INTERNATIONAL JOURNAL OF ADVANCES IN PHARMACY, BIOLOGY AND CHEMISTRY

Research Article

Effect of Ripen Mature and Ratio of Wood Apple's Pulp Supplemented with Sugar for *Feronia limonia Quach* wine Fermentation

Nguyen Thi Hong Tham, Nguyen Phuoc Minh.

Postharvest Center Argi-Aquaculture Faculty Tra Vinh University, Vietnam

ABSTRACT

Wood apple *Feronia limonia*(Vietnamese: Trai Quach) is a special fruit in Tra Vinh Province, Vietnam. Tourists can find *Trai Quach* (wood apple) sold everywhere along the sidewalk. *Trai Quach Feronia limonia*is also planted in many local gardens.Wood apple is chosen to produce wine because of its particular flavour and aroma. There are some factors needed to determine in experiments that influence to the fermentation and quality of wood apple wine.When the experiments are carried out, some quantitative analysing methodsare used to determine sensory and chemical characteristics.The results of these experiments are as follow: the fermentation culture for wood apple wine is strain of yeast *Saccharomyces cerevisae*; product's quality from wood apple is fermented after 4 days of preservation, wood apple pure is added with water proportion 20%, Brix 18, starting yeast proportion 1.5%, pH = 4, fermentation temperature 28-32^{0}C, fermentation time 9 days.

Keywords:*Feronia limonia, wood apple wine, saccharomyces cerevisae, fermentation.*

I. INTRODUCTION

Limonia acidissima (syn. *Feronia elephantum*, *Feronia limonia*, *Hesperethusa crenulata*, *Schinus limonia*) is the only species within the monotypicgenus*Limonia*. It is native in Bangladesh, India, Pakistan, Sri Lanka, and Vietnam. Vernacular names in English include: wood-apple, elephant-apple, monkey fruit, and curd fruit are the variety of common names in the languages of its nativehabitat regions.

Classification of *Limonia acidissima*as followsKingdom:*Plantae;* Order:*Sapindales;* Family: *Rutaceae;* Subfamily: *Aurantioideae;* Tribe: *Citreae;* Genus: *Limonia;* Species: *L. acidissima*


Figure 1. The wood apple fruit (a) and pulp (b)

Trai Quach has been a part of Tra Vinh for more than half a century. A *Quach* tree stands about seven to eight meters tall, has small leaves with a body of feroniella. Usually, a seven-year-old *Quach* tree will start producing fruit, which looks just like a gray plastic ball, with many tiny dots on it. When it matures, *Trai Quach* spreads a faint aroma, although not as strong as *Trai Thi*, which is still enough to fascinate and attract people.

On a hot and muggy day, by mixing *Trai Quach* with some sugar, milk and ice, we will have one unique refreshing drink. The combination of the irresistible smell and the sour taste of trai quach, along with the sweetness of sugar and milk can quickly make you forget the summer heat. In Cau Kedistrict Tra Vinh province, people also use *Trai Quach* to make a specialty wine. This wine will not only bewitch people with the signature aroma of *Trai Quach*, it will also balance blood pressure, remove body aches and helps your liver. *Trai Quach* is commonly sold at Cau Ke market, Bac Lieu or even in HCMC. In

Cambodia,*Quach* is planted widely. People there usually enjoy *Trai Quach* with peppers and salt; however, nowhere else uses *Quach* the same way as Vietnam.

The fruit is a hard-shelled many seeded berry with its pinkish brown aromatic sour – sweet pulp being the edible portion, the seeds embedded in it. It is an ideal tree to be exploited for growing in wasteland. Wood apple is a nutrient rich fruit which contains a surprisingly high amount of protein and low levels of sugar and carbohydrates compared with many other fruits. The wood apple is rich in Beta carotene, a precursor of vitamin –A which also contains significant quantities of the B vitamins such as thiamine and riboflavin and small amounts of vitamin C. Wood apple is useful in preventing and curing scurvy and in reliving flatulence. Mashed seedless pulp of the raw fruit is beneficial in the treatment of dysentry, diarrhoea and piles. Wood apple in the form of chutneys or sherbet is useful in treating hiccups.

