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Storage Stability and Nutrient Composition of Kombucha Incorporating *Prunus nepalensis* from Meghalaya

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ABSTRACT

Background: Kombucha is a beverage which is prepared by fermentation of sweetened tea with the help of a SCOBY (symbiotic culture of bacteria and yeast). It was first established in Japan from where its name was originally derived. Kombucha is known for its antioxidant and anti-inflammatory properties to promote health as it is rich in nutraceuticals. *Prunus nepalensis* is a minor fruit which is widely grown in Meghalaya, but it is not cultivated across the country, therefore, it is considered to be underutilized.

Methods: The aim of the present study was to develop a kombucha beverage by incorporating *Prunus nepalensis* and to analyse the nutrient composition and storage stability of the developed kombucha for a period of 60 days under refrigerated temperature based on parameters such as appearance (colour), taste, odour, pH value, acidity and microbial growth.

Result: The nutrient composition analysis of the developed kombucha revealed that the antioxidant content, total phenolic acids and total flavonoids content were found to be 40.53 mg GAE/100 g, 175.4 mg GAE/100 g and 170.6 mg/100 g, respectively. The alcohol content of the kombucha was observed to be 0.41%. The results showed that the developed kombucha is suitable for storage up to two months or probably even more, as it was found to be acceptable in terms of appearance, odour, taste, pH value and there were no microbes detected until the final day of analysis.

Key words: Acceptability, Antioxidant capacity, Beverage, Fermentation, Fruit, Incorporation, Minor, Product development.

INTRODUCTION

Kombucha is a fermented tea beverage which is prepared by using tea leaves, water, sugar and a Symbiotic Culture of Bacteria and Yeast (SCOBY). The term “Kombucha” is derived from two words, “Kombu” which refers to the person credited with introducing fermented tea as a beverage in Japan and “Cha” the Japanese word for tea (Kitwetcharoen *et al.,* 2023).

Kombucha possesses anti-cancer properties along with the prevention of liver diseases, boosting immunity, improving gastrointestinal ailments and regulating blood pressure in the body (Kapp and Sumner, 2019). Kombucha contains several types of micronutrients, lactic acid, glucuronic acid, vitamins, gluconic acid, antibiotics and polyphenols and these contribute to the health benefits that can be attained through the consumption of the beverage (Kushwaha *et al.,* 2024). It is responsible for the improvement in gut health due to the presence of probiotic and prebiotic bacteria (Batista *et al.,* 2022).

Prunus nepalensis is a minor fruit which is widely prevalent in Meghalaya and is locally known as Sohiong in the Khasi hills region of the state. It has a common name known as Khasi cherry and it belongs to the family species called Rosaceae. The fruiting season of *Prunus nepalensis* is during the months of June to August (Jha *et al.,* 2021). It resembles the round shape of a cherry with a seed on the inside. This fruit is grown extensively in Naga hills in Nagaland, specific parts of Arunachal Pradesh, Sibsa valley in Assam and in the Khasi and Jaintia hills region of Meghalaya (Thakur, 2023). The carbohydrate, nitrogen and phenol content of *Prunus nepalensis* is significantly influenced by the method and time of grafting, whereby tongue grafting is determined as the best grafting method (Mabel *et al.,* 2025).

Prunus nepalensis is an excellent source of natural substances like gallic acid, catechin, purpurin, reserpine, rutin, methyl gallate, ascorbic acid and tannic acid that contribute to the remarkable antioxidant properties of this cherry, which help to prevent ailments (Bagde *et al.,* 2024). As *Prunus nepalenisis* is rich in phytonutrients that enhance its liver-protecting and free radical scavenging activities, it was investigated that the solution extracted from the fruit comprises several therapeutic effects (Ale *et al.,* 2023). Being an excellent source of phytochemicals and biologically effective substances, *Prunus nepalensis* extract makes an appropriate flavouring ingredient in addition to the antioxidant and anti-inflammatory properties of kombucha beverage. Also, there are rarely any studies conducted on *Prunus nepalensis* kombucha Therefore, this

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study aims to develop a kombucha beverage that is enriched with *Prunus nepalensis*, accompanied with a main focus on evaluating its nutrient composition and assessing its storage stability over a specific period of time.

