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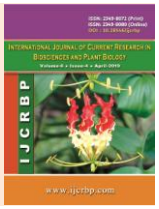

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Evaluation of physicochemical and sensory properties of wine from *Citrus maxima* fruit

Anjana Prakash[1], Ancy Philip[1], S. Archa[1], C. V. Archana[1], Arathy G. Nair[1], B. Arya[1], K. R. Sabu[2], B. R. Rajesh[1] and Pratap R. Chandran[1]*

[1]Department of Biotechnology and Research, K. V. M. College of Science and Technology, K.V.M. College Road, Kokkothamangalam P. O., Cherthala – 688 583, Alappuzha District, Kerala State, India
[2]Department of Chemistry, Arba Minch University, Abaya Campus, Arba Minch. P. B. No. 21, Ethiopia

*Corresponding author; e-mail: drpratapchandran@yahoo.co.in

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ABSTRACT

Wine production is a natural process that requires very little human intervention. *Saccharomyces cerevisiae* is the backbone of wine industry. Pomelo (*Citrus maxima*), considered as a citrus fruit provides number of surprising health benefits. It is native to South and South East Asia. The taste of pomelo is quite pleasant with the consistency of grape fruits without tart or tangy flavor. This work is aimed at producing wine from fermented pomelo fruit pulp. Primary and secondary fermentation of pomelo fruit lasted for 20 days, during which aliquots of samples were analyzed for pH, yeast count, alcohol content, specific gravity, poly phenol content, electrical conductivity, antioxidant property and citric acid content using standard procedures. The organoleptic assay of pomelo wine was also carried out after the completion of the fermentation tenure which gave a satisfactory result. The pH of fruit must during the fermentation period ranged from 6 to 4. Specific gravity of wine showed a decrease from 1.13 to 1.12. On the other hand alcohol content almost tripled and citric acid content increased from 2.56% to 4.99% during fermentation. As the fermentation progressed, the content of polyphenol showed a gradual decrease. The electrical conductivity of wine when observed after the completion of fermentation was found to be 897 mS and the total dissolved solids were found to be 484 ppm at 30°C. The antioxidant activity of the wine was evaluated using DPPH assay and the value was obtained as 86.3%.

Introduction

Wine is an alcoholic beverage made from fermented fruit juice (Johnson, 1989). Yeast (*Saccharomyces cerevisiae*) consumes the sugar in the fruit juice and converts it to ethanol and carbon dioxide. Primary fermentation last for approximately one week, during which, most of the sugar present in the fruit juice gets converted to ethanol (McGovern et al., 2013). The excess yeast cells are then removed from the juice along with other sediments and a slower secondary fermentation is allowed to proceed to develop the final flavour. Sugar can be added to get the desired alcohol content or to modify the flavour. Grapes were commonly preferred for wine production because grape juice aids the fermentation process without the addition of

sugars, acids, enzymes, or other nutrients. However, coconut sprout and fruits such as banana and pineapple were also subjected to fermentation to study the quality of wine formed (Mohan et al., 2018; Ajit et al., 2018).

Pomelo, *Citrus maxima* is a natural citrus fruit belonging to the family Rutaceae and is native to South East Asia. It tastes like a sweet, mild grape fruit. The pulp of pomelo contains considerable amount of sugar, thus making it suitable for wine production. Pomelo contains minerals and other components like vitamin C, potassium, dietary fiber, vitamin B6, magnesium, folic acid, vitamin B1, iron, vitamin A, beta carotene, bioflavonoid, antioxidants and enzymes. Consuming this fruit has several health benefits such as preventing infection, boosting immunity, promoting healing, preventing anemia, warding off cold and flu, fights cancer and ageing, helping in weight loss as well as digestion, preventing osteoporosis and muscle cramps, controlling blood pressure, etc. (Singh and Gupta, 2017).

