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The Microbiology and Traditional State of Fermented Beverage Handia: A Review

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ABSTRACT

Fermentation is one of the ancient techniques of food processing and is a common practice all across the world. Handia is a fermented rice beverage common to ethnic tribes of the eastern region of India. It is prepared by application of an amylolytic starter culture called Bhakhar which is a dough-like mixture of powdered rice and herbs and the exact composition of which varies from community to community. Malto-oligosaccharides produced by the action of amylolytic starter culture compensates for dehydration due to water-retaining capacity. The microbial flora and antioxidant activity of Handia are attributed to the type of rice and herbs used. The most common types of microorganism found in Handia are yeast-like *Saccharomyces cerevisiae* KpY, *Hanseniaspora guilliermondii* G1 and bacteria such as *Pediococcus lolli*, *Lactobacillus* sp., and *Bifidobacterium* sp., exhibiting antimicrobial activity against several bacteria viz. *Shigella dysenteriae* MB14, *Salmonella typhi*, *Micrococcus luteus*, *Staphylococcus aureus*, *Vibrio cholerae*, and *Escherichia coli* which are infamous enteropathogens. Antioxidant activity like free radical scavenging and chelating activity has been observed however specific antioxidant has not been identified till date. There are traditional claims of its therapeutic use in several acute and chronic diseases like fever, cholera and dysentery. The claimed health benefits of Handia and the presence of beneficial microflora in it, are suggestive of using it as probiotics.

Key words: Fermentation, Handia, Bhakhar, microbial flora, therapeutic use, Probiotics

Fermentation has been practiced globally since time immemorial. A vast variety of fermented food and beverages can be seen worldwide exhibiting heterogeneity due to different geographical areas where they are produced, the staple or by-product used for fermentation and the different traditions and cultural preferences prevailing in a particular region [1]. The popularity of fermentation and fermented products is indicated by the wide spectrum of foods marketed globally. Prevalence of fermentation at such a broad scale is attributed to the various benefits it provides such as preserving seasonal harvest leading to availability of food all the year round which in turn enhances food security [2]. Apart from preservation; new products with unique sensorial properties are produced by fermentation [3]. Combining fermentation activity with domestic and traditional activities seems to contribute to the livelihoods especially in

rural areas for income generation. Fermentation is mostly weather independent and by-products can be recycled into livestock fodder thus it makes maximum use of the produce [4].

The origin of fermentation process is not exactly known but it is believed to have been started by microbial interactions of acceptable nature. The earliest evidence documented about fermentation shows that over 9000 years ago fermented beverages were being used [5]. In Northern China, pottery jars containing preserved ancient organic materials were found in the Neolithic village of Jiahu, in Henan province; chemical analysis of the same was evident about the production of a beverage composed of rice, honey, and fruit [6]. Approximately at the same time, barley beer and grape wine were also beginning to be made in the Middle East. Several archaeological excavations led to the discovery of jars containing remains of wines that was 7000 years old [7]. Pieces of evidence from Archeological studies indicate the development of brewing with barley as a serious art approximately 6000 years ago in the valley of the Tigris and Euphrates, Nile, Indus and other civilizations [8].

Literary texts reveal a 3000-year-old history of fermentation in India with *Soma* Juice presumably being the first fermented product. It is a sweet-tasting drink prepared from plant juices prepared by Vedic Aryans [9]. Harrapans were familiar with Fermentation and distillation processes around 3200 to 1500 BC [10]. Mahdi Hasan [11] reported that the pre-Aryan Hill tribes seemed to prepare distilled liquor from

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fermented *Mahua*—flowers (*Bassia latifolia*). The knowledge of Fermentation among tribes has been passed upon generations and maintained as such.

Production of fermented food and beverages has become a part of indigenous cultural practices across the globe [12]. Studies suggest that the several inherent benefits of alcoholic beverages like providing relief from physical pain, retarding of food spoilage, killing harmful bacteria, were discovered by experience rather than education because of first hand witnessing of the effects by our ancient ancestors [13]. Fermented drinks were used to treat various ailments apart from being used for pain relief and alcohol was used as a solvent in dispensing medicinal herbs and spices by medicinal practitioners in ancient Egypt and Mesopotamia, China, Rome and Greece. Providing fermented beverages as a form of labour wages has also been a social practice [14]. It is reported that such ancient wonders as the Egyptian pyramids, Incan royal cities and vast irrigation networks were built with labourers generously rewarded with fermented beverages. [15].

