Cereal based Traditional alcoholic beverages of Lahaul and Spiti area of Himachal Pradesh

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Some cereal based traditional alcoholic beverages, Chhang, Lugari, Aara and Chiang consumed by people of Lahaul and Spiti area of Himachal Pradesh were documented and explored microbiologically. All these beverages were made by cooking their grains and then inoculating them with inocula called Phab/ Dhaeli. Chemical analysis of these products showed an acidic nature with pH in the range of 3.31-4.02 in undistilled samples, and 3.95-5.17 in distilled samples. Total soluble solids in all the undistilled samples were in the range of 14.58 to 18.56 °B, whereas in distilled samples these were in the range of 7.19-8.0 °B. Ethanol contents (%v/v) were 5-12 % in undistilled and 13-19% in distilled products. A wide variation in certain other chemicals constituents, viz. acetaldehyde, methanol, ester, n-propanol, etc. was observed in the distilled alcoholic beverages. Microbiological examination of these beverages and their source of inocula revealed the dominance of yeasts mainly from genus Saccharomyces and Endomyces. Bacteria encountered in these beverages were from genus Lactobacillus, Acetobacter, and Bacillus.

Keywords: Traditional alcoholic beverages, Fermentation, Cereals and indigenous knowledge

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Fermentation is the oldest known form of food biotechnology, which has been practiced for thousands of years by the ancient man as the potent tool for preserving foods and beverages. It is a process by which useful products are made by the application of microorganisms. The product may be one which is synthesized by the organism(s), the organisms themselves or a combination of the two. A product is considered fermented when one or more of its constituents are acted upon by microorganisms to produce a considerably altered final product, acceptable for human use. Microflora, either of natural flora or inoculated one, causes microbiological as well as biochemical changes, forming a new product with modified qualities especially, flavour and nutritional components. In tribal areas of Himachal Pradesh, several indigenous alcoholic beverages are consumed and traditionally prepared without much awareness about the role or involvement of microorganisms. Some of these indigenous products having cereals as the main substrate are extensively consumed by inhabitants of Lahaul and Spiti area of Himachal Pradesh. These products are primarily restricted to households and are prepared according to the traditional methods using simple equipments under unhygienic conditions with undefined micro flora. Therefore, it was essential to document and explore the specific micro flora associated with some of these products to evaluate their quality and consistency.

Methodology

Lahaul and Spiti district of Himachal Pradesh was selected as the main study area for traditional alcoholic beverages. Indigenous knowledge of these products was documented using Participatory Rural Appraisal Technique which consisted of discussion with key informants and focused group discussion with the groups chosen randomly from different residential areas of Lahaul and Spiti. The study was conducted in Hinsa, Chimrit, Tamlu, Khanjar, Karpat and Jundla villages of Lahaul and Hansa and Lari villages of Spiti. Samples of each product along with their source of inoculum were collected in sterilized containers/vials for processing of chemical and microbial analysis. Samples of each product were analyzed chemically by following standard techniques. Distilled samples of alcoholic products

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were analyzed for acetaldehyde, methanol, ester, n-propanol, iso-butanol and amyl alcohol by Gas Liquid Chromatography (NUCON-5700). Results were expressed in mg/100ml of the sample. Chemical constituents of undistilled samples could not be analyzed due to chocking of column.

For studying the microbial diversity, collected samples of each product were processed immediately on selected media, viz. Potato dextrose agar (PDA) for yeasts and molds, Lactobacillus isolation agar/de, Man, Rogosa, Sharpe (MRS) agar for Lactobacillus, Acetobacter isolation agar for Acetobacter and nutrient agar for the cultivation of other bacteria. The colonies of yeasts and bacteria exhibiting different morphology were selected and purified by culturing them on their respective selective media. Then each purified culture/ isolate was maintained on agar slants of their respective media and preserved at 4°C in refrigerator for further studies. The purified yeast isolates were identified on the basis of morphological (type, shape, colony and growth characteristics on potato dextrose agar/broth, mycelium, pseudomycelium, pellicle formation, growth at different temperature) and biochemical characteristics (assimilation/ growth in the presence of ethanol, potassium nitrate and cycloheximide and fermentation pattern for different sugars, viz. glucose, galactose, trehalose, salicin, starch, lactose, maltose, sucrose, raffinose, etc.).

