Research article

Improvement of Khmer Traditional Rice Liquor (Sraa Sor) Productivity Using Different Fermentative Conditions

CHIM CHAY*
Royal University of Agriculture, Phnom Penh, Cambodia
Email: chaychim@yahoo.com

CHAKRIYA NORNG
Royal University of Agriculture, Phnom Penh, Cambodia

DINA KOU
Royal University of Agriculture, Phnom Penh, Cambodia

HAMANO MITSURU
International Center for Agriculture Education, Nagoya University, Japan

Received 16 December 2011 Accepted 12 March 2012 (*: Corresponding Author)

Abstract The objective of this research was to establish suitable conditions for rice liquor (*Sraa sor*) in order to improve its productivity in rural areas. Five treatments of fermented rice conditions were used: cool temperature, ambient temperature, ambient temperature with plastic bag-covered pot, high temperature and room temperature condition. Data of temperature, productivity, pure ethanol and ethanol percentage was collected. The data was analyzed using SPSS version 15 in general linear model univariate to identify the significant level. The results of analysis indicated that fermentation temperature had a strong significance at ($p<0.05$). Fermented rice changeable temperature was caused by ambient temperature. So the ambient temperature is very necessary for fermented rice conditions. Yeast activity is reduced by low temperature. At temperature between 30-38 ℃ in the fermented rice condition, the productivity, pure ethanol and ethanol percentage had strong significance ($p<0.05$). Lowest productivity was observed in cool temperature condition. In summary, rice fermentation in room temperature was found to be the best condition, while ambient temperature with plastic bag-cover pot was possible in rural area for development of rice liquor productivity. However, the level of added water during fermentation and period of fermentation should be examined.

Keywords Khmer traditional rice liquor (*Sraa sor*), fermentative condition, alcoholic percentage

INTRODUCTION

Rice liquor (sraa sor) is obtained through traditional fermentation of steamed/boiled rice by *me sraa* (yeast) in Cambodia. The fermentation of steamed/boiled rice into rice liquor is a complex biochemical process, during which yeasts utilize sugars and other constituents of steamed/boiled rice as substrates for their growth, converting them to ethanol, carbon dioxide, and other metabolic end products that contribute to the chemical composition and sensory quality of the rice liquor. Several factors affect yeast growth during alcoholic fermentation, including cooking rice condition, sanitation of pots, and temperature of fermentation. However, rice liquor fermentation condition is the single most important aspect for ethanol productivity. The rice fermentation condition varies depending on season, especially cold season causes low productivity but this condition can be controlled and improved.

METHODS

Location

This experiment was conducted in the bio-processing lab of the Graduate School, Royal University of Agriculture. The experiment was conducted in April 2011, which is the hottest month in Cambodia.

Experimental designs

Five treatments of rice fermentation temperature conditions were done in 3 replicates (Fig. 1). Completely randomized experimental design was used. The fermentation conditions are shown below: 1) Fermented rice in cool temperature (CT): cool temperature was achieved by adding ice to the water in a plastic box. 2) Fermented rice in ambient temperature (AT): ambient temperature was a place with free air flow with a roof but no walls 3) Fermented rice in ambient temperature with plastic bag-covered pot (ATPC): the condition was similar to second treatment condition, but a plastic bag was used to cover the pot. 4) Fermented rice in room temperature (RT): this was placed in the bio-processing lab. And 5) fermented rice in high temperature (HT): the HT was achieved in a small room by turning on lamps throughout the fermentation.

CT AT ATPC HT RT

Fig. 1 Rice fermentation temperature in different conditions

Table 1 Experiment material for conducting the treatments

Fermented condition	Rep.	Rice amount (kg)	Yeast amount (g)	Amount of added water (kg)	Femented period (hr)
CT AT ATPC HT RT	3	1	25 (me sra Phùòng Trang)	4.6	96

Rice liquor processing

Rice preparation: Rice was weighed and washed with water 2 to 3 times to remove the rice brain and husks.

