International Journal of Advanced Biochemistry Research

ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating: 5.29
IJABR 2025; 9(4): 998-1003
www.biochemjournal.com
Received: 07-01-2025
Accepted: 10-02-2025

Madhusmita Malakar
Food Science and Technology Programme, Department of Horticulture, Assam Agricultural University, Jorhat, Assam, India

Pratikshya Dutta
Department of Applied Biology, University of Science & Technology Meghalaya, Ri-Bhoi, Meghalaya, India

Ananta Saikia
Food Science and Technology Programme, Department of Horticulture, Assam Agricultural University, Jorhat, Assam, India

Soumitra Goswami
Food Science and Technology Programme, Department of Horticulture, Assam Agricultural University, Jorhat, Assam, India

Madhuri Sinha
Department of Food Science and Nutrition, College of Community Science, Assam Agricultural University, Jorhat, Assam, India

Mriganka Shekhar Borah
Department of Agricultural Engineering, Assam University, Silchar, Assam, India

Corresponding Author:
Pratikshya Dutta
Department of Applied Biology, University of Science & Technology Meghalaya, Ri-Bhoi, Meghalaya, India

Assessment of quality parameters and sensory profiles of vinegar produced from black rice of Assam with herbal incorporation

Madhusmita Malakar, Pratikshya Dutta, Ananta Saikia, Soumitra Goswami, Madhuri Sinha and Mriganka Shekhar Borah

DOI: https://www.doi.org/10.33545/26174693.2025.v9.i4l.4217

Abstract
The present investigation was carried out to evaluate the efficiency of fermentation and production technology of vinegar prepared from black rice and to assess the quality of the product. The process involved malting and crushing in a malt grinder, then the yeast Saccharomyces cerevisiae strain NCIM3212 was used in fermentation to make black rice beer. Two strains of Acetobacter aceti, MTCC-3246 and MTCC-3347, were also used to ferment the acetic acid. Four treatment groups supplemented with local herbs and a control group devoid of herbs were evaluated. The rice was subjected to a thorough proximate analysis, which measured its levels of moisture, crude fat, protein, ash, and starch. Black rice beer's alcohol concentration, as determined by biochemical analysis, was 5.76%. Following acetic acid fermentation, the rice vinegar's acetic acid level varied between 3.77% and 4.73% (w/v). Among all the treatments, the vinegar prepared from black rice beer with herbs contained the highest acetic acid and phenol content (16.86 mg GAE ml-1) as well as lowest pH (4.4) level, when both strains of acetic acid bacteria were used. There was no evidence of microbial contamination in the vinegar samples. Moreover, based on the sensory profiling, this treatment was the most favourable, with the greatest overall acceptability scores.

Keywords: Fermentation, black rice, vinegar, herbs, Acetobacter

1. Introduction

Herbs have been integral to traditional healthcare systems for centuries, particularly in Indian Ayurveda and Chinese medicine, which have utilised medicinal plants for over 3000 and 2000 years, respectively [1]. Rich in bioactive compounds and antioxidants, herbs are known to help prevent or manage chronic diseases like diabetes, cancer, obesity, and cardiovascular disorders [2]. As their therapeutic potential became widely recognised, herbs found their way into culinary practices not only enhancing flavour and nutrition but also extending shelf life by inhibiting foodborne pathogens [3]. Herbal infusions in teas, dairy products, and fermented foods have shown numerous health benefits, including improved digestion and cardiovascular health [4, 5]. Fermentation further amplifies these effects by increasing the bioavailability of herbal compounds through microbial transformation [6].

Fermentation, one of the oldest food processing techniques, has deep cultural roots and has long been used to create products like beer, wine, yoghurt, pickles, and curd [7]. In Northeast India, ethnic communities traditionally produce rice beer using local herbs believed to have medicinal value. For instance, the Mising community brews *Apong* from Sali rice, while the Deoris and Ahoms produce *Sujen* and *Xaj*, respectively [8, 9].

