APPLIED AND ENVIRONMENTAL MICROBIOLOGY, May 1977, p. 1067-1073

Vol. 33, No. 5
Printed in U.S.A.

Indonesian Tapé Ketan Fermentation[1]

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Received for publication 22 November 1976

Indonesian tapé ketan is a fermentation in which a mold, *Amylomyces rouxii* Calmette (*Chlamydomucor oryzae* Went and Prinsen Geerligs), in combination with one or more yeasts such as *Endomycopsis burtonii* converts steamed rice to a sweet-sour, slightly alcoholic paste. A study was made to determine the biochemical changes that occur in the substrate during fermentation. It was found that the product was ready for consumption after fermentation at 30°C for 36 to 48 h. *A. rouxii* used about 30% of the total rice solids, resulting in a crude protein of 12% in 96 h, whereas the combination of the mold with *E. burtonii* reduced total solids by 50% in 192 h, causing crude protein to increase to 16.5%. Soluble solids increased from 5 to about 67% in 36 h and decreased to 12% at 192 h with *A. rouxii* alone, whereas soluble solids fell to about 8% at 192 h in the fermentation with both the mold and the yeast. The mold, by itself, reduced the starch content of the rice from 78 to 10% in 48 h and to less than 2% in 144 h. The mold plus yeast reduced the starch content to about 18% in 48 h; however the "starch" content did not fall below 6% even at 192 h, presumably because the yeast was producing glycogen, which was determined along with the residual starch. With both the mold and the mold plus yeast fermentations, reducing sugars increased from less than 1% to approximately 5% in 24 h and reached maximum concentration, 16 to 17%, between 36 and 48 h. *A. rouxii* by itself produced a maximum of about 5.6% (vol/vol) ethanol at 96 h. The highest concentration of ethanol (8%, vol/vol) was produced by the mold plus *E. burtonii* at 144 h. The mold by itself reduced the starting pH from 6.3 to about 4.0 in 48 h. The combination of the mold and yeast reduced the pH to 4.1 in 144 h. The mold increased total acidity to approximately 6.2 meq of H^+ per 100 ml, and the combination of the mold and yeast increased the total acidity to 7.8 meq of H^+ per 100 ml in 192 h. At 48 h there was practically no difference in the volatile acidity (0.20) for the combined fermentation compared with 0.26 meq of H^+ per 100 ml for the mold fermentation. The mold and at least one species of yeast were required to develop the rich aroma and flavor of typical Indonesian tapé.

Indonesian tapé ketan is a fermented, partially liquefied, sweet-sour, mildly alcoholic rice paste. It is prepared from glutinous rice which has been steamed, spread in thin layers on woven bamboo trays, and inoculated with ragi, dry flattened circular cakes, about 3 cm in diameter, prepared from rice flour and distinctive spices and containing the required organisms. The substrate is then covered, typically with a bamboo leaf, and allowed to ferment for approximately 24 to 48 h at ambient temperature (25 to 30°C) (3, 8, 11, 14).

Tapé ketan is produced throughout Indonesia on a home or cottage industry scale. It is consumed as a dessert or snack item without further processing after the fermentation. As an alternative substrate, peeled, diced, and steamed cassava tubers are used; this product is tapé ketella. Brem and arak, two other products related to the tapé fermentation, are prepared similarly but allowed to ferment longer, resulting in greater liquefaction of the rice. Both brem and arak are prepared from the liquid portion; the former is sun-dried and consumed as a solid "candy," and the latter is consumed as an alcoholic beverage (8, 14).

Went and Geerligs (14) were the first to isolate and identify the microorganisms thought to be essential for the fermentation. They noted that the tapé fermentation is dependent on the presence of at least one amylolytic filamentous fungus and one or more alcohol-producing yeasts. Numerous fungi and yeasts have been isolated while studying the microflora of tapé,

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ragi-tapé, and related fermentations of Southeast Asia (2–4, 11, 13). The presence of the fungus *Amylomyces rouxii* Calmette (5) (formerly *Chlamydomucor oryzae* Went and Prinsen Geerligs) appears to be essential for the production of tapé ketan; less is known about the contribution by the yeasts. Both *Endomycopsis burtonii* (= *E. chodati*) as reported by Djien (3) and *Candida lactosa* as reported by Dwidjoseputro and Wolf (4), when combined with *A. rouxii*, are capable of transforming steamed rice into good-quality tapé ketan.