- **Pan preparation:** Water was poured into the cooking pan and heated on the steaming plate (or table iron). The washed rice was placed on a cloth and put on the surface of steaming plate and spread to a uniform surface and then covered with a cloth
- **Steaming:** The rice was steamed for 30 minutes. The steaming rice was stirred using a bamboo stick, and sprinkled with water again and covered; the water was added to be boiled again. The same steps were repeated as above but the amount of water was reduced a little. After the water was boiled for the second time for 30 minutes, the fire gradually reduced and the steamed rice was taken out from a steaming pan.
- **Mixed rice with yeast:** The steamed rice was taken out from the pan and placed on a plastic mat. The steamed rice was spread thinly on the mat. Steamed rice was cooled at room temperature while the temperature was decreased between 35 to 38 ℃, suitable for mixing; yeast was sprayed on the surface of the steamed rice. After which the steamed rice was uniformly mixed with yeast. The steamed rice mixed with yeast was put in glass.

Fermented rice condition:

- **Fermentation without water:** Steamed rice mixed with yeast was put into a glass. The fermentation temperature and conditions were observed at six hours over a 48-hour.
- **Fermentation with water:** After 48-hour fermentation, water was added into the glass. Water was twice the amount of milled rice. The temperature was checked every six hours over 48 hours. The total time of rice liquor fermentation was 96-hour including fermented rice without and with water.
- **Distillation of rice liquor:** The liquid part of fermented rice was put at the bottom of distillation pan while solid fermented rice was put in the surface of the steaming table. The burned fermented rice was placed in the distillation pan until rice liquor was dropped. The rice liquor was dropped until one litter per kilogram of milled rice was obtained.

Data collection: Temperature of the fermented rice was taken every six hours using a thermometer. The results are shown in the Table 2 below.

Table 2 Temperature records of rice fermented and ambient conditions (in degrees C)

	First day				Second day				Third day				Fourth day				DD
Time (24hr)	06	12	18	24	06	12	18	24	06	12	18	24	06	12	18	23	06
Fermented Period (hr)	MT		10	16	22	28	34	40	46	52	58	64	70	76	82	88	94
	Temperature checking in fermented rice																

MT = Temperature of steamed rice mixed with yeast, DD = Distillation day

Ethanol percentage: Measure the degree of alcoholic percentage by hydrometer and look up the % alcohol on a table at 15 ℃.

Pure ethanol: Pure ethanol (kg) = ethanol volume (l) x ethanol percentage (%)/100

Productivity ethanol per kilogram of milled rice: Productivity (l/kg) = pure ethanol (l) / milled rice volume (kg).

Statistical Analysis

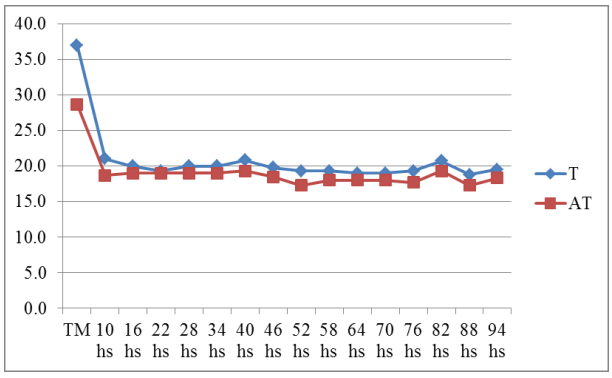
The data was analyzed using general linear model in the program SPSS version 15.0 for window XP. Multiple comparisons were conducted using Duncan at a significance level of $\alpha = 0.05$.

