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Fermentation of rambutan *Nephelium lappaceum* flesh to wine production

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Abstract
Rambutan is a special fruit in tropical region. We have conducted the fermentation of a new fruit wine using rambutan flesh. With the initial dry matter 15-17°Bx, we soak rambutant flesh with syrup 42°Brix at ratio 1:1, reduced sugar 372 mg/ml, acidity 2.36%, pH 3.67, tannin 0.24%. *Saccharomyces cerevisiae* is the suitable yeast for fermentation. The initial 20°Bx, sugar supplementation 60 g/litre, 10 days of fermentation, ethanol formation 10.08 degree, pH 3.36, total dry matter 11.2, total acidity formation 6.04 g/l are key parameters of fermentation.

Keywords: *Rambutan, Saccharomyces cerevisiae, fermentation, wine.*

1. Introduction
Rambutan (*Nephelium lappaceum*) is a medium-sized exotic fruit grown by a tree called rambutan. This fruit is relatively common to Malaysia, Thailand, the Phillippines, Vietnam, Borneo, and other regions of the country. It's a member of a Sapindaceae (a plant family). Rambutan is naturally red in color, but sometimes it can be found yellow or orange. The taste of this fruit is sweet and divine. Rambutan has benefits and similar content with orange and apples. Inside the fruit there are many important compounds such as vitamin C, iron, phosphorus, protein and carbohydrates, all the substances needed by the body every day. Rambutan fruit is known as a very low-fat. Most of the calories of rambutan, derived from carbohydrates. Institute of Medicine recommends 130 grams of rambutan for your daily consumption.
Rambutan is very rich in iron, which is needed to control the oxygen levels in the body. Iron helps prevent fatigue and dizziness caused by anemia. In addition, flesh rambutan also meets 4.3 percent of the daily phosphorus needs for the body. Phosphorus helps filter out waste in the kidneys, as well as necessary for the growth, maintenance, and repair of all tissues and cells. Rambutan is rich in sugar. Most of it is fructose and sucrose, but contains very little calories, only 60 calories in each piece. There is an abundance of vitamin C in rambutan, including potassium, iron, vitamin A, and a little calcium, magnesium, sodium zinc, niacin, fiber and protein. Rambutan contains ingredients as an antioxidant. The rambutan fruit, seeds and skin have powerful antioxidants called flavonoids. Several types of flavonoids are believed to reduce cholesterol, anti-cancer and anti-inflammatory. One of the compounds in rambutan skin is Gallic acid. These compounds act as free radical antidote because it helps protect the body from oxidative damage. Once again, this can be a help to fight cancer. Rambutan is rich in vitamin C. If a person consuming 10 to 12 rambutan, then he was taking 75-90 mg of ascorbic acid, more than twice the recommended amount in the daily menu. In addition to functioning as an anti-oxidant, vitamin C can prevent cell damage and helps the absorption of iron. This sweet fruit also has a small amount of copper. This substance is needed as a shaper of white blood cells and red blood cells. In addition, rambutan also contains iron which can prevent the occurrence of anemia. Rambutan also has fiber that can help a person avoid constipation. In addition, rambutan can also kill parasites in the intestines and helps relieve the symptoms of diarrhea.
The fruit are usually sold fresh. Processed rambutan products include rambutan in syrup, rambutan jam, dehydrated rambutan as well as frozen rambutan (Anchalee Sirichote et al., 2008; Fila W. O., et al., 2012; Y.L. Hor et al., 1990; Johnson J.T., et al., 2013; M.N. Latifah et al., 2009; Margaret Landrigan 1996; Ngamjit Lowithun and Sanguansri Charoenrein, 2009; Nurhuda et al, 2013; Suhaila Mohamed et al., 1988). There is no research mentioned to rambutant wine. Purpose of our research is to investigate different factors influencing to rambutant wine fermentation such as chemical composition of raw material (sugar, tannin, pH, dry matter); rambutant juice concentration and yeast species to ethanol formation, CO_2,

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residual sugar, yeast viability; wine quality in sub-fermentation; rambutant wine standard according to TCVN 7045–2002.

