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**Nguyen Phuoc Minh**
*Tra Vinh University, Vietnam*

**Correspondence:**
**Nguyen Phuoc Minh**
Tra Vinh University, Vietnam.

# Utilization of ripen star fruit for vinegar fermentation

## Nguyen Phuoc Minh

### Abstract
Fermentation of star fruit juice was conducted to produce vinegar using the yeast *Saccharomyces cerevisiae* and *Acetobacter aceti* bacteria. Results are as follows: ethanol fermentation: soluble dry matter 9.21 $^{o}$Brix, pH 4.3, yeast supplementation from $14.10^6$ cfu/ml to$16.10^6$ cfu/ml, fermentation time 7 days, ethanol concentration 7-8%. acetic acid formation by traditional method: ethanol concentration 7%, pH 3.5, *acetobacter* 15%, fermentation time 25 days, acetic acid achievement 43-48 gram/litre. Acetic acid formation by quick method: ethanol 7%, pH 3.5, *acetobacter* 20%, fermentation time 6-7 days.

**Keywords:** Star fruit, fermentation, Saccharomyces cerevisiae, Acetobacter aceti, vinegar.

### 1. Introduction
The star fruit of carambola (*Averrhoa carambola* L.) an attractive fruit of family *Oxalidaceae*, also known as 'Golden Star' had attained the status of a popular commercial crop in the Vietnam. Star fruit is green when unripe; the fruit vary from pale yellow to deep amber when ripe. It has four to six strongly pointed ridges that run from top to bottom, and the soft flesh is encased in a thin waxy, translucent skin (which is eaten together with the flesh). When the fruit is cut crosswise each slice is shaped like a star, hence its named, "star fruit." The fruit, which is mostly consumed fresh or as juice, is rich in vitamins A and C and it also has iron, and has high fiber content. The taste varies from sour to sweet, one way of distinguishing the sour variety from the sweeter ones is that the former has narrower ribs, while the latter have thicker, fleshy ones. Unripe star fruit is preserved in many parts of Southeast Asia and is used as a traditional remedy. Most carambola fruits are marketed in processed forms. It is edible and has numerous uses. The ripe fruit may be processed into fermented or unfermented drinks, jam or jelly, can be eaten fresh or as dessert. The unripe fruit may also be eaten as a vegetable (Campbell, 1986). The sweet type is processed into wine in Surinam (Lewis & Grocizam 1989). The fresh fruits of carambola (Averrhoa carambola L.) are used in jelly making, for garnishing salads and to prepare drinks. In some Asian countries, the green mature fruit is relished and consumed fresh and used in pickle preparations (Avinash G. Patil *et al.*, 2010).
Y. Duangjitcharoen *et al.*, (2009) investigated the potential use of probiotic *Lactobacillus plantarum* SS2 isolated from a fermented plant beverage: safety assessment and persistence in the murine gastrointestinal tract. In the present study, its in vivo safety and gastrointestinal survival following oral administration to mice was investigated. The acute toxicity test on ICR mice by force-feeding of a single dose of $10^9$ and $10^{12}$cells per mouse over 14 days after ingestion showed no adverse effects related to the normal behavior of the laboratory mouse. Although the weight gains of the dosed mice were lower than the control they were still within normal values. There were no significant differences in liver weight ratio or spleen weight index among tested mice and control mice and no evidence of bacteremia. Live SS2 cells labeled with a fluorescent dye (cFDA-SE) and administered orally at $10^9$ cells per mouse were shown to persist in the gastrointestinal tract for 7 days. Colonies similar to the SS2 were detected in fecal samples from the test mice even though the fecal lactic acid bacterial count showed no significant difference in any mice. The strain SS2 is therefore considered to be a possible alternative choice for an inoculum to produce fermented plant beverages.
Phonesavard Sibounnavong *et al.*, (2010) applied *Saccharomyces cerevisiae* for wine production from star gooseberry and carambola. The experiment was to produce wine from star gooseberry (Phyllanthus acidus (L) Skeels and carambola (Averrhoa carambola L.) by fermented with Saccharomyces cerevisiae for two weeks. Results showed that star gooseberry wine gave significantly higher total acid (%TA) than carambola wine at all formulations but the star gooseberry wine had lower acidity than carambola wine. Star gooseberry wine gave significantly higher in ethyl alcohol production (averaged 15.90%) than carambola wine (averaged 8.28%). Meanwhile, star gooseberry wine formulation 4 gave the highest ethyl

alcohol (23.12%), and followed by carambola wine formulation 4 (14.37%), star gooseberry wine formulation 3 (17.25%), star gooseberry wine formulation 2 (13.75%), star gooseberry wine formulation 1 (9.5%), carambola wine formulation 3 (8.75%), carambola wine formulation 2 (6.5%) and the lowest ethyl alcohol production in carambola wine formulation 1 (3.5%). The amount of ethyl alcohol was analysed in each formulation both in star gooseberry wine and carambola wine. It is demonstrated that all formulations of star gooseberry wine showed significantly higher amount of ethyl alcohol than all formulations of carambola wine.

Abdul Karim, et al. (2011) conducted fermentation of vinegar from star fruit *(averrhoa carambola).* The acetic fermentation was carried out for 120 hours in shake flasks with ethanol concentration of 4, 6 and 8% (v/v), inoculums size of 6, 8 and 10% or ml (v/v) and agitation of 150, 200 and 250 rpm. Two level factorial designs from Design Expeli are applied for experimental design and analyses of acetic acid and ethanol were conducted using ANOVA analysis to select the optimized results. The experiment showed that the optimum conditions for acetic fermentation is with 8% (v/v) of ethanol and 10% inoculums (v/v) and 250 rpm agitation speed. The vinegar obtained had a concentration of acetic acid of 2.855% which was higher than predicted value obtained from the selected fennented star fruit juice and the efficiency of acetic fermentation was 32.5%.

