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## Investigation of various factors influence to fermented guava beverage production

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### Abstract

Guava (*Psidium guajava L.*) is an important tropical fruit produced in Vietnam because of its sensory characteristics, specially its aroma and taste. Although wine technology is focused on grapes, the application of alcoholic fermentation in guava is a promissory alternative to generate value and social development in this country. The aim of this work was to evaluate the effect of different conditions to the fermented guava beverage production. We draw out some major results as follows: *saccharomyces cerevisiae* yeast, yeast  $47.10^6$  cfu/ml, initial dry matter for fermentation 17 °Bx, pH suitable 3.5, yeast ratio 3%, fermentation time 72 hours.

**Keywords:** Guava, *saccharomyces cerevisiae*, aroma, taste, beverage, fermentation

### 1. Introduction

Guava (*Psidium guajava L.*) is one of the most important commercial fruit crops in Vietnam consumed locally. It is a good source of ascorbic acid, pectin, sugars and certain minerals. Its skin and flesh colours vary from variety to variety depending on the amount and type of pigments. Guava, with its widely appreciated flavor and aroma, is able to compete in the market, either as guava juice or as mixtures with other juices or fermented guava beverage.

Grapes and apples have been widely applied to ferment beverages (Polychroniadou E. *et al.*, 2003) the use of other fruits, such as orange (Selli S. *et al.*, 2008), cacao (Dias DR *et al.*, 2007), mango (Kumar YS *et al.*, 2009), gabiropa (Duarte WF *et al.*, 2009), caja (Soufleros *et al.*, 2001), kiwi (Duarte *et al.*, 2010), and in the production of wine has been recently demonstrated.

Fermented guava (*Psidium guajava L.*) beverage is the product of anaerobic fermentation by yeast in which the sugars are converted into alcohol & carbon dioxide. Fermented guava beverage production from guava pulp or juice is reported (Gurvinder, 2011; Sevda SB, 2011; Sahota PP, 2013; Layam Anitha, 2014; Kaiser Younis, 2014). The aim of our research is to investigate various factors influence to fermented guava beverage production.

### 2. The Concept of Time Management

Time management has been described using many different terms including spontaneity, balance, flexibility, and having control over time (Lakein, 1973). Time management has also been characterized as a habit developed only through determination and practice (Simpson, 1978), as prioritizing and respecting those priorities (Soucie, 1986), and as setting priorities and scheduling tasks (Jordan *et al.*, 1989). Time management can also be considered as the process, by which an



**Fig 1:** Guava fruit

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**2. Material & Method**

**2.1 Material**

Guava fruits were purchased from local market in Mekong river delta, Vietnam. The fruits were washed with fresh water. Guava was crushed completely to make guava pulp and guava pulp was treated with pectinase enzyme to extract the juice.

**2.2 Research method**

**2.2.1 Microbial method**

- Proliferate: guava juice has sugar content 100 g/l, pH = 3.5.
- Ferment medium: guava juice has pH = 3.5.
- Count yeast cells: use counting cell and microscope.

**2.2.2 Chemical method**

- Determine total sugar and reduced sugar by Bertrand method.
- Determine total acidity by KOH 0.1N with phenolphthalein as indicator.
- Determine ethanol by distilling apparatus and refractometer 1E.
- Determine moisture content by drying to constant weight.
- Determine total ash by burning at 550 – 600 °C.
- Determine concentrated status by refractometer 1E.

**2.2.3 Sensory method**

- Evaluate turbidity, aroma and taste of semi-product and finished product.

**2.3 Statistical analyses**

- Use Microsoft Excel 2003 at 95% confidence level.

**3. Result & Discussion**

**3.1 Chemical compositions and sensory evaluation of guava fruit material**

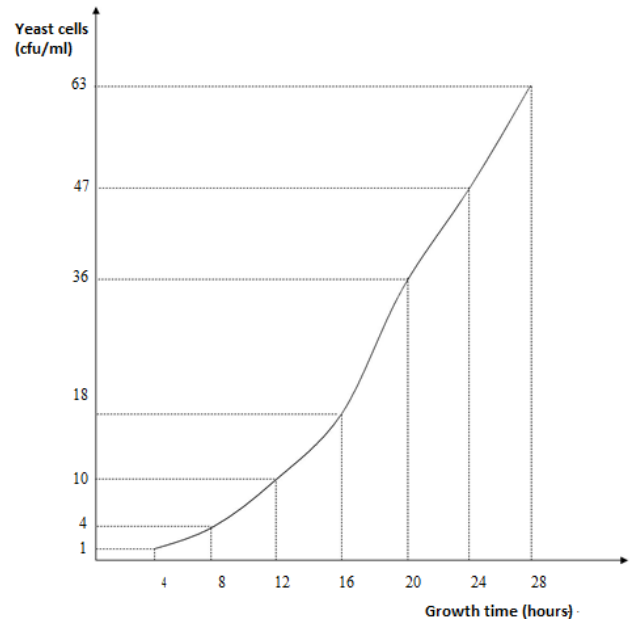
Guava fruit has moisture content: 86.3%; reduced sugar: 4.190 %; mineral: 1.895 %; acidity: 0.7 %; sensory characteristics with mild sweet flavour; status: quite soft; color: yellow green. We notice that moisture content in guava fruit is quite high (86.4%) so it's suitable for beverage production. However, because initial sugar is rather low (4.190%), it's necessary to add more sugar and water into fermentation fluid. These factors will strongly affect to production cost.

**3.2 Effect of proliferation time to number of yeast cells in proliferated fluid**

Yeast supplementation strongly affects to product quality: ethanol, residual sugar, color, aroma and taste. So we should examine the appropriate proliferation time.