While the production of herbal rice beer is relatively common, herbal rice vinegar made through a two-stage fermentation process involving alcohol and acetic acid fermentation is less explored. Historically, flavoured vinegar dates back to Babylonian times and has been used medicinally and culinarily for centuries [10]. Today, while fruit-based vinegars dominate globally, rice vinegar remains more prevalent in Asian countries like China and Japan [11, 12].

Given the abundance of medicinal herbs and high-yield rice varieties in Northeast India, there is significant potential to produce herbal rice vinegar with functional health benefits. In particular, black rice rich in anthocyanins, polyphenols, and dietary fibre offers a promising but underutilised substrate for such fermentation processes [13]. Leveraging indigenous knowledge alongside modern fermentation technologies could enable the development of a cost-effective and commercially viable herbal rice vinegar with therapeutic potential.

This study, therefore, aims to standardise the production process of vinegar using black rice and selected medicinal herbs, highlighting its scope for health benefits and economic value.

2. Materials and Methods

2.1. Sample Collection

2.1.1 Rice Variety

Black rice grains were procured from the Instructional-cum-Research Farm of Assam Agricultural University, Assam. This variety was selected as the primary substrate for vinegar production.

2.1.2 Microbial Strains

For alcoholic fermentation, *Saccharomyces cerevisiae* was obtained from the National Collection of Industrial Microorganisms (NCIM), National Chemical Laboratory, Pune. Two strains of *Acetobacter aceti*—MTCC-3246 and MTCC-3347—were used for acetic acid fermentation. These were acquired in freeze-dried ampoules from the Microbial Type Culture Collection (MTCC), ICAR-Institute of Microbial Technology, Chandigarh.

2.1.3 Herbal Mix

A blend of indigenous herbs traditionally used in ethnic fermentation practices was included in the starter culture for rice beer. The herbal composition was adapted and standardised from [14]. The plant species and their respective parts used are listed in Table 1.

Table 1: Plants used in the herbal mixture for rice beer starter culture

Sl. No	Local names	Scientific name	Part used	Amount (g)
1.	Anaros	*Ananas comosus*	leaves	0.35
2.	Bhilongoni	*Cyclosorus exlensa*	leaves	4.90
3.	Kopowdhekia	*Lygodium flexuosum*	leaves	3.50
4.	Kothalpat	*Artocarpus heterophyllus*	matured leaves	0.70
5.	Durun	*Leucas plukenetii*	leaves	0.35
6.	Tikaniborua	*Smilax perfoliata* Lour	leaves	0.35
7.	Dhopattita	*Clerodendrum viscosum*	leaves	0.35
8.	Bormanimuni	*Centella asiatica*	whole plant	0.35
9.	Mahudi	*Croton caudatus*	leaves	0.35
10.	Posotia	*Vitex negundo*	leaves, root, twigs	0.35
11.	Tejmori/Tejmoi	*Zanthoxylum nitidum*	root, stem, fruit	0.35
12.	Borbihlangani	*Pteridium aquilinum*	leaves	1.40
13.	Kotholua	*Palaquium obovatum*	leaves	0.35
14.	Chubaiatha	Not botanically identified	leaves	10.0

2.1.4 Chemicals

All chemicals and media used were of analytical grade and purchased from HiMedia Laboratories Pvt. Ltd. and Sigma-Aldrich, Mumbai.

2.2 Methods

2.2.1 Preparation of Rice Beer

Malting of black rice involved three steps: steeping (alternating soaking and draining for 48 hours), germination (at 30 °C and ~95% humidity), and kilning (at 40-50 °C for 24 hours until 4-6% moisture). The kilned grains were milled into fine powder.

Mashing and Fermentation

500 g of malted rice was mashed in water, gradually increasing the temperature from 43 °C to 77 °C in specific resting phases. After lautering and boiling, the wort was inoculated with 10 g of starter culture and fermented at 26±2 °C for 8 days. The endpoint was determined by stabilization of alcohol content.