Similarly, bacterial isolates were identified on the basis of morphological (cell shape, gram’s reaction, colony and growth characteristics on their respective media/ broth, motility, spore formation, growth at different temperatures and pH) and biochemical characteristics (citrate utilization, oxidase, catalase, hydrolysis of starch & arginine, production of gas from glucose, mechanism of fermentation in Gibson’s semi solid media, action on litmus milk, dextran production on sucrose agar, growth in presence of 2, 4 and 6% NaCl, fermentation pattern for different sugars).

**Results and discussion**

Various ethnic groups of Lahaul and Spiti area of Himachal Pradesh are consuming a variety of cereal based traditional alcoholic products including Chhang, Lugari (Chakti), Aara and Chiang (Table 1). These products were not properly looked earlier with respect to their method of preparation, consumption pattern, microbial diversity and other characteristics. Several such cereals based indigenous alcoholic products consumed in Darjeeling and Sikkim, Sub-Himalayan region, etc. have been documented. However, their method of preparation and other characteristics differ from region to region. As evident from the results (Table 1), phab and/or dhaeli were the main traditional inoculum applied for the preparation of these beverages. These inocula are available as dried white cakes/ granules in the adjoining region of the study area. Kullu (Fig. 1). Application of similar type of traditional inocula, phab, murcha, etc. has also been recorded in different regions for cereal based beverages.

These beverages were traditionally prepared from cooked cereal grains with an indigenous inoculum, phab/dhaeli under mesophilic conditions for variable fermentation duration. Documented methods of preparation are broadly in agreement with the methods described for other cereal based alcoholic products, except for minor variations, i.e. type of container, fermentation duration, processing of substrate, etc. As evident from the documented information, Chhang, Aara and Chiang are consumed as undistilled fermented products or as distilled products, whereas Lugari (Chakti) is consumed as undistilled only (Fig. 2). The distillation is carried out through traditional method of distillation (Fig. 3). These products are generally prepared in the summer months probably due to availability of congenial temperature for the fermentation of these products as reported by other workers, who found temperature as a sole and key factor for affecting fermentation processes. These products are regularly consumed, however, their production and consumption are augmented during special occasions or ceremonies or special get together known as Tagri/Gaji and engagement ceremonies. Similar type of social concomitants with different alcoholic beverages in different regions have been reported. Chhang, Chiang and Aara are much preferred over Lugari due to their better taste, flavour and shelf life. Not only that, these products also being exploited as a source of income in some of the households of the study area.