Wood apple fruit contains flavonoids, glycosides, saponins and tannins. There are reports that some coumarins and tyramine derivatives were also isolated from the fruits of *Limonia* (Ilango and Chitra, 2009). The fruit may be eaten raw but it has a resinuous taste and requires sweetening. The ripe fruit is used as a dessert and a source of beverage, cream and jellies (Adikaram et al., 1989). A stiff jelly could be made from these fruits but the flavour is somewhat hash and hence, is seldom used. The fruits can be mixed with guava to make good quality jelly (Hayes, 1970). The ripe fruit pulp makes excellent chutney and it also consumed afresh along with sugar.

Several outstanding researchers investigated wood apple production:

Narsing Rao et al. (2011) executed a preparation of wood apple (*Feronia limonia* L.) seed protein concentrate and evaluation of its nutritional and functional characteristics. Wood apple (Feronia limonia L.) seed protein concentrate (WSPC) was prepared and its properties were compared with the wood apple seed meal (WSM). The protein content was found to be 33.79 and 77 g/100 g in WSM and WSPC respectively. WSPC was good source of essential amino acids leucine, phenyl alanine, valine, iso-leucine and threonine. Protein extractability of WSPC showed optimum WSPC to water ratio of 1:50 (w/v), over 60 min. Maximum protein extractability (95 g/100 g) was observed at pH 12 and minimum (11 g/100 g) at pH 6. Protein precipitability was maximum (91 g/100 g) at pH 5.5. A higher buffer capacity of WSPC was observed in the pH range 6 - 2. SDS-PAGE of the WSM and WSPC showed 9 protein bands ranging from 205 kDa to 12 kDa. Higher water absorption, lower oil absorption capacity, stable foam and presence of essential minerals of WSPC favour its industrial application.

R. Vidhya and A. Narain (2011) developed the preserved products using under exploited fruit, wood apple (*Limonia acidissima*). This study was planned to utilize the preserved wood apple by preserving them as jam and fruit bar. Using wood apple preserved products like jam and fruit bar were developed, stored and quality parameters were assessed for a periods of 90 days. Organoleptic evaluation shows storage stability was good in both jam and fruit Bar with respect to flavour and consistency. Nutritive analysis shows reduction in Vitamin C, Calcium and Phosphorous in both jam and fruit bar during 90th day of storage. The acidic content of the preserved products decreased in both Jam (2.5%) and fruit bar (1.66%). No Significant change observed in TSS, pH, pectin and ash value for both jam and fruit bar during storage. Total sugar increased up to 0.68 and 0.89% and reducing sugar increased to 2.59 and 1.53% in both jam and fruit bar, respectively. The microbial loadof both jam and fruit bar was under the limit at the end of 90 days. Hence, the prepared jam and fruit bar was safe and fit for consumption.

Poongodi Vijayakumar, et al. (2013) surveyed the drying characteristics and quality evaluation of wood apple (*Limonia acidissima* L.) fruit pulp powder. The wood apple (*Limonia acidissima* L.) pulp with seed was dried by using hot air oven, tray dryer and solar drier, powdered and compared for its drying characteristics, fruit powder yield, physical and functional properties, least gelation concentration, nutritional composition, non-nutritional qualities and sensory profile. The wood apple pulp gets completely dried within 5-6 hours in all drying methods. The overall drying rate was significantly ($p<0.05$) high in hot air oven dried sample; dehydration ratio, rehydration ratio and co-efficient of rehydration were significantly high in tray dried sample at $p<0.01$. The total polyphenol content and antioxidant activity was significantly higher ($p<0.01$) in sun dried wood apple pulp powder. Nutrients get concentrated except vitamin C which was lost on drying. The titrable acidity and pH revealed the medium acidic nature of wood apple pulp powder. Organoleptically, the hot air oven dried sample was liked very much in terms of its appearance, color and flavor. Hence the wood apple pulp could be dried effectively using solar dryer, preserved as dried powder and value added for its industrial exploitation.

