

WINE PRODUCTION FROM VARIOUS UNDERUTILIZED AND NEGLECTED FRUITS IN KERALA

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ABSTRACT

Underutilized or neglected fruit trees are rich in vitamins, minerals, fibers and considered to be an important role in mitigating malnutrition and poverty in developing and under developed countries. The reasons for the lack of popularity of these fruit trees may be due to a variety of reasons including ignorance, lack of knowledge, availability, difficulty in harvesting and storage. Wine production has a traditional place in development of mankind and various cultures considered to be the oldest biotechnological approach. The quality of the wine is dependent on type of substrate, sugar content, strain of yeast, climatic and storage conditions. Lacking qualitative and quantitative data on wine production using underutilized and neglected fruit trees in Kerala, objective of this study were: (1) to identify and characterize importance sources of fruit trees for wine production and (2) to determine the value addition of the products after wine production. A total of 6 samples were collected from different regions of the Kerala from January 2015 to February 2015. The samples were fermented for 20 days and parameters like pH, density, acidity, volatile acidity, alcohol and vitamin C contents. The samples were subjected to sensory analysis by a panel of 10 trained judges. Highest alcohol contents were in the order Bilimbi (0.39 ± 0.014) > Java Apple > Ginger > Gooseberry > Coffee > Pepper (0.25 ± 0.009). Gooseberry samples showed highest vitamin C content (12.73 ± 3.60) while least with Ginger samples (1.13 ± 0.12). The overall quality was very high for Gooseberry followed by Bilimbi and Java Apple. Sour taste over dominate in Coffee, Ginger and Pepper samples. Value added products like Parchment Coffee and White Pepper almost doubles the income of farmers with the possibility of production of alcohol. Importance should be given to the propagation, processing and storage of various underutilized and neglected fruits.

Keywords: Value Addition, Underutilized Fruits, Volatile Acidity, Sensory Analysis

INTRODUCTION

Wine is one of the oldest, fermented, traditional, convenient alcoholic beverages of the mankind (Platt, 1955; Blandino *et al.*, 2003; Das *et al.*, 2012). Fermentation is considered as the oldest method producing and preserving food (Chavan and Kadam, 1989; Blandino *et al.*, 2003). The quality of the wine is influenced by the choice of the substrate, climate, soil type, sugar content, strain of yeast and storage conditions (Jones and Davis, 2000; Hale *et al.*, 1999; Esteves and Orgaz, 2001; Gil *et al.*, 1996; Jackson and Lombard, 1993). Around the globe a large choice of natural alcoholic beverage exist which vary from culture to culture and county to county depending on the substrate used. The most promising ones were Grappa (Italy), pulque (Mexico), Yu and Toddy (India), miki (Japan), Bushera (Africa) (Kim *et al.*, 1996; Bovo *et al.*, 2009; Escalante *et al.*, 2008; Singh and Singh, 2006; Chellapandian *et al.*, 1998; Teramoto *et al.*, 1998; Muyanjanja *et al.*, 2003; Platt, 1955). Cereal grains are rich in dietary proteins, carbohydrates, vitamins, minerals and considered to be an important cheap source for fermentation (Blandino *et al.*, 2003; Chavan and Kadam, 1989; Sanni *et al.*, 1999; Das *et al.*, 2012).

Underutilized or neglected fruit trees were considered to be an important role in mitigating malnutrition and poverty in developing and under developed countries. These fruit trees may be neglected due to ignorance, lack of knowledge, availability, difficulty in harvesting and storage (Sundriyal and Sundriyal, 2003; Badola and Aitken, 2010; Gebauer *et al.*, 2007). Most of these fruits are rich in vitamins, antioxidants, organic acids and phenolic contents (Pande and Akoh, 2010; Kelebek and Selli, 2011; Kong *et al.*, 2011; Salmanian *et al.*, 2014; Kalt *et al.*, 1999). The major underutilized fruit trees in Kerala

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includes Jack fruit (*Artocarpus heterophyllus*), Bilmi (*Averrhoa bilimbi* L.), Indian plum (*Flacourtia rukam*), Java apple (*Syzygium javanica*), Jamun (*Syzygium cumini*), Gosseberry (*Embllica officinalis*), Pomelo (*Citrus grandis*), Malay apple (*Syzygium malaccense*), Kokum (*Garcinia indica*), tamarind (*Tamarindus indica*), Malabar tamarind (*Garcinia gummi-gutta*) (Thomas, 1980; Raju and Reni, 2001; Mohan, 2004; Peter and Abraham, 2007; Mohan et al., 2007; Mathew et al., 2004; Kruijssen et al., 2009; Nair and Sreedharan, 1986; Jose and Shanmugaratnam, 1993; Peyre et al., 2006).

