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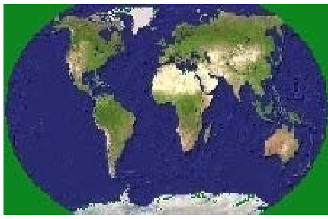

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Nguyen Phuoc Minh
Tra Vinh University, Vietnam

Saccharomyces sp. isolation and comparison of yeast growth between *Saccharomyces* sp. and *Saccharomyces cerevisiae* for papaya wine fermentation

Nguyen Phuoc Minh

Abstract
Papaya is a powerhouse of nutrients and is available throughout the year. It is a rich source of threes powerful antioxidant vitamin C, vitamin A and vitamin E; the minerals, magnesium and potassium; the B vitamin pantothenic acid and folate and fiber. In addition to all this, it contains a digestive enzyme-papain that effectively treats causes of trauma, allergies and sports injuries. All the nutrients of papaya as a whole improve cardiovascular system, protect against heart diseases, heart attacks, strokes and prevent colon cancer. The fruit is an excellent source of beta carotene that prevents damage caused by free radicals that may cause some forms of cancer. Papaya is a sugar crop with soluble saccharides in the form of glucose, fructose, sucrose and it's widely cultivated in several countries. Sugars represent that part of the fruits which is used by microorganisms for wine production. The aim of our research is to investigate yeast growth, ethanol formation in case of papaya juice fermentation by *Saccharomyces sp* naturally isolated on papaya skin and *Saccharomyces cerevisiae*. We also examine effect of the initial pH of papaya juice to fermentation speed on each yeast culture. We draw out some major points as follows: yeast naturally isolated on papaya fruit is *Saccharomyces* sp. and *Saccharomyces cerevisiae* not significantly different at 95% level in various conditions: pH .1, 4.1, 4.5. Maximum yeast density in fermentation is 10^{10} cfu/ml. With initial sugar content 22%, yeast ratio 2%, main fermentation time performs in 07 days. By this, ethanol formation is accumulated at 12%, not significantly different at 95% reliability level in various conditions: pH 3.1, 4.1, 4.5. We successfully isolated yeast species *Saccharomyces* sp. is the S*accharomyces cerevisiae*. So we can utilize this natural yeast for wine fermentation without purchasing S*accharomyces cerevisiae* in laboratory.

Keywords: *Saccharomyces cerevisiae,* papaya fruit, yeast isolation, fermentation, wine

1. Introduction
Papaya, botanical name Carica papaya, is a lozenge tropical fruit, often seen in orange-red, yellow-green and yellow-orange hues, with a rich orange pulp. The fruit is not just delicious and healthy, but whole plant parts, fruit, roots, bark, peel, seeds and pulp are also known to have medicinal properties. The many benefits of papaya owed due to high content of Vitamins A, B and C, proteolytic enzymes like papain and chymopapain which have antiviral, antifungal and antibacterial properties. Carica papaya can be used for treatment of a numerous diseases like warts, corns, sinuses, eczema, cutaneous tubercles, glandular tumors, blood pressure, dyspepsia, constipation, amenorrhoea, general debility, expel worms and stimulate reproductive organs and many, as a result Carica papaya can be regarded as a neutraceutical. Aravind *et al*. (2013) reviewed the pharmacological uses of Carica papaya and side/toxic effects. Carica papaya contains an enzyme known as papain which is present in the bark, leaves and fruit. The milky juice is extracted, dried and used as a chewing gum for digestive problems, toothpaste and meat tenderizers. It also contains many biological active compounds including chymopapain and papain which is the ingredient that aids digestive system, and again used in treatment of arthritis. The ripe papaya fruit is usually eaten raw, without the skin or seed, because of its high sugar content (59%) and thus could be used in wine production as any fruit with a good proportion of sugar may be used. Aysun Ozkan *et al*. (2011) studied antioxidant capacity of juice from different papaya (*Carica papaya* L.) cultivars grown under greenhouse conditions. Ayanaru *et al*. (1985) who showed that it has a capacity of generation of ethanol by microbial conversion of sugar in the papaya fruit. Fermentation is a relatively low energy preservation process which increases the self-life and decreases the need for refrigeration or other forms of food preservation technology. Wine is considered to be the oldest fermented alcoholic beverage.