Microorganisms involved in fermentation were first observed in the seventeenth century due to the development of high-quality lenses by a Dutch tradesman named Antonie van Leeuwenhoek [16]. Yeast was defined in the Dictionary of the English Language as "the ferment put into drink to make it work; and into bread to lighten and swell it." by Samuel Johnson in 1755. The conversion of sugar into alcohol or its oxidation into carbon dioxide was concluded in 1789 by the French chemist Antoine Lavoisier. In the eighteenth and nineteenth centuries, chemists worked hard to infer the nature of alcoholic fermentation through analytical chemistry and chemical nomenclature. In 1815 the French chemist Joseph-Louis Gay-Lussac observed that yeast was indispensable for alcoholic fermentation [17]. The role of yeast in fermentation was confirmed by, Charles Cagniard de la Tour, a French inventor in 1835 [18].

The modern understanding of the fermentation process comes from the work of the French chemist Louis Pasteur. He published a paper "Etudes sur la biere" in 1876 describing fermentation as "respiration without air" [19-20]. He also reported that undesirable microorganisms caused the souring of beer and wine [21]. In 1907 Eduard Buchner was awarded the Nobel Prize in Chemistry for showing that fermentation could occur in yeast extracts free of cells, thus introducing the concept of enzymes in the study of fermentation Biochemistry [22-23]. The complete glycolytic pathway i.e., sugar decomposition was explicated in 1940. The ancient knowledge has been combined with basic scientific knowledge and applied to modern production processes [24].

Fermentation has been observed to be useful in survival strategies. In Sudan, during the 1983-85 famine; it was found by the relief workers that people survived by preparing specific traditional fermented products, especially kawal (prepared by fermenting the green leaves of *Cassia obtusifolia* and used by certain tribes of Sudan as a meat substitute) [25]. Studies show that approximately 60% of fermented food in Sudan are famine or survival food held up by the fact that when a family becomes rich several fermented foods are no longer prepared [26].

Various fermented products have been used from ancient times, out of which most popular and widely accepted are alcoholic beverages. Fermented drinks have attracted consumer's interest due to the fact of them being minimally processed and thus having better nutritive quality and organoleptic properties in comparison to the use of modern food processing techniques and synthetic preservatives [27]. Cereal based fermented beverages are commonly found across the Asian countries namely: Sake prepared from rice by using

starter culture *chu* in China and *Koji* in Japan [28], *Takju* prepared from wheat using starter culture *nuruk* in Korea [29], *Banh men* prepared from rice in Vietnam [30] and India. Among all cereals, rice is the most popular due to its easy availability and low cost as a raw material [31]. The use of medicinal plants and herbs in the preparation of these drinks provide them with the nutritional and medicinal benefits as well as the fact of plants containing natural antibacterial agents, most probably contributes to the antimicrobial potency of the product [32].

In India various regions have different tribal communities having indigenous methodologies for processing and preparation of fermented beverages, of which, rice-based fermented beverages are more common. While the central theme of the preparation process being almost the same, the drinks differ in the composition of the starter cultures and nomenclature. The various rice-based fermented drinks across the different States of India are *Apong* by *Mishings* tribe in Arunachal Pradesh made by starter culture *Ipoh* [33], *Zutho* by *Angami Nagas* tribe of Nagaland using a starter culture called *Piazu* [34-35] *Sajpani* by *Ahom* tribe using a starter *Vekurpitha* [36] and *Jumai* prepared by *Boro* tribe using starter material *angkur* [37] in Assam. *Kaid* prepared by *Pnar* tribe of Meghalaya using a starter culture *Thait* [38]. *Handia* prepared by *oraon* tribes of Bihar and West Bengal using starter culture *Ranu* [39].