RESULTS AND DISCUSSION

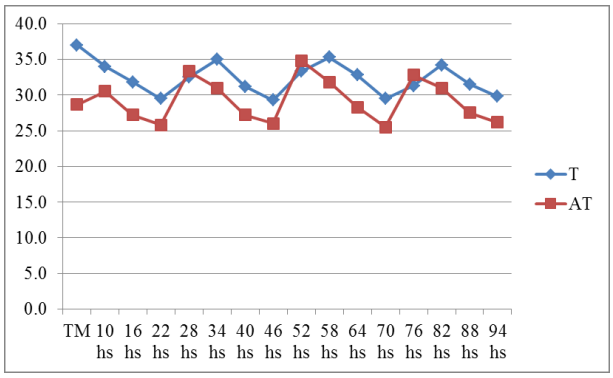
Fermented rice and ambient temperature

Fermented rice temperature had strong significance in different types of fermentation conditions ($P<0.05$). Fermented rice in cool temperature exhibited incomplete fermentation, as evidenced by continued yeast activity compared to the yeast activity observed in high temperature fermentation. Fermented rice in high temperature was more active than that in low temperature. Fermentable rice at low temperature was not completed because the period of fermentation was short, but it was complete at high temperature fermentation. The aroma of fermented rice at AT, ATPC and RT were good (sweet) but sour in CT and HT. Report by Fleet and Heard (1993) showed that temperature affects the rate of fermentation, with lower temperatures yielding slower fermenation rates and longer fermentation periods. At a low temperature, fermentation is exceedingly slow. But the low temperatures notably reduce the growth of acetic and lactic acid bacteria and they can facilitate the control of alcoholic fermentation (Ribereau-Gayon et al., 2002). On the other hand, fermented rice in high temperature condition was quite rapid with complete fermentation as compared to low temperature.

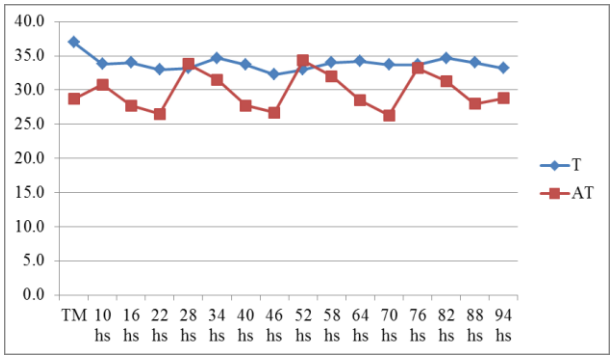
Fermented rice was variable depending on the ambient temperature (Fig. 2a). However, fermented rice in ambient temperature with plastic bag-covered pot was stable but ambient temperature varied according to the external temperature (Fig. 2b). The unstable temperature affected the yeast activities. Moreover, fermented rice in room temperature was stable even out and inside temperatures (35 to 38 degree Celcius). According to Fleet and Heard, (1993); Ribereau-Gayon et al., (2000) reported that temperature has an impact on yeast development and on fermentation kineties.



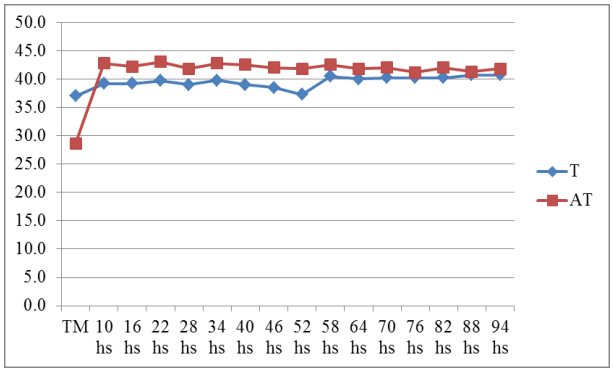
a) Fermented rice in cool temperature



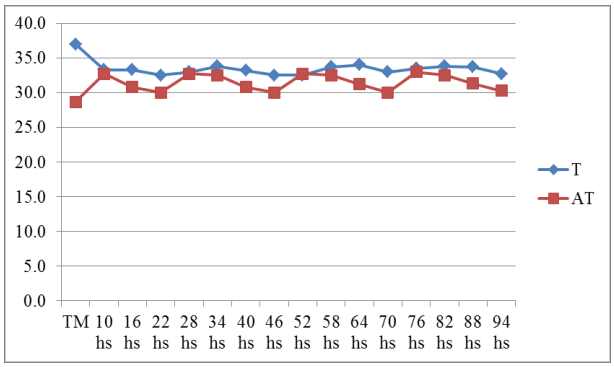
b) Fermented rice in ambient temperature



c) Fermented rice in ambient temperature with plastic bag-covered pots



d) Fermented rice in high temperature



e) Fermented rice in room temperature

Fig. 2 Fermented rice and temperature (in degree Celcius)

Based on these results, fermented rice in cool temperature was unsuitable as fermention temperature of rice liquor condition. Rice fermemntation in ambient temperature was impossible in cold season but suitable in hot season. On the other hand, fermented rice in ambient temperature with plastic bag-covered pot and room temperature were possible for fermented rice liquor in both cool and hot conditions or seasons. It was observed that fermented rice in ambient temperature with plastic bag-covered pot is a suitable way for producing rice liquor by rural farmers. It may be concluded that rice fermentation in room temperauture is the best way. The temperature between 30 to 38 degree Celcius of the rice fermentation condition was suitable for producing rice liquor in Cambodia within fermentation periods of 96 hours.

