Physicochemical and Sensory Properties of Honey Wine 'Mies' Fermented with Commercial Yeast

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Abstract

Mies is a traditional Tigrayan honey wine produced typically from honey, gesho, and water, prepared with varying ingredient proportions and requiring prolonged fermentation. To shorten this duration, commercial yeasts are increasingly being used. This study aimed to determine the optimal yeast inoculum size and honey-to-water ratio and to 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 3.515–3.74, titratable acidity of 3.562–5.584 g/L, and ethanol levels of 6.2–8.75%. All physicochemical and sensory parameters differed significantly ($p < 0.05$). Fermentation time ranged from 24 to 120 hours. Honey-to-water ratios significantly affected physicochemical properties, while sensory quality was influenced by both ratios and inoculum size. Overall, mies produced with 1:4.25 ratio showed superior sensory performance.

1. Introduction

Fermentation, a low-input process, has been used to provide inexpensive, safe, and nutritious foods for low-income people [41]. Alcoholic beverages are among the most consumed fermented foods in human dietary traditions. In Ethiopia, they are produced at the household level and consumed in considerable quantities [27]. These beverages also play an important role in enhancing food security, generating income, and preserving socio-cultural practices. Globally, their production makes also a substantial contribution to the world economy [44]. For these reasons, people across the world produce and consume traditional alcoholic beverages prepared from locally available materials through fermentation with indigenous methods [1, 44]. *Mies* is an indigenous honey wine originating from Tigray, produced primarily from honey, water, and the leaves and twigs of *gesho* (*Rhamnus prinoides L.*). This fermented alcoholic beverage is prepared and consumed during both religious and other social events, including weddings, birthdays, festivals, and funerals. Nowadays, it is increasingly common to find *mies* in resorts, grocery stores, hotels, and restaurants in urban and semi-urban areas, contributing to income generation. Accordingly, traditionally produced alcoholic beverages have an almost equal market share with modern alcoholic beverages [22, 27]. Ethiopia is one of the largest honey producers in Africa and ranks tenth globally [43]. Approximately 80% of Ethiopia's honey production is used for the preparation of *mies* [29]. Honey is a complex product containing about 200 substances, including sugars, enzymes, amino acids, organic acids, carotenoids, vitamins, minerals, aromatic substances, and biologically active compounds [23, 27, 36]. As a result, honey is known for nutritional and therapeutic values and is considered a medicinal food [35]. As a type of honey wine, *mies* can offer numerous health benefits due to its therapeutic and nutraceutical properties of antioxidants (polyphenols and flavonoids) attributed to the honey [34, 42], additional metabolites generated during fermentation through microbial enzymatic activity, and bioactive compounds extracted from *gesho* [28, 61]. It is also a source of nutrition,

consisting primarily of carbohydrates, with trace amounts of proteins and fats [11, 47]. Quality *mies* is yellow like in color, with a sweet-sour alcoholic flavor and a fizzy, cloudy appearance. Its ethanol content ranges from 5% to 13.16% (v/v) [11, 68]. The quality of honey is critical in *mies* production; however, many other factors can influence the product's quality. Tigrayan producers often prefer red or dark crude and aged honey due to its fermentability and the attractive yellowish color it imparts to *mies*. Studies have reported that dark honey varieties contain essential constituents crucial for fermentation and the metabolic processes of fermentative microorganisms [4, 49, 51]. Moreover, hydroxymethylfurfural is a critical safety issue; it should not exceed 40 mg/kg or 80 mg/kg for tropical honey used in production [21]. In *mies*, the leaves and stems of *gesho* (*Rhamnus prinoides L.*) contribute to a special aroma and flavor due to their bittering substances [8, 15]. Similarly, these components inhibit the growth of undesirable microorganisms and regulate the microbial dynamic in fermentation [3, 15]. In some areas, *tseddo* (*Rhamnus tseddo*) is used in *mies* production, either in combination with *gesho* or as a substitute. It is applied as crushed powder to produce a stronger *mies*, which can reduce the amount of honey needed and lower costs. Various roots, barks, herbs, and spices are also used to enhance flavor and potency of the *mies* [11], as well as for washing and seasoning production equipment. Therefore, the strength of *mies* may depend more on these additives than alcohol content to attract customers. *Mies*, as a cultural beverage, has been known for over 2000 years since the beginning of the Axumite kingdom [37], and it contributes indispensable economic, health, and socio-cultural significance. Despite the production is continued in an indigenous traditional manner, its fermentation occurs spontaneously through wild microorganisms naturally present in the raw materials and equipment used for production due to the absence of direct inoculation [11, 12, 28, 67]. Consequently, the production is labor-intensive, taking weeks to months and delays for marketing. Therefore, to reduce the length of fermentation, commercially available *Saccharomyces cerevisiae* is being used as an alternative for complete or partial inoculation of a starter culture in Tigray region, even though it is low-alcohol tolerant. In the modern world, commercial yeasts are used for mead production [48, 64], however the fermentation and quality vary depending on the nature and/or mass of honey, yeast strain, honey-must composition, supplementation, fermentation temperature and time [42, 44, 46, 52]. In addition, using unstudied inappropriate inoculum size of yeast may influence *mies* quality, because an excessive use can lower synthesis of desirable aromatic compounds and can cause other off-odors and off-flavors [33, 50]. Furthermore, the method of preparation, proportion ratios of ingredients, variety and dose of concoctions, and fermentation condition and duration varied among households. Consequently, the physicochemical and sensory properties of the final product may also vary, leading to inconsistencies. Therefore, this study was conducted aiming to determine the optimal inoculum size of yeast, ingredient variety and proportion ratios, preparation methods, and fermentation duration.