Till date studies have shown that the manufacturing of the drink involves the incorporation of diverse groups of flora and microbial culture. The diversity of microorganisms is sourced from the ingredients, utensils or environment involved in fermentation [40]. Variation is also observed regarding the choice of substrates, amylolytic starter cultures, methodologies, environment for the preparation, from community to community and is the main decisive factor for the final product. The selections depend on the topographical location of the tribal community, which in turn defines the biochemistry of the process and the microbes responsible for carrying out the fermentation. The biochemical composition of the substrate and microbes involved exhibit synergistic effect in defining the profile of the product [41].

Preparation of Handia

Handia is a local liquor of the states of Jharkhand, Orissa [42], Bihar, Chhattisgarh and West Bengal prepared by tribal groups like *Santhal*, *Ho*, *Baiga*, *Bhumij Munda*, *Gond* etc. It is a key element of the social, cultural and economic life of the indigenous people and thus considered a traditional drink [43]. The word *Handia* probably originated due to the use of large earthen pots known as *handi* in local language for fermentation.

Handia is prepared by fermentation of rice. The starter culture used in the process is known as *Bhakhar* or *Ranu*. The starter culture is a mixture of rice and different plant parts mixed in a certain ratio. Both rice and plant parts are sun-dried ground to fine powder form before mixing. The composition of *ranu* tablets is responsible for the microflora and organoleptic properties of the final consumable product.

To prepare *bhakhar* or *ranu* tablets, the powdered rice and plant parts are sieved separately to remove any large particles. The two powders are combined; sometimes crushed *ranu* tablets from previous batches are also mixed in very small amounts, and formed into a dough by adding water. The dough is rolled into small balls or disc weighing approximately 10 to 15 grams each, which are then kept covered on a mat of paddy straw or bamboo for two to three days until they are completely dry [44]. Properly dried *ranu* tablets have been observed to have

a shelf life of several months and at most up to a year if stored properly. Different kinds of plant parts are used according to the property of the plant to develop the characteristic taste and to

provide nutritional and medicinal property to Handia [45]. Few plants which are commonly used across India are listed in (Table 1).

Table 1 Plants and their parts used across India in rice beverage-starter cultures and their medicinal values Jharkhand

Name of plant	Plant part used	Medicinal value	References
<i>Woodfordia fruticosa</i> (L.) Kurz.	Roots	Used in treatment of rheumatism, dysentery, foot and mouth disease	[46]
<i>Ruellia tuberosa</i> (L.)	Roots	Used in kidney diseases and whooping cough, urinary diseases and stomach problems	[46]
<i>Andrographis paniculata</i> (Burm.f.) Wall. Ex Nees	Roots	Used in stomach ache, inflammation, pyrexia, and intermittent fevers	[46]
<i>Dioscorea bulbifera</i> (L.)	Roots	Used in diarrhea, dysentery and conjunctivitis	[46]
<i>Madhuca longifolia</i> (Koenig) MacBride var. <i>latifolia</i> Roxb.	Roots	used for diarrhea, fever, chronic tonsillitis, bleeding gums and diabetes	[46]
<i>Asparagus racemosus</i> Willd.	Root	Orissa Nutritive tonic and demulcent, Cures fever, leprosy, bronchitis and cough.	[47-48]
<i>Clerodendrum serratum</i> (L.) Moon	Root, Leaf	Roots used in fever, snakebite, asthma and cough.	[49]
<i>Coccinia grandis</i> (L.) Voigt	Root tuber	Ear pain, jaundice, blood dysentery and diabetes.	[50-51]
<i>Dipteracanthus suffruticosus</i> (Roxb.) Voigt	Root	Renal problem	[52]
<i>Rauwolfia serpentina</i> (L.) Benth.	Root	Used as antidote for snake bite, malaria	[52-53]
<i>Madhuca longifolia</i> (Koenig) MacBride var. <i>latifolia</i> Roxb.	Seed, Leaf, Bark	Seed oil used in rheumatism, leaf and bark used for diabetes	[52], [54]
<i>Calotropis gigantea</i> (L.)	Leaves	Assam Analgesic, antiseptic, anthelmintic, used in burns, ear and eye complaints	[55]
<i>Ochocloa coracana</i> Edgew.	Leaves	Used in Measles and pneumonia	[55]
<i>Saccharum officinarum</i> (L.)	Leaves	Used in constipation	[55]
<i>Cleodendrum Viscosum</i> Vent.	Leaves	Anthelmintic, hair tonic, used in malaria, headache and tumours	[55]
<i>Scoparia dulcis</i> (L.)	Shoots	Used in urinary trouble, eye trouble	[55]
<i>Cissampelos pareira</i> L.	Roots	West Bengal Wound healing, antidote, skin disorders, snake poison, blood purification, and anti-inflammatory properties	[56]
<i>Lygodium smithianum</i> C. Presl	Whole plant body	Treatment of jaundice	[57]
<i>Orthosiphon rubicundus</i> (D. Don) Benth.	Tubers	Treatment of vomiting, diarrhea, dysentery	[58]
<i>Terminalia alata</i> Roth.	Bark	Antibacterial, anti-cancer hepato-protective, and antifungal activity	[59]
<i>Ruellia tuberosa</i> L.	Whole plant body (tuber, bark, root)	diuretic, anti-diabetic, antipyretic, analgesic, antihypertensive, gastro-protective, and to treat gonorrhea	[60]