Abdul Karim *et al.*, (2011) used single stage stirred tank bioreactor production of star fruit *(Averrhoa carambola)* vinegar. Vinegar was produced in the lab from fermentation of local star fruit (Averrhoa carambola) juice in stirred tank bioreactor. The fermentation of star fruit juice into vinegar was produced by decomposing sugar contained in the fruit substrate through alcohol and acetic acid fermentation over a period of three days with the aid of two microorganisms, Saccharomyces cerevisiae and Acetobactor aceti. To Study the optimization of the fermentation process, the parameter conditions used in the tank bioreactor are agitation speed, aeration and the concentration of glucose used. The design of the experiment was conducted using Design Expert software. Analysis at every 12 hours interval was carried for ethanol concentration, reducing sugar level and acetic acid. The findings in this project can be applied to produce vinegar in large amount from our local tropical star fruit juice. The experiment showed that the optimum conditions for agitation was 300 rpm, aeration at 0.5 Lpm and glucose concentration (20%) consequently produced 1.63% vinegar.

Abdul Karim *et al.*, (2011) conducted a kinetic study on vinegar production using star fruit juice. A study was undertaken to produce Star fruit vinegar (Averrhoa carambola) juice through double stage fermentation with the use of microorganisms namely Saccharomyces cerevlszae and Acetobacrer acetl. The star fruit juice with optimum glucose concentratIOn (20%) was used and growth kinetic study of the cultures to optimize operational conditions ( agitation speed and inoculums size) in 2L bioreactor for production of star fruit vinegar was conducted .An experimental design using the Central Composite Design by Design Expert software, where the main factors Impeller speed(rpm) and moculums sizes (% inoculums) were optimized affecting the production of acetic acid in fruit Juice during fermentation. Sampling of the were done every 12 hours during 4 days of fermentation time to analyze cell concentration (OD 600 nm), Total Cell Number, cell dry weight, reducing sugar, ethanol and acetic acid production. On the different combination of factors for all 9 runs of experiment conducted, It was found optimum production of acetic acid was achieved with agitation speed of 250 rpm and 5% inoculums sizes where highest ethanol production of 17.8% or 150.34 g/L. and 2.76% acetic acid (TA) was obtained. This combination of parameters produces the highest specific growth rate, u and highest yield which are 0.859 and 0.342 g/g of the culture was obtained.

Sanjib K. Paul andJatindra K. Sahu (2014) studied the process optimization and quality analysis of carambola (*Averrhoa carambola L.*) Wine. The juice extracted from carambola (*Averrhoa carambola L.*) fruits was used for wine production after reconstituting with distilled water, 50% (*w/v*) sugar syrup and 0.1 N oxalic acid at different concentrations to attain a range of different independent processing parameters. The processing conditions i.e., pH, fermentation temperature, inoculum size, and total soluble solids (TSS) were optimized on the basis of percentage of ethanol production. At optimum processing conditions of pH = 4.5, temperature = 26 °C, inoculums size = 12% (*v/v*), and TSS = 24°B, the production of ethanol percentage in the wine was 12.15 ± 0.28% (*v/v*). At this optimum condition, the values of titratable acidity, total sugar, reducing sugar, and TSS were 0.76 ± 0.21% (*w/w*), 2.84 ± 0.22% (*w/w*), 2.65 ± 0.16% (*w/w*), and 4.6 ± 0.06°B, respectively. Color of the wine was observed to be light greenish yellow, but pH level which was 3.94 ± 0.17 was slightly higher than that of the acceptable limit. Sensory evaluation showed that the wine possessed very good taste, aroma, and clarity with moderately good body and aftertaste. It was also observed that the wine undergoes color changes during storage with more stability at lower storage temperature.

Purpose of our research is to utilize the available ripen star fruit in Mekong River Delta, Vietnam to ferment into a valuable vinegar.

## 2. Material & Method

### 2.1 Material

Star fruits are collected in rural area of Tra Vinh province, Vietnam. *Saccharomyces cerevisiae*, *Acetobacter aceti* strains are supplied from Pasteur Institute, HCM City, Vietnam.

### 2.2 Research method

#### 2.2.1 Tanin removal in star fruit juice

**2.2.1.1** Tanin removal by NaCl

Star fruit fruits are blanched with NaCl soulution at 1%, 2%, 3% in 90 $^{o}$C during 5 minutes. Then pulp is pressed and filtered by filter paper. Star fruit filtrate is then removed tanin.


**Fig 1:** Star fruit (Carambola)

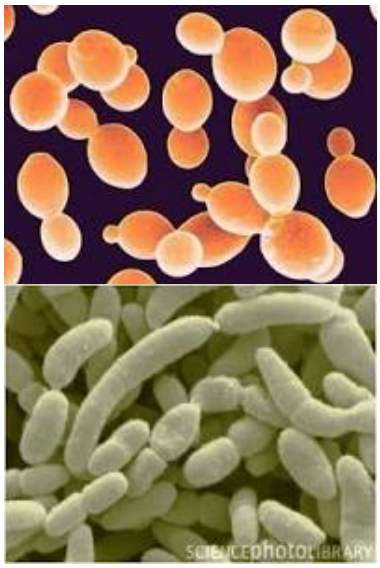

**Fig 2:** *Saccharomyces cerevisiae* and *Acetobacter aceti*

**2.2.1.2 Tanin removal by H2SO4 0.2N in different ratios**
Star fruit fruits are blanched with hot water at 70 $^{o}$C, supplemented with $H_2SO_4$ 0.2N with different ratios 10%, 15%, 20%, 25% compared to water volume. We continue keeping them in 5 minutes, cooling them quickly in distilled water, washing them carefully before pressing and filtering. Star fruit filtrate is then removed tannin.

**2.2.1.3 Tanin removal by $H_2SO_4$ 0.2N in different times**
Star fruit fruits are blanched with $H_2SO_4$ 0.2N to 70 $^{o}$C in different times 3 minutes, 4 minutes, 5 minutes, 6 minutes. We continue **c**ooling them quickly in distilled water, washing them carefully before pressing and filtering. Star fruit filtrate is then removed tannin.

**2.2.1.4 Tanin removal by gelatin**
Star fruit juice which is heated to 60$^{o}$C is treated with gelatin ratios 1.0%; 1.5%; 2.0%; 2.5%. Then we thoroughly mix it in 5 minutes, stable it in 60 minutes. The star fruit filtrate is then removed tannin.