The aim of our study is to investigate the effect of some factors influence to the fermentation and quality of wood apple (*Quach*) wine.

II. MATERIAL AND METHOD

2.1 Raw material

Wood apple (*Quach*) fruit is collected in local Tra Vinh province. Its pulp is processed and fermented in the laboratory of the post harvest center, agri-aquaculture faculty, Tra Vinh university, Vietnam.

2.2 Method

2.2.1 Experiment #1: Effect of the ripen mature of wood apple fruit to quality of Quach (wood apple) wine fermentation

a. Purpose is to determine the appropriate ripen mature for wine production.Ripen mature of wood apple fruit is crucial to wine fermentation owing to sugar content, acidity. These parameters affect to yeast growth. Finding ripen mature at the appropriate stage is very necessary and optimal during fermentation.

b. Experiment design: One factoe (A);2 replicates;5 roots; 10 units; A1: Ripen #1 (after being harvested); A2: Ripen #2 (after 2 days harvested); A3: Ripen #3 (after 4 days harvested); A4: Ripen #4 (after 6 days harvested); A5: Ripen #5 (after 8 days harvested)

c. Preparation: Choose 25 fruits having the same weight and divide into 5 groups (5 fruits each group), note from A1,A2,A3,A4,A5. Two replicates (25*2=50 fruits). Keep in normal temperature.

d. Performance: Divide fruits into two groups

Group # 1:Prepare samples and testing chemical reagents.

Date #1: take onefruit A1 and analyze total sugar and acidity, take 4 fruits A1 left to fermentation.

Date #3: take one fruit A2 and analyze total sugar and acidity, take 4 fruits A2 left to fermentation.

Date #5: take one fruit A3 and analyze total sugar and acidity, take 4 fruits A3 left to fermentation.

Date #7: take one fruit A4 and analyze total sugar and acidity, take 4 fruits A4 left to fermentation.

Date #9: take one fruit A5 and analyze total sugar and acidity, take 4 fruits A5 left to fermentation.

Group # 2:Prepare samples and materials for wine fer

entation. Total samples: 5. Sample volume: 2.5lit/sample. Fixed parameters: Pulp ratio 20%, pH 3.7 , Bx 20, yeast 1%.

e. Testing parameter:

*Wood apple pulp:*Total sugar, total acidity, sensory characteristics (color, aroma, taste).

Fermenting wort: pH, total sugar, alcohol, sensory characteristics of wine (color, aroma, taste, turbidity).

2.2.2 Experiment #2: Effect ofwood apple fruit juice ratio and sugar supplemented to quality of Quach (wood apple) wine fermentation

a. Purpose is to determine the water and sugar supplemented to wort for wine production. Each kind of fruit having different moisture content and chemical composition. This creates different fermenting media. Some kind of fruits are available for fermentation such as grape, but some fruits must be treated in advance such as rambutan, banana. Depending on variety of fruits, we have various strategies for yeast growthby creating the approriate moisture. So fermenting culture should be adjusted with favorable condition for a best wine fermentation.

b. Experiment design: Two factor (B, C);two replicates

Factor B: ratio of wood apple pulp (% pulp/total fermenting batch); B1 15%; B2 20%; B3 25%; B4 30%. Factor C: degree Bx; C1: 16^{o}Bx; C2 18 ^{o}Bx; C3 20 ^{o}Bx; C4 22 ^{o}Bx; Roots: 4*4=16; Unit: 16*2=32

c. Preparation:Choose wood apple fruit at ripen mature (4 days after being harvested)

d. Performance: Prepare sample and material for wine fermentation.Total samples: 16. Sample volume: 2.5lit/sample. Fixed parameters: pH 3.7, yeast 1%

e. Testing parameter:Total sugar; total acidity; pH; alcohol; sensory characteristics of wine (color, aroma, taste, turbidity)

***2.2.3 Experiment #3:* determine pH for fermenting wort**

a. Purpose:determine optimal pH value for yeast growth to get the best wine quality. Acidity in fermenting wort is very important for yeast growth. Ion H+ influences to charge of cell wall, increase or decrease permeability of specific ions. In some cases, it can inhibit enzyme activity on cell wall.