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. The color and physical quality of the raw honey were visually assessed. The honey was placed in a plastic container and transported to the Food Science and Postharvest Technology Laboratory at Mekelle University. *Gesho* leaves were purchased from the local market in Mekelle, where they are traditionally sold for use in alcoholic beverage production. Commercial instant dry yeast (Angel brand, *Saccharomyces cerevisiae*) and 20 liters of packaged water were also obtained from supermarkets 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 measured after the weighed sample were kept the whole night in oven using crucible at 100–110°C [10, 56]. The weight loss was taken as MC, which was calculated by the following formula

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

Analysis of Total soluble solids (TSS)

TSS was analyzed by digital Abbe-refractometer after it cleaned using distilled water and adjusted at zero 20°C. After an appropriate sample size was placed on the prism-plate, the reading that appeared on the screen was directly recorded as total soluble solids (°Brix) [56].

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, then taken in the beaker and inserted pH meter [10, 21].

Analysis of Ash content

It was determined by weighed 10 g sample in a silica crucible, and then heated in a muffle furnace for about 3 to 5 h at 500°C. It was cooled in a desiccator and weighed, reheated for half an hour, then cooled and weighed again. This was repeated consequently until the weight became constant [10, 56]. Weight of ash was calculated by the following formula

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

Analysis of hydroxymethylfurfural (HMF)

HMF content was determined using the White method with a UV–spectrophotometer [40]. Five grams of honey were weighed into a 50 mL beaker and transferred to a 50 mL volumetric flask. Subsequently, 0.5 mL of Carrez I solution and 0.5 mL of Carrez II solution were added. The flask was then filled to volume with distilled water and thoroughly homogenized. The solution was filtered through filter paper, discarding the first 10 mL of the filtrate. From the remaining filtrate, 5 mL was pipetted into each of four clean test tubes. To prepare the reference (blank) solution, 5 mL of sodium bisulfite solution was added to one of the tubes, while 5 mL of distilled water was added to the other tubes and mixed well. The absorbance of each solution was measured using 10 mm quartz cuvettes at wavelengths of 284 nm and 336 nm.

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

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

$$\text{Factor} = 149.7 = \frac{126}{16830} \text{x} \frac{1000}{10} \text{x} \frac{1000}{5}$$

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 Fermentation

The *birzi* (honey–water solution) was prepared by diluting 0.408 kg (P1), 0.235 kg (P2), and 0.165 kg (P3) of honey with 1 L of water. The mixture was stirred continuously until the honey dissolved completely, after which it was filtered to remove wax and other impurities. To the filtered *birzi* solutions, 0.024 kg (P1), 0.014 kg (P2), and 0.010 kg (P3) of pounded *gesho* (based on mean honey-to-*gesho* ratios) were added. Each experimental treatment was inoculated with 3%, 4%, or 5% (w/w) yeast [32], calculated regarding to the weight of honey used. A control treatment was prepared in the same manner as P2 but without yeast inoculation. This control was prepared two weeks earlier than the inoculated treatments to allow for spontaneous fermentation, ensuring that all samples reached readiness for sensory evaluation simultaneously. Fermentation was carried out in 2 L plastic containers for ease of handling, and the mixtures were stirred daily throughout the fermentation period. The temperature during fermentation ranged from 19.2 to 21.5°C. Upon completion of fermentation, the treatments were filtered and racked to

allow maturation. The matured *mies* were filtered again to remove sediments, wax, yeast flocculates, and degraded *gesho* leaves that had passed through the initial filter. Following these processes, the samples were prepared for sensory evaluation.

Yeast inoculation

Filtration & racking Stirring

2.5. Physicochemical Analysis of Must and *Mies*

The pH, total soluble solids (TSS), and titratable acidity (TA) of the must were measured prior to yeast inoculation and after *mies* fermentation.

Analysis of Degree of Brix/TSS

It was measured using digital Abbe refractometer, after calibrated according to the manufacturer's instructions at 20°C. The sample was placed on the prism, then readings was recorded as TSS [2, 64].

Analysis of pH

It was measured by immersing the glass electrode of a digital pH meter into 25 mL of each sample [30].

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 [10], and calculated as follows

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

Analysis of Alcohol (methanol)

It was determined by the distillation method using a *Malligand* apparatus, calibrated with standard alcohol. A small amount of *mies* was added to the upper part of the apparatus, which was then properly sealed. Subsequently, 100 mL of distilled water was added to the lower part of the *Malligand* condenser and brought to a boil. When the temperature reached 75°C, the reading displayed was recorded as the percentage of alcohol [30].

2.6. Sensory Evaluation

The sensory attributes of *mies* were evaluated by a panel of 20 trained panelists, consisting of MSc students and lecturers from the Department of Food Science and Postharvest Technology at Mekelle University. They 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 (1- Dislike extremely, 2- Dislike very much, 3- Dislike moderately, 4- Dislike slightly, 5- Neither like nor dislike, 6- Like slightly, 7- Like moderately, 8- Like very much and 9- Like extremely). 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 TSS, pH, TA, and ethanol content, as well as the sensory attributes, were performed using Minitab Statistical Software (version 21) at a 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 must 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