Correspondence:
Nguyen Phuoc Minh
Tra Vinh University, Vietnam

The term wine is applied to the product made by alcoholic fermentation by yeast of fruits or fruit juice, with an aging process. Wine has been known for thousand of years, covering the period of ancient civilization to modern times. It has been produced and enjoyed by many people from peasants to kings. It is produced by fermentation of juice of ripe grapes using *Saccharomyces cerevisae.*

Other fruits such as apples, berries and blackcurrants are sometimes also fermented. These however are referred to as fruits or country wine. In contrast to most foods and beverages that spoil quickly or that can spread diseases, wine doesnot spoil if stored properly. The alcohol in wine, ethanol is present in sufficient concentration to kill pathogenic microorganism, which makes to be considered to be safer to drink than water or milk.

Okoro, Casmir Emeka (2007) produce red wine from roselle *(Hibiscus sabdariffa*) and pawpaw (*Carica papaya)* using palm-wine yeast (*Saccharomyces cerevisiae*). S. Awe (2011) studied production and microbiology of pawpaw (Carica papaya L) wine. Investigations on the preparation of wine from papaya are reported (C. Maragatham and A. Panneerselvam, 2011). All the inoculum was given good result for papaya wine making using clarified juice, non clarified juice and pulp. Among this the wine prepared from either the clarified or non clarified papaya juice is highly acceptable using the inoculum pure culture and sediment of secondary fermentation. It is quite possible to utilize papaya fruits successfully to make an acceptable quality of wine as per the procedure developed. Idise Okiemute Emmanuel and Ofiyai Odoyo (2011) produced wine from pawpaw (*Carica papaya*). Pin-Rou Lee *et al*., (2011) examined the effect of fusel oil addition on volatile compounds in papaya wine fermented with *Williopsis saturnus* var. *mrakii* NCYC 2251. Pin-Rou Lee *et al*., (2012) demonstrated that yeast ratio was a critical factor for sequential fermentation of papaya wine by *Williopsis saturnus* and *Saccharomyces cerevisiae*. Pin-Rou Lee *et al*., (2013) studied the impact of the addition of fusel oil or amino acids on the fermentation by a mixed culture of *S. cerevisiae* and *W. saturnus* on the formation of volatile compounds and aroma profiles was assessed during papaya juice fermentation.

In traditional wine fermentation, people normally utilize yeast on fruit's skin. However, to ensure the stable fermentation it should be standardized, yeast isolation. Our research focuses on yeast isolation and growth comparison of S*accharomyces cerevisiae* and *Saccharomyces* sp. naturally isolated on papaya skin. It's very essential to select the best yeast species having good fermentation capability to produce high quality wine. That's the aim of this research.

2. Material & Method

2.1 Material

Ripe papaya fruits are collected in Vinh Long and Tra Vinh province, Vietnam. Yeast *Saccharomyces cerevisiae* is supplied from Pasteur Institute, HCM City, Vietnam.

Fig 1: Papaya (*Carica papaya*)

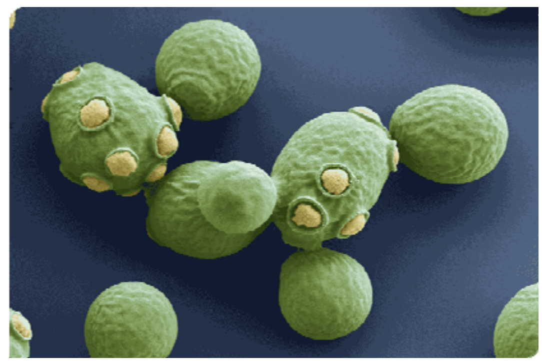

Fig 2: *Saccharomyces cerevisiae*

2.2 Research method

2.2.1 Experiment #1: yeast isolation

Papaya fruits are thoroughly cleaned by fresh water, chopped into small parts. Take one part put on petri dish with appropriate medium. Discard this fruit part, take petri dish into incubator to incubate at 37 ^{o}C in 24-48 hours to see colony forming. Yeast species is determined by visual observation to its colny under microscope. Then we select typical colonies (white, big egg shape) inoculating into culturing tube. In order to check purity of yeast which is just isolated, we examine traces of inoculation under microscope. Transfer the isolated yeast to new medium. Proliferation is executed inside the culturing tube to create mass, fermentation speed and species preservation.