**2.2.1.5 Tanin removal by $Ca(OH)_2$**
We use $Ca(OH)_2$ to adjust pH of star fruit filtrate (initial pH 4.31) to pH 4.7, 5.0, 5.5, 6.0. Then we can use $H_3PO_4$ to neutralize pH to the initial point. Leave it in 60 minutes and analyse tannin content.

**2.2.1.6 Tanin removal by NaCl 2% and gelatin**
Star fruit fruits are blanched with hot water at 90 $^{o}$C combined with NaCl 1.5%, 2.0%, 2.5% in 5 minutes. Then we rinse it thoroughly by clean water, press to take juice, heat to 60$^{o}$C, mix with gelatin 2% in 5 minutes, leave it in 60 minutes, analyse tannin content.

**2.2.2 Ethanol fermentation**
**2.2.2.5 Initial condition for ethanol fermentation**

**2.2.2.1 Yeast growth ability in star fruit juice**
Yeast strain used for experiment belongs to *Saccharomyces cerevisiae* proliferated in Tra Vinh university laboratory. Star fruit filtrate is heated in 100 $^{o}$C within 3 minutes, cooled to 85oC in 20 minutes, then quickly cooled under tap water. After getting the fermented fluid, we inoculate culture onto agar medium at pH 4.3 and soluble dry matter 9.21. After 24 hours, we check some parameters: total yeast cells, proliferated yeast cells, dead yeast cells.

**2.2.2.2 Soluble dry matter to the ethanol fermentation**
Star fruit filtrate after being removed tannin is adjusted to the appropriated soluble dry matter (9, 10, 11, 12, 13 and 14 $^{o}$Brix). We inoculate yeast strain into the prepared liquid medium. After fermentation, we check the soluble dry matter, pH, acidity, ethanol, sensory characteristics.

**2.2.2.3 Yeast ratio to the ethanol fermentation**
We prepare the fermentation batch as follows: filtrate after being removed tannin is supplemented with different yeast ratios (10-12 million cfu/ml, 12-14 million cfu/ml, 14-16 million cfu/ml, 16-18 million cfu/ml, 18-20 million cfu/ml). Then we inoculate the prepared yeast strain from the liquid medium. After fermentation, we check the soluble dry matter, pH, acidity, ethanol, sensory characteristics.

**2.2.2.4 Molasses and sacharoza supplementation to increase soluble dry matter in fermentation batch**
We prepare the fermentation batch as follows: filtrate after being removed tannin is adjusted different pH values (10, 11, 12, 13, 14 $^{o}$Brix). Then we inoculate the prepared yeast strain from the liquid medium. After fermentation, we check the soluble dry matter, pH, acidity, ethanol, sensory characteristics.

**Table 1:** Initial condition for ethanol fermentation from the star fruit juice

| Sample | Initial condition | | | | |
|---|---|---|---|---|---|
| | Soluble dry matter | pH | Yeast supplementation | Tanin removal | Star fruit pulp |
| **1** | 9.21 | 4.3 | No | No | No |
| **2** | 9.21 | 4.3 | No | No | Yes |
| **3** | 9.21 | 4.3 | No | Yes | Yes |
| **4** | 9.1 | 4.3 | Yes | Yes | Yes |

**2.2.3 Acetic acid fermentation by the traditional method**

**2.2.3.1 Effect of ethanol concentration to acetic acid formation**

Experiments are performed in the same conditions with the exception of ethanol concentration (7, 8, 9, 10 degree). Purpose of these experiments is to define the appropriate ethanol concentration for further fermentation. After 25 days, we check ethanol concentration, pH and acetic acid concentration.

**2.2.3.2 Effect of Acetobacter inoculum ratio to acetic acid fermentation**

Experiments are performed in the same conditions with the exception of Acetobacter inoculum (5, 10, 15%). After 25 days, we check ethanol concentration, pH and acetic acid concentration.

**2.2.3.3 Effect of pH to acetic acid fermentation**

Experiments are performed in the same conditions with the exception of pH (3.0, 3.5, 4.0, 4.5) of the fermentation batch. After 25 days, we check ethanol concentration, pH and acetic acid concentration.

**2.2.4 Acetic acid fermentation by feed-back method**

**2.2.4.1 Acetic acid fermentation by bacteria membrane forming**

**Table 2:** Survey the method of bacteria membrane forming

| Ratio of star fruit ethanol (%) | Ratio of Acetobacter (%) | Initial acetic acid concentration (g/l) |
|---|---|---|
| **95** | 5 | 30.0 |
| **90** | 10 | 29.4 |
| **80** | 20 | 28.0 |
| **70** | 30 | 27.0 |
| **65** | 35 | 26.5 |

After 24 hours, we measure acetic acid accumulation until its concentration over 30 gram per litre.

**2.2.4.2 Effect of ethanol concentration to acetic acid fermentation**

When we have a good bacteria membrane, we add star fruit ethanol (5%, 10%, 15%, 20%) to investigate the fermentation. The fermentation will last until acetic acid concentration after being supplemented ethanol equal to the acetic acid concentration before being supplemented ethanol.

**2.3 Sensory evaluation**

Sensory evaluation is conducted by TCVN 3215 – 79.

**2.4 Statistical analysis**

All data are processed by ANOVA (Startgraphics) to check the significant difference via LSD

**3. Result & Discussion**

**3.1 Tanin removal in star fruit juice**

**3.1.1 Tanin removal by NaCl**

**Table 3:** *Tanin removal by NaCl*

| Temperature (°C) | Time (minutes) | NaCl (%) | Residual tannin (g/l) | Tannin loss (%) |
|---|---|---|---|---|
| **90** | 5 | 1 | 1.974 | 59.9 |
| **90** | 5 | 2 | 1.580 | 67.9 |
| **90** | 5 | 3 | 1.500 | 69.5 |

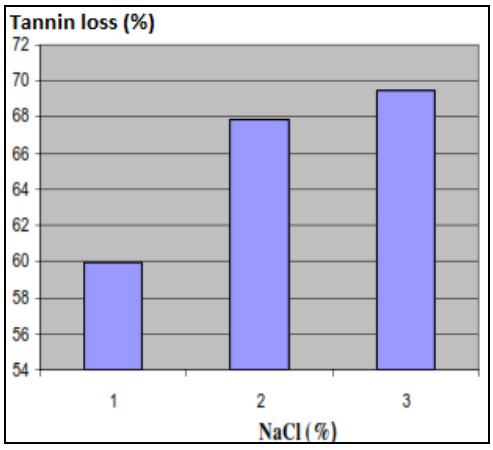


**Fig 3:** Tanin removal by NaCl

We choose NaCl 2% for further experiments.

