Evaluation of Nutritional and Biochemical aspects of Po:ro apong (Saimod) - A home made alcoholic rice beverage of Mising tribe of Assam, India

Devid Kardong*, Khirod Deori2, Kaushal Sood3, RNS Yadav4, TC Bora4 & BK Gogoi6
1-4Department of Life Sciences, Dibrugarh University, Dibrugarh-786004; Assam
5,6Biotechnology Division, North East Institute of Science and Technology, Jorhat-785006, Assam, India
E-mail: kardongdevid@yahoo.co.in

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Po:ro apong (saimod) is a socio-culturally important alcoholic rice beverage unique to the Mising Tribe of Assam in India. The various steps involved in the preparation of saimod and its nutritional aspects were evaluated through biochemical analysis of the finished product. About 16 (sixteen) different plant species having medicinal values are used for preparation of the epop (yeast starter). The finished product (po:ro apong) is reported to have medicinal values and finds applications in homely treatment of various ailments. The use of burnt ash of rice husk and rice straw during the preparation of this beverage is unique. Biochemical analysis shows that the finished product is acidic beverage and contains almost all metabolically important nutrients with the detectable amount of amylase enzymes. The alcohol content in the consumable finished product is significantly higher (7.52% - 18.5% v/v) as compared to other popular rice beer. The microbial load in different stages of the po:ro fermentation was recorded as 549 x10<sup>7</sup> cfu/gm in epop followed by 414 x 10<sup>7</sup> in ripe mash and 8,700 ± 6.332 cfu/mm<sup>3</sup> in filtrate product (po:ro apong).

Keywords: Po:ro Apong, Yeast starter, Mising, Alcohol, Biochemical, Epop

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The North Eastern region of India is well known for production of household liquors, which is associated with the region’s rich indigenous knowledge system that is extricably linked to its socio-cultural, environmental and institutional contexts. Sufficient information is available on the traditional knowledge of making alcoholic beverages in Northeast India. These knowledge systems are both tacit and explicit that has been codified into words or transferred from one generation to another through ages, thereby suggesting a sense of common or communal ownership amongst different communities. Though, different tribes of the region use more or less similar substrates for fermentation, the methods for preparation of wine and other alcoholic beverages varies as all of them follow their own indigenous protocols employing different starter cultures. The po:ro apong is a very popular home made alcoholic rice beverage unique to the Mising tribe of upper Assam. Though, a similar beverage is prepared and consumed as Ennog by Adi tribes of Arunachal Pradesh. It is blackish to reddish brown, nearly transparent liquid with pleasant aroma and taste. The consumption of this particular beverage begins from spring and preferably during the summer and autumn seasons because of its reported cooling effect. It is prepared by fermentation of cooked rice (Oriza sativa) mixed with burnt ash of rice husk and straw. The starter material for fermentation is a homely prepared yeast culture (epop in Mising dialect) maintained in a starchy (rice) medium mixed with a number of powdered plant/ plant parts that are thought to contribute medicinal properties and aroma to the finished product. The po:ro apong is also used in various ailments including dysentery, body pain, etc. Suitable dose of po:ro helps in increasing the perspiration and thereby expelling the extra heat from the body and hence this drink is used preferably during the hot summer season.

Preparation of epop (starter culture)

The epop (Fig. 1) is prepared from rice, preferably glutinous, soaked in water for about 2 hrs., ground together and mixed properly with semidried powder of leaves or the whole plant/ plant parts which is then kneaded to specific shape (usually oval) followed by...
the transfer (inoculation) of microbial consortium with desired quality from the old stock of previous batch culture. The quality referred here is the property of the individual microorganisms that contribute to the aroma, taste and texture of the finished product (po:ro apong). Various steps involved in the preparation of epop are given below (Flow chart 1).