Wine is associated as a part of the diet reduces cardiovascular diseases, anticancer agents and high antioxidant capacity (Guerrero et al., 2009; Soleas et al., 2002; Yadav et al., 2009). The antioxidant capacity is attributed by bioactive compounds especially polyphenols especially anthocyanins (Rivero-Pérez et al., 2008; Carluccio et al., 2003; Soleas et al., 2002; Rice-Evans et al., 1997). Given lacking qualitative and quantitative data on wine production using underutilized and neglected fruit trees in Kerala, objective of this study were: (1) to identify and characterize importance sources of fruit trees for wine production and (2) to determine the value addition of the products after wine production.

MATERIALS AND METHODS

Study Area

Kerala state covers an area of 38,863 km² with a population density of 859 per km² and spread across 14 districts. The climate is characterized by tropical wet and dry with average annual rainfall amounts to $2,817 \pm 406$ mm and mean annual temperature is 26.8°C (averages from 1871-2005; Krishnakumar et al., 2009; Figure 3).



Figure 1: Various Underutilized Fruits from Kerala, Bilimbi (*Averrhoa Bilimbi* L.); Top Left, Jackfruit (*Artocarpus Heterophyllus* Lam.); Top Right, Cashew Nut (*Anacardium Occidentale*); Middle Left, Indian Plum (*Flacourtia Rukam*); Middle Right, Java Apple (*Syzygium Javanica*); Bottom Left, Jambul (*Syzygium Cumini*); Bottom Right

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Figure 2: Various Underutilized Fruits from Kerala, Pomelo (*Citrus Maxima*); Top Left, Malay Rose Apple (*Syzygium Malaccense*); Top Right, Indian Plum (*Flacourtia Rukam*); Middle Left, Gooseberry (*Emblica Officinalis*); Middle Right, Tamarind (*Tamarindus Indica*); Bottom Left, Malabar Tamarind (*Garcinia Gummi-Gutta*); Bottom Right

Sample Collection

Samples of various underutilized and neglected fruit trees were collected based on an elaborative literature survey as well as traditional knowledge. A total of 6 samples were collected from different regions of the Kerala from January 2015 to February 2015. Locations of the sample collection areas were recorded using a Trimble Geo explorer II (Trimble Navigation Ltd, Sunnyvale, California) and data were transferred using GPS Pathfinder Office software (Trimble Navigation Ltd, Sunnyvale, California).

Bilimbi (*Averrhoa Bilimbi L.*)

Bilimbi, is a fruit-bearing tree of the genus *Averrhoa*, family Oxalidaceae. It is a close relative of carambola tree. Tree reaches 5-10 m in height. Its trunk is short and quickly divides up into ramifications. Bilimbi leaves, 3-6 cm long, are alternate, imparipinnate and cluster at branch extremities. There are around 11 to 37 alternate or sub opposite oblong leaflets.

Java Apple (*Syzygium Javanica*)

Syzygium javanica is a tropical tree, bell shaped fruit, colours ranging from white, pale green, red, purple and crimson. Flowers are used to treat fever and diarrhea, the principal component is tannin. The plant need long dry season and ripening of fruit from May to June in several time periods.

