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Research Article

Biochemical properties, quality characteristics, and fermentation dynamics of metheglin affected by the addition of *Bergenia ciliata*

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Abstract

Herbs can be used to enhance the quality of beverages therapeutically and organoleptically. This study explored the potential for creating medicinal mead by incorporating local and indigenous herbs into fermentation. Specifically, the effects of adding *Bergenia ciliata* rhizome to honey must at 5 concentrations, i.e., 0, 0.25, 0.5, 0.75, and 1% w/v during fermentation on the physicochemical composition, antioxidant, antimicrobial, and α-amylase inhibitory activity, and organoleptic quality of the resulting metheglins were explored at 5% level of significance. The study found that concentrations of rhizome at or above 0.5% decreased fermentation rate, and the optimal concentration for the best overall properties was 0.5% or less. The incorporation of *B. ciliata* rhizome significantly increased the therapeutic properties of the metheglins, as indicated by increased total phenolic content, DPPH radical scavenging activity, antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli*, and α-amylase inhibitory activity. Minor changes were observed in volatile acidity and higher alcohol content at higher rhizome concentrations, but overall, the physicochemical properties of the metheglins were largely unaffected. These findings have important implications for promoting the commercial cultivation of precarious Nepalese medicinal plants and honey production.

Keywords

bergenia ciliata, herbal wine, metheglin, fermentation dynamics and kinetics, therapeutics properties, honey wine

Abbreviations

ANOVA – analysis of variance, DMAB – p-dimethyl amino benzaldehyde, DPPH – 2,2-diphenyl-1-picryl-hydrazyl-hydrate, GAE – gallic acid equivalent, HSD – honestly significant difference, MSA – mannitol sugar agar, RO – reverse osmosis, RSA – radical scavenging activity, TPC – total phenolic content, TSS – total soluble solid, UV – ultraviolet

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Introduction

Metheglin is one of the many styles of mead, which contains spices or herbs. Mead is an alcoholic product generally having 8-18% (v/v) ethanol made by yeast to the honey water must, followed by a period of fermentation time (Iglesias et al. 2014). Fermented honey must is one of the oldest fermented beverages. The production of mead has been known since ancient times.

Using herbs in alcoholic beverages offers numerous benefits, including antimicrobial, anti-cancerous, antioxidant, and other medicinal properties that positively impact physical and mental well-being. Studies have also shown that using herbs in alcoholic drinks can reduce hypertension and enhance overall body performance (Usman and Jawaid 2012). If medicinal herbs are used, metheglin produced might potentially be a medicinal beverage. Various herbs and spices play an important role in alcoholic beverage production. They are used as enhancers, preservatives, and antioxidant sources (Yuwa-Amornpitak et al. 2012). The alcohol from the fermented sugar in mead, paired with acids, acts as an excellent solvent or vehicle for extracting some of the beneficial components of spices/herbs with nutritional and medicinal value (Payne 2016). Metheglin, being a type of mead, not only causes less impact on the body's systems than distilled spirits, but it also comes with other nutritional benefits and elements that help the body break it down and process it.

Bergenia ciliata, commonly called Pakhanbedh, is a perennial rhizomatous creeping herb on rocks ledges with stout. The part used is the rhizome. In the Himalayan region, many rural communities use *B. ciliata* to treat various diseases. For centuries, the rhizome of *B. ciliata* has been used for curing pulmonary infections, leucorrhea, piles, and for dissolving bladder and kidney stones (Ahmad et al. 2018; Yadav 2016). It is popular in the hilly eastern region of Nepal for its many health benefits and is infused with alcohol to produce "pakhanbedh raksi."

Herbal wines have gained popularity as people increasingly seek luxury and health benefits from their food and beverage choices. Despite the growing demand for herbal fermented beverages like herbal wine and metheglin, research on their quality and production is limited. Challenges faced in mead and metheglin production include fermentation delays and lack of product uniformity due to factors such as honey variety, bioactive herb components, temperature, and pH. Insufficient technical knowledge and research hinder the growth and marketing of metheglin production.

Nepalese herbs, known for their therapeutic properties, can be incorporated directly into mead, offering a unique and indulgent experience. Local and indigenous herbs can be utilized to extract their full potential and create a luxurious product for distribution. In addition, studying the kinetics of honey must with different proportions of rhizome of *B. ciliata* fermentation can help understand the interaction of yeast with the chemical constituent of honey and *B. ciliata*, which helps to identify the chance of stuck fermentation and eliminate them. Also, with knowledge of fermentation kinetics, the process can be optimized. The present study explores the feasibility of using *B. ciliata* rhizome (also called "Pakhanbedh") to prepare metheglin and meet the demand for diverse, healthy products.

The hypothesis upon which this study was predicated posits that incorporating *Bergenia ciliata* into the mead fermentation process could potentially impede yeast growth and metabolism due to the herb's reported antimicrobial properties. Moreover, the bioactive components present in the herb may be extracted during the production of metheglin, thereby imbuing the resulting beverage with therapeutic properties. Additionally, it is posited that the herb's presence may alter the metheglin's chemical composition, including its volatile constituents and sensory profile.

Overall, the combination of herbs' medicinal properties and mead's nutritional benefits makes metheglin an appealing and potentially therapeutic beverage. Therefore, this study aims to investigate the fermentation kinetics, oenological, organoleptic, and potential health benefits of Pakhanbedh-infused metheglin.

Materials and Methods

Raw Materials. Brassica honey from *Apis cerena* fed on *Brassica napus* was obtained from a honey farm named "API enterprises" located at Cancer Gate, Bharatpur - 7, Chitwan, Nepal (Google plus code 7MV6MC79+42), which was farmed there locally. The herb (rhizome of *Bergenia ciliata*) was

bought from the local market of Dharan (Google plus code 7MR9R76P+GF), Nepal, which was locally grown, cleaned and air-dried in Namche (Google plus code 7MV8WJ42 + J6).