The survey data were collected from 82.8% of mothers with over two years of experience in *mies* production for marketing. About 59.4% of them had completed grade five or above. According to the survey results, approximately 55.2%, 13.8%, and 30% of producers used red, yellow, and mixed, crude aged honey, respectively. Most of them purchased the honey directly from beekeeping farmers, while some obtained it from honey shops. It is evident that honey, water, and the leaves and/or stems of *gesho* are the core ingredients commonly used in *mies* production. However, the results indicated that *tseddo* (*Rhamnus tseddo*) serves as a secondary alternative, either replacing *gesho* or being used in combination with it in varying proportions in Adigrat City. Accordingly, traditional *mies* production involve not only the main ingredients but also various other concoctions. In this context, the quality of *mies* can be significantly influenced not only by the ratios of the main ingredients but also by the types and amounts of additives. Approximately 65.5% of *mies* producers use a combination of *gesho* and *tseddo*, 27.6% use only *gesho*, and 6.9% use only *tseddo*. It is noted that *tseddo* is believed to be more potent than *gesho*, which could be a primary reason for the significant variation in the ratios of the main ingredients. As a result, the proportion of honey, water, and *gesho* was found to be 4.25 ± 1.8 liters of water and 0.06 ± 0.029 kg of *gesho* per kilogram of honey. The use of *gesho* parts also varied; 79.3% of producers used the leaves, 3.4% used the stems, and 10.3% used both leaves and stems, either in whole form or pounded, which were added to the *birzi* either immediately or a few days later. Other additives, such as legumes, turmeric (*Curcuma longa*), and rhubarb root (*Rheum rhabarbarum*) in coarse or powdered form, are also used to enhance color, flavor, and fermentation, particularly when white honey is used. The use of legumes in mead fermentation is intends to supply nitrogen, an essential nutrient for the metabolism and growth of microorganisms [63]. Some respondents also acknowledged the use of other concoctions but were unwilling to disclose them, considering them their own special ingredients for enhancing quality, to be used secretly, although often perceived by others as adulteration.

Furthermore, 58.6% of responses showed that, depending on the honey type and fermentation temperature, *mies* fermentation takes from one to three months. Consequently, most producers encounter marketing challenges due to delays. Therefore, although the use of commercial yeast to shorten the fermentation period is often considered immoral and an adulteration practice by those producers, about 48.3% of them used commercial yeast either as a partial or complete starter culture, or alternatively employed *embula* (a back-slop starter culture) derived from residues of previously produced *mies* and other fermented products. It is important to note that the use of *S. cerevisiae* cannot produce a high yield of alcohol that satisfies consumers due to its low alcohol tolerance capacity (Santos et al. 2008). Therefore, it is mainly used to accelerate fermentation while allowing natural microbes to continue until the desired alcohol content is achieved. For this reason, producers prefer crude aged honey to facilitate microbial adaptation [65]. In some cases, *S. cerevisiae* is utilized because it is effective in producing sweet *mies* quickly during holidays. Additionally, survey data indicated that the quality of *mies* may not be determined solely by the types and proportions of ingredients. It is also influenced by differences in ingredient preparation and production procedures, the materials and methods used for washing and fumigating equipment, the sources and parts of the ingredients, as well as fermentation and aging conditions, including temperature and duration. In production, once the must is prepared and fermentation begins, it is agitated daily or every other day to prevent settling, and this continues until fermentation is complete. Around 20.7% of producers retail *mies* immediately without aging, while 13.8% allow it to mature for more than one month, believing that this stabilizes the components, increases alcohol content, and enhances flavor. Leaves of *grawa* (*Vernonia amygdalina Del.*), ash and detergents are commonly used for washing utensils, whereas *gesho* and olive wood are primarily used for fumigating, although variations are observed. All these variations depend on individual knowledge, skills, and access in *mies* production [16, 30]. In this study, the common ingredients and usual methods of mies production were applied to produce *mies* on a laboratory scale, as some ingredients and technical variations were observed.

3.2. The Physicochemical Properties of Honey and Must

Although fresh honey is not preferred by producers, this study employed freshly harvested honey to observe the possible effects of *S. cerevisiae* without interference from wild fermentative microbes. Moreover, a controlled study was necessary because the aged honey preferred for *mies* traditional production is believed to be colonized and adapted by wild microbes, which may vary in load, thus could make study irrational. The physicochemical properties of the honey were analyzed to ensure safety and quality. In particular, hydroxymethylfurfural (HMF) concentration and moisture content are critical quality indicators for honey and honey wines [25, 38]. However, all the measured parameters met the criteria specified by the Codex Alimentarius standard [21] for honey.

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

Item	Parameters				
Honey	MC (%)	TSS (^{O}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)**	**^{O}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.***

Based on the indigenous knowledge of *mies* producers, red or dark crude aged honey varieties are preferred. It is also scientifically revealed that the quality of mead production depends on the honey variety [11, 44, 53], which is attributed to higher nutrient content and pH [48]. Thus, color serves as an indicator of honey quality, related to phenolic compounds, flavonoids, organic acids, and mineral content [7, 36, 44], which plays a vital role in fermentation and in the quality of honey wine. Minerals act as cofactors in sugar conversion during fermentation [48], and are found in higher concentrations in dark honey varieties [9, 44, 48]. Amino acids (nitrogen) and vitamins are also essential for yeast metabolism [4], and their quantities may likewise depend on honey color. Honey is acidic and limited in some nutrients; therefore, in mead production, optimal yeast growth can be stimulated by adding inorganic salts to increase pH and supplement vitamins and nitrogen [49, 64]. Given the aforementioned evidence, darker honey is of higher quality and provides essential nutrients required for fermentation [49]. Accordingly, differences in honey composition affect the physicochemical properties of honey wine [11]. It has been reported that dark honey contains sufficient minerals and vitamins and has a higher pH, which creates optimal fermentation conditions for mead [48, 49]. Therefore, this study used a red honey variety, considering its compositional quality to be suitable for fermentation and *mies* quality. Moreover, before inoculating yeast into the prepared must, pH, titratable acidity, and degrees of brix were analyzed. Profiling these physicochemical properties was necessary to note their changes during fermentation, and significant variations were observed. Similarly, it was important to identify the most suitable must for *S. cerevisiae*, particularly concerning pH and sugar concentrations. As the pH of the must decreases during fermentation, it affects yeast fermentation efficiency [49, 53, 58]. Additionally, musts with high sugar concentrations are difficult to ferment and may cause fermentation to stall in traditional mead-making without adjustments [53, 66].

