounds extracted from *gesho* [24, 54]. Nutritionally, *mies* consists primarily of carbohydrates with trace amounts of proteins and fats [9, 41]. High-quality *mies* is yellowish in color, sweet sour with an alcoholic flavor, and exhibits a fizzy, cloudy appearance. Its ethanol content typically ranges from 5% to 13.16% (v/v) [9, 59]. The quality of honey is critical for successful *mies* production. Producers in Tigray often prefer red or dark, crude, and aged honey due to its superior fermentability and the desirable yellow kind it imparts to the final product. Dark honey varieties have been reported to contain essential constituents that support fermentation and microbial metabolism [4, 43, 45]. However, hydroxymethylfurfural (HMF) presents a safety concern and should not exceed 40 mg/kg, or 80 mg/kg for tropical honey [18]. *Gesho* contributes characteristic bitterness, aroma, and antimicrobial properties that regulate microbial dynamics during fermentation [3, 7, 13]. In some areas, *tseddo* (*Rhamnus tseddo*) is used either as a substitute or in combination with *gesho* to produce a stronger beverage while reducing honey masses and requirements. Additional roots, barks, herbs, and spices are also incorporated to enhance flavor, potency, and sanitation of equipment [9]. Thus, perceived strength and consumer preference may depend not only on ethanol content but also on these additives. Historically, *mies* dates back over two millennia to the era of the Kingdom of Aksum [32], underscoring its sociocultural importance. Traditionally fermentation occurs spontaneously through wild microorganisms present in raw materials and utensils, without controlled inoculation [9, 10, 24]. This process is labor-intensive, often requiring weeks to months, thereby delaying marketing. To shorten fermentation time, commercially available *S. cerevisiae* has increasingly been applied for partial or complete inoculation, despite its limited alcohol tolerance. While commercial yeasts are employed in mead production [42, 57], fermentation performance depends on honey characteristics, yeast strain, must composition, supplementation, temperature, and duration [36, 38, 40]. Inappropriate inoculum size may also adversely affect aroma synthesis and lead to off-flavors [29, 44]. Moreover, variations in preparation methods, ingredient ratios, additive types and dosages, and fermentation conditions among households contribute to inconsistencies in physicochemical and sensory properties. Therefore, this study aimed to determine the optimal yeast inoculum size, ingredient variety and proportion, preparation method, and fermentation duration to improve the quality and consistency of *mies*. Thereby, their effect on the physicochemical properties and sensory acceptance.

2. Materials and Methods

2.1. Honey, Gesho and Yeast Sources

Five kilograms (5 kg) of fresh red honey from modern hives were purchased from beekeeping farmers in Ahferom Wereda, Central Tigray Zone. *Gesho* leaves, commercial instant dry yeast (Angel brand, *Saccharomyces cerevisiae*) and 20 liters of packaged water were purchased from the local market in Mekelle City.

2.2. Survey Data Collection

Important information about the indigenous production methods of *mies* was collected through a survey (questionnaire) from 29 respondents. The survey aimed to identify the variety of ingredients, their proportions or doses commonly used in *mies*, and to apply the usual baseline methods in production. The survey was conducted in five cities and towns of Tigray: Mekelle, Adigrat, Hagere Selam, Abyi Adi, Axum, and Shire, as these are among the primary locations for *mies* production. Data were purposefully collected from households primarily engaged in *mies* production for marketing purposes. The questionnaire was prepared in Tigrigna (the local language) to facilitate comprehension for literate respondents; oral interviews were conducted with those unable to read.

2.3. Physicochemical Analysis of Honey

Analysis of Moisture content (MC): MC was determined by oven-drying a weighed sample in a crucible at 100-110 ℃ overnight [8, 49]. The weight loss was recorded as MC and calculated using the following formula:

$$\text{Moisture (\%)} = \frac{\text{Weight of fresh sample} - \text{Weight of dry sample}}{\text{Weight of fresh sample}} \times 100 \quad (1)$$

Analysis of Total soluble solids (TSS): TSS were measured using a digital Abbe refractometer, cleaned with distilled water and calibrated to zero at 20 ℃. A drop of sample was placed on the prism plate, and the reading displayed was recorded directly as °Brix [49].

Analysis of pH: It was determined by pH meter calibrated with buffers at pH 4 and 7. Sample solution was prepared by dissolving 10g of honey in 75ml of distilled water, and then taken in the beaker and inserted pH meter [8, 18].

