

Quality of Rice Wine as Affected by Rice Polishing Ratio

L.N. Kadawatha Arachchige, C.K. Illeperuma¹, L.B. Wewegama¹ and
M.H.W. Gunawardhane²

Postgraduate Institute of Agriculture
University of Peradeniya
Peradeniya, Sri Lanka

ABSTRACT. *The effect of rice polishing ratio on rice wine quality was studied using the local cultivar Bg 358 polished at 90, 80, 70 and 60%. Proximate composition of rice of different polishing ratios altered depending on the degree of polishing. Higher rate of fermentation and higher number of volatile constituents were found in wine made using rice polished at 60 and 70% than those compared to 80 and 90%. The average ethanol content of rice wine of different polishing ratios varied from 12.8 to 13.5% at ambient conditions (temperature of $28.5 \pm 0.5^{\circ}\text{C}$ and relative humidity of $71.6 \pm 8\%$). Degree of saccharification of rice starch by koji (fungal culture) was found to be high with increasing polishing ratio as noted in the presence of higher residual sugar level in wine of polishing ratio at 60 and 70% than at 80 and 90%. Thus, the degree of rice polishing has influenced the quality of rice wine.*

INTRODUCTION

Rice wine has achieved a unique position among alcoholic beverages due to its characteristic flavor and conspicuous brewing process compared to fruit wines (Steinkraus, 1996). The brewing involves saccharification of rice starch by koji, the fungal mycelia of *Aspergillus oryzae* grown on rice and simultaneous alcoholic fermentation by yeasts. The enzymes produced by *A. oryzae* such as α -amylases and glucoamylases saccharify the rice starch into fermentable sugars (Anto *et al.*, 2005; Yoshizawa, 1982), which in turn are utilized by yeast.

Quality of the raw materials used in rice wine production determines the quality of rice wine. Rice being the principle raw material would have a greater impact on rice wine quality. Presence of excessive levels of fat, protein and minerals, predominantly present in the bran and germ of the rice grain, would result in over growth of koji mould, thereby lowering the liquor quality (Japan International Corporation, 2002). Moreover, phytates (inositol hexaphosphate) present in rice grains would hinder the synthesis of flavor enhancing substances by yeasts (Furukawa *et al.*, 2003). Therefore, rice is polished to remove such unnecessary constituents prior to brewing (Furukawa *et al.*, 2003). In Japan a range of rice wine types are produced using Japonica varieties with different polishing ratios.

1 Department of Food Science and Technology, Faculty of Agriculture, University of Peradeniya, Sri Lanka.

2 Food Research Unit, Department of Agriculture, Peradeniya, Sri Lanka.

Ordinary type and 'daiginjo' type rice wines are produced using rice grains polished at 70 and 40-50%, respectively (Thu, 2003). The objective of this study was to find out the effect of polishing ratio of an Indica rice variety, Bg 358, on quality of rice wine.

MATERIALS AND METHODS

Procurement of rice samples

Rice samples of the local cultivar Bg 358 was obtained from the Rice Research and Development Institute (RRDI), Bathalagoda, Sri Lanka. The samples were polished at 90, 80, 70 and 60% separately (10, 20, 30 and 40% of the brand was removed, respectively) using a testing mill (Satake grain testing mill, model 553328, Hiroshima) at the Institute of Post Harvest Technology, Anuradhapura, Sri Lanka.

Analysis of proximate constituents

Moisture, ash, crude protein and crude fat of rice samples were determined according to the standard methods (AOAC, 1995).