Pomelo fruit usually has a pale green to yellow colour. It is considered as the largest fruit in the citrus family, averaging a size larger as 30cm in diameter and a weighs of 1-2 kg. The climatic conditions of tropic zone are suitable for the growth of pomelo (Kyndt et al., 2010). In general wine is an un distilled beverage which is so nutritive and even more tasty than the raw fruit itself. Upon fermentation the physicochemical and sensory qualities such as taste, flavor etc. of the fruit are modified due to the formation of certain compounds such as alcohol, organic acids, amino acids, etc. Fermentation for the production of beverages like wine depends on the ability of the yeast to convert sugar contents of the substrate to alcohol and esters. Moreover, the species of yeasts that develop during the fermentation determine the final characteristics (flavour, taste, aroma etc.) of the product. Wine has a greater acceptance than other ordinary drink (Yang and Wiegand, 1949). It is a beverage with greater shelf life and the alcohol present in it has antipathogenic effect (Oddbins, 2012). The organic content present in fruit pulp gives it characteristic odor, taste, smell, and other health benefits. Hence the present study was aimed to estimate the chemical constituents and the quality of wine produced from pomelo fruit using *S. cerevisiae* and spontaneous fermentation.

Materials and methods

Microorganism used

S. cerevisiae obtained from the culture collection of K.V.M College of Science and Technology, Cherthala, Alappuzha, Kerala, India was used for fermentation.

Collection of sample

Pomelo fruit (*C. maxima*) sample was obtained from Alappuzha district. About 1 kg of the fruit was taken, cleaned and the fruit pulp was extracted.

Inoculum preparation

Sterilized glucose yeast broth was inoculated with *S. cerevisiae* in a rotary shaker at 60 rpm for 24 hours at 30°C. Then it was centrifuged at 6000rpm at 4°C for 10 minutes for cell separation. The pre-inoculum was prepared by washing the cell twice and resuspending in normal saline to attain a concentration of 10^8 cells/ml. 10ml of pre-inoculum was then transferred into 250 ml Erlenmeyer flask containing 100ml pomelo juice. This mixture was used as inoculum. The incubation of the mixture was then carried out in a shaking incubator at 60rpm and then kept overnight at 30°C.

Preparation of must and fermentation

Crushed pomelo fruit pulp was used as the substrate for the production of wine. About 100 g of sugar and 250 ml water were mixed together to form a solution. To the sugar solution crushed pomelo fruit pulp (1 kg) was added and mixed well. A control was also prepared by mixing pomelo fruit pulp and sugar solution. The must as well as control were autoclaved at 121°C for 15 minutes at 15 lbs and then cooled. The prepared inoculum of *S. cerevisiae* was added to the sample except the control followed by thorough mixing and kept at room temperature for fermentation.

The fermentation was carried out for 20 days in dark. During fermentation at an interval of 5 days, the sample and control were filtered by sieving through four layers of muslin cloth. The filtrate was used for conducting various analytical tests and the residues were discarded. The wine jar was

immersed in water bath at 68-70°C in order to stop fermentation after the completion of the 20th day.

pH

pH of the wine samples were checked using a digital pH meter (Eutech CyberScan pH 510) pre-calibrated with buffers of pH 4 and 7 (Ochai and Kolhatkar, 2008).

Estimation of yeast cell count

The increasing number of yeast cells (*S. cerevisiae*), increases the turbidity of the sample. Optical density is parameter widely used to estimate the number of cells in a culture. Five ml of the wine sample was taken in a test tube along with a suitable blank. The absorbance was read using spectrophotometer at 600 nm. The density of yeast cell at 600 nm is around 10^7 cells per ml.

Determination of Total phenolic content

The total phenolic content in wine was estimated by Folin-Ciocalteau method (Singleton and Rossi, 1965). About 1 ml of the sample was taken as an aliquot. To this 1 ml of Folin-Ciocalteau reagent was added and mixed for 3 minutes after which 1.0 ml of saturated sodium carbonate solution was added and the final volume was made up to 10 ml with distilled water. The same procedure was repeated for the control too after which both tubes were kept in dark for 30 minutes. The colour intensity was then measured at 760 nm against the control which was taken as the blank. Calibration curves were drawn using gallic acid standard and total phenol content (TPC) were calculated.