Making the po:ro apong (Saimod)

About 100 gm of powdered starter culture (epop) is sufficient to cause fermentation of 3 kg cooked rice. The warm cooked rice is properly mixed with the ash powder of straw and rice husk in such a way that a thin layer of ash is formed around each rice grain (Flow chart 2). This rice-ash mixture is now sprinkled and mixed properly with the starter culture (epop) and the whole mass is taken into a clean dry container (preferably earthen pot) then plugged with dry straw or fresh leaves of fern (Pteridium sps.) and fermentation is allowed to for about 20 days at around 30°C-35°C. The fermented ripe mash called po:ro arouk is a black, rough, wet solid mass reduced in volume (Fig. 2).

Extraction of finished product (po:ro apong) from the fermented mash

There is an interesting traditional way of monitoring the fermentation and maturity stages of good quality ferment. When the fermentation is set on right, a mild fruity sweet aroma within first 2nd or 3rd days followed by the characteristic strong fruity pungent odor appears after 8 to 10 days of fermentation. The characteristic odor of the fermenting broth of po:ro apong attracts a kind of fly typically small in size, resembling the fruit fly Drosophila sps. (To be identified). The appearance of maggot of such flies and their subsequent disappearance without affecting the brew probably because of increasing alcohol concentration may be used as a traditional indicator of maturity of quality fermentation. After twenty days of fermentation, the po:ro is extracted through filtration. The extraction process is an age old filtration technique accomplished with the help of specially designed cone made of split bamboo. The drink is extracted by slowly pouring warm water on top of fermented mash (po:ro arouk/ponyok) after putting it into a cone shaped apparatus (tasuk) (Fig. 3) made of bamboo, internally wrapped with banana or Canna leaves. The semi-transparent liquid collected through bottom of the apparatus is used as the drink (Fig. 4).
The left out mash is used as an excellent fodder for pig and cattle. A high level traditional skill is required for the filtration process to obtain the desired reddish brown color of the finished product (Fig. 5). However, the filtration process is a time consuming crude technology which is in utmost need of modern technological aid.

Statement of the problem

Studies have shown that the starter culture is a consortium of microbial concoction, most of which are yeasts, fungus (molds) and bacteria\(^5\). Although all of them may not equally important, yet these organisms contribute individually to the overall nutritional facts on the finished product (apong)\(^5, 6, 8\). Since, the crude filtrate of the brew constitutes the consumable part; the contamination by the harmful microbes is of great concern to the health and hygiene of the consumers. Moreover, involvement of a large number of plant species having medicinal values during preparation of starter is a time tested value of traditional knowledge system of the community\(^9, 10, 11\). Further, evaluation of the food value through analysis of its biochemical composition is an essential step towards the assessment of its commercial exploitation\(^12\). The present investigations is an attempt to document the scientific background of the preparation method of po:ro apong and major biochemical parameters of the finished product.

Methodology

The samples of ‘po:ro apong’ were collected under sterilized condition from local households of the Mising community of the district of Dibrugarh in upper Assam. The decanted product po:ro apong (consumable liquid) having suitable aroma, flavor and texture was also collected and used as sample for further analysis. Total carbohydrate content was estimated by Anthrone method\(^13\). A standard curve was prepared using D-glucose solution (0.1mg/ml) and the values were expressed in mg/ml po:ro apong. Crude protein content was estimated by Folin-Phenol method\(^14\). The reducing sugar by standard biochemical method using 3,5-di-nitrosalisylic acid (DNS) reagent with slight modification\(^15\). The total amino acid content was determined by standard biochemical method using ninhydrin reagent. A standard curve was prepared with suitably diluted Lysine (5mg/50ml) solution and result was expressed in mg/ml. \(pH\) was determined by Elico make digital \(pH\) meter. Titratable acidity was expressed as % of lactic acid of sample and was calculated by titrating it against 0.1N NaOH following standard method\(^16\). Opacity of the decanted product was expressed in terms of the optical density measured with the help spectrophotometer (Systronics-109). The total solid materials in the product mg/ml (dry) was determined by evaporating the liquid and other volatile compounds at 110\(^\circ\)C for 2 hrs. The alcohol content was determined sin distillate from po:ro apong using chromic acid and was expressed as % \(v/v\)\(^16\). The microbial load in the finished product was determined with the help of haemocytometer and was expressed in cfu/mm\(^3\) volume. The same, in starter (epop) and ripe mash (po:ro aruk), was determined by standard dilution plate technique and expressed as cfu/gm. The names of plant species used in preparation of starter culture were listed by interacting with the villager expert concern and by literature survey\(^17\). The values given are mean of 3 samples each in triplicate and ± SD (wherever necessary).