**Table 1:** Effect of proliferation time to number of yeast cells in proliferated fluid

Sample	Initial sugar content (10 <sup>0</sup> Bx)	pH	Temperature (°C)	Proliferation time (h)	Yeast cells (million)
1	10 <sup>0</sup> Bx	3.5	30	4	1
2	10 <sup>0</sup> Bx	3.5	30	8	4
3	10 <sup>0</sup> Bx	3.5	30	12	10
4	10 <sup>0</sup> Bx	3.5	30	16	18
5	10 <sup>0</sup> Bx	3.5	30	20	36
6	10 <sup>0</sup> Bx	3.5	30	24	47
7	10 <sup>0</sup> Bx	3.5	30	28	63



**Fig 2:** Effect of proliferation time to number of yeast cells

After 4 hours of proliferation, yeast density in proliferated juice batch is 10<sup>6</sup> cfu/ml. After 8 hours of proliferation, yeasts proliferate quickly (4.10<sup>6</sup> cfu/ml) and reach 47.10<sup>6</sup> cfu/ml after 24 hours. And at 28 hours they reach to 63.10<sup>6</sup> cfu/ml. So we choose proliferation time 24 hours for further experiments.

**3.3 Effect of pectinase enzyme supplementation**

**3.3.1 Effect of pectinase supplementation to guava juice**

Pectinase plays important role in guava juice extraction. It hydrolysis fruit pulp to increase extraction recovery and less turbidity.

**Table 2:** Effect of pectinase supplementation to guava juice extraction

Sample	Initial guava pulp (g)	Pectinase (ml)	Temperature (°C)	Treatment time (minutes)	Juice volume (ml)	Sensory score of guava juice turbidity	Concentrated degree of guava juice (°Bx)
1	200	0.0	30	90	150.0	1.76	6.0
2	200	0.2	30	90	200.0	2.08	6.5
3	200	0.3	30	90	230.5	3.52	6.9
4	200	0.4	30	90	232.0	2.88	7.0
5	200	0.5	30	90	234.5	2.40	7.0

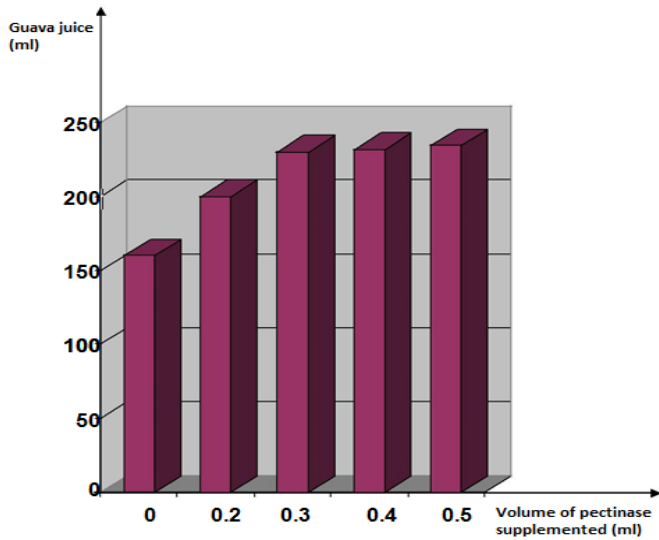


Fig 3: Volume (ml) of guava juice received when supplementing pectinase

All experimental samples in figure 3 are incubated at 40 °C in 90 minutes. Without pectinase treatment, guava juice is very low 160 ml/200 g. When supplementing 0.2 ml pectinase, extracted juice will increase dramatically to 200ml, 1.25 higher than the control sample. Increasing 0.3 ml pectinase extracted juice comes to 230.5 ml. If we continue adding 0.4 (232 ml) and 0.5 (234.5) ml of pectinase, extracted juice ascends slightly. So we either choose 0.2 ml pectinase enzyme for further experiments.

**3.3.2 Effect of pectinase supplementation to guava juice turbidity**

After extraction, guava juice will come to incubation before sensory evaluation. We check its turbidity. Main purpose of incubation is to hydrolyze protopectin, make tissue to be soft which is easily for extraction and clearance. In order to determine pectinase volume, we established a board of 5 specialists to evaluate. Average score from 5 specialists is then multiplied with the important factor 0.8.

Table 3: Sensory score of guava juice supplemented enzyme

Sample	Pectinase (ml)	Scores of specialists							
		Turbidity					Average score without important factor	Important factor	Average score with important factor
		A	B	C	D	E			
1	0.0	2	2	3	2	2	2.2	0.8	1.76
2	0.2	3	2	3	2	3	2.6	0.8	2.08
3	0.3	5	4	5	4	4	4.4	0.8	3.52
4	0.4	4	4	3	4	3	3.6	0.8	2.88
5	0.5	2	4	3	3	3	3.0	0.8	2.40

