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Research article

Process standardization and characterization of *Mies*: Ethiopian honey wine

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ABSTRACT

Mies is a delicious honey wine traditionally processed in Ethiopia and Eritrea. This study aimed to investigate the standardization and characterization of high-quality *Mies*. The ingredients for *Mies* preparation were collected, and three formulations were created by varying the amounts of *Gesho* and honey. First, *Birzi* was made by dissolving honey in a 1:5 ratio (honey to water) and fermenting it at 22 °C for five days. Next, coarsely ground *Gesho* was added to the *Birzi* in plastic tanks, where it fermented for 14 days. The fermented mixture was then filtered through a clean white cotton cloth into a new plastic tank. The filtrate (*Mies*) was seasoned and fermented for an additional two days, after which its physicochemical, nutritional, sensory, and microbial properties were analyzed using standard methods. As fermentation time increased, the pH values of the three *Mies* formulations decreased from the first to the twenty-first day. The study revealed that as fermentation time increased, so did the temperature and alcohol levels in all three formulations. Formulation three (F3) was selected by the panelists as the highest quality *Mies*, with a temperature of 22.50 °C after 21 days. The alcohol content of F3 rose from 2.93 % on the first day to 5.72 % by the twenty-first day. The titratable acidity and total soluble solids of F3 were measured at 3.50 g/L and 26.24 °Brix, respectively. The nutritional contents of F3 included lipid (0.13 %), protein (0.10 %), and carbohydrates (3.02 %). The total energy of F3 was found to be 73.91 %. The study revealed that potassium (K) was the most abundant element in F3 and its ingredients, followed by phosphorus (P), while manganese (Mn) and zinc (Zn) were detected in the lowest amounts. Lactic acid bacteria (LAB) were identified as the predominant microbes in F3. Standardizing production procedures for Ethiopian honey wine can enhance commercialization and scalability, while its distinct flavors and fragrances can boost demand, profitability, and potential market entry. To improve the consistency and quality of honey wine production, further research is required to identify species-specific microbial profiles using molecular tools, optimize production parameters, and address concerns related to preparation.

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Abbreviations

g/L	Gram Per Liter
g	Gram
h	Hours
LAB	Lactic Acid Bacteria
MRS agar	de Man Rogosa and Sharpe agar
MIT	Mekelle Institute of Technology
NaOH	Sodium Hydroxide
PLC	Private Limited Company
PDA	Potato Dextrose Agar
W/V	Weight Per Volume
W/W	Weight Per Weight

1. Introduction

Humans have been consuming alcohol since prehistoric times in many regions of the world [1]. Globally, each region produces and enjoys unique types of alcoholic beverages, including traditional fermented beverages made locally using indigenous knowledge in spontaneous processes [2]. Ethiopians consume various fermented alcoholic beverages like *Mies, tella, borde, shamita, korefe, cheka, ogol, booka,* and *keribo*, but maintaining their quality becomes challenging [3,4]. These fermented beverages are typically made from locally available ingredients using traditional processes [4–6].

Honey wine, known as *Mies* in Tigrinya, *tej* in Amharic, and *daadhii* in Oromiffa, has been consumed in Ethiopia and Eritrea since prehistoric times. In Tigrai, *Mies* is using for large events like weddings, commencement ceremonies, breaking fasts, and cultural and religious holidays like *Meskel* (finding of the True Cross), *Lidet* (Christmas), *Timket* (Epiphany), *Ashenda* (a typical and special females holiday in Tigrai), among others [2]. *Mies* is especially prevalent in Agame, specifically in Adigrat and its surroundings, during the celebration of the finding of the True Cross. *Mies* is served to people in long naked bottles called *berelle* (made of glass) to drink while they are eating *tihlo* – a special and popular barley-based dish in the Eastern Zone of Tigrai. Besides, *Mies* holds significant cultural value in Tembien Abyi Addi, especially during holiday festivities like *Ashenda* and many other religious and cultural holidays.

Mies is made using honey, water, and *Gesho* (*Rhamnus prenoides*) stems or leaves [1,7]. Prior *Mies* preparation, fermentation containers are smoked with olivewood (*Olea europea*) stems to reduce microbial contamination and to give a pleasant aroma. *Mies* is a fermented beverage made using natural methods without the use of starter cultures or advanced techniques. *Mies* has yellow color, sweet alcoholic flavor, and effervescent and cloudy appearances with residual yeast cells, unfermented substrates, and other microorganisms. *Mies* producers use various preparation methods and additives, such as barks or roots of specific plants or herbal components, to enhance flavor and attract customers [7,8]. Undetermined doses of additives may pose health risks to consumers [8,9]. The practices and oxidation reactions can cause *Mies* to become soured quickly, resulting in a decrease in its quality [10].

Indigenous knowledge of *Mies* preparation is limited to specific locations and skilled women, resulting in poor transmission and a primitive process that can be easily spoiled. The study of *Mies* is limited, leading to a lack of comprehensive and reliable data on the

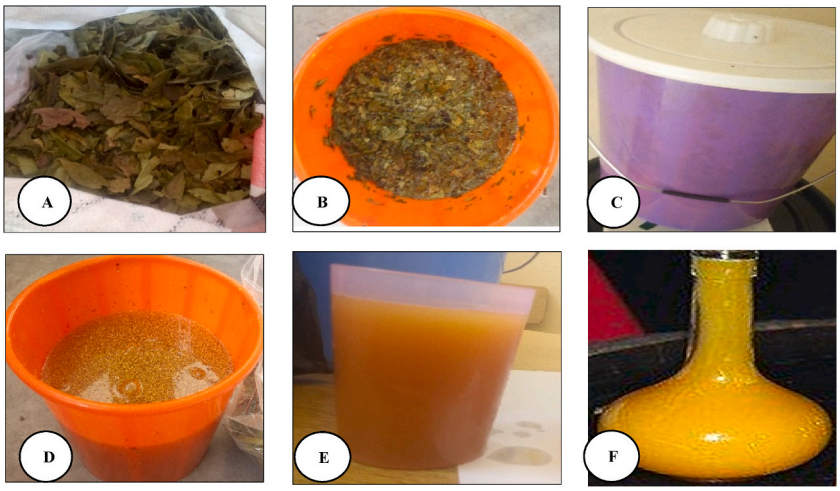

Fig. 1. Raw Materials Used for *Mies* Preparation, Intermediate Product (*Birzi*) and *Mies* (A) Dry leaves of *Gesho* (which contain a naphthalene glucoside compound called Geshoidin, serving as a bittering, coloring, flavoring, and antiseptic agent against microbial flora rather than yeast); (B) Coarsely pounded *Gesho*; (C) Plastic tank containing honey (which consists of about 200 different substances, with the main constituents being fermentable carbohydrates and minor components such as minerals, proteins, vitamins, lipids, organic acids, amino acids, aroma compounds, flavonoids, phenolic acids, 5-hydroxymethylfurfural pigments, waxes, pollen grains, enzymes, and other phytochemicals, making it one of the healthiest substrates for the preparation of honey wine); (D) *Birzi*; (E) *Mies*; (F) *Mies* containing *Berelle*.