In the fermentation stage of handia first of all, desired amount of washed raw dehusked rice is put in boiling water, in enough quantity that water is completely absorbed by the time rice is 90-95% cooked. The cooked rice is then taken off from the flame and spread on a mat for cooling [61].

The vessel specific for fermentation is heated and the ranu tablets are put in that hot vessel before mixing in the cooked rice. This heating process probably sterilizes both, the vessel and the outer surface of ranu tablets. Apart from direct heating, smoking is used to kill undesirable airborne pathogenic microbes as the CO and CO₂ gasses present in the fumes produced by burning rice straw inactivates their functional enzymes [62].

After cooling, crushed ranu tablets are mixed properly into the cooked rice. A little amount of ranu powder is first sprinkled in the inside of the empty fermenting vessel, the ranu mixed cooked rice is then placed in the fermenting vessel up to almost three fourth full. It is then covered and is not necessarily completely anaerobic. A visible collapse in the level of rice and

flowing of dark straw-colored liquid on tilting of the vessel and a peculiar smell is generally observed after the fourth or fifth day and considered as the completion of fermentation [63]. The time required for fermentation depends on and varies by the change in temperature. Handia preparation in summer takes three to four days, whereas in winter it takes five to six days. After completion of fermentation, rice grain is gelatinized due to microbial activities. The fermented material is taken out and sieved for removal of particulate matter; the filtrate is further diluted with drinking water before consumption [64].

Beneficial aspects

Numerous reports have revealed a variety of microbial flora to be involved in the preparation of various fermented beverages. Maji *et al.* [65] reported a wide microbial consortium in handia, identification and molecular characterization of the microorganisms showed the presence of yeast as well as bacteria. Yeast species were identified as *Saccharomyces cerevisiae* KpY, *S. cerevisiae* H15, *S.*

cerevisiae H17, *S. cerevisiae* 18VSL, *Hanseniaspora guilliermondii* G1, *H. guilliermondii* G4, *Pichia kudriavzevii* H21L, *Candida glabrata* H3, *C. glabrata* H8, *C. glabrata* H11, *C. glabrata* H12 and *C. tropicalis* 18VLL. The bacterial species identified were *Brevibacillus agri*, *Leuconostoc mesenteroides* and *Kocuria* sp.

Lactic acid bacteria strains were isolated from Hamei and Marcha- starter cultures for preparation of various indigenous alcoholic beverages of North east India and were identified as *Pediococcus pentasaceus*, *Lactobacillus brevis* and *Lactobacillus plantarum*. LAB strains showed strong acidifying ability [66].