Analysis of Ash content: It was determined by weighing 10 g of sample into a silica crucible and incinerating it in a muffle furnace at 500 ℃ for 3–5 h [8, 49]. The residue was cooled in a desiccator, weighed, reheated for 30 min, and reweighed until a constant weight was obtained. Ash percentage was calculated as follows:

$$\text{Ash (\%)} = \frac{Weight\ of\ sample\ after\ ashing}{Weight\ of\ fresh\ sample\ taken} \times 100 \quad (2)$$

Analysis of hydroxymethylfurfural (HMF): HMF content was determined by the White method using a UV–spectrophotometer [34]. Five grams of honey were dissolved in a 50 mL volumetric flask, treated with 0.5 mL each of Carrez I and Carrez II solutions, diluted to volume with distilled water, homogenized, and filtered (first 10 mL discarded). From the filtrate, 5 mL aliquots were transferred into test tubes; sodium bisulfite solution was added to one as a blank, while distilled water was added to the others. Absorbance was measured at 284 nm and 336 nm using 10 mm quartz cuvettes.

$$HMF(mg/kg) = \frac{(A284 - A336) \times 149.7 \times 5}{Weight\ of\ sample\ taken} \quad (3)$$

Where: A284 = absorbance reading at 284 nm; A336 = absorbance reading at 336 nm; 5 = nominal mass of sample.

$$Factor = 149.7 = \frac{126}{16830} x \frac{1000}{10} x \frac{1000}{5} \quad (4)$$

Where: 126 = molecular weight of HMF, 16830 = molar absorption coefficient of HMF at 284 nm, 1000 = mg/g 10 = cL/L, 5 = nominal test portion weigh.

2.4. Birzi and Must Preparation and Mies Fermentation

Birzi (honey-to-water solution) was prepared by diluting 0.408 kg (P1), 0.235 kg (P2), and 0.165 kg (P3) of honey in 1 L of water, stirred until fully dissolved, and filtered to remove wax and impurities. *Gesho* was added at 0.024 kg (P1), 0.014 kg (P2), and 0.010 kg (P3) based on the mean honey-to-*gesho* ratio. Each treatment was inoculated with 3%, 4%, or 5% (w/w) yeast relative to honey weight [28]. The control (P2 without yeast) was prepared two weeks earlier to allow spontaneous fermentation and synchronize sensory evaluation. Fermentation was conducted in 2 L plastic containers at 19.2–21.5 ℃ with daily stirring. After fermentation, samples were filtered, racked, matured, and re-filtered prior to sensory evaluation.

2.5. Physicochemical Analysis of Must and Mies

Analysis of Degree of Brix/TSS: It was measured using digital Abbe refractometer, after calibrated according to the manufacturer's instructions at 20 ℃. The sample was placed on the prism, then readings was recorded as TSS [2, 57].

Analysis of pH: It was measured by immersing the glass electrode of a digital pH meter into 25 mL of each sample [26].

Analysis of Titratable acidity (TA): For TA determination, 10 mL of samples was titrated with a standardized solution of 0.1 N sodium hydroxide, and employed 3 to 5 droplet of phenolphthalein as an indicator. The results were expressed as tartaric acid content in accordance with Official Method 962.12 [8], and calculated as follows:

$$TA(\%) = \frac{0.1N\ NaOH\ (mL) \times Equivalent\ weight\ of\ acid}{Volume\ of\ sample\ (mL)} \quad (5)$$

Analysis of Alcohol (methanol): It was determined by the distillation method using a *Malligand* apparatus calibrated with standard alcohol. A measured volume of *mies* was placed in the upper chamber, while 100 mL of distilled water was added to the lower condenser and heated to boiling. When the temperature reached 75 ℃, the alcohol percentage was recorded directly from the scale [26].

2.6. Sensory Evaluation

Sensory attributes of *mies* were evaluated by a panel of 20 trained assessors, consisting of MSc. students and lecturers. The panelists, aged 27 to 40 years, were healthy and reported no allergies to alcohol. Samples were served in pure white glasses on designated tables, and panelists rinsed their mouths with potable water before each new serving. Evaluations were for aroma, taste, flavor, appearance, and overall acceptability, using a nine-point hedonic scale. Naturally fermented *mies* was included as the reference sample for sensory analysis.

2.7. Data Analysis

Survey data were analyzed using SPSS software (version 20) at a 95% confidence level to generate descriptive statistics (means and standard deviations). The statistical analyses of physicochemical and sensory attributes, were performed using

Minitab Software (version 21) at 5% significance level. Tukey's HSD test ($P < 0.05$) was applied to determine significant differences among treatment means. All experiments were conducted in duplicate following a completely randomized design (CRD). Data was collected for raw honey, and for each *birzi* and *mies* treatment in triplicate. The results are expressed as means ± standard deviations and percentages.