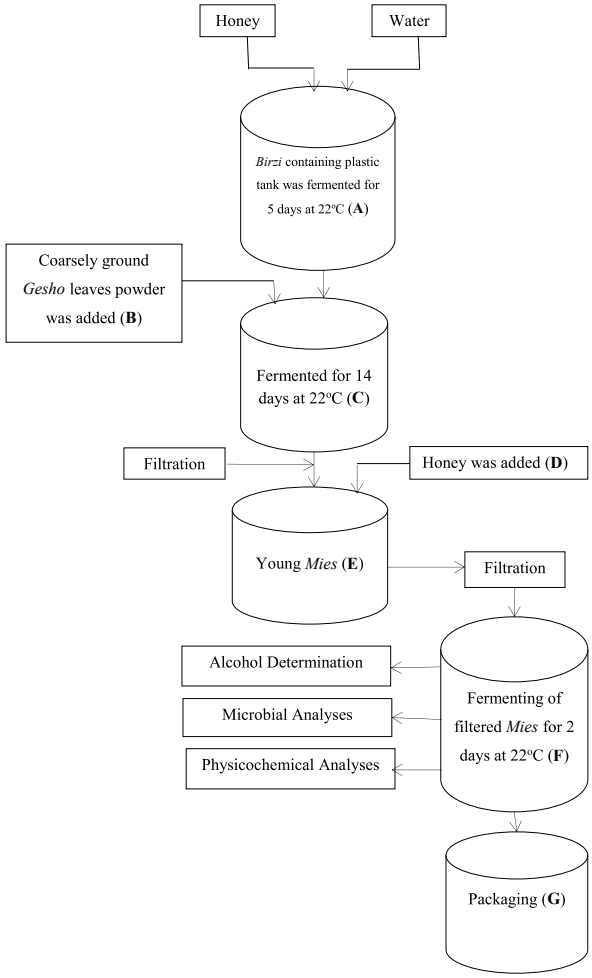

Fig. 2. Experimental workflow of *Birzi* and *Mies* preparation.

standardization and quality characterization of *Mies* processes [7]. The study aimed to standardize and characterize *Mies* through nutritional, physiochemical, and microbe analysis, highlighting the need for modernization and providing a foundation for further research, as standardization can enhance social and economic benefits.

2. Materials and methods

2.1. Sample collection

A total of 3 kg of dried *R. prinoides* leaves (Fig. 1A) and 20 kg of fresh honey were obtained from the Hahayle and Hagere Selam markets in the Central Zone of Tigrai (Fig. 1C). The leaves were coarsely ground (Fig. 1B) and stored in airtight plastic bags at 4 °C for further analysis [11].

2.2. Birzi and Mies Preparation

Twenty liters of purified packed water were procured from a shop in Mekelle, and nine plastic tanks were prepared for *Birzi* preparation, fermentation, filtration, and seasoning. The plastic tanks were washed with tap water and *Vernonia amygdalina* Del (*Grawa* in Tigrinya) leaves, and then dried upside down. The tanks were smoked/fumigated with *Gesho* and olive wood splinters for 10–15 min [12]. A clean dry stick served as a stirrer, and a filter made of white cotton cloth (*Shash* in Tigrinya) was used for the *Birzi* fermentation process. *Birzi* was prepared by dissolving honey in a ratio of 1:5 (honey: water) and fermented at 22 °C for 5 days in a brightly lit classroom (Fig. 1D). After fermentation, the honey wax was discarded from the *Birzi*.

Gesho (0.05 kg) was added to the *Birzi* in the plastic tanks. The tanks were tightly covered with clean cloth and left to ferment for 14 days at 22 °C, away from direct sunlight. During fermentation, the tanks were stirred daily with a clean dry stick to homogenize the mixture. The young *Mies* was then filtered through a clean white cotton cloth into a new plastic tank and fermented for an additional

Table 1
Three formulations of *Mies*.

Formulations	Ratio of Ingredients		
	Gesho	Honey	Water
Formulation (F_1)	0.05 Kg	0.4 Kg	2 L
Formulation (F_2)	0.04 Kg	0.5 Kg	2 L
Formulation (F_3)	0.05 Kg	0.5 Kg	2 L

two days to produce matured *Mies* for consumption by consumers using *Berelle* (Fig. 1E & F). The matured *Mies* was then stored in various containers in a refrigerator at 4 °C for further investigations (Figs. 1 and 2) [13].

Honey was dissolved in water (this is called *Birzi*) and fermented for 5 days (A); *Gesho* powder was added to the plastic tank containing *Birzi* and fermented for 14 days, after which it was filtered into a new tank (at this stage, it is called young *Mies*) (B & C); To enhance the flavor of *Mies*, honey was added to the young *Mies* and then filtered (D & E); The filtrate (young *Mies*) was fermented for 2 days to become matured *Mies* (ready to drink) (F); The ready-to-drink *Mies* was packed tightly in a new plastic tank to keep the beverage fresher, prolong its shelf life, prevent spoilage, and reduce oxidation reactions, which can lead to souring in a short period of time (G).

2.3. Proportion of ingredients used for Mies Preparation

To determine the best proportions of *Gesho* and honey, three *Mies* formulations were prepared and evaluated by the judges (Table 1).

2.4. Physiochemical and nutritional analyses of Mies and its ingredients

2.4.1. Determination of pH and temperature of Mies

Briefly, 25 mL of *Mies* was pipetted into a beaker, and the temperature and pH were measured using a digital pH meter [14,15].

2.4.2. Determination of titratable acidity

Briefly, 15 mL of *Mies* taken from the 21st day of fermentation was mixed with 0.5 mL of a 5 % phenolphthalein indicator in a conical flask, ensuring thorough mixing. The conical flask was titrated with 0.1N NaOH against a white background, continuing the titration until a faint pink color appeared. The pink solution was then mixed with 0.2 mL of alkali, with the first burette reading serving as the endpoint [14]. The titratable acidity was measured in grams per liter (g/L). The total titratable acidity of *Mies* was determined using the following formula: Titratable acidity = (0.1N NaOH (mL) × equivalent weight of acid (mL))/volume of Mies (mL).