2.2.2 Production of Black Rice Vinegar

A loopful of 36-hour-old *A. aceti* cultures (MTCC-3246 and MTCC-3347) was inoculated into a medium containing 1% glucose and 0.2% peptone and incubated at 30±2 °C for 36 hours. This was added to the fermented rice beer to initiate acetic acid fermentation under submerged conditions [15]. The process was continued until the vinegar reached ≥4% acetic acid concentration.

2.2.3 Filtration and Clarification

The vinegar was filtered using Whatman No. 1 filter paper. A 2.5 g/L bentonite solution was used for clarification, followed by refrigeration at 4 °C for 7 days to remove remaining haze and sediment.

Table 2: Treatment and Composition of Black Rice vinegar

Vinegar	Composition
A	Black rice beer + herbs + strain 1
B	Black rice beer + herbs + strain 2
C	Black rice beer + herbs + strain 1 &strain 2
H	Black rice beer without herbs and strains (control)

2.3 Laboratory methods

2.3.1 Biochemical analysis of the rice

Moisture, ash, fat [16], protein (via micro-Kjeldahl method), and starch [17] were analyzed in black rice grains.

2.3.2 pH

pH was measured using a digital pH meter (Mettler-Toledo, Mumbai) for both rice beer and vinegar.

2.3.4 Acetic acid content

The acetic acid content of the developed rice vinegar was determined by titration. The total acetic acid percent was calculated by the following formula [18]:

$$\% \text{ Acetic acid} = \frac{\text{Titre value} \times \text{Normality of alkali} \times \text{Equivalent wt.of acid} \times \text{Vol.make up} \times 100}{\text{Volume of sample taken for estimation} \times \text{Wt.or Vol.of sample taken} \times 1000}$$

2.3.5 Alcohol content
Alcohol content (% v/v) in rice beer and vinegar was measured using the spectrophotometric method described by [19]. Absorbance readings were taken at the specified wavelength, and values were calculated using a standard calibration curve

2.3.6 Total Soluble Solids (TSS)
TSS was measured using a digital MA871 refractometer (ATAGO, Tokyo) and expressed in degrees Brix (°Bx)

2.3.7 Tristimulus Colour (CIE L* a* b* parameter)
CIE L*, a* and b* values for the developed vinegar sample were determined by Hunter Lab Colour Quest XE colourimeter and the H* and C* values were estimated using the following equation:

Hue (H*) = Tan^{-1} b/a

Chroma (C*) = $\sqrt{a^2 + b^2}$

2.3.8. Sensory evaluation
The sensory quality of the vinegar was assessed by a panel of 15 semi-trained members using a 9-point hedonic scale (1 = dislike extremely, 9 = like extremely). Parameters evaluated included appearance, aroma, taste, and overall acceptability.
The Completely Randomized Design (CRD) method was used for statistical data analysis with three times replication.

3. Results and Discussion
3.1 Properties of the Rice
The amount of moisture, crude protein, crude fat, ash, and starch of the rice variety used for the development of rice vinegar is presented below:

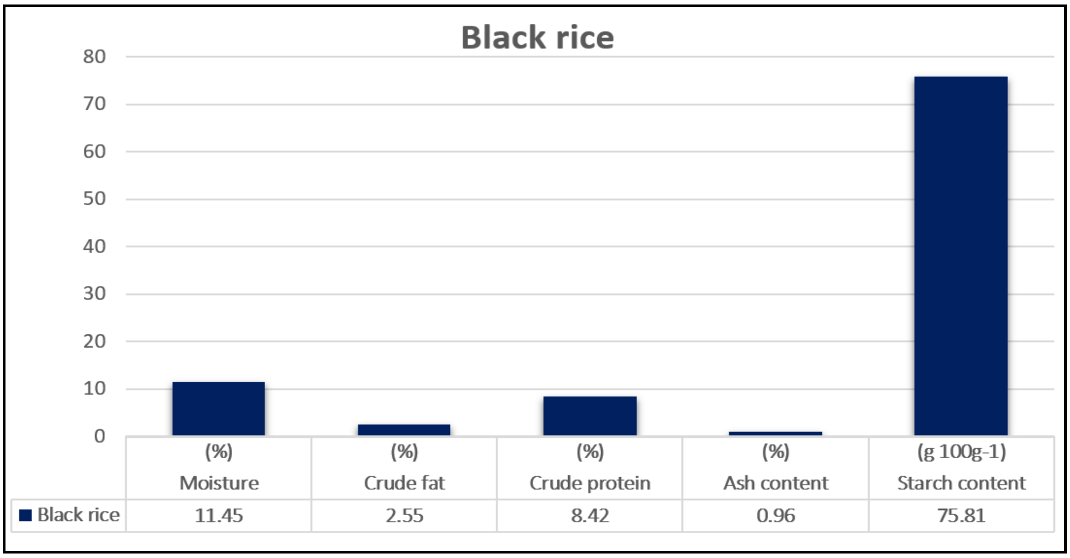

Fig 1: Proximate analysis of the rice variety

3.2 Biochemical Analysis of the Developed Rice Beer
The data on pH, acidity, alcohol content, and total soluble contents are presented below in Table 3.

Table 3: Biochemical composition of the developed rice beers

Beers	pH	Acidity %	Alcohol content % (v/v)	TSS (°Bx)
Black Rice beer	4.52	0.66	5.76	3.47

The properties of raw materials have a profound influence on the final product. The starch content of black rice was in the range similar to barley (62-77%), which makes it a suitable ingredient for beer production.
The brewing industries consider pH and total acidity to be two of the most significant parameters that influence the chemical stability and the organoleptic characteristics such as colour and flavour of the developed beer [20]. The pH of the developed black rice beer was 4.52, which was slightly higher than the range of 3.90 to 4.20 preferred in commercial beer. This difference in pH might be the result of using a different variety of yeasts. It has also been reported that the size of the batch has a significant influence on the pH of the final product [21]. The addition of hops and adjuncts in commercial beer production might also have a role in the final pH.
Total Soluble Solids (TSS) of the developed black rice beer was 3.76°Bx. Yeasts can only utilize selected lower molecular weight sugars, such as glucose, fructose, maltose, sucrose, and maltotriose. The conversion depends on the enzymes released by the yeasts to break down starch substrates into reducing sugars [22].
In the present study, the alcohol content of black rice beer was somewhat higher than the popular commercial beer brands "Budweiser" and "Corona," which have alcohol concentrations of 4.2 to 5% [23]. Across the period of alcoholic fermentation, the sugar content decreased gradually, and alcohol content increased. During this time, starch was converted to fermentable sugars, leading to alcohol production. This is reflected in the gradual depletion of sugar during alcoholic fermentation [24]. The alcohol level depends on the fermenting efficiency of yeast strains, and *Saccharomyces cerevisiae* species have shown better fermentation ability [24].

3.3 Biochemical Analysis of the Developed Rice Vinegars
The data on pH, acidity, alcohol content and total soluble solids content of black rice vinegars are presented in Table 4

Table 4: Biochemical composition of the vinegars developed from black rice

Vinegar	pH	Acidity %	Alcohol content % v/v	TSS (°Bx)
A	3.30±0.10	4.40±0.09	0.70±0.02	1.20±0.04
B	3.46±0.15	4.43±0.12	0.66±0.01	1.10±0.04
C	3.30±0.10	4.73±0.02	0.55±0.01	0.98±0.01
H (control)	4.30±0.17	3.77±0.47	0.80±0.01	2.20±0.10
S Ed (±)	0.11	0.20	0.01	0.05
CD = (0.05)	0.26	0.45	0.03	0.11

The rice vinegars were developed by fermenting the rice beers, and their biochemical and sensory parameters were analyzed to determine the soundness of acetic acid fermentation. The efficiency of fermentation differs with the type of bacterial strains used [25].

