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Comparative study of Chhyang, an ethnic fermented beverage of Nepal

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Chhyang, also known as Kodo ko jaanr, is a traditional drink of the Mangarantis, one of Nepal's ethnic groups. It is one of the most nutritious beverages. Because of its high calorie count, vitamin content, and presence of helpful lactic acid bacteria and yeast, the mildly alcoholic sweet-flavored drink is considered food rather than an alcoholic beverage. Traditional ingredients for its manufacture include barley or ragi. Ragi or finger millet (*Eleusine coracana*) is a traditional substrate for the manufacturing of Chhyang. In the present study, three different types of Chhyang were produced using three different substrates rice, finger millet, and maize. Marcha was added at a rate of 1.8% in the form of powder uniformly over the surface of cooked and cooled mashes, followed by 48 hours of aerobic fermentation followed by 19 days of anaerobic fermentation. A chemical, microbiological and sensory analysis of the produced Chhyang samples was conducted. Results showed that the fermented beverages contain 4-8% alcohol, 0.35-0.70% acidity, 3.5-5.0 g/100L aldehyde, 26-70 g/100L ester, 0.70-1.0% reducing sugar, 8.0-14% higher alcohol, 0.08-0.15% methanol, respectively. From the sensory evaluation almost all the panelist detected very high amount of alcohol in rice-based Chhyang, while slightly acidic (sourness) was found in sample maize-based Chhyang. Sample rice-based Chhyang received the highest score for color. Similarly, in terms of aroma, and taste rice-based Chhyang received the highest score.

Keywords: Chhyang, Fermentation mangarantis, Marcha traditional beverage

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Ethnic foods represent the culinary traditions and heritage of specific cultural groups, utilizing locally available plants and animals[1]. Chhyang, a nutritious alcoholic beverage, is a prime example, deeply rooted in the Himalayan cultures of Nepal, India, Bhutan, and Tibet[2,3]. Chhyang is known to be the most effective remedy for countering the harsh cold of mountainous areas. It is believed to possess various healing qualities for ailments like the common cold, fevers, allergic rhinitis, and alcohol dependence, among others. Among the traditional drinks of the region, Chhyang, with its mildly alcoholic and sweet flavor, is regarded more as a food item due to its high calorie count, vitamin content, and the presence of beneficial lactic acid bacteria and yeast, rather than just an alcoholic drink[4,5]. Chhyang's production is relatively straightforward and affordable, contributing to its widespread popularity in Nepal. Its unique, mild flavor appeals to a broad range of consumers. Younger generations enjoy its easy-to-drink nature, comparing it to juice, while older generations appreciate its traditional strength[6].

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Chhyang is known locally as a vital alcoholic drink crafted by the tribal communities of Darjeeling and is essential for all social events. It is made mostly from finger millets and rice, which gives it a sweet, tart, and acidic flavor. The rice-based alcoholic beverage referred to as Bhaati ko Jaanr or Chhyang is a key part of social life in the Eastern Himalayas (Nepal, India, and Bhutan) and is seen as crucial by tribal groups in Darjeeling, featuring prominently in their community gatherings. Commonly called an indispensable drink, it is mainly made from finger millet, although rice is also utilized, resulting in the beverage known as "Bhaati ko Jaanr" or Chhyang, which has a delightful sweet, sour, and slightly acidic taste[7]. This affordable, calorie-rich, and low-alcohol beverage is made from steamed sticky rice and is a typical element of the regional cuisine[8]. The fermentation period for this drink can change depending on the season, lasting 3 days in summer and 10 days in winter. To achieve higher alcohol content, this duration can be lengthened as needed. Once fermentation is finished, the contents of the pots are emptied and moved to wooden barrels, after which water is added until the contents are fully submerged.

The mixture is left to sit for 2-5 h, and the liquid is filtered out 2 to 4 times. The liquid is consumed after a day, while the solid residue is utilized as feed for animals[5]. It is rich in calcium, phosphorus, and vitamins including riboflavin, niacin, cyanocobalamin, and pantothenic acid, as well as living beneficial microorganisms, and has a caloric[9].