2.4.3. Determination of total soluble solids

The refractometer was calibrated according to the manufacturer's instructions to measure the total soluble solids of *Mies* (HRO32-T, Germany). The electrode was submerged in the *Mies* sample taken from the 21st day of fermentation and the refractometer reading was recorded in °Brix [16].

2.4.4. Determination of alcohol content

The alcohol content of *Mies* was assessed using a Maligand apparatus. The Maligand apparatus was calibrated with standard alcohol, and a small amount of *Mies* was added to the second line of the apparatus, which was then closed properly. One hundred milliliters of distilled water were added to the Maligand's condenser and boiled, allowing the total alcohol content of the inserted *Mies* to be determined by raising the line to 75 °C [17].

2.4.5. Determination of moisture content

Gesho and honey samples were dried in a furnace at 100 °C for 1h, then placed in desiccators for 30 min, weighed, and placed in crucibles (W_2). The *Mies* samples were dried at 110 °C for 1h, and then cooled in desiccators at room temperature before being weighed (W_2). *Mies* samples were cooled, weighed, and moisture content of *Mies* and ingredients was calculated using an equation used by previous studies [18,19]. Moisture content was analyzed with a moisture content analyzer. The moisture content was calculated as: Moisture content (%) = (W_1-W_2)/W_1*100. Where, W_1 = weight of sample before drying; W_2 = weight of sample after dried. The experiment was done in triplicates.

2.4.6. Determination of crude ash content

The ash content of *Gesho*, honey, and *Mies* was calculated using a previously reported equation after being weighed, heated, and cooled [19,20]. Crude ash content of *Gesho* was determined as: Ash content of *Gesho* (%) = (Weight of ash (g)/Weight of *Gesho* (100 g))*100, honey was calculated as: Ash content of honey (%) = (Weight of ash (g)/Weight of honey (100 g))*100, and *Mies* was calculated as: Ash content *Mies* (%) = (Weight of ash (g)/Weight of *Mies* (100 g))*100.

2.4.7. Determination of carbohydrates

Briefly, 100 mg of *Mies* was digested with 2.5N HCl for 3 h, then cooled, neutralized with Na2CO3, and centrifuged to reach a final volume of 100 mL. Test tubes were used to introduce working standards (0.1 mg/mL concentration) and prepared *Mies*, with varying concentrations (0.2, 0.4, 0.6, 0.8, and 1 mL). Distilled water was added to each tube until a total volume of 1 mL was reached. A 1 mL phenol solution and 96 % H_2SO_4 were mixed in the tubes, shaken, and heated in a water bath for 20 min, with absorbance measured at 490 nm. The carbohydrate content of the samples was determined by the absorbance of 0.2 mL of the test solution [21] and calculated as follows: Total carbohydrate (%) = ((glucose (mg))/(volume of sample)) * 100. Since protein + lipid + ash + moisture + fiber + carbohydrate = 100, the carbohydrate content in the *Mies* and its ingredients was calculated according to a previous study [22].

2.4.8. Determination of proteins

Total quantity of proteins were determined using Kjeldahl method [18]. A 2g *Mies* sample was digested with 15 mL of concentrated H_2SO_4 at 410 °C for 45 min, followed by treatment with sodium hydroxide-sodium thiosulfate solution. Protein was distilled into a boric acid solution, titrated with 0.2N HCl, and calculated by multiplying by a factor (6.25). Crude protein (%) = N (%)* Factor (6.25). Experiment was conducted in triplicates, and mean values were recorded.

2.4.9. Determination of lipids

The gravimetric determination of the total lipid content of *Mies* was conducted [23]. A 2g of sample was solubilized in 2 mL of alcohol, hydrolyzed with 10 mL of HCl at 70–80 °C for 40 min, extracted with petroleum ether, evaporated, and dried at 100 °C for 90 min [24]. Crude lipid was calculated by the formula: Crude lipid (%) = (X-F)/W*100. Where, X – Weight of the beaker with fat; F – Weight of the beaker and W–Weight of the beaker with sample.

2.4.10. Determination of crude fiber

To determine the crude fiber of a sample, AOAC (Association of Official Analytical Chemists) procedure was used. A 2g *Mies* solution was boiled with 100 mL of 0.023 M H_2SO_4, filtered, washed, and transferred to a conical flask for further analysis. A 100 mL of hot 0.312 M NaOH was added into the flask, boiled for 30 min, and filtered quickly. Then the residue was washed with acetone, dried in an oven, cooled in a desiccator, and weighed in a crucible (W_2). The crucible and its content were burned in a muffle furnace at 550^{o}C for 2h and cooled in a desiccator and reweighed (W_3) [21,25]. Crude fiber was calculated using the formula: Crude Fiber (%) = (W_2-W_1)/W_1*100. Where, W_1 = Weight (g) of sample; W_2 = Weight of (g) of insoluble matter and W_3 = Weight of (g) of ash.

2.4.11. Elemental analysis

Atomic absorption spectrometry was used to determine the quantity of elements [26,27]. Atomic absorption spectrometry was calibrated using standard solutions of elements, and Ca, Mg, Mn, Cu, Fe, and Zn (each mg/100g) were quantified after *Mies* digestion. A 5g of *Mies* sample was digested with 20 mL of aqua regia (1/4 HNO_3, 3/4 HCL), then dried in an oven and filtered into a 50 mL volumetric flask [28]. Presence of Na and K and P and N in *Mies* was analyzed by flame photometry and spectrophotometry, respectively [25].

2.4.12. Determination of calorie

The caloric value of *Mies* and its ingredients were calculated by multiplying total carbohydrate, fat, and protein quantities by coefficients 4, 9, and 4, and reported in kilocalories [29]. Energy was calculated as: Energy = (% total protein x 4 + % total carbohydrates) x 4 + (% total fats) x 9.

2.5. Dominant microbes analyses

Briefly, 25 mL of *Mies* was mixed with 225 mL of saline solution and homogenized. Serial dilution was performed and dominant microorganisms were determined using pour plating method [30,31]. Whereas, the total aerobic mesophilic bacteria were evaluated based on the previous standard microbiological [32].

2.5.1. Lactic acid bacteria (LAB)

The sample was serially diluted and 1 mL was spread-plated on pre-dried de Man Rogosa and Sharpe (MRS) agar. The MRS-agar plates were incubated anaerobically at 37 °C for 48h. LAB colonies were identified using colony morphology, Gram staining, and biochemical tests [33,34].

