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Optimization of fermentation parameters for production of malted and alcoholic beverage from Kodo and little millet

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ABSTRACT

Three worts from combinations (50:75, 50:50 and 75:50) of Kodo millet, *Paspalum scrobiculatum* and little millet, *Panicum sumatrense* were taken to study the effect of the combination of two millets on the quality of the beverage. Worts were analysed and fermented with *Saccharomyces cerevisiae*. Optimization of the parameters for fermentation (inoculum level and fermentation period) was carried out for good quality beverage production from the combination of both the millets. Among the inoculum levels used (0.06, 0.08 and 0.1%) the pH and residual reducing sugars of beverage were found to decrease up to 0.1 per cent inoculum. The alcohol content increased as inoculum level increased up to 0.1 per cent. Similarly in the case of different fermentation periods (5, 7 and 10 days), decrease in the pH and residual reducing sugars was observed up to tenth day and alcohol content increased significantly. Among the three combinations little/Kodo millet (50:50) was found to be best in all aspects when tested organoleptically.

Keywords: Millet; wort; yeast; beverage; fermentation

INTRODUCTION

Kodo millet, *Paspalum scrobiculatum* is a perennial cereal that is considered a minor grain of southcentral India. In India it is grown mostly in Andhra Pradesh, Maharashtra, Karnataka, Tamil Nadu and Uttar Pradesh. The grain is covered with a horny seed coat of brick red colour which needs to be removed before cooking. It is rich in glutamic acid, alanine, leucine and serine but deficient in lysine. In comparison to finger, barnyard and pearl millet, Kodo millet has the highest free radical quenching potential indicating possible useful antioxidant activity (Hedge et al 2005).

Little millet, *Panicum sumatrense* is one of the important minor millets indigenous to Indian subcontinent (Nirmalakumari et al 2010). It is grown extensively in the tropics and is a staple food for the low income group in some

countries of the world. It is the food and fodder crop grown on a limited scale as poor man's crop capable of withstanding both drought and water logging. The nutritional value of little millet makes it the preferred food for those who sustain themselves for the whole day on the single morning meal. It is grown throughout India but has received very little attention from plant breeders.

Malts can be produced from any cereal grain or millet however the most researched and used cereal grain is barley. Among the tropical cereals the most investigated grain is sorghum and has resulted in the research assessment of the full potential of brewing with sorghum. However several other grains including wheat, rye, triticale and oats continue to be malted commercially for both traditional and practical reasons but no detailed studies have been carried out on millets. Indeed research studies have shown that millet could be used in brewing European type lager beer. Other studies have suggested that millet malt wort filtered faster than sorghum malt wort and produced beers that had better foam properties than beers brewed from sorghum malt . Moir (1992) attributed beer quality to colour, clarity, foam, appearance and flavour. Comparative studies of barley, sorghum and millet showed that beer brewed from millet malt met these qualities. In the present study the fermentation parameters for the production of malted and alcoholic beverage from Kodo and little millet grains were optimized.

MATERIAL and METHODS

The fermentation parameters were optimized using the standardised wort prepared from the combination of Kodo and little millet grains to get healthy fermentative food product. The standardisation of malting and mashing of Kodo and little millet grains had already been done in Department of Food Science and Technology, University of Pondicherry (Sethi 2013). The standardised wort obtained from Department of Food Science and Technology, University of Pondicherry was boiled for 1 hour; after 30 minutes of boiling, sugar was added as an adjunct to make up the brix to 20°. The wort was cooled, filtered and pH was adjusted to 4.5-5.0. After that wort was pitched with yeast, *Saccharomyces cerevisiae* and the prepared alcoholic beverage was pasteurized at 65°C, cooled and then stored.