b. Experiment design: onefactor D, two replicates; D1: pH 3.4; D2: pH 3.7; D3: pH 4.0; D4: pH 4.3; D5: pH 4.6; Roots: 5; Units: 5*2=10

c. Preparation:Choose Quach fruit at ripen mature (4 days after being harvested); prepare wort with pulp ratio 20%, Brix 18.

d. Preformance: Prepare sample and material ready for wine fermentation. Total samples: 5. Sample volume: 2.5lit/sample. Fixed parameters: yeast 1%, Bx 18, juice ratio 20%.

e. Testing analysis:Total sugar; total acidity; pH; alcohol; sensory characteristics of wine (color, aroma, taste, turbidity).

2.2.4 Experiment #4: determine yeast ratio inoculated

a. Purpose:determine yeast ratio inoculated for Quach wine fermentation.Yeast ratio is also a vital parameter for wine fermentation. If yeast is less, fermenting period will be long and contaminated. If yeast is much, fermenting period will be short but expensive.In this experiment, we use *Saccharomyces cerevisiae* in dry particle with specific aroma.

b. Experiment design:One factor E; two replicates; E1 yeast ratio: 0.5%; E2 yeast ratio: 1.0%; E3 yeast ratio: 1.5%; E4 yeast ratio: 2.0%; E5 yeast ratio: 2.5%; Roots: 5; Units: 5*2=10

c. Preparations:Choose wood apple fruit at ripen mature (4 days after being harvested; prepare fermenting wort: pulp 20%, Brix 18, pH=4.

d. Performance: Prepare sample and material ready for Quach wine fermentation. Total samples: 5.Sample volume: 2.5lit/sample. Fixed parameters: Bx 18, pulp 20%, pH 4.0.

e. Testing analysis:Total sugar; total acidity; pH; alcohol; sensory characteristics of wine (color, aroma, taste, turbidity).

2.2.5 Experiment #5: determine the main fermenting duration

a. Purpose:determine the shortest fermenting duration while maintaining *Quach* wine quality. We must verify the main fermenting stage.After this, almost yeast willbe died and should be filtered out of liquid to avoid contamination. If the ripen stage stops too soon, nutrients left much and alcohol too little so it is not enough to prevent microorganism contaminated.

b. Experiment design: One factor F; two replicates; F1: 6 day fermentation; F2: 7 day fermentation; F3: 8 day fermentation; F4: 9 day fermentation; F5: 10 day fermentation; Roots: 5; Units: 5*2=10

c. Preparation:Choose wood apple fruit at ripen mature (4 days after being harvested); prepare wort: pulp 20%, Brix 18, pH=4, yeast 1.5%

d. Performance: Prepare sample and material ready for *Quach* wine fermentation. Total samples: 5.Sample volume: 2.5lit/ simple. Fixed paramters: Bx 18, pulp 20%, pH 4, yeast 1.5%.

e. Testing analysis:Total sugar; total acidity; pH; alcohol; sensory characteristics of wine (color, aroma, taste, turbidity)

2.3 Statistical analysis

Collect sample after 10 days fermented. Verify aroma, color, turbidity, alcohol in Quach wine. Filter solid after fermentation. All data are summarized and analysed by Excel and Statgraphic software.

III. RESULT AND DISCUSSION

3.1 Effect of ripen mature of *Quach* (wood apple) fruit:

Right after harvest, wood apple fruit has the light brown pulp, stringen acidity, tannin and strong flavour. After 2 days of preservation, pulp will become dark brown, sweeter, less acid, more flavour. From the 4 days afterward, 80% of pulp gets black brown, sweet, less acid, less tannin, specific aroma. After 8 days, 100% of fruits have black brown pulp, soft, and less glutinous and all of 3/10 fruits become decay.

From our observation, fruits after 4, 6 and 8 days give the best alcohol and sensory characteristics (figure 2). There is no significant difference of wine sample used fruits after 4,6 or 8days. So we choose fruits at ripen mature (4 days)for further experiments.