3.3. Total Soluble Solid (TSS) of *Mies* in Every Day of Fermentation

The concentration of TSS in the treatments was recorded daily until stabilization, in order to determine the final stage of *mies* fermentation. This is important because the honey-must composition and the mass of inoculated yeast influence the length of the fermentation period [42, 64]. As a result, the TSS concentration of treatments prepared with less honey (approximately 15.14 °Brix) was reduced to around 26.67% within the first 24 hours, irrespective of the mass of the inoculated yeast, and no significant changes were observed thereafter. This indicates that the physicochemical properties of the must were favorable to the yeasts, in addition to being low in sugar, which was insufficient to sustain fermentation longer. An increase in yeast population leads to a reduction in sugars and an increase in ethanol content [30].

Table 2

The daily recorded TSS of the *mies* treatments prepared with three honey-to-water proportion ratios and fermented by three inoculum sizes of yeasts.

Treatments	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]	*	*	*	*
Treatments	**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.

Among the remaining treatments, the decrease in TSS continued for nearly 120 hours. This shows that the variations were positively correlated with the amounts of honey and the mass of yeast used. Consequently, as expected, treatments with less honey and more yeast stabilized first, while those with more honey and less yeast stabilized last. Approximately 46.5% to 68% of the total consumable sugar in treatments with initial concentrations of 21.27 °Brix and 32.58 °Brix respectively was consumed by the time fermentation halted. The variations in daily recorded TSS values were clearly influenced by yeast inoculum size (cell population) within groups of treatments, well as the differences in sugar concentration attributed to the honey caused difference between groups of treatments. A similar report noted that nearly 54% of a 23 °Brix must was consumed within 24 hours [64], in agreement with this study's findings. The slight variation from that report could be attributed to differences in yeast mass and strain type, fermentation conditions, and honey or honey-must composition.

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

The end of fermentation was noted when the TSS value became constant. Observing the final fermentation time of *mies* fermented with *S. cerevisiae* was important to assess the effect of inoculum size on fermentation duration and to compare it with spontaneously fermented *mies*. Accordingly, the inoculum size of the employed *S. cerevisiae* had only a slight influence on the final fermentation time of the treatments. All *mies* treatments prepared from 1:6 (P3) honey-to-water ratios (w/v), which had low sugar concentrations of about 15.14 °Brix, completed fermentation in around 24 hours (Table 2). As a result, variation in inoculum size in the treatments with the same honey concentration (P3Y1, P3Y2, and P3Y3) did not show significant differences, as nearly all sugar was consumed within the first 24 hours. This showed a reduction of 24 hours compared with the shortest mead fermentation time reported previously (48 hours) [64]. In contrast, treatments with 1:2.45 (P1) honey-to-water ratios (w/v), with higher sugar concentrations of about 32.58 °Brix, took approximately 72 to 120 hours, depending on inoculum sizes of yeast (Table 2). Overall, fermentation time was directly correlated with honey concentration and inoculum sizes. Treatments with less honey and more yeast fermented fastest, while those with more honey and less yeast took longer. The fermentation times reported by Vatti [64], are consistent with some findings of this study, although the longest duration observed here was 24 hours shorter than the 144-hours fermentation reported by Pereira et al. [49]. Fermentation time can be reduced by increasing inoculum size; however, excessive increases may adversely affect aromatic compounds [50]. Various strains of *Saccharomyces* are commercially available to shorten mead fermentation, but the rate of fermentation also depends on added nutrients and adjunct supplements [49, 64], as well as the nature and mass of honey, yeast strain, honey-must composition, temperature and other fermentation conditions [52, 44, 46, 53]. In contrast, spontaneously fermented *mies* required about 24 days to complete fermentation, far longer than the treatments with *S. cerevisiae*. This was similar to survey results as it lasting from one week to three months. The primary cause of this delay is spontaneous fermentation [28], as wild microbes require extended adaptation and growth in an acidic, high-sugar medium [53, 66].

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

In this study, laboratory-scale *mies* was fermented with *S. cerevisiae* and by spontaneous fermentation (control). The *mies* treatments were prepared with three different honey-to-water ratios and fermented using three inoculum sizes of commercial *S. cerevisiae*. The objective was to evaluate the physicochemical properties, sensory attributes, and fermentation time of the treatments. Accordingly, total soluble solids (TSS), pH, titratable acidity (TA), and ethanol (EA) were analyzed.