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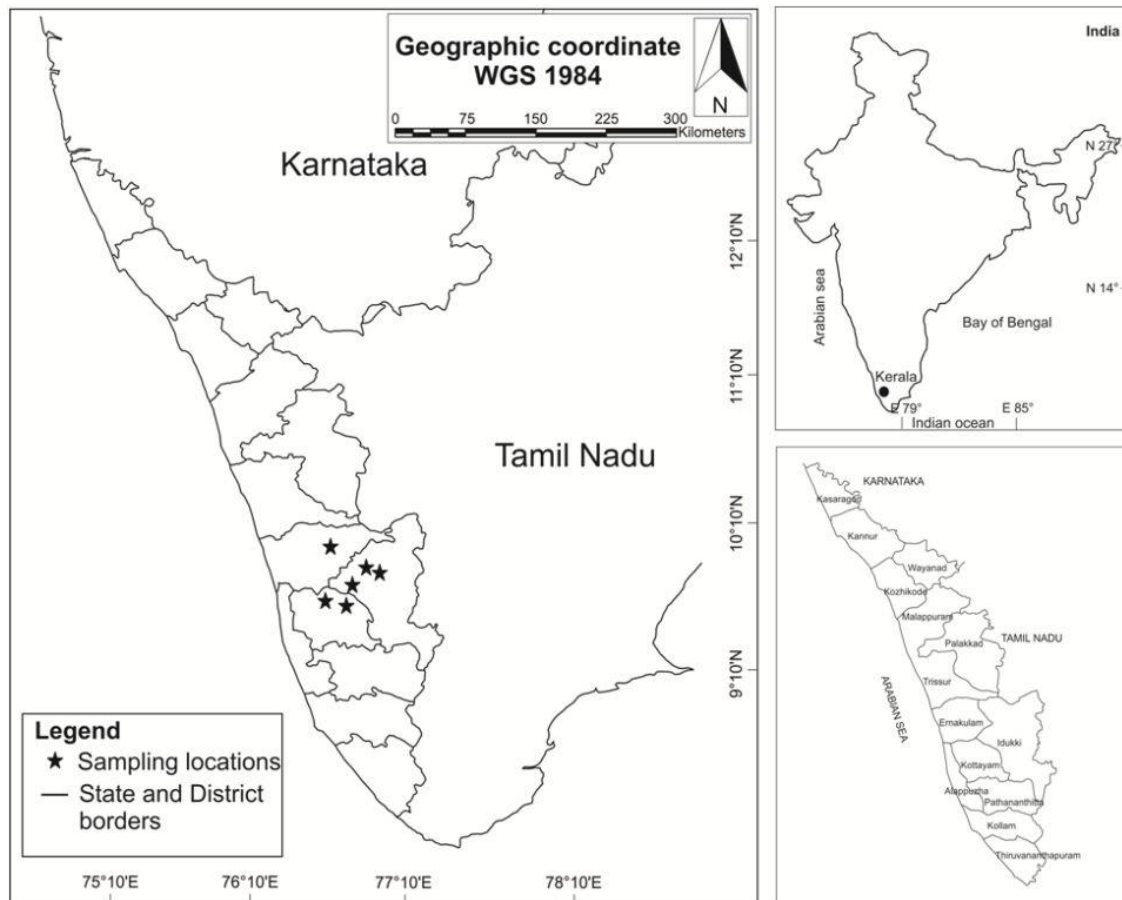


Figure 3: Map of Kerala Showing the Various Sample Collection Points

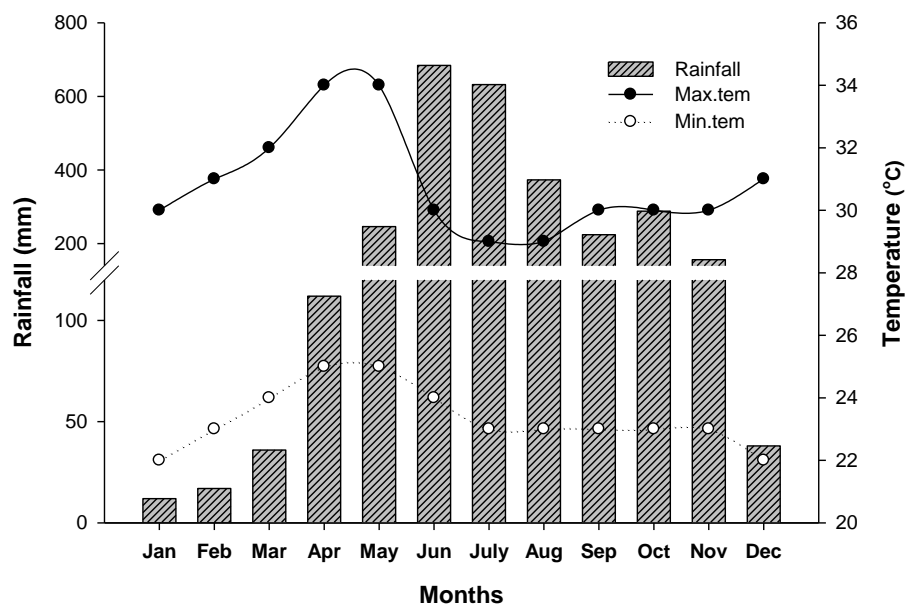


Figure 4: Mean Monthly Rainfall (mm), Maximum and Minimum Temperatures (°C) in Kerala, India (1871-2005; Krishnakumar et al., 2009)

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Figure 5: Value Added Products after Wine Production: Coffee Fruits; (Top Left), Pepper Fruits; (Top Right), Coffee Fruits before Fermentation; (Middle Left), Pepper Fruits before Fermentation; (Middle Right), Parchment Coffee after Fermentation (Bottom Left), White Pepper after Fermentation; (Bottom Right)

Gooseberry (Emblca Officinalis).

