RESEARCH PAPER
J. Food Sci. Technol. Nepal, Vol. 6 (31-35), 2010
ISSN: 1816-0727

Solid Versus Semi-solid Fermentation of Finger Millet (*Eleusine coracana* L.)

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Effects of solid versus semi-solid fermentations on the chemical and organoleptic qualities of finger millet Jand were studied. Millet was fermented under solid and semi-solid states by using defined fermentation starter and the Jand was subjected to chemical and sensory analyses. Results indicated that except on moisture and alcohol contents, semi-solid fermentation reflected a significant effect (p<0.05) on the chemical characteristics of millet Jand. TSS, acidity and ester contents increased substantially in semi-solid fermentation as compared to solid-state one. Millet fermented with 50% water addition had more than 2-folds higher total acidity than that of solid-state fermented and every 50% increase in water addition nearly doubled the fixed acidity of the products. No remarkable improvement on the chemical and sensory quality of millet Jand was found by using semi-solid state fermentation.

Keywords: Semisolid fermentation, Defined starter, Millet *Jand*, Chemical and sensory quality

Introduction

Food fermentation is regarded as one of the oldest methods of food processing and preservation. More than anything else, man has known the use of microbes for the preparation of food products for thousands of years and all over the world a wide range of fermented foods and beverages contributed significantly to the diets of many people (Achi, 2005). Alcoholic beverages have played an important role in human spiritual and cultural life both in Eastern and Western societies. Unlike in Europe and Middle East, where indigenous alcoholic beverages are produced primarily from fruit, alcoholic beverages in the Asia-Pacific region are produced from cereals and serve as an important source of nutrients. Beverages vary from crystal clear (e.g. *Sake)* to turbid thick products e.g. *Takju* in Korea (Yoon, 1993). Indigenous alcoholic beverages not only add nutrients to the local diet, but also play an important part in the local custom. Traditional alcoholic beverages are offered to perform the religious practice to pray family God.

Jand is a Nepalese indigenous fermented beverage prepared by solid-state fermentation of starchy materials, particularly finger millet (*Eleusine coracana*), locally called as *Kodo* using *Murcha* (a traditional fermentation starter). It is one of the socially and culturally accepted mild alcoholic beverages and is presumably nutritionally superior to other alcoholic beverages, although its exact nutritional status is still unexplored. The term *Murcha* is a Nepali word and the different ethnic communities of the region call it by their own dialect such as *Khesung* by Limbu, *Bharama* by Tamang, *Bopkha* by Rai and *Buth/Thanbum* by *Lepcha* (Karki and Kharel, 2007). *Kodo ko Jand* is the most commonly fermented alcoholic beverages prepared from dry seeds of finger millet locally called as *Kodo* in the Eastern Himalayan regions of Darjeeling hills and Sikkim in India, Nepal and Bhutan. *Jand* is a common name for all alcoholic beverages in Nepal. In the oriental method of *Jand* making, the millet is cleaned, dehusked, winnowed, steeped in water and cooked. The cooked cereal is cooled and *Murcha* is added (1-2% by wt) followed by aerobic fermentation (biomass development) for 2-3 days and packed in earthen pot for alcoholic fermentation for at least 7 days (Thapa and Tamang, 2004).

Several traditional food fermentations e.g. *Nigeriam Ogi,* Japanese *Sake*, have been upgraded to high technology production system because of the strong research on traditional fermentation technology. But the production of indigenous fermented foods is still largely a traditional family art done in homes in a crude manner consequently the production has not increased substantially more than a cottage industry (Odufa, 1985).

Although the technology of *Jand* making has been practiced since antiquity, its production is still limited to home scale in Nepal. It has many shortcomings as a result of which the final product quality is the mercy of the *Murcha* and environmental conditions used during processing. It has a great potential of being commercialized, both in domestic and foreign markets, provided its quality (safety and nutritional value) is enhanced, which calls for a sound research and development work. Much literature is available on the traditional alcoholic fermentation of starchy raw materials under solid-state conditions using both traditional as well as defined fermentation starters (starters prepared by using pure microbial cultures isolated from traditional starters (Subba, 1985; Verma, 1991; Venkataramu and Basappa, 1993; Bhandari, 1997, Rajbhandari, 1999 and Upadhyaya, 2005) but information regarding semi-solid fermentation is scanty (Cai and Nip, 1990; Dung *et al.,* 2007). In Nepal, all traditional cereal-based alcoholic beverages are produced under solid-state fermentation and, hence this work was undertaken aimed at investigating the effect of semi-solid fermentation on the

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quality of finger millet *Jand* (millet beer) using defined fermentation starter prepared from *R. oryzae* and *S. cerevisiae*.