MATERIALS AND METHODS

The development of *Prunus nepalensis* kombucha was conducted in the Food, Nutrition and Dietetics laboratory of Assam down town University, Guwahati, Assam. The lab experiments for the formulation and development of *Prunus nepalensis* kombucha were performed in the food Laboratory of Food, Nutrition and Dietetics from Aug 2023 to Oct 2024. All of the other analytical tests were conducted in AGT Biosciences Laboratory, situated at Guwahati Biotech Park, Amingaon, Guwahati, Assam.

Procurement of sample

The *Prunus nepalensis* fruit incorporated in this study was collected from Sohryngkham a village located in the Mawryngkneng subdivision of the East Khasi Hills district in the state of Meghalaya, India. This area was primarily selected because it is known for the abundant growth of *Prunus nepalensis* trees, making it an excellent area for sourcing organic and high-quality fruits for this study. The total amount of *Prunus nepalensis* fruits procured from this area was about 1000 grams.

Analysis of nutritive value of *prunus nepalensis*

The nutritive value of *Prunus nepalensis* fruit (about 50 grams for proximate composition analysis) was analysed based on several parameters, including moisture content, total ash value, crude carbohydrate, crude protein, crude fat, crude fiber contents, along with the estimation of vitamins and minerals. The analysis of all the parameters was carried out according to the FSSAI Manual of Analysis for Fruits and calculations were done by applying specific formulas which are appropriate for each of the parameters (Rashidinejad and Ahmmed, 2024).

Development of kombucha incorporating *Prunus nepalensis*

The first fermentation of unflavoured kombucha was done by using an amalgamation of ingredients like tea leaves, water, sugar and kombucha SCOBY. This was kept to ferment for about 7-10 days. For the second fermentation, flavourings were added to the unflavoured kombucha and it was again kept to ferment for 3-4 days. The developed kombucha was prepared by following a specified method for making kombucha with minor changes in the fermentation process (Jakubczyk *et al.,* 2022; Zubaidah *et al.,* 2018). This was then followed by formulation of four treatment samples of the kombucha for the purpose of organoleptic evaluation.

Formulation of kombucha treatment samples

A total of four treatment samples, each with three replicates, were formulated in varying compositions of unflavoured kombucha and *Prunus nepalensis* extract. Table 1 indicates the amount of unflavoured kombucha and *Prunus nepalensis* juice in the four formulations based on their ratios (Vargas *et al.,* 2021). The Control sample is comprised of 100% plain or unflavoured kombucha. The ratio of unflavoured kombucha and *Prunus nepalensis* extract for K1 sample was 60:40; for K2 sample the ratio was 70:30 and for K3 sample the ratio was 80:20. All of the formulated treatment samples were further fermented for about 3 days so that the flavours will be imparted evenly (de Miranda *et al.,* 2022).

Organoleptic evaluation

Organoleptic evaluation was conducted using the 9-point Hedonic scale method by semi-trained panelists of 30 members. This was done after formulating and bottling the developed kombucha (Nicolas *et al.,* 2010; Tomar *et al.,* 2022).

Physical parameters of developed kombucha

The pH value of the developed kombucha was determined by using digital pH meter. The total titratable acidity was measured by using a standard method of titration of acid in the beverage with standardized NaOH solution. Total soluble solids were measured by using Atago hand-held refractometer and reducing sugar content was determined by using Lane and Eynon method with Fehling's solution. The alcohol content of the developed kombucha was also measured using Anton Paar DMA 4500 M instrument (Bellut *et al.,* 2018).

Analysis of nutritional composition of *prunus nepalensis* kombucha

The nutritional composition of *Prunus nepalensis* kombucha was conducted based on different parameters such as antioxidants, phenolic acids (as polyphenols), total flavonoids, vitamin C and vitamin E. The antioxidants and phenolic content of the developed kombucha were measured using UV-Vis Spectrophotometer at 765 nm, total flavonoid content was determined spectrophotometrically at a wavelength of 510 nm and vitamins C and E were measured by using the HPLC method (Saimaiti *et al.,* 2022).

