Nonwaxy Rice for *Tapuy* (Rice Wine) Production

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ABSTRACT

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A study was undertaken to determine the feasibility of substituting nonwaxy rice for waxy rice in an improved *tapuy* (rice wine) process using high-saccharifying fungi and high-ethanol-tolerant yeast. Waxy and low-amylose (12–20%) milled rices showed the highest *tapuy* yield, ethanol content, and ethanol conversion, followed by intermediate-amylose (20–25%) rice and then high-amylose (>25%) rice. Weight and starch content of the residual mash were positively correlated with amylose content. Properties of *tapuy* aged for one month showed significant differences in taste panel scores, but only color scores were correlated with amylose content. Low- and intermediate-amylose rices could be used in place of waxy rice in the improved *tapuy* production process without significant change in *tapuy* yield and quality; ethanol recovery was slightly lower for intermediate-amylose rice.

Traditonally, waxy (glutinous) milled rice, often pretoasted, is used for the preparation of Philippine rice wine (Tanimura et al 1977, Cordero-Fernando 1984). Red waxy rice is preferred over nonpigmented waxy rice. Waxy rice is also preferred in the preparation of traditional rice wines such as the Indonesian *brem* (Saono et al 1982) and the Japanese *mirin* (Oyashiki et al 1985). Low-amylose milled rice is used in producing Japanese rice wine sake (Yoshizawa and Kishi 1985) and as a U.S. rice adjunct in beer brewing (Teng et al 1983). Low-amylose nonwaxy rice soaked at 65°C and steamed at 135°C gave a *mirin* yield and quality comparable to waxy rice steamed at 100°C (Oyashiki et al 1985).

Philippine rice wine, known as *tapuy* in the Ifugao and nearby provinces where it is prepared and consumed, is made from waxy rice mixed with powdered rice starter called *bubod* (Sakai and Caldo 1985a). Although the method of preparation varies from one locality to another (Tanimura et al 1977), the general procedure involves the same basic steps: overnight steeping of rice kernels in water, draining, cooking (or steaming) for 15–45 min, adding starter, saccharifying, fermenting, and harvesting. The same method is followed in producing *pangasi* (Sakai and Caldo 1985b), another traditional Philippine rice wine in northwestern Mindanao, *brem* in Indonesia, *ba-si-de* in Vietnam, and *tapay* in Malaysia (Sakai and Caldo 1985a).

Studies of the traditional method (using *bubod*) (Tanimura et al 1977, Sanchez et al 1984) showed that there is variability of microbial load in the starter, which causes variations in the efficiency of alcoholic fermentation (Sanchez et al 1984). This led to the study on local strains of *Aspergillus oryzae, Rhizopus oryzae, Saccharomycopsis,* and *Saccharomyces cerevisiae* (Sanchez et al 1984). The microorganisms were used either in pure form or grown on cooked rice (*koji*) to optimize process parameters of rice wine production. Improvement of the process was achieved by altering critical steps, including the following: addition of water during steaming at the rate of 1:3, rice to water; extending steaming time from 15–45 min to 60 min; and use of pure mold and yeast cultures at amounts that vary with the temperature and time of saccharification. Highest alcohol yield was obtained using these modifications, with waxy rice producing the highest amount of 16–18%. The process was optimized further by devising a saccharification and fermentation system in which flowing humid air was introduced to the saccharifying rice to maintain the moisture content of the substrate (Chay et al 1986).

A survey of *tapuy* made from 14 Philippine rice varieties differing in amylose content showed that alcohol content ranged from 12.0 to 13.3% and that all commercial varieties can be used for *tapuy* production (Coronel et al 1981). Black and white waxy rices gave higher wine yields than nonwaxy rice for Indonesian *brem* production (Aryanta 1980). Total extract yield of a brewing adjunct mash decreased with an increase in gelatinization temperature of U.S. milled rice and was highest for a waxy variety (Teng et al 1983).

In view of the rather variable information on the varietal type most suitable for rice wine production and the relatively high price of waxy rice in the Philippines, a study was undertaken to determine the feasibility of substituting nonwaxy rice for waxy rice using the improved traditional method (Sanchez et al 1984).

MATERIALS AND METHODS

Nine International Rice Research Institute derived (IR) rough rices were obtained from crops at the Institute farm in 1985. Inga brown (dehulled) rice was obtained from the 1985 crop of Yanco Agricultural Institute, NSW, Australia. Preliminary trials in 1985 used eight IR rices; a later study utilized four varieties representing the various amylose types. Rough rices were dehulled in a Satake THU-35A dehusker, and the brown rice was milled in a Satake TM-05 pearler to 10% bran-polish removal.