Table 3 Ethanol production per kilogram of milled rice

Fermented condition	Milled rice (kg)	Ethanol % (%)	Pure ethanol (L)	Liquor (L)	Productivity (L/kg)
CT	1	9.2[b]	0.09[b]	1	0.09[b]
AT	1	31.4[a]	0.31[a]	1	0.31[a]
ATPC	1	33.4[a]	0.33[a]	1	0.33[a]
HT	1	29.0[a]	0.29[a]	1	0.29[a]
RT	1	33.2[a]	0.33[a]	1	0.33[a]

CT = fermented rice in cool temperature, AT = fermented rice in ambient temperature, ATPC = Fermented rice in ambient temperature with plastic bag-covered pot, RT = fermented rice in room temperature and, HT = fermented rice in high temperature

Table 4 Temperature of fermented rice in different fermentation conditions (degree C)

Fermentation without water

	Mixed yeast		F 10 hrs		F 16 hrs		F 22 hrs		F 28 hrs		F 34 hrs		F 40 hrs		F 46 hrs	
	TM	AT	T	AT	T	AT	T	AT	T	AT	T	AT	T	AT	T	AT
CT	37.0	28.7	21.0[c]	18.7[c]	20.0[c]	19.0[d]	19.3d	19.0[d]	20.0[c]	19.0[c]	20.0[c]	19.0[c]	20.8[d]	19.3[d]	19.8[d]	18.5[d]
AT	37.0	28.7	34.0[b]	30.5[b]	31.8[b]	27.2[c]	29.5c	25.8[c]	32.5[b]	33.3[b]	35.0[b]	31.0[b]	31.2[c]	27.2[c]	29.3[c]	26.0[c]
ATPC	37.0	28.7	33.8[b]	30.8[b]	34.0[b]	27.7[c]	33.0b	26.5[c]	33.2[b]	33.8[b]	34.7[b]	31.5[b]	33.7[b]	27.7[c]	32.3[b]	26.7[c]
HT	37.0	28.7	39.2[a]	42.8[a]	39.2[a]	42.2[a]	39.7a	43.0[a]	39.0[a]	41.8[a]	39.8[a]	42.8[a]	39.0[a]	42.5[a]	38.5[a]	42.0[a]
RT	37.0	28.7	33.3[b]	32.7[b]	33.3[b]	30.8[b]	32.5b	30.0[b]	33.0[b]	32.7[b]	33.8[b]	32.5[b]	33.2[b]	30.8[b]	32.5[b]	30.0[b]
P <.05			**	**	**	**	**	**	**	**	**	**	**	**	**	**

Fermentation with water

	F 52 hrs		F 58 hrs		F 64 hrs		F 70 hrs		F 76 hrs		F 82 hrs		F 88 hrs		F 94 hrs	
	T	AT	T	AT	T	AT	T	AT	T	AT	T	AT	T	AT	T	AT
CT	19.3[c]	17.3[c]	19.3[c]	18.0[c]	19.0[c]	18.0[d]	19.0[d]	18.0[d]	19.3[d]	17.7[c]	20.7[c]	19.3[c]	18.8[d]	17.3[d]	19.5[d]	18.3[d]
AT	33.3[b]	34.8[b]	35.3[b]	31.8[b]	32.8[b]	28.3[c]	29.5[c]	25.5[c]	31.3[c]	32.8[b]	34.2[b]	31.0[b]	31.5[c]	27.5[c]	29.8[c]	26.2[c]
ATPC	33.0[b]	34.3[b]	34.0[b]	32.0[b]	34.2[b]	28.5[c]	33.7[b]	26.3[c]	33.7[b]	33.2[b]	34.7[b]	31.3[b]	34.0[b]	28.0[c]	33.2[b]	28.8[c]
HT	37.3[a]	41.8[a]	40.5[a]	42.5[a]	40.0[a]	41.8[a]	40.2[a]	42.0[a]	40.2[a]	41.2[a]	40.2[a]	42.0[a]	40.7[a]	41.3[a]	40.7[a]	41.8[a]
RT	32.5[b]	32.7[b]	33.7[b]	32.5[b]	34.0[b]	31.2[b]	33.0[b]	30.0[b]	33.5[b]	33.0[b]	33.8[b]	32.5[b]	33.7[b]	31.3[b]	32.7[b]	30.3[b]
P <.05	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**

