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# Research

# Abstract

**Haria** is a rice-based fermented beverage that is popular among tribal and low income people in lateritic West Bengal and East-Central India. The principal ingredient of this beverage is low grade boiled rice (*Oryza sativa* L.), which is mixed with a traditional starter, called **bakhar**, and fermented within a heat-sterilized earthen pot for 3–4 days. The main aim of this study was to investigate the ethnobotanical importance and traditional process of **har**ia preparation. The method adopted for this study was based on interactive questionnaires and laboratory experiments. It was found that the pH decreased during the course of fermentation with increased titratable acidity of 1.42%. The alcohol content was 2–3% (v/v) in the consumable beverages. This documentation will be useful for further exploitation of **haria** as a health drink.

# Introduction

Cereal-based foods are very popular throughout the world and have a large impact on human diet and health. In India and Africa, 80% or more of the average diet is cereal-based products (Adebayo et al. 2010). Traditional fermented foods are prepared from most common types of cereals such as rice, corn, wheat, millet, and sorghum (Das et al. 2011). Fermented foods are prepared by the action of microorganism(s), either naturally fermented or by adding starter culture(s), which modify the substrates biochemically and organoleptically into edible products, and are thus generally palatable, safe, and nutritious (Simango 1997, Steinkraus 1983). During the course of fermentation, a group of metabolites such as lactic acid, alcohol, enzymes, antimicrobial substances, aromatic compounds, and organic acids are produced, which enrich the quality of products in respect to availability of nutrients and therapeutic potentials (Steinkraus 1983).

Global interest on rice and its fermented product is increasing due to their caloric value, unique guality, and high acceptability. Rice is a good source of carbohydrates (77-89%) and energy (1460-1560 KJ) (FAO 1993). It also provides a moderate amount of protein (6.3-7.1%), though it is devoid of lysine (Steinkraus 2002). There are many popular rice fermentation procedures used to make it more nutritious (i.e., enrichment with essential amino acids and removal of phytic acid, a major anti-nutrient in rice), easily digestible (as microbial enzymes predigest it), and acquire therapeutic properties (antimicrobial peptides, antioxidants, etc.) and synbiotic properties (lactic acid bacteria and bifidobacteria can easily grow in it; occurrence of both oligosaccharide and probiotic make it a synbiotic food) (Steinkraus 2002). Rice beer is a nutritious and energy-enriching fermented beverage recognized worldwide though mostly found in a few tropical areas of Asian countries. A comparative list of most popular rice beers is listed in Table 1.

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Name & type of rice beer	Substrate	Starter material	Reported from	Reference
Apong (white)	Boiled rice	Ipoh (yeast)	Arunachal Pradesh, India	Tiwari & Mahanta 2007
Ennog (black)	Boiled rice + burned rice husk	Ipoh (yeast)	Arunachal Pradesh, India	Tiwari & Mahanta 2007
Zutho	Whole sprouted rice + unhulled sprouted rice	Piazu	Nagaland, India	Teramoto <i>et al</i> . 2002, Das <i>et al</i> . 2012
Tai Ahom	Rice ( <b>Bora</b> variety)	Vekurpitha (yeast + leaves of some ethnomedicinal plants)	Assam, India	Saikia <i>et al.</i> 2007
Sujen	Rice + husk + medicinal plants + charcoal	Mod pitha (natural starter)	Assam, India	Deori <i>et al.</i> 2007
Bhaati Jaanr	Glutinous rice	<b>March</b> (mixed culture of yeasts, filamentous molds, lactic acid bacteria)	Eastern Himalayan regions of Nepal, India, & Bhutan	Tamang & Thapa 2006
Tape Ketan	Glutinous rice	Ragi	Indonesia	Steinkraus 1983
Miso & Shoyu	Mold rice, soybean	Aspergillus flavus Link, Zygosaccharomyces rouxii (Boutroux) Yarrow	Japan, China, Malaysia, Thailand	Steinkraus 1983

Table 1. Some common rice beers produced in Asia.