2.2.2 Experiment #2: Fermentation speed affected by Saccharomyces sp. from papaya fruit and Saccharomyces cerevisiae from bread

Papaya is pressed to collect juice and pulp, pulp removed to get the clear juice. Papaya fruit will then be mixed with sugar to get the appropriate soluble dry matter (^{o}Brix). This juice is then be pasteured by $NaHSO_3$ 122 mg/l, wait for 30 minutes and add yeast 2%. In each 12 hours of fermentation, sample is analysed to verify pH, density, CO_2 content, ethanol, and ^{o}Brix. Microbial testing is performed on petri dish. Experiments are randomly arranged with 2 factors. Factor A: pH (A1: 3.7; A2: 4.1; A2: 4.5). Factor B: yeast ratio by strain B1 (*Saccharomyces* sp.*)*; strain B2 (*Saccharomyces cerevisiae)*

2.3 Statistical analysis
All data are processed by Excel 2003 and ANOVA (Startgraphics) to check the significant difference via LSD.

3. Result & Discussion
3.1 Experiment #1: Yeast isolation
After isolation, we have selected the most specific yeast species having the best fermentation capability. They are naturally presented on papaya skin, having oval shape and white color. After isolation, we define *Saccharomyces* sp. belongs to *Saccharomyces cerevisiae*.

3.2 Experiment #2: Comparision of yeast growth by two yeast species
3.2.1 Effect of pH to yeast growth, ºBrix and ethanol formation when fermenting papaya juice by *Saccharomyces sp.*

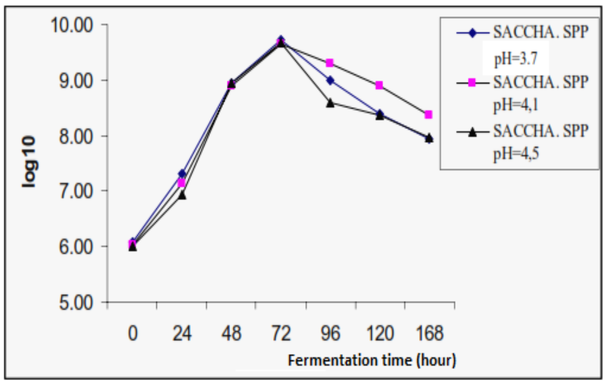

Fig 3: Effect of pH to yeast growth during fermentation

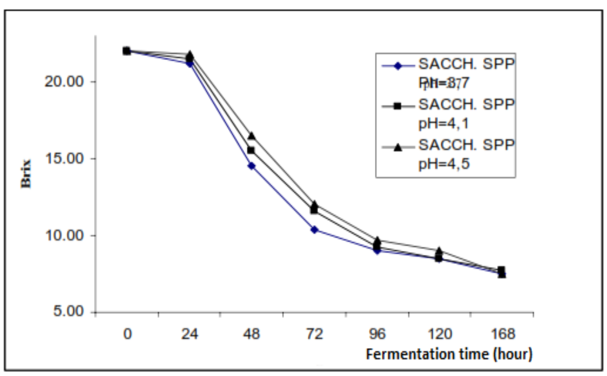

Fig 4: Effect of pH to oBrix during fermentation

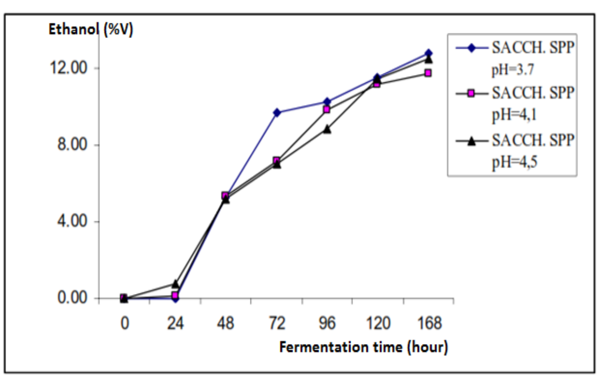

Fig 5: Effect of pH to ethanol formation during fermentation

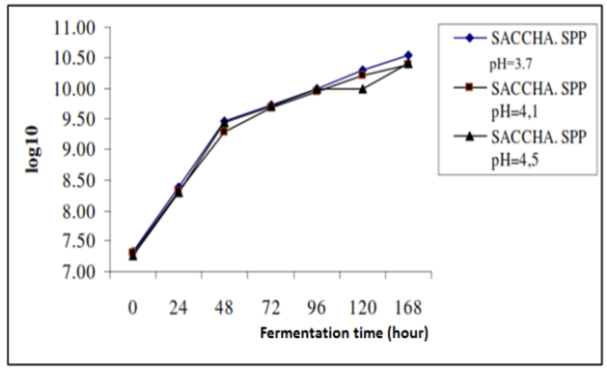

Fig 6: Effect of pH to speed of yeast growth during fermentation

3.2.2 Effect of pH to yeast growth, ºBrix and ethanol formation when fermenting papaya juice by *saccharomyces. cerevisiae*