Results and discussion

Fermentation of starter and the po:ro apong

Most of the domestic fermentation of alcoholic rice beverages in Asian nations involves use of one or more plant ingredients to enrich and determine product quality of the drink\(^4, 18\). Generally, these plant ingredients which are believe to enrich the product quality are used in preparation of the epop. The present study suggests that various plant ingredients are used to fulfill different requirements for improving the finished product\(^19\). Some ingredients however are used as preservative\(^20\) while some others are used as antibacterial and flavouring agent\(^4, 10, 19\). Mishing tribe generally uses at least 16 different herbs with different interpretations. This number may vary from place to place within the tribe itself. The preparation of epop and the po:ro apong is somewhat similar with babud preparation reported from Phillipines\(^12\) and the Ipoh preparation by Adi tribe of Arunachal Pradesh, India\(^3\). The plant ingredients in the epop on one hand might be adding essential micronutrients and minerals\(^21\) to the finished product as well as enrichment of the nutrient medium for the useful microbial population while on the other hand may also act as antibacterial agents\(^3\). Table 1 shows the list of plant species commonly used by Mising tribe in fermentation of starter culture (epop). Notably most of them are reported to have medicinal values or antimicrobial properties\(^10, 11\). The use of plant species
(viz. Scoparia dulcis, Cyclosorus sp., Adhatoda zeylenica, Zanthoxylum hamiltonianum, Costus speciosus, Naravelia zeylanica, Melothrea heterophylla.) as essential ingredients during preparation of epop may add phenolics and other compounds which in turn might have role to play in changing microbial population during perpetuation of the process parameters since time immemorial7,8. Though such reports are still scanty but yet their equivocal effects cannot be nullified7,9. Secondly, the use of the burnt ash with cooked rice prior to the addition of starter is very unique to this particular beverage. It is assumed that this layer of ash around each rice grain help in controlling the activity of microorganisms during fermentation. However, further studies are needed to explain its technological basis.

Biochemical analysis of po:ro apong

The fermentation is a traditional process of bio enrichment of food with proteins, essential amino acids and vitamins and thus enhancing the nutritive value of the raw material by the microbial activity5,6. Table 2 shows the physico chemical properties and major nutritional composition of po:ro apong. The finished product is an alcoholic drink with attractive taste, texture and color maintaining an acidic range of pH= 4.06 ± 0.1 which is helpful in exclusion of bacterial populations8. The Titratable acidity was calculated to be 0.53 (% Lactic acid). The data also shows the major macronutrients in the po:ro apong. The presence of these macronutrients is quite useful in energy metabolism as it can meet energy requirement of the cell to a certain extent. The high level of free amino acids (2.43 ± 0.12 mg/ml) and also the reducing sugars (3.33 ± 0.2 mg/ml) along with the total protein (1.05 ± 0.06 mg/ml) and carbohydrate (46.62 ± 4.3 mg/ml) are found to be significant from the metabolic point of view. This finding is corroborating the fact of metabolic enrichment during the fermentation process by various
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In such composite fermentation, the synthesis of vitamins of microbial origin cannot be ruled out. The alcohol content in po:ro apong generally ranges between 7.52%-18.5% the upper limit of which is relatively higher as compared to other rice beer such as Japanese Sake (12%), Sikkim’s Kodo ko jaanr (4.8±1.90 %), Korean takju (7%) and Indonesian brem bali (6-14 %) and Sura (15%). The lower limit of alcohol content in saimod is usually variable and goes still lower depending on the filtration process. The amylases are the only detectable enzymes in the finished product (po:ro apong) which is expected to have produced by amylolytic microbes in the epop (Fig. 6). However, persistence of the activity of the enzyme in high alcohol content is not clear. The microbial load is reduced to 8,700 ± 6.332 cfu / mm$^3$ in the finished product (po:ro apong) as compared to 414x10$^7$ cfu /gm in the ripe mash and 549x10$^7$ cfu /gm in the epop (starter culture). The reduction of microbial load is due to the rising alcohol concentration towards the end of the fermentation process. Similar results were observed in natural and controlled fermentation of Kinema$^8$. The present study revealed that, though three major types of microbial populations viz. Molds, yeast and bacteria were initially observed and isolated from the epop, only two kinds of microbial population