3. Results and Discussion

3.1. Results of the Survey

Survey data were obtained from 82.8% of mothers with more than two years of experience in commercial *mies* production, of whom 59.4% had completed grade five or above. Regarding raw materials, 55.2%, 13.8%, and 30% of producers used red, yellow, and mixed crude aged honey, respectively, mainly purchased directly from beekeepers, while others sourced it from honey shops. Honey, water, and *gesho* (leaves and/or stems) were identified as the core ingredients. However, *tseddo* (*Rhamnus tseddo*) is widely used in Adigrat either as a substitute for *gesho* or in combination with it. About 65.5% of producers used both *gesho* and *tseddo*, 27.6% used only *gesho*, and 6.9% used only *tseddo*. Since *tseddo* is considered more potent than *gesho*, substantial variation exists in ingredient ratios. The mean proportion was 4.25 ± 1.8 L of water and 0.06 ± 0.029 kg of *gesho* per kilogram of honey (mean ± SD). The parts of *gesho* used also varied; 79.3% used leaves, 3.4% stems, and 10.3% both, either whole or pounded, added immediately or after a few days. Additional ingredients such as legumes, turmeric (*Curcuma longa*), and rhubarb root (*Rheum rhabarbarum*) were incorporated in coarse or powdered form to improve color, flavor, and fermentation, particularly with white honey. Legumes serve as nitrogen sources essential for microbial growth [56]. Some respondents acknowledged secret additives intended to enhance quality, though often perceived by others as adulteration. Fermentation commonly lasted one to three months (58.6%), depending on honey type and temperature, causing marketing delays. Although some producers consider commercial yeast use immoral, 48.3% applied commercial yeast or *embula* (backslop starter) especially for sweet *mies* during holidays. *S. cerevisiae* has limited alcohol tolerance [47], and is mainly used to initiate rapid fermentation, allowing natural microbial to complete the process. Thus, producers prefer crude aged honey to support microbial adaptation [58]. Quality variation was attributed not only to ingredient types and ratios but also to preparation methods, equipment sanitation using (*grawa leaves* (*Vernonia amygdalina Del*.), *weira (Olea europaea* subsp. *Cuspidate*, ash, detergents, and *gesho*), fermentation practices, aging duration, and individual skill [14, 26]. In this study, commonly used ingredients and traditional methods were applied at laboratory scale while considering observed variations.

3.2. The Physicochemical Properties of Honey and Birzi

Freshly harvested red honey was deliberately selected to evaluate the fermentation performance of *S. cerevisiae* under controlled conditions, thereby eliminating interference from indigenous wild microbial commonly associated with aged honey used in traditional *mies* production. Physicochemical characterization confirmed substrate safety and quality, with particular emphasis on hydroxymethylfurfural (HMF) and moisture content as critical quality indices [21, 33]. All measured parameters complied with the standards of the Codex Alimentarius Commission [18], validating the honey's suitability for controlled fermentation experiments.

Table 1. *Physicochemical properties of honey, and must prepared with three honey-to-water proportions ratios.*

Item	**Parameters**				
Honey	**MC (%)**	**TSS (°Brix) (%)**	**PH**	**HMF (mg/Kg)**	**Ash (%)**
	18.8±0.1	**81.2±0.1**	**3.93±0.006**	**3.57±0.16**	**0.17±0.02**
Must	pH	TA (g/L)	°Brix (%)		
P1	3.81±0.006 [c]	4.43±0.04 [a]	32.58±0.188 [a]		
P2	3.85±0.01 [b]	3.51±0.1 [b]	21.27±0.071 [b]		
P3	3.91±0.006 [a]	3.17±0.05 [c]	15.14±0.031 [c]		
C	3.85±0.006 [b]	3.54±0.057 [b]	21.50±0.1 [b]		

Note: Data are expressed as mean ± SD. All analyses were done in triplicate. Mean values in the same column with different superscript are significantly different at p<0.05. P1 (1: 2.45); P2 (1: 4.25); P3 (1: 6.05); & C (1: 4.25) honey-to-water ratios (w/v) respectively (before yeast inoculated), where; P1=proportion ratio one, P2=proportion ratio two, P3=proportion ratio three and C= control.

Prior to yeast inoculation, the *birzi* was analyzed for pH, titratable acidity, and °Brix to record physicochemical properties and fermentation-induced changes. These parameters are essential for determining the suitability of the medium for *S. cerevisiae*, particularly regarding acidity and sugar concentration.

3.3. The Total Soluble Solid of Mies Treatments in Every Day of Fermentation

Total soluble solids (TSS) were recorded daily until stabilization to determine fermentation completion, as must composition and yeast inoculum size influence fermentation kinetics [36, 57]. Low-honey treatments (15.14 °Brix) showed rapid TSS reduction within 24 h and stabilized early, whereas the higher initial concentrations (21.27–32.58 °Brix) continued fermenting up to 120 h, consuming 46.5-68% of fermentable sugars.

Accordingly, fermentation rate was positively correlated with honey concentration and yeast biomass, consistent with enhanced sugar depletion and ethanol production at higher cell populations [26]. These results align with report of Vatti [57], with minor variations attributable to strain, inoculum level, and must composition differences.