All the alcoholic products (distilled as well as undistilled) were found to be acidic in nature with pH ranging from 3.31 to 4.02 in undistilled and 3.95 to 5.17 in distilled products. The acidic nature of these products is probably due to the production of organic acids during the process of fermentation by acid producing microorganisms, as the fermentation is carried out under unhygienic and uncontrolled
<table>
<thead>
<tr>
<th>Product</th>
<th>Chhang</th>
<th>Lugari (Chakti)</th>
<th>Aara</th>
<th>Chiang</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw material</td>
<td>Wheat</td>
<td>Rice</td>
<td>Barley</td>
<td>Wheat</td>
</tr>
<tr>
<td>Inoculum</td>
<td>*Phab or Dhaeli</td>
<td>Phab</td>
<td>Phab or Dhaeli</td>
<td>Phab or Dhaeli</td>
</tr>
<tr>
<td>Quantity of preparation</td>
<td>Quantity of preparation depends upon the availability of raw material, capacity of container and temperature</td>
<td>Quantity of preparation depends upon the availability of raw material, capacity of container and temperature</td>
<td>Quantity of preparation depends upon the availability of raw material, capacity of container and temperature</td>
<td>Quantity of preparation depends upon the availability of raw material, capacity of container and temperature</td>
</tr>
<tr>
<td>Raw material cultivated or purchased</td>
<td>Raw material is purchased</td>
<td>Raw material is purchased</td>
<td>Raw material is purchased</td>
<td>Raw material is purchased</td>
</tr>
<tr>
<td>About 1 kg of wheat yields approx 750 ml of Chhang &amp; about 400 ml of distilled liquor</td>
<td>About 1 kg of rice yields approx 900 ml of Lugari</td>
<td>About 1 kg of barley yields approx 800 ml of Aara and about 450 ml of distilled liquor</td>
<td>About 1 kg of wheat yields approx 750 ml of Chiang and about 400 ml of distilled liquor</td>
<td></td>
</tr>
<tr>
<td>Cereal grains are washed and soaked in water (1:1) for 8-10 hrs</td>
<td>Rice grains are washed and soaked in a drum containing water (1:2) for 20-24 hrs</td>
<td>Cereal grains are washed and soaked in water (1:1) for 15-20 hrs</td>
<td>Cereal grains are washed and soaked in water (1:1) for 10-12 hrs</td>
<td></td>
</tr>
<tr>
<td>Cooked for 1-2 hrs in an open vessel</td>
<td>Not cooked</td>
<td>Cooked for 2-3 hrs in an open vessel</td>
<td>Cooked for 1-2 hrs in an open vessel</td>
<td></td>
</tr>
<tr>
<td>Cooled, powdered phab is sprinkled on cooked grains (for about 2-3 kg wheat, 1-2 granules or cakes are added)</td>
<td>Powdered phab is mixed with the grains (for about 2-3 kg rice, 2-3 granules or cakes are added)</td>
<td>Cooled, powdered phab is sprinkled on cooked grains (for about 2-3 kg barley, 3-4 granules or cakes are added)</td>
<td>Cooled, powdered phab is sprinkled on cooked grains (for about 2-3 kg wheat, 3-4 granules or cakes are added)</td>
<td></td>
</tr>
<tr>
<td>Mixed and piled on bags</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Kept for fermentation (25-35°C, 2-3 days). To maintain its temperature, bags are wrapped in blanket</td>
<td>Mixture is kept in warm room for fermentation (25-35°C, 15-30 days)</td>
<td>Mixed and kept for fermentation (25-35°C, 3-4 days) in a container containing water (for about 50 kg barley, 30-35 L of water)</td>
<td>Mixed and kept for fermentation (25-35°C, 2-3 days) in a container containing water (for about 50 kg wheat, 30-35 L of water)</td>
<td></td>
</tr>
<tr>
<td>Transferred to a container or plastic vessel containing water (for about 50 kg wheat approximately 30 L of water)</td>
<td>Liquid part is squeezed out from the solid part with a bamboo basket or a cloth</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Fermented for about 15-20 days in warm room</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Undistilled product is known as Chhang</td>
<td>Collected liquid product is locally known as Lugari (Chakti)</td>
<td>Fermented product obtained is locally called as Aara</td>
<td>Fermented product is locally called as Chiang</td>
<td></td>
</tr>
<tr>
<td>Solid part left is used as cattle feed</td>
<td>Solid part left is used as cattle feed</td>
<td>Solid part left is used as cattle feed</td>
<td>Solid part left is used as cattle feed</td>
<td></td>
</tr>
</tbody>
</table>

Contd.
Several researchers have also observed a strong acidity in their cereal based alcoholic beverages. The total soluble solids were 14.58-18.56 °B in undistilled samples and 7.19-8.0 °B in distilled sample. The higher TSS values in these products could be due to the continuous hydrolysis of starchy compounds by varied micro flora encountered in these products as most of the collected samples were 2-3 weeks old. As evident from the analysis of distilled samples for various chemical constituents, all the samples had excess of all constituents, viz acetaldehyde, n-propanol, iso-butanol and amyl-alcohol (Table 2). Beside this, in a sample of Chhang, methanol (9.6 mg/100 ml) and esters (58 mg/100 ml) were also detected, thereby indicating a very crude system of distillation being followed for the preparation of these beverages (Fig. 3). In fact, this traditional system of distillation has no comparison to the alcohol being produced in proper distilleries, where a set of column is used to remove all such impurities by taking distillation cuts of all low boilers and high boilers. In this case, no cuts are being followed. Chemical constituents like, ester, acetaldehyde, n-propanol, iso-butanol, amyl-alcohol,
etc. are associated with flavour and aroma of fermented products, but in very low concentrations. However, it is not yet known which compounds are primarily responsible for flavour characteristics in the documented alcoholic beverages. Therefore, more studies under controlled conditions are needed, before any effective conclusion could be made.