2.5.2. Enterobacteriaceae, coliforms and enteric pathogens

A 0.1 mL sample was spread-plated on violet red bile glucose agar and incubated at 32 °C for 24 h. Purple/pink colonies surrounded by purple halos were considered as group of enterobacteriaceae [35]. To assess the presence of coliforms and enteric pathogens in *Mies*, 1 mL diluted *Mies* solution was spread-plated on MacConkey agar, supplemented with crystal violet, NaCl, and bile salts (0.15 %), and incubated at 37 °C for 24 h to detect coliforms and enteric pathogens [30].

2.5.3. Cultural and biochemical characterization of yeast

The sample (1 mL) was plated onto potato dextrose agar and incubated at 25 °C for 5 days, followed by purification using the streak plate method and stored at 4 °C for further analysis. The study examined the cultural characteristics of yeast isolates on PDA, including

Table 2
Effect of fermentation time on the pH of Three *Mies* formulations.

Weeks	Formulations	Days						
		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Week 1	F_1	3.98 ± 0.06^{a}	3.97 ± 0.00^{j}	3.94 ± 0.06^{r}	3.92 ± 0.00^{xx}	$3.90 \pm 0.06^{bbb*}$	3.85 ± 0.06^{bc}	3.85 ± 0.06^{bcc}
	F_2	4.12 ± 0.00^{b}	3.89 ± 0.06^{kk}	3.88 ± 0.00^{ss}	3.86 ± 0.00^{xx}	$3.82 \pm 0.00^{bbb*}$	3.81 ± 0.00^{cd}	3.80 ± 0.00^{cc}
	F_3	4.23 ± 0.00^{c}	4.19 ± 0.00^{kk}	4.17 ± 0.00^{ss}	4.17 ± 0.00^{xx}	4.15 ± 0.00^{bbb}	4.08 ± 0.00^{cd}	4.06 ± 0.00^{cc}
Week 2	**Formulations**	**Day 8**	**Day 9**	**Day 10**	**Day 11**	**Day 12**	**Day 13**	**Day 14**
	F_1	3.83 ± 0.00^{d}	3.82 ± 0.00^{l}	3.82 ± 0.00^{tt}	$3.82 \pm 0.00^{y*}$	3.82 ± 0.00^{cc}	3.82 ± 0.00^{ca}	3.82 ± 0.00^{cf}
	F_2	3.85 ± 0.00^{e}	3.85 ± 0.00^{m}	3.85 ± 0.00^{u}	$3.84 \pm 0.00^{y*}$	3.83 ± 0.00^{bb}	3.83 ± 0.00^{cb}	3.83 ± 0.00^{bg}
	F_3	3.99 ± 0.01^{f}	3.96 ± 0.06^{n}	3.96 ± 0.00^{tt}	3.96 ± 0.00^{y}	3.90 ± 0.00^{aa}	3.89 ± 0.00^{ac}	3.87 ± 0.00^{ah}
Week 3	**Formulations**	**Day 15**	**Day 16**	**Day 17**	**Day 18**	**Day 19**	**Day 20**	**Day 21**
	F_1	3.78 ± 0.00^{g}	3.78 ± 0.00^{o}	3.76 ± 0.06^{v}	$3.76 \pm 0.00^{aa*}$	3.75 ± 0.00^{b}	3.74 ± 0.00^{ba}	3.73 ± 0.00^{bn}
	F_2	3.76 ± 0.00^{h}	3.74 ± 0.00^{p}	3.73 ± 0.06^{ww}	$3.72 \pm 0.06^{aa*}$	3.72 ± 0.00^{cb}	3.72 ± 0.00^{ce}	3.72 ± 0.00^{cm}
	F_3	3.89 ± 0.00^{i}	3.88 ± 0.00^{q}	3.84 ± 0.06^{ww}	3.84 ± 0.00^{aa}	3.83 ± 0.00^{cb}	3.83 ± 0.00^{ce}	3.82 ± 0.00^{al}

Values with similar superscript letters within columns indicate no statistically significant difference ($P > 0.05$) in pH among the three formulations, while values with similar superscript letters followed by an asterisk (*) indicate a statistically significant difference ($P \leq 0.05$). Values with different superscript letters within columns indicate there is a statistically significant difference ($P \leq 0.05$) in pH among the three formulations. Values are presented as mean ± standard deviation of triplicates.

their ability to utilize carbon and nitrogen sources, D-glucose, fructose, maltose, galactose, lactose, and sucrose, and gas production during carbohydrate fermentation. Yeast isolates were identified based on morphological and biochemical tests at the genus level [35]. The study employs morphological and biochemical tests to identify yeast isolates at the genus level, aiming to understand their diversity and metabolic capabilities. Analyzing sugar utilization and fermentation gas production could offer insights into industrial applications and biotechnological applications.

2.6. Sensory evaluation of Mies

A total of 30 people (20 men and 10 women), who have indigenous knowledge of the qualities of the best *Mies*, were purposely selected to test the sensory characteristics of the beverage. The attributes were evaluated using a 9-point Hedonic Scale, where '9′ stands for 'Like Extremely,' '8′ for 'Like Very Much,' '7′ for 'Like Moderately,' '6′ for 'Like Slightly,' '5′ for 'Neither Like nor Dislike,' '4′ for 'Dislike Slightly,' '3′ for 'Dislike Moderately,' '2′ for 'Dislike Very Much,' and '1′ for 'Dislike Extremely.' The participants were also interviewed regarding the physiological characteristics of *Mies*, such as color, odor, flavor, and any possible side effects affecting the quality of the best *Mies*.

2.7. Data analyses

Quantitative data was collected three times from various measures, counts, tests, and experiments. SPSS Ver. 20 was used to process the data and perform relevant descriptive and inferential statistics. All data were analyzed using analysis of variance (ANOVA) with a p-value of less than 0.05. Mean (±SD) comparisons were made after ANOVA using the least significant difference. The quantitative data processing results were supplemented with qualitative data gathered from both microscope and visual observations [36].

3. Results and discussion

3.1. Physicochemical analyses of Mies

The safety and quality of beverages are significantly influenced by their physicochemical and proximate compositions, which help reduce microbial load and enhance organoleptic properties. An increase in ethanol content in beverages leads to a higher yeast population and a decrease in total carbohydrates. High moisture and pH levels in beverages encourage microorganism growth, resulting in reduced nutritional quality and an increased risk of foodborne illnesses. Probiotic microorganisms can mitigate these issues by competing for nutrients in the host's body. Consuming probiotic beverages helps maintain the balance of gut microbiota, reducing foodborne illnesses and improving digestive health. Additionally, probiotics enhance the nutritional value of fermented beverages by promoting the growth of beneficial bacteria. Incorporating probiotics into one's diet can positively impact gut health and overall well-being [35].