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

Treatments	TSS (%)	pH	TA (g/L)	EA (%)
P1Y1	18.45 ± 0.07^{a}	3.74 ± 0.014^{a}	5.587 ± 0.053^{b}	8.65 ± 0.07^{b}
P1Y2	18.05 ± 0.21^{a}	3.734 ± 0.021^{a}	5.662 ± 0.053^{b}	8.61 ± 0.14^{b}
P1Y3	17.45 ± 0.35^{a}	3.73 ± 0.014^{a}	5.775 ± 0.106^{b}	8.70 ± 0.01^{b}
P2Y1	7.15 ± 0.07^{b}	3.54 ± 0.014^{c}	4.125 ± 0.00^{c}	8.65 ± 0.07^{b}
P2Y2	6.95 ± 0.21^{b}	3.525 ± 0.021^{c}	4.325 ± 0.035^{c}	8.74 ± 0.14^{b}
P2Y3	6.70 ± 0.14^{b}	3.515 ± 0.007^{c}	4.362 ± 0.088^{c}	8.75 ± 0.07^{b}
P3Y1	4.05 ± 0.07^{d}	3.673 ± 0.021^{b}	3.562 ± 0.053^{d}	6.24 ± 0.01^{c}
P3Y2	4.12 ± 0.00^{d}	3.675 ± 0.007^{b}	3.635 ± 0.049^{d}	6.20 ± 0.00^{c}
P3Y3	3.95 ± 0.07^{d}	3.665 ± 0.007^{b}	3.562 ± 0.053^{d}	6.25 ± 0.07^{c}
C	5.50 ± 0.14^{c}	3.435 ± 0.021^{d}	8.05 ± 0.091^{a}	9.72 ± 0.14^{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.

These parameters are essential for the general acceptance of alcoholic beverages in relation to their sensory attributes [47]. All these physicochemical properties were statistically significant different at $p < 0.05$ (Table 3). Consequently, variations were observed between groups of treatments prepared with different honey masses. Unexpectedly, the masses of yeast employed in the fermentation process did not cause variations in any of these physicochemical properties.

3.5.1. Total Soluble Solids (TSS) of the *Mies*

TSS represent the concentration of sugars that influence the sweetness of the product. In the present study, the TSS of the treatments ranged from 3.95 ± 0.07% for P3Y3 to 18.45 ± 0.07% for P1Y1. These corresponded to 26.67% (P3Y3) and 56.56% (P1Y1) of the unconsumed sugars remaining at the final stage of fermentation, respectively. The variation in TSS among treatments appears to have been primarily due to the proportion of honey employed, although it depended more strongly on the alcohol tolerance capacity of the yeasts than on either the amount of honey added or the level of yeast inoculation [54, 64]. Alcohol intolerance of the yeasts could significantly affect TSS variation and result in high levels of unfermented sugar residues, owing to the inhibitory properties of alcohol on viable cells responsible for converting sugars into ethanol during fermentation [54]. Nevertheless, the role of osmotic stress induced by high sugar concentration and low pH can't be overlooked, even though the pH exhibited considerable complexity. In treatments with lower TSS values, the residual sugars were likely non-fermentable [50]. The final TSS of the *mies* treatments showed a direct correlation with the initial sugar concentration (°Brix) of the must. Several studies have reported that mead fermentation depends on multiple factors, of which yeast efficiency, physicochemical properties, and nutrient composition of must are particularly critical [18, 19, 42, 69]. However, in the present research, with respect to nutritional composition, an inverse correlation was observed: yeast performance was limited more by alcohol intolerance than by nutrient availability in the *birzi*. Treatments containing larger proportions of honey were higher in essential nutrients required by yeasts. As a result, *mies* treatments rich in minerals, vitamins, amino acids (nitrogen), sterols, and fatty acids, the nutrients critical for yeast growth and the regulation of fermentation kinetics [4, 42, 49, 58], were expected to have lower TSS and higher alcohol content. Moreover, as the fermentation of honey wines depends on honey-must composition [42], yeasts can osmotically stress when the pH of the must falls below 4.0 and due to the high concentration of sugars surrounding the yeast cell membrane [18, 19, 57]. In this context, it can be inferred that when the yeasts were highly alcohol-tolerant and not osmotically stressed, treatments remained with high sugar concentrations would have exhibited reductions in TSS. Consequently, *mies* treatments with the highest alcohol content also retained higher concentrations of unfermented sugar. This outcome was directly related to both the amount of honey employed and the initial °Brix of the must. Overall, the TSS values of all treatments fell within the residual sugar range (2.5–27.8%) established by mead standards [59]. These results also confirmed both similarities and slight differences compared with previously reported findings [6, 55, 62, 64]. Such variation may be attributed to differences in the type of honey used, properties and composition, supplementation, yeast strain and inoculation mass, as well as other fermentation conditions [6, 48, 50, 64].

3.5.2. pH of the *Mies*

pH is a measure of the strength of organic acids in honey wine and plays a critical role in its flavor and stability. Among the treatments, the lowest pH value was observed in P2Y3 (3.515 ± 0.007), while the highest was found in P1Y1 (3.74 ± 0.014) (Table 3). It was anticipated that treatments prepared with larger amounts of honey would exhibit the lowest pH values owing to their higher organic acid content.

