THE PREPARATION OF BREM RAGI - AN IMPROVED METHOD

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ABSTRACT

The ragi made by the improved method, using pure cultures of the selected molds and yeasts, CB3-RM1 and AU3-K3, had a much higher counts of fermentative yeast (10^7 and 10^8) but lower counts of mold (10^4 both) than the commercial ragi from Solo. This commercial ragi had a true yeast counts of 10^5 and amylolytic mold counts of 10^5. No filamentous yeast and no lactic-acid bacteria were present in both of the new ragi, whereas in the commercial ragi these micro-organisms were present in high numbers, 10^8 of filamentous yeast and 10^4 of lactics.

Brem wine made from CB3-RM1 and AU3-K3 ragi had a quite higher ethanol % (10.83 and 10.68%), lower reducing sugar % (7.43 and 5.17%), lower ml of titratable acidity (6.82 and 6.22 ml), higher pH values (4.84 and 4.97) and higher formol nitrogen % (0.112 and 0.0105%) as compared to the commercial ragi's wine. The wine made from the commercial ragi had an ethanol of 8.70%, reducing sugar of 8.02%, ml. T.A. of 10.03, pH of 4.19 and formol nitrogen of 0.0070%.

Brem wine produced from CB3-RM1 ragi had a grape aroma and more desirable than the commercial one. However, brem wine from AU3-K3 was not prefered due to its strong fusel-oil aroma.

INTRODUCTION

Ragi is an Indonesian traditional starter for the fermentation of starch-rich substrates, like casava, glutinous rice and ordinary rice, into fermentation products of tafu, brem-wine, brem dry-cake, badek and arak beras.

The ragi preparation is done by the traditional method. It is made from rice flour mixed with aromatic plant materials such as garlic, galanga rhizome, pepper, red chillies, cinnamon, etc, and same water into a dough. Afterwards it is malded into flat balls, inoculated with powdered ragi, and sundried after incubation or a layer of rice straw. This method of ragi preparation could not control the microorganisms coming from the ragi ingredients, hence, the quality of the fermentation produced from such ragi would vary greatly. The microflora of ragi is numerous and variable (Dwidjosepoetro 1970, Saono *et al.* 1974, Hadisepoetro *et al.* 1979, Toyota and Kozaki 1978).

From an earlier experiment (Saono 1983), it was proved that spices were not essential for making ragi if the preparation condition is under control and pure cultures of productive microorganisms were applied. And spices could even cause countamination of undesirable microorganisms into the ragi.

The purpose of this study is to prepare ragi for brem wine fermentation using an improved method by incorporating selected strains of molds and yeasts.

MATERIALS AND METHODS

Amylomyces rouxii strains AU3 and CB_3 were isolated from ragi collected from Krasak-ageng, Pekalongan, and Kebon-pedes, Sukabumi, respectively. *Saccharamyces cerevisiae* strains RM_1 and K_3 were isolated from ragi produced in Solo.

Pure cultures of *Rhizopus formosaensis* was obtained from FIRDI, Taiwan, and *Saccharamyces cerevisiae* Taiken from Lab. of Applied Microbiology, Tokyo Univ. of Agriculture.

Ragi making by improved method: Firstly, the seed for ragi was made by heating of 20 g rice flour in a petri-dish in oven at 105°C for 18 hours, and after cooling, it was inoculated with one slant of one week mold culture on potato dextrose agar scraped by spatula in 9 ml of sterile distilled water, and 3 loupe of 3 day old yeast culture on yeast extract malt extract agar suspended into another 9 ml of sterile water containing no or 0.2 g saccharose. Then, they were mixed thoroughly with a spoon and incubated at 30°C for *R. formosaensis* and 25—28°C for the ragi molds AU3 and CB3, for a period of 2—3 days; with 1 day in closed condition and 2 days in slightly open condition. The mold covered rice dough was dried in drying oven at 42—44°C for 2 days. This process was done aseptically. This seed ragi was used to inoculate a larger batch of 500 g of rice flour at the rate of 5 g of seed ragi and 450 g of water with 5 grams of cane sugar that had been cooked. These ingredients were mixed and spread on an aluminium foil into thin layer of 0.5—1.0 cm thick, covered with an aluminium foil and kept at room temperature for 3 days with 1 day covered and 2 days slightly open. Prior to drying, the molded dough was cut into squares of 3 cm × 3 cm to facilitate the drying process.