Haria (in Bengali) or handia (in Hindi) is an inexpensive beverage prepared from low grade rice and consumed as a staple food by tribal people in lateritic West Bengal and in many areas of East-Central India. During any festival, ceremonious occasion, rituals, social feast, marriage feast, death feast, etc., sharing of haria by the tribal (Adivasi) community is a traditional culture. Apart from these, other tribal peoples (e.g., Lodha, Kheria (Sabar), Munda, Santal, Kohl, Oraon, Mahali, Bhumij) of all ages in this area regularly consume this drink. These groups of people mostly live near forests and depend upon this traditional beverage as a staple food as well as medicine. They also consume it as an energy-enriching drink. Traditionally, this home-based beverage is prepared within an earthen pot by a large number of women using their indigenous knowledge. The traditional starter used for the haria preparation is bakhar (in Bengali) or ranu tablet (in Hindi). Bakhar is a mixture of old ferments (containing microbial inoculums), parts of different plants (fresh root sand leaves), and rice dust (Dhal et al. 2010). Some are also economically dependent upon this local product. In this regard, many small-scale cottage industries have developed in West Bengal, India.

To date, no analytical research has been conducted on **haria** to establish it as a fermented product and to evaluate its composition and health effects. The present paper deals with the traditional preparation procedure of this ethnic beverage while at the same time documenting some of its physico-chemical and ethnomedicinal characteristics.

## Methods

#### Data collection about the ethnic preparation of haria

Household surveys and focus group discussions were employed for this study. The present documentation of haria preparation is based on data collected from different villages of West Midnapore and Bankura districts, West Bengal, India (Figure 1), through questionnaires (Appendix 1), insight observation, as well as laboratory investigation. After acquiring prior consent, a total of 120 tribal people (70 women and 50 men) including producers, sellers, and consumers ranging in age from 20 to 60 years were involved in the interviews and discussions. A questionnaire (Appendix 1) was first translated into the local languages (Bengali and Santali) and pre-tested in front of the local people who have sufficient knowledge about the haria preparation. The questionnaire was then revised and modified for better flow and consistency. The survey was conducted through face-to-face interaction with either heads of households or knowledgeable adults in the cottage industries. Focus group discussions were comprised of 10 people (5 males and 5 females). During the data collection, especially while conducting the interviews, observations were made and comments of responders and other people were noted.

After thorough conversation and field observation, **haria** preparation can be broadly divided into following steps: sterilization of the earthen pot, boiling of rice grain, addition of starter culture (**bakhar**), incubation at room tem-



**Figure 1**. West Bengal, India, with (**A**) West Midnapore and (**B**) Bankura districts.

perature (for 3–4 days), and dilution of glutinous fermented material with drinking water for final consumption.

#### Plant collections

During the process of interviewing, plant used in **bakhar** production were identified by those being questioned. The identity of plant species was confirmed with voucher specimens which were deposited at the Department of Microbiology, Vidyasagar University.

#### Haria sample collection and analysis

**Haria** samples were collected from different villages of West Midnapore and Bankura districts of West Bengal, India, for further experiments by diluting 100 g with distilled water (2:1, w/v). The pH of the fermented substrate was measured by a glass probe digital pH meter (ELICO, India). The total titratable acidity (equivalent to percent of lactic acid) was determined by the standard titration procedure according to AOAC (1990). Briefly, 10 g of sample was dissolved in 90 ml of  $CO_2$ -free distilled water and then titrated by 0.1N NaOH. Phenolphthalein (0.1% w/v in 95%

ethanol) was used as an indicator. The amount of acid produced was calculated as percent (%, w/v) of lactic acid according to the following formula:

Total tritratable acidity (% of lactic acid) = [ml of 0.1 (N) NaOH × normality of NaOH × mol wt of lactic acid]/(ml of sample × 10)

Alcohol content during the fermentation (0 to 4<sup>th</sup> day) was measured by the dichromate oxidation method (AOAC 1990). Briefly, 15 ml of sample was oxidized by 25 ml of  $K_2Cr_2O_7$ . This reaction mixture was then incubated at 30°C for 30 minutes and absorbance was read at 600 nm ( $\lambda$  max).