However, the lowest pH occurred in the treatment prepared with a moderate amount of honey. This indicates that pH variation was influenced more by sugar concentration and alcohol content than by organic acid levels, which are typically associated with pH reduction. Although alcohol itself does not directly lower pH, its production is linked to the depletion of sugars and the concurrent synthesis of certain organic acids, both of which can decrease pH [19]. As sugar concentration decreases, the effect of organic acids on pH becomes more pronounced; conversely, high sugar concentrations and the buffering capacity of the growth medium can mask acidification [30, 42, 49]. From this perspective, the observed pH variations in *mies* were not primarily attributable to organic acids. Instead, the results suggest that sugar concentrations, in conjunction with the introduced alcohol content, exerted a stronger influence. It is important to note that pH is not directly equivalent to acidity, as buffering effects of acids and the presence of minerals can alter the relationship [24]. In this study, the highest pH values were associated with treatments containing the highest TSS or sugar residues and alcohol contents, whereas the lowest pH values were observed in treatments with intermediate TSS and elevated alcohol levels. Furthermore, the pH values of the *mies* treatments decreased compared with the initial pH of the must, likely due to the production of organic acids such as acetic, citric, lactic, and succinic acids synthesized by yeasts during fermentation [19, 28, 33, 58]. The pH values observed in this study (3.515–3.74) fall within the range previously reported for *tej* samples (3.07–4.90) [11]. They are partly consistent with naturally fermented *tej* (3.29–3.73) [16], and generally higher than values reported in other studies (3.4–3.5) [25]. Such variation may be attributable to the greater production of organic acids by acid and alcohol-tolerant lactic acid bacteria and other wild yeasts involved in spontaneous fermentation [16, 28]. Differences in yeast inoculation mass, strain characteristics, supplementation, and the composition and acidity of the honey used could also contribute to these variations [14, 48, 64]. Finally, the pH of spontaneously fermented *mies* differed significantly from that of treatments fermented with commercial yeast, most likely for the reasons outlined above [16, 28].

3.5.3. Titratable Acidity (TA) of the *Mies*

TA represents both the organic acids naturally present in honey [44], and those produced during fermentation [19]. Acidity is a critical quality parameter in mead [64], as it contributes to the desired sweet–sour balance, enhances flavor stability, and helps prevent spoilage of *mies* [30]. In the present study, the lowest TA values were observed in P3Y1 and P3Y3 (3.562 ± 0.053 g/L), whereas the highest value was recorded in P1Y3 (5.775 ± 0.106 g/L) (Table 3). These results were directly related to the initial acid concentrations of the must. Differences in TA were primarily attributed to the amount of honey used in *birzi* preparation; however, concentrations increased further after fermentation. All TA values of the *mies* treatments fell within the range reported for *tej* (0.1–1.03 g/100 mL) [11]. They were higher than those commonly reported for mead [14, 62], but lower than acidity values found in some *tej* samples (4.2–11.6 g/100 mL) [16]. Such discrepancies are likely due to similar factors as those discussed in relation to pH variation [14, 16, 28, 64]. Previous studies have shown that mead fermentation with high sugar concentrations can increase the levels of organic acids such as acetic, citric, succinic, and lactic acids [28, 33, 58, 62]. In the current study, TA increased from 3.17 ± 0.055 g/L in P3 *birzi* to 3.562 ± 0.053 g/L in P3Y1 and P3Y3 *mies*, and from 4.43 ± 0.033 g/L in P1 *birzi* to 5.775 ± 0.106 g/L in P1Y3 *mies*, the

lowest and highest post-fermentation values, respectively (Tables 1 and 3). Thus, must with lower initial acidity corresponded to *mies* with lower final acidity, and vice-versa, showed a consistent trend. Similarly, earlier work has reported that the TA of must with approximately 23 °Brix increased from 4 g/L to 6.7–7.6 g/L in the final meads [49]. Conversely, the naturally fermented *mies* showed a markedly higher acidity (8.05 ± 0.091 g/L) compared with all other treatments, indicating a significant increase relative to *mies* fermented with commercial yeasts. This variation is likely explained by the same factors identified for pH, including the activity of acid and alcohol-tolerant microorganisms, yeast strain differences, and fermentation dynamics [16, 28].

3.5.4. Alcohol Content of the *Mies*

Alcohol content is the most important quality parameter of all alcoholic beverages. In this study, the alcohol content of the *mies* treatments ranged from 6.2 ± 0.00% (P3Y2) to 8.75 ± 0.07% (P2Y3) (Table 3). Treatments produced with honey-to-water ratios of 1:2.45 and 1:4.25 (w/v) did not show significant differences. When the honey ratio exceeded 1:4.25 (w/v), alcohol content did not increase proportionally. Since the initial sugar level in the must typically determines the final ethanol concentration [44, 50], higher honey ratios would normally be expected to yield higher alcohol levels. However, no positive correlation was observed between honey mass and alcohol content in *mies* production with *S. cerevisiae* at ratios above 1:4.25 (w/v). Although ethanol production has also been linked to the mass of yeast inoculation [64], in this study alcohol yield did not correlate directly with either the honey concentration or the size of the yeast inoculum. Thus, increasing both honey proportion and yeast inoculum did not result in higher ethanol yields. As discussed in relation to TSS, several stress factors likely limited yeast efficacy: low alcohol tolerance, ethanol toxicity, osmotic stress from high sugar concentrations, pH values below 4.0, and suboptimal fermentation temperatures [18, 19, 50, 64, 69]. These conditions restrict ethanol production in *mies* treatments prepared with higher honey concentrations, leaving large amounts of residual sugars unfermented. Ethanol toxicity is considered a primary cause of low alcohol yields, as it decreases yeast cell viability [54]. The low phytosterol content of honey musts can make *Saccharomyces* particularly susceptible to ethanol stress [64]. In addition, acetic acid has an inhibitory effect on yeast cell membranes due to changes in the pH of the product in mead fermenting with high sugar [58, 64]. Previous studies have further suggested that commercially available yeast strains, when used as starter cultures, may be poorly adapted to the stressful conditions of honey fermentation, resulting in incompatibility and reduced ethanol productivity [13, 20]. Consequently, in this study, *mies* treatments with higher residual sugar contents did not produce ethanol levels exceeding 8.75 ± 0.07% (the maximum yield observed). Compared with prior research, approximately 70% of the alcohol content recorded here fell between findings reported in *tej* [11, 28]. However, all values were lower than those reported in other studies [16, 25, 33, 49]. Nonetheless, the alcohol content of all *mies* treatments remained within the standards for mead [60]. Like the TA findings, the control *mies* exhibited significantly higher alcohol content than the other treatments. These variations may be attributed to ethanol, osmotic, and acid tolerance strains of *Saccharomyces* and *Lactobacillus*, which can produce higher ethanol levels with lower residual sugar contents [16, 28], as well as to the other factors discussed for TA, TSS, and pH.