Table 2. *The daily recorded TSS of the mies treatments prepared using three honey-to-water proportion ratios and fermented with three yeast inoculum sizes.*

Treat-ments	Daily recorded TSS values (%)						
	Day 1 (0 hrs)	**Day 2 (24 hrs)**	**Day 3 (48 hrs)**	**Day 4 (72 hrs)**	**Day 5 (96 hrs)**	**Day 6 (120 hrs)**	**Day 7 (144 hrs)**
P1Y1	32.62±0.283[a]	23.81±0.212[a]	21.32±0.212[a]	19.75±0.113[b]	18.90±0.085[b]	18.45±0.042[b]	18.45±0.042[b]
P1Y2	32.38±0.141[a]	22.4±0.707[ab]	20.90±0.424[a]	19.34±0.141[b]	18.05±0.057[c]	18.05±0.057[b]	*
P1Y3	32.75±0.071[a]	20.91±0.141[b]	19.41±0.17[b]	18.70±0.071[c]	17.45±0.071[d]	17.45±0.071[c]	*
P2Y1	21.26±0.141[b]	9.6±0.424[c]	8.22±0.028[c]	7.15±0.028[d]	7.15±0.028[e]	*	*
P2Y2	21.21±0.1[b]	8.80±0.071[cd]	7.63±0.042[cd]	6.95±0.071[d]	6.95±0.071[e]	*	*
P2Y3	21.35±0.424[b]	8.00±0.707[d]	6.80±0.283[d]	6.80±0.283[d]	*	*	*
P3Y1	15.11±0.042[c]	4.21±0.042[e]	4.05±0.028[e]	4.05±0.028[e]	*	*	*
P3Y2	15.15±0.071[c]	4.12±0.071[e]	4.12±0.071[e]	*	*	*	*
P3Y3	15.17±0.042[c]	3.95±0.141[e]	3.95±0.141[e]	*	*	*	*
Treat-ments	Day 1 (0 hrs)	Day 2 (24 hrs)	Day 3 (48 hrs)	Day 4 (72 hrs)	Day 23 (528hrs)	Day 24 (552 hrs)	Day 25 (576 hrs)
C	21.5±0.466[b]	21.5±0.424[b]	21.5±0.321[a]	21.45±0.141[a]	5.42±0.141[a]	5.50±0.42[a]	5.48±0.425[a]

Note: Data are expressed as mean ± SD. All analyses were done in triplicate. Mean values in the same column with different superscript are significantly different at $p<0.05$. P1Y1; 1: 2.45, 24 & 12.2, P1Y2; 1: 2.45, 24 & 16.3, P1Y3; 1: 2.45, 24 & 20.4, P2Y1; 1: 4.25, 14 & 7.0, P2Y2; 1: 4.25, 14 & 9.4, P2Y3; 1: 4.25, 14 & 11.7, P3Y1; 1: 6, 10 & 5.0, P3Y2; 1: 6, 10 & 6.6, P3Y3; 1: 6, 10 & 8.2, and C; 1: 4.25, 14 & 0 (no yeast), of honey-to-water ratios (w/v), grams of gesho and yeast, respectively, where P = ratio of honey to water, Y = mass of yeast, and C = control.

3.4. Effect of the Inoculum Size of *S. Cerevisiae* on Mies Fermentation Time Reduction

The inoculum size of *S. cerevisiae* had minimal effect on final fermentation time. *Mies* treatments with 1: 6 honey-to-water ratios (P3; 15.14 °Brix) completed fermentation almost in 24 h, with almost no observable differences among P3Y1, P3Y2 and P3Y3, and reduced 24 h compared with the 48 h reported by Vatti [57]. In contrast, high-sugar treatments (1: 2.45 ratio or 32.58 °Brix) required 72 to 120 h depending on yeast inoculum. These results align partially with Vatti [57], and were 24 h shorter than the 144 h reported by Pereira et al., [43]. Fermentation can be accelerated by increasing inoculum size, though excessive levels may negatively affect aroma [44]. Fermentation rate also depends on yeast strain, nutrient supplementation, honey composition and mass, must characteristics, temperature, and other processing conditions [6, 38, 40, 43, 46, 57]. In contrast, spontaneous fermentation required about 24 days, consistent with survey reports of 1 week to 3 months, due to slow adaptation and growth of wild microbials in acidic, high-sugar media [24, 46].

3.5. Effect of Honey-to-Water Ratios and Inoculum Sizes of *S. Cerevisiae* on the Physicochemical Properties of Mies

Physicochemical parameters are critical determinants of alcoholic beverage quality and sensory acceptance [41]. In this study, all measured properties differed significantly ($p < 0.05$) among treatments, primarily due to variations in honey concentration. However, yeast inoculum size did not produce significant differences in any physicochemical attribute.