The change in texture of the rice grain was checked using an inverted microscope (Magnus MS24) (4× magnification) during the course of fermentation.

#### Statistical analysis

All laboratory experiments were repeated five times, and the results were combined as the mean ± standard deviation (SD). Data were further analyzed using oneway ANOVA and Duncan's multiple range test (Duncan 1955) in Sigma Plot 11.0 (USA) to determine the significant relationship between the means.

## Results

A survey was conducted among tribal people of West Midnapore and Bankura districts to understand the traditional processes and knowledge of **haria** preparation, which is schematically represented in Figure 2. The procedure in both household and cottage industries is the same. The home-based beverage is generally prepared by rural women (mostly by senior members of the family). The rural folk historically employed trial and error methods for improving the quality of this drink over time. The knowledge thus gathered is now maintained and passed on. The principal ingredient of this beneficial health drink is low grade rice (*Oryza sativa* L.), which is very low in cost compared to other rice grains.

#### Procedure overview

A clean, sterile earthen pot is used as a fermenter. Rice grains are boiled to a char in water, and the mixture is spread over a mat in open air to cool and dry. **Bakhar** starter is added to this, and the mixture is then poured into the pot and the lid closed. The mixture is incubated at room temperature for 3 (during summer) to 4 days (during winter) in a closed, dark room. The earthen pots are kept in an upright and immobile condition. Any physical disturbance during fermentation is entirely prohibited. The texture of the rice grain changes notably during fermentation, losing its compactness, melting, and developing a glutinous appearance (Figure 3). Finally, the



Figure 2. Traditional haria production process in lateritic West Bengal, India.

ferment is diluted with drinking water (~1:6) and sieved with a fine cloth. The cream-colored, buttermilk-like filtrate is then consumed, often with some spicy vegetables. The detailed, stepwise traditional process of **haria** preparation is as follows.

#### Sterilization of earthen pot

Earthen pots (Figure 4A) are cleaned using a bundle of rice straw and sterilized by direct heating and smoking (Figure 4B). The rice straw is burned for fume generation.

#### Preparation of substrates

The low grade rice is used as a sole substrate for **haria** preparation. Impurities in the rice grains are removed manually. These grains are then vigorously boiled with an appropriate quantity of water (rice:water; 1:1.2) in a large vessel up to the point of charring. Presumably, boiling kills the pathogenic and undesirable microbes in the rice grains, as well as enhances the availability of substrate to the starter culture by softening and increasing the surface area of the grains. The parboiled grain is locally known as **bhat**. This boiled rice is air-dried under a shade by spreading on a clean persimmon-leaf mat to remove the excess water and to separate the boiled rice grains.

#### Addition of starter culture

The traditional starter used for preparation of **haria** is **bakhar** (Figure 4C). **Bakhar** is typically made of different plant parts. The major plants used for **bakhar** preparation were *Cissampelos pareira* L. roots, *Diospyros melanoxy-lon* Roxb. bark & leaves, *Lygodium smithianum* C. Presl. whole plants, *Orthosiphon rubicundus* (D.Don) Benth. tubers, *Ruellia tuberosa* L. tubers, bark, and roots, and bark of *Terminalia alata* Roth. These plant parts are considered essential for **bakhar** activity. Without these, the ferment will rot. In general, **bakhar** is kept in a dry place. Usually, 2–3 g (2 g during summer and 3 g during winter) of starter dust is mixed with 200 g of parboiled rice and then fermented for **haria** preparation (Figure 4D). Therefore, **haria** can be put into a unique category of rice-based beverage that is blended with an herbal mixture.