This investigation was conducted to determine the basic biochemical changes that occur during a typical tapé ketan fermentation under pure culture conditions. An attempt was made to understand the changes that occur in the substrate as a result of the presence of the mold *A. rouxii* and the mold in combination with eight yeasts that had been isolated from ragi-tapé, with principal emphasis on *E. burtonii* (3, 4).

MATERIALS AND METHODS

Rice. White polished rice (Carolina Brand) was used in all studies except for those on thiamine production, where unenriched rice was used as substrate.

Cultures. The microorganisms used in this study are listed in Table 1. Stock cultures of the yeasts were maintained on Difco malt extract slants. *A. rouxii* was maintained in Sabouraud dextrose broth. All cultures were grown at 30°C and stored at 5°C between transfers.

Preparation of inocula. The yeast inocula were prepared by introducing 5 ml of sterile water onto slants that had been streaked 24 h earlier and gently vortexing to form a cell suspension. Two milliliters of the suspension was distributed over the rice substrate surface.

After 5 days of incubation, which allowed for adequate development of the aerial mycelium of *A. rouxii*, mycelial mats were aseptically transferred to a covered, sterile, fritted-glass crucible. The mats were washed and transferred to an Omnimixer cylinder and comminuted for 5 min at a moderate speed with the cylinder immersed in an ice bath. Two milliliters of the inoculum was applied uniformly over the rice substrate.

TABLE 1. *Cultures used in the studies*

Organisms	Culture collection no.	Source
Amylomyces rouxii (formerly *C. oryzae*) (5)	NRRL A-17, 199	K. S. Djien[a]
Endomycopsis burtonii	NRRL-Y-7143	K. S. Djien
E. fibuligera	NRRL-Y-7145	NRRL[b]
Candida lactosa	NRRL-Y-7170	NRRL
C. melinii	NRRL-Y-7171	NRRL
C. parapsilosis	NRRL-Y-7172	NRRL
Hansenula anomala	NRRL-Y-7174	NRRL
H. malanga	NRRL-Y-7175	NRRL
H. subpelliculosa	NRRL-Y-7176	NRRL

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[b] Northern Regional Research Laboratory, Peoria, Ill.

Preparation of substrate. Nalgene specimen dishes (105 by 43 mm), fitted with covers, were used as the substrate containers. Forty grams of rice and 100 ml of water were added to each dish. The rice was steamed for 15 min and subsequently autoclaved for 15 min at 121°C.

Sample preparation. A sample consisted of the entire contents of a dish. For wet analyses, dishes were removed from the incubator, covered, and frozen at −20°C until the analyses could be performed. Prior to analysis, the sample was briefly thawed at room temperature and subsequently homogenized using an Omnimixer. Where dried samples were required for analyses, the sample was removed from the incubator, frozen at −40°C, and freeze-dried. A Wiley mill equipped with a 20-mesh screen was used to reduce the freeze-dried sample to a uniform powder.

Analytical methods. Changes in pH were followed using a Beckman Zeromatic pH meter. Total acidity was determined by titration to pH 8.2. Volatile acids were determined by steam distillation of 5-ml samples using a micro-steam distillation Juffmann-Comes (Milan, Italy) apparatus. The distillate was titrated to pH 8.2 with 0.02 N NaOH.

Total solids of the fermented product were determined by drying weighed samples of the homogenate at 50°C in a vacuum oven for 24 h. Nitrogen was determined on weighed samples by the micro-Kjeldahl method of analysis (1), with total crude protein estimated by the conversion factor 6.25. Soluble solids and soluble nitrogen were determined likewise on the supernatant after centrifugation of the homogenate at 14,000 × *g* for 15 min in a Sorvall centrifuge, model SS-3. Soluble nitrogen is reported as a percentage of total nitrogen.

Soluble reducing sugars were determined as D-glucose by a colorimetric copper method (12). Approximately 20 ml of the above supernatant was passed through a Dowex 50W-X8 column to remove enzymatic activity. The last 5 ml of the eluate was retained, from which a sample was diluted to achieve a reducing sugar concentration of 0.05 to 3.0 mg/ml.