***Table 3.** Physicochemical properties of the mies treatments prepared using three honey-to-water proportion ratios and fermented with three yeast inoculum sizes.*

Treatments	TSS (%)	pH	TA (g/L)	EA (%)
P1Y1	18.45 ±0.07ᵃ	3.74 ±0.014ᵃ	5.59 ±0.053ᵇ	8.65 ±0.07ᵇ
P1Y2	18.05 ±0.21ᵃ	3.73 ±0.021ᵃ	5.66 ±0.053 ᵇ	8.61 ±0.14ᵇ
P1Y3	17.45 ±0.35ᵃ	3.73 ±0.014ᵃ	5.77 ±0.106 ᵇ	8.70 ±0.01ᵇ
P2Y1	7.15 ±0.07ᵇ	3.54 ±0.014ᶜ	4.12 ±0.00ᶜ	8.65 ±0.07ᵇ
P2Y2	6.95 ±0.21ᵇ	3.52 ±0.021ᶜ	4.32 ±0.035ᶜ	8.74 ±0.14ᵇ
P2Y3	6.70 ±0.14ᵇ	3.51 ±0.007ᶜ	4.36 ±0.088 ᶜ	8.75 ±0.07ᵇ
P3Y1	4.05 ±0.07ᵈ	3.67 ±0.021ᵇ	3.56 ±0.053ᵈ	6.24 ±0.01ᶜ
P3Y2	4.12 ±0.00ᵈ	3.67 ±0.007ᵇ	3.63 ±0.049ᵈ	6.20 ±0.00ᶜ
P3Y3	3.95 ±0.07ᵈ	3.66 ±0.007 ᵇ	3.56 ±0.053ᵈ	6.25 ±0.07ᶜ
C	5.50 ±0.14ᶜ	3.43 ±0.021ᵈ	8.05 ±0.091ᵃ	9.72 ±0.14ᵃ

Note: Data are expressed as Mean ± SD. All analyses were done in triplicate. Mean values in the same column with different superscript are significantly different at p<0.05. P1Y1; 1: 2.45, 24 & 12.2, P1Y2; 1: 2.45, 24 & 16.3, P1Y3; 1: 2.45, 24 & 20.4, P2Y1; 1: 4.25, 14 & 7.0, P2Y2; 1: 4.25, 14 & 9.4, P2Y3; 1: 4.25, 14 & 11.7, P3Y1; 1: 6, 10 & 5.0, P3Y2; 1: 6, 10 & 6.6, P3Y3; 1: 6, 10 & 8.2, and C; 1: 4.25, 14 & 0 (no yeast), of honey-to-water ratios(w/v), gram of gesho and yeast respectively, where; P=proportion ratio of honey & water, Y=mass of yeast & C=control.

3.5.1. Total Soluble Solids of the *Mies*

Total soluble solids (TSS), represents residual sugar content and sweetness, ranged from 3.95 ± 0.07% (P3Y3) to 18.45 ± 0.07% (P1Y1), corresponding to 26.67% and 56.56% unconsumed sugars at fermentation completion, respectively. Final TSS was directly correlated with initial °Brix of the *birzi* and the proportion of honey used. However, variation among treatments was governed more strongly by yeast alcohol tolerance than by honey concentration or inoculum size [47, 57]. Ethanol toxicity likely inhibited viable yeast cells [47], limiting sugar conversion and resulting in elevated residual sugars. Osmotic stress caused by high sugar concentrations and low pH (<4.0) may also have constrained yeast metabolism [15, 16, 51]. Although honey-rich treatments contained higher levels of essential nutrients (minerals, vitamins, amino acids, sterols, and fatty acids), critical for yeast growth and fermentation regulation [4, 36, 43], yeast performance appeared more limited by alcohol intolerance than nutrient availability. Treatments with lower TSS likely contained predominantly non-fermentable sugars [44]. Given that mead fermentation depends on yeast efficiency, physicochemical conditions, and must composition [15, 36, 60], highly alcohol-tolerant and osmotically resilient strains would be expected to achieve greater TSS reduction at high sugar levels. All treatments fell within the residual sugar range for mead (2.5–27.8%) [52], and were broadly consistent with previous study [48], with variations attributable to honey type, supplementation, yeast strain, inoculum size, and fermentation conditions [42, 57].

3.5.2. pH of the *Mies*

pH is a key indicator of organic acid strength in honey wine and critically influences flavor, microbial stability, and overall quality [26]. In this study, pH values ranged from 3.51 ± 0.007 (P2Y3) to 3.74 ± 0.014 (P1Y1). Although higher honey proportions were expected to yield lower pH due to greater organic acid content, the lowest pH was observed in a moderately concentrated treatment. This indicated that pH variation was influenced more by sugar concentration and alcohol production than by intrinsic acid levels. While ethanol itself does not directly reduce pH, sugar depletion and the synthesis of organic acids during fermentation contribute to acidification [16]. As sugars decline, the effect of organic acids becomes more pronounced; conversely, high sugar concentrations and buffering capacity may mask acidity [26, 36, 43]. Thus, pH variation was not solely attributable to organic acids but to the combined effects of residual sugars and ethanol formation. Moreover, pH does not directly equate to titratable acidity due to buffering effects and mineral composition [20]. All treatments showed decreased pH relative to the initial *birzi*, likely due to yeast-derived organic acids such as acetic, citric, lactic, and succinic acids [24, 29, 51]. Observed values (3.51–3.74) were within reported ranges for *tej* [9, 14], but varied slightly from other studies, likely due to differences in microbial flora, yeast strain, inoculum level, honey composition, and fermentation conditions [5, 12, 57].