In figure3, we recognize the acidity decreased and sugar increased by time (at room temperature).In the first three days, sugar increases slightly so we must supplement more saccharose. From day 4^{th}, sugar changes dramatically compared the the first 3^{rd} day. So wood apple at the 4^{th} day ripen can be used for fermentation because saccharose supplementation will be at least. At harvesting period, wood apple fruits are ripen profusely and vigorously, they should be preserved and fermented gradually without affecting to wine quality. Acidity is decreased gradually, at the 3^{rd} day with pulp ratio 20%, pH 2.9-3.6; at the 4^{th} day, pH 3.7-3.9. So at the 4^{th} day, pH of pulp is suitable for yeast growth.

4.2. Effect of pulp ratio and Bx

Bx and sugar content are two elements affecting to yeast growth. The more sugar is, the more alcohol receives. However, yeast only grows well in medium with 10-18% sugar, slowly ferment at 25% sugar and stop at 30% sugar.

According to table 1 and figure 4, average alcohol at different pulp ratio and Bx: at 20% pulp, the highest alcohol and significantly statistical difference we notify at Bx 20; then Bx 18 and Bx 16.

At 20% pulp, we see the reduced sugar as in figure 6.

On figure 5, Bx 22 strongthly inhibits yeast growth. Bx 20 is very suitable for yeast fermentation. While comparing Bx 20 and Bx 18, we don't see any significantly difference. So we decide to choose Bx 18 for further experimentS.

4.3 Effect of pH to wine quality:

Yeast can grow beyond pH range 4 – 6. However at pH 3.0-3.5, yeast still can grow. On this research, we examine the optimal pH value for fermentation.

On figure 7, we see that pH 4.0 and 4.3 show the highest alcohol. So we select pH 4.0 for further expriments.

4.4 Effect of yeast ratio supplementation to alcohol accumulation

At yeast ratio 0.5% and 1% at the 9th day, the reduced sugar left in whole some. On figure 8, when increasing yeast ratio, we receive more alcohol. The highsest alcohol notified at yeast ratio 2.0. However yeast ratio 1.5 and 2.5% are not significantly different in alcohol accumulation. So we select yeast ratio 1.5% for further experiments.

4.5 Effect of the main fermenting duration to wine quality.

Apart from physic-chemical analysis, we also monitor the current of CO_2. Solubility of CO_2in alcohol is triple to water. During fermentation, alcohol and CO_2will be dissolved into liquid. CO_2will quickly absorb on yeast surface. When these bubbles increase their volume, this will create a lift for yeast to float. When bubbles collapse, yeast will then be submerged into fermenting wort.During fermentation, alcohol accumulates in liquid. At the final stage of the main fermentation, yeast become weak and dead. Liquid should be filtered to get clear appearance. In the first 4th days, bubbles move very quickly, but slowly in the 5th day and completely cease in the 9th day. After filtration, clear liquid should also enter a minor fermentation at least 3 months at temperature 8±2^{o}C so that it can change malic acid into lactic acid.Based on initial parameters at pulp20%, Bx 18, pH = 4, yeast ratio 1.5%we get some key results: on the 6th day, alcohol formation happens slowly but the highest alcohol content is on the 8th day. Liquid will get clear, but moving of yeast is weakly. From the 5th day, yeast movement will get slowly and slowly. On the 9th day, the main fermentation should be stopped.

IV. CONCLUSION

During our experiment, we consider *Quach* (wood apple) as a natural fruit available for wine fermentation with good quality and specific aroma. In the future, it's necessary to execute some more researches such as: determine some components in pulp (polyphenol, protein....) to choose the optimal ripen mature; verify the optimal fermenting temperatures; and examine malolactic fermentation to get the good*Quach* wine quality.