Marcha is a type of mixed culture that is traditionally prepared and is available for commercial use. It is utilized in the making of various ethnic fermented drinks from the Himalayas, including Bhaati Jaanr and Kodo ko jaanr[8]. While rice is the most widely used option, other cereals such as finger millet and maize are also utilized, particularly in rural and ethnic communities. Nevertheless, there is a lack of comparative information regarding the effects of these various substrates on the physicochemical, microbial, and sensory characteristics of Chhyang[10]. The absence of such data hampers efforts toward scientific validation, quality improvement, and potential commercialization of this ethnic beverage. Without a clear understanding of how different raw materials affect fermentation outcomes, it is difficult to ensure consistent quality or meet broader consumer and safety expectations. Therefore, this study aims to address these gaps by comparing Chhyang prepared from rice, finger millet, and maize under controlled fermentation using the same starter culture.

Methodology

Sample collection

Elderly and knowledgeable local residents in the Pokhara Valley, located in Kaski district, were chosen due to their extensive experience in producing three distinct varieties of Chhyang using finger millet, rice, and maize as substrates. Therefore, raw materials were collected from the local area of Pokhara Valley. After collection, the samples were packed in enclosable low density polyethylene plastic pouches, taking care to avoid cross contamination and stored in bulk in the refrigerator until needed. *Murcha* cakes were mixed uniformly and placed in clean polyethylene plastic pouches avoiding the contamination and the samples were carefully sent to Pokhara Bigyan Tatha Prabidhi Campus, Pokhara.

Materials and Methods

Biochemical analysis

Ragi, or finger millet (*Eleusine coracana*), is a traditional substrate for the manufacturing of Chhyang. In the present study, three different types of Chhyang were produced using three different cereals finger millet, rice and maize respectively. After the production of Chhyang the samples were packed in sterile bottles and aseptically brought to the laboratory for final biochemical analysis.

Determination of alcohol content

Alcohol contents were determined by pycnometer method as described by FSSAI (2016), taking 200 mL of Chhyang and 200 mL of water in a 500 mL distillation flask where 200 mL distillate (*Raksi*) was collected. To determine the percentage of alcohol by volume, the distillate's apparent density was compared to the standard chart[11].

Determination of acidity, pH and TSS

For determining acidity, 10 mL sample was taken in a beaker which was titrated against 0.1 N NaOH using phenolphthalein indicator and acetic acid was determined and pH of the samples was determined by the digital pH meter of and standardized with standard buffer at 25°C[11]. TSS of different samples was measured by using the refractometer (Hanna make, Portugal). The refractometer was thoroughly washed with distilled water and then wiped with soft tissue after each test.

Determination of aldehyde content

Aldehyde content was determined using the method described by[11], and the results were reported in g per 100 L of absolute alcohol as acetaldehyde. In brief, 50 mL of distilled liquor was placed in a 250 mL Iodine flask and 10 mL of sodium bisulphite solution was added. The flask was then placed in a dark room for 30 min, shaking constantly. It was filled with 25 mL of standard iodine solution, and the surplus iodine was back titrated against standard thiosulphate solution using a starch indicator until it reached a bright green endpoint. The blank was run in the same manner, using 50 mL of distilled water. The difference in titer value in milliliters of sodium thiosulphate solution resulted in an equivalent aldehyde content.

Aldehyde expressed as acetaldehyde, g per 100 L of absolute alcohol is:

$$\text{Aldehydes (g aldehyde/100 L abs. alcohol)} = (V \times 0.0011 \times 100 \times 1000 \times 2) / V_1$$

Where, V_1 = alcohol % by volume, V = the difference in titer between the blank and sample, measured in mL of sodium thiosulphate solution.

Determination of reducing sugar

Reducing sugar was determined using the Lane and Eynon method on spent wash recovered following alcohol distillation, and the values were represented as percent of dextrose[11].

Determination of ester content

Ester value of distillate was determined as per[11]. The values were expressed in g per 100 L. Briefly, 10 mL standard NaOH was added to the neutralized distillate and refluxed in the steam bath for an hour. Then it was cooled and unspent alkali was back titrated against standard sulphuric acid. Blank titration was simultaneously carried out taking 50 mL of distilled water instead of the distillate in the same way. The difference in the titer value in milliliters of standard sulphuric acid gave the equivalent ester. Esters expressed as ethyl acetate, g per 100 L of absolute alcohol is:

Esters (g ethyl acetate/ 100 L abs. alcohol =

$$(V \times 0.0088 \times 100 \times 1000 \times 2) / V_1$$

Where, V = difference of the titer value of standard H_2SO_4 used for blank and sample, in ml V_1 = alcohol % by volume