The mold and yeast counts were done by plating method on yeast extract malt extract agar medium (this medium was better for counting ragi molds than potato dextrose agar by giving higher counts and restricted growth of colonies and not too fast spreading of mycelial growth) after quantitative dilution of the finely ground ragi, aseptically. Incubation was done at 27°C for 1 day for mold and 2 days for yeast.

Brem making from improved ragi: 100 g of black glutinous rice and 150 g of water in a container was autoclaved at 15 p.s.i. for 15 minutes, cooled, and inoculated by 250 mg of ragi containing mold and yeast. Incubated at 30°C (for ragi containing *R. formosaensis* and "B" ragi) and 25—27°C (for ragi containing AU3 and CB3 molds) for 7 days. Then the saccharified rice was pressed to squeez out the liquor. The liquor was tranfered into a screw-capped

jar for further alcoholic fermentation at 25°C for 14 days. The brem wine was aged for 2 months at refrigerator temperature (5—8°C). Analyses were done for ethanol % (gas chromatography after ether-pentane (2:1) extraction), reducing sugar % (Somogy method), formol nitrogen, ml of total acidity, and pH (Horiba pH meter M7).

RESULTS AND DISCUSSION

Ragi by the Improved Method

During the preparation of ragi, it was observed that *R. formosaensis* and *S. cerevisiae* strains grew well at 30°C and the aeration during incubation needed to be controlled by closing the petri-dish for 1 day and then opening it slightly for 2 days. However, the ragi molds AU3 and CB3 needed a lower temperature of 25—27°C for good growth. It was also noticed that the addition of sugar was harmful to the CB3-RM1 ragi; it produced a watery ragi after 3 days of incubation period. But, the presence of sugar did not cause any harm to the ragi containing *R. formosaensis* and the wine yeasts.

Level of heat treatment given to the rice flour had an effect on the sporulation of *R. formosaensis*. Flour thar had been heated at 105°C for 6 hours did not cause the growing mold to sporulate, while the heat treatment at 120°C for 4 hours which caused browning of the flour resulted in sporulation of this mold after 3 days of incubation at 30°C. And plating of both flour on glucose yeast extract malt extract agar showed no bacterial growth nor noticeable odor. Therefore, for ragi making the extend of heating at 105°C for 6 hours would be adequate.

The microbial counts of ragi are presented in Table 1. It is shown here that the number of fermentative yeasts were quite high in improved ragi than in the commercial ragi, but the mold counts were lower. However, in an earlier experiment (Saono, 1983) it was proved that even at a low counts (10^2) W2 mold had a higher saccharifying and liquifying activities than at higher counts

Table 1. Microbial counts of improved ragi and the B ragi, per gram of ragi.

Ragi	Mold	Yeast		Lactic acid bacteria
		True	Filamentous	
R.for-S.sake	2×10^4	6.4×10^5	0	0
R.for-S.steinberg	2×10^4	1.1×10^5	0	0
R.for-S.taiken	3×10^3	2.1×10^7	0	0
CB3-RM1	2×10^4	1.9×10^7	0	0
AU3-K3	8×10^4	1.7×10^8	0	0
B ragi	5×10^5	1.0×10^5	2.2×10^8	4×10^4

(10^4). Because, in a suitable medium or substrate a Mucoraceous mold can spreads rapidly.

In the commercial ragi, a great number of filamentous yeast were found besides lactic-acid bacteria. The presence of these microorganisms may be undesirable since they both could lower the pH of the brem wine too much.

The numbers of fermentative ragi yeasts K3 and RM1 in the improved ragi were much higher than the wine yeasts. It seemed that the development in rice flour of ragi yeasts was better than the wine yeasts.

Table 2 shows that the brem produced by ragi CB3-RM1 and AU3-K3 had better alcohol content than the brem produced by ragi *R. formosaensis - S. cerevisiae* steinberg, sake or taiken, and also "B" ragi. The degree of alcohol production may be due to the combined action of the yeast and the mold.