### 3.1.2 Tanin removal by gelatin

**Table 4:** Tanin removal by gelatin

| Temperature (°C) | Time (minutes) | Gelatin (%) | Residual tanin (g/l) | Tanin loss (%) |
|---|---|---|---|---|
| **60** | 5 | 1.0 | 1.70 | 65.4 |
| **60** | 5 | 1.5 | 1.60 | 67.4 |
| **60** | 5 | 2.0 | 1.58 | 67.9 |
| **60** | 5 | 2.5 | 1.66 | 66.2 |

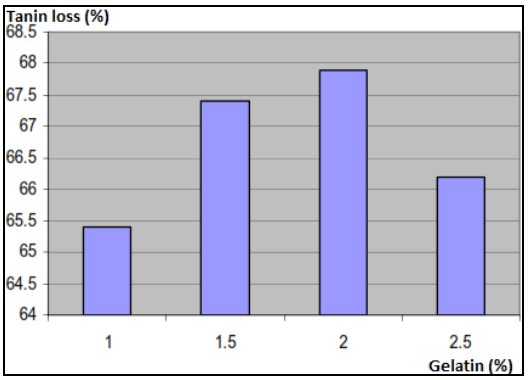


**Fig 4:** Tanin removal by gelatin
We choose gelatin 1% for further experiments

### 3.1.3 Tanin removal by Ca $(OH)_2$

**Table 5:** Tanin removal by Ca $(OH)_2$

| pH | Time | Residual tannin (g/l) | Tanin loss (%) |
|---|---|---|---|
| **4.31 (initial)** | 60 | 4.92 | 0.00 |
| **4.70** | 60 | 2.30 | 53.25 |
| **5.00** | 60 | 2.01 | 59.14 |
| **5.50** | 60 | 1.70 | 65.40 |
| **6.00** | 60 | 1.72 | 65.04 |

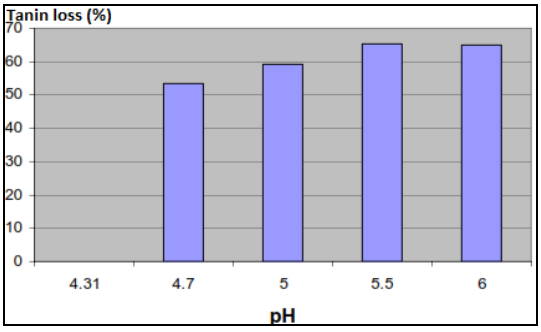


**Fig 5:** Tanin removal by Ca $(OH)_2$
The highest tannin removal is noted at pH 5.5 – 6.0

### 3.1.4 Tanin removal by H2SO4 0.2N in different ratios

**Table 6:** Tanin removal by $H_2SO_4$ 0.2N in different ratios

| Temperature (°C) | Time (minutes) | %$H_2SO_4$ 0.2 N | Residual tannin (g/l) | Tannin loss (%) |
|---|---|---|---|---|
| **70** | 5 | 10 | 3.28 | 33.33 |
| **70** | 5 | 15 | 3.24 | 34.14 |
| **70** | 5 | 20 | 2.99 | 39.22 |
| **70** | 5 | 25 | 2.90 | 41.05 |

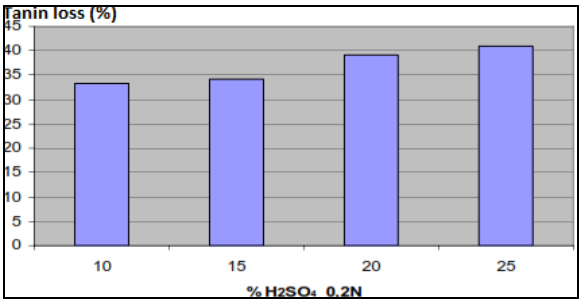


**Fig 6:** Tanin removal by $H_2SO_4$ 0.2N in different ratios

At $H_2SO_4$ 0.2N 25%, tannin removal gets the highest efficiency but %) but it's not safe for product so we don't choose this method.

### 3.1.5 Tanin removal by $H_2SO_4$ 0.2N in different times

**Table 7:** Tanin removal by $H_2SO_4$ 0.2N in different times

| Temperature (°C) | Time (minutes) | %$H_2SO_4$ 0.2N | Residual tannin (g/l) | Tanin loss (%) |
|---|---|---|---|---|
| **70** | 3 | 100 | 1.85 | 62.4 |
| **70** | 4 | 100 | 1.68 | 65.85 |
| **70** | 5 | 100 | 1.56 | 68.29 |
| **70** | 6 | 100 | 1.45 | 69.52 |

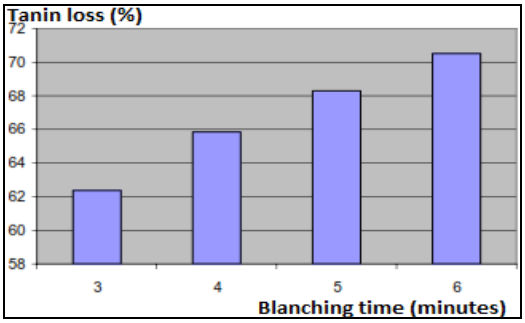


**Fig 7:** Tanin removal by $H_2SO_4$ 0.2N in different times

With blanching time 6 minutes, we get the highest tannin removal (69.52%) but it's not safe for product so we don't choose this method.