Starch was isolated quantitatively from the freeze-dried product by the procedure of Hassid and Neufeld (7) as outlined by Joslyn (9). From appropriately diluted samples of the starch solutions, starch was determined colorimetrically as D-glucose by reaction with anthrone in 95% H_2SO_4 (10).

Ethanol was separated and determined quantitatively by gas chromatography. The gas chromatographic instrument used was a Beckman model GC-5 equipped with a flame ionization detector. A coiled stainless-steel column, 3 feet by 1/8 inch (outer diameter) (ca. 91.4 by 0.32 cm) with a wall thickness of 0.02 inch (0.05 cm), was fitted into the injection port to allow on-column injection. The column packing

was Porapak Q-S, 100/120 mesh. The column oven was maintained at 115°C, with the detector oven and the injector port maintained at 235 and 200°C, respectively. The flow rate of the carrier gas (nitrogen) was maintained at 25 ml/min. The instrument sensitivity was set at 2×10^{-2} A, full scale.

A Hewlett-Packard integrator, model 3370A, was connected in series to the gas chromatographic instrument to determine the peak areas. A visual record of the chromatogram was achieved by use of a Beckman 10-inch recorder (1 mV, 0.5 inch [1.27 cm]/min).

Standard aqueous solutions containing 0.25, 0.50, 0.75, 1.00, 1.25, and 1.50% (vol/vol) ethanol were prepared. A 2.0% (vol/vol) acetone solution was used as the internal standard. Chromatographic peak areas were determined by electronic integration, and the peak area ratio, ethanol-internal standard, was calculated. Retention times (T_r) were 1.01 and 1.78 for ethanol and acetone, respectively.

A 1.0-ml amount of homogenized, fermented product was transferred to a gas-tight centrifuge tube and diluted with an equal volume (1.0 ml) of the internal standard solution. The tube was stoppered, and the contents were mixed well and centrifuged for 5 min at 2,500 rpm. From the supernatant, a 2.5-μl sample was injected into the column with a no. 701 Hamilton syringe. The percentage of ethanol obtained was then multiplied by the corresponding dilution factor.

Thiamine was determined by the thiochrome procedure (6). A phosphatase solution (Mylase P, prepared from *Aspergillus oryzae*) was used to aid in the conversion of bound thiamine to its free form, with activated Decalso used in the purification procedure. Unenriched polished rice was used for this portion of the study.

RESULTS AND DISCUSSION

Tapé ketan produced by fermenting steamed rice with *A. rouxii* (formerly *C. oryzae* [5]) in combination with *E. burtonii* had a sweet, slightly acid taste with a mildly alcoholic flavor. These were the organisms that Djien (3) isolated and reported as capable of producing a typical, good-flavored tapé. Between 36 and 48 h of fermentation at 30°C, the product was soft and juicy and ready for consumption, the individual rice grains having become a cohesive, yet porous, mass. Beyond 48 h the rice substrate was largely liquified, and the product could either be consumed directly or used in the production of brem or arak.

The mycelium of *C. oryzae* was first visible after 24 h of fermentation at 30°C. After an additional 12 to 24 h, the rice grains were almost totally covered with fine, white mycelium. Since *A. rouxii* does not form free spores, no discoloration was observed.

The development of yeast was first noticed after 36 to 48 h as small, white, unevenly distributed patches on the surface of the rice. Only very slight, localized liquefaction of the rice occurred when inoculated with *E. burtonii* alone.

In rice fermented with *A. rouxii*, starch decreased only slightly (78 to 73%) during the first 24 h but decreased sharply to about 12% through 48 h, reaching less than 2% after 144 h (Fig. 1). The decrease in starch reflects the extent to which liquefaction of the substrate had occurred. With the mold and the yeast combined, the decrease in starch to 48% was slightly more rapid during the first 36 h (Fig. 2); but the total amount of starch remained higher (about 8%) beyond 96 h, presumably as a result of yeast-produced glycogen which would be expected to be recovered as "starch" in the analysis (7).