Materials and Methods

Materials: Finger millet (brown variety) was collected from local market of Dharan. Fermentation starter was prepared using yeast (*Saccharomyces cerevisiae*) and mold (*Rhizopus oryzae*) isolated from traditional starter (*Murcha*) in PDA supplemented with 100ppm of chloramphenicol. Yeast was grown in molasses broth of 6ºBx TSS for 3 days at 27 ºC and kept in refrigerator until used. Mold bran was prepared by inoculating isolated mold in sterile wheat bran, incubating for 5 days at 30 ºC, drying at 24 ºC for 12hr and then packed in sterile glass bottle. Yeast (from molasses broth) and mold (from mold bran) were added to sterile rice flour, thoroughly mixed with the addition of required amount of sterile distilled water and the dough was divided into small balls. The balls were flattened, placed in a sterile petri plate, incubated at 30 ºC for 3 days and dried at 40 ºC for 24hr.

Fermentation of finger millet: Finger millet (*Eleusine coracana)* (brown variety) was cleaned, dehusked, winnowed and washed with water. The millet was steeped in water for two hours, washed again, cooked for 20min at 121 ºC, and cooled to room temperature. Defined fermentation starter (prepared from rice using pure cultures of *S. cerevisiae* and *R. oryzae*) was mixed to the cooked millet at the rate of 1% (m/m), and kept for 2 days at 30ºC for bio-mass development (aerobic fermentation). For solid-state fermentation, the biomass developed millet was tightly packed into plastic containers whereas for semi-solid state fermentations, millet was filled into plastic containers and previously boiled and cooled water was added to the containers at the rate of 50% and 100% (v/m) for semi-solid$_1$ and semi-solid fermentations respectively. All containers were then capped tightly and kept at 28±1 ºC for 15 days for alcoholic fermentation.

Preparation of millet Jand: For the preparation of millet *Jand*, 1.5 parts (by vol) of previously boiled and cooled water was added to each part (by wt) of biomass developed millet used for alcoholic fermentation. In the case of semi-solid fermented millet, the volume of water added previously during the start of alcoholic fermentations was subtracted from that of total volume of water to be added. The mixture was gently macerated, allowed to stand for 20 min and strained through muslin cloth with gentle pressing. The *Jand* (millet beers) so obtained were subjected to chemical and organoleptic analyses.

Analytical procedure: Total soluble solids (TSS) was determined by using hand refractometer (0-32ºBx, Hanna Instrument, Portugal) and alcohol content was determined by visible spectrometric dichromate oxidation method (Metertek SP-870, Spectrophotometer, Metertech Inc., Taiwan) as described by Zoecklein *et al.,* (1997). The pH was determined using portable digital pH meter (Hanna Instrument, Portugal). Total, fixed and volatile acidities and ester contents were determined as per Kirk and Sawyer (1991). Reducing sugar was determined by Nelson-Somogyi method as per Sadasivam and Manickam (1996). Moisture content was determined by oven drying method according to Ranganna (1986).

Sensory evaluation: Sensory evaluation of the millet brew (*Jand)* was performed using Hedonic Rating Test as per Ranganna (1986). Ten semi-trained panelists were asked to evaluate the products in terms of color, taste and smell on a scale of nine-points ranging from "Dislike extremely" (1) to "Like extremely" (9).

Statistical analysis: The experiment was conducted in Complete Randomized Design (CRD) with three replications. The experimental data were analyzed using one-way ANOVA as per Buysse *et al*., (2007) at 5% level of significance and the means were compared by LSD method.

Results and Discussion

The effects of solid and semi-solid state fermentations using defined fermentation starter on the physico-chemical quality of millet *Jand* were analyzed and the results are shown in Table 1.