Determination of higher alcohol

Higher alcohol content was determined using the titrimetric method, as described in[11]. The ester determination solution was transferred to a separatory funnel, and 50 mL of distilled water was added. It was saturated with sodium chloride and then extracted four times with 40, 30, 20, and 10 mL of carbon tetrachloride. All of the extracts were combined and rinsed three times with saturated sodium chloride solution and twice with saturated sodium sulfate solution. The filtered extract was treated with 50 cc of oxidizing mixture. The mixture was refluxed for two hours, then cooled and rinsed with 50 mL of distilled water. After that, 50 milliliters of water were used to move it to the distillation assembly. After distilling about 100 mL, it was found that no charring occurred. Phenolphthalein indicator was used to titrate the distillate against standard NaOH. The same procedure was used for a blank, substituting 50 milliliters of distilled water for the liquor's distillate.

Higher alcohol expressed as amyl alcohol, in g per 100 liters of abs. alcohol is:

Higher alcohol =

$$(V \text{ x } 0.0088 \text{ x } 100 \text{ x } 1000 \text{ x } 2) / (V_1 \text{ x } V_2)$$

Where, V = difference of titer value of Std. alkali used for blank and sample, in ml, V_1 = Volume of sample taken for estimation, V_2 = alcohol % by volume

Determination of methanol

The method described in[11] was used to determine methanol content. About 40 milliliters of distillate were obtained from 50 milliliters of material in a straightforward still. After diluting 1 mL of distillate with 5 mL of distilled water, the mixture was thoroughly shaken. Separate 50 mL stoppered test tubes were filled with 1 mL of this solution, 1 mL of distilled water (for the blank), and 1 mL of each of the methanol standards. The tubes were then placed in an ice-cold water bath. Two milliliters of KMnO4 reagent were added to each test tube and left for half an hour. After adding a little amount of sodium bisulphite to decolorize the mixture, 1 milliliter of chromotropic acid solution was added and thoroughly mixed. After adding 15 milliliters of sulfuric acid gradually while whirling, the mixture was kept in a hot water bath at 80 degrees Celsius for 20 min. The liquid was cooled when the color changed from violet to red, and a 1 cm cuvette cell was used to measure the absorbance at 575 nm. The following is the methanol content in grams per 100 liters of absolute alcohol: Methanol = $(A_2 \text{ x } C \text{ x } D \text{ x } 1000 \text{ x } 100 \text{ x } 100) / (A_1 \text{ x } S)$.

Where, A_2 = absorbance of sample solution, C = concentration of methanol std. solution, D = dilution factor for sample solution, A_1 = absorbance of methanol std. solution.

Microbiological analysis

Total yeast and mold count

For the enumeration of yeast and mold, serial dilution was carried out according to standard microbiological techniques[12]. One gram of the sample was mixed with 9 mL of sterile distilled water to obtain a 10^{-1} dilution, followed by serial dilutions up to 10^{-8}. From selected dilutions (10^{-4}, 10^{-6}, and 10^{-8}), 0.1 mL aliquots were spread onto PDA plates using the spread plate technique. Plates were incubated at 27°C for 5 days, after which colonies were counted.

Total lactic acid bacteria (LAB) count

Serial dilution was also performed to enumerate LAB, as described by[13]. One gram of the sample was homogenized in 9 mL of sterile distilled water to achieve a 10^{-1} dilution, followed by further dilutions up to 10^{-8}. From 10^{-4}, 10^{-6}, and 10^{-8} dilutions, 1 mL aliquots were poured into sterile petri dishes, followed by addition of Plate Count Agar (PCA) using the pour plate method. Plates were incubated at 35°C for 48 h. Only plates with 25–250 colonies were used to ensure accuracy.

The yeast and mold and LAB counts were calculated using the same formula as:

N = Average colony count (Volume plated x Dilution factor)

Where, N = Colony-forming units (CFU) per gram of sample

Sensory analysis and data analysis

Sensory evaluation of prepared Chhyang was also done using 13 semi-trained panelists consisting students and teachers on a nine-point hedonic rating (9 = like extremely, 1 = dislike extremely) for color, aroma, taste, sourness and overall acceptability. They were asked to rate the product according to their liking or disliking following 9 points hedonic rating test as described by[14]. Data was statistically processed by SPSS (version 20) for Analysis of Variance (ANOVA). Data analyses on sensory quality of Chhyang from different starter were statistically processed by One Way Analysis of Variance (ANOVA). The physicochemical properties of Chhyang were similarly processed for ANOVA.