The %Brix values were quite high; this %Brix indicates all the soluble solids present in the brem, including the unfermented sugars.

The acidity of brem could be seen from the ml NaOH titration value and pH value. The ml T.A. of brem from improved ragis were much lower and the pH quite higher than the brem produced by "B" ragi. Perhaps this higher acidity by "B" ragi was due to the lactic-acid bacteria and fillamentous yeasts present in this ragi as shown in an earlier experiment.

The formol nitrogen in brem produced by the improved ragis were quite higher than in brem by "B" ragi, and quite lower than in Japanese sake. Formol nitrogen indicates the amino type nitrogen or amino acids present in the brem. The amino acid are formed during fermentation from the protein in the rice substrate by the attack of protease from mycoflora, and, amino acids on the other hand are used up by the yeast for its growth and in the reactions to form higher-alcohols and esters.

Table 2. Characteristics of the brem wine made from improved ragi, compared to the B ragi, bubod and sake.

Ragi	Ethancl %	Reducing sugar %	% Brix	Formol N %	Ta ml	pH
R.for-S.sake	7.91	9.85	17.7	0.0154	6.82	4.81
R.for-S.steinb	7.28	4.92	14.0	0.0154	7.87	4.81
R.for-S.taiken	9.82	10.44	19.0	0.0211	5.82	5.05
CB3-RM1	10.83	7.43	15.8	0.0112	6.82	4.84
AU3-K3	10.68	5.17	14.2	0.0105	6.22	4.97
B ragi	8.70	8.02	17.5	0.0070	10.03	4.19
Philippine bubod*	12.5-19.1	0 - 5.44	8-9 —	0.0045-0.0257	6.55-22.49	3.3-4.9
Japanese sake**	15.0	4.20	—	0.0288	1.52	—

* Tanimura *et al.* 1977; Del Rosario 1980.

** Hayashida *et al.* 1968.

The results of the aroma components analysis given in Table 3 shows that the total analysable aroma components was highest in CB3-RM1 brem (1952.32 ppm) and lowest in "B" ragi (1089.75 ppm). Ethyl acetate was quite high in "B" ragi and CB3-RM1 brems, iso-butyl alcohol was distinctively high in CB3-RM1 and Rfor-sake brems, and an unknown compound*, iso-amyl alcohol and ethyl caproate were very high in CB3-RM1 and AU3-K3 brems. Propyl alcohol was not detected in the brems, whereas methyl alcohol was present only in Rfor-steinberg and Rfor-sake only.

Cronk *et al.* (1979) studied the higher alcohols produced by ragi micro-organisms in tape ketan fermentation; the largest amount of fusel oil produced were iso-amyl alcohol and iso-butanol, but no n-propanol was detected. *Amylomyces rouxii* produced 275 ppm, *A. rouxii - Endomycopsis fibulgera* 558 ppm, *A. rouxii-Candida* sp. 618 ppm, and *A. rouxii-Hansenula* sp. 248 ppm of ethyl acetate after 8 days.

The famous Batavia arak, a distilled ricewine from fermentation by ragi, was highly demanded in Europe (Raffles 1830; Prinsen Geerligs 1905). This arak contained high amount of volatile acids and esters, aldehyde, furfural, and higher-alcohols as analysed by Prinsen Geerligs (Table 3). And, De Kruyff (1909) suggested the yeasts responsible for the good aroma of Batavia arak to be the now called *Saccharomyces pombe* and *Willia indica,* whereas in the lower quality Cheribon arak were *S. cerevisiae* and *Hansenula anomala.*

Brem, similar to Cronk's tape ketan has a much higher concentration of higher-alcohols than sake, this may distinguish the brem wine from sake. Brem is made of completely different substrate and fermented by different micro-organisms, also the method of preparation differed from that of sake. The higher-alcohols or fusel-oil in brem wine may have been derived from the proteins in the unpolished rice, through the action of the proteases of the microorganisms.

The brem wine produced from CB3-RM1 ragi had a nice grape juice aroma and made it desirable. However, the brem wine from AU3-K3 was less acceptable due to its strong aroma. And, the flavor of the brem wine made from *R. formosaensis* and the wine yeasts *S. cerevisiae* steinberg, taiken and sake were all prefered than the commercial ragi "B".