Part of the milled rice was converted into a flour with a Udy cyclone mill with 60-mesh sieve for chemical analysis: amylose content by iodine colorimetry (Juliano et al 1981), starch content by the glucoamylase-glucose oxidase method (Perez et al 1971), gel consistency by measuring the gel length of 100 mg of milled rice in 2 ml of 0.2*N* KOH in 100 × 11 mm i.d. test tubes (Cagampang et al 1973), and protein content by the micro-Kjeldahl method using the factor 5.95. Gelatinization temperature was measured in duplicate from digestion of six milled rice grains in 10 ml of 1.7% KOH for 23 hr at 30°C (Little et al 1958). Residual mash was also analyzed for starch and amylose content.

At the Institute of Food Science and Technology, a duplicate 800 g of milled rice was washed with water three times, steeped in water for 3 hr, and steamed for 1 hr with three times its weight of water (2,400 g). The boiled rice (water content 75–80%) was cooled and spread on bamboo trays, inoculated with 1% starter consisting of 10^7/ml of *Rhizopus oryzae* spores, and incubated for 20 hr. This was followed by inoculation with 1% of 10^{12}/ml *Saccharomycopsis capsularis* cells and incubation for 12–15 hr. The saccharified rice was transferred to a 4-L widemouthed glass jar and inoculated with 1% starter of 10^{12}/ml *Saccharomyces cerevisiae* cells and incubated for 16 days. After fermentation, the *tapuy* was filtered to obtain the rice wine and residual fermenting mash.

Ethanol was extracted by collecting 90 ml of distillate from 100 ml of *tapuy* in a 100-ml graduated cylinder, and adding distilled water to 100 ml. The solution was cooled to 15°C, and ethanol content (by volume) was read with an alcohol hydrometer. *Tapuy* yield was taken as volume per 800 g of milled rice. Alcohol yield was converted to weight using a density value of 0.789. Reducing sugars were determined by the method of Somogyi (1952) and total sugars by the method of Dubois et al (1956). Total titratable acidity was determined by titration with standard 0.1*N* NaOH and total

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soluble solids by hand refractometer after boiling for 5 min in a water bath. The pH values were measured with a pH meter. Amino N was measured by the formol method. Residual mash was analyzed for titratable acidity and total and reducing sugars.

Samples of *tapuy* aged one month were presented to eight taste panelists in sets of three randomly selected samples each round. This was done repeatedly until each of the 10 samples was analyzed 10 times. The unbiased panelists consisted of personnel from the University of the Philippines at Los Baños complex, all of whom drink wine but were not familiar with *tapuy*. Each sample was rated independently based on a five-point scale for color, aroma, flavor, astringency, acidity, and general quality.

Data were subjected to analysis of variance (Snedecor and Cochran 1980). Analysis was followed by Duncan's (1955) multiple range tests for taste panel scores for *tapuy* properties.

RESULTS AND DISCUSSION

Samples selected for the study were two waxy, three low-amylose, two intermediate-amylose, and three high-amylose milled rices (Table I). Only the intermediate- and high-amylose rices showed intermediate gelatinization temperature (GT, alkali-spreading value 4–5). Gel consistency was soft (>60 mm) for waxy and low-amylose rices, medium (41–60 mm) for intermediate-amylose rices, and most varied for high-amylose rices. Protein content was lowest for the high-amylose rices as a group and highest for waxy rice. Among these five properties, only amylose and protein contents were significantly correlated (r = −0.68* [* = 0.05 level of significance]).

Wine yields after 16 days fermentation were highest for waxy rices and IR24 and lowest for high-amylose rices (r = −0.91** [** = 0.01 level of significance]) (Table II). Wine yields were also positively correlated with protein content (r = 0.68*), probably because protein and amylose contents were negatively correlated. Ethanol content of *tapuy* was significantly negatively correlated with amylose content (r = −0.67*) and positively with starch content (r = 0.74*). The pH ranged from 4.6 to 5.0 and was not correlated with starch properties. Total soluble solids correlated negatively with alkali spreading value (r = −0.80**) and were highest for IR62 and IR36. Total titratable acidity also correlated positively with amylose content of milled rice (r = 0.86**) and negatively with *tapuy* pH (r = −0.81**). Amino N content of *tapuy* showed a narrow range, and differences correlated negatively with gel consistency (r = −0.70*). Reducing and total sugars were similar in all *tapuy* samples and were not simply correlated with properties of raw rice.