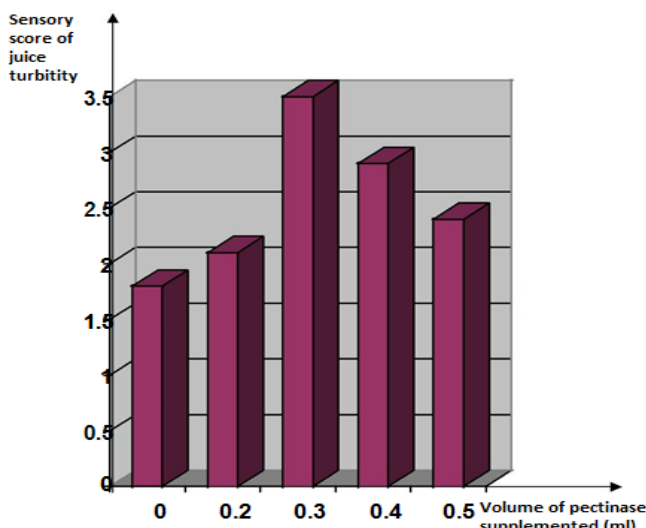


Fig 4: Effect of pectinase supplementation to guava juice turbidity

**3.3.3 Effect of pectinase supplementation to guava juice concentration**

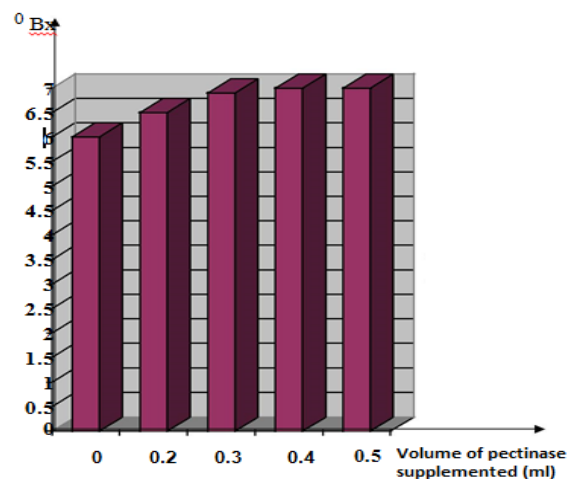


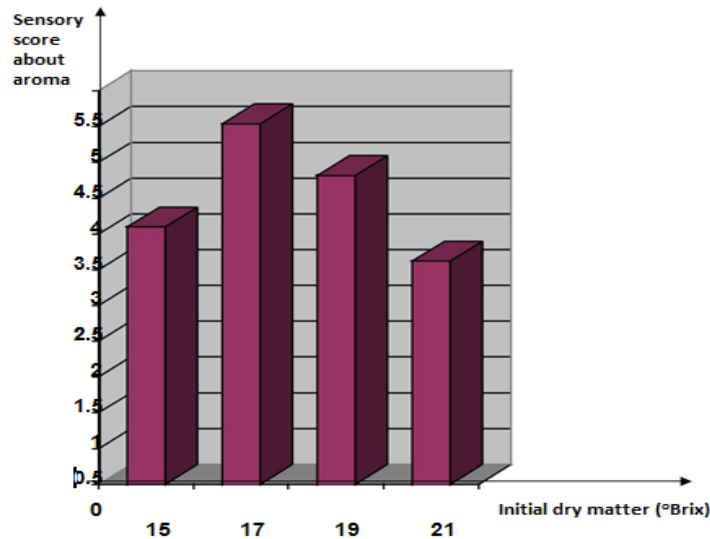
Fig 5: Effect of pectinase supplementation to concentrated degree of guava juice

Control sample has the lowest average score (1.8) owing to its high turbidity. Sample supplemented 0.2 ml pectinase, guava juice color is brighter but still slightly turbid. Sample supplemented 0.3ml pectinase has the highest sensory score (3.5) owing to its good color and clearness. Samples supplemented 0.4 ml and 0.5 ml pectinase enzyme have sensory score a little bit lower than sampled supplemented 0.3 ml pectinase in spite of their higher extraction volume compared to sample 0.3 ml pectinase. So supplementing with 0.3 ml pectinase into 200 g of guava pulp is optimal for further researches.

Initial concentrated degree of raw material sample (without supplementing enzyme) is rather low (0.6). When adding pectinase, the concentrated degree will dramatically increase from 6.0 to 6.5 which is equivalent to 0.2 ml pectinase enzyme. Continue increasing enzyme to 0.3 ml, concentrated degree also go up but slowly (6.9 to 7.0). So 0.3 ml pectinase into 200 g guava pulp is appropriated.

**Table 4:** Sensory score about product aroma of experiment to determine sugar content for fermentation

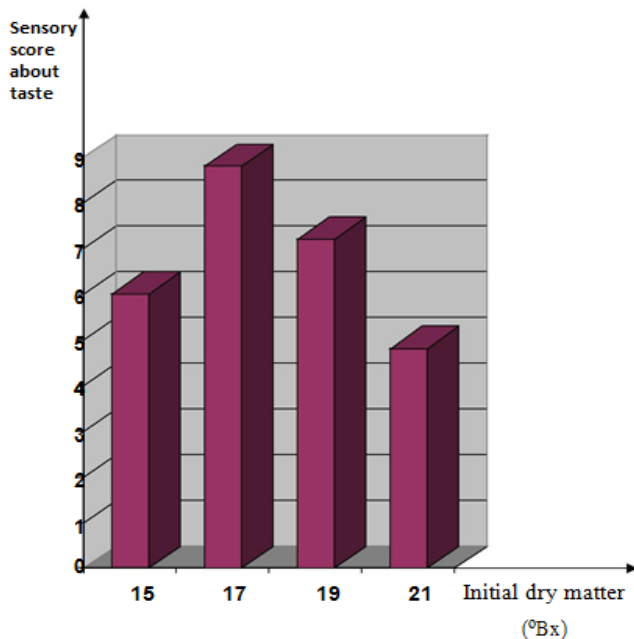
Sample	Initial sugar content (°Brix)	Sensory score of specialists							
		Aroma							
		A	B	C	D	E	Average score without important factor	Important factor	Average score with important factor
1	15	3	4	3	2	3	3.0	1.2	3.60
2	17	4	5	4	4	4	4.2	1.2	5.04
3	19	3	4	5	3	4	3.6	1.2	4.32
4	21	3	2	3	2	3	2.6	1.2	3.12



**Fig 6:** Effect of sugar content to product aroma

**Table 5:** Sensory score about product taste of experiment to determine sugar content of fermentation

Sample	Initial sugar content (°Brix)	Sensory score of specialists							
		Taste							
		A	B	C	D	E	Average score without important factor	Important factor	Average score with important factor
1	15	2	4	3	3	3	3	2	6
2	17	5	4	5	4	4	4.4	2	8.8
3	19	4	4	3	4	3	3.6	2	7.2
4	21	2	3	2	3	2	2.4	2	4.8



**Fig 7:** Effect of sugar content to product taste

Sugar content reaches 15°Bx, guava juice has sensory score with important factor about turbidity 2.4, aroma 3.6 and taste 6.0. When increasing sugar content to from 15°Bx to 17°Bx, guava juice get the highest sensory score with important factor (turbidity 3.52; aroma 5.02 and taste 8.8). However, if continue increasing 17 °Bx to 21 °Bx sensory score trends to decrease, especially more turbidity, bad flavour. So initial sugar content 17°Bx and pH = 3.5 are chosen for further experiments.