### 3.1.6 Tanin removal by combination of NaCl 2% and glegatin

**Table 8:** Tanin removal by combination of NaCl 2% and glegatin

| NaCl (%) | Gelatin (%) | Tanin removal efficiency (%) |
|---|---|---|
| **2** | 1.5 | 81.13 |
| **2** | 2.0 | 76.27 |
| **2** | 2.5 | 73.06 |

At NaCl 2% and gelatin 1.5% we get the highest tannin removal efficiency (%). So we choose this method for further experiments.

### 3.2 Ethanol fermentation

### 3.2.1 Proliferation of Saccharomyces cerevisiae in the star fruit juice

**Table 9:** Proliferation of *Saccharomyces cerevisiae* in the star fruit juice

| Time (hours) | Total cells ($x10^6$) | Budding cells ($x10^6$) | Dead cells ($x10^6$) |
|---|---|---|---|
| **0** | 0.24 | 0.00 | 0.00 |
| **24** | 28.25 | 4.32 | 1.86 |
| **48** | 133.20 | 17.51 | 4.66 |
| **72** | 172.35 | 23.06 | 8.25 |
| **96** | 198.75 | 18.14 | 10.92 |
| **120** | 201.10 | 15.63 | 12.17 |

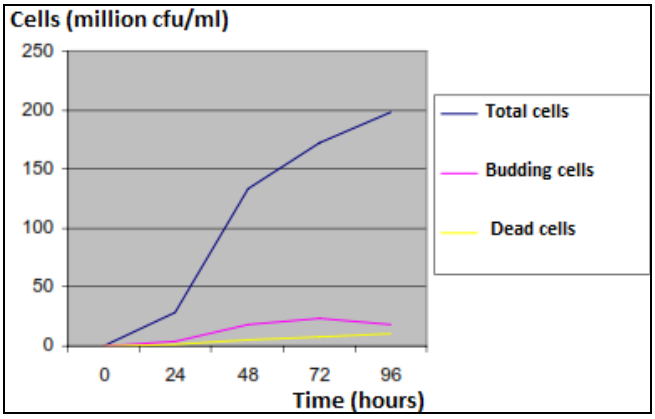


**Fig 8:** Proliferation of *Saccharomyces cerevisiae* in the star fruit juice

From figure 8, we notify some key points as follows:
- From 0 – 24 hours, yeast at the adaption stage
- From 24 – 48 hours, yeast at the growth stage
- From 48 – 72 hours, yeast at the stable stage
- After 72 hours, yeast at the dead stage.

So we choose the yeast at 24 – 72 hours to inoculate into the fermentation batch.

### 3.2.2 Effect of total soluble dry matter to the ethanol fermentation

**Table 10:** Effect of total soluble dry matter to the ethanol fermentation

| Sample | Initial factors | | | After 7 days of fermentation | | | |
|---|---|---|---|---|---|---|---|
| | oBrix | pH | Yeast cell (million/l) | oBrix | pH | Acidity (g/l) | %V ethanol |
| **1** | 9 | 5.5 | 14-16 | 5.4 | 3.5 | 3.6 | 7.00 |
| **2** | 10 | 5.5 | 14-16 | 5.2 | 3.4 | 3.3 | 7.08 |
| **3** | 11 | 5.5 | 14-16 | 5.0 | 3.4 | 3.6 | 8.93 |
| **4** | 12 | 5.5 | 14-16 | 4.7 | 3.6 | 3.1 | 10.00 |
| **5** | 13 | 5.5 | 14-16 | 5.3 | 3.4 | 3.2 | 9.84 |
| **6** | 14 | 5.5 | 14-16 | 5.9 | 3.4 | 3.2 | 9.92 |

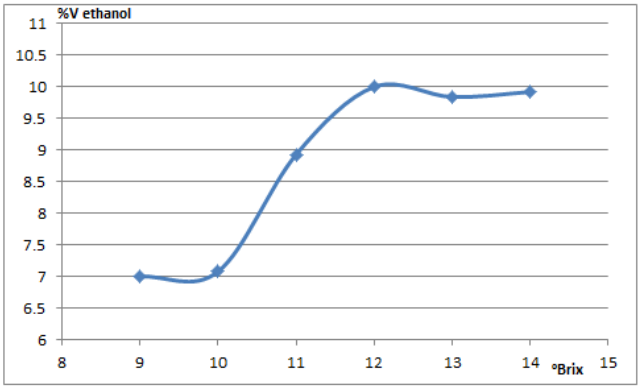


**Fig 9:** Effect of total soluble dry matter to the ethanol fermentation

Optimal soluble dry matter for ethanol fermentation is 12 °Brix.

### 3.2.3 Effect of pH to ethanol fermentation

**Table 11:** Effect of total soluble dry matter to the ethanol fermentation
**(Fermentation time o hour, yeast ratio 14-16 million cells/l)**

| Sample | pH | Acidity (g/l) | Dry matter (%) | %V ethanol |
|---|---|---|---|---|
| **1** | 4.0 | 2.24 | 12 | 0 |
| **2** | 4.5 | 2.23 | 12 | 0 |
| **3** | 5.0 | 2.12 | 12 | 0 |
| **4** | 5.5 | 2.10 | 12 | 0 |
| **5** | 6.0 | 2.00 | 12 | 0 |

**Table 12:** Effect of total soluble dry matter to the ethanol fermentation
**(Fermentation time 3 days, yeast ratio 14-16 million cells/l)**

| Sample | pH | Acidity (g/l) | Dry matter (%) | %V ethanol |
|---|---|---|---|---|
| **1** | 3.5 | 2.65 | 7.1 | 0 |
| **2** | 4.1 | 2.67 | 7.2 | 0 |
| **3** | 4.4 | 2.75 | 6.9 | 0 |
| **4** | 4.2 | 2.55 | 6.3 | 0 |
| **5** | 4.6 | 2.61 | 7.1 | 0 |

**Table 13:** Effect of total soluble dry matter to the ethanol fermentation
**(Fermentation time 5 days, yeast ratio 14-16 million cells/l)**

| Sample | pH | Acidity (g/l) | Dry matter (%) | %V ethanol |
|---|---|---|---|---|
| **1** | 3.3 | 2.75 | 5.9 | 0 |
| **2** | 3.9 | 2.78 | 5.8 | 0 |
| **3** | 3.8 | 2.79 | 5.3 | 0 |
| **4** | 3.7 | 2.59 | 5.2 | 0 |
| **5** | 4.0 | 2.63 | 5.8 | 0 |