Table 1: Average alcohol after fermentation at different pulp ratio and Bx

Factor B / FactorA C	B1 15%	B2 20%	B3 25%	B4 30%	Average alcohol at different pulp ratio
C1 (Bx=16)	10.75	12.00	12.25	11.50	11.60^{b}
C2 (Bx=18)	13.75	14.75	13.75	14.00	14.10^{a}
C3 (Bx=20)	14.25	15.00	14.50	13.75	14.37^{a}
C4 (Bx=22)	14.00	14.50	13.75	13.25	13.87^{a}
Average alcohol at different Brix	13.20^{b}	14.10^{a}	13.56^{ab}	13.12^{b}	

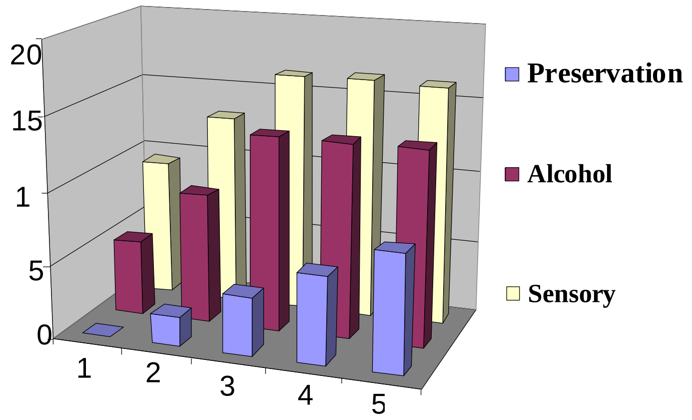

Figure 2. Effect of wood apple ripen mature to alcohol and sensory characteristics of *Quach* wine