Storage stability analysis of the developed *prunus nepalensis* kombucha

The storage stability analysis of *Prunus nepalensis* kombucha beverage was carried out for a period of 60 days under refrigerated temperature of 4°Cat five intervals, *i.e.* 0th day, 15th day, 30th day, 45th day and 60th day (Kaul and Rehal, 2025). The storage stability was determined based

Table 1: Formulations of *Prunus nepalensis* Kombucha treatments.

Sample code	Unflavoured Kombucha (mL)	*Prunus nepalensis* extract (mL)
Control sample (100%)	500	-
K1 sample (80%/20%)	400	100
K2 sample (70%/30%)	350	150
K3 sample (60%/40%)	300	250

on various parameters such as appearance (colour), odour, taste, pH value, acidity of the kombucha and microbial growth of coliform bacteria, *Escherichia coli, Staphylococcus aureus, Salmonella* sp. and *Listeria monocytogenes*, using colony count method (Fatima *et al.,* 2024).

Statistical analysis

All of the data collected for organoleptic evaluation were statistically analyzed using descriptive statistics such as mean and standard deviation. The collected data were calculated and are indicated as mean ± SD (standard deviation) as described by (Hasani *et al.,* 2018).

RESULTS AND DISCUSSION

The results obtained in the present study are compared with the findings of previous studies which are mainly focused on indigenous and well-known fruits, including their nutritive value of the fruit, organoleptic evaluation, physical parameters, nutritional composition and storage stability of developed products. The main purpose for comparison of these parameters is to determine the overall quality of the developed product in the present study in terms of its health-promoting properties and its taste scores, in contrast to other similar researches that have been done in previous years.

Table 2: Nutritive value of *Prunus nepalensis*.

Parameters	Amounts
Moisture content	53.531% by wt.
Ash value	1.59 g/100 g
Crude carbohydrate	26.63 g/100 g
Crude protein	0.86 g/100 g
Crude fat	Not detected
Crude fiber	40.35 g/100 g
Sodium	38.75 mg/100 g
Potassium	479.19 mg/100 g
Calcium	78.87 mg/100 g
Manganese	0.80 mg/ 100 g
Phosphorus	109.65 mg/100 g
Iron	4.54 mg/100 g
Zinc	1.88 mg/100 g
Vitamin A	18.76 mcg/100 g
Vitamin C	40.76 mg/100 g
Vitamin E	0.54 mg/100 g
Antioxidants (as polyphenols)	89.65 mg GAE/100 g
Total phenolic content	0.57 mg/100 g
Total flavonoids	0.92 mg/100 g

Nutritive value of *Prunus nepalensis*

From Table 2, it was observed that *Prunus nepalensis* mainly consists of 53.531% of moisture content, 1.59 g of ash value, 26.63 g of crude carbohydrate, 0.86 g of crude protein, 40.35 g of crude fiber and crude fat was not detected in this fruit.

A similar study indicated that the moisture content of *Prunus nepalensis* fruit was 88.50% (Mandal *et al.,* 2017). Similar data was reported by Vivek *et al.,* (2018) where the ash value of *Prunus nepalensis* was recorded 1.03 g (Vivek *et al.,* 2018). The amount of carbohydrate observed by Chaudhuri *et al.* (2015) was about 33.01±0.11 mg/100 mg in *Prunus nepalensis* powder, which has been freeze-dried using the pulp of the fruit (Chaudhuri *et al.,* 2015)..The vitamin composition of *Prunus nepalensis* in the present study was found to contain 18.76 mcg of vitamin A and 0.54 mcg of vitamin E and vitamin C was 40.76 mg, which is similar to a study conducted by Chyne *et al.* (2019) where it was reported that the highest amount of vitamin C was found in *Prunus nepalensis*, which was estimated at 43.45 ± 1.44 mg/100 g (Chyne *et al.,* 2019). Rymbai *et al.* (2016) indicated that the amount of phosphorus was 115 mg, potassium was 485.01 mg, iron was 9.60 mg and manganese was 7.70 mg and these findings are observed to be similar to the findings in the present study (Rymbai *et al.,* 2016).