Figure 3. Changes in rice grain texture during the course of traditional haria fermentation in lateritic West Bengal, India: (A) first day; (B) second day; (C) third day; and (D) fourth day.



Figure 4. Traditional haria production steps in lateritic West Bengal, India: (A) earthen fermentation pots; (B) batch sterilization of earthen pots; (C) bakhar (starter culture); (D) mixing inoculum; (E) filling inoculated substrate into earthen pot; (F) incubation in earthern pots; (G) post-fermentation; (H) dilution with drinking water and sieving with fine cloth; and (I) serving haria in bowl.

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### Fermentation

Fermentation is performed within the earthen pot. The inoculated boiled rice is packed in the pot, and the pot is tightly closed with its earthen lid (Figure 4E), presumably creating an anaerobic environment. This is essential for anaerobic fermentation of rice with the starter microbes. The pot is then incubated at room temperature for 3–4 days depending on the season (Figure 4F).

#### Extraction and filtration

After completion of fermentation, rice grain is gelatinized due to microbial activities (Figure 3). The fermented material is taken out of the pot (Figure 4G) and transferred into a fine cloth. The glutinous material is diluted with drinking water and sieved using the cloth (Figure 4H). The weak, cream-colored, buttermilk-like filtrate (Figure 4I) is then drunk by the people with some spicy vegetables (Figure 5). This type of dilution and sieving is done for removal of non-fermenting ingredients as well as particulate matter and for proper mixing.

During **haria** preparation, it was observed that the pH of the ferment decreased significantly (p < 0.05) from 6.81 (initial) to 3.61 (Day 4) (Table 2). The titratable acidity (equivalent to lactic acid) increased significantly (p < 0.05) from 0.01% (initial) to 1.42% on Day 4 of fermentation (Table 3). The alcohol content was also seen to increase (p < 0.05) from 3.4% (Day 1) to 11.0% (v/v) towards the end (Day 4) of fermentation (Table 2). This was due to the presence of alcohol producing yeast which converted the available simple sugars into alcohol by the process of anaerobic fermentation. After dilution with drinking water, the alcohol content decreased to 2–3% (v/v) only during consumption.

#### Medicinal uses

From our survey it was found that **haria** is applied as a remedy for many degenerative and infectious diseases. Those surveyed believe that it can protect them from many gastrointestinal ailments, particularly dysentery, diarrhea, amebiosis, acidity, and vomiting. Apart from these, tribal gurus (native physicians) prescribe it as a skin, eye, hair, and heart protective agent.



Figure 5. Drinking traditional haria as a finished product in lateritic West Bengal, India.

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**Table 2**. Physico-chemical changes during **haria** preparation in lateritic West Bengal, India. Values are the mean ( $\pm$ SD) of pH (F = 199309.760; df = 5, 24; p < 0.05), titratable acidity (F = 18560.393; df = 5, 24; p < 0.05), alcohol content (F = 316649.661; df = 5, 24; p < 0.05). Values within a column followed by different superscripts are significantly different according to ANOVA (Duncan's multiple range tests).

Fermentation time (days)	<b>pH</b> ± 0.01	Titratable acidity (% lactic acid)	Alcohol content at 30°C (%, v/w raw ferment)
0	6.81ª	0.01 <sup>e</sup> ± 0	Oe
1	3.76 <sup>b</sup>	1.06 <sup>d</sup> ± 0.01	$3.4^{d} \pm 0.1$
2	3.68°	1.25° ± 0.01	$6.3^{c} \pm 0.2$
3	3.66 <sup>d</sup>	1.38 <sup>b</sup> ± 0.02	8.8 <sup>b</sup> ± 0.2
4	3.61 <sup>e</sup>	1.42 <sup>a</sup> ± 0.02	11.0ª ± 0.3

## Discussion

Based on this work, it can be stated that **haria** is a locally brewed rice beer. After the desired period of microbial acitivity, it is found to contain fermented rice residues, different reactive metabolites, and alcohol.