Fig 1: Rambutant fruit

2. Material and Method

2.1 Material

Rambutant fruits are purchased in Mekong river delta, Vietnam. *Saccharomyces cerevisiae* is supplied from Pasteur Institute, HCM City, Vietnam.

2.2 Research method

Extraction of rambutant juice by soaking with syrup: soaking rambutant flesh with syrup at ratio 1:1 within 6 months to extract rambutant juice. Dilute this juice to 18; 20; 22°Bx before fermentation.

2.3 Testing method

Determine pH by pH meter
Determine dry matter by refractometer
Determine organic acid by titration wiht NaOH 0.1N
Determine reduced sugar by acid dinitrosalicylic (DNS) methiod
Determine tannin by titration with $KMNO_4$
Determine yeast cell density by counting chamber
Determine yeast growth by optical density at 700 nm

2.4 Statistical analysis

All data are processeed by Excel 2003.

3. Result and Discussion

3.1 Chemical composition in rambutant syrup

Table 1: Chemical composition in rambutant

Parameter				
Dry matter (°Bx)	**Reduced sugar (g/l)**	**Acidity (%)**	**pH**	Tannin (%)
42 ±0.90	372±1.95	2.36±0.12	3.67±0.10	0.24±0.02

Through table 1, we see the high dry matter concentration in rambutant syrup so it's necessary to dilute this fluid before fermentation. Acidity (2.36%) and tannin (0.24%) in rambutant syrup are suitable for fermentation

3.2 Effect of rambutant syrup concentration and yeast to wine fermentation:

We investigate different rambutant syruo concentration 18, 20, 22°Bx at temperature 28°C. Testing parameters includes yeast cell density, yeast viability, ethanol, pH, acidity, residual sugar, °Bx.

3.2.1 Yeast cell density during 10 days of fermentation

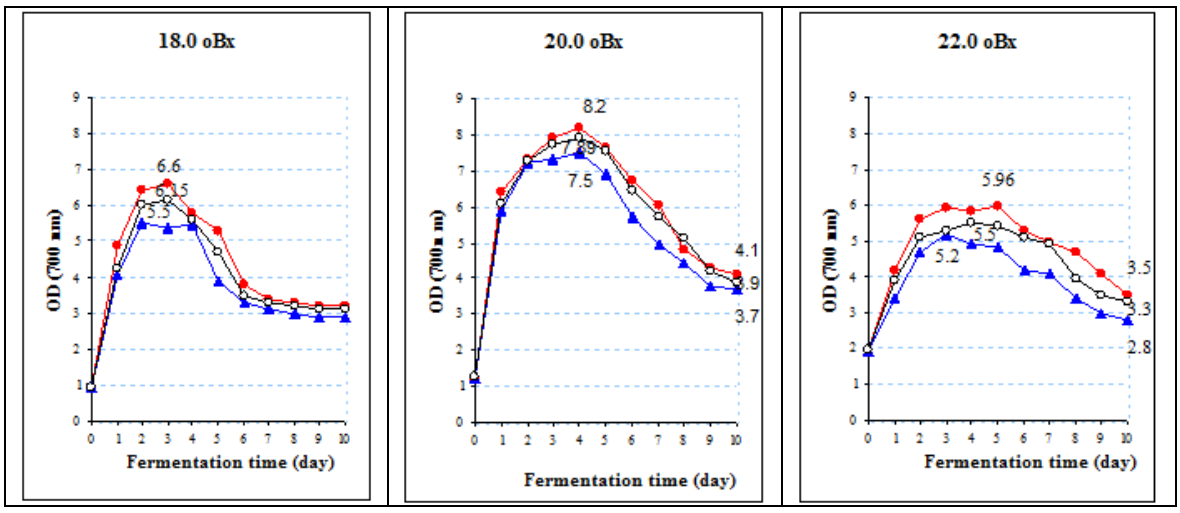

Fig 2: Yeast cell density during the main fermentation: (●) species TVA; (▲) species SLS); (❑) species TVA + SLS

At 20°Bx all three yeast species show the highest density after 96 hours of fermentation.