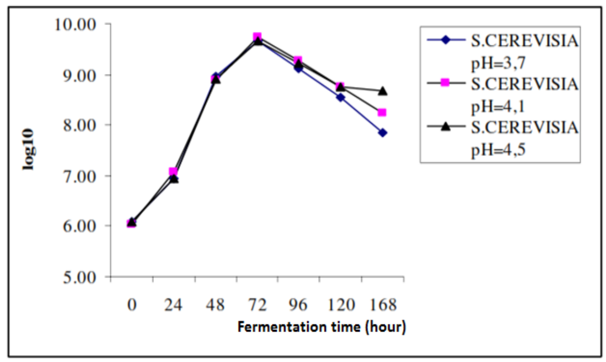

Fig 7: Effect of pH to yeast growth during fermentation

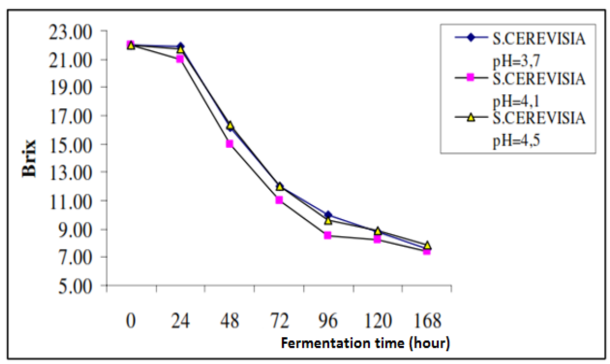

Fig 8: Effect of pH to oBrix during fermentation

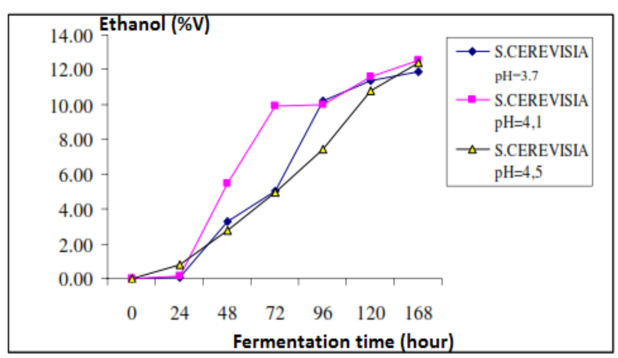

Fig 9: Effect of pH to ethanol formation during fermentation

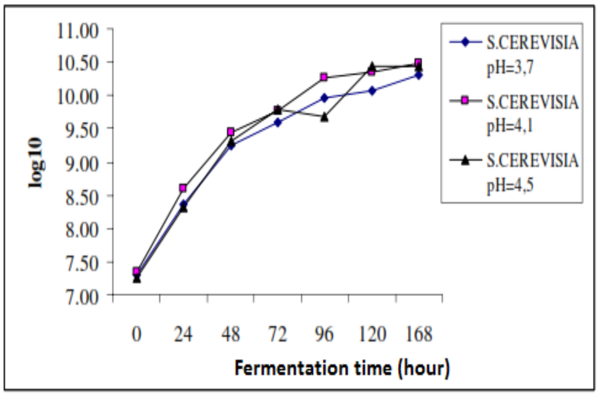

Fig 10: Effect of pH to speed of yeast growth during fermentation

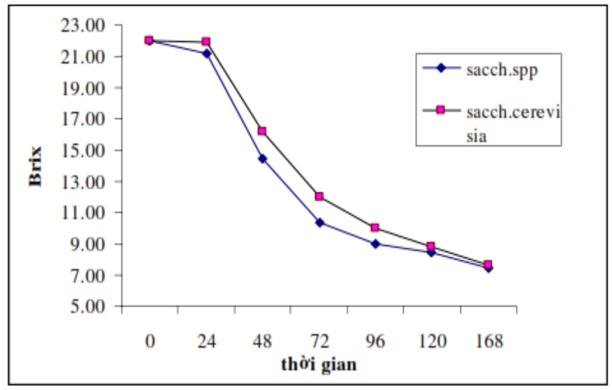

Fig 12: Reduction of Brix by *Saccharomyces. cerevisiae* and *Saccharomyces* sp.at pH=3.7