3.6. Sensory attributes of the *Mies*

The sensory evaluation results of the *mies* treatments are shown in Table 4. All sensory attributes of the *mies* treatments; aroma, flavor, taste, color (appearance), and overall acceptance, showed significant differences at $p < 0.05$. Variations were noted both in-between and within treatment groups, owing to the honey mass and the inoculum size of yeasts, respectively, despite some observed complexities. The aroma of mead depends on alcohols, esters, fatty acids, carbonyl compounds, and volatile phenolic compounds [64]. The aroma of P2Y1 and P2Y2 received the highest scores, followed by the control (C), while P3Y3 received the lowest score. Accordingly, the results showed that *mies* treatments produced from a moderate mass of honey and fermented with the lowest inoculum size were rated higher for aroma acceptance. Aroma compounds present in the mead directly influence the flavor [64], and flavor also arises from acids [44]. Therefore, the treatments that showed superiority in aroma also scored highest in flavor were C, P2Y1, P2Y2, and P2Y3, respectively. In contrast, the lowest scores and least preferred treatments were P3Y3, P3Y2, and P3Y1, respectively, with no significant variation. In this study, unlike aroma, the acid content did not show a direct correlation with the flavor results. Instead, these results may have been more strongly influenced by the combined effect of sugar residues, alcohols and mass of the inoculums. Previous studies have also noted that an exaggerated inoculum size can lower the production of desirable aromatic compounds and produce off-flavors and off-aromas [33, 50]. However, the effect of varying inoculum sizes was not observed in the treatments prepared with a 1:6.05 honey-to-water ratio. Regarding taste, the highest scores were given to P2Y1, followed by P2Y2 and P2Y3, which showed significant differences from C. The lowest scores, with no significant variation, were observed in P3Y3, P3Y2, and P3Y1, respectively. Taste comprises acidity, sweetness, astringency, and strength of the beverage [64]. The content of residual sugars significantly affects taste and quality [38]; thus, consumers may prefer mead with a sweeter taste [31]. However, because the sweetness of mead correlates with ethanol content [57], the *mies* treatments with the highest alcohol content and medium sugar residues showed superiority.

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

Treatments	Aroma	Taste	Flavor	Appearance (color)	Over all acceptance
P1Y1	6.500 ± 0.827^{c}	7.350 ± 0.671^{cde}	6.800 ± 0.523^{d}	8.000 ± 0.649^{a}	6.950 ± 0.686^{c}
P1Y2	6.150 ± 0.745^{cd}	7.200 ± 0.696^{de}	6.600 ± 0.681^{d}	8.050 ± 0.605^{a}	6.800 ± 0.616^{c}
P1Y3	5.650 ± 0.813^{de}	7.050 ± 0.686^{e}	6.400 ± 0.681^{d}	8.100 ± 0.641^{a}	6.750 ± 0.716^{c}
P2Y1	8.200 ± 0.768^{ab}	8.400 ± 0.681^{ab}	8.350 ± 0.671^{ab}	7.850 ± 0.671^{a}	8.200 ± 0.616^{ab}
P2Y2	8.050 ± 0.826^{ab}	8.100 ± 0.718^{abc}	7.900 ± 0.553^{bc}	8.000 ± 0.649^{a}	7.900 ± 0.718^{b}
P2Y3	7.600 ± 0.883^{b}	7.850 ± 0.671^{bcd}	7.600 ± 0.754^{c}	7.850 ± 0.671^{a}	7.800 ± 0.523^{b}
P3Y1	5.250 ± 0.851^{e}	4.900 ± 0.968^{f}	5.250 ± 0.716^{e}	5.600 ± 1.046^{b}	5.300 ± 0.801^{d}
P3Y2	5.000 ± 0.858^{e}	4.900 ± 0.912^{f}	5.000 ± 0.795^{e}	5.500 ± 1.051^{b}	5.050 ± 0.759^{d}
P3Y3	4.950 ± 0.945^{e}	4.850 ± 0.813^{f}	4.950 ± 0.759^{e}	5.400 ± 1.188^{b}	5.300 ± 0.923^{d}
C	8.650 ± 0.489^{a}	8.650 ± 0.587^{a}	8.800 ± 0.410^{a}	8.200 ± 0.768^{a}	8.650 ± 0.587^{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, variations in the appearance of the treatments were not observed within groups. The *mies* treatments prepared with 1:6.05 honey-to-water ratios (w/v) (P3Y1, P3Y2, and P3Y3) scored the lowest and differed significantly from the others. The control (C) received the highest score, followed by P1Y3 with no significant variations. Products derived from honey are typically cloudy and colored due to residues of substrates and fermenting microbes [11]. As a result, the *mies* prepared with more honey were expected to receive the highest scores, as this is directly correlated with the amount of substrate used for preparation. However, possibly due to the expected color of the *mies*, no significant