With both the mold and the mold plus yeast fermentations, reducing sugars increased from less than 1% to approximately 5% in 24 h and reached maximum concentration, 16 to 17%, between 36 and 48 h. Beyond 48 h the concentration of reducing sugars leveled off in the mold fermentation and decreased in the combined fermentation due to use of the glucose by the yeast.

The pH of rice fermented with *A. rouxii* decreased only slightly during the first 24 h, fol-

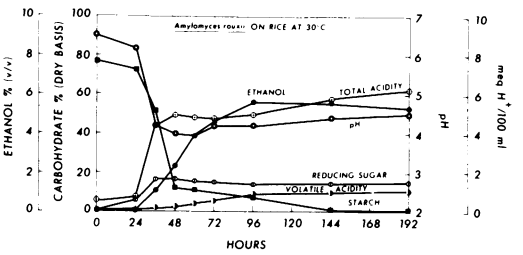

FIG. 1. *Changes in starch, reducing sugars, ethanol, pH, and total and volatile acidity occurring in rice fermented with A. rouxii at 30°C.*

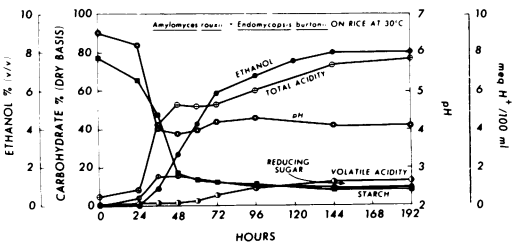

FIG. 2. *Changes in starch, reducing sugars, ethanol, pH, and total and volatile acidity occurring in rice fermented with A. rouxii in combination with E. burtonii at 30°C.*

lowed by a sharp decrease from 6.3 to 4.0 during the next 24 h. The pH then gradually increased to 4.5 with continued fermentation. Tapé ketan produced by *A. rouxii* in combination with *E. burtonii* had a pH of 3.9 at 48 h. Beyond 48 h there was some fluctuation, but the pH remained less than 4.3 throughout the 192 h. A pH of approximately 4.0 is considered typical for the fermented product (3).

The total acidity of the rice fermented by *A. rouxii* increased from 0.52 to 4.94 meq of H^+ per 100 ml and to 5.32 meq of H^+ per 100 ml for the combined fermentation within 48 h. At 192 h, total acidity for the mold fermentation was about 6.25 meq of H^+ per 100 ml, and it was nearly 8 meq of H^+ per 100 ml for the combined fermentation (Fig. 1 and 2).

At 48 h, there was essentially no difference in the volatile acidity for the mold fermentation, 0.26 meq of H^+ per 100 ml, compared with 0.20 for the combined fermentation. At 96 h, the volatile acidity was nearly 1 meq of H^+ per 100 ml in both fermentations. As fermentation continued, there was a slightly higher (1.40 meq of H^+ per 100 ml) volatile acidity in the combined fermentation at 144 to 192 h.

After an initial 24-h lag, rice fermented by the mold alone contained 2.3% (vol/vol) ethanol at 48 h. At 96 h a maximum concentration of 5.6% (vol/vol) ethanol was achieved. Thereafter, the concentration decreased slightly, due presumably to further metabolism or loss by evaporation.

The concentration of ethanol in rice fermented with *A. rouxii* in combination with *E. burtonii* was 2.7% (vol/vol) at 48 h, increased to 6.4% at 72 h, and reached a maximum concentration of 8.0% (vol/vol) at 144 h.

Endomycopsis fibuligera, *Candida lactosa*, *C. melinii*, and *C. parapsilosis*, each in combination with *A. rouxii*, had increased the ethanol concentration a minimum of 1.0% (vol/vol) above that produced by *A. rouxii* alone at 48 h (Table 2). At 96 h there were smaller differences between the ethanol concentrations of the products fermented by these same yeasts and *E. burtonii*. At 144 h, however, the highest concentration of ethanol was produced by *E. burtonii* plus the mold.

Ethanol concentrations similar to those produced by *A. rouxii* in combination with *E. burtonii* were also produced in combination with *Hansenula anomala*, *H. malanga*, and *H. subpelliculosa* at 48 h. However, by 96 h the ethanol concentrations of the rice fermented by these combinations were markedly lower than the concentrations present in the rice fermented by the other yeasts plus the mold. Ethanol concentrations resulting from these species of *Hansenula* were nearly as low or lower than that present in the rice fermented by the mold alone.