**Table 14:** Effect of total soluble dry matter to the ethanol fermentation
**(Fermentation time 7 days, yeast ratio 14-16 million cells/l)**

| Sample | pH | Acidity (g/l) | Dry matter (%) | %V ethanol |
|---|---|---|---|---|
| **1** | 3.4 | 3.21 | 5.8 | 8.36 |
| **2** | 3.9 | 3.11 | 5.6 | 8.60 |
| **3** | 3.8 | 3.06 | 5.0 | 9.26 |
| **4** | 3.6 | 3.04 | 4.8 | 10.01 |
| **5** | 4.1 | 3.26 | 5.0 | 9.76 |

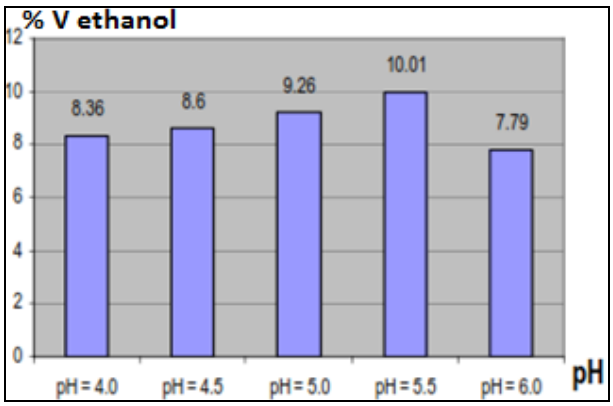


**Fig 10:** Effect of pH to ethanol fermentation
At pH 5-6, the ethanol formation is achieved maximum. So we choose this pH for ethanol fermentation.

### 3.2.4 Effect of yeast ratio to ethanol formation

**Table 15:** Effect of yeast ratio to ethanol formation

| Sample | Initial factors | | | After 7 days of fermentation | | | |
|---|---|---|---|---|---|---|---|
| | oBrix | pH | Yeast cell (million/l) | oBrix | pH | Acidity (g/l) | %V ethanol |
| **1** | 12 | 5.5 | 10-12 | 5.3 | 3.5 | 3.9 | 9.26 |
| **2** | 12 | 5.5 | 12-14 | 5.2 | 3.4 | 3.7 | 9.34 |
| **3** | 12 | 5.5 | 14-16 | 4.8 | 3.5 | 3.1 | 10.17 |
| **4** | 12 | 5.5 | 16-18 | 4.9 | 3.4 | 3.4 | 10.09 |
| **5** | 12 | 5.5 | 18-20 | 5.3 | 3.4 | 3.6 | 9.43 |

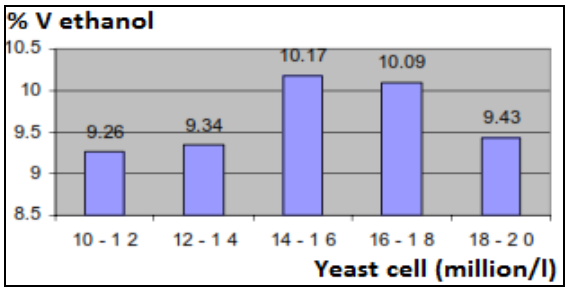


**Fig 11:** Effect of yeast ratio to ethanol formation
With yeast ratio 14-16 million per litre, we get the highest ethanol concentration.

### 3.2.5 Molasses and sacharoza supplementation to increase soluble dry matter in fermentation batch

**Table 16:** Effect of molasses supplementation to increase soluble dry matter in fermentation batch

| Sample | Initial factors | | | After 7 days of fermentation | | | |
|---|---|---|---|---|---|---|---|
| | oBrix | pH | Yeast cell (million/l) | oBrix | pH | Acidity (g/l) | %V ethanol |
| **1** | 10 | 5.5 | 14-16 | 5.5 | 3.8 | 4.0 | 7.00 |
| **2** | 11 | 5.5 | 14-16 | 5.3 | 3.6 | 3.9 | 8.08 |
| **3** | 12 | 5.5 | 14-16 | 4.8 | 3.5 | 3.9 | 9.92 |
| **4** | 13 | 5.5 | 14-16 | 4.9 | 3.7 | 3.8 | 9.01 |
| **5** | 14 | 5.5 | 14-16 | 5.3 | 3.7 | 4.1 | 8.93 |

**Table 17:** Effect of sacharoza supplementation to increase soluble dry matter in fermentation batch

| Sample | Initial factors | | | After 7 days of fermentation | | | |
|---|---|---|---|---|---|---|---|
| | oBrix | pH | Yeast cell (million/l) | oBrix | pH | Acidity (g/l) | %V ethanol |
| **1** | 10 | 5.5 | 14-16 | 5.3 | 3.2 | 3.7 | 7.39 |
| **2** | 11 | 5.5 | 14-16 | 5.1 | 3.3 | 3.6 | 8.31 |
| **3** | 12 | 5.5 | 14-16 | 4.6 | 3.4 | 3.5 | 10.01 |
| **4** | 13 | 5.5 | 14-16 | 4.9 | 3.2 | 3.4 | 9.76 |
| **5** | 14 | 5.5 | 14-16 | 5.3 | 3.1 | 4.0 | 9.10 |