3.5.3. Titratable Acidity of the *Mies*

Titratable acidity (TA) reflects both intrinsic honey acids [38], and organic acids synthesized during fermentation [16], and is a critical determinant of mead quality, contributing to sweet sour balance, flavor stability, and microbial safety [26, 57]. In this study, TA ranged from 3.56 ± 0.053 g/L (P3Y1/P3Y3) to 5.77 ± 0.106 g/L (P1Y3), corresponding closely with the initial acidity of the *birzi*. Fermentation increased acidity in all treatments: P3 must rose from 3.17 ± 0.055 g/L to 3.56 ±0.053 g/L, while P1 increased from 4.43 ± 0.033 g/L to 5.77 ±0.106 g/L, indicating a direct relationship between initial and final acidity. All TA values fell within reported ranges for *tej* (0.1–1.03 g/100 mL) (Bahiru et al., 2001), exceeded values commonly reported for mead [12, 55], yet were lower than some *tej* samples (4.2–11.6 g/100 mL) [14]. Increased acidity is consistent with the formation of fermentation-derived organic acids such as acetic, citric, succinic, and lactic acids, particularly in high-sugar musts [24, 29, 51]. Similar increases have been reported for musts of about 23 °Brix, rising from 4 g/L to 6.7–7.6 g/L in final meads [43]. Notably, spontaneously fermented *mies* exhibited significantly higher TA (8.05 ±0.091 g/L), likely due to the activity of acid and alcohol-tolerant indigenous microorganisms and differences in fermentation dynamics [14, 24].

3.5.4. Alcohol Content of the *Mies*

Alcohol content, a principal quality attribute of alcoholic beverages, ranged from 6.2 ±0.00% (P3Y1/P3Y2) to 8.75 ± 0.07% (P2Y3). No significant differences were observed between honey-to-water ratios of 1: 2.45 and 1: 4.25 (w/v), subsequently increasing honey beyond 1: 4.25 did not proportionally rise ethanol yield. Although initial sugar concentration typically determines final ethanol levels [38, 44], no positive correlation was found between honey concentration, inoculum size, and alcohol production. The limited ethanol yield suggests that yeast performance was constrained by stress factors mentioned in TSS, including ethanol toxicity, low alcohol tolerance, osmotic stress from high sugar concentrations, pH below 4.0, and possible suboptimal fermentation conditions [15, 16, 57, 60]. In addition to ethanol toxicity, the low phytosterol content of honey must may increase *saccharomyces* susceptibility to ethanol stress [57]. Moreover, acetic acid accumulation under high-sugar conditions can impair membrane integrity [51, 57]. Commercial starter strains may also be inadequately adapted to honey fermentation stress, limiting productivity [11, 17]. Consequently, treatments with high residual sugars did not exceed 8.75% of alcohol. Values were comparable to ranges reported by Bahiru et al. [9] and Fentie et al. [24], though lower than some studies [14, 43]. All results remained within mead standards [53]. Spontaneously fermented *mies* exhibited higher alcohol content, likely due to stress-tolerant indigenous strains [14, 24].

3.6. Sensory attributes of the Mies

The sensory evaluation results of the *mies* treatments are presented in Table 4. All evaluated sensory attributes, aroma, flavor, taste, color (appearance), and overall acceptability, showed statistically significant differences ($p < 0.05$). Variations were observed both between and within treatment groups, which can be attributed to differences in honey concentration and yeast inoculum size, respectively, despite some observed complexities. Range of volatile compounds, including alcohols, esters, fatty acids, carbonyl compounds, and volatile phenolic [5, 57], influence aroma of mead. In this study, treatments P2Y1 and P2Y2 achieved the highest aroma scores, following to control (C), whereas P3Y3 received the lowest score. These findings indicate that *mies* produced with a moderate honey concentration and a low yeast inoculum size were more favorably rated for aroma. Aroma compounds directly contribute to flavor perception [57], while flavor is also influenced by organic acids [38]. Accordingly, treatments that exhibited superior aroma, namely C, P2Y1, P2Y2, and P2Y3 also received the highest flavor scores. In contrast, P3Y3, P3Y2, and P3Y1 were the least preferred treatments in terms of flavor, with no significant differences among them. Interestingly, unlike aroma, acid content did not show a clear correlation with flavor outcomes in this study. Instead, flavor differences may have been more strongly influenced by the combined effects of residual sugars, alcohol content, and inoculum size. Previous studies have reported that excessive inoculum size can reduce the formation of desirable aromatic compounds and lead to the development of off-flavors and off-aromas [29, 44]. However, such effects were not evident in treatments prepared with a 1: 6.05 honey-to-water ratio, suggesting that formulation factors may modulate the influence of inoculum size. In terms of taste, the highest scores were recorded for P2Y1, followed by P2Y2 and P2Y3, all of which differed significantly from the control. The lowest scores, with no significant variation, were observed for P3Y3, P3Y2, and P3Y1. Taste perception in mead encompasses attributes such as acidity, sweetness, astringency, and overall strength [57]. Residual sugar content plays a critical role in determining taste and overall quality [33], and consumer preference often leans toward sweeter products [27]. Furthermore, sweetness in mead is associated with ethanol content [50]. In this study, treatments with higher alcohol content and moderate residual sugar levels were generally preferred, indicating a balance between sweetness and alcohol as a key determinant of taste acceptability.