Table 1. Effect of semi-solid fermentation on the chemical characteristics of finger millet *Jand.*

Parameters	Values*		
	Solid state	Semi-solid$_1$	Semi-solid$_2$
Moisture (% m/v)	96.33 ^{a} (1.07)	96.21 ^{a} (0.18)	96.28 ^{a} (0.23)
Total soluble solids (ºBx)	3.27 ^{a} (0.06)	3.87 ^{b} (0.23)	4.03 ^{b} (0.06)
pH	4.17 ^{a} (0.12)	3.83 ^{b} (0.23)	3.37 ^{c} (0.12)
Total acidity, as lactic acid (% m/v)	0.33 ^{a} (0.01)	0.72 ^{b} (0.01)	1.24 ^{c} (0.01)
Fixed acidity, as lactic acid, (% m/v)	0.26 ^{a} (0.02)	0.50 ^{b} (0.001)	1.00 ^{c} (0.01)
Volatile acidity, as acetic acid (% m/v)	0.048 ^{a} (0.03)	0.143 ^{b} (0.001)	0.156 ^{b} (0.002)
Alcohol (% v/v)	6.20 ^{a} (0.72)	7.13 ^{a} (0.3)	6.91 ^{a} (0.17)
Reducing sugar as dextrose, % m/m	1582 ^{a} (128.10)	71 ^{b} (6.50)	69 ^{b} (5.10)
Esters, as ethyl acetate (mg/100 ml)	10.60 ^{a} (0.90)	13.30 ^{b} (0.70)	18.50 ^{c} (0.40)

* Means followed by different superscripts in a row are significantly different ($p<0.05$) by LSD. Figures in the parentheses are standard deviations. Semi-solid$_1$ and Semi-solid$_2$ are the semi-solid state fermentations using 50% and 100 % water addition respectively to biomass developed millet during alcoholic fermentation.

Semi-solid state fermentation showed significant effects ($p<0.05$) on the total soluble solids (TSS), pH, acidities, ester and reducing sugar contents of the finger millet *Jand* (millet brews) whereas alcohol and moisture contents were not affected. The average moisture contents of millet *Jand* prepared by solid- and semi-solid state fermentations lied in the range of 96.21 to 96.33% (m/v) and the values were not significantly different ($p>0.05$) from each other. TSS values were 3.27, 3.87 and 4.03 ºBx for solid state, semi-solid$_1$ and semi-solid$_2$ fermented *Jand* respectively. Semi-solid fermentations incurred significantly higher TSS compared to control (solid state); however, the values between semi-solid fermentations were not different. The pH values decreased with increasing water addition and the values were significantly different from one another. Semi-solid fermentations significantly increased ($p<0.05$) the total acidity contents of millet brews compared to control. Millet fermented with 50% water addition had more than 2-fold total acidity than that of solid-state fermented one. Similarly, increasing water level increased the fixed acidity of millet *Jand*. Furthermore, every 50% increase in water addition nearly doubled the fixed acidity in the millet brew. The volatile acidity of millet *Jand* prepared by semi-solid state fermentations were significantly higher compared to control, whereas the values between semi-solid fermentations did not differ significantly. From Table 1, it can be envisaged that semi-solid fermentation had a profound effect on volatile acidity than those of total and fixed acidities.

The alcohol contents in the *Jand* prepared from solid, semi-solid$_1$ and semi- solid$_2$ fermentations were 6.2, 7.13 and 6.91% (v/v) respectively, but the values were not statistically different. Reducing sugar contents decreased with increase in water addition during alcoholic fermentation. Solid-state fermented *Jand* contained higher reducing sugar than those of semi solid$_1$ and semi solid$_2$ fermented ones, however, the values between the later two were not significantly different. The lower reducing sugar contents in semi-solid fermentation could not be explained owing to the lack of relevant information. The ester contents were 10.6, 13.3 and 18.5 mg/ 100 ml *Jand* prepared by solid, semi solid$_1$ and semi solid$_2$ fermentations respectively and the values were significantly different from each other.

Due to the lack of relevant information, the results of this study could not be compared. However, similar result of alcohol content (6.8% v/v) but higher values pH and total acidity were reported by Subba, (1985) in traditionally prepared millet *Jand*. According to Mongar (2001), the pH, total acidity (%m/v as lactic acid), reducing sugar (% m/v as dextrose) and alcohol (% v/v) contents in millet *Jand* fermented by using *Murcha* were 4.5, 0.6, 1.52 and 4.03 respectively. The pH and reducing sugar contents seemed comparable, but the acidity was quite high and alcohol content was low than that of solid-state fermented millet samples of this study. Osti (2004) found similar alcohol content (6.68% v/v) in millet *Jand* fermented by using starter made from *Rhizopus* and *Saccharomyces* spp. Cai and Nip (1990) reported analogous effect of semisolid state fermentation on glucose in taro fermentation using starter containing R. *tankinensis*, *R. oryzae*, *R. chinensis* and *S. cerevisiae*, but contrary to our finding, they reported a significantly higher yield of alcohol in semisolid fermentation than that of solid state fermentation. Similar results of pH, and total acidity, but a lower value of alcohol (4.8%, v/v) in traditionally prepared *Jand* was also reported by Thapa and Tamang (2004).