3.2.2 Yeast viability and total yeast cells during the main fermentation

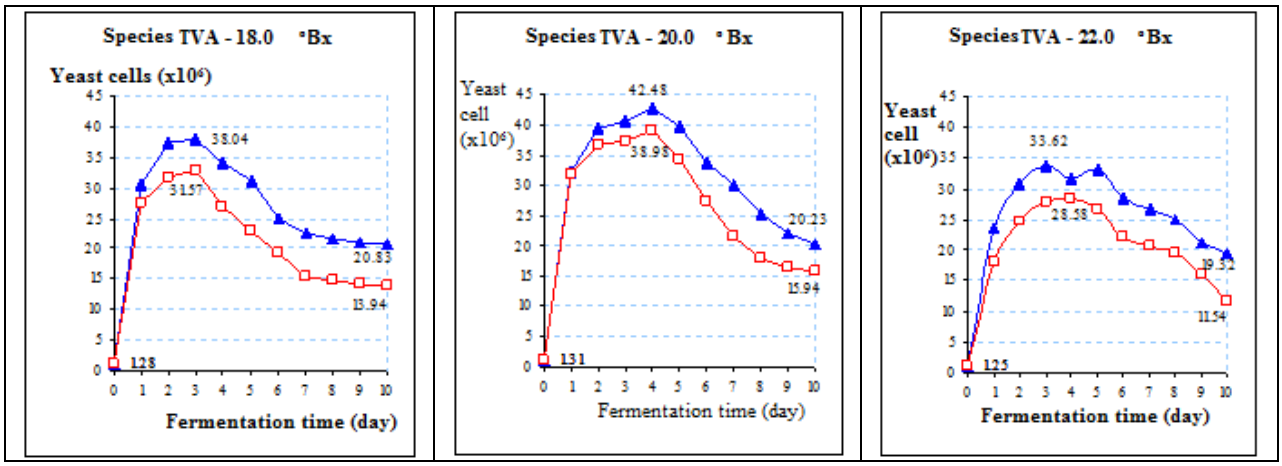

Fig 3: Yeast vialibility and total yeast cells during 10 days of fermentation by TVA species: (▲) total cells; (❑) yeast viability

From figure 3, we see the yeast growth is strong in the first 2 days. Yeast viability at 22°Bx is 5.44*10^6 CFU/ml, at18°Bx 2.97*10^6 CFU/ml, at 20°Bx 0.22*10^6 CFU/ml. The highest yeast cell viability is presented at 20°Bx (37.28*10^6 CFU/ml) in the 4th day; 18°Bx (32.82*10^6 CFU/ml) at the 3rd day; 22°Bx (28.58*10^6 CFU/ml) at the 3rd day.