Preparation of yeast starter

A pure culture of *Saccharomyces cerevisiae* isolated from naturally fermenting coconut sap (Wellala *et al.*, 2004) was used for rice wine production. The isolate was maintained on PDA (Oxoid) slants at 11^oC. The culture medium was prepared in an Erlenmeyer flask, containing 100 ml of 0.1 M Potassium dihydrogen orthophosphate (BDH Laboratories); glucose (BDH Laboratories) 5 g; peptone (Difco) 1 g; and yeast extract (Difco) 1 g. Few drops of 0.1 M HCl (Merck) were used to adjust the final pH to 4.5. The culture medium was autoclaved (Hirayama, HV-50, Japan) at 121^oC for 15 min. A loopfull of yeast cells from the PDA slants was inoculated in the above medium and incubated at room temperature (28.5±0.5^oC) on an orbital shaker (SO1, Bibby Sterlin, UK) at 100 rpm to obtain about 1 x 10⁷ cells/ml. Cell counts were determined using a Haemocytometer (Superior, West Germany).

Preparation of tane-koji and koji

An indigenous strain of *A. oryzae* isolated from bread (Sooriyamoorthy *et al.*, 2004) was used to prepare tane-koji (Yoshizawa, 1982). The spores of pure culture of indigenous *A. oryzae* were sprinkled on rice, which was washed, soaked, steamed for 1h and cooled to about 30-35^oC. The mixed rice was then incubated (Yamato, IC 600, Japan) at 35^oC for 5-6 days for abundant spore formation. The incubated rice was ground, packaged in low-density polyethylene pouches and stored at -18^oC. In the preparation of koji, tane-koji (1 g) was sprinkled on steamed rice (Bg 358 polished at 90, 80, 70 and 60%), which had been prepared as for tane-koji and incubated at 35^oC for two days. During incubation, the heap of rice grains was mixed after 24 h. On the second day of incubation, grains looked like white frost with uniform growth of the fungal mycelia were packaged in airtight plastic boxes and stored at -18^oC.

Koji driven fermentation of rice

Batch fermentation was carried out using Bg 358 polished at 90, 80, 70 and 60% as the treatments in a completely randomized design with triplicate. Spring water obtained from the Kandy District was mixed with potassium metabisulfite to make a 100 ppm solution. Steamed rice, koji, spring water and the yeast starter were added in five successive additions every other day as reported by Yoshizawa (1982). The samples were allowed to ferment in ambient conditions (temperature of $28.5 \pm 0.5^\circ\text{C}$ and relative humidity of $71.6 \pm 1.8\%$) until a constant weight was reached. At the end of fermentation, the content was filtered through cheesecloth followed by a second filtration through Whatman No 1 filter papers using a Buchner funnel. The filtrate was filled into pre-sterilized bottles, loosely capped and pasteurized in a water bath at 65°C (Kodama *et al.*, 2002) for 20 min. The wine samples were analyzed for pH, TSS, total sugars and ethanol content and headspace constituents as given below.

Rate of fermentation

Rate of ethanol production (g/100 g of rice) in each day was used as a criterion for the determination of fermentation rates of the wine samples made using rice polished at 90, 80, 70 and 60%. The ethanol content was calculated by:

$$P = W (M_E/M_C)$$

where P is the concentration of ethanol (g/100 g of rice), W is the drop in weight due to the loss of carbon dioxide (g) and M_E and M_C are the molecular weights of ethanol and carbon dioxide respectively (Tabera *et al.*, 1985).

Analysis of physico-chemical properties of rice wine

The wine samples made using rice polished at four polishing ratios were analyzed in triplicate for pH using a pH meter (IM - 40S TOA Electronics, Japan) and total soluble solids (TSS) using a refractometer (Atago N-1E, Japan). Ethanol content was measured in triplicate according to the standard gas chromatographic method described in AOAC (1995) as follows. Isopropanol solution (0.2% v/v) was used as the internal standard. An alcohol standard solution (10% v/v) was diluted with the internal standard solution (1:100). Similarly, wine samples were also diluted with the internal standard solution (1:100). An aliquot of 2 μl of each diluted sample was injected to gas chromatograph (Shimadzu GC-14B, Tokyo, Japan) equipped with a flame ionization detector. The column (2 m x 5 mm x 2.6 mm) used was a Carbopack BAW (Sigma-Aldrich, USA). The injector and detector temperature was held at 150°C . Oven temperature was maintained at 105°C . Flow rate of the Nitrogen carrier gas was maintained at 15 ml/min. The response ratio (RR) of alcohol peak area: Isopropanol peak area of each sample was determined and the ethanol content was calculated by: % Alcohol = $(RR^1 \times 10)/RR^0$ where RR^0 is the response ratio of diluted alcohol standard solution and RR^1 is the response ratio of the diluted wine sample.