Specific gravity

Estimation of specific gravity was done using specific gravity bottle (Ranganna, 1986). A 50 ml specific gravity bottle was washed with distilled water and dried in an oven and cooled at room temperature, weighed and noted as W1. It was then filled with distilled water and surface of the bottle was cleaned using cotton and was weighed again and the value was recorded as W2. After rinsing the bottle with 10ml of pomelo wine it was filled up to the brim with wine and weighed and the weight was noted as W3. The specific gravity of the wine sample was calculated using the formula:

$$\text{Specific gravity} = \frac{W3-W1}{W2+W1}$$

Estimation of alcohol

The estimation of alcohol was done using the iodoform test (Kulandaivel and Janarthanan, 2012). About 1ml of wine sample containing alcohol was taken in a test tube and 4 drops of 1 N sodium hydroxide was added to it. Concentrated solution of iodine was then added drop by drop until a faint yellow colour persisted. The tubes were allowed to stand for a minute and added excess amount of sodium hydroxide solution if excess colour developed. Shook the mixture well and allowed to stand for 2-3 minutes. A yellow coloured precipitate that was formed was found to settle at the bottom. Removed the precipitate at room temperature and weighed and calculated the amount of alcohol present in the sample.

Determination of antioxidant activity

The antioxidant activity of wine was estimated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay (Soares et al., 1997). The method is based on the scavenging of DPPH by antioxidants, which upon reduction reaction decolorizes the methanol solution of DPPH. If DPPH radical accept an electron or hydrogen from antioxidant molecules it becomes a stable diamagnetic molecule and will change the colour to yellow. Lower the absorbance of the reaction mixture, higher the free radical scavenging activity. The wine sample measuring 5 ml was mixed with a solution of 5 ml of 0.06 mM DPPH in methanol and incubated in dark for 30 minutes. The absorbance was measured at 520 nm. The capability to scavenge the DPPH radical was calculated using the equation:

$$\text{RSA } \% = \frac{[1-(Abs\ control-Abs\ sample)]\times 100}{Abs\ control}$$

Estimation of reducing sugar

The concentration of reducing sugar was analysed according to the following method (Saqib and Whitney, 2011). DNS reagent was prepared by dissolving 1 g of DNS and 30 g of sodium potassium tartaric acid in 80 ml of 0.5 N sodium hydroxide solutions. For the complete dissolution

of reagents the solution was kept at 45°C and cooled down to room temperature and made up to 100 ml with distilled water. Then the solution was kept at 4°C for two weeks. In order to estimate the reducing sugar 0.4 ml of DNS reagent was added to 0.1ml of sample and kept at 95°C for 5 minutes in a water bath for incubation, after which the absorbance was measured spectroscopically at 540 nm.

Titratable acidity

Titratable acids represent the sum of all acids in the wine and titratable acidity corresponds to the percentage of citric acid and the latter was determined by the method of AOAC (1995). Titration with indicator phenolphthalein is usually done to estimate the content of citric acid in wine.10 ml of wine sample was pipetted into a conical flask, few drops of the indicator was then added. It is then titrated against the solution of sodium hydroxide taken in the burette till the colour of the wine sample changed to pink.

Weight of citric acid in 1000 ml of sample =

$$\frac{\text{normality} \times \text{volume} \times \text{equivalent weight}}{1000}$$

Determination of electrical conductivity

Total dissolved ionic solids (TDS) such as salt and minerals increase the electrical conductivity (EC) of a solution. The EC and TDS were determined using electrical conductivity meter (Systronics 308).

FTIR spectroscopy

Fourier Transform Infrared Spectrophotometer (FTIR) is a powerful technique used to identify the chemical bond present in a compound. By interpreting IR spectrum obtained, the chemical bonds in a compound can be analysed. The tablet for spectroscopy (FTIR Shimadzu prestige21) were prepared in agate mortar, by mixing a drop of wine sample with KBr (1:100 p/p) absorption spectra were measured between 400 and 4000 cm^{-1}.

Organoleptic assay

After the completion of 20th day of fermentation, wine samples collected from the fermentation flask were filtered and subjected to sensory evaluation. The panel comprised of different people recruited from members of the staff committee. The aim of choosing different panelist was because of difference in the sensibility of various assessors. The characters such as taste, odour, flavor, clarity and the overall acceptance of wine were assessed by 5 point hedonic scale (Espinoza et al., 2005).

Results and discussion

Wine is an important fermented product made from fruits since ancient times. Consuming wine on a regular basis results in health benefits and also it has a long shelf life. Throughout the fermentation period wine retains the acidic nature. Acidity increased during the fermentation period and the pH ranged from 5.7 to 4.5 (Table 1). The content of alcohol present in pomelo wine also showed an increasing trend that ranged from 0 to 7%. As the fermentation proceeds the yeast cell count decreased to 2.35×10^6 from 8.1×10^6. Using spectrometer the initial value was measured to be 8.10×10^6 on the first day, then it attained a maximum value of 9.20×10^6on the 5th day and then decreased to 2.35×10^6on the 20th day (Fig. 1).