Yeast and yeast nutrients. The active wine yeast used for pitching and fermentation was *Saccharomyces cerevisiae*, from Fermentis SAFŒNO™ SC 22. The yeast nutrient used was Fermentis SpringFerm™, a multi-purpose fermentation activator made of partially autolyzed yeast with organic nitrogen, sterols, minerals, and vitamins.

Chemicals. The chemicals used in this study were trademarked and procured from Sigma Aldrich except for the MSA (HiMedia M118-500G) and MacConkey agar (HiMedia M081B-100G).

Preparation of metheglin. Total soluble solids (TSS) of 23°Bx was maintained by adding Reverse Osmosis (RO) processed water to honey and was measured with a hand refractometer. The pH was maintained at 3.7 using citric acid and was measured with a digital pH meter. The yeast nutrient was added at 0.2 $g.L^{-1}$ to the must. Triplicates of five must with different herb concentrations (0, 0.25, 0.5, 0.75, and 1% w/v) were prepared with granules of size (500-2500 µm) diameter after hammer milling the rhizome. The must was pasteurized with gradual heating at 65±0.5℃ for 15 min on an open pan heating system and cooled to 24℃. Yeast was activated/rehydrated with mildly heated water, and pitching was done at 10^5 $cfu.ml^{-1}$ for all musts. Fermentation was carried out in plastic jars of volume 2.25 L, which were sealed with an airlock system to create an anaerobic condition. The jars were shaken for the first two days to dissolve oxygen for yeast growth. The temperature of fermentation was between 20-29℃. Fermentation was stopped after gassing ceased, and there was no significant change in TSS, as shown in Fig. 1(a). After fermentation, the clear metheglin was drawn off using a sterilized food-grade silicon tube. After fining with bentonite, it was bottled in a 750 ml Bordeaux bottle, leaving 2.5 cm headspace, and pasteurized by heating the bottles to 65±0.5℃ for 15 min and then cooled to room temperature. A bottle with water was filled and kept with other bottles during pasteurization to keep a record of the temperature.

Determination of total soluble solid (TSS). The TSS of the honey, must, mead, and metheglins were determined using a hand refractometer (Hanna Instrument, Portugal) at 20℃ after degassing the samples. The results were expressed as °Bx.

Determination of reducing sugar. The percentage of reducing sugar in samples was determined by the Lane and Eynon method, according to Kirk and Sawyor (1991). The Fehling factor was calculated with the help of invert sugar. Invert sugar was taken in a burette, and a known volume of Fehling solution (A+B) was taken in a conical flask. This is titrated at a temperature of 65-70℃. Titration is continued till it acquires a very faint blue colour. At this stage, 3 drops of methylene blue indicator were added. The dye was immediately reduced to a colorless compound, and the endpoint was indicated by the change of blue colour to brick red. A similar procedure was applied to all the samples after filtration with the Whatman filter paper (grade 42). The reducing sugar was calculated as dextrose in $g.L^{-1}$.

Determination of pH. pH of honey, must, mead, and metheglins were determined by the digital pH meter of Deluxe pH meter, model LT-10, brand Labtronic™(IndiaMART InterMESH Ltd., Noida, Uttar Pradesh, India).

Determination of total acidity, fixed acidity, and volatile acidity. The total-, fixed- and volatile acidity were determined as per Kirk and Sawyor (1991). Total and fixed acidities were expressed in % w/v as lactic acid, while volatile acidity was expressed in % w/v as acetic acid. The sample was mixed with an equal volume of distilled water to determine the volatile acidity. Subsequently, the mixture was boiled until the volume was reduced by half before titration. Titration of the samples with standardized NaOH was done to assess their acidity.

Preparation of distillate from mead and metheglins. Transferred 200 ml of neutralized fermented honey musts to a 500 ml distillation flask with 25 ml of distilled water and pumice stone. Distilled for 35 min, collected the distillate in a 200 ml flask. Adjusted the volume with distilled water and mixed well.

Determination of ethanol content. The percentage of alcohol by volume was determined according to AOAC (2007) using a pycnometer.

Determination of ester content. The titrimetric method determined the total esters content per Kirk and Sawyor (1991). A hundred ml of the distillate was neutralized with 0.1 M NaOH, and 10 ml of 0.1 M NaOH solution was added. It was refluxed for 1 h using glass beads, cooled, and titrated with 0.05 M H_2SO_4 solution. Similarly, a blank was also run using 100 ml of distilled water instead of the distillate. Total esters content was expressed as an ethyl acetate in g.100 L^{-1} of alcohol calculated using Equation 1.

$$Total\ esters,\ g\ ethyl\ acetate.100\ L^{-1}alc. = \frac{880\ x\ V}{S} \quad (1)$$

where, V = (Blank titer – Sample titer), ml

S = Alcohol content in the distillate, % (v/v)

Determination of aldehyde content. The titrimetric method determined the total aldehyde content per FSSAI (2015). Fifty ml of the distillate of liquor was taken in a 250 ml iodine flask, and 10 ml of bisulfite solution was added. Kept the flask in a dark place for 30 min with occasional shaking. Twenty-five ml of standard iodine solution was added, and back-titrated excess iodine was against standard thiosulphate solution using a starch indicator to a light green endpoint. A blank was run using 50 ml of distilled water similarly. The difference in titer value in milliliters of sodium thiosulphate solution gives the equivalent aldehyde content expressed as acetaldehyde as given by equation 2.

$$\frac{V \times 0.0011 \times 100 \times 1000 \times 2}{V1},\ g.100\ L^{-1}\ alcohol \quad (2)$$

V = difference in titer of blank and sample in ml

V_1 = alcohol percentage by volume.

Determination of fusel oil (higher alcohol). Higher alcohol was determined by DMAB spectrophotometric method as per AOAC (2007) and expressed as milligrams per liter of metheglin, $mg.L^{-1}$.