Undistilled as well as distilled alcoholic samples were also analyzed for their ethanol content (Table 3). In undistilled samples of Chhang, Lugari, Aara and Chiang, ethanol concentrations were 8.16, 5.38, 11.54 and 7.53% (v/v), respectively. In distilled samples, the concentrations had elevated to 13.76, 18.78 and 13.94% (v/v) in Chhang, Aara and Chiang, respectively. Lugari was never distilled and was consumed only in undistilled form. These observations are in good agreement with the findings of other researchers, who also reported an ethanol content of 7-8% (v/v) in fermented cereal grains, which then increased to 15-16% on distillation\textsuperscript{10}. Based on morphological and biochemical characteristics, 6 strains were found to be from genus Saccharomyces, 4 from undistilled samples, one from phab and one from dhaeli. However, one strain from dhaeli was from genus Endomyces (Table 4). Similar
dominance of these genera in other cereal based beverages has also been noticed earlier. Two isolates, *Saccharomyces fermentati* and *Endomyces fibuliger* were observed only in the source of inoculum and not in undistilled products meaning thereby that they were outnumbered in the fermenting mixture. This might be due to the dominance of *Saccharomyces cerevisiae* in the fermenting substrate owing to their superior fermentative ability, tolerance to high pH (3-6), ethanol concentration (7-8%) and high temperature as already reported.

It is observed that the threshold concentrations of ethanol required to halt the growth of fermenting microorganisms is nearly at 6.9-11.3% (w/v). The high concentration of ethanol in these products is thus an exhibition of high ethanol tolerance ability of these local yeast isolates. A variable ethanol tolerance ability of yeast isolates obtained from *Chhang* prepared in Sub-Himalayan region was also noticed earlier. Most of the yeast isolates from genus *Saccharomyces* were capable of showing growth at a temperature range of 15-42°C. However, one isolate of genus *Endomyces*, showed growth at a temperature range of 4-37°C. A wide temperature range of 15-35°C for *Saccharomyces* species was also reported earlier. It has been reported that some of yeast isolates from starter culture could grow at 4-5°C as observed in the study also in case of *Endomyces*. Bacterial isolates encountered in undistilled products were identified on the basis of morphological and biochemical characteristics. These were mainly from genera *Lactobacillus*, *Acetobacter* and *Bacillus* (Table 5). The above results are corroborated by earlier findings which also encountered similar genera in their undistilled products. However, no such strains were encountered in *phab/dhaeli* samples. Thus these bacterial strains are probably the natural contaminants, which come from the staple and surroundings and are probably responsible for desirable flavour and aroma of the product as suggested earlier. However, excessive population of these bacterial strains may result in the production of a poor quality product or in spoilage of whole product. Most of the lactic acid bacteria can tolerate high acidity of 3.5-5.0 and alcohol content up to 18%, whereas as *Acetobacter* can tolerate a pH of 4.0-5.5 and alcohol up to 14-15%, and *Bacillus* a pH of up to 6.0 and low alcohol content. Availability of conducive/optimum environment in the documented alcoholic beverages might be one of the important reasons for the presence of these microorganisms. A close association between *Lactobacillus* and *Saccharomyces* species during fermentation under high acid and low pH conditions was reported. Similar association was noticed in the study also. *Lactobacillus* dominates in the early stages of fermentation and creates conditions conducive for the growth of *Saccharomyces*, which are then ultimately responsible for production of ethanol.

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### References

### Table 5—Predominating bacterial flora in traditional beverages

<table>
<thead>
<tr>
<th>Product</th>
<th>Isolate Code</th>
<th>Identified Bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Chhang, Aara, Chiang</em></td>
<td>LS-I</td>
<td><em>Lactobacillus plantarum</em></td>
</tr>
<tr>
<td><em>Chhang, Aara, Lugari</em></td>
<td>AS-I</td>
<td><em>Acetobacter acetii</em></td>
</tr>
<tr>
<td><em>Aara Chiang, Lugari</em></td>
<td>BS-I</td>
<td><em>Bacillus subtilis</em></td>
</tr>
</tbody>
</table>
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