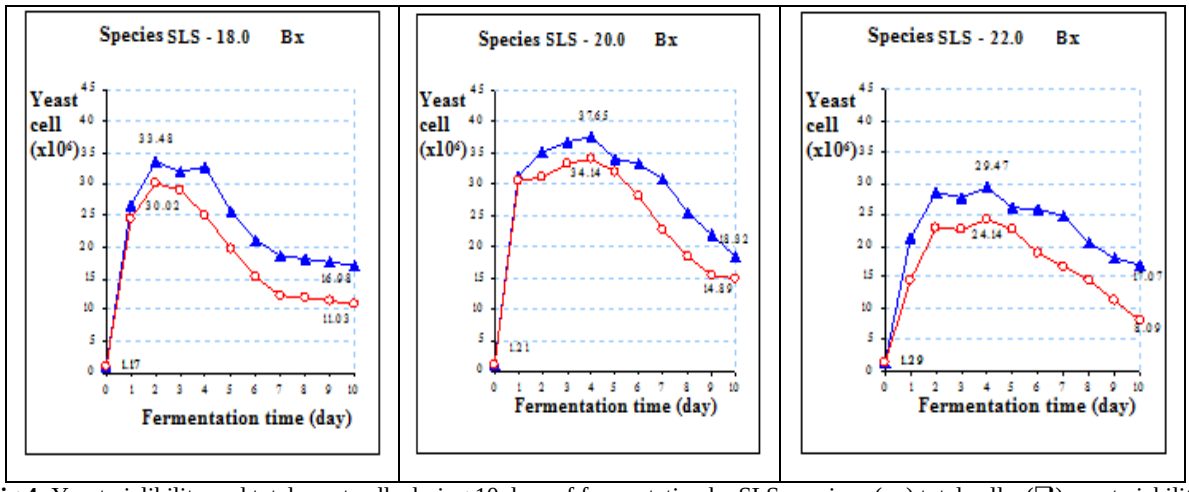

Fig 4: Yeast vialibility and total yeast cells during 10 days of fermentation by SLS species: (▲) total cells; (❑) yeast viability

After the first day, the yeast viability at 22°Bx (6.63*10^6 CFU/ml); at 18°Bx (2.02*10^6 CFU/ml), at 20°Bx (0.78*10^6 CFU/ml). The highest yeast viability of SLS species is presented at 20°Bx (34.14*10^6 CFU/ml) in the 4th day, 18°Bx (30.02*10^6 CFU/ml) in the 2nd day, 22°Bx (24.14*10^6 CFU/ml) in the 4th day.

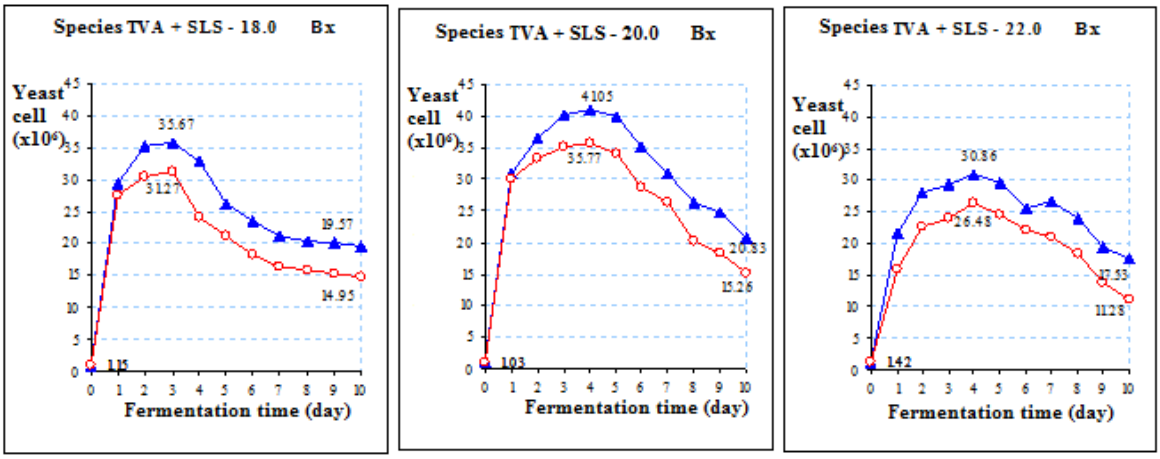

Fig 5: Yeast vialibility and total yeast cells during 10 days of fermentation by TVA + SLS species: (▲) Total yeast; (❑) Yeast viability

With a combination of TVA+SLS, yeast grows well in the first two days. After the first day, yeast viability at 22°Bx is 5.49*10^6 CFU/ml; at 18°Bx is 2.03*10^6 CFU/ml; at 20°Bx is 0.77*10^6 CFU/ml. The highest yeast viability is presented at 20°Bx (35.77*10^6 CFU/ml) in the 4th day, at 18°Bx (31.27*10^6 CFU/ml) at the 2nd day; 22°Bx (26.48*10^6 CFU/ml) at the 4th day.