## Sterilization of earthen pots

Sterilization by direct heating and smoking using rice straw results from fumes containing both CO and  $CO_2$  gases which kill undesirable airborne pathogenic microbes by inactivation of their functional enzymes (Damar & Balaban 2006). Direct heating (incineration) is one of the established methods for killing the microorganisms. Thus, the pot becomes fully sterilized.

## Addition of starter culture

The microbial inoculums in **bakhar** ultimately act on the rice grain for conversion of the starchy material to simple sugar and alcohol. The plant parts in **bakhar** may increase the shelf-life of the microbes, acting as biopreservatives (see Table 3). Apart from these, the plant parts contain many bioactive compounds (Table 3). Researchers identified these compounds by spectrophotometric assays, high performance thin layer chromatography, fourier transform infrared spectroscopy, gas chromatography, and mass spectrometry from these plant parts (Halliwell 1997, Mallavadhani *et al.* 2004, Manikandan & Doss 2010, Ramıreza *et al.* 2003, Srivastava *et al.* 1999, Tadayuki *et al.* 1990).

## Fermentation

Generally, in cereal-based food fermentation processes, lactic acid bacteria, mold, and yeast play principal roles (Tamang & Thapa 2006). We also found that a diverse group of microbial consortia including lactic acid bacteria, bifidobacter, and yeast dominate in the **haria** (data not shown). For their growth and activity, an anaerobic environment is essential.

## Extraction and filtration

The microbial starch-degrading enzyme systems play a major role in the process of rice gelatinization, which directly help in the further growth and metabolism of microbes (Tamang & Thapa 2006).

The decrease of pH during fermentation is likely due to utilization of free sugars (Efiuvwevwere & Akona 1995, Mugochi *et al.* 2001) and generation of organic acids (e.g., lactic acid and other) during the course of microbial growth (Nyanga *et al.* 2008). Similar results were also found in **bhaati jaanr** (Tamang & Thapa 2006), but total titratable acidity of **haria** was higher than that of **bhaati jaanr** (0.20% on Day 4 and 0.17% at the end of fermentation) (Tamang & Thapa 2006) (Table 2). On the contrary, both pH and total titratable acidity of **tape ketan** vary from 4.2–4.5 and 5.74–8.11%, respectively, after 6 days of fermentation (Cronk *et al.* 1977) (Table 2), which are much higher than **haria**.

#### Medicinal uses

For generations, haria has been consumed by the tribal people of India as a staple food as well as for its ethnopharmacological benefits. It is likely that the accumulated bioactive compounds and probiotic organisms present in haria act as treatment agents. The plant constituents used in the haria preparation may also enhance the therapeutic potential of this ethnic beverage. The early brewers made conscious selections of varieties of biological materials following trial and error methods, observations, and empirical reasoning for the improvement of their health and treatment of other physical and mental ailments (Pushpangadan et al. 2012). Therapeutic use of haria has been naturally improved by the tribal people through intuitive exploration and has been standardized by the group effort of many generations. Now, brewers are mostly following their indigenous knowledge without further modification using modern science and technology.