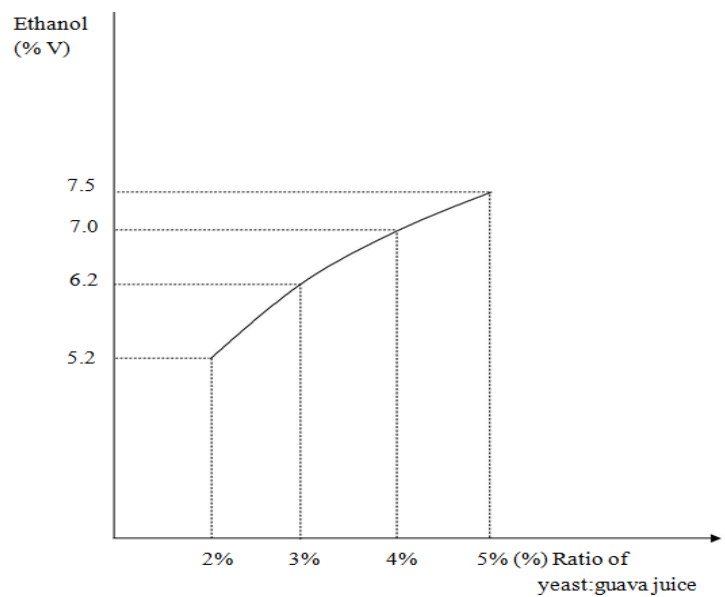
### 3.4 Effect of sugar content in guava juice fermentation batch

Sugar content strongly affect to product ethanol, aroma and taste.

#### 3.4.1 Effect of sugar content in guava juice to chemical compositions and sensory characteristics of fermented guava beverage

**Table 6:** Summary of experiments determining sugar content in fermentation

Initial sugar content (°Brix)	pH	Yeast ratio (%)	Yeast density (cfu/ml)	Fermentation time (days)	Fermentation temperature (°C)	Ethanol (%V)	Average sensory score with important factor
15	3.5	3	47.10 <sup>6</sup>	3	30	5.5	12.0
17	3.5	3	47.10 <sup>6</sup>	3	30	6.2	17.3
19	3.5	3	47.10 <sup>6</sup>	3	30	7.0	14.2
21	3.5	3	47.10 <sup>6</sup>	3	30	7.2	9.80



**Fig 8:** Effect of yeast ratio to product ethanol

The more sugar content is, the more fermentation time we get. Sugar content increases from 15<sup>0</sup>Bx to 19<sup>0</sup>Bx; ethanol in fermented beverage also increases 5.5 to 7.2. However sugar

#### 3.4.2 Effect of sugar supplementation to sensory characteristics of fermented guava beverage

After checking chemical characteristics for samples at 4 sugar contents: 15<sup>0</sup>Bx, 17<sup>0</sup>Bx, 19<sup>0</sup>Bx, 21<sup>0</sup>Bx; and pH = 3.5, these

content increases to 21<sup>0</sup>Bx, ethanol decreases slightly. This phenomenon is explained owing to high osmosis pressure by high dry mater leading to disorder yeast metabolism.

ones will be evaluated sensory. Turbidity, aroma and taste strongly affect to product quality. A board of 5 specialists is established to verify these parameters in fermented guava beverage.

**Table 7:** Sensory score about product turbidity in experiment determining sugar content supplemented

Sample	Initial sugar content (°Brix)	Sensory score of specialists							
		Turbidity					Average score without important factor	Important factor	Average score with important factor
		A	B	C	D	E			
1	15	2	4	3	3	3	3	0.8	2.40
2	17	4	5	4	5	4	4.4	0.8	3.52
3	19	3	4	3	4	3	3.4	0.8	2.72
4	21	2	2	3	2	3	2.4	0.8	1.92

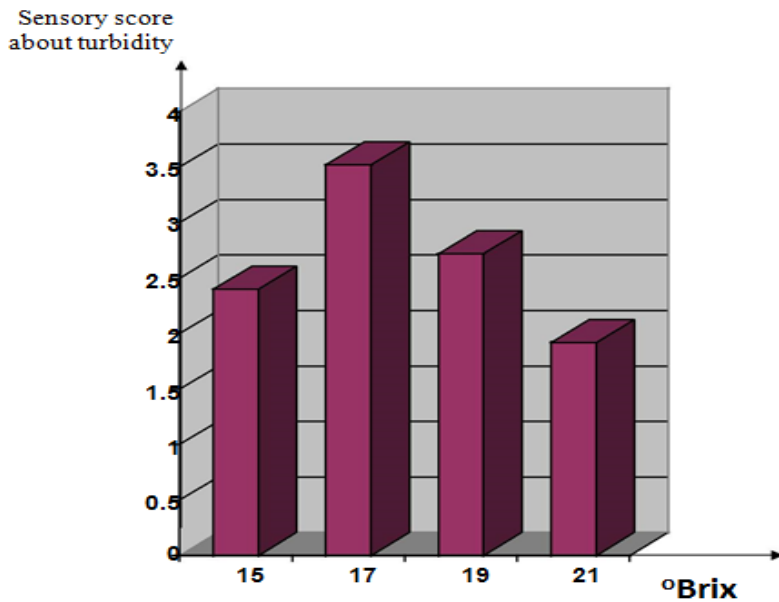


Fig 9: Effect of sugar content to product turbidity

Table 8: Sensory score about product aroma in experiment determining sugar content supplemented

Sample	Initial sugar content (°Brix)	Sensory score of specialists							
		Aroma					Average score without important factor	Important factor	Average score with important factor
A	B	C	D	E					
1	15	3	4	3	2	3	3.0	1.2	3.60
2	17	4	5	4	4	4	4.2	1.2	5.04
3	19	3	4	5	3	4	3.6	1.2	4.32
4	21	3	2	3	2	3	2.6	1.2	3.12