In Sabouraud medium culture, figure 3 expresses the yeast proliferation. Meanwhile figure 6 expresses total yeast under microscope because we can't distinguish the living cells or dead cells. From above figure we see in the first 24 hours of fermentation, sugar concentration is still high because of low yeast density and its biomass. After 24 hours, growth speed of yeast happens strongly (logarithm). Figure 3 shows the maximum yeast density in range 48-72h, yeast density 10^{10} cfu/ml. Then the living cells will be reduced. This phenomenon can be explained by two reasons: (1) low sugar residue creates competitivity among yeast cells (10^{10} cfu/ml); (2) high ethanol content limits yeast growth. Living yeast cells will be decreased after 72 hours. Sugar content decreases slowly so ethanol formation increases step by step. Actually, figure 5 shows the ethanol formation highly increasing from 48-72h, and then slowly increases owing to low sugar content. During fermentation, pH comes to lowest level at 48h, because the main products of fermentation are ethanol and organic acids. After 48 hours, pH increase gradually because some microorganisms can use acid for energy. In fact after 96 hours, sugar residue is very low and pH increases quickly because acid is consumed in replace for sugar to supply energy for microorganism growing. From above figures, we see the growing speed of yeast and ethanol produced by *Saccharomyces* sp. is noticed at pH 3.7. The growing speed of yeast and ethanol produced by *saccharomyces cerevisiae* tis noticed at pH 4.1.

3.2.3 Growth comparision of two yeast species *saccharomyces. sp* and *saccharomyces. cerevisiae* in different pH values

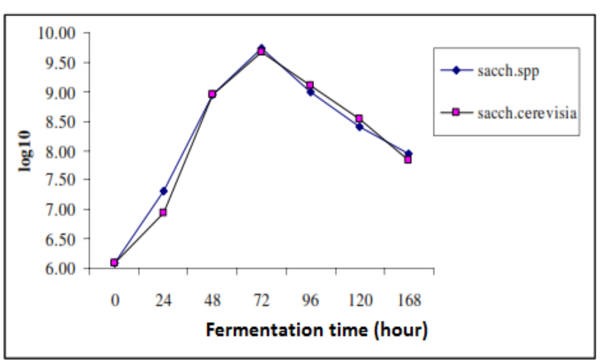

Fig 11: Growing speed of *Saccharomyces* sp. and S*accharomyces cerevisiae at* pH=3.7

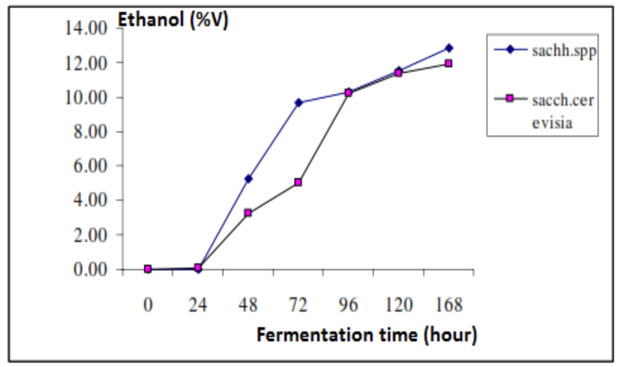

Fig 13: Comparision of ethanol formation by *Saccharomyces* sp. *and Saccharomyces cerevisiae at* pH=3.7

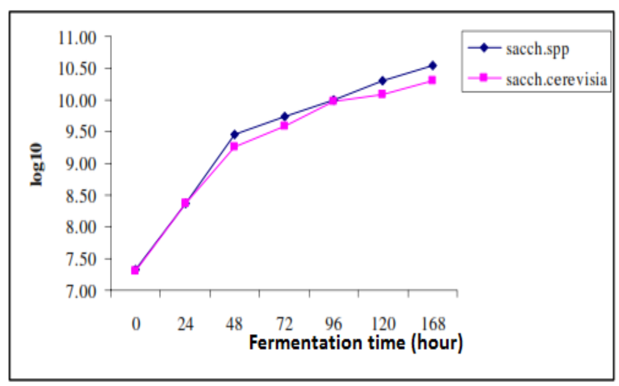

Fig 14: Comparision of yeast growth by *Saccharomyces* sp. and *Saccharomyces cerevisiae* at pH=3.7