Sha *et al.* [67] studied the bacterial and fungal communities in marcha and thiat which are traditionally prepared amylolytic starters used for the production of various ethnic alcoholic beverages in Sikkim and Meghalaya, states of India. Characterization of bacterial community depicts phylum *Proteobacteria* is the most dominant in both marcha (91.4%) and thiat (53.8%), followed by *Firmicutes*, and *Actinobacteria*. Estimates of fungal community composition showed *Ascomycota* as the dominant phylum. The presence of *Zygomycota* in marcha distinguishes it from the thiat. Identification of mold and yeast in a rice wine starter culture of Cambodia called medombae, has shown presence of *Rhizopus oryzae* and *Mucor* species for mold; *Candida tropicalis*, *Saccharomyces cerevisiae* and *Saccharomycopsis* species for yeast [68]. Fermentation yeast was isolated and identified as being a strain of *Saccharomyces cerevisiae*, from a rice beer zutho a fermented product in Nagaland India [69].

Panda *et al.* [70] isolated *Enterococcus faecium* and *E. faecalis* from Handia. These strains showed ability to grow in temperature 10 to 42°C and pH \geq 9.5 they exhibited tolerance towards physiological concentration of bile salt and 8.0% NaCl. Roy *et al.* [71] reported variation in degree of intoxication and amount of alcohol present in Handia prepared by varying source of native origin of Bhakhar from different areas of West Bengal.

Microorganisms found in handia were screened and bacterial species namely *Bifidobacterium* sp. (MKK4), *Pediococcus lolli* (MKK21), and *Lactobacillus* sp. (MKK37) which showed potential probiotic characteristics out of which *Bifidobacterium* sp. had the highest cumulative probiotic score of 82% auto-aggregation, 53% cell surface hydrophobicity and 66.3% biofilm producing capability under conditions of nutrient depletion. Along with stability in simulated acid and bile solutions it also exhibited antimicrobial activity against gram positive bacteria viz. *Shigella dysenteriae*, *Salmonella typhi*, *Micrococcus luteus*, *Staphylococcus aureus*, *Vibrio cholerae*, and *Escherichia coli* [72].

Contribution of medicinal plants toward the antimicrobial property of fermented drinks has also been reported. In the region of West Garo hills indigenous fermented rice beverages use a starter rice cake Wanti, containing

medicinal plants *Scoparia dulcis* and *Leucas lavandulaefolia* which showed excellent antibacterial activity against *Bacillus cereus* [73]. Effective antioxidant activity of Handia and its concentrate has also been reported but the specific antioxidant component has still not been identified [74]. Study on antibacterial activity of microorganisms isolated from Handia is suggestive of formation of bacteriocin like inhibitory substances, but require further research for confirmation and detailed characterization [75].

The tribes firmly believe that apart from serving the purpose of beverage, brewed liquor aid in remedy to several acute and chronic diseases. Studies show the nutritional importance of Handia in terms of having a considerable amount of protein and carbohydrate content along with lower alcoholic content (0.78%-1.38%) and its ability to compensate water loss during heavy physical labour particularly during summer [76]. It is used as a nutritional supplement as tribals get 5-10% of their daily nutrients from handia [77]. The Maria tribe of Bastar use it as a light tranquillizer [78]. Gond tribe of Surguja district use ranu tablets to treat cholera [79].

Socio-economic status

Among the tribes, handia is a key element in the day to day life. It is served to guests in every social event like marriage, birth, death rituals and cultural functions. It is also used as a gift while house visiting as a symbol of affection. The guests are also welcomed by serving them Handia (<https://indroyc.com/2017/09/02/handia/>). It is a source of income for landless people commonly tribal women because of the process mainly referred to as kitchen work [80]. Although beneficial, profuse drinking habits cause behavioural changes. Handia acts as a mood stimulant and also provides energy when consumed in little amounts but excessive drinking causes intoxication leading to ethological changes like aggression and abusiveness [81].

CONCLUSION

Fermentation is a universally used food processing method. Benefits of which include food preservation and improved nutritional quality. Handia is considered as poor man's beer due to the simplicity in its preparation and Ingredients. Traditionally it is claimed to help in treatment of diseases like jaundice and also protects from sun-stroke but medical documentation is not available. Micro flora exhibiting potential probiotic activity and antimicrobial property have been obtained from Handia. Lactic acid bacteria have been reported to provide several health benefits such as improved digestion of lactose control of intestinal infections and serum cholesterol. These attributes manifest handia as a popular drink in rural areas. The results are suggestive of using Handia as probiotics.

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