3.1.1. Effect of fermentation time on the pH of three Mies formulations

The pH values of the three *Mies* formulations, F_1 (3.98–3.73), F_2 (4.12–3.72), and F_3 (4.23–3.82), decreased from the 1st day to the 21st day due to increased fermentation and storage time (Table 2). Lactic acid bacteria (LAB) convert carbohydrates into organic acids, causing a decrease in pH. As they grow, lactic and acetic acids accumulate, leading to a more acidic environment. LAB initiate glycolysis, producing pyruvate, which is then converted into lactic acid. Other acidic compounds, such as acetic or formic acid, may

Table 3
Effect of fermentation time on the temperature of three *Mies* formulations.

Weeks	Formulations	Days						
		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Week 1	F_1	21.63 ± 0.06^{a}	22.00 ± 0.00^{ae}	21.13 ± 0.11^{ag}	22.10 ± 0.10^{a}	20.87 ± 0.06^{a}	21.70 ± 0.00^{a}	21.20 ± 0.00^{b}
	F_2	21.80 ± 0.00^{a}	21.87 ± 0.06^{ae}	21.03 ± 0.06^{bc}	22.10 ± 0.10^{a}	20.73 ± 0.06^{a}	21.67 ± 0.06^{b}	21.23 ± 0.06^{b}
	F_3	21.76 ± 0.58^{b}	21.70 ± 0.00^{ae}	21.23 ± 0.06^{bc}	22.13 ± 0.15^{a}	20.87 ± 0.06^{a}	21.63 ± 0.06^{ab}	21.33 ± 0.06^{a}
	Formulations	**Day 8**	**Day 9**	**Day 10**	**Day 11**	**Day 12**	**Day 13**	**Day 14**
Week 2	F_1	21.67 ± 0.06^{bb}	22.00 ± 0.00^{ba}	21.20 ± 0.00^{ad}	22.23 ± 0.06^{a}	20.87 ± 0.06^{a}	21.63 ± 0.06^{a}	21.23 ± 0.06^{a}
	F_2	21.80 ± 0.00^{bb}	21.86 ± 0.06^{ba}	21.03 ± 0.06^{dh}	22.00 ± 0.00^{b}	20.83 ± 0.06^{a}	21.60 ± 0.00^{a}	21.27 ± 0.06^{a}
	F_3	21.77 ± 0.06^{c}	21.70 ± 0.00^{ba}	21.23 ± 0.06^{dh}	22.10 ± 0.17^{ab}	20.90 ± 0.00^{a}	21.67 ± 0.06^{a}	21.26 ± 0.12^{a}
	Formulations	**Day 15**	**Day 16**	**Day 17**	**Day 18**	**Day 19**	**Day 20**	**Day 21**
Week 3	F_1	21.43 ± 0.06^{ab}	21.70 ± 0.00^{bf}	$21.60 \pm 0.00^{aa*}$	20.67 ± 0.06^{a}	21.63 ± 0.06^{a}	22.03 ± 0.06^{a}	22.47 ± 0.06^{a}
	F_2	21.43 ± 0.06^{ac}	21.80 ± 0.00^{bf}	21.53 ± 0.06^{aa}	20.70 ± 0.00^{a}	21.50 ± 0.06^{a}	22.07 ± 0.06^{a}	22.37 ± 0.06^{b}
	F_3	21.37 ± 0.06^{ad}	21.73 ± 0.06^{b}	$21.60 \pm 0.00^{aa*}$	20.73 ± 0.06^{a}	21.60 ± 0.00^{a}	21.23 ± 0.06^{a}	22.50 ± 0.00^{a}

Values with similar superscript letters within columns indicate no statistically significant difference ($P > 0.05$) in temperature among the three formulations, while values with similar superscript letters followed by an asterisk (*) indicate a statistically significant difference ($P \leq 0.05$). Values with different superscript letters within columns indicate a statistically significant difference ($P \leq 0.05$) in temperature among the three formulations. Values are presented as mean ± standard deviation of triplicates.

Table 4
Titratable acidity and total soluble solids of three *Mies* formulations on the 21st day.

Formulations	Titratable acidity (g/L)	Total soluble solids (°Brix)
F_1	0.02 ± 0.00^{a}	$18.83 \pm 0.29^{b*}$
F_2	3.00 ± 0.00^{a}	$16.24 \pm 0.02^{b*}$
F_3	3.50 ± 0.00^{a}	26.24 ± 0.03^{b}

Values with the same superscript letters within columns indicate a significant difference in titratable acidity and total soluble solids among the three formulations, whereas superscript letters followed by an asterisk (*) indicate no significant difference. Values with different superscript letters within columns indicate a statistically significant difference ($P \leq 0.05$) in titratable acidity and total soluble solids among the three formulations. Values are presented as mean ± standard deviation of triplicates.

also contribute to the pH drop. The availability of fermentable sugars and the buffering capacity of the growth medium also influence the decline in pH. Low-pH beverages can cause acidosis, a negative health effect that hinders nutrient absorption and increases tumor cell survival. Additionally, low pH interferes with heavy metal detoxification, cell repair, and energy production, making it crucial to maintain a balanced pH level. Consuming alkaline foods and beverages, like fruits and vegetables, can help neutralize acidity and promote a healthy pH balance [27].

In contrast, high acidity in *Mies* is necessary to prevent a flat taste and spoilage, as well as to maintain good color and flavor [14]. The pH value of high-quality *Mies* (F3) decreased from 4.23 on the 1st day to 3.52 on the 21st day of fermentation. In contrast, previous studies reported that the pH value of *Mies* increased from 3.56 to 4.45 [17] and from 3.07 to 4.90 [37] during fermentation. Variations in ingredients may be linked to factors such as fermentation time, ingredient ratio, and the quality of the ingredients. The study utilized red yellow honey with a pH value of 4.23, which aligns with reports within the quality regulation level of 3.4–6.1. The acidity of the honey may contribute to its excellent resistance to microorganisms and its natural flavor. This study emphasizes the importance of understanding the impact of various factors on the finished honey wine product. It reveals how ingredient variations affect fermentation and overall quality, allowing researchers to improve production processes and ensure that consumers receive consistent, high-quality honey wine [38].