Scientific name (Voucher number)	Part used	Bioactive chemicals	Ethnomedicinal properties
Cissampelos pareira L. (VUM036/11)	Roots	Cissampelo flavone has been isolated from the aerial parts of the plant (Ramireza <i>et al.</i> 2003). Pareitropone has been isolated from its roots, plus cissampareine, a-bisbenzyl isoquinoline alkaloid (Morita <i>et al.</i> 1955).	Wound healing, antidote, fistula, pruritis, skin disorders, snake poison, blood purification, and anti-inflammatory properties (Sankaranarayanan <i>et al.</i> 2010).
<i>Diospyros melanoxylon</i> Roxb. (VUM068/11)	Bark, leaf	Flavones, pentacyclic triterpines (Mallavadhani <i>et al.</i> 2004).	Antimicrobial properties (Mallavadhani et al. 2004), antihyperglycemic activity (Jadhav <i>et al.</i> 2009), antiplasmocidal effects against <i>P. falciparum</i> (Kantamreddi & Wright <i>et al.</i> 2008).
Lygodium smithianum C. Presl (VUM015/09)	Whole body	<i>Lygodium</i> sp. contains antheridiogens (Tadayuki <i>et al.</i> 1990), lygodinolide (Achari <i>et al.</i> 1986), O-P-coumaryl dryocrassol, tecto-quinone, kaempferol, kaempferol-3-beta- D-glucoside, beta-sitosterol, and stigmasterol (Halliwell 1997).	Treatment of jaundice (Kumar 2002).
Orthosiphon rubicundus (D. Don) Benth. (VUM027/11)	Tubers	-	Treatment of vomiting, diarrhea, dysentery (Sinha & Lakra 2007).
<i>Ruellia tuberosa</i> L. (VUM097/11)	Whole plant body (tuber, bark, root)	Lycopene, carotenoid, tocopherol, saponin, flavonoids (Manikandan & Doss 2010).	Anti-nociceptive, anti-inflammatory properties (Alam <i>et al.</i> 2009), diuretic, anti-diabetic, antipyretic, analgesic, antihypertensive, gastro-protective, and to treat gonorrhea (Lans 2007).
<i>Terminalia alata</i> Roth. (VUM089/11)	Bark	Terpene glycoside, chalchone glycoside, flavanone (Srivastava <i>et al.</i> 1999).	Antibacterial, anti-cancer hepato- protective, and antifungal activity (Shinde & Wadje 2011).

**Table 3**. Ethnomedicinal and biochemical properties from literature for some plant parts used in **bakhar** preparation in lateritic West Bengal, India.

# Conclusion

This study for the first time emphasizes the traditional process for haria preparation as well as its microbial association, nutritional quality, and ethnomedicinal importance. The fermentation makes the rice more nutritious as microbes partially digest the substrate into simple sugars and facilitate bioavailability (e.g., of lactic acid, alcohol, minerals, bioactive compounds). Lactic acid content in haria has several beneficial health effects like immunostimulation, cholesterol reduction, endocrine secretion stimluation, stress remover, and brain stimulation and shows protective roles for intestinal mucosa, flora, and overall intestinal function (The Cory Holly Institute 2005). A mild dose of alcohol reportedly reduces the risk of heart disease, gallstone formation, and diabetes and gives many other psycho-social benefits (Baum-Baicker 1985). Thus, the health promoting effect of lactic acid and

a mild dose of alcohol, probiotic organisms, and bioactive substances from microbes and plant materials likely make **haria** a healthy nutritive drink. A detailed evaluation of microbial associations, their ethnopharmacological effects, and safety of consumption should be conducted. These are needed for quality improvement of this indigenous fermented beverage.

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**Appendix 1**. Semi-structured questionnaire, used for data collection on the production, ethnobotanical and related information in community use and knowledge for **haria** preparation in lateritic West Bengal, India.

Demographic information Haria collection site: Date: Collection number: Location of interview: Name of the respondent: Age & sex: Profession: Habitat profile:

#### Preparation process

- 1. What is the traditional method for haria preparation?
- 2. Types of container (fermenter) is used in haria preparation?
- 3. What kind of substrate and starter culture is used?
- 4. What is the composition of the starter culture?
- 5. What temperature and duration are optimum for fermentation?
- 6. Is this brought to the market (daily/weekly/monthly)?
- 7. What traditional beliefs are associated with the haria preparation?

#### Other ethnobotanical information

- 1. Are there diseases treated?
- 2. What is the name of the collected plant?
- 3. What parts of the plant are used for **bakhar** preparation?
- 4. If you do not use the plant parts, then what will be its quality?
- 5. What are benefits of haria consumption?
- 6. Are there any side effects?