Determination of methanol content. Methanol content was determined by the chromotropic acid colorimetric method as per OIV (2020) and expressed in $mg.L^{-1}$ of metheglin.

Determination of total phenolic content (TPC). Total phenolic content was determined as per Prior et al. (2005) using the Folin-Ciocalteau method. Different concentrations of gallic acid standard solution (0, 25, 50, 100, and 200 ppm) were prepared. One ml of the filtered (Whatman 42 filter paper) metheglin was diluted to 10 ml with distilled water. One ml of the diluted metheglin was pipetted into a test tube, and 2 ml of distilled water and 0.5 ml of Folin-Ciocalteu reagents were added. After 3 min, 2 ml of sodium carbonate solution (20%) was added, mixed thoroughly, and incubated at room temperature for 1h, after which the absorbance was measured at 765 nm against a reagent blank. The same process was applied to measure the absorbance of gallic acid standard solutions, and the standard curve was plotted. The total phenolic content in the metheglin was calculated from the standard curve prepared, and the result was expressed as mg of gallic acid equivalent (GAE). 100 ml^{-1} metheglin.

Determination of antioxidant activity. The antioxidant activity of the product was determined by the DPPH method as per Sing et al. (2008). Briefly, the metheglin was filtered through Whatman No. 42 filter paper. One ml of the filtered metheglin was diluted to 10 ml with methanol. One ml of the diluted metheglin was taken in a test tube, and 4 ml of 0.004% methanolic solution of DPPH was added. Then the test tube was left for 30 min in the dark, and absorbance was measured at 517 nm using a UV-vis spectrophotometer Labtronics, model LT-291 (Uvsar India Pvt. Ltd, Ghaziabad, India). Similarly, a blank was also run using methanol instead of the sample. The DPPH scavenging activity was calculated by using Equation 3.

$$\frac{(Blank\ absorbance - Sample\ absorbance) \times 100}{Blank\ absorbance} \quad (3)$$

Determination of antimicrobial activity. Antibacterial activity of prepared products against Gram-positive bacteria, *Staphylococcus aureus*, and a Gram-negative bacteria, *Escherichia coli*, was performed by well diffusion technique per Deans and Ritchie (1987). The *E. coli* in MacConkey agar and *S. aureus* in mannitol salt agar (MSA) were isolated from raw cow milk from the local farm of Dharan. The *S. aureus* was identified based on Gram staining (+ve), colony morphology, oxidase (+ve), and catalase test (+ve). The *E. coli* was identified based on gram staining (-ve), colony morphology, catalase (+ve), and oxidase (-ve) tests. Media was prepared by dissolving 5.6 g of nutrient agar and 2.6 g of nutrient broth in 200 ml of distilled

water in the flask. The nutrient broth was taken approximately 7-8 ml per test tube. All the apparatus and media, viz. Petri plates, tips, and normal saline used in the activity were autoclaved for 20 min at 1215℃. After sterilization, nutrient agar was poured into the Petri plates and allowed to solidify. The culture was actively grown in nutrient broth for 16 h at 375℃. 100 µL liquid culture of each microbe was spread on each nutrient agar plate to create a bacterial lawn of the respective microbe. 6 wells with a diameter of 6 mm were punched in a nutrient agar plate, and 100 µL mead and metheglin samples were added to the bored wells in each plate under aseptic conditions as well as 10% (v/v) pure ethanol was also loaded in one of the wells. The plates were left for 30 min at room temperature for the diffusion of the test samples before being incubated at 375℃for 24 h, after which the diameter of zones of inhibition (in mm) was measured using a vernier caliper. Analyses were carried out in triplicate.

Determination of the number of yeast cells. The number of yeast cells was determined using a hemocytometer. For dilution, 0.5% sulfuric acid was used as a diluent to deflocculated cells, and serial dilution was performed when required. Budded yeast cells were counted as one cell if the bud was less than half the mother cell's size, and both cells were counted if the bud was equal to or greater than half the mother cell's size. Counting no fewer than 75 cells on the entire 1-mm 2 x 9 ruled area and no more than 48 cells in one of the 25 squares was recommended to obtain an accurate yeast cell count. The correct observation was considered if the counts from both sides of the slide were agreed to within 10%. The number of cells per milliliter was calculated using Equation 4.

$$Number\ of\ cells.ml^{-1} = Total\ cells\ in\ central\text{-}25\text{-}square\ ruled\ area \times dilution\ factor \times 10^4 \quad (4)$$

Determination of pancreatic α-amylase inhibitory activity. α-amylase inhibitory activity of the prepared metheglins was carried out according to Ademiluyi and Oboh (2013) with minor modification. The reaction mixture containing 500 µL sodium phosphate buffer (100 mM, pH = 6.9), 100 µl α-amylase (2 Unit.ml^{-1}, i.e., 1 unit = 1 µmol.min^{-1}), and 200 µl of the sample was pre-incubated at 37°C for 20 min. Then, the 200 µL of 1% soluble starch (100 mM phosphate buffer pH 6.9) was added as a substrate and incubated further at 37°C for 30 min; 1000 µL of the DNS color reagent was then added and boiled for 10 min. The reaction mixture was diluted after adding 10 ml of distilled water, and absorbance was measured at 540 nm. In parallel, control was created with the addition of water as a sample, and each experiment was performed in triplicates. The results were expressed as percentage inhibition, calculated using Equation 5.

$$Inhibitory\ activity,\ \% = \frac{Ac - As}{Ac} \times 100 \quad (5)$$

where, As is the absorbance in the presence of the test substance, and Ac is the absorbance of control.