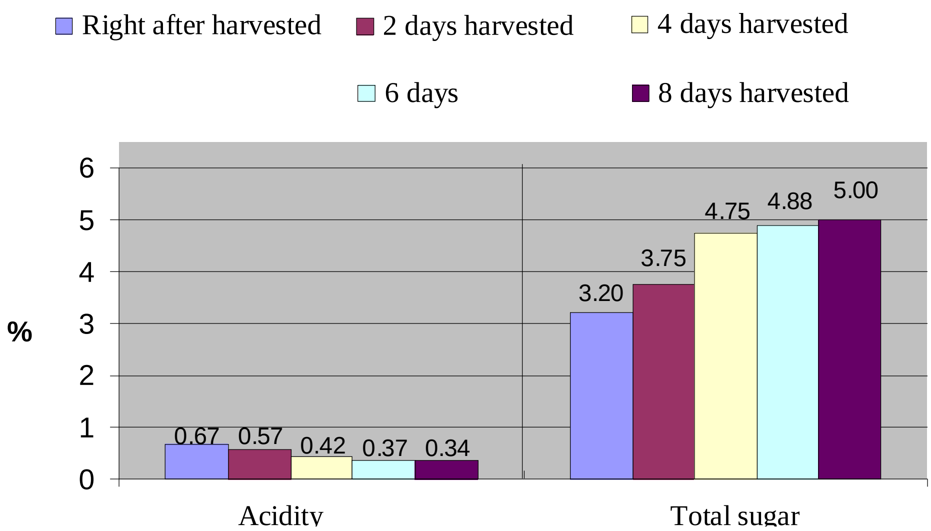

Figure 3. Change of acidity and sugar by preservation duration

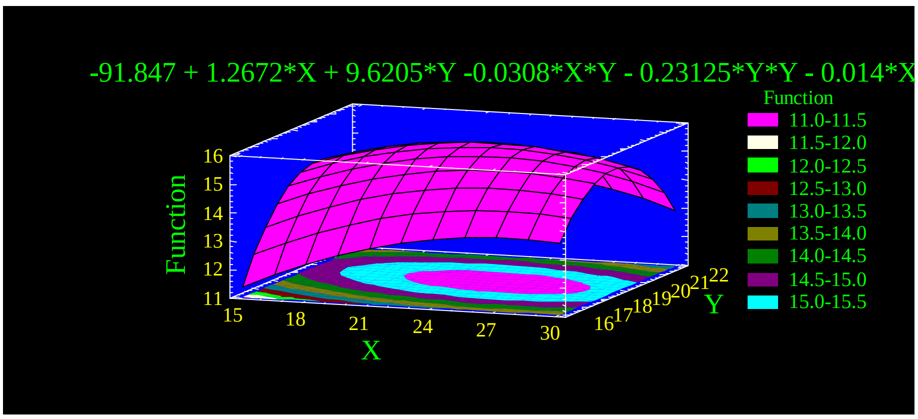

Figure 4. Diagram of wine sensory evaluation at different pulp ratio and Bx

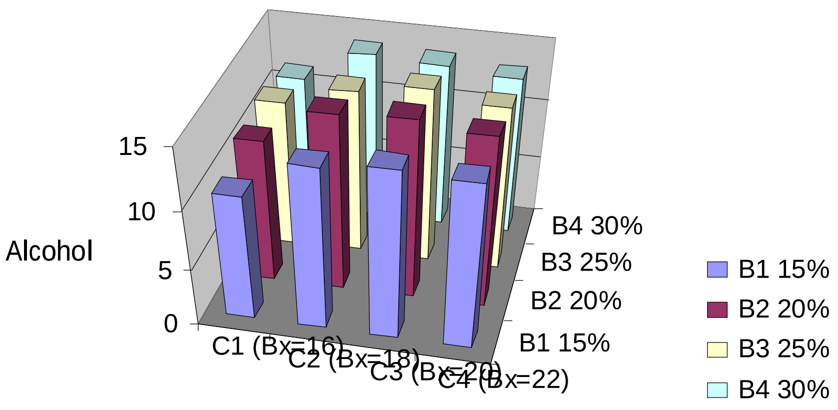

Figure 5. Effect of pulp ratio and Bx to alcohol

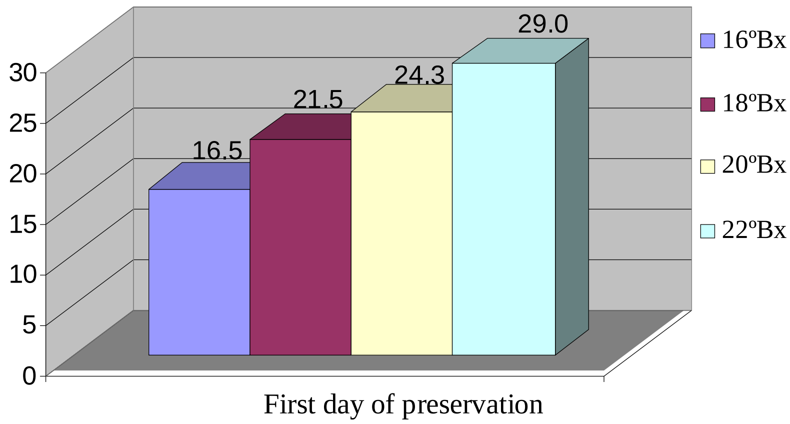

Figure 6. Reduced sugar at different Bx of wort

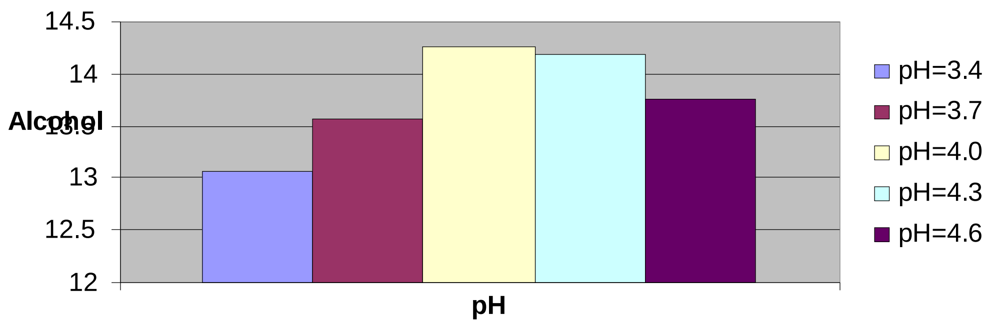

Figure 7. Effect of pH to alcohol accumulation during fermentation

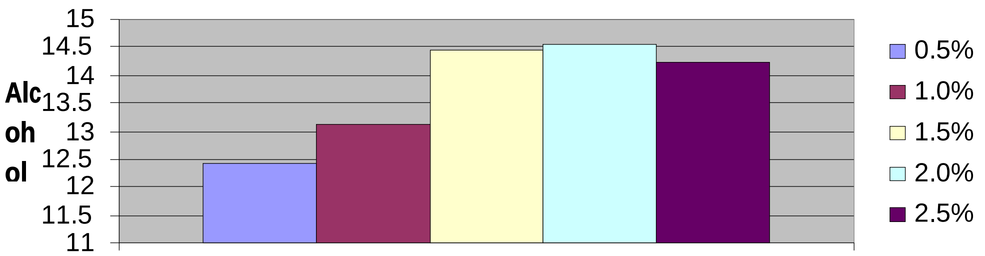

Figure 8. Effect of yeast ratio to wine fermentation

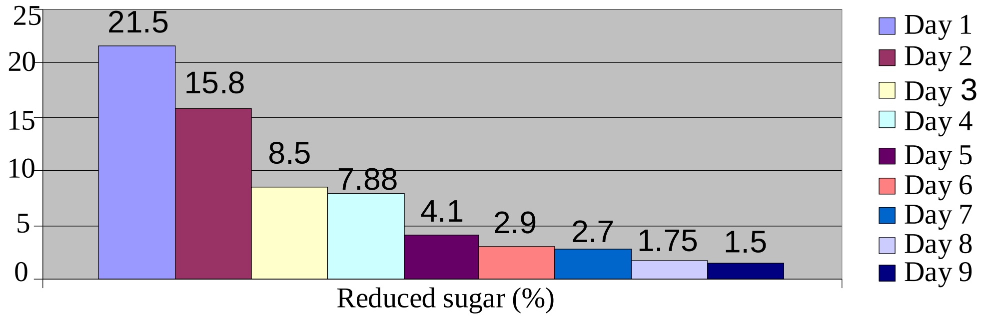

Figure 9. Change of reduced sugar by fermenting time

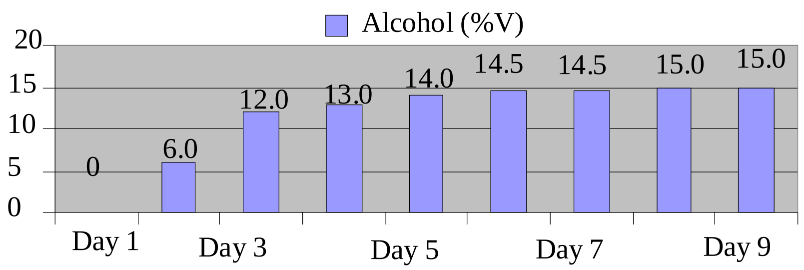

Figure 10. Alcohol formation during fermentation

REFERENCES

1. Adikaram NKB, Yamuna Abhayawardhane, Ratnayake Bandara. BM, Leslie Gunatilaka AA, Kithsiri Wijeratne EM. Antifungal activity, acid and sugar content in the wood apple (Limonia acidissima) and their relation to fungal development. Plant Pathology 1989;38: 258-265.
2. Hayes WB, (1970). Fruit growing in India. Third edition. Kitabistan, Allahabad.
3. Ilango K and Chitra V. Antidiabetic and antioxidant activity of Limonia acdissimia linn. in alloxan induced rats. Der Pharmacia Lettre 2009;1: 117-125.
4. Narsing Rao G, Prabhakara Rao PG. and Govardhana Rao D. Preparation of wood apple (Feronia limonia L.) seed protein concentrate and evaluation of its nutritional and functional

characteristics. International Food Research Journal 2011;18: 949-955.
5. Poongodi Vijayakumar, Punitha TK and Banupriya L. Drying characteristics and quality evaluation of wood apple (Limonia acidissima L.) fruit pulp powder. Int. J. Cur. Tr. Res2013; 2: 147-150.
6. Vidhya R and Narain A. Development of preserved products using under exploited fruit, wood apple (Limonia acidissima). American Journal of Food Technology 2011;6: 279-288.