Total residual sugars were determined in triplicate by the Anthrone test (Khoshkhoo *et al.*, 1994) as follows. Anthrone reagent was prepared by adding 76 ml of conc. Sulphuric acid and 150 mg of Anthrone powder into 30 ml of distilled water. Wine sample was diluted 1:10 times with distilled water. An aliquot of 10 μl of the diluted sample was then mixed with 90 μl of distilled water followed by 3 ml of Anthrone reagent and

heated for 10 min. at 80°C. The solution was then cooled to room temperature and the absorbance was measured using a spectrophotometer (A1075, Shimadzu Corporation, Kyoto, Japan) at 620 nm.

The volatile composition of the samples was determined in triplicate using headspace gas chromatography (Akiyama *et al.*, 1978). Glass vials with self-sealing septa containing 5 ml rice wine were allowed to stand at 50°C for 30 min. in a water bath. Headspace gas (3 ml) was withdrawn using a gas tight syringe and injected into the Gas Chromatograph (Shimadzu model GC - 14B) equipped with a flame ionization detector and Carbopack BAW column. Flow rate of the Nitrogen carrier gas was maintained at 40 ml/min. Oven temperature was maintained at 105°C. Both injector and detector temperatures were 105°C.

Statistical analysis

The results of pH, TSS, alcohol content and residual sugar level of the wine samples made using rice polished at four different polishing ratios were analyzed using the SAS: 6.12 statistical software by the SAS-ANOVA procedure and mean comparison was done by the SAS-Least significant difference (LSD) procedure ($p < 0.05$). Results of volatile composition of wine headspace, proximate constituents and rate of ethanol production during fermentation were expressed as mean value \pm SD using the Microsoft Excel Data Analysis.

RESULTS AND DISCUSSION

Proximate composition

Proximate constituents of the rice polished at 90, 80, 70 and 60% are given in Table 1. Ash, protein and fat contents decreased with increase in polishing ratios, which is in agreement with the findings reported by Kodama and Yoshizawa (1977). When degree of polishing increased from 90 to 60%, the ash, protein and fat contents approximately reduced by 77, 32 and 69%, respectively. However, the ash, protein and fat contents of 70 and 60% polished rice were similar and were somewhat close to the proximate constituents of the ordinary brewing rice where ash, protein and fat contents were 0.2, 6 and 0.1%, respectively (Kodama and Yoshizawa, 1977). The presence of excessive levels of ash, protein and fat is reported to be undesirable due to over growth of the koji mould as the rice becomes richer in nutrients (Steinkraus, 1996). Such koji would leads to overheating of the fermentation medium and produce off color and flavor in the liquor (Japan International Corporation, 2002).

The initial moisture content of polished rice grain is very much crucial in the brewing process. This is due to absorption of moisture to a level of about 25-30% during washing and soaking of rice. Rice with lower polishing ratio absorbs lower amount of water during washing and soaking. This would negatively affect the brewing process as the rice becomes too hard after steaming causing deterioration of the koji mould. Such rice is considered unsuitable, as it is difficult to make koji (Japan International Corporation, 2002). According to Yoshizawa (1982) the initial moisture content of polished rice should not exceed 14% in order to achieve an optimum level of moisture after washing and soaking.

Based on the proximate composition, rice polished at 70 and 60% were found to be more suitable than rice polished at 90 and 80% for rice wine production.

Table 1. Proximate constituents rice polished at different ratios.