variation was observed among the treatments prepared with 1:2.45 and 1:4.25 honey-to-water ratios (w/v). Furthermore, variations in overall acceptance were observed among groups of treatments, except for those prepared with 1:4.25 honey-to-water ratios (w/v). The highest score was given to C, followed by P2Y1, P2Y2, and P2Y3, while P3Y2 received the lowest score. However, similarities were observed among the other treatments regardless of honey-to-water ratios or yeast mass. In general, with respect to the honey-to-water ratios, the *mies* prepared with moderate honey were highly preferred in all sensory attributes. Regarding the inoculum sizes used in fermentation, the smallest size gave better results for the sensory attributes. Ultimately, the spontaneously fermented *mies* (C) received the highest scores for all sensory attributes except appearance. This could be due to the various volatile compounds produced by wild yeasts and lactic acid bacteria species, which can positively influence sensory attributes [16, 28]. Additionally, the use of commercial yeasts as starter cultures can result in a loss of distinctive characteristics due to their inability to adapt to stressful fermentation conditions [13, 20]. Moreover, mead fermentation with single-strain cultures can also reduce product uniqueness [45].

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. The honey-to-water ratio and the inoculum size determined the duration of fermentation. As a result, *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 by the fermentation efficiency of the yeast, rather than by the inoculum size. These variations were influenced by the yeast's low alcohol tolerance and osmotic stress response, which links to the pH and sugar concentration of the must. Consequently, *mies* produced with more honey retained a higher percentage of unfermented sugars. As the honey-to-water ratio increased above 1-to-4.25 (22.27 °Brix) (w/v), ethanol content did not increase further. As a result, the ethanol levels in *mies* produced from both the 1:2.45 (32.58 °Brix) and 1:4.25 (22.27 °Brix) (w/v) ratios were equivalent. Similarly, titratable acidity showed a positive correlation with the amount of honey used; it also increased with the must. content. In contrast, pH exhibited a more complex pattern. The findings for total soluble solids and titratable acidity correlated with the honey mass. Unlike the physicochemical properties, the sensory attributes were affected by both the yeast inoculum size applied during fermentation and the physicochemical characteristics of the final product attributable to the honey used. Alcohol and residual sugar contents were determined for the sensory acceptance. The influence of inoculum size on sensory attributes was evident, as the smallest inoculum size showed slightly better outcomes. However, using *S. cerevisiae* exhibited significant reduction in fermentation time, did not completely resemble to naturally fermented *mies* in sensory attributes. Nevertheless, *mies* produced with a 1:4.25 (w/v) honey-to-water ratio and fermented with a low inoculum size were preferred.

Declarations

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Author Contributions

Gebrehiwot Gidey Gebrekrstos: conceptualization, data curation, formal analysis, investigation, methodology, validation, visualization, writing original draft, and writing review and editing. Hagos Hailu Kassegn, Teklebrhan Welday Atsbha, Ftwi Gebremedhin Kidane, Kidanemariam Tesfay Zenebe: data curation, formal analysis, investigation, methodology, review and editing, project administration, and supervision.

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Availability of data and materials

All relevant data generated from the survey-based research on the traditional production process and raw materials used for mies among the Tigreayan community are included in this article in the form of detailed descriptive text. In addition, comprehensive data on the physicochemical properties and sensory attributes of *mies* are presented within this paper.

Ethics Statement

The local Ethics Committee of the University of Mekelle Research Academy reviewed and waived ethical approval for this study, following its established guidelines, although the experiment was not conducted on human subjects. This decision was influenced by the reflective nature of the study and the fact that all procedures were part of standard care. The research protocol received approval from the Research Ethics Committee of Mekelle University Research Academy. All activities of the study were carried out in line with the ethical standards set forth by the committee.

Informed Consent Statement

Informed consent was obtained from all subjects involved in the study.

Conflicts of Interest

The authors declare no conflicts of interest.

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Figures

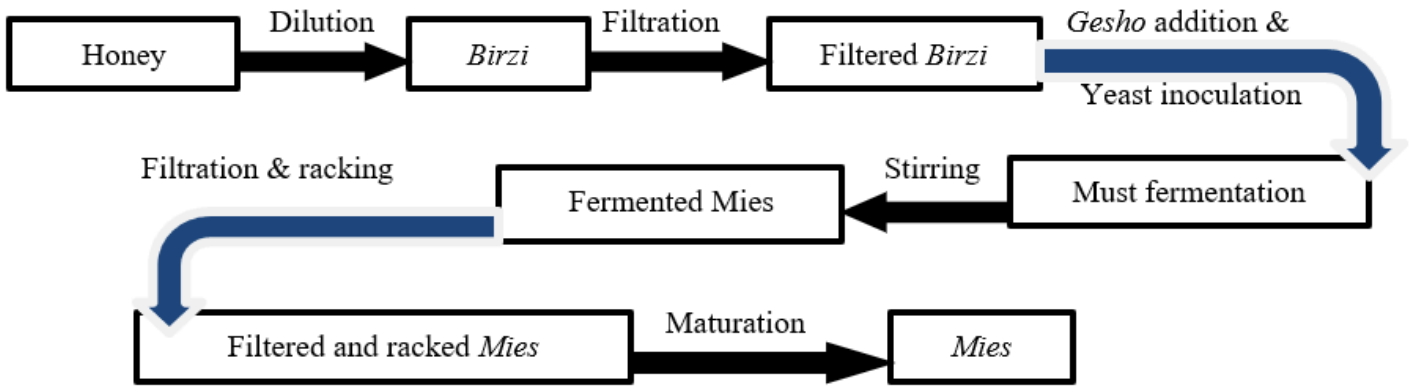

Figure 1

Flow diagram for *mies* production and fermentation using commercial yeast