The reducing sugars were estimated by 3, 5-dinitrosalicylic acid method (Miller 1959). The pH of samples was recorded by using the pH meter equipped with an electrode (Model 1595414, Cyber Scan, Eutech instruments, Netherlands). Standard solutions of pH 4.0 and 9.0 were used as reference. The total soluble solids of the samples were measured by using hand refractometer (ERMA Inc REF-106b, Tokyo, Japan) and the results were expressed in °Brix. Alcohol content was estimated by Pycnometer method (Anon 2012). Visual color was

measured using a Hunter colorimeter (ColorFlex-CX 2748, Hunter Associates Laboratory, Reston, VA) in terms of L (lightness), a^* (redness and greenness) and b^* (yellowness and blueness). The instrument was calibrated with a standard black and white tile followed by measurement of samples. Five point hedonic scale (1- Dislike extremely; 2- Like moderately; 3- Like much; 4- Like very much; 5- Like extremely) was selected which was based mainly on the physical characteristics and taste (Mohanty et al 2006). The organoleptic properties of the prepared samples were evaluated by a panel of ten judges who were acquainted with beer and similar beverages either occasionally or frequently.

RESULTS and DISCUSSION

The pH of the wort prepared from three different combinations was found to be similar. The TSS of the wort made from the various combinations differed significantly as indicated in Table 1. The TSS of the wort made from the combination little/ Kodo (75:25) was found to have highest value of 11.83°Brix. This could be due to the release of more sugars from the combination little/Kodo millet (75:25) during mashing.

A significant difference was found in the values of reducing sugars of the worts prepared from different combinations. The highest amount of reducing sugars ie 47.63 mg/g was obtained from the wort prepared from the combination of little/Kodo millet (75:25). The high sugar content can be the result of high degradation efficiency of endogenous enzymes as well as release of sugars during boiling of worts.

pH was found to decrease with increase in the inoculum level in the study. The lowest pH (4.26) was recorded in the beverage treated with 0.1 per cent inoculum level while the highest pH (4.35) was recorded at 0.06 per cent inoculum level in all the combinations. The decrease in pH with increase in inoculum level could be due to the depletion in the reducing sugars and the production of organic acids during the early stages of fermentation. The lowest (4.22) pH was recorded in the combination little/Kodo millet (75:25) and highest (4.31) in the combination little/Kodo millet (25:75) at 0.1 per cent alcohol content. There was an increase in the alcohol content of beverage with increase in inoculum level. Maximum alcohol (3.0%) was obtained at an inoculum level of 0.1 per cent. Similar work was done by Okafor and Aniche (1987) who used 1 ml of yeast suspension containing 2.25 mg of yeast, *S uvarum* for pitching 12 litres of wort prepared from sorghum and recorded an alcohol content of 3.09 per cent. Similarly Agu and Obanu (1991) produced beer from Nigerian millet using yeast, *S uvarum* at 3 g fresh weight of yeast as inoculum per litre of wort and obtained an alcohol content of 2.39 per cent. Among the three combinations little/ Kodo millet (75:25) recorded the highest alcohol content (3.41%) at an inoculum level

Table 1. Chemical analysis of wort prepared from Kodo and little millet for beverage production

Millet combination	pH	TSS (°Brix)	Reducing sugar (mg/g)
Little/Kodo (75:25)	6.0 ± 0.01[a]	11.83±0.76[a]	47.63 ± 0.72[a]
Little/Kodo (50:50)	6.01 ± 0.01[a]	10.66± 0.57[b]	45.45 ± 0.94[b]
Little/Kodo (25:75)	6.02 ± 0.00[a]	9.40±0.36[c]	41.18 ± 0.28[c]

The values represented are mean ± SD
Mean values in the same column not sharing the same superscript differ significantly at $p < 0.05$

of 0.1 per cent. With increase in inoculum level residual reducing sugars decreased significantly. The inoculum level of 0.06 per cent recorded the highest residual reducing sugars (8.93 mg/g) while the least residual reducing sugars (7.97mg/g) were recorded in beverage pitched with 0.1 per cent inoculum level and in the combination little/ Kodo millet (75:25). This difference may be due to the initial sugar concentration of wort, efficiency of fermentation and the capacity of yeast strains for sugar uptake which might be maximum at higher yeast population.