We can see the slow speed of yeast growth in the first 24h, and increase dramatically in 24-72 hours. The more sugar is consumed by yeast, the more sugar produces and pH decreases strongly. However, there is not statistically significant difference at 95% level regarding to growth speed, sugar residue, ethanol accumulation, pH reduction in fermented fluid which is done by two different yeast species in different pH values.

Table 1: ºBrix, and yeast growth by different methods

Parameter	Yeast culture					
	Saccharomyces sp.			*Saccharomyces cerevisiae*		
	pH=3.7	pH=4.1	pH=4.5	pH=3.7	pH=4.1	pH=4.5
ºBrix	13.3[a]	13.7143[a]	14.0714[a]	14.0714[a]	13.31[a]	14.0714[a]
Yeast amount (inoculating method)	920.443[a]	903.586[a]	518.814[a]	661.429[a]	939.986[a]	903.586[a]
Yeast amount (counting method)	10508.7[a]	7975.71[a]	7502.71[a]	6750.0[a]	11317.4[a]	9761.14[a]

Table 2: Ethanol formation after fermentation

Parameter	*Saccharomyces* sp.			*Saccharomyces cerevisiae*		
	pH=3.7	pH=4.1	pH=4.5	pH=3.7	pH=4.1	pH=4.5
Ethanol (%)	12.82	11.73	12.31	11.91	12.51	12.38

3.3 Quality of papay wine

Table 3: Sensory score of papaya wine

Parameter		pH = 3.7			pH = 4.1			pH 4.5		
		Color	Aroma	Taste	Color	Aroma	Taste	Color	Aroma	Taste
Time	1	7	8	8	6	7	7	6	7	6
	2	7	7	6	9	8	6	8	7	7
Total correct answers		14	15	14	15	15	13	14	14	13

Table 4: Summary of differentiation in wine by two evaluations

Differentiation	Value
Seldom	98
Rather	25
Much	7
Too much	1

From sensory evaluation, we see the papaya wine fermented by *Saccharomyces* sp. and *Saccharomyces cerevisiae* has narrow differentiation, especially aroma and taste in different pH value at reliability µ=99.9%. From that, we can conclude these yeast species are the same.

4. Conclusion

Papaya helps in the prevention of diabetic heart disease. Papaya lowers high cholesterol levels as it is a good source of fiber papaya effectively treats and improves all types of digestive and abdominal disorders. It is a medicine for dyspepsia, hyperacidity, dysentery and constipation. Papaya helps in the digestion of proteins as it is a rich source of proteolytic enzymes. Even papain-a digestive enzyme found in papaya is extracted, dried as a powder and used as an aid in digestion. Ripe fruit consumed regularly helps in habitual constipation. It is also reported that papaya prevents premature aging. It may be that it works because a poor digestion does not provide enough nutrients to our body. The fruit is regarded as a remedy for abdominal disorders. The skin of papaya works as a best medicine for wounds. Even you can use the pulp left after extracting the juice from papaya as poultice on the wounds. The enzymes papain and chymopapain and antioxidant nutrients found in papaya have been found helpful in lowering inflammation and healing burns. That is why people with diseases (such as asthma, rheumatoid arthritis, and osteoarthritis) that are worsened by inflammation, find relief as the severity of the condition reduces after taking all these nutrients. Papaya contributes to a healthy immune system by increasing your resistance to coughs and colds because of its vitamin A and C contents. Papaya included in your diet ensures a good supply of vitamin A and C that are highly essential for maintaining a good health. *Carica papaya* constituents exhibit alkaline combination, as with borax or potassium carbonate and they have showed good results in treatment of warts, corns, sinuses, eczema, cutaneous tubercles and other hardness of the skin, and also injected into indolent glandular tumors to promote their absorption. Green fruits of papaya are used to treat high blood pressure, dyspepsia, constipation, amenorrhoea, general debility, expel worms and stimulate reproductive organs. Above are all reason that papaya (Carica papaya) was chosen for this study. It is quite possible to utilize papaya fruits successfully to make an acceptable quality of wine in the tropical region.

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