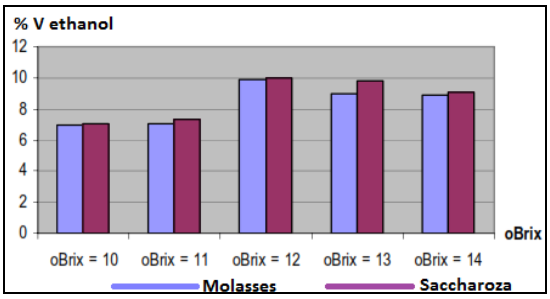


**Fig 12:** Effect of molasses and sacharoza supplementation to increase soluble dry matter in fermentation batch

### 3.2.6 Survey initial conditions for ethanol fermentation

**Table 18:** Initial conditions for ethanol fermentation

| Sample | Initial conditions | | | | | After 7 days of fermentation | | | |
|---|---|---|---|---|---|---|---|---|---|
| | °Brix | pH | Added Yeast | Tanin removal | Star fruit pulp | °Brix | pH | Acidity (g/l) | %V ethanol |
| **1** | 9.21 | 4.3 | No | No | No | 6.6 | 3.1 | 3.9 | 6.00 |
| **2** | 9.21 | 4.3 | No | No | Yes | 6.8 | 3.0 | 4.0 | 7.24 |
| **3** | 9.21 | 4.3 | No | Yes | Yes | 6.3 | 3.3 | 3.9 | 7.04 |
| **4** | 9.21 | 4.3 | Yes | Yes | Yes | 5.3 | 3.8 | 3.6 | 8.00 |

Ethanol fermentation is optimal at pH 5.5, soluble dry matter 12 oBrix, yeast ratio 14-16 million cells/litre.

Ethanol formations by two supplementations are nearly the same. However, samples supplemented by molasses have higher acidity than samples supplemented saccharoza. On the sensory characteristics (color and aroma), samples supplemented by molasses have lower value than samples supplemented saccharoza.

### 3.3 Vinegar fermentation by static method

#### 3.3.1 Effect of ethanol content to acetic acid formation

**Table 19:** Effect of ethanol content to acetic acid formation

| Sample | Initial condition | | | After 25 days of fermentation | | |
|---|---|---|---|---|---|---|
| | Ethanol (%) | pH | *Acetobacter* ratio (%) | Ethanol (%) | pH | Acetic acid (g/l) |
| **1** | 7 | 3.5 | 10 | 0.5 | 2.3 | 43.06 |
| **2** | 8 | 3.5 | 10 | 0.6 | 2.1 | 45.00 |
| **3** | 9 | 3.5 | 10 | 0.5 | 2.3 | 45.00 |
| **4** | 10 | 3.5 | 10 | 0.5 | 2.2 | 46.80 |

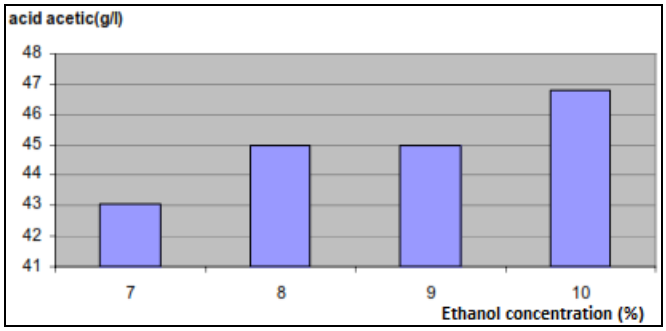


**Fig 13:** Effect of ethanol content to acetic acid formation

At ethanol concentration 10%, the acetic acid formation is maximum. However, there is no significant difference. To get 10% ethanol, the initial soluble dry matter should be 12 oBrix so we have to add more sugar inconvenient. In contrast, with ethanol 7%, there is no need to add more soluble dry matter. The initial dry matter in the star fruit 9.31% is enough to produce 7% ethanol. So we choose ethanol 7% for vinegar fermentation.

#### 3.3.2 Effect of Acetobacter aceti ratio to vinegar fermentation

**Table 20:** Effect of *Acetobacter aceti* ratio to vinegar fermentation

| Sample | Initial condition | | | After 25 days of fermentation | | |
|---|---|---|---|---|---|---|
| | Ethanol (%) | pH | *Acetobacter* ratio (%) | Ethanol (%) | pH | Acetic acid (g/l) |
| **1** | 7 | 3.5 | 5 | 1.0 | 2.5 | 39.0 |
| **2** | 7 | 3.5 | 10 | 0.6 | 2.2 | 43.5 |
| **3** | 7 | 3.5 | 15 | 0.5 | 2.1 | 46.0 |

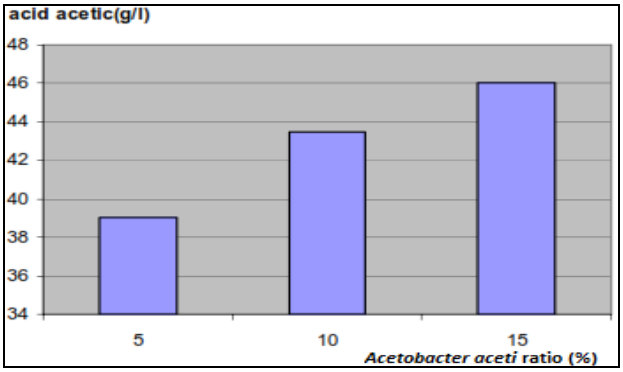


**Fig 14:** Effect of *Acetobacter aceti* ratio to vinegar fermentation

We choose *Acetobacter aceti* ratio 15% to vinegar fermentation

### 3.3.3 Effect of pH to vinegar fermentation

**Table 21:** Effect of pH to vinegar fermentation

| Sample | Initial condition | | | After 25 days of fermentation | | |
|---|---|---|---|---|---|---|
| | Ethanol (%) | pH | *Acetobacter* ratio (%) | Ethanol (%) | pH | Acetic acid (g/l) |
| **1** | 7 | 3.0 | 15 | | 1.9 | 48.0 |
| **2** | 7 | 3.5 | 15 | | 2.0 | 48.1 |
| **3** | 7 | 4.0 | 15 | | 2.1 | 48.8 |
| **4** | 7 | 4.5 | 15 | | 2.1 | 49.2 |

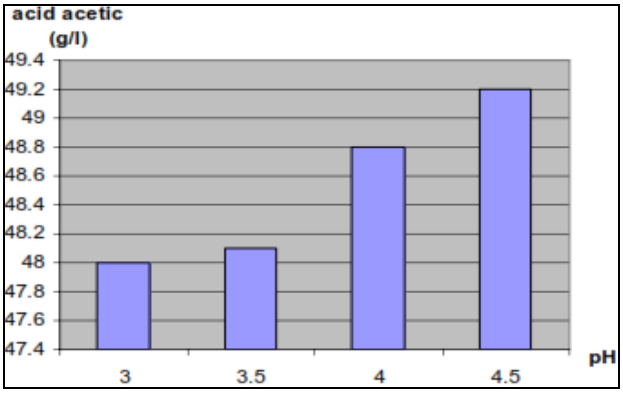


**Fig 15:** Effect of pH to vinegar fermentation

Acetobacter aceti can oxidise ethanol to form acetic acid at pH 4.5. However, we choose pH 3.5 for convenience (the final pH of the ethanol fermentation). So optimal conditions for vinegar fermentation can be elaborated as follows: ethanol 7%, pH 3.5, Acetobacter aceti 15%, fermentation time 25 days, acetic acid 43-48 gram/l.