Sensory evaluation. The prepared samples were subjected to sensory evaluation for consumer acceptability. The samples were served in clean wine glasses in a quiet environment. Sensory attributes (such as appearance, odor, in-mouth sensation, finish, and overall quality) were evaluated using 7 points hedonic rating test ranging from faulty (1) to exceptional (7) as described by Jackson (2002) with the help of 15 semi-trained panelist with age ranging from 22-54, who were teachers and students of food technology who were familiar with alcoholic beverages. Before the sensory evaluation, two hours seminar on the method of wine sensory was provided to the panelists.

Statistical analysis. All the data obtained in this work was analyzed by the statistical program known as GenStat (Genstat Discovery Edition 12 2009). ANOVA on the data was conducted, and the treatment means were compared by Tukey HSD test at a 5% significance level to determine whether the samples differed significantly from each other. A superscript was assigned to each of the mean values in descending order. MS-Excel 2016 was also employed for general calculations, graphs, and diagram construction.

Results and Discussion

Chemical analysis of honey. The analysis of the honey used in the product preparation is given in Table 1.

Fermentation dynamics of honey must with different concentrations of *B. ciliata*. The TSS generally represents the sugar content of the honey

Table 1. Analysis of the honey

Parameter	Value
TSS, °Bx	81.00 ± 0.00
Acidity, % as lactic acid	0.21 ± 0.02
pH	4.60 ± 0.00
Moisture, %	17.61 ± 0.28
Total ash, %	0.17 ± 0.02
Reducing sugar, % as dextrose	77.00 ± 0.40
Total phenolic content, mg GAE.100 g^{-1}	68.00 ± 0.23

*Values in the above table are the means of three determinations

must, as seen from the honey analysis. From the presented graph in Fig. 1(a), for all the samples, the slope is highest for up to day 4, meaning the rate in change of TSS was highest during that course of fermentation, which is highest being a 43% decrease in sample 0% and lowest being 34% decrease in sample 1%.

Since the values for TSS and sugar content in honey must are similar, as seen from the chemical analysis of honey, and the sugar in honey is mainly reducing sugars, the rate of change in the reducing sugar content is very similar to the rate of change in TSS content as observed in Fig 1 (b). On the last day of fermentation, there was a 77% and 70% decrease in reducing sugar content for herb concentrations 0% and 1% (w/v), respectively.

The acidity increased during the initial fermentation days for up to 9 d, which increased approximately 37-43% (min-max%) of the initial value, after which it slowed down, and the total increase was approximately 51-60% of the initial value. The rise in acidity is due to the increase in volatile (acetic acid) and non-volatile acid (succinic acid and lactic acid) production by yeast metabolism during alcoholic fermentation, as suggested by Thoukis et al. (1965). The graphical presentation of the changes in acidity for all 5 samples is shown in Fig. 1(c).

Ethanol is formed by the conversion of sugar in must to ethanol through the metabolism of sugar by yeast. The ethanol production rate was high at the initial phase of fermentation for up to 12 d, where most of the ethanol (>85%) was produced in the samples, after which the rate decreased during the later fermentation phase. The ethanol yield of the five samples, 0, 0.25, 0.5, 0.75, and 1% w/v for this fermentation, were found to be 47.7, 48.1, 47.1, 48, and 49.8%, respectively. Since the ethanol is converted from the reducing sugar in the honey must, the pattern for the rate of ethanol production is somewhat inverse to the rate of reducing sugar depletion, as shown in Fig. 1(d).

The yeast growth (increase in the number of yeast cells) increased exponentially during the first five days of fermentation, which was an over 500-fold increase in the number of cells in all the samples, after which the growth was slowed down, then the yeast entered the stationary phase which is similar to what Pereira et al. (2013) found in their study. Sample 0.25% exhibited a growth increase of 1100 times, while sample 1% showed a growth increase of 700 times after 21 d. The former displayed the largest growth, while the latter displayed the smallest growth among the five samples. The graphical presentation of the changes in yeast number regarding fermentation days is presented in Fig. 1(e).

From the presented data, we can conclude that the *B. ciliata* concentration significantly affected the yeast growth dynamics affecting the metabolism of sugar, acidity, and ethanol production during the alcoholic fermentation of the honey must. Increasing the concentration decreased the cell number and growth rate as well as substrate utilization and product formation except for concentration 0.25% (w/v), which might be due to low antimicrobial activity at the given concentration and supplement of yeast growth factors or there might not be a significant difference between control and sample 0.25% so, more study may help to get on a rigid conclusion. This can be described as *B. ciliata* being a powerful antimicrobial herb (Khan et al. 2018; Singh et al. 2017).

Effect on total soluble solid (TSS) and reducing sugar. The reducing sugars are the primary food source for yeast growth and ethanol precursor in fermentation. The honey is rich in reducing sugar (>90% of total sugar), so no amylase source or hydrolysis treatment is needed for honey fermentation as in starchy commodities. Since reducing sugar is the major soluble solid in honey, TSS and reducing sugar correlate highly, as shown in the data in Table 2.