Gooseberry is a deciduous tree of the family Phyllanthaceae. The fruit is nearly spherical, light greenish yellow, quite smooth and hard on appearance, with six vertical stripes or furrows. The tree is small to medium in size, reaching 8 to 18 m in height, with a crooked trunk and spreading branches. The branchlets are glabrous or finely pubescent, 10-20 cm long, usually deciduous; the leaves are simple, subsessile and closely set along branchlets, light green, resembling pinnate leaves. The flowers are greenish-yellow. The taste of Indian gooseberry is sour, bitter and astringent, and it is quite fibrous.

Pepper (Pepper Nigrum)

Pepper is often described as the "king of spices," and it shares a place on most dinner tables with salt. The word pepper originated from the Sanskrit word pippali, meaning berry. The various species of Piper are grown mostly as woody shrubs, small trees, and vines in the tropical and subtropical regions of the world. The hot taste sensation in pepper comes from a resin called chavicine in the peppercorns. Peppercorns

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also are the source of other heat-generating substances, including an alkaloid called piperine, which is used to add the pungent effect to brandy, and an oil that is distilled from the peppercorns for use in meat sauces. As a natural medicinal agent, black pepper in tea form has been credited for relieving arthritis, nausea, fever, migraine headaches, poor digestion, strep throat, and even coma. It has also been used for non-medical applications as an insecticide. Black pepper is a favorite spice of cooks because of its dark color and pungent aroma and flavor.

Coffee (*Coffea Canephora*)

Coffee, beverage brewed from the roasted and ground seeds of the tropical evergreen coffee plant of African origin. It is consumed either hot or cold by about one-third of the people in the world, in amounts larger than those of any other drink Its popularity can be attributed to its invigorating effect, which is produced by caffeine, an alkaloid present in green coffee in amounts between 0.8 to 1.5 % for the Arabica varieties and 1.6 to 2.5 % for Robusta.

Ginger (*Zingiber Officinale*)

Ginger is widely used in traditional Indian cuisines and used various ayurvedic and folk medicine preparations. Used for the treatment of throat infections, diarrhea, jaundice and various digestive disorders.



Figure 6: Fermentation of various Underutilized and Neglected Fruit Trees; (Top Left), Java Apple (*Syzygium Javanica*); (Top Right), Java Apple (*Syzygium Javanica*) Mixed with Ginger; (Middle Left), Cleaned and Sliced Ginger Rhizome; (Bottom Left), Pepper Fermentation; (Bottom Right), Gooseberry (*Emblica Officinalis*) in Fermentation Flask

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Yeast Culture

The yeast culture (*Saccharomyces cerevisiae*) were originally procured from MTCC (Microbial Type Culture and Gene Bank), Chandigarh, India; maintained on yeast malt extract agar (YMEA, HiMedia Laboratories, Mumbai, India) and re-cultured after every three months.

Wine Production

All the substrates were washed two times with distilled water to remove dirt and other impurities. Java Apple fruits were cut open, stones removed and divided into many pieces (minimum four pieces). Half of the samples were heat treated for 20 minutes at 80°C. Ginger samples were soaked in distilled water, outer skin peeled and cut into small pieces. In the case of pepper both ripe and unripe fruit bearing spikes were selected. The spike bearing fruits were directly added to the fermentation vessel. The fermentation vessels were made of porcelain (12L x 14W x 12H) and procured from a local dealer.

Fully ripe coffee fruits were selected and half of the samples were crushed by hand and transferred to fermentation vessel. In the case of Indian gooseberry, no pre-processing is done. The substrates, sugar and water were added in three treatments for each substrate. Treatment 1 with substrate sugar water ratio of 2:1:1, while treatment 2 and 3 with 1:1:1 and 1:1:2 respectively. Fermentation was initiated by the addition of active yeast culture of *Saccharomyces cerevisiae* (5%) at a temperature of $22 \pm 1^\circ\text{C}$. The fermentation process was initiated by transferring the 48 h grown yeast inoculum to various substrates containing porcelain jars (5 liter capacity) and was incubated at $22 \pm 2^\circ\text{C}$ in darkness for 20 days. The samples were stirred with a wooden stick for 2 minutes daily for the mixing and uniform fermentation.