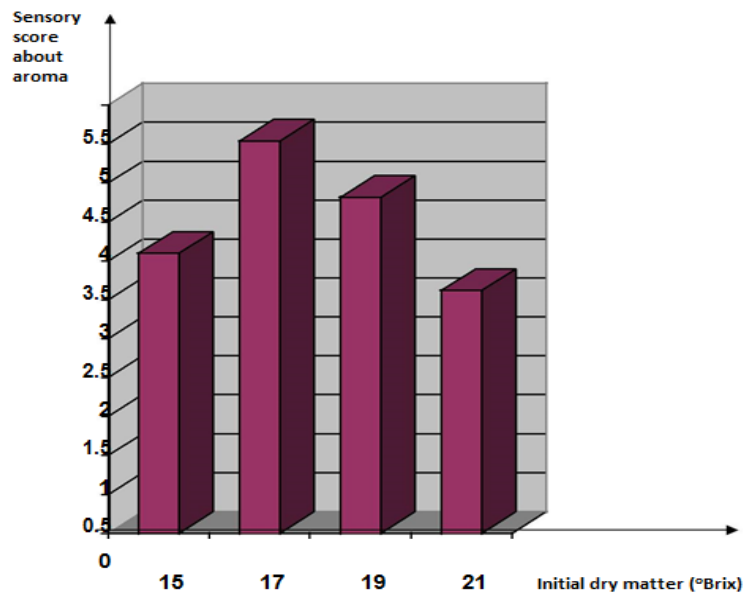


Fig 10: Effect of sugar content to product aroma

Table 9: Sensory score about product taste in experiment determining sugar content supplemented

Sample	Initial sugar content (°Brix)	Sensory score of specialists							
		Taste					Average score without important factor	Important factor	Average score with important factor
A	B	C	D	E					
1	15	2	4	3	3	3	3	2.0	6.0
2	17	5	4	5	4	4	4.4	2.0	8.8
3	19	4	4	3	4	3	3.6	2.0	7.2
4	21	2	3	2	3	2	2.4	2.0	4.8

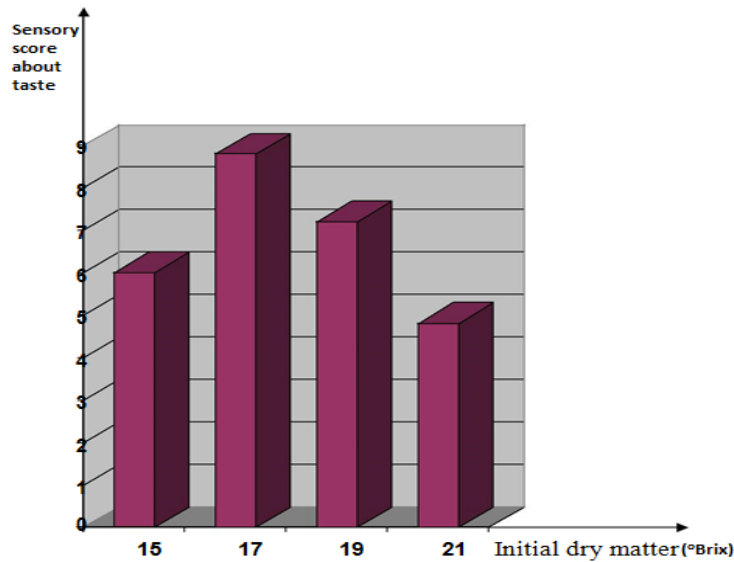


Fig 11: Effect of sugar content to product taste

Sugar content reaches 15<sup>0</sup>Bx the sensory score with important factor about turbidity 2.4, aroma 3.6 and taste 6.0. If sugar content comes from 15<sup>0</sup>Bx to 17<sup>0</sup>Bx, the highest sensory score about turbidity, aroma and taste is noticed at 17<sup>0</sup>Bx (turbidity 3.52; aroma 5.02 and taste 8.8). However, if we continue increase sugar content from 17<sup>0</sup>Bx to 21<sup>0</sup>Bx sensory score becomes decrease. At 21<sup>0</sup>Bx, fermented guava gets more turbidity and worse flavour. So we choose initial sugar content 17<sup>0</sup>Bx and pH = 3.5 for further studies.

**3.5 Effect of yeast supplementation**

Yeast numbers strongly affect to product ethanol and fermentation time

**3.5.1 Effect of yeast ratio to chemical and sensory characteristics of fermented guava beverage**

Table 10: Summary of experiment determining yeast ratio for fermentation

Initial sugar content (°Brix)	pH	Yeast ratio (%)	Yeast density (cfu/ml)	Fermentation temperature (°C)	Fermentation time (day)	Average sensory score with important factor	Ethanol (%V)
17	3.5	2	47.10 <sup>6</sup>	30	3	9.9	5.2
17	3.5	3	47.10 <sup>6</sup>	30	3	16.9	6.2
17	3.5	4	47.10 <sup>6</sup>	30	3	14.9	7.0
17	3.5	5	47.10 <sup>6</sup>	30	3	11.6	7.5

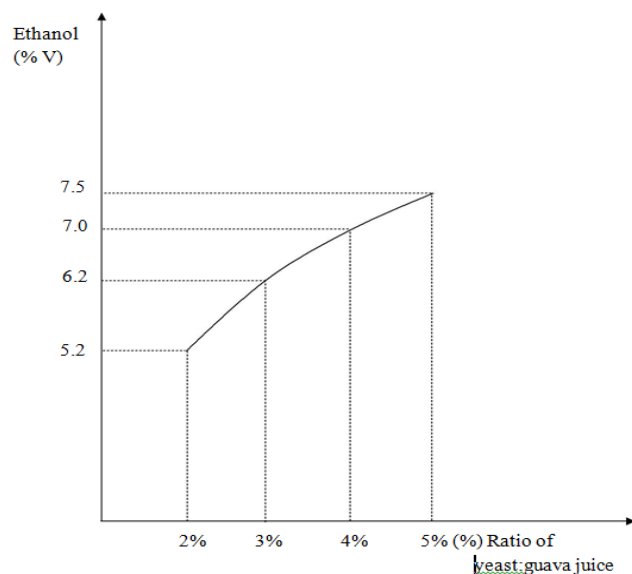


Fig 12: Effect of yeast ratio to product ethanol

The more yeast ratio is, the more ethanol we get. However, too much yeast also create bad effects such as turbidity and bad flavour. We fix sugar content so if adding more yeast, ethanol is nearly stable. At yeast 2%, ethanol we get ethanol at 5.2%.