*TM = Temperature during mixed steamed rice with yeast, AT = Ambient temperature, T = Temperature in fermented rice, F = Fermentation period,Hr = Hour, * Significant, ** High significant*

The degree of ethanol percentage, pure ethanol and productivity were affected by fermentable temperature condition, as shown in Table 4. The degree of ethanol percentage, pure ethanol and productivity were strongly significant under different fermentation temperature conditions. Fermented rice condition in CT had the lowest productivity. Fermented rice condition in AT, RT, HT and ATPC showed similar production, but AT condition, suitable for hot season, losing its fermentative abilities in cool season. ATPC and RT produced stable liquor even in a cool or hot season. Fermented conditions were affected by the degree of ethanol percentage, pure ethanol and ethanol production. The alcohol yield is generally lower at elevated temperatures, which has been related to a drop in the ethanol yield and a reduction of substrate (Ribereau-Gayon et al., 1975; Llaurado, 2002; Torija et al., 2003).

Virender et al. (2000) showed that typical yield of alcohol from molasses is 0.225 l/kg when molasses contains 45% sugar, only 70-75% of the sugar get fermented to alcohol and the remaining sugars go into spent wash. On the other hand, in typical grain fermentation the alcohol yield is >0.36 l/kg of grain, containing 60% of starch which corresponds to 85% of conversion of sugar to

alcohol. An addition of sorghum can potentially give good yield of alcohol of about 0.38 to 0.39 litre of absolute alcohol per kilogram of grain provided and the process is optimized from 0.32 to 0.33 litre.

CONCLUSION

In summary, the fermention temperature affected the yeast activity, causing a decrease in the productivity of pure ethanol and ethanol percentages. The fermented rice in ambient temperature with plastic bag-covered pots and in room temperature were suitable temperatures for rice liquor production.

Temperatures between 30 to 38 ^{o}C were the best rice fermentation conditions. However, the amount of added water in fermented rice and period of fermented should be evaluated further.

REFERENCE

Fleet, G.H. and Heard, G.M. 1993. Yeast growth during fermentation. Wince Microbiology and Biotechnology, ed. G. H. Fleet Switzerland: Harwood Academic Publishers, 27-54.

Gina, J. 2004. Temperature at which beer is fermented. http://hypertextbook.com/facts/2004/GinaJohn.shtml.

Killian, E. and Ough, C.S. 1979. Fermentation ssters-formation and retention as affected by fermentation temperature. Am. J. Enol. Vitic. 30, 301-305.

Llaurado, J. M., Rozes, N., Bobet, R., Mas, A. and Constanti, M. 2002. Low temperature alcoholic fermentation in high sugar concentration Grape Must. J. Food Science, 67, 268-273.

Peterson, A. 1995. Production of fermentable extracts from cereals and fruits. Fermented Beverage Production (Lea, A.G.H, and Piggot, J.R., eds). London, UK, 1-31.

Ribereau-Gayon, J., Peynaud, E., Ribereau-Gayon, P. and Sudraud, P. 1975. Sciences Et Techniques Du Vin, Traité dÓenology IIParis: Dunod.

Ribereau-Gayon, P., Dubourdieu, D., Doneche, B. and Lonvaud, A. 2000. Handbook of Enology. The Microbiology of Wine and Vinifications Vol. I. West Sussex, England.

Robert, W. 2004. Mashing and fermentation. Heineken Brewing kettles, Amsterdam. http://running_on_alcohol.tripod.com/id20.html.

Sheorain V., Banka, R. and Chavan, M. 2000. Ethanol production from Sorghum. Technical and Institutional Options for Sorghum Grain Mold Management: Proceedings of an International Consultation, 228-239.

Torija, M.J., Rozes, N., Poblet, M., Guillamon, J.M. and Mas, A. 2003. Effects of fermentation temperature on the stain population of Saccharomyces Cerevisiae. Int. J. Food Microbiol, 80, 47-53.