TABLE I
Physicochemical Properties of 10 Raw Milled Rices[a] Used for *Tapuy* Production and Their Correlation Coefficients

Variety Name	Amylose Type	Amylose Content (% db)	Starch Content (% wb)	Alkali-Spreading Value	Gel Consistency (mm)	Protein Content (% wb)
IR29	waxy	3.2	83.5	7.0	76	8.3
IR65	waxy	3.0	80.4	6.2	100	9.0
IR24	low	13.9	80.8	6.5	70	8.2
IR43	low	17.8	81.5	7.0	61	8.1
Inga	low	15.8	80.1	6.2	73	7.7
IR48	int	21.7	77.2	7.0	42	8.6
IR64	int	22.8	81.8	5.0	53	6.7
IR42	high	25.6	82.0	7.0	30	6.6
IR62	high	26.9	79.6	5.0	91	7.3
IR36	high	27.6	71.8	4.2	38	7.8
LSD (5%)		0.4	1.0	0.2	7.1	0.4

[a] Moisture content 12 ± 0.5%.

Ethanol recovery based on either dry matter or starch content was negatively correlated with amylose content (r = −0.86** and −0.85**) (Table II). Waxy and low-amylose rices had the highest ethanol recoveries, and high-amylose rices the lowest. Despite its higher level of reducing and total sugars, IR48 had ethanol recovery comparable to that of IR64. Ethanol recoveries both on dry matter and starch bases were correlated (r = 0.97**). Alcohol recovery for *brem* was also lower for nonwaxy than for waxy rice (Aryanta 1980).

Dry weight of residual mash and volume of lees were positively correlated with amylose content of milled rice, but the relationship was more significant for residue weight (r = 0.84** and 0.65*) (Table III). In addition, starch content in the residual mash correlated positively with milled rice amylose content (r = 0.88**) as did moisture content of mash (r = 0.90**). Starch content of residual mash ranged from 1.4 to 1.9% in waxy rice, 4.2 to 18.6% in low-amylose rice, and 30.5 to 38.2% in intermediate- and high-amylose rices. Amylose content in dry residual mash also increased with amylose content of milled rice (r = 0.88**). It corresponded to 58 to 92% of starch content of mash (mean 72.6%). By contrast, amylose content of milled rice was 3.0–27.6% (Table I) and 3.3–33.8% on starch basis (mean 19.9%). Titratable acidity and reducing and total sugars of the mash were similar and did not correlate with milled rice properties. Among the low-amylose rices, Inga and IR43 had more residual mash starch than IR24, and among high-amylose rices, IR62 and IR36 had more residual mash starch than IR42. These differences were not consistently reflected in the ethanol recovery in *tapuy* (Table II). Teng et al (1983) reported that the total extract yield of a brewing-adjunct mash decreased with an increase in gelatinization temperature of U. S. milled rice and was highest for a waxy rice. U.S. rices with low gelatinization temperature have a low amylose content; those with intermediate gelatinization temperature have an intermediate amylose content.

TABLE II
***Tapuy* Processing Characteristics of 10 Milled Rices and Their Correlation with Starch Properties and Protein Content**

Variety Name	Amylose Type	Wine Yield[a] (ml)	Ethanol Content (%, v/v)	pH	Total Soluble Solids (°Brix)	Total Titratable Acidity[b]	Amino N (%)	Reducing Sugars (%)	Total Sugars (%)	Ethanol Conversion Wt. %	Ethanol Conversion % of Starch
IR29	waxy	2,365	12.9	4.80	3.3	10.2	0.03	0.07	0.14	34.2	36.0
IR65	waxy	2,350	12.2	4.80	3.3	11.3	0.03	0.12	0.13	32.2	35.2
IR24	low	2,330	12.4	4.98	3.0	10.6	0.04	0.08	0.10	32.4	35.3
IR43	low	2,230	12.6	5.00	3.2	11.4	0.04	0.10	0.12	31.5	34.0
Inga	low	2,210	12.9	4.82	3.2	11.2	0.03	0.06	0.09	31.9	35.1
IR48	int	2,120	12.0	4.70	3.3	16.0	0.04	0.21	0.25	28.5	32.5
IR64	int	2,065	12.4	4.60	3.5	16.8	0.03	0.08	0.10	28.7	30.9
IR42	high	1,890	11.4	4.65	3.4	18.7	0.04	0.12	0.15	24.1	25.9
IR62	high	1,875	11.6	4.75	3.9	19.5	0.03	0.09	0.11	24.4	26.9
IR36	high	1,840	10.6	4.65	3.9	20.2	0.04	0.09	0.11	21.8	26.8
LSD (5%)		97	0.5	0.04	0.3	0.2	ns[c]	ns	ns	2.7	2.9

[a] Yield expressed as milliliters of wine per 800 g of rice at 12% mb.
[b] Milliliters of 0.1 N NaOH per 10 ml.
[c] ns = Not significant.