If yeast increases to 3%, ethanol increases to 6.2 %. If the yeast ratio is 4% or 5%, ethanol flavour will press special product flavour. So this yeast ratio 3% is appropriated to get the good product quality.



**3.5.2 Sensory value of fermented guava beverage at ideal yeast ratio**

In order to verify our comment at yeast ratio 3%, we establish

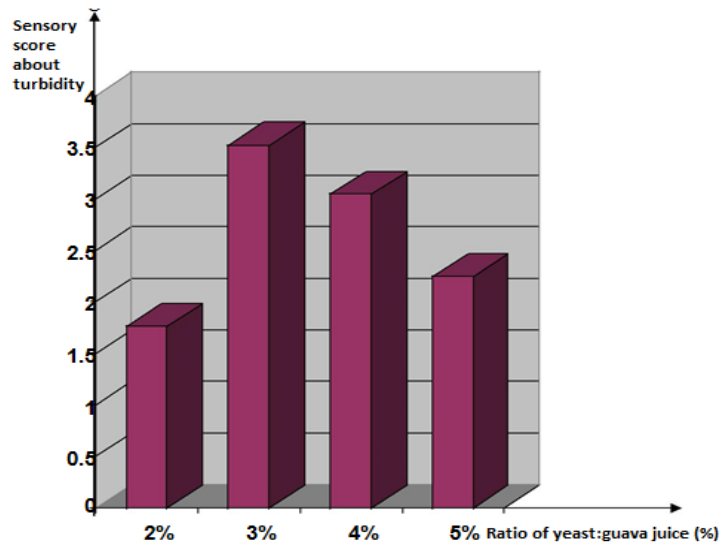
a board of 5 specialists to evaluate sensory of product after being fermented at 2%, 3%, 4%, 5% yeast.

**Table 11:** Sensory score about product turbidity of experiment determining yeast ratio for fermentation

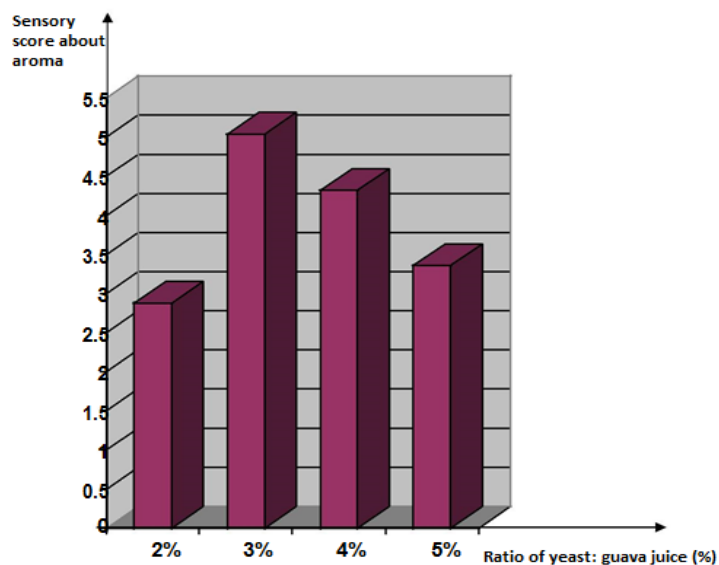
Initial sugar content (°Brix)	pH	Yeast ratio (%)	Sensory score of specialists							
			Turbidity							
			A	B	C	D	E	Average score	Important factor	Average score with important factor
17	3.5	2%	2	2	2	3	2	2.2	2	1.76
17	3.5	3%	4	5	5	4	4	4.4	2	3.52
17	3.5	4%	4	3	3	5	4	3.8	2	3.04
17	3.5	5%	3	4	3	2	2	2.8	2	2.24

**Table 12:** Sensory score about product aroma of experiment determining yeast ratio for fermentation

Initial sugar content (°Brix)	pH	Yeast ratio (%)	Sensory score of specialists							
			Aroma							
			A	B	C	D	E	Average score	Important factor	Average score with important factor
17	3.5	2%	2	2	3	2	3	2.4	1.2	2.88
17	3.5	3%	4	5	4	4	4	4.2	1.2	5.04
17	3.5	4%	3	4	5	4	4	3.6	1.2	4.32
17	3.5	5%	3	4	3	2	2	2.8	1.2	3.36