Method of preparation

The detailed process and steps followed for the preparation of three different Chhyang prepared using three substrates finger millet, rice and maize. The detail method of preparation of Chhyang is given in (Fig. 1).

1. Collection of raw materials: Good quality of substrates finger millet, rice and maize was collected from the local village of Pokhara Valley, Nepal.
2. Sample preparation: After collection, the samples were packed in enclosable low density polyethylene plastic pouches, taking care to avoid cross contamination and stored in bulk in the refrigerator until needed. Murcha cakes were mixed uniformly and placed in clean polyethylene plastic pouches avoiding the contamination.
3. Preparation of raw materials: Substrates like rice, millet and maize were cleaned and washed with water. Water was boiled in three different vessels for three substrates respectively and then added into the boiled water and cooked under the same cooking conditions till soft consistency and cooled to room temperature.
4. Cooking of grains: The purpose of cooking the grains is to convert the starch into amylose and to denature the protein in the grains. Steaming also destroys the contaminating micro-organisms. Cooking of grains was performed in an open vessel. The cooked grains were spread onto a muslin cloth and allowed to air cooled.
5. Inoculation: Murcha was added at the rate of 1.8% in the form of powder uniformly over the surface of the cooked and cooled mashes. After the addition of Murcha powder, it was mixed intimately with mash. After inoculation of Murcha powder, the mashes were left for biomass build up at room temperature for 48 h. at aerobic condition with loose covers.
6. Fermentation: After 48 h, optimum biomass was seen in visible puffy colonies. Then the mass was transferred into a plastic vessel and closed tightly

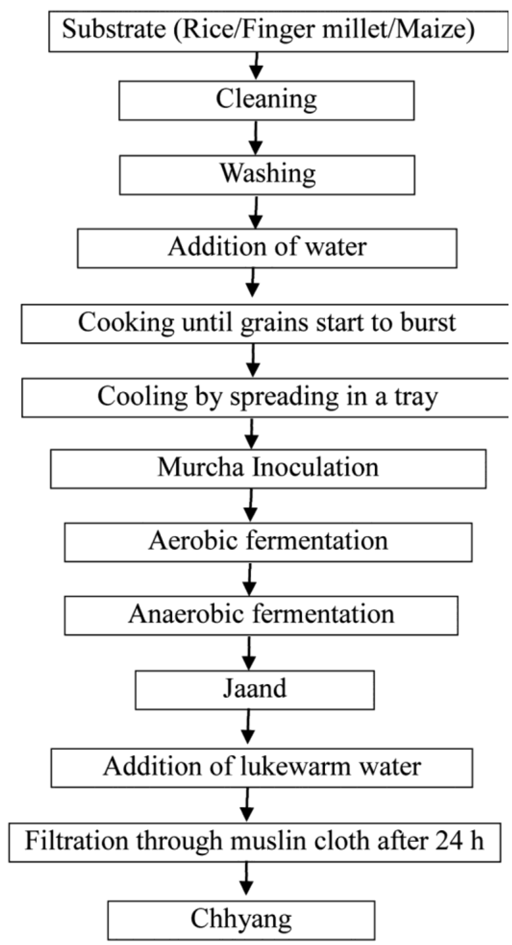

Fig. 1 — Flow sheet of preparation of Chhyang

with a lid and left undisturbed at room temperature for 21 days for alcohol fermentation.

Chemical used

Sodium hydroxide (NaOH), phenolphthalein indicator, sodium bisulphite, iodine solution, sodium thiosulphate, starch indicator, sulphuric acid (H_2SO_4), sodium chloride (NaCl), carbon tetrachloride (CCL_4) sodium sulphate, oxidizing mixture, potassium permanganate ($KMnO_4$), chromotropic acid, standard buffer solution.

Results and Discussion

The process of preparing Chhyang included continuous stirring, and once cooking was completed, the grains appeared to be split open. Additionally, a slight slimy texture was noted in the cooked grains. Since rice is the softest grain, it cooked more thoroughly and expanded more than the other grains because of starch gelatinization. Marcha was added in the form of powder at the rate of 1.8 % of the weight of dry/raw grains used uniformly over the surface of cooked and cooled mash. The fermentation was done for 48 h under aerobic condition, a fruity smell developed was found in all samples. After anaerobic fermentation for 19 days a fruity and optimal balance flavour was developed in rice-based Chhyang. Fermentation enhances the flavor while simultaneously boosting the nutritional content regarding protein, calcium, fiber, and B vitamins, as well as improving *in vitro* protein digestibility and reducing the amounts of anti-nutrients present in food grains[14-5]. Three different samples of Chhyang prepared from finger millet, rice and maize and analyzed for physiochemical analysis which is presented in (Table 1 below).