In a follow-up fermentation to determine the esters content of *tapuy* before and after six months of aging, IR29, Inga, IR64, and IR62 were again processed. IR64 mash showed the fastest separation of the liquid phase during fermentation; however, its *tapuy* yield or ethanol content was not exceptionally different (Table IV). Wine yield decreased with increase in amylose content of milled rice. IR62 had the lowest ethanol content but Inga showed the lowest total solids content. Ethanol recovery was inversely correlated with amylose content. Weight and starch in the residue mash increased proportionately with amylose content. The volume of lees was higher than that in Table III for all four varieties.

Theoretical ethanol recovery from starch is 56.9%, based on two molecules of ethanol per glucose repeating unit of starch. The highest ethanol recovery was 36.0% of starch for IR29 *tapuy*; the lowest was 26.8% for IR36 *tapuy* and 26.9% for IR62 *tapuy*. After correction for total sugars in the *tapuy* and for starch and sugars in the residue, actual ethanol recovery was 36.2% of starch for IR29, 28.2% for IR36, and 28.3% for IR62. These values correspond to 63.6% efficiency for IR29 *tapuy*, 49.6% for IR36, and 49.7% for IR62.

The undigested or residual-mash starch, which increases with amylose content of milled rice, must be mainly retrograded amylose (Table III). The fraction of boiled and retrograded corn starch resistant to β-amylase-pullulanase action was reported to have 66% amylose compared with 27% amylose for raw corn starch (Matsunaga and Kainuma 1986). In studies on response of glucose and insulin in blood plasma to ingested cooked rice, Juliano and Goddard (1986) showed that high amylose content may explain the slower rate of glucose response in the variety Newrex, which has high amylose and an intermediate gelatinization temperature. Starch lipids were not an important factor because starch lipids of high-amylose rices were not higher than those of intermediate-amylose rice. In addition, Holm et al (1983) reported that rats readily digest and absorb the amylose-lipid complex.

Taste panel scores of one-month-old *tapuy* from the 10 varieties showed sample differences in five properties and general quality (Table V). IR65 had the highest aroma score and IR62 the lowest. IR65 *tapuy* also had the best color score, and IR48 the lowest. Flavor scores were highest for IR62 and lowest for IR65. All samples showed moderate to slight astringency, least for IR48 and most for IR24. Similarly, the highest acidity scores were obtained by IR43 and Inga and the least by IR36 and IR42, the most acidic. IR36 had the highest total titratable acidity in freshly fermented unaged *tapuy*, followed by IR42 (Table II). Total titratable acidity of *tapuy* and acidity scores were negatively correlated ($r = -0.72$*). Total titratable acidity of residue and acidity scores were also negatively correlated ($r = -0.84$**).

The general quality of the samples ranged from fair to good and was highest for IR64 and lowest for IR65. The panel scores for IR64 *tapuy* were high except for color. Among the panel scores, only color scores were negatively correlated with amylose content ($r = -0.77$**) and positively correlated with gel consistency ($r = 0.81$**). The lowest general quality scores were obtained by IR65. The IR65 *tapuy* had good panel scores for all except flavor and astringency. Significant correlation coefficients among the panel scores were between flavor scores with astringency ($r = 79$**) and general quality ($r = 0.67$*).

Aroma score was highest for *tapuy* from waxy rice IR65 (Table V). Since aroma is derived from nonstarch lipid fatty acids in sake (Yoshizawa and Kishi 1985), aroma would be expected to be highest in waxy milled rice, which has the highest content of nonstarch lipids among amylose types (Juliano and Goddard 1986). However, *tapuy* from IR29, the other waxy rice sample, was no different in aroma score from *tapuy* of nonwaxy samples except for IR62 *tapuy*.

TABLE III
Volume of Lees and Properties of Residual Mash from *Tapuy* Production and Their Correlation with Milled Rice Starch Properties and Protein Content