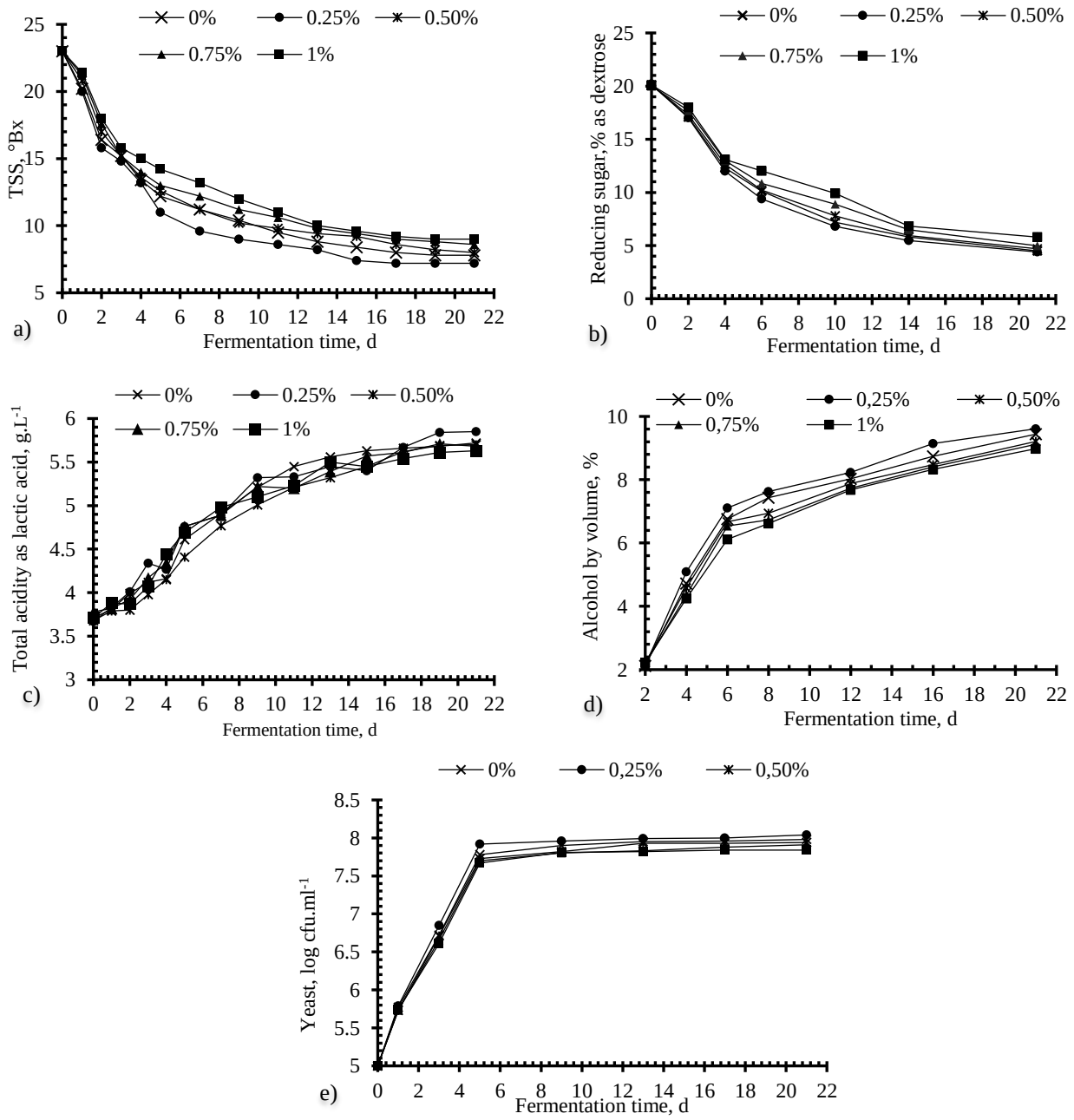


Figure 1. Fermentation dynamics of (**a**) TSS depletion, (**b**) reducing sugar, (**c**) total acidity (% lactic acid), (**d**) ethanol formation, and (**e**) yeast growth over the course of fermentation

Effect on pH, total titratable, fixed, and volatile acidity. Total titratable acidity and fixed is expressed as lactic acid and volatile as acetic acid. The data for pH and acidities are presented in Fig. 2. There was no significant difference ($p \geq 0.05$) in herb addition on the products' pH, titratable and fixed acidity. Volatile acidity decreased on adding herbs at higher concentrations ≥ 1%. This might be due to the lower metabolism of yeast at higher herb concentrations since yeast produces volatile acetic acid during fermentation. Steinkraus and Morse (1973) reported acetic acid in honey wines as 0.014-0.078 g.100 ml^{-1}, corresponding with our data of 0.04±0.004 g.100 ml^{-1}. So, we can conclude that the herb has no acidic or basic properties which affect the acidities profoundly.

The pH for the five metheglins were 3.63, 3.56, 3.56, 3.60, and 3.60 for herb concentrations 0, 0.25, 0.5, 0.75, and 1% (w/v), respectively.

Table 2. Final TSS and reducing sugar content of the metheglins

***B. ciliata* concentration, % w/v**	**TSS, °Bx**	**Reducing sugar, as dextrose g.L^{-1}**
0.00	$7.53^{c} \pm 0.23$	$45.21^{bc} \pm 2.49$
0.25	$7.27^{c} \pm 0.12$	$44.58^{c} \pm 3.55$
0.50	$8.13^{b} \pm 0.12$	$47.03^{b} \pm 1.78$
0.75	$8.60^{a} \pm 0.20$	$51.78^{a} \pm 1.51$
1.00	$8.86^{a} \pm 0.16$	$58.29^{a} \pm 2.25$

[a, b, c] - indices showing significant differences ($p < 0.05$) between the mean values in the columns

Effect of different concentrations of *B. ciliata* on volatile constituents of produced metheglins. Volatile constituents, viz., alcohol, total esters, total aldehydes, higher alcohols, and methanol contents of samples, were analyzed. Esters, aldehydes, volatile acids, and higher alcohols are mainly responsible for giving the nose to the fermented beverage. The data for volatile constituents of analyzed metheglins are given in Table 3.