The TSS of the three different substrates (rice, finger millet and maize) based Chhyang was found to be (7.87, 5.97, and 3.1) ºBx as shown in (Table 1). Continuous fermentation for up to 21 days the TSS decreased significantly in all samples due to the conversion of sugars to alcohol and other products. The TSS of rice based alcoholic beverages varied from 5 to 10% depending upon the variety of rice used[15]. A strong positive correlation ($r = 0.98$, $p<0.01$) was found between initial TSS and final alcohol content, confirming that a higher initial sugar concentration results in greater alcohol yield. These findings are consistent with the general understanding that higher initial sugar content leads to increased ethanol production during fermentation. The pH decreased during the fermentation in all three samples and reached to 3.9, 3.6, and 3.4 in (rice, finger millet, and maize) based Chhyang. The study by[16] indicated that the final pH of rice beer was 4.18. Similarly[4] found that kodo ko jaanr had a pH of 3.5, while[8] reported that bhaatejaanr also had a pH of 3.5. These findings provide strong support for the (Table 1) mentioned above. Acidity plays a key role in the flavor and microbial stability of fermented beverages. The titratable acidity (as % acetic acid) was highest in maized-based Chhyang 0.70%, followed by finger millet 0.54% and rice-based 0.35 %.[15]. Reported the total acidity content of rice based alcoholic beverages ranges from 1.2 to 2% depending upon the variety of rice used. We may conclude that the total acidity of all the samples was somewhat lower, which could be attributed to variation in Marcha's quality due to the presence of diverse molds, yeast, and bacteria. The increase in acidity and subsequent drop in pH during cereal fermentation was most likely due to the utilization of free sugars by yeasts and LAB[8].

The alcohol percentage was highest in the substrate rice (7.45%) and lowest in maize-based (4.5%). This variation was statistically significant ($p<0.05$), highlighting the influence of substrate composition on fermentation efficiency. These results align with previous studies on Nepalese homebrewed alcoholic beverages, which reported ethanol concentrations ranging from 1% to 18.9% for non-distilled beverages like Chhyang[17]. According to[18] the ethanol concentration in homebrewed alcohol is 5.2% ranging

Table 1 — Bio-chemical analysis of rice-based, finger millet-based and maize based-Chhyang

Parameters	Rice based Chhyang	Finger millet based Chhyang	Maize based Chhyang
TSSº Bx	7.87±0.11	5.97±0.05	3.1±0.1
pH	3.9±0.02	3.6±0.02	3.4±0.02
Acidity%	0.35±0.01	0.54±0.02	0.70±0.01
Alcohol%	7.45±0.15	5.5±0.01	4.5±0.10
Aldehyde g/100 L	4.04±0.09	5.03±0.07	3.53±0.07
Esters g/100 L	40.02±0.52	70.02±0.60	26.02±0.30
Reducing sugar %	1.0±0.05	0.78±0.02	0.70±0.05
Higher alcohols g/100 L	10.51±0.17	13.51±0.22	8.01±0.22
Methanol g/100 L	0.10±0.01	0.15±0.01	0.08±0.01

*The values in the table are the mean of the triplicates ± standard deviation

Table 2 — Microbial analysis of rice-based, finger millet-based and maize based-Chhyang

Sample	Yeast and mold count	LAB count (CFU/g)
Rice-based	1.99×10^4	8.27×10^3
Finger millet-based	1.11×10^5	2.53×10^4
Maize-based	6.27×10^4	2.20×10^4

from 1% to 18.9% for non-distilled alcohols (Jaand/ Chhyang/Tongba) and 14% for distilled alcohol (local Raksi). Aldehydes contribute to the aroma and flavor of fermented beverages. Finger millet based Chhyang contains the highest concentration of aldehyde 5.03 g/100 L, followed by rice based 4.04 g/100 L, and maize based 3.53 g/100 L. Likewise, finger millet based Chhyang also had the highest concentration of esters 70.02 g/100 L, followed by rice based (40.02 g/100 L) and maize based (26.02 g/100 L). Esters are known for their fruity and floral notes. The reducing sugar content was low in all Chhyang samples, indicating that most of the fermentable sugars were utilized during fermentation. The reducing sugar content of rice-based, finger millet-based and maize-based Chhyang were found to be 1.00%, 0.78% and 0.70% respectively. Similar data of reducing sugar of (1±0.08%) was seen in kodo ko jaanr[8]. As per[19], sugar utilization is crucial for achieving desired alcohol content and flavor in fermented beverages. The reducing sugar content decreased significantly during fermentation due to yeast metabolism.