3.1.2. Effect of fermentation time on the temperature of three Mies formulations

The study found no significant difference in the temperature of fermented *Mies*, with maximum and minimum recorded temperatures of 22.50 °C and 20.67 °C, respectively (Table 3). A balanced fermentation temperature can enhance the drink's shelf life by inhibiting harmful bacteria and ensuring the full flavor and quality potential. Brewers can avoid off-flavors and spoiled batches by carefully monitoring and adjusting the temperature during the fermentation process. Proper temperature regulation is critical for producing high-quality and consistent products, minimizing disruptions and extending shelf life. Investing in temperature control systems outweighs the costs, improving product quality, consumer satisfaction, and potentially increasing sales [38].

3.1.3. Titratable acidity and total soluble solids of the three Mies formulations

The titratable acidity of *Mies* on the 21st day of fermentation was found to be much lower than previously reported, ranging from 0.02 to 3.50 g/L (Table 4). In contrast, an earlier study documented titratable acidity within the range of 1 g/L to 1.03 g/L [35]. Our analysis revealed a significantly lower total soluble solids range of 18.83–26.24, compared to the previous report's 3877 % increase

Table 5
Effect of fermentation time on the alcohol content of *Mies*.

	Formulations	Fermentation time		
		1st day	15th days	21st day
Alcohol Content	F_1	2.80 ± 0.00^a	4.03 ± 0.06^b	5.30 ± 1.00^c
	F_2	2.30 ± 1.00^a	3.12 ± 0.01^b	4.09 ± 0.03^c
	F_3	2.93 ± 0.11^a	4.62 ± 0.06^b	5.72 ± 0.02^c

Values with similar superscript letters within columns indicate there is no statistically significant difference ($P > 0.05$) in alcohol content among the three formulations. Values with different superscript letters within columns indicate a statistically significant difference ($P \leq 0.05$) in alcohol content among the three formulations. Values are presented as mean ± standard deviation of triplicates.

Table 6
Nutritional content and total energy of *Mies* and its ingredients.

Samples	Percentage of Nutritional Content (%) and Total Energy						
	Moisture	Ash	Lipid	Protein	Fiber	Carbohydrate	Energy
Gesho	7.90 ± 0.02^c	8.15 ± 0.42^a	2.43 ± 0.05^a	1.24 ± 0.03^a	18.53 ± 0.47^a	61.73 ± 0.92^b	13.5 ± 3.94^b
Honey	27.39 ± 0.10^b	0.77 ± 0.42^c	0.81 ± 0.02^b	0.99 ± 0.02^b	1.00 ± 0.00^b	69.03 ± 0.05^a	287.40 ± 0.27^a
Mies (F_3)	97.1 ± 0.26^a	1.04 ± 0.14^b	0.13 ± 0.02^c	0.10 ± 0.03^c	0.00 ± 0.00^c	3.02 ± 0.01^c	73.91 ± 0.51^c

Values with similar superscript letters within columns indicate a statistically significant difference ($P \leq 0.05$) in nutritional content and total energy among the *Mies* ingredients, whereas similar superscript letters followed by an asterisk (*) within columns indicate no statistically significant difference ($P > 0.05$) in titratable acidity and total soluble solids among the *Mies* ingredients. Values with different superscript letters within columns indicate a statistically significant difference ($P \leq 0.05$) in titratable acidity and total soluble solids among the *Mies* ingredients. Values are presented as mean ± standard deviation of triplicates.

[39]. The study shows that variations in total soluble solids (TSS) and total dissolved solids (TDS) in beverage samples significantly affect the product's flavor and quality. TSS influences sweetness, acidity, and overall flavor balance, while TDS affects mouthfeel, texture, and aftertaste. Understanding these variables helps producers make informed decisions and ensures consistency. Acidity and pH in alcoholic drinks are closely linked, with pH indicating acidity. Regulating these factors is crucial for determining flavor, preservation, microbiological stability, and overall quality, enabling manufacturers to create beverages with optimal taste, texture, and shelf life.

3.1.4. Effect of fermentation time on the alcohol content of three Mies formulations

The alcohol content of *Mies* exhibited a positive correlation with the duration of storage, as shown in Table 5. Specifically, the alcohol content for F_1 was recorded as 2.80 % on the 1st day, 4.03 % on the 15th day, and 5.30 % on the 21st day. These values fall within the ranges reported in previous studies, which were from 4 % to 11.5 % (v/v) [10] and from 2.7 % to 21.7 % [7]. In high-quality *Mies*, the increase in alcohol content over time is attributed to the incorporation of honey, which serves the dual purpose of preserving the beverage's acidity and augmenting its alcoholic content.

This study underscores the finding that the alcohol content of high-quality *Mies* increases with fermentation time, reflecting observations in the alcohol content of Soursop wine during fermentation [14]. This research confirms that fermentation time plays a key role in shaping the alcohol content of high-quality *Mies*. According to the study, fermentation time significantly influences the alcohol content of high-quality honey wines; the longer the fermentation process, the more time yeast has to convert sugars into alcohol, resulting in higher alcohol content. To achieve the desired alcohol levels, honey winemakers must monitor and control fermentation time.

The length of fermentation also influences the flavor profile and overall quality of honey wine, making it an important factor in the winemaking process. The study highlights the significance of fermentation time in affecting the alcohol content, flavor, and overall quality of high-quality *Mies*, offering practical insights for producers to enhance their processes. The fermentation of *Mies* results in a decrease in pH and an increase in acidity, which are directly linked to the rise in alcohol content and significantly impact the flavor, consistency, and overall quality of the beverage.

3.2. Nutritional content and total energy of Mies and its ingredients

Table 6 provides a comprehensive overview of the nutritional composition and total energy content of *Mies* and its ingredients. The moisture content in dried *Gesho*, honey, and *Mies* was measured at 7.90 %, 27.39 %, and 97.1 %, respectively. Notably, the moisture content in *Gesho* was slightly lower than the previous report from Adet Agriculture Research Center (10.125 %) [40]. While the moisture content in honey closely resembled an earlier study's findings of 26.51 % [41], it was higher than another report's value of 16.7 % [42]. The moisture content in *Mies* was 97.1 %, similar to the moisture content observed in honey and coconut milk blend wine, which ranged from 89.83 % to 96.98 % [42].

The study determined the ash content in honey to be 0.77 %, slightly higher than a previous study's result of 0.35 % [42]. *Gesho* exhibited an ash content of 8.15 %, aligning with the standard values recommended by the WHO [21]. The prepared *Mies* had an ash

Table 7
Elemental analysis of *Mies* formulation three and its ingredients.