The ethanol content decreased significantly ($p < 0.05$) after the herb concentration increased from 0.5% to that of the control. This is due to the low sugar consumption rate and growth rate of yeast in high *B. ciliata* concentration. The ester content of the fermented products does not differ from each other and with control significantly ($p \geq 0.05$). Pereira et al. (2019) reported ester content in mead to be 30-65 mg. L^{-1}, and concentration is affected by the strain of yeast used, to which our data corresponds, which is 46.28±6.21 mg.L^{-1}. So, adding *B. ciliata* does not affect the ester content of metheglin. The aldehyde content of *B. ciliata* added metheglins showed no significant difference ($p \geq 0.05$) between the control and each other.So, the addition of *B. ciliata* does not affect the aldehyde content of fermented honey must. Some authors stated carbonyl content in meads produced by *S. cerevisiae* in concentrations between 5 and 30 mg.L^{-1} (Pereira et al. 2013; Roldán et al. 2011). Steinkraus and Morse (1973) stated that the acetaldehyde concentration in meads usually ranges between 18.2 and 125.5 mg.L^{-1}. But aldehyde content varies with

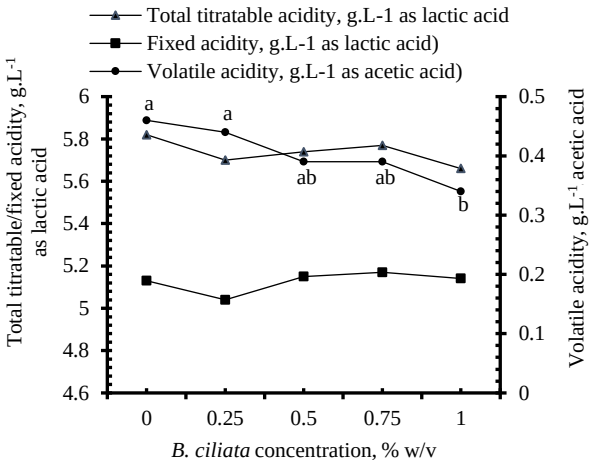


Figure 2. Different acidities of produced metheglins

the type of honey and yeast strain. Since our yeast strain and honey type differ from those stated in their study, the data might not correspond with them. Also, the difference is not myriad.

The methanol content of *B. ciliata* added metheglins showed no significant difference ($p \geq 0.05$) between the control and each other. The methanol content of mead is very low since there is almost no pectin compound in honey, and pasteurization of the must deactivates any pectinase present in honey or herb. Also, it has been found S. cerevisiae doesn't produce pectinase enzyme.

The higher alcohol content increased slightly by adding *B. ciliata* to the honey must. This might be due to the stress given by *B. ciliata* to yeast. The higher alcohol in mead ranges from 90 to 350 mg/L (Mendes-Ferreira et al. 2010; Pereira et al. 2013; Roldán et al. 2011), which corresponds with our data. The higher level may be due to the rising of temperature during day time, leading to higher alcohol formation since high temperature favors higher alcohol production during fermentation (Albertazzi et al. 1994).

Effect on total phenolic content (TPC). The TPC content of metheglins was significantly different ($p < 0.05$) from each other, and increasing the *B. ciliata* concentration increased the TPC content in produced metheglins, as shown in Fig. 3(a).

Table 3. Volatile constituents of *B. ciliata* added metheglin.

***B. ciliata* concentration, % w/v**	**Ethanol content, % v/v**	**Ester content, g ethyl acetate. 100 L^{-1} alc.**	**Aldehyde, g acetaldehyde.100 L^{-1} alc.**	**Methanol, $mg.L^{-1}$**	**Higher alcohol, $mg.L^{-1}$**
0.00	$9.44^{a} \pm 0.10$	$48.32^{a} \pm 3.31$	$201.43^{a} \pm 10.55$	$28.90^{a} \pm 8.87$	$311.42^{b} \pm 9.40$
0.25	$9.61^{a} \pm 0.11$	$45.45^{a} \pm 5.34$	$198.72^{a} \pm 6.89$	$31.16^{a} \pm 2.41$	$337.3^{ab} \pm 13.14$
0.50	$9.21^{b} \pm 0.14$	$50.26^{a} \pm 6.21$	$206.16^{a} \pm 5.46$	$33.42^{a} \pm 7.12$	$333.52^{ab} \pm 9.82$
0.75	$9.12^{bc} \pm 0.11$	$43.76^{a} \pm 7.67$	$201.12^{a} \pm 17.83$	$31.56^{a} \pm 3.46$	$354.76^{a} \pm 18.98$
1.00	$8.98^{c} \pm 0.08$	$44.2^{a} \pm 5.32$	$205.53^{a} \pm 3.46$	$30.19^{a} \pm 5.56$	$352.89^{a} \pm 5.73$

$^{a, b, c}$ - indices showing significant differences ($p < 0.05$) between the mean values in the columns

Water and ethanol present in the fermenting must act as a solvent to extract the polyphenols from the herb. Mead (0%), the control in the study, also had TPC content that got along with the honey used to make mead and metheglins since honey contains phenolic compounds. The data shows that adding just 0.25% to 1% w/v of herb increases the musts TPC content by > 140% to > 630%, respectively. So, we can conclude that inducing *B. ciliata* to a fermenting must significantly increase the TPC content of the final resulting metheglin present in the fermenting must act as a solvent to extract the polyphenols from the herb. Mead (0%), the control in the study, also had TPC content that got along with the honey used to make mead and metheglins since honey contains phenolic compounds. The data shows that adding just 0.25% to 1% w/v of herb increases the must TPC content by > 140% to > 630%, respectively. So, we can conclude that inducing *B. ciliata* to a fermenting must significantly increase the TPC content of the final resulting metheglin.

Effect on antioxidant activity. The % DPPH radical scavenging activity (RSA) of metheglins were significantly different ($p < 0.05$) from each other, and increasing the *B. ciliata* concentration increased the value of produced metheglins. % RSA is increasing similarly with the TPC content of respective metheglins, as shown in Fig. 2(a), so we can say the phenolic compounds from the herb *B. ciliata* are primarily responsible for the antioxidant activity in the metheglin and looking at the pattern of the graph for TPC and %RSA in Fig. 2 (a), we can establish they are highly correlated as stated by Agnihotri et al. (2014). Mead (0%), the control in the study, also had % RSA activity that got along with the honey used to make mead and metheglins. So, fermented beverages made by incorporating the herb into the must can significantly increase the antioxidant activity in the final fermented product, and increasing the concentration of the herb increases the antioxidant activity.

