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Short Note

Development of Value Added Beverage From Under Utilized Millets

G. Gayathry and K. Jothilakshmi*
*Department of Food Science and Nutrition,
Home Science College and Research Institute, Madurai - 625 104

A value added alcoholic beverage was produced from under-utilized millets such as kodo millet and little millet by fermentation. The biochemical changes of alcoholic beverage was analysed at different time intervals from 0 h to 192 h. It was found that the Total Soluble Solids (TSS) content was 7.4% to 11.2% from 0 h to 192 h. The pH was recorded as 6.8 at 0 h and it declined to 4.1 at 96 h, and it reached 4.5 at 192 h. Total Titrable Acidity was in the range of 3.0 % to 5.87 %. Reducing sugar content was maximum at 96 h and reduced gradually from 6.42 % to 6.12 % in 144 h to 192 h respectively. The alcohol content was 10.9 % (V/V) at 192 h. The content of alcohol increased to 71 % from 48 h to 192 h. The prepared alcoholic beverage was organoleptically evaluated for flavour, pleasant or bitter taste, sweet or sourness on a five point hedonic scale and the developed product scored very good category with good flavor, sweet, less sour and pleasant taste. The present study proved that alcoholic beverage can be developed from millets by fermentation.

Key words: Alcoholic beverage, TSS, TTA, alcohol, fermentation

Alcoholic beverages can be prepared from different cereals such as rice, corn, sorghum, millet, barley, wheat and rye using mold and yeasts by fermentation (Hesseltine, 1991). In North-Eastern states of India, particularly in Sikkim an amylolytic fermented, alcoholic beverage called *marcha* is prepared traditionally by the local people (Tsuyoshi *et al*., 2005).

People in some parts of The Great Himalayas of India, prepare an alcoholic product called *kodo ko jaanr* using finger millet by wild fermentation (Thapa and Tamang, 2004). In the production of alcohol from starchy materials, the complex starch is converted into sugars by mold and further fermented by yeasts to produce alcohol. In most of the Western countries, fungal enzymes are used and in the far-East countries the saccharification of starch is accomplished with mold and yeast.

High quality sweetened alcoholic beverages can be produced from rice fermented for 36 to 48 h at 30ÚC using a combination of *Amylomyces rouxii* and *Endomycopsis fibuliger*. In the present study an attempt was made to develop alcoholic beverage from less utilized millets that were locally available. Alcoholic beverage production was standardized using little millet (*Panicum miliare*) and kodo millet (*Paspalum scrobiculatum*) by inoculating pure cultures of *Candida krusei* (1%), *Amylomyces rouxii* (0.5%) and *Rhizophus oryzae* (0.5%).

*Corresponding author email: gayasaro@yahoo.co.in

Materials and Methods

Processing of millets and development of alcoholic beverage

Samples of little millet and kodo millet were purchased from local grocery market at Madurai, Tamilnadu. The raw materials were sorted, sieved and cleaned to remove moldy discoloured grain and other extraneous matter. The dehusked millets were soaked for one hour, the excess water was drained off and sterilized at 120^0 C for 15 minutes in flat bottomed flasks. Then it was inoculated with *Amylomyces rouxii* (0.5%), *Rhizophus oryzae* (0.5%) and *Candida krusei* (1%) (Verma *et al*., 2000). The contents were mixed well and kept for fermentation for 7 days. After 7 days the contents were taken and squeezed using sterilized muslin cloth and filtered. Then it was pasteurized at 60^0C for one hour and stored in pasteurized bottle to obtain a sweetened alcoholic beverage.

Cultures for the development of alcoholic beverage from Millets

Mold cultures such as *Amylomyces rouxii* (SGK 2) and *Rhizophus oryzae* (PGJ 1) were isolated from traditional fermented foods namely ragi porridge and ragi kali respectively. The isolates were characterised microscopically and were deposited at Microbial Type Culture Collection (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh, India, with strain accession numbers of MTCC 6586 and MTCC 6584 respectively. Two molds such as *Amylomyces rouxii* (SGK 2) and *Rhizophus oryzae*

(PGJ 1) were grown separately in sterilised Potato dextrose broth. Two molds were taken from the potato dextrose broth and transferred to potato dextrose agar slants separately and incubated at 35°C for 3 days. Then the mycelium was taken from the surface of the agar slants and inoculated in 50 ml of a medium containing 1.0% glucose, 0.67% yeast extract, Calcium carbonate (1.25 g) and 0.5% casamino acids in a 100 ml Erlenmeyer flask and incubated for three days at 35°C. The mycelial mat obtained after three days of incubation were transferred to sterilised rice flour for the development of spore based inoculum of the two molds taken separately. The spore based inoculum obtained separately for the two mold *Amylomyces rouxii* (SGK 2) and *Rhizophus oryzae* (PGJ 1) were used as starters for beverage fermentation at the rate of 0.5% respectively. The standard yeast culture *Candida krusei* 2526 was obtained from MTCC and was mass multiplied in sterilised Malt Yeast Extract agar medium and it was used at the rate of 1% for the development of alcoholic beverage (Hesseltine *et al*., 1976)