At the end of the fermentation, the fermented solution was centrifuged at 5,000 rpm for 20 minutes to separate the yeast cells and allowed to sediment for 10 more days to settle the unfermented solid materials. After 10 days, the solution was siphoned to separate the two phases of wine (solid and liquid). The filtered wine was stored at 4°C in tight PE bottles to avoid air contact.

Acidity and Volatile Acidity Determination

The acidity of the wine samples were determined using 0.1 N NaOH (sodium hydroxide) and 1% phenolphthalein indicator (Boulton, 1980). The measurements were made for 5, 10, 15 and 20 days respectively. Total acidity and volatile acidity were determined using the following equations

$$\text{Total Acidity} = \frac{(\text{Volume of alkali used (ml)} \times \text{Normality of alkali} \times 7.5)}{(\text{Weight of the sample in g})}$$

$$\text{Volatile Acidity} = \frac{(\text{Volume of alkali used (ml)} \times \text{Normality of alkali} \times 6.0)}{(\text{Weight of the sample in g})}$$

Estimation of the Alcohol Content

The alcohol of the samples was measured using alcoholmeter (Leimco, Mumbai, India) and the measurements were made for 5, 10, 15 and 20 days respectively.

Estimation of the Vitamin C Content

Vitamin C standard were prepared using two pharmaceutical samples procured from a local drug store. The tablets were finely grounded using a pestle and mortar stored in plastic vials till analysis. Standard stock solutions were prepared by dissolving the finely powered samples to give a concentration of 1 mg per ml. The titration values from the standard solution were used for the further calculations.

The Vitamin C contents were measured using the redox titration method of potassium dichromate with starch as indicator (Roe and Kuether, 1943; Bessey and King, 1933). When iodine solution is a titrant, vitamin C is oxidized to form dehydroascorbic acid while the iodine is reduced to iodide ions. When all vitamin C has finished, the excess iodine solution will react with starch solution to form blue-black color as endpoint of titration. The formation of a pale blue color was considered as the end point of titration.

Measurement of pH and Density

The pH measurements were taken using a portable pH meter while density measurements were done using a digital bench balance.

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Table 1: Properties of the Various Underutilized Fruits Used in the Study

General Name	Scientific Names	Common Uses
Bilimbi	<i>Averrhoa bilimbi L.</i>	Pickles, juices, food preparations
Java Apple	<i>Syzygium javanica</i>	Fruit, juice
Gooseberry	<i>Emblica officinalis</i>	Pickles, food preparations, juice
Pepper	<i>Pepper nigrum</i>	Spice, food preparations
Coffee	<i>Coffea canephora</i>	Beverage
Ginger	<i>Zingiber officinale</i>	Pickles, juice, food preparations

Sensory Analysis

The sensory analysis of different wines was also conducted to evaluate the aroma and acceptance by a panel of 10 trained judges. Panelists were asked to score for various parameters including sweet, smoky, sour, fermented, alcoholic like, smoky and overall quality of the wine (Rai *et al.*, 2010; Joshi *et al.*, 2013).

Profit Analysis of Value Added Products

The value added products produced from Coffee and Pepper were calculated using the local market price comparison of the products (Parchment Coffee and White Pepper) with the raw normal products.

Statistical Analysis

The survey results were analyzed and descriptive statistics were done using SPSS 12.0 (SPSS Inc., an IBM Company, Chicago, USA) and graphs were generated using Sigma Plot 7 (Systat Software Inc., Chicago, USA).

RESULTS AND DISCUSSION

pH and Density

pH values of the solution in various fermentation stages varied across days of production as well as type of substrate used. Least pH values at the time of maturity were found in Gooseberry wine (3.18 ± 0.02). The density of the wine made with different substrates also changes with time of maturity. In general the density of the solution decreased as fermentation progress. On maturity, the least density wine was Java Apple (0.990 ± 0.003) and high density was from Gooseberry (0.989 ± 0.002).