Table 1. Physicochemical analysis.

Physico-chemical parameters	Day 0	Day 5	Day 10	Day 15	Day 20
pH	5.7	5.5	5.1	4.8	4.5
Alcohol content (%)	Nil	2.8	5.2	6.5	7
Specific gravity (g/cm^3)	Nil	1.13	1.13	1.12	1.12
Titratable acidity (g/L)	Nil	2.56	2.97	3.8	4.99

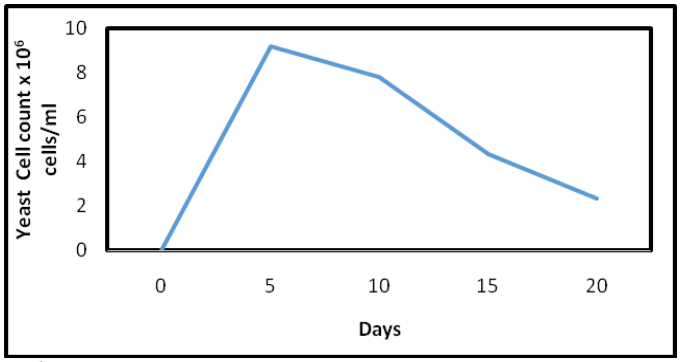

Fig. 1: Graph showing the yeast cell count in the wine sample.

During the course of fermentation, specific gravity of pomelo wine decreased from 1.13 to 1.12. As the day passes by, the citric acid content increased. The initial value was found to be 2.56% on the 5th day and it increased to 4.99% on the final day. Citric acid content showed an increasing trend during fermentation. From the estimation we get a decreased value from polyphenol content from 2.49 to 1.97 (Fig. 2). During wine ageing the decrease in phenolic content was observed due to polymerization of phenolic components present in the wine. Antioxidant content of the pomelo wine was evaluated using DPPH assay and the value was obtained as 86.31. Concentration of the reducing sugars declined as the fermentation proceeded. Reduction in sugar content was due the production of alcohol by the yeast cell. Electrical conductivity of the wine was read immediately after the completion of the 20th day using conductivity TDS meter and it was found to be 897 mS. This value is used to estimate the extent of dissolved solids and it was found to be 484ppm at 30°C.

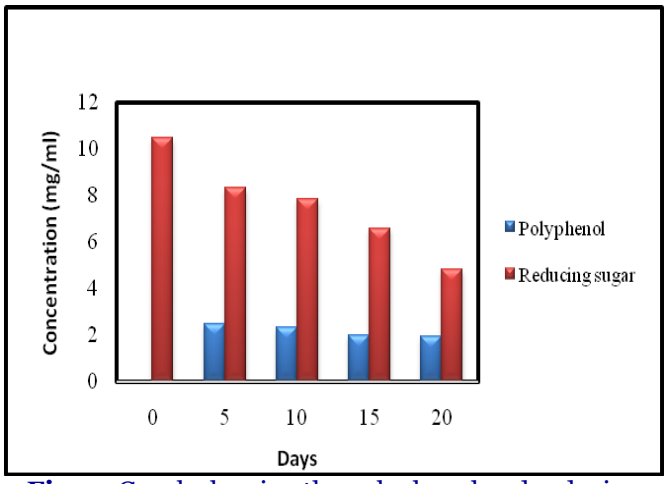

Fig. 2: Graph showing the polyphenol and reducing sugar content.

Decreasing trend of the pH found during the course of fermentation is due to organic acid production by the yeast (Okemini and Igwebike-Ossi, 2017). Specific gravity of wine decreased after fermentation. This helps to reduce the soluble solids in the wine and hence alcohol content increases during fermentation. Yeast present in wine utilized the sugar content and thereby reduces the soluble solids (Onwuka and Awam, 2001). Titratable acidity increases due to the metabolic activity of yeast present in the must (Akubor et al., 2003). The organoleptic assay was carried out on the completion of the fermentation process. A panel of people having varying sensibility was selected to test characters such as taste, odor, flavor, clarity and overall acceptance of wine. Estimation of organoleptic characters showed that the pomelo wine has a good acceptance (Fig. 3).