3.2.3 Effect of soluble dry matter during 10 days of fermentation

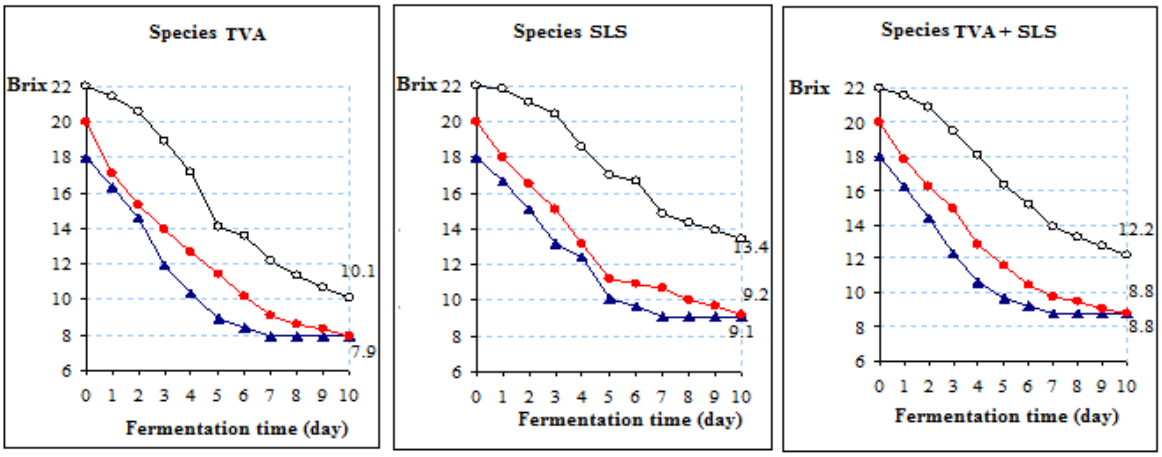

Fig 6: Change of the soluble dry matter during 10 days of fermentation: (▲) 18.0°Bx; (●) 20.0°Bx; (❑) 22.0°Bx
A combination of two species TVA+SLS is ideal for fermentation.

3.2.4 Change of acidity during 10 days of fermentation

Table 2: Change of acidity during 10 days of fermentation

Day	Acidity (g/l)								
	Species TVA			Species SLS			Species TVA+SLS		
°Bx	18	20	22	18	20	22	18	20	22
1	5.19	5.25	5.07	5.21	5.26	5.13	5.16	5.08	5.27
2	5.54	5.42	5.31	5.68	5.61	5.38	5.54	5.47	5.53
3	6.13	5.71	5.48	6.32	6.15	5.51	5.98	5.83	5.76
4	6.37	6.13	5.66	6.49	6.74	5.69	6.47	6.04	5.94
5	6.84	6.49	5.9	6.75	6.98	5.94	6.82	6.39	6.05
6	7.13	6.57	6.01	7.24	7.07	6.08	7.18	6.51	6.17
7	7.31	6.72	6.13	7.63	7.16	6.23	7.45	6.63	6.28
8	7.31	6.83	6.19	7.63	7.22	6.35	7.45	6.72	6.4
9	7.31	6.89	6.31	7.63	7.29	6.47	7.45	6.79	6.48
10	7.31	6.92	6.37	7.63	7.33	6.58	7.45	6.84	6.52

At 18°Bx, acidity is highest (7.63%).