Table 4. *The score of sensory attributes of mies treatments prepared using three honey-to-water proportion ratios and fermented with three yeast inoculum sizes.*

Treatments	Aroma	Taste	Flavor	Appearance (color)	Over all acceptance
P1Y1	6.50 ± 0.827^{c}	7.35 ± 0.61^{cde}	6.80 ± 0.52^{d}	8.00 ± 0.64^{a}	6.950 ± 0.686^{c}
P1Y2	6.15 ± 0.745^{cd}	7.20 ± 0.69^{de}	6.60 ± 0.68^{d}	8.05 ± 0.60^{a}	6.80 ± 0.61^{c}
P1Y3	5.65 ± 0.813^{de}	7.05 ± 0.68^{e}	6.40 ± 0.68^{d}	8.10 ± 0.64^{a}	6.75 ± 0.71^{c}
P2Y1	8.20 ± 0.768^{ab}	8.40 ± 0.68^{ab}	8.35 ± 0.67^{ab}	7.85 ± 0.67^{a}	8.20 ± 0.61^{ab}
P2Y2	8.05 ± 0.826^{ab}	8.10 ± 0.71^{abc}	7.90 ± 0.55^{bc}	8.00 ± 0.64^{a}	7.90 ± 0.71^{b}
P2Y3	7.60 ± 0.883^{b}	7.85 ± 0.67^{bcd}	7.60 ± 0.75^{c}	7.85 ± 0.67^{a}	7.80 ± 0.52^{b}
P3Y1	5.25 ± 0.851^{e}	4.9 ± 0.96^{f}	5.25 ± 0.71^{e}	5.60 ± 1.04^{b}	5.30 ± 0.80^{d}
P3Y2	5.00 ± 0.858^{e}	4.90 ± 0.91^{f}	5.00 ± 0.79^{e}	5.50 ± 1.05^{b}	5.05 ± 0.75^{d}
P3Y3	4.95 ± 0.945^{e}	4.85 ± 0.81^{f}	4.95 ± 0.75^{e}	5.40 ± 1.18^{b}	5.30 ± 0.92^{d}
C	8.65 ± 0.489^{a}	8.65 ± 0.58^{a}	8.80 ± 0.41^{a}	8.20 ± 0.76^{a}	8.65 ± 0.58^{a}

Note: Data are expressed as Mean ± SD. All analyses were done in triplicate. Mean values in the same column with different superscript are significantly different at p<0.05. P1Y1; 1: 2.45, 24 & 12.2, P1Y2; 1: 2.45, 24 & 16.3, P1Y3; 1: 2.45, 24 & 20.4, P2Y1; 1: 4.25, 14 & 7.0, P2Y2; 1: 4.25, 14 & 9.4, P2Y3; 1: 4.25, 14 & 11.7, P3Y1; 1: 6, 10 & 5.0, P3Y2; 1: 6, 10 & 6.6, P3Y3; 1: 6, 10 & 8.2, and C; 1: 4.25, 14 & 0 (no yeast), of honey-to-water ratios(w/v), gram of gesho and yeast respectively, where; P=proportion ratio of honey & water, Y=mass of yeast & C=control.

Unlike other sensory attributes, no significant differences in appearance were observed within treatment groups. However, treatments prepared with a 1: 6.05 honey-to-water ratio (P3Y1, P3Y2, and P3Y3) received significantly lower appearance scores compared to others. The control sample achieved the highest score, followed by P1Y3, with no significant difference between them. Honey-based products are typically characterized by cloudiness and coloration due to residual substrates and microbial activity [9]. Although higher honey content was expected to enhance appearance due to increased substrate concentration, no significant differences were observed among treatments with 1: 2.45 and 1: 4.25 ratios, possibly reflecting consumer expectations regarding the typical appearance of *mies*. Regarding overall acceptability, significant differences were observed among treatment groups, except for those prepared with a 1: 4.25 honey-to-water ratio. The highest overall acceptance was recorded for the control, followed by P2Y1, P2Y2, and P2Y3, while P3Y2 received the lowest score. Nevertheless, similarities in acceptability were noted among several treatments regardless of honey concentration or yeast inoculum size. In general, *mies* produced with a moderate honey-to-water ratio were preferred across most sensory attributes. Similarly, lower yeast inoculum sizes resulted in more favorable sensory outcomes. Notably, the spontaneously fermented *mies* (control) achieved the highest scores in all sensory attributes except appearance. This may be attributed to the diverse range of volatile compounds produced by wild yeasts and lactic acid bacteria, which can enhance sensory complexity and overall quality [14, 24]. In contrast, the use of commercial starter cultures may limit product distinctiveness due to reduced adaptability under fermentation stress conditions [11, 17]. Additionally, fermentation with single-strain cultures has been reported to diminish product uniqueness [39].