The vinegars developed from black rice using both strains of *Acetobacter* (Vinegar F) contained the highest acetic acid concentration, whereas the control samples, which were fermented naturally by wild *Acetobacter*, reported the lowest. This might be attributed to the presence of two different strains that resulted in the conversion of more alcohol to acetic acid compared to the controls. The amount of acetic acid production is also dependent on the bacteria's ability to utilize sugar, tolerate alcohol, and endure high acetic acid levels. In another study in Thailand by [27] rice vinegars developed from rice berry and black glutinous rice reached acetic acid levels of 3.63-4.07% after 21 days of fermentation.

The pH of the developed vinegar ranged between 3.30-4.30, higher than commercial vinegar (2.70-3.15) [28]. This may be due to accumulation of other volatile organic acids such as tartaric, propionic, citric, succinic, lactic, and butyric acids, which enhance vinegar aroma and taste [29]. Vinegars developed with both *Acetobacter* strains and herbs had the lowest pH. This may result from *A. aceti* converting more alcohol to acids and producing additional acids [30].

Residual alcohol remained highest in the controls due to inefficient wild *Acetobacter* strains. Evaporation of volatile alcohol could also reduce levels in developed vinegars. The variation in alcohol concentrations may also be attributed to differences in the efficiency of various bacterial strains [31, 32].

In the current study, some sugars remained unfermented, resulting in TSS values from 2.20 to 0.97°Bx. The highest TSS found in the controls may be due to the absence of efficient acetic acid bacteria. [27] reported higher TSS (5.10-10.17°Bx) in rice vinegar.

3.4 Colour of the Developed Rice vinegars

The data on colour evaluation of the vinegars developed from black rice are given in Table 5.

Table 5: Colour of the vinegars developed from black rice

Vinegar	CIE L´	CIE a´	CIE b´	Hue (H´)	Chroma (C´)
A	71.37±0.01	2.12±0.01	22.02±0.01	84.47±0.005	22.15±0.01
B	71.08±0.01	-0.02±0.005	11.28±0.01	-89.96±0.007	11.28±0.01
C	81.07±0.01	1.18±0.01	12.34±0.05	84.65±0.05	12.38±0.01
H (control)	57.66±0.01	4.43±0.01	27.88±0.01	80.97±0.01	28.24±0.01
S Ed (±)	0.01	0.01	0.02	0.02	0.01
CD = (0.05)	0.03	0.02	0.05	0.05	0.01

There was a significant difference in the colour studies of the developed rice vinegars. The microbial strains can significantly affect the colour intensity [26]. In all the vinegars, the colour was comparatively light as indicated by L´ values higher than 50 moving towards white from gray. Similarly, a´ and b´ values in all the vinegars (except E) were in the first quadrant with H´ in the range of 80.97 to 84.47. Colour values (a´ and b´) of vinegar E fell into the second quadrant with H´ of-89.96 (equivalent to 180.00-89.96 = 90.04). C´ or chroma (saturation) of colour in all the vinegars were low in the range of 11.28 to 28.24 which was also supported by higher L´ values with a lighter colour

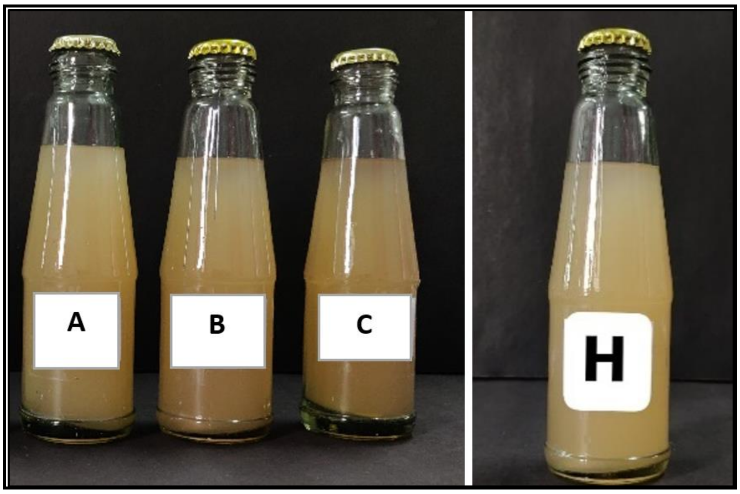

Fig 2: Developed vinegars from black rice (A, B, C, H), where H is control

3.5 Sensory Evaluation of the Developed Rice Vinegars
The data for sensory evaluation of the developed rice vinegars from bora rice and black are presented in Table 6, respectively.