Constituent (%)	Polishing ratio			
	90%	80%	70%	60%
Moisture	16.75±0.19	14.17±0.11	13.47±0.20	12.55±0.23
Ash	0.70±0.05	0.41±0.04	0.19±0.05	0.16±0.04
Protein	9.80±0.33	7.38±0.42	6.68±0.23	6.64±0.27
Fat	1.24±0.05	1.09±0.02	0.43±0.04	0.39±0.04

Note: Values represent the mean ± SD (n=3).

Rate of fermentation indicated by rate of alcohol production was higher when rice polished at 70 and 60% was used compared to 90 and 80% (Fig. 1). This suggests that higher degree of rice polishing would positively affect the brewing quality of rice wine, which is attributed to the increased rate of saccharification of highly polished rice. Moreover, highly polished rice absorbs more moisture during washing, soaking and steaming thus starch becomes highly susceptible for saccharification by koji mould (Japan International Corporation, 2002). Based on the rate of alcohol production, rice polished at 70 and 60% were found to be better than rice polished at 90 and 80% for rice wine production.

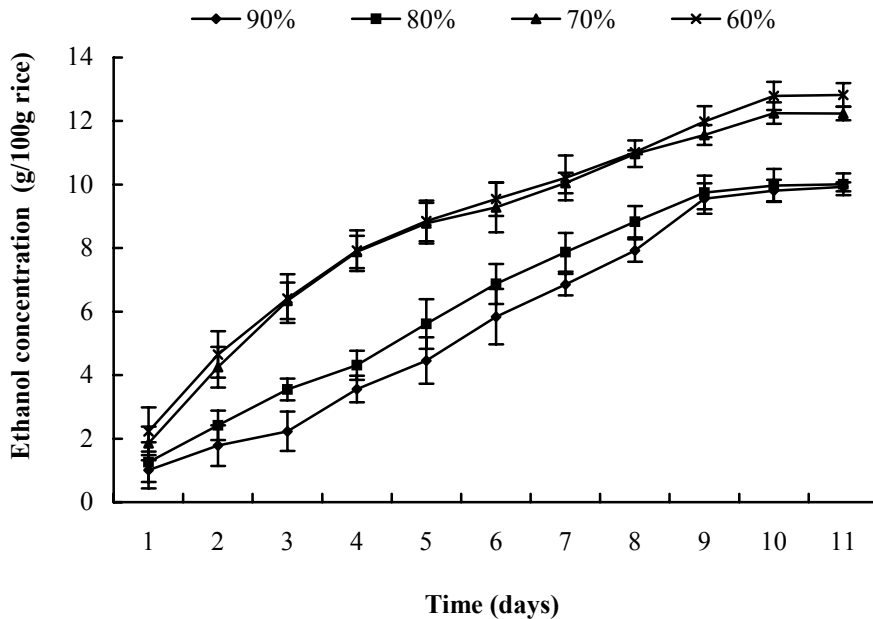


Fig. 1. Rate of ethanol production during rice fermentation as affected by rice polishing ratio.

Physico-chemical properties of rice wine produced using rice polished at 90, 80, 70 and 60% are given in Table 2. Polishing ratio did not affect the pH and TSS of rice wine as revealed by non-significant difference in pH and TSS of the wine samples. However, total sugar content increased significantly with increase in degree of rice polishing. This may be due to the increased rate of saccharification attributed to the optimum mycelial growth of koji mould in the presence of optimum levels of minerals, fat and protein contents of highly polished rice (Japan International Corporation, 2002). The ethanol content of rice wine increased with the increase in degree of rice polishing. This may be due to availability of fermentable sugars resulted by increased rate of saccharification of highly polished rice as revealed by about 4% total sugars in rice wine produced using rice polished at 70 or 60%.

Table 2. Physico-chemical properties of rice wine as affected by rice polishing ratio.

Parameter	Polishing ratio			
	90%	80%	70%	60%
pH	4.16 ^a	4.19 ^a	4.17 ^a	4.17 ^a
Ethanol (% v/v)	12.84 ^b	13.16 ^{ab}	13.54 ^a	13.51 ^a
TSS (°Brix)	5.80 ^a	5.77 ^a	5.90 ^a	5.83 ^a
Total sugar (% w/v)	3.65 ^b	3.77 ^b	4.18 ^a	4.20 ^a

Note: Means in rows with different letters are significantly different ($p < 0.05$).