Effect on antimicrobial activity. The antimicrobial activity of the produced metheglins was assessed with the well diffusion method, and two food pathogens, i.e., *Staphylococcus aureus* and *Escherichia coli* were taken for the study. Since ethanol also exhibits an inhibitory effect on microorganisms, 10% (v/v) ethanol was used as a control. The zone of inhibition produced from metheglins were significantly different ($p < 0.05$) from each other, and increasing the *B. ciliata* concentration increased the value of produced metheglins, as shown in Fig. 3(b). Mead also had an inhibitory effect on *S. aureus* and *E. coli* because of the honey, which contains antibiotics properties as stated by many authors (Amit et al. 2005; Moussa et al. 2012; Sherlock et al. 2010; Taormina et al. 2001). Also, from the data, ethanol at 10% v/v has some antimicrobial properties. So, *B. ciliata* incorporated metheglin and potentially other alcoholic beverages can suppress pathogens in the gut. Also, it might preserve fermented products from spoilage-causing microbes and might act as a preserving fluid for herbs or fruits. So, we can conclude that the inhibitory effect of metheglins is due to ethanol, herb components, and honey antimicrobial activity.

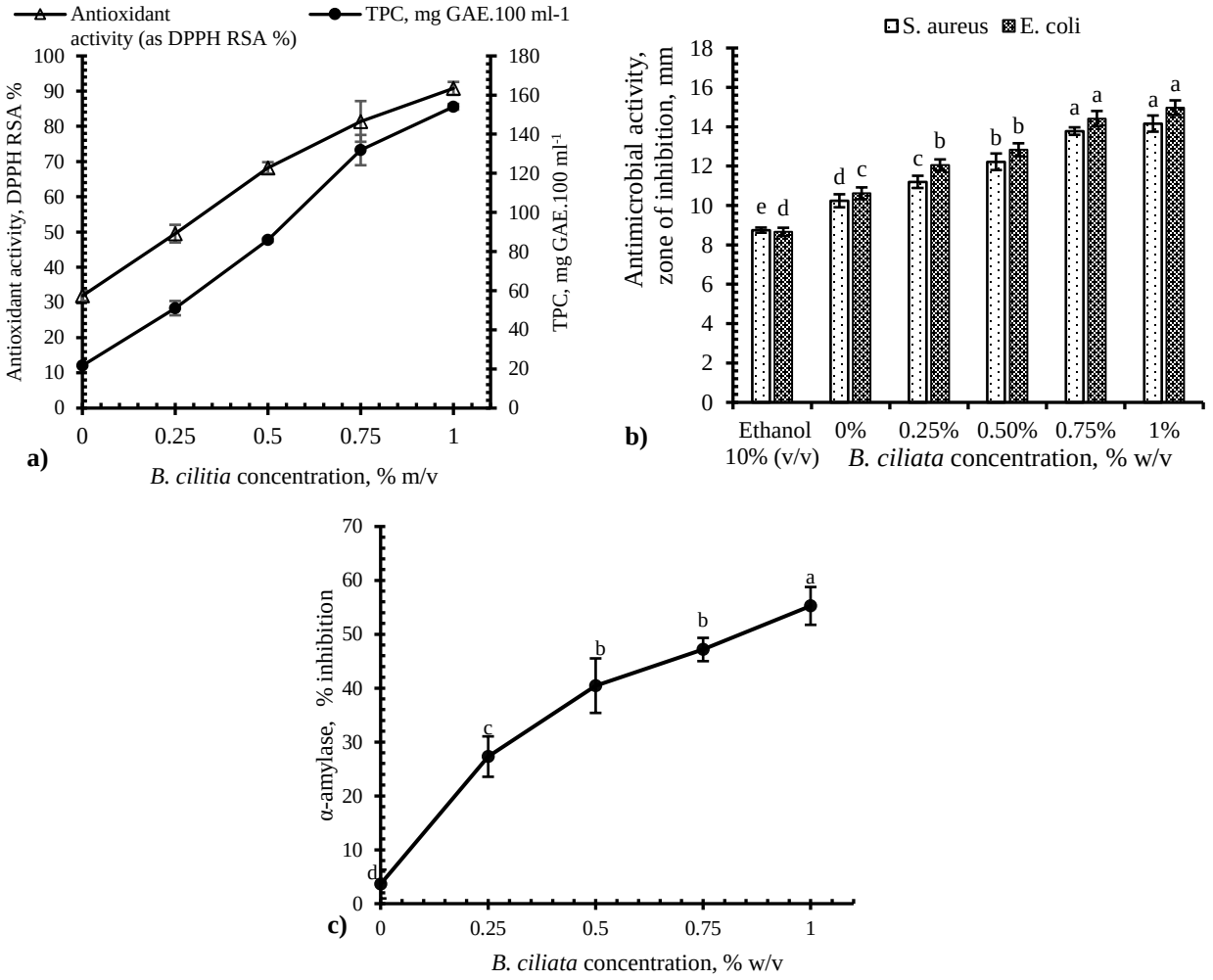


Figure 3. Effect of different *B. ciliata* concentration of metheglins on (**a**) TPC content and % DPPH RSA, (**b**) antimicrobial activity, (**c**) α-amylase inhibitory activity

α-amylase inhibitory activity. The α-amylase inhibitory activity was assessed using porcine pancreatic α-amylase. Incorporating the herb *B. ciliata* significantly boosted ($p < 0.05$) the inhibitory effect on porcine pancreatic α-amylase. So, this might also slow down the breakdown of starch in the human intestine, leading to the slow formation of glucosides for glucosidase to work on, resulting in low glucose levels, thus having an anti-diabetic effect. Increasing the herb concentration increased the inhibitory effect on amylase with a maximum inhibition at herb concentration of 1%, at which the inhibitory effect increased over 100% than at 0.25% herb concentration, as shown in Fig. 3(c). Using *B. ciliata* in a starchy material for fermentation might cause slow fermentation due to inhibition of amylase enzyme and slow growth rate of microbes in higher concentrations of herb. Bhandari et al. (2008) reported two active compounds (-)-3-O-galloylepicatechin and (-)-3-O-galloylcatechin to have dose-dependent enzyme inhibitory activities against porcine pancreatic α-amylase which probably got extracted by water and ethanol during fermentation in the metheglins and are responsible in our case also for the inhibitory action.