The rice fermented in part by *H. anomala* and *H. subpelliculosa* developed a strong odor of ethyl acetate, which might decrease palatability to some consumers. The esterification of acetic acid with ethanol to form ethyl acetate accounts partially for the decreased concentrations of ethanol observed. Also, these species of *Hansenula* may produce less ethanol than *Endomycopsis* and *Candida* species. No ethyl acetate aroma was produced by *H. malanga*.

Temperature also influenced the concentration of ethanol present in tapé ketan (Table 3). In product fermented with *A. rouxii* alone and in combination with *E. burtonii*, the concentration of ethanol at 36 h increased as incubation temperature was raised from 25 to 35°C. There was also an increase in the concentration of ethanol for both fermentations at 48 and 96 h with a temperature increase from 25 to 30°C. Maximum ethanol production (8.5%, vol/vol) was achieved by the mold plus yeast incubated at 25°C for 144 h.

TABLE 2. *Ethanol production and accumulation by A. rouxii alone and in combination with various yeasts on rice at 30°C*

Organisms	Ethanol (%, vol/vol)[a]			
	24 h[b]	48 h	96 h	144 h
A. rouxii	<0.1	2.3	5.6	5.6
A. rouxii + *E. burtonii*	<0.1	2.7	6.8	8.0
A. rouxii + *E. fibuligera*	<0.1	3.7	7.0	7.7
A. rouxii + *C. lactosa*	<0.1	3.3	7.4	7.3
A. rouxii + *C. melinii*	<0.1	3.4	7.3	7.5
A. rouxii + *C. parapsilosis*	<0.1	3.6	7.2	7.6
A. rouxii + *H. anomala*	<0.1	2.6	3.2	3.7
A. rouxii + *H. malanga*	<0.1	2.8	5.6	6.0
A. rouxii + *H. subpelliculosa*	<0.1	2.6	4.2	4.2

[a] Each entry is a mean of triplicate analyses.
[b] Length of fermentation.

TABLE 3. *Influence of temperature on ethanol production and accumulation by A. rouxii alone and in combination with E. burtonii on rice*

Organisms	Temp (°C)	Ethanol (%, vol/vol)[a]			
		36 h[b]	48 h	96 h	144 h
A. rouxii	25	0.2	2.0	5.3	5.6
	30	1.4	2.7	5.3	5.2
	35	1.9	3.2	3.5	3.5
A. rouxii + *E. burtonii*	25	0.2	2.4	6.0	8.5
	30	1.3	3.0	6.9	7.2
	35	1.5	2.4	5.1	4.6

[a] Each entry is a mean of triplicate analyses.
[b] Length of fermentation.

An incubation temperature of 30°C appears optimal for maximum production and accumulation of ethanol in tapé fermented for 48 h by *A. rouxii* and *E. burtonii*. However, to achieve the maximum concentration of ethanol for the production of arak, 144 h at 25°C would be most effective.

In rice fermented by *A. rouxii*, total solids changed little during the first 36 h; by 48 h there was a 10% reduction in solids and by 96 h there was nearly a 30% reduction (Fig. 3). Beyond 96 h no further decrease in total solids was observed. A similar reduction in total solids was observed for the combined fermentation in the initial 48 h (Fig. 4); however, beyond 48 h, the loss of solids was greater for the combined fermentation than for the fermentation by the mold, approximately 40% of the solids having been hydrolyzed by the combined action of the mold and yeast, between 96 and 144 h (Fig. 4).

Soluble solids increased sharply, from approximately 5 to 67% during the first 36 h of the fermentation with *A. rouxii* alone and in combination with *E. burtonii*. The decrease in soluble solids beyond 48 h reflects the use of sugars for the production of ethanol. The decrease was greater with the yeast present, with soluble solids finally reaching a level of about 40% (192 h).