Table 6: Sensory evaluation of the vinegars developed from black rice

Vinegar	Colour	Aroma	Taste	Sourness	Overall acceptability
A	7.00	6.17	6.58	6.29	7.05
B	7.52	6.76	6.82	7.00	7.11
C	7.70	6.58	6.41	6.70	7.00
H (control)	7.23	6.70	6.41	6.35	6.41
S Ed (±)	0.29	0.42	0.35	0.34	0.24
CD = (0.05)	0.59	0.84	0.70	0.90	0.48

Sensory assessment of the rice vinegars revealed that the vinegar developed from black rice using both the *Acetobacter* strains showed promising acceptance among the developed vinegars. Vinegar C (black rice vinegar) demonstrated favourable results regarding taste, sourness, aroma, colour, and overall acceptance, indicating its potential for consumer acceptance.

4. Conclusion
The present study establishes that malted black rice can be effectively used for developing herbal rice vinegar with desirable acidity and flavours. The successful production of vinegar from black rice beers demonstrates the product's promising commercial significance, as various rice beers are traditionally made by different indigenous communities in North-East India. The scientific procedure standardised in this investigation can be followed to ferment these rice beers into vinegar with herbal incorporation.
Furthermore, other local rice varieties can be utilised for vinegar production. The incorporation of herbs enhances the therapeutic value of the final product, which requires further monitoring. More locally available herbs known for their health benefits could be leveraged for vinegar production. To improve the final product's flavour, there is an opportunity to introduce unique fruit flavours, preferably from seasonal fruits, into the vinegars.
Although the product has high commercial potential in the North-East region, it is crucial to analyse the shelf-life and stability of the final product under various periods and storage conditions. Enhanced filtration and clarification could further enhance the appearance of the final product.