Biochemical Characterization of alcoholic beverage from millets

Ten ml of beverage was taken and the pH was determined by digital pH meter calibrated with standard buffer solutions. Total Soluble Solids (TSS) was determined using Hand Refractometer. Total Titrable Acidity (TTA) was determined by titrating the beverage with 0.1 N sodium hydroxide to end point using phenolphthalein as indicator. The reducing sugar content was determined by dinitrosalicylic acid method (DNS) (Miller, 1972). Alcohol content of the prepared beverage was assessed at 48 h interval by using alcohol meter. The organoleptic evaluation was done using 5 point hedonic scale used especially for alcoholic foods and beverages (Shrestha *et al*., 2002).The scale and the scores or category is as follows.

1. No flavour, no taste – Poor
2. Less flavour, less taste – Average
3. Alcoholic flavour, bitter taste – Good
4. Good flavour, sweet, less sour, pleasant taste, less bitter – Very good
5. Very sweet, less sour, pleasant taste with good flavour, no bitterness - Excellent

All the determinations were done in triplicates and the mean values were recorded.

Statistical analysis

The results of the experiments were subjected to statistical scrutiny as per the methods detailed by Panse and Sukhatme (1985) using AGRES software package. Critical differences were worked out at 5% probability level and presented.

Results and Discussion

The results of the biochemical characteristics of the developed alcoholic beverage are presented in Table 1. It was recorded that the alcoholic beverage from the millets fermented by *Candida krusei* (1.0%) + *Amylomyces rouxii* (0.5%) + *Rhizophus oryzae* (0.5%) recorded pH of 6.8 at 0 h and it declined to 4.1 at 96 h, and it reached 4.5 at 192 h of fermentation. The acidic nature of the product may be due to production of organic acid, especially lactic acid by the molds. Several researchers has indicated the acidic nature of cereal based alcoholic beverages (Basappa, 2002 : Muyanja *et al*., 2003). It was noted that the TSS content was 7.4% to 11.2% from 0 h to 192 h.The increased TSS value is due to the continuous hydrolysis of starchy compounds present in millets. A research work carried out to study the biochemical characteristics of traditionally produced alcoholic beverage *lugari, Chaang, Aara, Chiang* of Himachal Pradesh has indicated that the TSS ranged from 14.58 ÚB to 18.56 ÚB (Kanwar *et al*., 2011). The results of the present study relating to TSS is in close proximity with those of the above mentioned research work. Total Titrable Acidity increased from 3.0 % to 5.87 % when the period of fermentation extended. Reducing sugar content was maximum at 96 h and reduced gradually from 6.42 % to 6.12 % in 144 h to 192 h respectively. It is clear from the investigation that the alcohol content increased to 71 % during fermentation from 48 h to 192 h and fungal cultures in combination with Candida yeast could yield higher amount of alcohol and a good quality alcoholic beverage. The alcoholic beverage was organoleptically accepted with the sensory evaluation score or category of very good. The results of this study demonstrate that

Table 1. Biochemical properties of alcoholic beverage at different intervals

Time (h)	pH	TSS(%)	TTA(%)	Reducing sugar (%)	Alcohol content% (V/V)
0	6.80	7.40	3.00	0.27	-
48	4.30	8.20	4.18	9.31	3.8
96	4.10	9.70	4.21	10.97	8.1
144	4.30	10.10	5.43	6.42	8.3
192	4.50	11.20	5.87	6.12	10.9
SED	0.0558	0.0474	0.0070	1.9005	1.7608
CD	0.1243	0.1057	0.0156	4.2347	3.1642

appreciable quantities of alcohol are produced during the fermentation of millets by the isolated cultures such as *A. rouxii* SGK 2 and *R. oryzae* PGJ 1 in combination with *Candida krusei*. Similar studies carried out by Cronk *et al*. (1979) has indicated that *A. rouxii* and *Endomycopsis fibuliger* produced alcohol of 72 mg/liter at 32 h and 558 mg/ liter at 192 h. Laopaiboon, (2009) has reported that alcoholic beverage can be produced from sorghum using wild yeasts with the alcohol content of 4.9 g $l^{-1}hr^{-1}$. Similarly the present experiment demonstrate the possibility of obtaining alcoholic beverage from fermentation substrates such as little millet and kodo millet which are underutilised in this fast food world. The present study also envisages the feasibility of using alternate substrates for alcohol production using composite cultures of mold and yeast.

Conclusion

The results of the present study demonstrate that appreciable quantities of pure alcohol can be produced from minor and major millets by amylolytic fermentation with mold and yeast. Direct fermentation of starch using molds offers an alternative to the conventional multistage process using commercial amylases for liquefaction and saccharification followed by fermentation with yeast. From this experiment it is evident that single step bioconversion of cooked millets into a alcoholic beverage is possible by coculturing of *Amylomyces rouxii*, *Rhizophus oryzae* and *Candida krusei.* Further in the fast food world, usage of millets are highly neglected and use of alternative substrates for alcoholic beverage production open for a wide area of nutritional research.

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