On a dry-weight basis, the total crude protein of the *A. rouxii*-fermented rice increased approximately 1% between 36 and 48 h (Fig. 3). Crude protein continued to increase by nearly 3.5% during the following 48 h. This increase reflects the corresponding decrease in total solids. As a result of a greater decrease in total solids in the rice fermented by the mold and yeast occurring after 48 h (Fig. 4), the increase in total crude protein was also greater than that for the rice fermented by the mold alone. Total crude protein exceeded 16% at 192 h for the combined fermentation versus 12% for the rice fermented without the yeast.

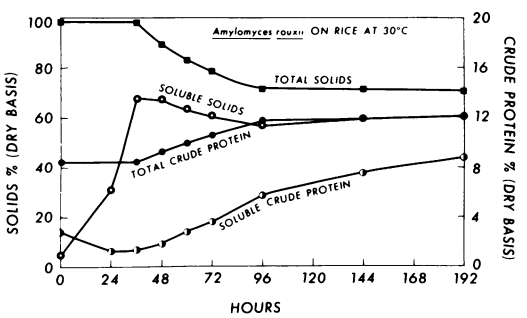

FIG. 3. *Changes in total and soluble solids and total and soluble crude protein occurring in rice fermented with A. rouxii at 30°C.*

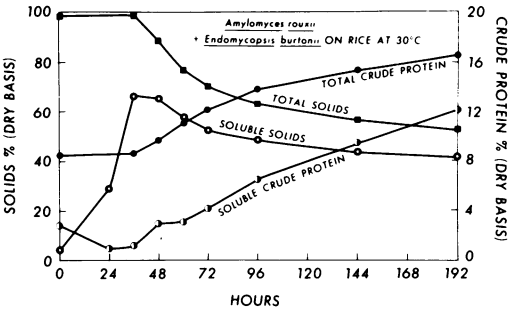

FIG. 4. *Changes in total and soluble solids and total and soluble protein occurring in rice fermented with A. rouxii in combination with E. burtonii at 30°C.*

With *A. rouxii*-fermented rice, the soluble crude protein decreased during the first 24 h and thereafter continually increased. The rapid assimilation of nitrogen by the developing mold during the early stages of the fermentation may account for the initial loss in the soluble protein. At 192 h, soluble crude protein for the mold was about 9% versus 12% in the mold plus yeast fermentation.

Changes in total and soluble solids, total and soluble crude protein, reducing sugars, pH, and total and volatile acidity were in general similar for rice fermented with *E. fibuligera*, *C. lactosa*, *C. melinii*, and *C. parapsilosis* (Table 4). Total solids decreased at a slower rate in rice fermented with *H. anomala*, *H. malanga*, and *H. subpelliculosa*, and consequently the percentages of total and soluble proteins at each interval were also lower. In contrast, the percentage of soluble solids at each interval was higher.

Higher concentrations of reducing sugars were attained with the *Hansenula* species than with the *E. fibuligera* and the *Candida* species studied. There were no appreciable differences in pH. For all yeasts studied, total and volatile acidity followed similar patterns of increase through 96 h. At 96 h, and also at 192 h, the *Hansenula* species produced higher levels of volatile acidity than that observed for other yeasts. Total acidity was appreciably higher only in rice fermented for 192 h with *H. anomala* and *H. subpelliculosa*.

In rice fermented with *E. burtonii* plus *A. rouxii* (Fig. 4), there was less decrease in total solids, which would account for a lower percentage of crude protein compared with fermentations involving certain yeasts in Table 4. The highest concentration of both soluble solids and reducing sugars occurred in rice fermented with *E. burtonii* plus *A. rouxii*. In typical tapé ketan fermented for 48 h, there were no appre-

TABLE 4. *Changes in total and soluble solids (TS; SS), total and soluble crude protein (TCP; SCP), reducing sugars (RS), pH, and total and volatile acidity (TA; VA) occurring in rice fermented with A. rouxii in combination with various yeasts*[a]