			Dry Residual Mash[a]					
Variety Name	Amylose Type	Volume of Lees (ml)	Weight (g)	Starch Content[b] (g)	Amylose Content (g)	Total Titratable Acidity[c]	Reducing Sugars (%)	Total Sugars (%)
IR29	waxy	90	29.2	0.4 (0.1%)	0.3	60	0.52	1.36
IR65	waxy	130	26.2	0.5 (0.1%)	0.4	61	0.65	1.63
IR24	low	130	31.2	1.3 (0.2%)	1.2	60	0.48	1.28
IR43	low	160	35.5	4.2 (0.6%)	2.9	70	0.74	1.31
Inga	low	175	33.8	6.3 (1.0%)	4.7	72	0.72	1.41
IR48	int	218	46.5	14.2 (2.2%)	8.4	70	1.34	1.71
IR64	int	230	46.2	14.5 (2.2%)	10.8	68	0.68	1.54
IR42	high	125	81.9	25.3 (3.9%)	18.8	88	1.08	1.63
IR62	high	185	74.9	27.3 (4.3%)	19.0	81	0.49	0.96
IR36	high	186	68.8	26.3 (4.6%)	15.2	85	0.42	1.17
LSD (5%)		34	6.4	1.7 (0.2%)	0.2	ns[d]	ns	ns

[a] Moisture content 83.2–87.1% (mean 85.4%).
[b] Percent of milled rice starch in parentheses.
[c] In milliliters of 0.01 *N* NaOH per 10 g.
[d] ns = Not significant.

TABLE IV
Confirmatory Data of *Tapuy* Production on Four Varieties of Milled Rice[a]

				Ethanol Recovery (%)		Dry Residue Mash		
Variety Name	Wine Yield (ml/800 g rice)	Ethanol Content (%, v/v)	Total Soluble Solids (°Brix)	Weight Basis	Starch Basis	Weight (g)	Starch (g)	Volume of Lees (ml)
IR29	2,516	12.6	3.8	35.5	37.4	25.8	2.1	122
Inga	2,274	12.8	2.6	32.8	36.0	41.8	6.6	252
IG64	2,072	12.8	3.3	29.7	32.0	62.2	14.0	240
IR62	1,928	12.0	3.2	26.0	28.8	75.8	19.8	300
LSD (5%)	132	0.6	0.2	2.8	3.4	14.0	3.0	ns

[a] Properties of raw milled rice presented in Table I. Amylose type: IR29 waxy, Inga low, IR64 intermediate and IR62 high. A sample size of 850 g was actually used for IR29 and Inga but data were calculated for 800 g of rice at 12% moisture.
[b] ns = Not significant.

TABLE V
Mean Scores by Eight Panelists of One-Month-Old *Tapuy* from 10 Milled Rices

Variety Name	Amylose Type	Mean Taste Panel Scores[a]					
		Aroma[b]	Color[c]	Flavor[d]	Astringency[e]	Acidity[f]	General Quality[g]
IR29	waxy	3.3 ab	3.9 bc	2.9 ab	3.7 a	3.2 ab	3.3 ab
IR65	waxy	3.6 a	4.4 a	2.4 c	3.3 bc	3.2 ab	2.9 c
IR24	low	3.1 b	3.9 bc	2.5 c	3.0 c	3.3 ab	3.2 abc
IR43	low	3.2 ab	4.0 b	2.7 abc	3.3 bc	3.4 a	3.3 ab
Inga	low	3.1 b	4.0 b	2.7 abc	3.3 abc	3.4 a	3.1 bc
IR48	int	3.2 b	3.4 d	2.9 ab	3.7 a	3.1 abc	3.2 ab
IR64	int	3.4 ab	3.6 cd	2.9 a	3.6 ab	3.3 a	3.4 a
IR42	high	3.1 b	3.5 d	2.5 c	3.5 ab	2.9 bc	3.3 ab
IR62	high	3.0 c	3.8 bcd	3.0 a	3.6 ab	3.1 abc	3.3 ab
IR36	high	3.4 ab	3.6 d	2.7 bc	3.4 abc	2.8 c	3.1 bc

[a] Means in a column followed by the same letter are not significantly different at the 5% level by Duncan's (1955) multiple range test. Each panelist evaluated the samples 10 times.

[b] Aroma: 5 = strong, 4 = moderate, 3 = slight, 2 = just detectable, 1 = none.

[c] Color: 5 = straw almost colorless, 4 = slightly yellowish, 3 = dull yellow, 2 = yellow, 1 = intense yellow.

[d] Flavor: 5 = strong, 4 = moderate, 3 = slight-moderate, 2 = slight, 1 = none.

[e] Astringency: 5 = none, 4 = slight, 3 = slight-moderate, 2 = moderate, 1 = strong.

[f] Acidity: 5 = none, 4 = weak, 3 = weak-moderate, 2 = moderate, 1 = high.

[g] General quality: 5 = very good, 4 = good, 3 = fair, 2 = poor, 1 = very poor.

CONCLUSION

In the improved *tapuy* production process, low- and intermediate-amylose milled rice may be used in place of waxy milled rice without significant change in *tapuy* yield and quality; ethanol recovery was slightly lower for intermediate-amylose rice.

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