"B" ragi wine had the highest ethyl acetate content, which might be attributed to the *H. anomala* present in that ragi. Cronk *et al.* (1979) experiment showed that *Hansenula* sp. produced very high concentration of ethyl acetate, 354—369 ppm, in tape ketan fermentation. According to Kunkee and Amerine (1970), *Saccharomyces* sp., *Torulaspora* sp. and *Torulopsis* sp. generally produced ethyl acetate at low rate, whereas *Kloeckera apiculata, S.*

* That has a retention time very close to iso-amyl alcohol.

ludwigii, and especially *Pichia* sp. and *Hansenula* sp. produced ethyl acetate at high rate. However, too high ethyl acetate content in wine, over 225 ppm, was considered to give an aroma of spoiled fruit (Kramer and Twigg 1966).

Table 3. The concentration of aroma components (ppm) in brem-wine produced by improved ragi, compared to Roda Mas R ragi.

Aroma components	Roda Mas R	Rfor-stein	Rfor-sake	Rfor-taiken	AU3-K3	CB3-RM1
Acetaldehyde	18.33	17.98	27.71	46.13	35.35	86.83
Acetone	6.45	16.84	30.21	34.82	19.37	54.44
Ethyl-acetate	92.58	15.54	20.70	21.89	19.29	60.83
Methyl-alcohol	–	21.76	36.83	–	–	+
Propyl-alcohol	–	–	–	–	–	–
iso-Butyl alcohol	397.58	272.95	650.38	299.01	344.41	737.61
Unknown	166.58	184.91	206.84	187.75	252.54	301.30
n-Butyl alcohol	–	–	–	–	–	–
iso-Amyl alcohol	157.97	151.91	150.33	171.16	430.82	234.69
Unknown	0.70	1.33	1.36	0.94	1.06	0.91
Ethyl-caproate	114.60	241.14	178.68	229.76	429.88	317.69
Unknown	2.37	2.51	1.17	4.19	1.15	0.21
iso-Butyl-caproate	4.77	4.57	0.09	0.07	–	+
Unknown	1.37	1.79	1.10	0.11	+	1.82
Unknown	0.33	0.28	–	–	–	–
n-Caprilic acid	60.59	126.75	38.89	68.12	18.65	35.51
Unknown	–	1.07	0.14	–	–	–
Pelargonic acid	0.40	0.63	4.62	4.97	+	–
Unknown	+	9.36	–	8.65	–	–
Unknown	0.10	6.68	11.82	–	1.47	4.02
Unknown	2.46	0.45	–	–	–	–
Unknown	2.35	–	–	–	0.58	0.41
Ethyl-caprate	0.30	0.90	0.58	0.90	0.05	+
Unknown	1.73	0.29	+	0.21	–	–
Unknown	2.35	0.68	0.33	–	0.44	3.45
Unknown	0.09	0.87	0.49	2.28	3.98	3.13
Unknown	5.34	5.14	1.67	0.96	1.77	5.04
Unknown	4.97	3.46	7.27	0.50	1.20	–
Ethyl-phenyl-acetate	13.67	19.40	24.22	10.56	60.75	18.90
Ethyl-laurate	0.83	0.28	3.06	–	2.24	0.91
Unknown	30.88	57.38	77.57	101.55	126.04	84.61
Unknown	0.06	–	–	–	–	–
Total	1089.75	1166.85	1476.06	1194.31	1751.04	1952.32

Improved method of ragi preparation using pure cultures of productive stains of molds and yeasts isolated from ragi, made it possible to elminate undesirable microorganisms coming into the ragi, and hence, produced a better quality of brem wine.

Besides the essential microorganisms the saccharifying *Amylomyces rouxii* or *Rhizopus* sp. and alcohol-ester producing yeast *Saccharomyces cerevisiae*, other microorganisms with a desirable flavor could be added, such as *Pediococcus pentosaceus* with a pleasant lactic acid taste and also *Hansenula* sp. with a high ethyl-acetate producing capacity.

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