Contents	*Mies* and Ingredients result (mg/100g)		
	Mies	*Gesho*	Honey
Nitrogen (N)	1.72 ± 0.56^{a}	19.78 ± 0.45^{a}	15.91 ± 0.38^{a}
Phosphorus (P)	69.88 ± 0.13^{c}	87.89 ± 0.28^{b}	75.76 ± 0.12^{b}
Potassium (K)	122.99 ± 0.39^{c}	183.24 ± 0.53^{c}	246.00 ± 0.39^{b}
Sodium (Na)	9.37 ± 0.62^{a}	3.30 ± 0.36^{d}	5.00 ± 3.90^{d}
Calcium (Ca)	24.57 ± 1.60^{a}	19.4 ± 0.10^{e}	17.4 ± 0.26^{e}
Magnesium (Mg)	3.33 ± 0.20^{b}	4.57 ± 0.25^{f}	4.57 ± 0.25^{d}
Copper (Cu)	0.18 ± 0.01^{c}	0.33 ± 0.03^{g}	2.78 ± 0.28^{g}
Manganese (Mn)	0.02 ± 0.00^{c}	0.11 ± 0.05^{g}	0.56 ± 0.02^{h}
Zinc (Zn)	0.06 ± 0.01^{c}	0.16 ± 0.00^{g}	0.18 ± 0.25^{h}
Iron (Fe)	0.21 ± 0.02^{c}	1.98 ± 0.90^{h}	0.56 ± 0.03^{h}

Values with similar superscript letters within columns indicate no statistically significant difference ($P > 0.05$) in the elements among *Mies* Formulation Three (F_3) and its ingredients. In contrast, values with different superscript letters indicate a statistically significant difference ($P \leq 0.05$) in the elements among *Mies* Formulation Three (F_3) and its ingredients. Values are presented as mean ± standard deviation of triplicates.

content of 1.04 %.

Regarding lipid content, *Gesho* showed a lipid content of 2.43 %, lower than findings from previous research [21], while honey's lipid content was 0.81 %. The lipid content in *Mies* was 0.13 %, consistent with previous reports ranging from 0.03 % to 0.62 % [43]. The fiber content of *Mies*, honey, and *Gesho* was 0 %, 1 %, and 18.53 %, respectively. The carbohydrate content of *Mies*, *Gesho*, and honey was 3.02 %, 61.73 %, and 69.03 %, respectively (Table 6). A previous study reported that the mean total carbohydrate content of *Mies* samples from various production units ranged between 1.49 and 3.73 mg/mL [44], and it was observed that the carbohydrate content in *Mies* was lower compared to other traditional alcoholic beverages in Ethiopia [45].

The nutritional content of *Mies*, including total protein (0.35 %), crude fat (0.35 %), carbohydrate (3.58 %), and total ash (0.04 %), was detected at higher amounts in previous studies [5,35,46].

The study found that the protein content in *Gesho* (2.43 %) was significantly lower compared to a previous study (8.5 %) [47]. The protein content in honey (0.99 %) slightly differed from an earlier report (0.5 %) [48]. The protein content in *Mies* (0.10 %) was not statistically significant compared to other fermented alcoholic beverages in Ethiopia. The highest energy content (kilocalories/100g) was detected in honey (287.40) followed by *Mies* (73.91) and *Gesho* (13.5). The substantial energy content in honey and *Mies* suggests that *Mies* could serve as a significant source of energy for consumers. In this study, the energy content of honey did not significantly differ from prior findings. The study discovered that both *Mies* and honey are high in energy, implying that *Mies* could be a beneficial energy source for consumers. Although honey's energy content was similar to that of a previous study, *Mies* had slightly more energy than previously reported, suggesting that it may be even more valuable than initially thought. This indicates that *Mies* and honey are potent natural energy boosters [49].

3.3. Elemental analysis

Table 7 provides insights into the elemental composition of *Mies* and its components, highlighting their richness in various elements, including nitrogen, potassium, calcium, sodium, magnesium, copper, manganese, zinc, and iron. These elements are essential for maintaining bodily functions and overall health. Understanding their interactions in the body may help maximize the nutritional benefits of *Mies* consumption, potentially leading to tailored dietary recommendations for specific health conditions or populations. Potassium (K) emerged as the most abundant element, with concentrations of 122.99 in *Mies*, 183.24 in *Gesho*, and 246.00 in honey. Phosphorus (P) came in second, with concentrations of 69.88 in *Mies*, 87.89 in *Gesho*, and 75.76 in honey. Manganese (Mn) was detected at the lowest levels in both *Mies* (0.02) and *Gesho* (0.11), while zinc (Zn) exhibited the lowest concentration in honey (0.18). Nitrogen levels were notably higher in *Gesho* and honey compared to *Mies*. In *Gesho*, no significant differences were observed for potassium, sodium, calcium, magnesium, copper, manganese, iron, and zinc [50]. Essential macronutrients such as calcium (Ca), potassium (K), and magnesium (Mg) play crucial roles in animal nutrition, cell repair, human bone and tooth development, red blood cell formation, and various bodily functions. Manganese, found in small amounts in *Mies*, is particularly important for managing diabetes, underscoring its significance in food nutrition [51].

3.4. Dominant microbes analyses

The high microbial load of lactic acid bacteria (LAB) and yeast plays a significant role in the beverage, food, and pharmaceutical industries by generating essential chemicals, safe starter cultures, probiotics, biocatalysts, ethanol, enzymes, and health-promoting compounds [52]. Yeast-produced enzymes are not only environmentally friendly but also cost-effective, making them suitable for various fermentation industries [35].

In the later stages of fermentation, LAB and yeast gradually eliminate pathogenic microbes, thereby reducing the formation of undesirable products such as carcinogenic amino acids. Morphological identification of yeasts and bacteria using selective media

Table 8
Morphological characteristics of dominant microbes found in *Mies*.

Name of Microbes	Shape	Color	GS	CT	CT	CFU/mL
Aerobic mesophilic bacteria	Cocci & bacilli	Cream	+	+	Smooth	$3.9*10^5$
Lactic acid bacteria	Cocci & rods	Purple	+	–	Irregular	$5.65*10^5$
Enterobacteriaceae	Cocci (oval)	Pink	–	+	Smooth	10.60
Pathogenic bacteria	ND	ND	ND	ND	ND	0.00
Yeasts	Oval	Creamy	ND	–	Smooth	$6.7*10^5$

NB: ND indicates that not detected; GS: Gram Staining; CT: Catalase Test; CS: Colony Structure.

Table 9
Carbohydrate fermentation test.