3.2.5 Redisual sugar after 10 days of fermentation

Table 3: Residual sugar after 10 days of fermentation

Day	Residual sugar (mg/ml)								
	Species TVA			Species SLS			Species TVA+SLS		
°Bx	18	20	22	18	20	22	18	20	22
0	159	181	204	157	180	201	161	179	203
1	105	122	172	123	138	179	112	125	178
2	73	90	148	89	99	156	81	97	142
3	61	76	132	68	81	143	63	82	123
4	47	64	123	57	70	117	46	68	112
5	31	52	92	41	62	105	34	54	101
6	24	39	81	32	53	99	28	41	97
7	17	32	73	26	46	92	24	37	85
8	17	25	64	26	39	81	24	32	77
9	17	19	57	26	31	73	24	27	70
10	17	17	51	26	28	67	24	24	63

At 20°Bx sugar reduces dramatically. At 18°Bx after 7 days sugar reduces 9.35 times from 159 mg/l to 17mg/ml. At 22°Bx after 10 day sugar reduces 3.78 times.

3.2.6 CO_2 emission and etanol formation during 10 days of fermentation

CO_2 emission is a critical parameter to determine fermentation intensity.

Table 4: CO_2 emission during 10 days of fermentation

Day	CO_2 emission (mg/ml)								
	Species TVA			Species SLS			Species TVA+SLS		
°Bx	18	20	22	18	20	22	18	20	22
1	0.78	0.97	0.52	0.64	0.83	0.31	0.71	0.92	0.43
2	2.03	2.34	1.24	1.61	2.02	0.98	1.82	2.04	1.02
3	3.57	4.05	2.46	2.94	3.45	1.36	3.22	3.68	1.94
4	4.84	5.25	3.68	4.03	4.37	2.71	4.47	4.97	2.25
5	5.62	6.1	4.87	4.76	5.08	3.49	5.03	5.34	3.59
6	6.07	6.32	5.53	5.32	5.53	3.68	5.34	5.61	4.41
7	6.19	6.65	5.87	5.48	5.74	4.75	5.58	5.83	5.02
8	6.2	6.78	6.31	5.48	5.86	5.06	5.59	6.02	5.47
9	6.2	6.93	6.49	5.48	5.99	5.41	5.59	6.15	5.72
10	6.2	7.05	6.72	5.48	6.02	5.67	5.59	6.26	5.87

At 20°Bx, CO2 emission is optimal, yeast TVA species can produce CO_2 maximum 7.05mg/ml; following TVA+SLS 6.26mg/ml, and finally SLS 6.02mg/ml.

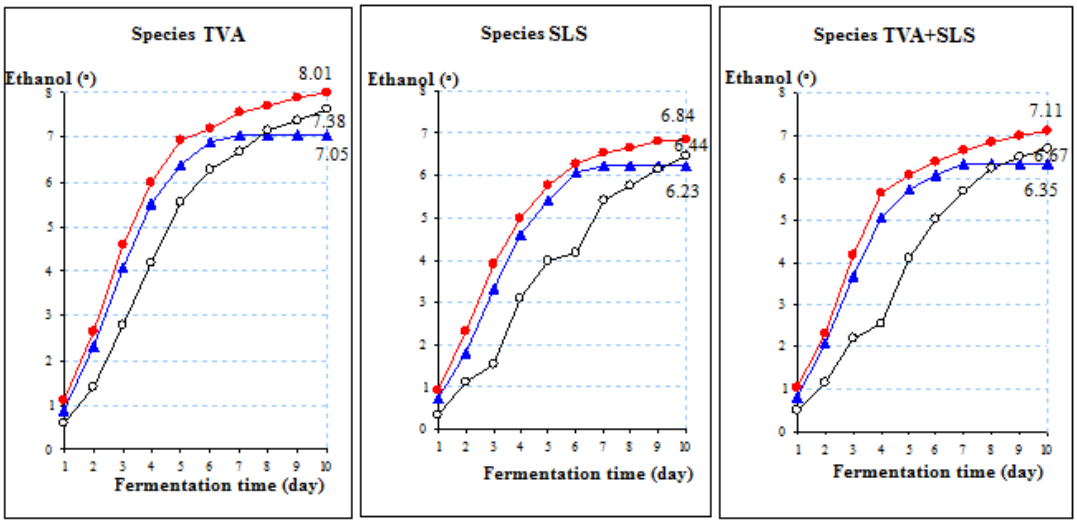

Fig 7: Ethanol formation after 10 days of fermentation: (▲) 18.0°Bx; (●) 20.0°Bx; (❑) 22.0°Bx