Table 2: pH Value of the Various Wine Samples with Different Days of Maturity

Sample	Days of Maturity			
	5	10	15	20
Bilimbi	3.30 ± 0.08	3.61 ± 0.11	3.42 ± 0.04	3.26 ± 0.06
Java Apple	3.20 ± 0.14	3.51 ± 0.08	3.56 ± 0.03	3.46 ± 0.02
Gooseberry	3.79 ± 0.07	3.47 ± 0.05	3.33 ± 0.13	3.18 ± 0.02
Pepper	4.23 ± 0.06	4.33 ± 0.07	3.92 ± 0.03	3.46 ± 0.08
Coffee	4.65 ± 0.05	4.69 ± 0.03	4.33 ± 0.09	3.82 ± 0.05
Ginger	3.89 ± 0.08	3.53 ± 0.03	3.55 ± 0.02	3.36 ± 0.06

Numbers represent means \pm one standard deviation (SD) of the mean

Table 3: Density of the Various Wine Samples with Different Days of Maturity

Sample	Days of Maturity			
	5	10	15	20
Bilimbi	0.999 ± 0.001	0.996 ± 0.002	0.991 ± 0.001	0.988 ± 0.001
Java Apple	0.998 ± 0.002	0.995 ± 0.001	0.993 ± 0.002	0.990 ± 0.003
Gooseberry	0.997 ± 0.001	0.994 ± 0.003	0.991 ± 0.002	0.989 ± 0.002
Pepper	0.999 ± 0.001	0.996 ± 0.002	0.994 ± 0.001	0.991 ± 0.002
Coffee	0.998 ± 0.001	0.997 ± 0.001	0.995 ± 0.002	0.993 ± 0.001
Ginger	0.999 ± 0.001	0.997 ± 0.001	0.994 ± 0.002	0.993 ± 0.002

Numbers represent means \pm one standard deviation (SD) of the mean

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Table 4: Total Acidity of the Various wine Samples with Different Days of Maturity (g/l Tartaric Acid)

Sample	Days of Maturity			
	5	10	15	20
Bilimbi	4.12 ± 0.007	4.25 ± 0.013	4.77 ± 0.016	5.36 ± 0.018
Java Apple	4.26 ± 0.005	4.73 ± 0.018	4.92 ± 0.011	5.69 ± 0.026
Gooseberry	3.82 ± 0.009	6.22 ± 0.007	6.97 ± 0.013	8.26 ± 0.015
Pepper	3.54 ± 0.005	3.72 ± 0.004	3.96 ± 0.011	4.06 ± 0.022
Coffee	3.12 ± 0.007	3.22 ± 0.004	3.87 ± 0.017	3.96 ± 0.019
Ginger	4.32 ± 0.006	6.12 ± 0.011	7.26 ± 0.012	7.83 ± 0.018

Numbers represent means ± one standard deviation (SD) of the mean

Table 5: Volatile Acidity of the Various wine Samples with Different Days of Maturity (% A.A)

Sample	Days of Maturity			
	5	10	15	20
Bilimbi	0.21 ± 0.004	0.29 ± 0.011	0.35 ± 0.012	0.39 ± 0.014
Java Apple	0.23 ± 0.003	0.27 ± 0.015	0.31 ± 0.011	0.37 ± 0.016
Gooseberry	0.25 ± 0.009	0.31 ± 0.014	0.33 ± 0.007	0.35 ± 0.012
Pepper	0.19 ± 0.004	0.21 ± 0.011	0.23 ± 0.003	0.25 ± 0.009
Coffee	0.22 ± 0.002	0.24 ± 0.010	0.27 ± 0.002	0.28 ± 0.019
Ginger	0.27 ± 0.003	0.29 ± 0.013	0.33 ± 0.002	0.36 ± 0.017

Numbers represent means ± one standard deviation (SD) of the mean

Table 6: Alcohol Content of the Various Wine Samples with Different Days of Maturity (% Alcohol)

Sample	Days of Maturity			
	5	10	15	20
Bilimbi	0.21 ± 0.004	0.29 ± 0.011	0.35 ± 0.012	0.39 ± 0.014
Java Apple	0.23 ± 0.003	0.27 ± 0.015	0.31 ± 0.011	0.37 ± 0.016
Gooseberry	0.25 ± 0.009	0.31 ± 0.014	0.33 ± 0.007	0.35 ± 0.012
Pepper	0.19 ± 0.004	0.21 ± 0.011	0.23 ± 0.003	0.25 ± 0.009
Coffee	0.22 ± 0.002	0.24 ± 0.010	0.27 ± 0.002	0.28 ± 0.019
Ginger	0.27 ± 0.003	0.29 ± 0.013	0.33 ± 0.002	0.36 ± 0.017