Rice fermented with:	Time (h)	TS (%)	SS (%)	TCP (%)	SCP (%)	RS (%)	pH	TA (meq of H^+/100 g)	VA (meq of H^+/100 g)
Unfermented	0	98.7	2.2	8.4	7.8	0.1	6.8	3.00	0.26
A. rouxii + E. fibuligera	36	92.8	48.3	8.6	1.1	10.9	4.3	3.40	0.36
	48	79.1	49.5	10.0	1.6	11.8	4.2	4.18	0.62
	96	50.5	32.9	16.1	5.8	7.9	4.3	5.23	0.81
	192	43.9	30.9	18.4	10.6	7.0	4.4	5.81	0.85
A. rouxii + C. lactosa	36	92.8	47.2	8.8	1.1	11.2	4.3	3.42	0.33
	48	78.1	46.4	10.4	1.6	10.8	4.3	3.95	0.68
	96	54.5	37.0	14.8	5.6	9.0	4.4	5.33	0.80
	192	47.4	33.3	17.0	10.4	8.0	4.5	5.74	0.99
A. rouxii + C. melinii	36	93.3	46.6	8.8	1.1	10.9	4.4	3.33	0.31
	48	77.7	47.8	10.2	1.6	11.4	4.2	4.01	0.55
	96	55.7	37.5	14.6	6.8	9.2	4.5	5.11	0.71
	192	43.7	30.7	17.7	11.4	7.5	4.5	5.73	0.91
A. rouxii + C. parapsilosis	36	93.5	45.6	8.6	1.1	10.4	4.4	3.20	0.36
	48	80.0	49.5	10.2	1.6	11.9	4.2	4.48	0.52
	96	54.2	36.8	14.9	6.0	9.0	4.3	5.67	0.71
	192	51.2	37.0	16.5	10.0	8.9	4.4	6.44	0.95
A. rouxii + H. anomala	36	96.3	52.1	8.2	0.9	11.2	4.4	3.38	0.33
	48	84.6	54.1	9.4	1.2	13.0	4.4	3.64	0.65
	96	67.9	51.5	11.8	4.1	12.9	4.4	5.69	1.88
	192	57.0	46.0	14.0	7.7	11.4	4.3	8.11	3.68
A. rouxii + H. malanga	36	96.4	51.8	8.2	1.0	10.2	4.2	3.85	0.28
	48	85.8	62.7	9.4	1.2	12.9	4.2	4.20	0.47
	96	67.4	51.4	12.1	4.2	12.7	4.4	5.68	1.33
	192	58.7	48.7	13.8	8.0	11.9	4.4	6.85	1.58
A. rouxii + H. subpelliculosa	36	96.2	62.3	8.4	1.0	12.4	4.2	3.83	0.33
	48	87.0	60.5	9.1	1.1	14.4	4.3	3.77	0.49
	96	64.0	48.8	12.6	3.8	12.4	4.3	6.24	1.55
	192	50.4	39.2	15.6	8.0	10.1	4.3	7.05	2.78

[a] Each entry is a mean of triplicate analyses.

TABLE 5. *Changes in the thiamine concentration of rice fermented with A. rouxii alone and in combination with E. burtonii at 30°C*

Fermentation with:	Thiamine (mg/100 g)[a]			
	0 h[b]	48 h	72 h	192 h
A. rouxii	0.04	0.07	0.10	0.12
A. rouxii + E. burtonii	0.04	0.11	0.12	0.12

[a] Each entry is a mean of duplicate analyses.
[b] Length of fermentation.

TABLE 6. *Thiamine concentration of rice fermented for 48 h with A. rouxii in combination with various yeasts at 30°C*

Organisms	Thiamine (mg/100 g)[a]
Unfermented rice	0.04
A. rouxii + C. lactosa	0.13
A. rouxii + C. melinii	0.13
A. rouxii + C. parapsilosis	0.13
A. rouxii + H. anomala	0.12
A. rouxii + H. malanga	0.11
A. rouxii + H. subpelliculosa	0.12

[a] Each entry is a mean of duplicate analyses.

ciable differences between the rice fermented with *A. rouxii* separately and that fermented by the mold in combination with these various yeasts, or with *E. burtonii*. Organoleptically, the products prepared with the *Candida* species, *E. fibuligera*, and *H. malanga* were palatable but lacked the desirable flavor associated with tapé ketan prepared by using *E. burtonii*.

The thiamine content of the rice increased nearly three times as a result of fermentation

by *A. rouxii* in combination with *E. burtonii* (Table 5). The fermentation of rice by other yeasts, each in combination with *A. rouxii*, also resulted in similar increases in the thiamine concentration (Table 6).

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