It was observed that the pH was notably influenced by fermentation period (Table 3). Highest pH (3.40) was obtained at fifth day of fermentation that decreased gradually up to tenth day. The lowest pH (3.35) was obtained on tenth day. The decrease in pH along with fermentation could be due to the efflux of H+ ions as a byproduct of the transport system and by organic acid production. Results are also in agreement with those of Okafor and Aniche (1987) who obtained a pH 5.0 at the first day of fermentation which decreased further and recorded a final pH 4.0 on the fifth day of fermentation. Among the different combinations little/Kodo millet how to write (25:75) showed the highest pH (3.42) on 10th day of fermentation. The highest alcohol content (4.50%) was observed in beverage fermented for 10 days in all three combinations of two millets which was at par with the 7th day of fermentation (4.48%). This can be attributed to the fact that longer fermentation can further deplete glucose, maltose and fermentable carbohydrates increasing alcohol content up to certain extent beyond which there is no significant difference as sugars get exhausted.

Okafor and Aniche (1987) reported that alcohol content of sorghum wort increased progressively from day one to fifth day of fermentation and the final beer gave maximum alcohol content of 3.09 per cent on the fifth day of fermentation. Among the different combinations little/Kodo millet (75:25) gave the highest alcohol content (5.09%) on 10th day of fermentation. The highest residual reducing sugars were

Table 2. Effect of inoculum level on alcohol content, pH and residual reducing sugar

Millet combination	Amount of yeast (%)								
	0.06			0.08			0.1		
	Alcohol content (%)	pH	Residual reducing sugar (mg/g)	Alcohol content (%)	pH	Residual reducing sugar (mg/g)	Alcohol content (%)	pH	Residual reducing sugar (mg/g)
Little/Kodo (75:25)	2.63	4.31	8.44	3.11	4.26	7.99	3.41	4.22	7.54
Little/Kodo (50:50)	2.28	4.35	8.89	2.64	4.32	8.41	2.98	4.26	7.92
Little/Kodo (25:75)	2.0	4.39	9.45	2.31	4.36	8.94	2.63	4.31	8.45
Mean	2.30	4.35	8.93	2.68	4.31	8.44	3.00	4.26	7.97

Values are means of three observations

Table 3. Effect of fermentation period on alcohol content, pH and residual reducing sugar

Millet combination	# days of fermentation at 0.1%								
	5			7			10		
	Alcohol content (%)	pH	Residual reducing sugar (mg/g)	Alcohol content (%)	pH	Residual reducing sugar (mg/g)	Alcohol content (%)	pH	Residual reducing sugar (mg/g)
Little/Kodo (75:25)	4.42	3.35	5.21	5.05	3.32	4.78	5.09	3.30	4.77
Little/Kodo (50:50)	3.86	3.38	5.57	4.44	3.35	5.11	4.47	3.33	5.09
Little/Kodo (25:75)	3.40	3.48	5.99	3.95	3.44	5.45	3.96	3.42	5.42
Mean	3.89	3.40	5.59	4.48	3.37	5.11	4.50	3.35	5.09

Values are the means of three observations

obtained on fifth day (5.59 mg/g). The least residual reducing sugars were recorded on the tenth day of fermentation (5.09 mg/g) which did not show any significant difference with seventh day (5.11 mg/g). The difference could be due to the initial concentration of sugars in the wort, sugar utilizing efficiency of yeast strains and the conversion of reducing sugars to alcohol with time. Goode and Arendt (2003) obtained reducing sugars of 0.166 g/100 ml from beer made from grist containing unmalted sorghum and malted barley. Difference was also reported among the different combinations with regard to reducing sugars. Least amount of residual reducing sugars (4.77 mg/g) was obtained from beverage fermented with combination little/Kodo millet (75:25) on the 10th day of fermentation.