Numbers represent means ± one standard deviation (SD) of the mean

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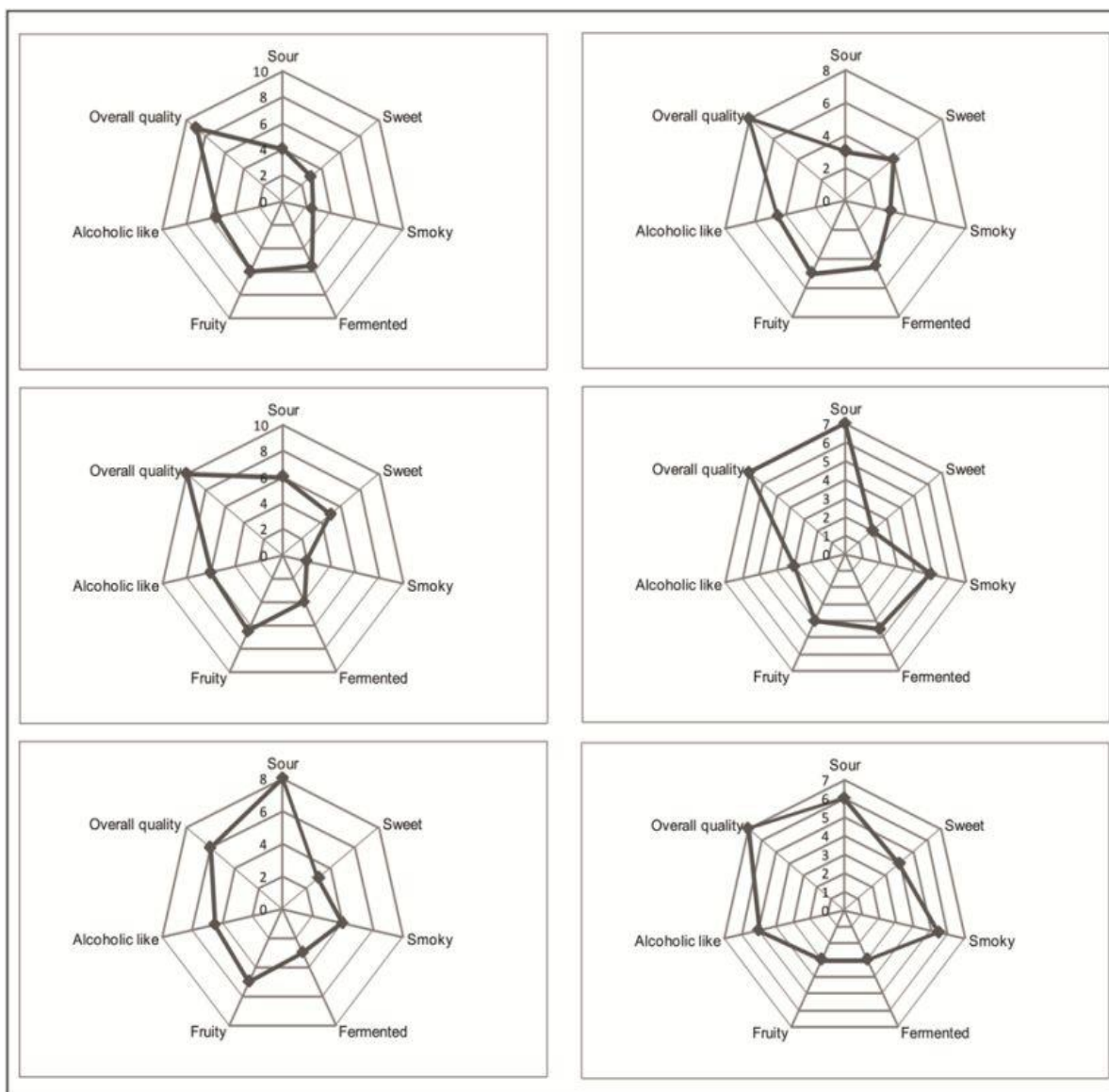


Figure 7: Sensory Evaluation of Various Kind of Wines: Bilimbi; (Top Left), Java Apple; (Top Right), Gooseberry; (Middle Left), Pepper; (Middle Right), Coffee; (Bottom Left), Ginger; (Bottom Right)

Total Acidity and Volatile Acidity

Total acidity was found high on Gooseberry (8.26 ± 0.015) and least on Coffee (3.96 ± 0.019) on 20 days of fermentation. Volatile acidity was maximum on Bilimbi (0.39 ± 0.014) after 20 days of fermentation.

Alcohol and Vitamin C Content

Highest alcohol contents were in the order Bilimbi (0.39 ± 0.014) > Java Apple > Ginger > Gooseberry > Coffee > Pepper (0.25 ± 0.009). Gooseberry samples showed highest vitamin C content (12.73 ± 3.60) while least with Ginger samples (1.13 ± 0.12).