Name of sugars	Microbial Isolates	
	Lactic Acid Bacteria	Yeasts
Lactose	+	-
Glucose	+	+
Fructose	+	+
Galactose	+	+
Sucrose	-	+
Dextrose	-	-
Maltose	+	+

revealed that LAB, which are Gram-positive, catalase-negative, and capable of fermenting lactose, fructose, glucose, galactose, and maltose, were predominant (Tables 8 and 9). LAB, crucial for the safety, nutritional value, shelf life, and acceptability of various foods, were identified as the dominant microbes in the study [34]. Table 8 illustrates a smooth, creamy white colony on PDA media capable of fermenting various carbohydrates. The study's findings align with yeast characteristics reported in previous research [53,54].

Evidence suggests that LAB potentially produce antimicrobial components such as acetic acid, higher concentrations of CO_2, H_2O_2, diacetyl, pyroglutamic acid, and bacteriocins. These components inhibit the growth of pathogenic bacteria and yeast species, potentially reducing the number of yeasts in the study [55–57]. Notably, pathogenic bacteria were not reported in *Mies* samples, which may be attributed to the inhibitory effects of its ingredients (*Gesho* and honey) [35]. LAB and yeasts are likely responsible for producing lactic acid and flavor components, respectively [58].

In general, the presence and types of microorganisms in traditional fermented beverages are influenced by the physicochemical properties and proximate composition of the fermenting substrates. In this study, the absence of pathogenic bacteria in *Mies* could be attributed to the role of LAB and yeasts in lowering the pH, suppressing the growth of unwanted microbes, enhancing the organoleptic properties of the fermenting mash, and producing beneficial compounds [35].

The study emphasizes the importance of a well-balanced microbial community in traditional fermented beverages for health and preservation. It suggests that understanding the interactions and contributions of microorganisms in fermentation can help determine the potential benefits of including fermented foods and beverages in our diets. This research opens up new opportunities for developing functional foods that promote both overall health and taste.

3.5. Sensory evaluation of Mies

The third formulation emerged as the most successful trial during the evaluation conducted by knowledgeable judges with indigenous expertise in assessing the qualities of the best *Mies* (Table 10). Recognizing that sensory quality serves as the ultimate measure of product success, sensory analysis encompasses a range of powerful and sensitive tools to gauge human responses to various products, including foods. During the evaluation, each assessor was presented with approximately 250 mL of *Mies* in a *berlle*. The majority of the judges reflected on the sensory qualities, noting a balance of sweetness and slight bitterness in the prepared samples. The sensory analysis results revealed that over 90 % of the judges expressed a preference for F3, indicating its favorable aroma and flavor. This preference is likely attributed to the well-proportioned ingredients, emphasizing the significant impact of ingredients on aroma and flavor.

In light of these findings, it is evident that the raw materials used in drink production and the type of yeast present during fermentation played a crucial role in defining the mouth feel parameters. The success of the third formulation in the sensory evaluation underscores the importance of ingredient selection and fermentation conditions in achieving a desirable sensory experience in *Mies* [17]. The sensory evaluation of the third formulation demonstrates the significance of these factors in enhancing the overall sensory experience. Further optimization of ingredient ratios and fermentation times could yield even better sensory attributes. Exploring different fermentation techniques or incorporating new ingredients may also improve the product's overall sensory profile. These findings highlight the importance of continuous improvement and innovation in *Mies* production to meet consumer preferences and market demands. The judges praised the formulation's harmonious blend of *Gesho* and honey, emphasizing the importance of ingredient proportions and precision in the brewing process.

Table 10
Sensory analysis of three formulations of *Mies* using a 9-point hedonic scale.

9-Point Hedonic Scale	Sensory evaluation	Sensory Attributes														
		F_1					F_2					F_3				
		C	T	A	MF	OA	C	T	A	MF	OA	C	T	A	MF	OA
9	Like extremely	8.91	8.85	8.72	8.93	8.95	8.10					8.7			8.65	8.21
8	Like very much							7.89	7.42	7.92	7.75		7.65	7.34		
7	Like moderately															
6	Like slight															
5	Neither like nor dislike															
4	Dislike slight															
3	Dislike moderately															
2	Dislike very															
1	Dislike extremely															

C: Color; T: Taste; A: Aroma; MF: Mouth feel; OA: Overall acceptability; F_1, F_2, F_3: Formulation 1, 2 and 3, respectively.

4. Conclusion

The study underscores the inconsistent quality of *Mies*, a honey wine, stemming from variations in preparation techniques, ingredient proportions, and unregulated fermentation processes. It emphasizes the necessity of standardizing and characterizing preparation procedures to enhance overall quality. Moreover, the research highlights the importance of modernizing and industrializing *Mies* production through comprehensive analysis encompassing nutritional, physicochemical, and microbial aspects. Optimal quality *Mies*, exemplified by formulation F_3, is achieved through a specific blend of *Gesho*, honey, and water, with fermentation temperatures averaging between 20.67 °C and 22.50 °C. Lactic acid bacteria and yeasts emerge as the primary microorganisms driving the fermentation process. The study offers valuable insights into standardizing *Mies* preparation and advocates for further investigations to delineate dominant microbial species, establish standardized production protocols, and effectively tackle production challenges. It emphasizes the importance of balancing physicochemical and microbial factors in *Mies* fermentation, suggesting that identifying critical parameters, such as fermentation temperature and microbial populations, can help improve product quality. Additionally, the study implies that further research could standardize production practices for *Mies*, enabling producers to make informed decisions and establish optimal conditions for consistency. This research highlights the potential for advancements in *Mies* production that benefit both producers and consumers.

CRediT authorship contribution statement

Weleba Muesho Gebremichael: Writing – original draft, Software, Resources, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Kiros Hagos Abay:** Writing – review & editing, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization. **Desta Berhe Sbhatu:** Writing – review & editing, Supervision, Project administration, Methodology, Data curation, Conceptualization. **Goitom Gebreyohannes Berhe:** Resources, Funding acquisition, Formal analysis, Conceptualization. **Gebreselema Gebreyohannes:** Writing – review & editing, Visualization, Validation, Supervision, Data curation, Conceptualization.

Data availability statement

Data will be made available on request.

Ethical and consent approval

The study involved participants who were informed about the study's objectives and the absence of health risks associated with alcohol consumption. They provided their consent and were assured that their data would remain private. Throughout the study, participants were closely monitored for signs of distress or negative health effects, and any concerns were promptly addressed by the research team. Participants had the option to withdraw without repercussions, ensuring ethical conduct.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:Weleba Measho reports financial support was provided by Mekelle University. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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