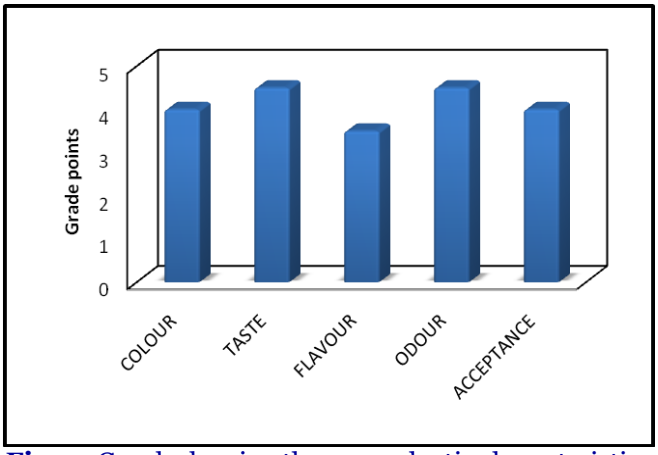

Fig. 3: Graph showing the organoleptic characteristics of the wine sample.

The *C. maxima* fruit juice before fermentation contains, as is evidenced from the FTIR spectrum, some of the marker bands of glucose, fructose and sucrose which correspond to the spectral range between 900 and 1400 cm inverse. The bands in the region 900 and 1153 cm inverse are assigned to C-O and C-C stretching modes while those in the 1199-1400 cm inverse region are due to O-C-H, C-C-H and C-O-H bending vibrational modes of the carbohydrates. Higher sucrose level in the juice is evidenced by the intense marker band of sucrose at 993 cm inverse. Regarding the functional groups of citric acid, which is a tricarboxylic acid, the FTIR spectrum shows O-H stretching bands at 3275-3298 cm inverse region along with C-H stretching at 2920-2924 cm inverse. Correspondingly C=O stretching is expected at about 1717-1732 cm inverse, which is not well evidenced. However, at the same time, strong C=O stretch has been observed at 1641 cm inverse and 1581 cm inverse. Similarly, -CH2_ and or -CH3 stretching vibrations are observed at 1413 cm inverse, 1348 cm inverse and 1274 cm inverse. Nevertheless, the C-O-H or C-O-R vibrations at 1011, 1026 and 1045 cm inverse are missing, which clearly indicates that the citric acid molecules are not existing in the free state or even in the esterified state, rather there is a possibility of them existing in the citrate salt form (either of calcium or magnesium) (Fig. 4).

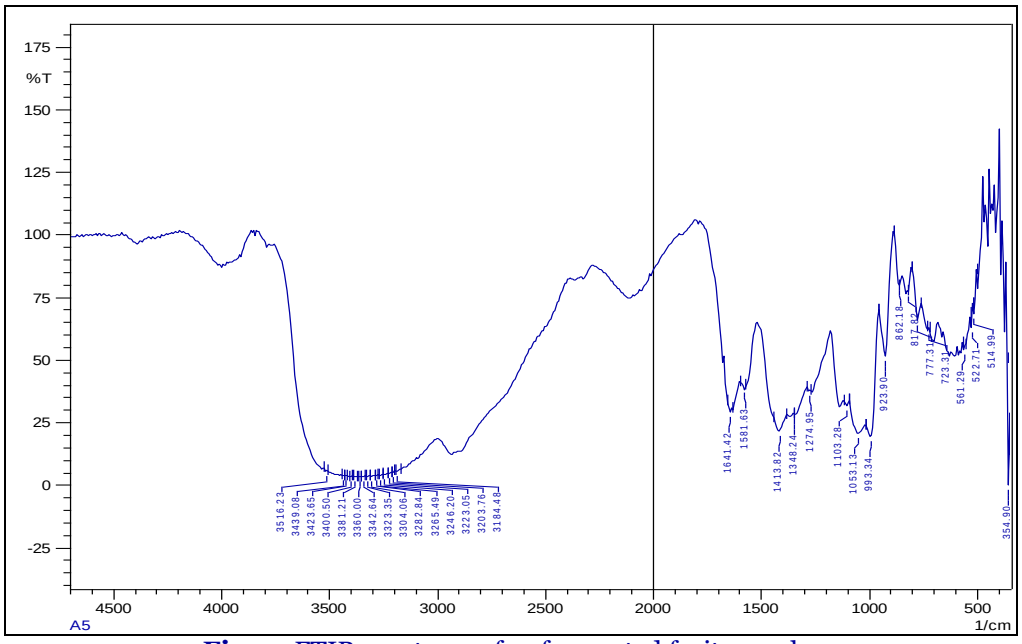

Fig. 4: FTIR spectrum of unfermented fruit sample.