In the analysis of volatile compounds, headspace of the wine samples heated at 50°C for 30 min. was directly injected to the gas chromatograph (Shibamoto, 1984). Relative abundance of the volatile compounds and the representative gas chromatograms of wine samples made using rice polished at 90, 80 70 and 60% are given in Table 3 and Figure 2, respectively. Identities of the major peaks of the headspace constituents numbered on the chromatograms are also given in Table 3. Ethanol, being the most abundant volatile constituent in alcoholic beverages, contributes to more than 90% of the headspace composition (Uzochukwu *et al.*, 1999). Higher number of headspace volatile compounds as revealed by the number of major peaks and those are in traces (Table 3) was detected in rice wine made using rice polished at 70 and 60% than at 90 and 80%. This difference in peak numbers may have been compensated by higher increase in ethanol concentration in headspace of wine made using rice polished at 90 and 80% than at 70 and 60%. Methanol was detected in the headspace of all the wine samples in minute quantities, which is reported to be present in the volatile profiles of rice as a trace constituent (Akiyama *et al.*, 1978). Acetaldehyde is one of the commonly present volatile compounds, which contributes to pleasant flavor at lower concentration and possesses a flavor threshold of 100-125 ppm in wine. Both fruit wines and grain wines contain acetaldehyde synthesized as a result of glucose metabolism of the yeasts (Liu and Pilone, 2000). Ethyl acetate and isoamyl alcohol were also detected in the headspace of rice wine, which are reported to be important flavor compounds in wines (Uzochukwu *et al.*, 1999; Wang *et al.*, 2004).