Effect of different concentrations of *B. ciliata* on organoleptic properties of produced metheglins. The product's appearance improved with increasing concentration of *B. ciliata* up to 0.75%, giving it a golden yellowish hue, which enhanced its aesthetic value. Also, increasing the herb concentration increased the clarity of the product, which might be due to the higher polyphenol content helping the sedimentation process. The optimum range for the best appearance of metheglin was found to be 0.25-0.50%. However, the high intensity of colour was less appreciated by panellists, i.e., the product of concentration 1%, as shown in Fig. 4.

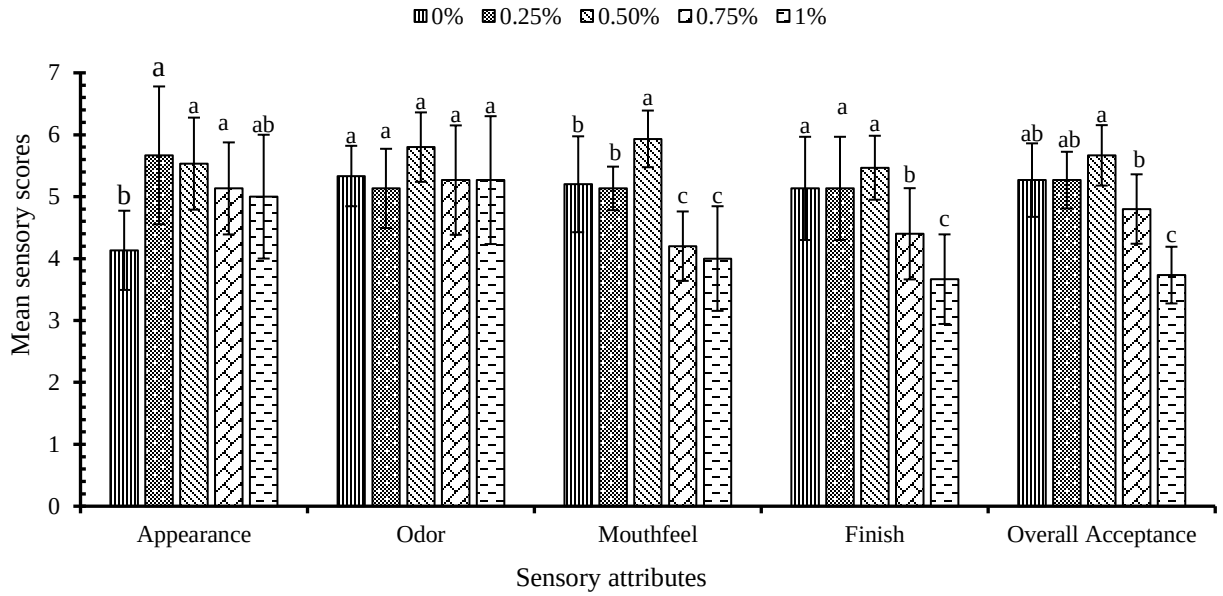


Figure 4. Effects of different concentrations of *B. ciliata* on the organoleptic quality of metheglins. Values on the top of the bars bearing similar superscripts are not significantly different at a 5% level of significance. Vertical error bars represent ± standard deviation of scores given by panelists.

Adding *B. ciliata* did not significantly affect ($p \geq 0.05$) the product's odor at any given concentration. This is because the dried rhizome of *B. ciliata* is not an aromatic herb and does not impart any odor to the product. Also, there is not a large difference in the concentration of the volatile constituents in produced metheglin

The product's mouthfeel improved by increasing the concentration of *B. ciliata* up to 0.5%, after which it decreased. This is because the polyphenols extracted from the herb gave slight astringency, which is desirable at low concentrations but undesirable at high concentrations.

The aftertaste or finish of the product improved with increasing concentration of *B. ciliata* up to 0.5%, after which it decreased. This is because the phenolic compounds extracted from the herb gave a bitter note to the product at high concentrations, which was found to be undesirable.

From this, we can conclude that *B. ciliata* enhances the appearance factor of the produced metheglin at a concentration < 0.75% and is found optimum at 0.25-0.5%. The herb doesn't affect the odor of the produced metheglins up to 1%. Both mouthfeel and finish are enhanced up to 0.5%. The best product for mouthfeel among the five samples is found at a concentration of 0.5%, after which the bitter note increased, which was detrimental to the results. So for sample 0.5%, the overall acceptance among the five samples had the highest positive rating. So, from an organoleptic point of view, increasing the concentration of the herb above 0.5% would be considered unpalatable

Conclusions

Increasing *B. ciliata* concentration in the honey must decrease the yeast growth and sugar consumption rate leading to slower fermentation, so using the herb after fermentation is complete might be a better-suited option for faster fermentation. 0.5% of the herb enhanced the appearance and mouthfeel of the final metheglins. Increasing the herb above this concentration negatively impacts appearance, mouthfeel, and aftertaste, especially increasing bitterness in the resulting fermented honey beverage. Incorporating *B. ciliata* enhances the metheglin's antioxidant, antimicrobial, and α-amylase inhibiting activity. Since B. ciliata is a medicinal herb, the product may be considered a medicinal beverage. The addition of B. ciliate at the rate of 0.5% (w/v) to the honey must significantly enhances the bioactive properties without scarifying its chemical and sensory attributes. Hence, we can utilize the *Bergenia ciliata* in producing fermented beverages.

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