Prunus nepalensis is a good source of antioxidants which was found to be 89.65 mg GAE, total phenolic content was found to be 0.57 mg and total flavonoids was about 0.92 mg, which is in alignment with the total flavonoids content reported in prunus pulp (Kuna *et al.,* 2018). The antioxidant activity of *Prunus nepalensis* evaluated by Zubaidah *et al.,* (2018) and Jennifer and Surya (2024) through DPPH was found to be 20 mg/mL, which is about 91.2±0.39% and the total phenolic content was revealed to be 8.32±0.18 mg GAE/100 g dm (Jennifer and Surya, 2024; Zubaidah *et al.,* 2018). It has been revealed that the integrated nanoparticles obtained from the phytochemicals found in *Prunus nepalensis* can probably be beneficial for curing cancer (Biresaw and Taneja, 2020).

Organoleptic evaluation

After conducting the organoleptic evaluation of the kombucha, all the data obtained were assessed and compared by using the mean acceptability scores (Table 3).

Table 3: Mean acceptability scores of the developed *prunus nepalensis* kombucha.

Product code	Appearance (Mean±SD)	Sweetness (Mean±SD)	Colour (Mean±SD)	Flavour (Mean±SD)	Taste (Mean±SD)	Aroma (Mean±SD)	Overall acceptability (Mean±SD)
Control	6.96±0.98	5.73±1.09	6.73±0.96	6.63±1.22	6.86±1.25	6.6±1.22	6.83±0.89
K1	7.4±1.28	5.56±1.76	7.7±0.9	6.36±1.72	6.33±1.69	6.6±1.08	7.13±0.95
K2	7.53±1.17	6.1±1.57	8.03±0.79	6.6±1.60	6.66±1.55	6.76±1.38	7.3±1.03
K3	7.9±0.7	6.23±1.70	8.13±0.76	6.93±1.69	6.9±1.93	6.93±1.61	7.53±0.99

It was found that K3 sample was the most acceptable among the four formulations in terms of overall acceptability, which was composed of 300 mL of *Prunus nepalensis* extract and 250 mL of the unflavoured kombucha. K2 was found to be highest in terms of appearance, sweetness and colour, as compared to the control and K1 sample. A similar study on noni juice extract kombucha was done by Jennifer and Surya (2024), in which the control sample was observed to be the most acceptable, where the acceptability score of the control sample was found to be higher than that of the treatment samples (Jennifer and Surya, 2024). In a study conducted by Aideel and Nurain (2023), the formulation of kombucha containing moderate amounts of dried orange and pomegranate peel powders were the most acceptable as compared to the formulations containing low and high amounts of both the powders (Aideel and Nurain, 2023).

Physical parameters of *prunus nepalensis* kombucha

From Table 4, it was observed that the pH value of the kombucha was 2.85. Total titratable acidity, total sugars and total soluble solids were 0.38 mg, 4.65 g and 4.70%, respectively.

Here, the pH value, total titratable acidity and total soluble solids of the developed kombucha were found to be in alignment with the data reported in a study conducted by Arunkumar (2024) (Arunkumar, 2024). The alcohol content of the developed kombucha was found to be 0.41% and this was found to be similar with the alcohol content of red dragon fruit kombucha cascara which was observed to be 0.63% during the second fermentation of 2 days (Muzaifa *et al.,* 2022). *Prunus nepalensis* and red dragon fruit are both known to be rich in bioactive compounds and provide several health benefits.

Table 4: Physical parameters of Prunus nepalensis kombucha.

Parameters	Amounts
pH value of the kombucha	2.85
Total titratable acidity	0.38 mg/100 mL
Total sugars	4.65 g/100 mL
Total soluble solids	4.70% by wt.
Alcohol content	0.41%

Table 5: Nutritional composition of developed Kombucha by incorporating *Prunus nepalensis.*

Parameters	Amounts
Antioxidants	40.53 mg GAE /100 mL
Total phenolic acids (as polyphenols)	175.4 mg GAE/100 mL
Total flavonoids	170.6 mg/100 mL
Vitamin C	4.65 mg/100 mL
Vitamin E	Not detected

Nutritional composition of *prunus nepalensis* kombucha

The antioxidant content of the developed kombucha shown in Table 5 was found to be 40.53 mg GAE, total phenolic acids analysed as polyphenols were 175.4 mg GAE and total flavonoids content was 170.6 mg.