Sensory Evaluation Analysis

The sensory evaluation of the wine samples varied widely. The overall quality was very high for Gooseberry followed by Bilimbi and Java Apple. Sour taste over dominate in Coffee, Ginger and Pepper samples.

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Profit Analysis

The local market price for the White Pepper was INR 1350/Kg (US \$ 21.7) and Parchment Coffee INR 170/Kg (US \$ 2.7). The Local market price for raw Pepper was INR 640/Kg (US \$ 10.3) and Whole Coffee beans INR 70/Kg (US \$ 1.1). The fermentation of Coffee and Pepper doubles the value addition of the products and farmers may get benefited from these extra income sources. Calculating the sale from the wine also further increases the profit of the farmers.

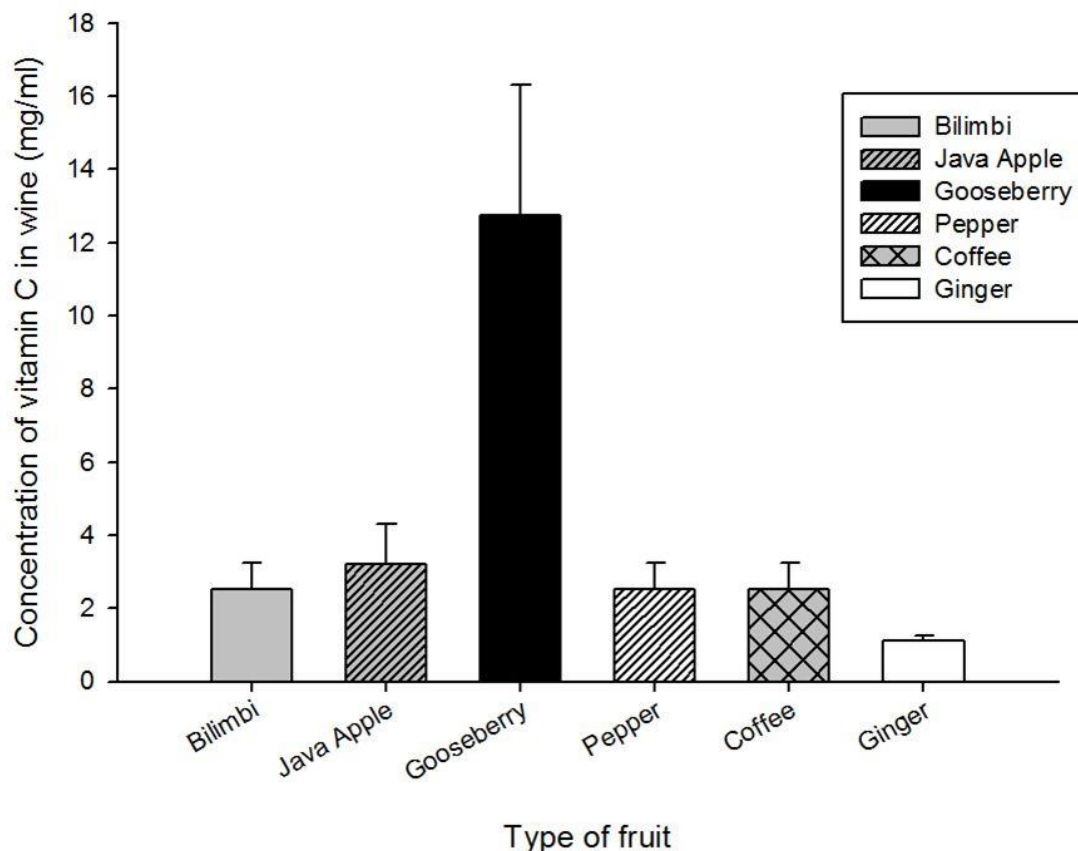


Figure 8: Concentration of Vitamin C (n=3, Mean \pm One Standard Deviation) in Various Wine Samples

Conclusion

The research work highlights the important fermentation capabilities of various neglected and underutilized fruits in Kerala. These underutilized fruit trees can be fully explored to the grass root level. However, elaborate research should be conducted to reveal the other nutritional parameters (protein, carbohydrates, fats, fibers, minerals and other vitamins) for the effective utilization and biodiversity preservation these species. Value addition also substantially reduces the cost of the famers and increase their profit.

ACKNOWLEDGEMENT

The authors are grateful for the cooperation of the management of Mar Augsthinose college for necessary support. Technical assistance from Binoy A Mulantra is also acknowledged. We also thank farmers for proving some samples used in the study.

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