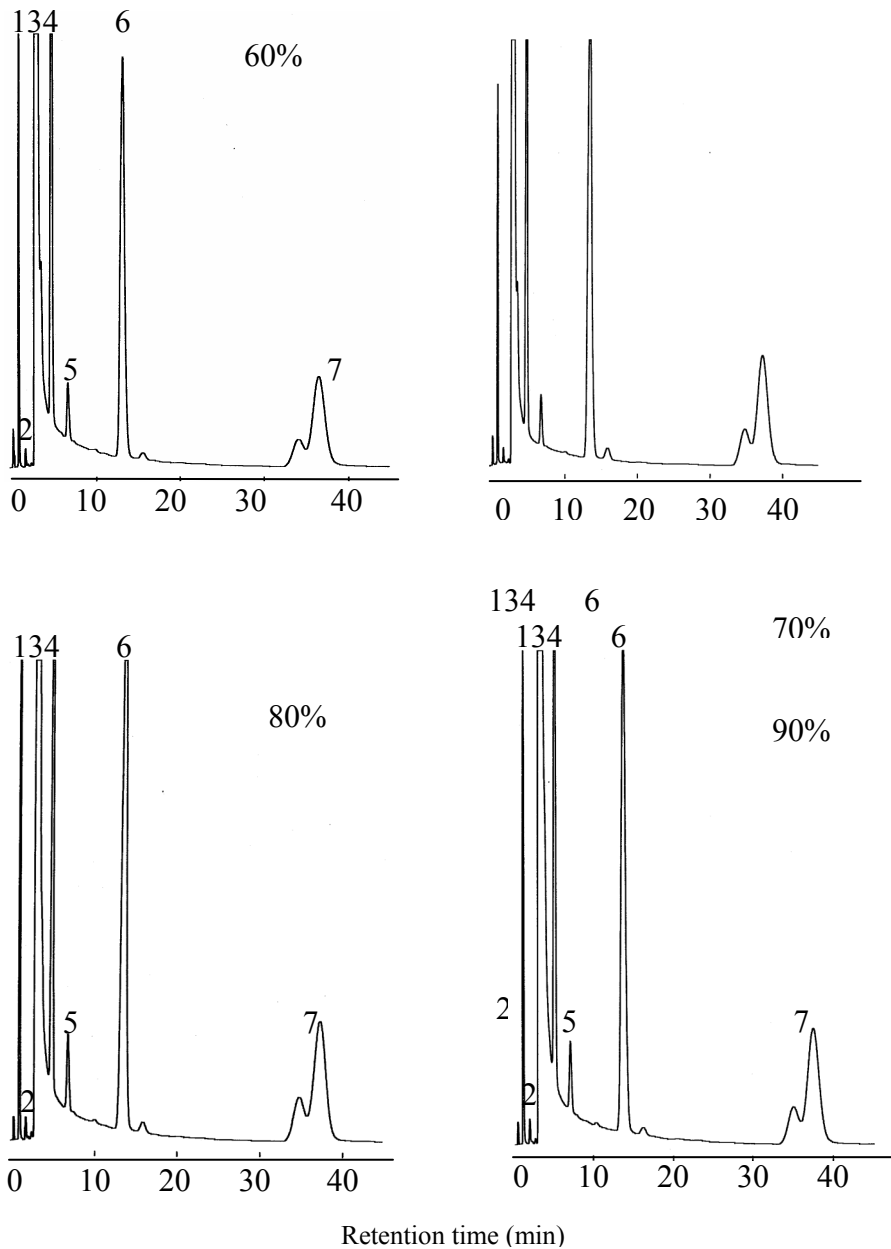


Fig. 2. Headspace GC chromatograms of wines made using rice polished at 90, 80, 70 and 60% (detector response vs. time).

Table 3. Relative abundance of GC headspace constituents of rice wine made using rice polished at different ratios.

Peak No.	Identity	Peak Area			
		90%	80%	70%	60%
1	Acetaldehyde	0.25±0.01	0.31±0.00	0.15±0.00	0.13±0.01
2	Methanol	0.02±0.01	0.02±0.00	0.01±0.00	0.01±0.01
3	Ethanol	95.30±0.18	95.20±0.23	94.60±0.10	94.10±0.22
4	Unknown 1	0.69±0.16	0.63±0.14	0.82±0.11	0.81±0.15
5	Unknown 2	0.36±0.07	0.39±0.05	0.27±0.06	0.29±0.07
6	Ethyl acetate	1.16±0.05	1.14±0.05	1.88±0.05	1.91±0.06
7	Isoamyl alcohol	0.98±0.17	1.04±0.20	1.50±0.21	1.48±0.24
Trace constituents					
		0.22±0.02 (09)*	0.24±0.02 (11)*	0.20±0.01 (14)*	0.17±0.02 (14)*

Note: Values represent the mean ± SD (n=3); *Mean number of peaks.

Rice wine made using rice polished at 60 and 70% had higher headspace concentrations of ethyl acetate and isoamyl alcohol that would positively contribute to the overall aroma of rice wine. Polishing of rice has a positive impact on rice wine aroma and is organoleptically superior than wine made using less polished rice (Japan International Corporation, 2002).

Furthermore, the studies of Furukawa *et al.* (2003) have shown that most of the aroma-enhancing compounds are synthesized from middle chain fatty acids through the metabolic pathways of yeast. Inositol, which is an inherent constituent of rice, reduces the production of middle chain fatty acids thereby, hindering the synthesis of aroma-enhancing compounds. Therefore, polishing of rice to about 70% or more would improve the aroma by inositol removal. These results suggest that rice polished at 60 and 70% contribute more to the liquor quality than rice polished at 80 and 90%.

CONCLUSIONS

Rice wine made using rice of the cultivar Bg 358 polished at 70 and 60% showed higher rates of fermentation, higher alcohol and sugar contents and more number of headspace volatile constituents than rice wine produced using rice polished at 90 and 80%. Based on the proximate composition of rice and physico-chemical properties and headspace volatile constituents of rice wine, rice polished at 60 and 70% was found to be better than rice polished at 90 and 80% for wine production.

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