### 3.4 Vinegar fermentation by feed-back method

### *3*.4.1 Vinegar fermentation by bacteria membrane forming

**Table 22:** Vinegar fermentation by bacteria membrane forming

| Sample | % ethanol | % Actobacter | Initial acidity (g/l) | Acidity after 24 hours | Acidity after 36 hours | Acidity after 48 hours | Acidity after 60 hours | Acidity after 72 hours |
|---|---|---|---|---|---|---|---|---|
| **1** | 95 | 5 | 6.1 | 9.1 | 12.5 | 14.9 | 16.9 | 19.7 |
| **2** | 90 | 13 | 11.3 | 14.3 | 19.1 | 22.1 | 23.3 | 25.8 |
| **3** | 85 | 15 | 16.6 | 19.6 | 23.1 | 25.2 | 27.2 | 30.9 |
| **4** | 80 | 20 | 19.6 | 23.6 | 27.4 | 29.6 | 31.6 | 33.2 |

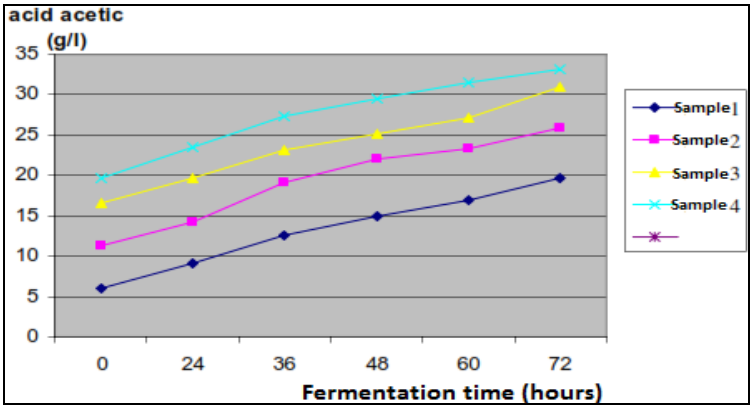


**Fig 16:** Vinegar fermentation by bacteria membrane forming

After 60 hours of fermentation with 20% acetobacter aceti, the vinegar fermentation happens quickly so we choose this fermentation time for further experiments.

### 3.4.2 Effect of the initial Acetobacter aceti ratio to vinegar fermentation

**Table 23:** Effect of the initial *Acetobacter aceti* ratio to vinegar fermentation

| Fermentation time (days) | Acetic acid (g/l) | | | | |
|---|---|---|---|---|---|
| | Sample #1 | Sample #2 | Sample #3 | Sample #4 | Sample #5 |
| **0** | 30.0 | 29.4 | 28.0 | 27.0 | 26.5 |
| **1** | 30.0 | 29.4 | 28.0 | 27.0 | 26.5 |
| **2** | 31.0 | 30.7 | 29.7 | 28.7 | 27.8 |
| **3** | 34.7 | 34.8 | 34.0 | 33.1 | 33.0 |
| **4** | 39.0 | 39.0 | 39.4 | 38.8 | 38.2 |
| **5** | 44.2 | 44.0 | 46.1 | 45.0 | 44.7 |
| **6** | 47.0 | 48.0 | 53.2 | 53.0 | 52.1 |
| **7** | 48.9 | 50.0 | 59.5 | 58.9 | 58.4 |
| **8** | 48.0 | 51.0 | 60.9 | 59.1 | 58.8 |
| **9** | 47.0 | 50.0 | 58.6 | 58.2 | 57.6 |

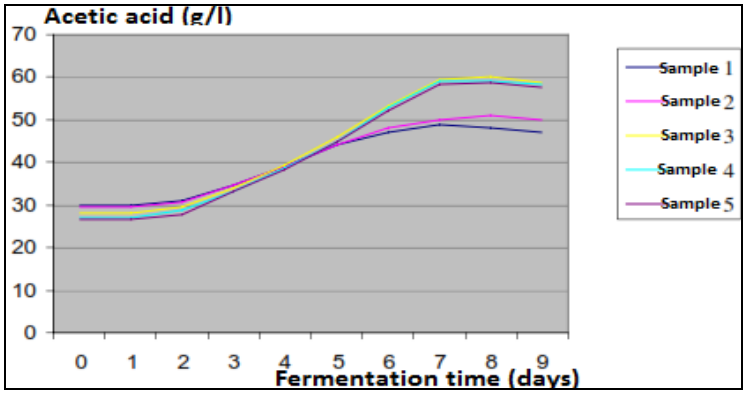


**Fig 17:** Effect of the initial *Acetobacter aceti* ratio to vinegar fermentation

The fermentation time 8-10 days with *Acetobacter* ratio 20%, pH 3.5, ethanol 7% is adequate for vinegar fermentation

## 4. Conclusion

Vinegar has been used as a seasoning in cooking since ancient times. Vinegar is a solution of acetic acid produced by a two-step bioprocess. In the first step, fermentable sugars are transformed into ethanol by the action of yeast. In the second step, acetic acid bacteria oxidize the ethanol into acetic acid in an aerobic process. One of the most used systems is the traditional method, also called the superficial, surface or Orleans method. It is a static method that is traditionally employed for the manufacture of high-quality vinegars. At the moment, the most common technology used in the vinegar industry is the feed-back method to increase the speed of the acetic acid biological reaction. Vinegar from star fruit is very suitable for most Vietnamese people owing to its healthcare effects.

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