After 10 days of fermentation, TVA species creates opimal etanol at 20°Bx (8.01°); fllowing TVA+SLS at 20°Bx (7.11°). At 18°Bx, the main fermentation stops after 7 days of fermentation.

3.2.7 Change of biochemical characteristics in wine after the sub-fermentation

We conduct the sub-fermentation during 20 days at 11-12°C to accomplish the wine fermentation.

Table 5: Biochemical characteristics in wine before and after the sub-fermentation

Time	Dry matter (°Bx)	Species	°Bx	Sugar (g/l)	Acidity (g/l)	Ethanol (%v/v)	*pH*
Before the sub-fermentation	18	TVA	7.9	17	7.31	7.05	*3.18*
		SLS	9.1	26	7.63	6.23	*3.13*
		TVA+SLS	8.3	20	7.45	6.35	*3.16*
	20	TVA	7.9	17	6.92	8.01	*3.22*
		SLS	9.2	28	7.33	6.84	*3.18*
		TVA+SLS	8.3	20	6.84	7.11	*3.23*
	22	TVA	10.1	51	6.37	7.38	*3.29*
		SLS	13.4	67	6.58	6.44	*3.26*
		TVA+SLS	11.4	57	6.52	6.67	*3.27*

After the sub-fermentation	18	TVA	7.9	17	7.23	7.05	*3.19*
		SLS	9.1	26	7.5	6.23	*3.15*
		TVA+SLS	8.3	20	7.36	6.35	*3.17*
	20	TVA	7.9	17	6.79	8.01	*3.24*
		SLS	9.2	28	7.21	6.84	*3.19*
		TVA+SLS	8.3	20	6.72	7.11	*3.25*
	22	TVA	10.1	51	6.28	7.38	*3.31*
		SLS	13.4	67	6.46	6.44	*3.28*
		TVA+SLS	*11.4*	*57*	*6.45*	*6.67*	*3.28*

Soluble dry matter, residual sugar, ethanol are nearly stable, this demonstrates that the main fermentation is stopped.

3.3 Effect of rambutan syrup concentration (24; 26; 28ºBx) to yeast species TVA in fermentation

3.3.1 Soluble dry matter concentration and residual sugar during fermentation

Table 6: Soluble dry matter concentration and residual sugar after 10 days of fermentation

Day	Soluble dry matter (ºBx)				Residual sugar (g/l)		
ºBx	24	26	28		24	26	28
3	14.1	13.9	13.8		75	77	74
3	18.1	19.9	21.8		115	137	154
4	16.8	18.4	20.5		103	123	135
5	15.6	17.1	18.3		89	108	119
6	14.5	15.7	17.4		77	93	108
7	13.6	14.3	16.9		68	77	99
8	12.8	13.2	16.3		59	67	92
9	12.1	12.3	15.8		51	56	86
10	11.4	11.5	15.3		44	46	80
11	10.8	11.3	14.9		37	43	76
12	10.3	11.2	14.6		31	42	73

The soluble dry matter and sugar decrease dramatically from 3 - 7 days of fermentation. The residual sugar. With initial 26ºBx, soluble dry matter decreases maximum 1.78 time (from 19.9 to 11.2ºBx); sugar decreases 3.26 times (from 137 to 42 g/l). With initial 28ºBx. Soluble dry matter decreases 1.49 times (from 21.8 to 14.6ºBx); sugar decreases 2.1 times (from 154 to 73 g/l).