**Fig 13:** Effect of yeast to product turbidity



**Fig 14:** Effect of yeast to product aroma

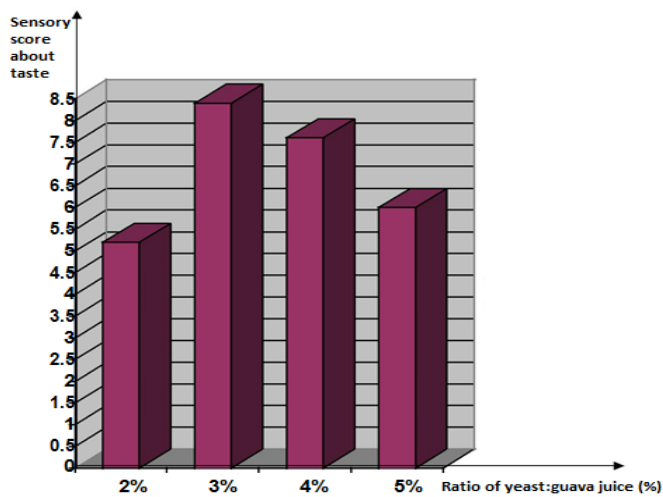


**Table 13:** Sensory score about product taste of experiment determining yeast ratio for fermentation

Initial sugar content (°Brix)	pH	Yeast ratio (%)	Sensory score of specialists							
			Taste							
			A	B	C	D	E	Average score	Important factor	Average score with important factor
17	3.5	2%	3	3	2	3	2	2.6	2	5.2
17	3.5	3%	4	4	5	4	4	4.2	2	8.4
17	3.5	4%	4	4	3	4	3	3.8	2	7.6
17	3.5	5%	2	4	3	3	3	3.0	3	6.0

**Table 14:** Summary of experiment determining fermentation time

Time (days)	Initial sugar content (°Brix)	pH	Yeast ratio (%)	Yeast density (cfu/ml)	Temperature (°C)	Ethanol (%V)	Average score with important factor	Residual sugar (g/l)
2	17	3.5	3	47.10 <sup>6</sup>	30	4.2	10.48	8.3
3	17	3.5	3	47.10 <sup>6</sup>	30	6.2	18.16	5.5
4	17	3.5	3	47.10 <sup>6</sup>	30	7.1	13.88	4.3
5	17	3.5	3	47.10 <sup>6</sup>	30	7.4	14.16	3.7

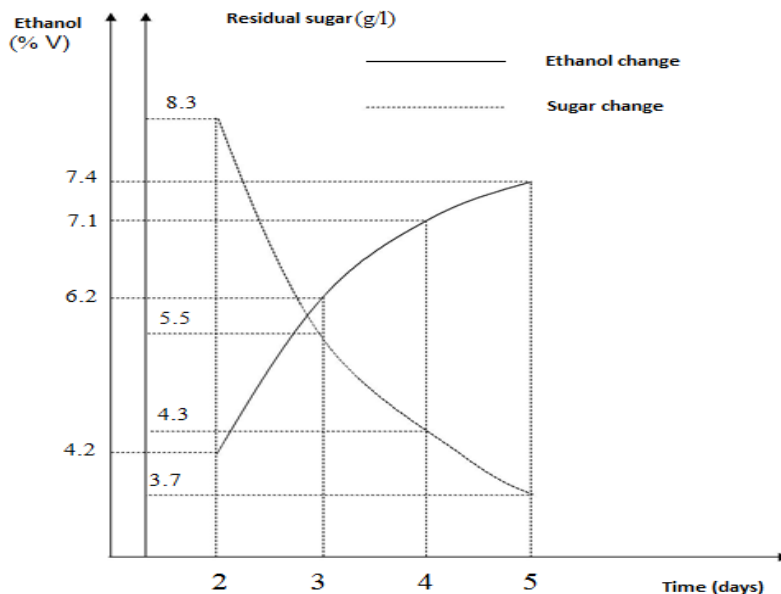


**Fig 15:** Effect of yeast to product taste

Yeast ratio at 2% shows low sensory score. When increasing from 2% to 5%, we get better product quality, especially at 3%. So this value is selected for further researches.

**3.6. Effect of fermentation time**

**3.6.1 Results of chemical and sensory evaluation by fermentation time**



**Fig 16:** Effect of fermentation time to product ethanol

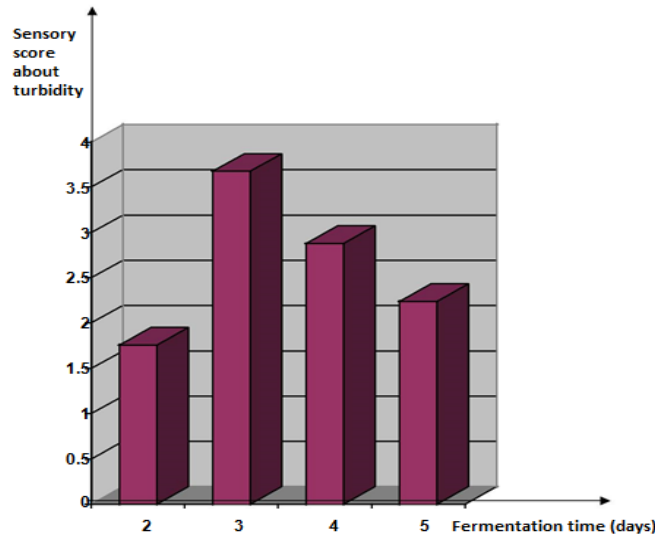
When increasing fermentation time from 2 to 5 days, ethanol formation will also increase from 4.2 to 7.4 degree. However, this formation speed happens quickly in the first 2 days. At the

third day, residual sugar is 5.5 g/l and down to 3.7 g/l at the 5<sup>th</sup> day. So we select 3 days for further studies.