Organoleptic evaluation results revealed that among three samples, sample C was more preferred and its overall acceptability was high as compared to other samples (Table 4). Sample C got highest scores in attributes like appearance, colour, flavour and mouth-feel. Sample A got low scores in appearance and colour as the beverage was too light in colour. Chemical analysis was done for the beverage which was most liked by the sensory panelists ie sample C which was made from the combination of Kodo and little millet in the ratio of 50:50. Results of the experiment are presented in Table 5.

The reduction in pH, TSS, residual reducing sugars and increase in alcohol content during fermentation showed good fermentative potential of Kodo and little millets. Colour values of wort and selected beverage (little/Kodo millet 50:50) was analysed by ColorFlex instrument and results are given in Table 6. Beverage was found to be of lighter colour than wort.

CONCLUSION

The quality of a good alcoholic beverage depends on the extent to which sugars are utilized by the yeast and hence the condition of a complete fermentation is of prime importance. Also a good quality alcoholic beverage depends on the amount of alcohol present and this results in a longer shelf-life. Reducing sugars are prime component of fermentation after consumption of which alcohol is produced. Some non-utilized sugars remain as residual reducing sugars in beverage which in high amounts decrease market quality of alcoholic beverage. In the present study best beverage was obtained at the inoculum level of 0.1 per cent as maximum alcohol content was found at this level for all three combinations of two millets and fermentation period of 7 days was found to be optimum as no significant differences were found in alcohol content, pH and residual reducing sugars between 7th and 10th day of fermentation. Among all the three combinations little/Kodo millet (50:50) was found best in all aspects when

Table 4. Sensory attributes of Kodo and little millet malted and alcoholic beverage

Sensory attribute	Sample A	Sample B	Sample C
Appearance	4.0 ± 0.74[a]	4.1 ± 0.66[a]	4.7 ± 0.48[b]
Colour	3.2 ± 0.63[a]	4.2 ± 0.78[b]	4.5 ± 0.53[c]
Aroma	4.1 ± 0.56[a]	3.7 ± 0.48[b]	4.2 ± 0.42[a]
Acidic taste	4.8 ± 0.42[a]	4.8 ± 0.42[a]	4.8 ± 0.42[a]
Mouth feel	3.9 ± 0.74[a]	3.5 ± 0.53[b]	4.7 ± 0.48[c]
Flavour	4.0 ± 0.81[a]	3.4 ± 0.52[b]	4.6 ± 0.52[c]
Astringency	2.9 ± 0.66[a]	3.1 ± 0.67[a]	3.0 ± 0.56[a]
General quality	3.8 ± 0.78[a]	3.3 ± 0.48[b]	4.6 ± 0.52[c]
Total score	30.7	30.1	35.0

Values are the mean ± SD of actual panellists' scores (n=10), Mean values in the same row not sharing the same superscript are significantly different at $p < 0.05$.
Sample A= Little/Kodo millet (75:50), Sample B= Little/Kodo millet (25:75), Sample C: Little/Kodo millet (50:50)

Table 5. Chemical parameters of malted and alcoholic beverage, little/Kodo millet (50:50)

Parameter	Value
pH	3.33 ± 0.01
TSS (°Brix)	8.50 ± 0.50
Residual reducing sugar (mg/g)	5.04 ± 0.01
Alcohol content (%)	4.56 ± 0.07

Table 6. Colour values for wort and beverage of little/Kodo millet (50:50)

Sample	L	a*	b*
Wort	3.97	0.22	-0.91
Beverage	3.99	0.33	-1.26

tested organoleptically. Also the selected combination ie 50:50 when tested was found to have appreciable amount of alcohol content ie 4.56 per cent. Thus from the present study it can be concluded that Kodo and little millets can be used for preparation of malted and alcoholic beverage production.

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