5. References

1. Shahrajabian MH, Sun W, Cheng Q, Singh RB. Traditional Herbs and Foods with Medicinal Value. New York: Nova Science Publishers; 2020.
2. Sharma M, Gupta A, Prasad R. A review on herbs, spices and functional food used in diseases. Int J Res Rev. 2017;4:103-108.
3. El-Sayed SM, Youssef AM. Potential application of herbs and spices and their effects in functional dairy products. Heliyon. 2019 doi:10.1016/j.heliyon.2019.e01989.
4. Chomchalow N. Production of herbs in Asia: an overview. AU J Technol. 2002;6:95-108.
5. Oraon L, Atanu J, Prajapati P, Suvera P. Application of herbs in functional dairy products-a review. J Dairy Vet Anim Res. 2017;5:109-115.
6. Hussain A, Bose S, Wang J-H, Yadav MK, Mahajan GB, Kim H. Fermentation, a feasible strategy for enhancing bioactivity of herbal medicines. Food Res Int. 2016;81:1-16.
7. Ray RC, Joshi V. Fermented foods: past, present and future. In: Ray RC, Didier M, editors. Microorganisms and Fermentation of Traditional Foods. Boca Raton (FL): CRC Press; 2014. p. 1-36.
8. Das AJ, Deka SC, Miyaji T. Methodology of rice beer preparation and various plant materials used in starter culture preparation by some tribal communities of North-East India: a survey. Int Food Res J. 2012;19:101-107.
9. Deka D, Sarma GC. Traditionally used herbs in the preparation of rice-beer by the Rabha tribe of Goalpara district, Assam. Indian J Tradit Knowl. 2010;9:459-462.
10. Budak NH, Aykin E, Seydim AC, Greene AK, Seydium ZBG. Functional properties of vinegar. J Food Sci. 2014;79:754-764.
11. Chen C, Chen F. Study on the conditions to brew rice vinegar with high content of γ-amino butyric acid by response surface methodology. Food Bioprod Process. 2009;87:334-340.
12. Zou X, Li Y, Shi J, Huang X, Zhao J. Traditional vinegars identification by colorimetric sensor. Proced Chem. 2012;6:20-26.
13. Moirangthem K, Jenkins D, Ramakrishna P, Rajkumari R, Cook D. Indian black rice: a brewing raw material with novel functionality. J Inst Brew. 2019;126:35-45.
14. Kalita S. Standardization of Starter Culture for Rice Beer (Xaaj) Fermentation [M.Sc. thesis]. Jorhat: Assam Agricultural University; 2019.
15. Kaur S, Phutela R. Fermentative production of rice vinegar using *Pichia kudriavzeii* and *Acetobacter aceti* cultures. Agril Res J. 2015;52:92.
16. AOAC. Official Methods of Analysis. 16th ed. Gaithersburg: Association of Official Analytical Chemists; 1995.
17. Kodandaram RD, Bhotmange MG. Isolation of starch from rice (*Oryza sativa* L.) and its morphological study using scanning electron microscopy. Int J Agric Food Sci Technol. 2013;4:859-866.
18. Tan SC. Vinegar Fermentation [MSc thesis]. Lafayette: University of Louisiana; 2005.
19. Sumbhate S, Satish N, Goupale D, Tiwari A, Jadon RS. Calorimetric method for the estimation of ethanol in alcoholic-drinks. J Anal Tech. 2012;1:1-6.
20. Pai TV, Sawant SY, Ghatak AA, Chaturvedi PA, Gupte AM, Desai NS. Characterization of Indian beers: chemical composition and antioxidant potential. J Food Sci Technol. 2013;53:1414-1423.
21. Burger M, Glenister PR, Becker K. Factors affecting the pH of beer. Proc Annu Meet Am Soc Brew Chem. 1952;10.
22. Nout MJR, Aidoo KE. Asian fungal fermented food. In: Hofrichter ME, editor. Industrial Application. Berlin: Springer; 2010. p. 29-58.
23. Anon. Calories, carbs and alcohol. 2021. Available from: https://www.realbeer.com/edu/health/calories [Accessed 6 March 2021].
24. Dung NTP, Rombouts FM, Nout MJR. Characteristics of some traditional Vietnamese starch-based rice wine fermentation starters (men). LWT. 2007;40:130-135.

25. Lea AGH. Cider vinegar. In: Downing DL, editor. Processed Apple Products. Switzerland: Springer Nature; 1989. p. 279-301.
26. Sharma AK, Sawant SD, Singh PN. Evaluation of fermentation efficiency of yeast strains and their effect on quality of young wines. Indian J Microbiol. 2012;52:495-499.
27. Pokkaew R, Manangsatit P. The comparison of two rice varieties on quality of rice vinegar. In: Proceedings of the 20th Food Innovation Asia Conference 2018 (FIAC 2018); 2018 June 14-16; Bangkok, Thailand. p. 420-426.
28. Jamaludin MA, Amin A, Fadzlillah NA, Kartika B, Othman R, Sani S, Ramli A. Study on physiochemical properties and the halalness of commercially marketed vinegar in Malaysia. Int Food Res J. 2017;24:S428-S435.
29. Liu F, He Y, Wang L. Comparison of calibrations for the determination of soluble solids content and pH of rice vinegars using visible and short-wave near infrared spectroscopy. Anal Chim Acta. 2008;610:196-204.
30. Yun JH, Kim JH, Lee JE. Surface film formation in static-fermented rice vinegar: a case study. Microbiol. 2019;47:250-255.
31. Spinosa WA, Santos Júnior Vd, Galvan D, Fiorio JL, Gomez RJHC. Vinegar rice (*Oryza sativa* L.) produced by a submerged fermentation process from alcoholic fermented rice. Food Sci Technol. 2015;35:196-201.
32. Kocher GS, Kalra KL, Phutela RP. Comparative production of sugarcane vinegar by different immobilization techniques. J Inst Brew. 2006;112:264-266.