### 3.6.2 Results of product sensory by fermentation time

**Table 15:** Sensory score about product turbidity of experiment determining fermentation time

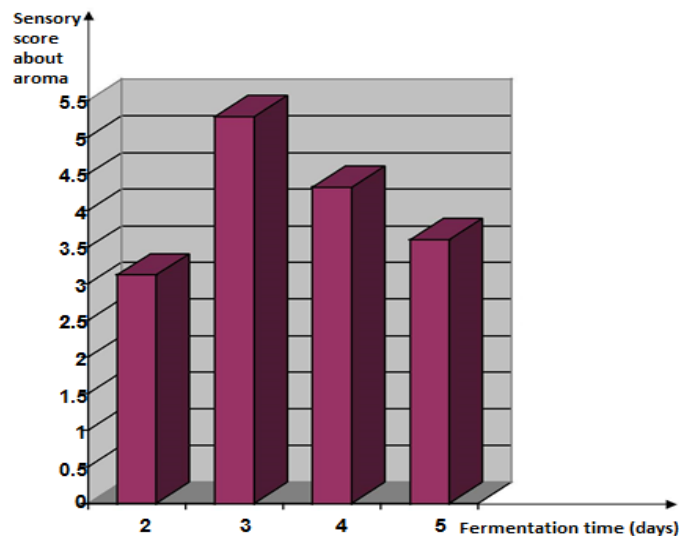
Sample	pH	Initial sugar content (°Brix)	Yeast ratio (%)	Fermentation time (days)	Sensory score of specialists							
					Turbidity					Average score	Important factor	Average score with important factor
					A	B	C	D	E			
1	3.5	17	3	2	2	2	2	3	2	2.2	0.8	1.76
2	3.5	17	3	3	5	4	5	4	5	4.6	0.8	3.68
3	3.5	17	3	4	4	4	3	3	4	3.6	0.8	2.88
4	3.5	17	3	5	3	3	2	2	4	2.8	0.8	2.24



**Fig 17:** Effect of fermentation time to product turbidity

**Table 16:** Sensory score about product aroma of experiment by fermentation time

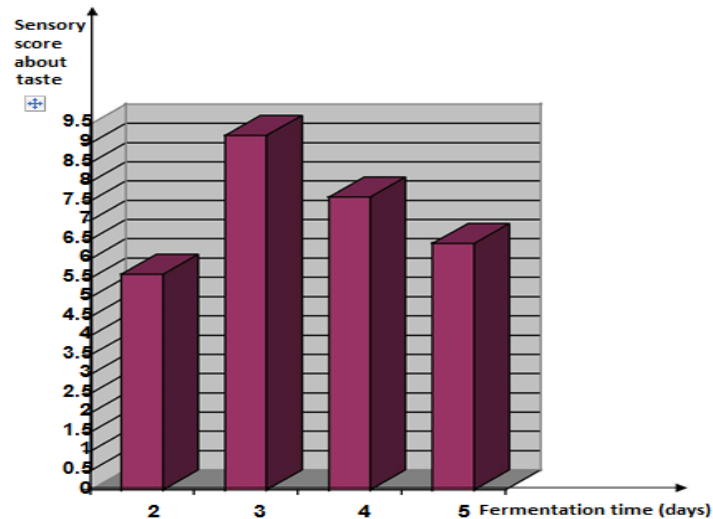
Sample	pH	Initial sugar content (°Brix)	Yeast ratio (%)	Fermentation time (days)	Sensory score of specialists							
					Aroma					Average score	Important factor	Average score with important factor
					A	B	C	D	E			
1	3.5	17	3	2	3	2	3	3	3	2.6	1.2	3.12
2	3.5	17	3	3	5	4	4	5	4	4.4	1.2	5.28
3	3.5	17	3	4	4	3	3	4	4	3.6	1.2	4.32
4	3.5	17	3	5	4	3	2	3	3	3.0	1.2	3.6



**Fig 18:** Effect of fermentation time to product aroma

**Table 17:** Sensory score about product taste of experiment by fermentation time

Sample	pH	Initial sugar content (°Brix)	Yeast ratio (%)	Fermentation time (days)	Sensory score of specialists							
					Taste					Average score	Important factor	Average score with important factor
					A	B	C	D	E			
1	3.5	17	3	2	2	3	4	3	2	2.8	2	5.6
2	3.5	17	3	3	5	4	5	4	5	4.6	2	9.2
3	3.5	17	3	4	4	5	3	4	3	3.8	2	7.6
4	3.5	17	3	5	3	3	4	3	3	3.2	2	6.4

**Fig 19:** Effect of fermentation time to product taste

Guava juice prepared for fermentation has pH = 3.5, initial sugar content 17° Bx (equivalent to 170 g sucrose / litre of juice) and then ferment at 30 °C. At the 3<sup>rd</sup> day of fermentation, beverage has sensory score with turbidity 3.68, aroma 5.28 and taste 9.2. Total sensory score for these parameters is 18.16.

### 3.7 Fermented guava beverage evaluation

After finding parameters for production, we take samples to analyse microorganism and sensory evaluation including a board of 5 specialists for three parameters: turbidity, aroma and taste.

**Table 18:** Sensory score of product turbidity, aroma and taste

Parameter	Scores of specialists					Total scores	Average score	Important factor	Average score with important factor
	A	B	C	D	E				
Turbidity	4	3	5	3	4	19	3.8	0.8	3.04
Aroma	3	5	4	4	5	21	1.2	1.2	5.04
Taste	4	4	5	5	4	22	4.4	2.0	8.80

Comparing to standard regulated by TCVN 3215 – 79, nutrient compositions, and initial characteristics change not much. It remains flavour except color. Turbidity of beverage is low.

### 3.8 Production cost of fermented guava beverage

We estimate production cost for 100 litres of fermented guava beverage

**Table 19:** Production cost for 100 litres of fermented guava beverage

No	Description	Unit	Unit price (VND)	Quantity	Amount (VND)
1	Guava	Kg	5,000	110	550,000
2	Refine sugar	Kg	13,000	23	299,000
3	Yeast	Litre	5,000	3	15,000
4	Citric acid	Kg	180,000	0.03	540
5	Electricity	Kwh	1,600	150	240,000
6	Water	M <sup>3</sup>	2,500	2	57,000
7	Distilled water	Litre	6,000	35	210,000
8	Others				100,000
<b>Total</b>					1,471,540

This value is based on 100 litres of fermented guava beverage. So production cost for 1 litre of this beverage will be 14,715 VND.

#### 4. Conclusion

Guava has great potential for extensive commercial use because of its ease of culture, high nutritive value and popularity of processed guava products. Demand of beverage consumption is increasing rapidly. This research can be considered as a minor contribution into this success to diversify Vietnamese fruits, acquiring for most consumers.

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