The total phenolic content analysed in the present study was found to be higher than the total phenolic content observed in black grape kombucha which was about 151.37 mg GAE (Tomar, 2023). This comparison is made to determine the potential antioxidant activity and identify which out of these two kombucha beverages has more functional properties. An increase in the total phenolic content was reported in *Prunus nepalensis* juice upon fermentation due to the prevalence of lactic acid bacteria, which metabolizes the bioactive compounds, leading to the formation of additional phenolic acids (Vivek *et al.,* 2019). The vitamin C content of the *Prunus nepalensis* kombucha was 4.65 mg, while vitamin E was not detected. Similar results were observed in the vitamin C content found in kombucha developed by using jujube powder which was about 4.15 mg (La Torre *et al.,* 2024). This comparison indicates the effect of incorporating fruits in fresh and powder form on the nutrient composition of the developed kombucha and to determine which beverage retains or provides more amounts of vitamin C after fermentation.

Storage stability analysis of *prunus nepalensis* kombucha

As shown in Table 6, the pH value of the developed kombucha was decreased slightly from 2.97 on the 0th day to 2.78 on the 60th day of analysis, although the level of acidity was found to be increased.

The pH of kombucha developed by using Brazillian coffee cascara was also observed to be slightly reduced from 3.7 to 3.5 which was analysed from the 0th day to the 9th day of analysis (Sales *et al.,* 2023). Here, *Prunus nepalensis* kombucha is compared with Brazillian coffee kombucha to

Table 6: Storage stability of developed Kombucha by incorporating *Prunus nepalensis.*

Parameters	Temperature	0th day	15th day	30th day	45th day	60th day
Appearance (colour)	25°C	Reddish colour	Reddish colour	Reddish colour	Reddish colour	Reddish colour
Odour	25°C	Acceptable	Acceptable	Acceptable	Acceptable	Acceptable
Taste	25°C	Acceptable	Acceptable	Acceptable	Acceptable	Acceptable
pH value	25°C	2.97	2.94	2.87	2.83	2.78
Acidity% by wt.	25°C	0.62	0.64	0.67	0.69	0.72
Coliform count, cfu/ml	25°C	<1	<1	<1	<1	<1
E.coli, MPN/ml	25°C	Not detected	Not detected	Not detected	Not detected	Not detected
Salmonella sp., per 25ml	25°C	Absent	Absent	Absent	Absent	Absent
S.aureus cfu/ml	25°C	<1	<1	<1	<1	<1
Listeria monocytogenes	25°C	Absent	Absent	Absent	Absent	Absent

evaluate the changes in the acidity levels that take place over time upon storage which in turn indicates the stability of the developed product. Similar observations were made in a study conducted by da Silva *et al.* (2024) in which the reduced pH and high acidity of kombucha was found to be due to the production of organic acids (da Silva *et al.,* 2024). Overall, the developed kombucha in the present study was found to be acceptable in terms of appearance, odour and taste till the final day of analysis and there was no microbial growth found to be present in the beverage.

The present study expresses its originality in incorporating a traditional fermented tea beverage with a minor fruit as the major ingredient that has not been associated with the traditional process of fermentation. The analysis of the nutrient composition and storage stability of the developed kombucha brings about innovative ideas and concepts with respect to combining the basic ingredients of kombucha together with unique fruits and herbs that will result in new perspectives and outcomes for developing flavoured fermented beverages.

CONCLUSION

In conclusion, we indicate that the kombucha having the highest amount of *Prunus nepalensis* extract (250 ml) was the most acceptable product in terms of overall acceptability. *Prunus nepalensis* contains high potassium, phosphorus and antioxidant levels which are beneficial for health. Additionally, the developed kombucha was found to be rich in total flavonoids and total phenolic content; the developed kombucha contained low alcohol content of 0.41% which is considered to be at an acceptable range for kombucha beverages. The storage stability analysis of the kombucha showed that the developed kombucha can be stored for a period of 60 days or maybe even more taking into account the pH of the beverage being stable throughout the period of analysis with no undesirable changes in its appearance, odour and taste and no prevalence of microbes, making it acceptable and safe for consumption upon storage under refrigerated temperatures. We reckon that the findings of the present study will be valuable to future research that will be conducted regarding the development, nutrient analysis and storage study of kombucha by utilizing other minor fruits so that such fruits can be popularized across the world for their health-promoting properties.

Conflict of interest

All authors declared that there is no conflict of interest.

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