3.3.2 CO_2 emission and ethanol formation during fermentation

Table 7: CO_2 emission and ethanol formation after 10 days of fermentation

Day	CO_2 emission (mg/ml)				Ethanol formation (º)		
ºBx	24	26	28		24	26	28
3	4.03	4.05	4.06		4.59	4.6	4.61
4	4.69	4.96	4.89		5.33	5.63	5.56
5	5.37	5.74	5.67		6.10	6.52	6.44
6	6.05	6.55	6.32		6.88	7.44	7.18
7	6.62	7.27	6.84		7.52	8.26	7.77
8	7.06	7.82	7.36		8.02	8.89	8.36
9	7.48	8.33	7.83		8.5	9.47	8.90
10	7.84	8.69	8.22		8.9	9.88	9.34
11	8.09	8.81	8.45		9.19	10.01	9.60
12	8.32	8.87	8.54		9.45	10.08	9.70

At 26 ºBx, yeast species TVA produces CO_2 8.87 mg/ml; following 28ºBx yeast produces CO_2 8.54 mg/ml; finally 24 ºBx CO_2 8.32mg/ml. This demonstrates that 26 ºBx is suitable for yeast species TVA fermentation. At 26 ºBx, ethanol formation is maximum (10.08º); following 28 ºBx (ethanol 9.70º); finally 24 ºBx (ethanol 9.45º).

3.3.3 Change of acidity during fermentation

Table 8: Change of acidity after 10 days of fermentation

Day	Acidity (g/l)		
°Bx	**24**	**26**	**28**
3	5.68	5.70	5.71
4	5.73	5.73	5.71
5	5.84	5.78	5.73
6	5.95	5.86	5.76
7	6.02	5.92	5.79
8	6.14	5.98	5.84
9	6.27	6.03	5.87
10	6.46	6.07	5.9
11	6.37	6.13	5.9
12	6.48	6.13	5.9

At 24 °Bx acidity is 6.48 g/l; at 26 °Bx acidity is 6.13 g/l; at 28 °Bx acidity is 5.9 g/l.

3.3.4 Change of biochemical characteristics during the sub-fermentation

The sub-fermentation is executed at 11-12°C to accomplish the rambutan wine. After 20 days of sub-fermentation, we get some biochemical characteristics of wine.

Table 9: Biochemical characteristics of rambutan wine after the sub-fermentation

Time	Syrup (°Bx)	Wine (°Bx)	Sugar(g/l)	Acidity (g/l)	Ethanol (%v/v)	*pH*
Before sub-fermentation	24	10.3	31	6.48	9.45	*3.28*
	26	11.2	42	6.13	10.08	*3.35*
	28	14.6	73	5.9	9.7	*3.39*
After sub-fermentation	24	10.3	31	6.32	9.45	*3.31*
	26	11.2	42	6.04	10.08	*3.36*
	28	*14.6*	*73*	*5.85*	*9.7*	*3.40*

From table 9 we see that the soluble dry matter, residual sugar, ethanol are stable.

3.3.5 Rambutan wine quality after the main fermentation

Table 10: Rambutan wine quality after the main fermentation

Yeast species	Average sensory score							
	°Bx	**Turbidity**	**Flavor**	**Ethanol**	**Sweet**	**Sour**	**Tannin**	Others
TVA	24	4.2^{a}	2.3^{b}	2.1^{b}	1.2^{c}	2.9^{a}	2.0^{a}	2.8^{b}
	26	4.1^{a}	3.4^{a}	3.2^{a}	2.3^{b}	2.9^{a}	2.3^{a}	3.9^{a}
	28	3.9^{a}	3.7^{a}	2.7^{ab}	3.6^{a}	2.7^{a}	2.4^{a}	1.9^{c}

From table 10, we clearly notice that rambutan wine is acceptable for human consumption.

4. Conclusion

Rambutan wine is a new approach to utilize this delicious fruits in Mekong river delta to produce a value-added product. Actually we have successfully investigated different factors influencing to rambutan fermentation such as yeast species, initial soluble dry matter

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