The higher alcohol concentration was found in sample finger millet-based Chhyang13.52 g/100 L, followed by rice based with 10.52 g/100 L and maize-based 8.02 g/100 L. Higher alcohols can contribute to the flavor complexity of fermented beverages but can also be undesirable at high concentrations. At last, methanol levels were low in all samples, with the concentrations of (1.0, 1.5 and 0.8) g/100 L in the sample rice-based, finger millet-based and maize-based Chhyang respectively. These levels are below the safety limits for alcoholic beverages.

Three different samples of Chhyang prepared from finger millet, rice and maize and analyzed for microbiological analysis which is presented in (Table 2 below).

Plate count Agar for LAB count was incubated at 27ºC for 24-120 h, while Potato Dextrose Agar for yeast and mold was incubated at 30ºC for 24-72 h. One milliliter of each sample was plated at a dilution of 108. In comparison to the other samples, rice-based Chhyang had the greatest microbial count (cfu/g). The count was comparable to that reported in Poko, a rice-based traditional fermented food product from Nepal[20]. Similarly, yeast and mold count in terms of cfu/g was highest in maize-based Chhyang. According to[5], yeast and mold count has been found to be more than the bacteria count in Marcha and the similar observation can be seen in the prepared Chhyang as well. The higher microbial counts observed in millet Chhyang suggests that millet may provide a more favorable environment for the growth of these microorganisms. This could be due to its nutrient profile, which might be richer in certain growth factors. The lower microbial counts in rice Chhyang might indicate that rice provides a less nutritive profile compared to millet. These differences in microbial populations could contribute to the variations observed in the chemical composition and sensory attributes of the Chhyang samples.

Fig. 2 — Final Chhyang samples prepared from three different substrates
Here (a)*= Rice based Chhyang, (b)*= Finger millet based Chhyang and (c)*= Maize-based Chhyang

Sensory evaluation

In the sensory evaluation almost all the panelist detected very high amount of alcohol in rice-based Chhyang, while slightly acidic (sourness) was found in sample maize-based Chhyang. Sample rice-based Chhyang received the highest score for color 7.50, while other two receive 7.15 in finger-millet Chhyang and 6.07 in maize-based Chhyang which is as shown in (Fig. 2). Similarly, in terms of aroma, and taste rice-based Chhyang received the highest score (7.30 and 7.40). The higher levels of aldehydes and esters in liquor may contribute to its distinct flavor. The sourness scale was slightly similar in all samples (6.25, 6.50, and 5.90) in rice, finger millet and maized based. Rice-based Chhyang contains medium amount of aldehyde and esters when compared to the other two samples. The overall acceptability score was higher in sample rice-based Chhyang with the hedonic scale 7.30.

Conclusions

Fermented drinks can be kept for extended durations without preservatives because of the creation of natural preservatives such as acid and alcohol. The current study compared the preparation of Chhyang utilizing three distinct substrates: rice, finger millet, and maize. It can be concluded that rice-based Chhyang is a superior substrate in comparison to the other after 21 days of fermentation and product analysis. To increase the product's appeal as a commercial beverage and to extend its shelf life, natural or artificial flavors, colors, or preservatives may be added. The Mangarantis, one of Nepal's ethnic groups, traditionally drink Chhyang, but the findings of this study indicate that it may be made in various communities across the nation.

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Author Contributions

The first author conducts all laboratory experiments with assistance from the second author, while the co-author and third author contributes to the article preparation and provides chemicals and equipment. The fourth and fifth authors offer general support for the article and provide valuable feedback on the manuscript.

Conflict of Interest

The authors disclosed no possible conflicts of interest.

Prior Informed Consent

Not relevant. This study did not include human participants, surveys, interviews, or the acquisition of personal information.

Ethics Approval

Since this study did not include human or animal participants and just entailed the production and laboratory examination of Chhyang samples, ethical approval was not necessary.

Use of Artificial Intelligence (AI)

No AI tools were employed.

Data Availability

Data will be made available by the corresponding author upon reasonable request.

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