Sensory evaluation of millet Jand: Millet *Jand* prepared by solid and semi-solid state [50% water addition, (semi-solid$_1$) and 100% water addition (semi-solid$_2$)] fermentations were subjected to sensory evaluation in terms of color, taste and smell using nine points hedonic method and the results are depicted in Figure 1. Statistical analysis revealed that semi-solid fermentation had a significant effect on the organoletpic properties of the millet *Jand*. Mean sensory scores for taste were found to be 8.4, 7.2 and 6.2 for solid, semi-solid$_1$ and semi- solid$_2$ fermented samples respectively. Increasing water level decreased the taste preference and the scores were statistically different from each other. Higher acidity seemed to have a negative effect on the taste preference. The taste preference of the *Jand* prepared by solid, semi-solid$_1$ and semi-solid$_2$ fermentations were rated as "Liked very much", "Liked moderately" and "Liked slightly" by the panelists.

The average color preference scores were 8.4, 7.4 and 7.2 for *Jand* prepared by solid, semi-solid$_1$ and semi-solid$_2$ fermentations respectively. Solid state fermentation produced significantly better colored *Jand* compared to semi-solid ones while the mean color scores between semi-solid fermented samples were not significantly different. Millet *Jand* prepared by solid state fermentation was "Liked very much" whilst those prepared by semi-solid fermentation were "liked moderately" by the panelists based on color preference. The mean scores for smell were 8.2, 7.7 and 6.8 for *Jand* prepared by solid, semi-solid$_1$ and semi-solid$_2$ respectively. The smell scores between solid and semi-solid$_1$ did not differ but those between solid and semi-solid2 and between semi-solid$_1$ and semi-solid$_2$ were significantly different. Although esters are responsible for the smell of the alcoholic beverages, higher contents are thought to be detrimental to the smell. Hence, low smell score for semi-solid$_2$ fermented product could be due to the presence of higher ester content (Table 1).

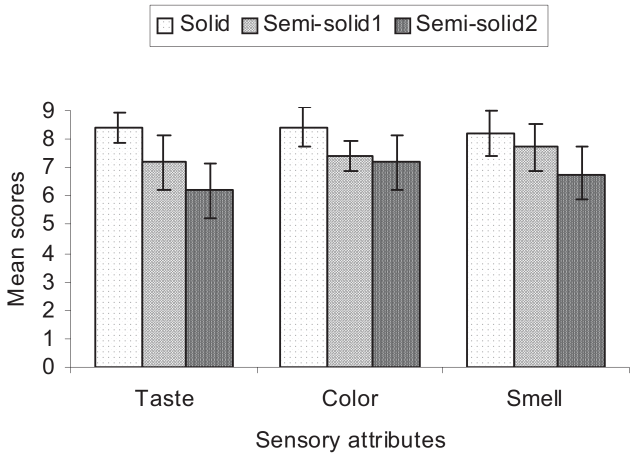

Figure 1. Effect of semi-solid state fermentation on the sensory quality of millet *Jand*

*Means ± SD, bars having different letters for any quality attributes are significantly different ($p<0.05$). Semi-solid$_1$ and Semi-solid$_2$ are the semi-solid state fermentations using 50% and 100 % water addition respectively to biomass developed millet during alcoholic fermentation.

Semi-solid state fermentations unacceptably increased the acidity resulting in substantial lowering in the taste, color and smell preference of the millet beer over the control (solid state fermentation). Alcohol content, one of the most important parameters of alcoholic beverages, was not significantly improved by semi-solid fermentations of finger millet. Moreover, semi-solid fermentation incurred a substantial increase in the ester content in the final product, which was beyond the acceptable limit as judged by the panelists. Hence, solid state fermentation could be regarded as the best method of finger millet fermentation than that of semi-solid fermentation.

Conclusion

Except moisture and alcohol contents, semi-solid fermentation had a significant effect on the chemical and organoleptic qualities of millet *Jand*. Substantial increase in TSS; total, fixed and volatile acidities, and ester contents occurred in semi-solid fermentation compared to solid state. Taste, color and smell of the product were largely impaired by semi-solid fermentation with profound impact on taste. No remarkable improvement on the overall quality of the finger millet *Jand* was found using semi-solid fermentation over solid state one.

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