4. Conclusion

The traditional method of *mies* production, as well as the basic ingredients, varied among households. Both secret and widely shared additives are used to improve potency and quality. As expected, in fermentation length, the *mies* prepared with less honey and more yeast fermented the fastest, while those with more honey and less yeast took longer. The physicochemical properties of the treatments were influenced by the honey mass and fermentation efficiency of the yeast, rather than the inoculum size. It is due to yeast's low alcohol tolerance, and osmotic stress response nature, which links to the pH and sugar concentration of the must. As a result, the *mies* with high alcohol content, also retained higher percentage of unfermented sugars. Therefore, since the honey-to-water ratio increased above 1-to-4.25 (22.27 °Brix) to 1-to-2.45 (32.58) (w/v), the ethanol content did not increase further. Accordingly, the ethanol levels of the *mies* treatments produced from both the 1: 2.45 and 1: 4.25 (w/v) ratios were equivalent. The findings for total soluble solids and titratable acidity correlated with the honey masses. In contrast, pH exhibited a more complex pattern. Unlike the physicochemical properties, the sensory attributes were affected by both the yeast inoculum size and physicochemical characteristics of the final product attributable to the honey used and yeast activities. The influence of inoculum size on sensory attributes was evident, as the smallest inoculum size showed slightly better outcomes. In line with, according to the panelists result, the association of alcohol and residual sugar contents were more determinant of the sensory acceptance. As a result, *mies* produced with a 1: 4.25 (w/v) honey-to-water ratio and fermented with the lesser inoculum size were preferred. Using *S. cerevisiae* significantly reduced the fermentation time, however, the *mies* did not completely resemble to naturally fermented in sensory attributes. These findings help producers determine the appropriate honey-to-water proportion and yeast inoculum size for reducing fermentation time through the application of commercial yeast, and provide useful information for optimizing and standardizing mies production.

Abbreviations

S. cerevisiae	Saccharomyces Cerevisiae
SD	Standard Deviation
TSS	Total Soluble Solids
TA	Titratable Acidity
MC	Moisture content
HMF	Hydroxymethylfurfural
°Brix	Degree of Brix

Acknowledgments

The authors would like to acknowledge Adigrat University for fianicial support. College of Dry Land Agriculture and Natural Resources, Mekelle University is also kindly acknowledge for space and laboratory facility allowance.

Author Contributions

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Funding

Adigrat University funded for this research.

Data Availability Statement

The data supporting the outcome of this research work has been reported in this manuscript.

Conflicts of Interest

The authors declare no conflicts of interest.

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Biography

Gebrehiwot Gidey Gebrekrstos is a lecturer in the Department of Food Science and Postharvest Technology at the College of Agriculture and Environmental Sciences, Adigrat University. He completed his B.Sc. Degree in Food Science and Post-harvest Technology in 2017, and his M.Sc. Degree in Food Processing Technology from Mekelle University in 2024. His academic and research interests focus on food processing technology, traditional fermented foods and beverages, nutrition, food microbiology, food quality and safety, food preservation and postharvest technology. He has participated in academic research and community service activities related to food science and technology. In recent years, he has been involved in research activities focusing on traditional Ethiopian fermented beverages and food processing technologies. He also actively participates in academic conferences, seminars, and professional collaborations in the fields of food science and food technology.

Research Field

Gebrehiwot Gidey Gebrekrstos: Food processing technology, traditional fermented foods and beverages, nutrition, food microbiology, food quality and safety, food preservation, and postharvest technology.

Hagos Hailu Kassegn: Cereal processing, fermentation and germination processing, food quality and safety, Innovations for food tradition, nutrient dense food formulations.

Teklebrhan Welday Atsbha: Food processing technology, food microbiology and food safety, traditional and functional foods, postharvest technology and storage science, nutritional and phytochemical analysis, industrial and food biotechnology, natural antimicrobials and food preservation.

Ftwi Gebremedhin Kidane: Food microbiology and safety, food processing technology, traditional fermented foods and beverages, nutrition, and food preservation.

Kidanemariam Tesfay Zenebe: Nutrition, traditional fermented foods and beverages, food processing technology and food preservation, and postharvest technology.