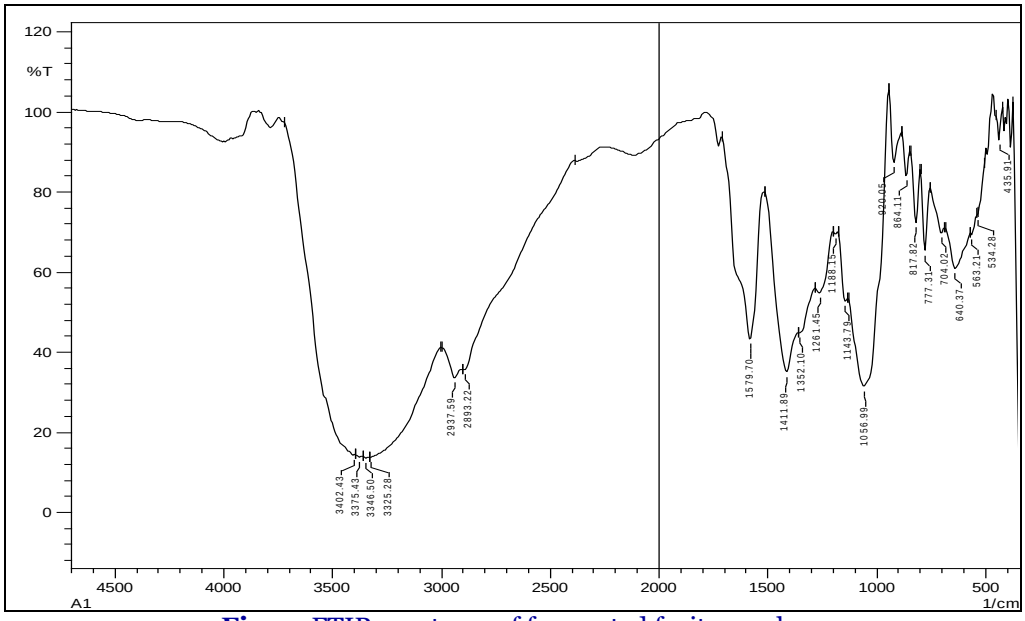

Fig. 5: FTIR spectrum of fermented fruit sample.

The most notable change observed after fermentation is the appearance of a strong absorption band at 1056 cm inverse due to the formation of ethyl alcohol, which corresponds to the C-O stretching vibrations, along with O-H stretching vibrations around 3400 cm inverse and C-H stretching vibrations at about 2937 cm inverse. More important is the diminishing of the strength of the marker band at 993 cm inverse due to sucrose which indicates the conversion of sucrose to ethyl alcohol during fermentation. Other peaks related to C=O stretching vibrations at 1579 cm inverse and -CH2- and or -CH3 stretching vibrations at 1411cm inverse, 1352 cm inverse and 1261 cm inverse remain intact, which indicates that the citrate components are unaffected by the process of fermentation. Broader peaks between 3325 cm inverse and 3300 cm inverse are due to the presence of -OH stretching, which could be a combined contribution from citrate hydroxyl and water hydroxyl, both showing strong intermolecular hydrogen bonding (Fig. 5).

Conclusion

After 20 days of fermentation wine from pomelo with nutritional values was obtained and it is a good alcoholic beverage providing numerous health benefits. It contains moderate amounts of antioxidants and polyphenols which can protect cell from damage during oxidative stress and also protect against variety of disease such as heart disorder, cancer etc. Citric acid content is also high. Citric acid gives energy for cellular functions. The sensory evaluation such as taste, smell, flavour also gave acceptable value. So the pomelo wine is good for use and it has better beneficial effects on health. Normally the pomelo fruit has a slight bitter and sour taste and so people avoid eating raw fruit. But from the sensory evaluation of pomelo wine we received a positive response, so the wine is more acceptable than the raw fruit.

Conflict of interest statement

Authors declare that they have no conflict of interest.

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