Table 2—Biochemical analysis of saimod (Po:ro) apong.
The data represents the mean of three samples having more or less similar taste and texture

A. Physico-chemical properties
i) State : Liquid
ii) Colour : Dirty black to Yellowish/ reddish brown.
iii) Taste : Strong taste with mild fruity smell.
iv) Opacity : Almost clear
  Optical density : 0.036 ± 0.003 (after centrifugation)
  Optical density : 0.217 ± 0.011 (before centrifuge)
v) Total solid content : 0.492 ± 0.07 mg/ml
vi) pH : 4.06 ± 0.1
vii) Titratable acidity : 0.53% lactic acid
B. Nutrient composition
i) Total Carbohydrate : 46.62 ± 4.3mg/ml
ii) Reducing Sugar : 3.33 ± 0.2mg/ml
iii) Total Protein : 1.05 ± 0.06mg/ml
iv) Free amino acids : 2.43 ± 0.12mg/ml
v) Ethanol content : 7.52% - 18.5%
C. Enzymes
i) Proteases : Not detected
ii) Lipase : Not detected
iii) Amylase : 2.38 ± 0.06Uml$^{-1}$min$^{-1}$
D. Microbial load
i) Starter culture (epop) : 549x10$^7$ cfu /gm
ii) Ripe mash : 414x10$^7$ cfu /gm
iii) Finished product : 8,700± 6.332 cfu / mm$^3$
N.B. The data represents the mean of three observations ± SD. (as applicable).

Figs. (1—6): (1) E pop; (2) Fermented ripe mash; (3) The ripe mash is being put into the cone for filtration; (4) The conical apparatus made of bamboo is replaced by aluminium sheet; (5) Po:ro apong with its actual color and texture and (6) Amylolytic activity resolved with starch-iodine reaction
were finally detected in the finished product, viz. one low fermenting yeast (data not included in this paper) and other rod shaped amylolytic bacteria which indicates the batch by batch occurrence and elimination cycle of different micro-flora during fermentation process.\(^8\) The decrease in microbial load in the finished product is encouraging. However, the presence of the same may be risky for both consumers and the shelve life of the finished product\(^12\). In a previous study it was found that the presence of amyl alcohol (fusel) which might be produced by certain microbial fermentation causes unattractive odor of the finished product\(^7\). Hence, characterization and identification of the microbes present in the finished product is an essential step towards the standardization of the process parameter. Moreover, use of discarded part of \textit{po:ro aruk} as fodder for cattle is an example of optimum resource utilization.\(^4\).5.

Conclusion

The biochemical composition of \textit{po:ro apong} indicates that it is an alcoholic food rather than being simply an alcoholic beverage. The nutrients present in the consumable finished product can provide energy besides its soothing effect and other medical properties to the consumer. The unique preparation process involving the powdered ashes of burnt rice straw/husk may prove to be useful in developing new technology for sustainable maintenance of process parameters of the fermentation.

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