PREPARATION OF JAMUN (*Syzigium cumini* L.) WINE WITH ANTIMICROBIAL PROPERTIES

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PHK 922

DIVISION OF HORTICULTURE
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PREPARATION OF JAMUN (*Syzigium cumini* L.) WINE WITH ANTIMICROBIAL PROPERTIES

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Thesis submitted to the

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For the award of the degree of

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In

PLANTATION, MEDICINAL, AROMATIC AND SPICE CROPS

BENGALURU **JULY, 2011**

AFFECTIONATELY DEDICATED
TO

My Parents, Brothers, Sister

&

my Family

DIVISION OF HORTICULTURE
UNIVERSITY OF AGRICULTURAL SCIENCES
GKVK, BENGALURU- 560 065.

CERTIFICATE

This is to certify that the thesis entitled **"Preparation of Jamun (*Syzigium cumini* L.) wine with antimicrobial properties"** submitted in partial fulfillment of the requirements for the award of the degree of **MASTER OF SCIENCE (HORTICULTURE)** in **PLANTATION, MEDICINAL, AROMATIC AND SPICE CROPS** of the University of Agricultural Sciences, Bengaluru, is a bonafide record of research work done by Mr. **MAHANTESH J.S., ID No. PHK 922** during the period of his study in the university under my guidance and supervision and that no part of this thesis has been submitted for the award of any degree, diploma, associateship, fellowship or other similar titles.

Bengaluru
July, 2011

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BENGALURU

JULY, 2011 **(*Mahantesh J. S.*)**

PREPARATION OF JAMUN (*Syzigium cumini* L.) WINE WITH ANTIMICROBIAL PROPERTIES

ABSTRACT

A study on the preparation of Jamun wine with antimicrobial properties was carried out at the Department of Horticulture, Gandhi Krishi Vignan Kendra, Bangalore during 2010-2011. The experiment was carried out with 5 treatments and 4 replications using Completely Randomised Design.

For the first time, whole fruits were used for the preparation of Jamun wine with repeated extractions. The wine was prepared from whole fruits in three batches using 24^{o}B sugar syrup and 0.025, 0.05, 0.075 and 0.1% Kutaja (*Holarrhena antidysentrica* bark) powder. The biochemical analysis of the prepared wine was carried out after three months of storage. The wine extracted from first batch was more acceptable and had optimum pH (3.19), Total Soluble Solids (8.53^{o}B), Anthocyanin (64 mg / 100 ml), Total Sugars (6.95%), Reducing sugars (5.84%), Alcohol content (8.04%), Titratable acidity (0.67%), Tannin content (126 mg/ 100 ml) and Phenolic content (234.5mg/ 100 ml), which was recorded in wine fortified with 0.075% Kutaja powder.

The results revealed that the wine developed from all the three batches were acceptable and wine extracted from first batch fortified with 0.075% Kutaja powder was the best. The antimicrobial property of Kutaja powder in wine was also studied. Microbial inhibition was not observed in any of the three microorganisms tested (*Escherichia coli, Staphylococcus aureus* and *Salmonella typhi*) suggesting a different mode of action of Kutaja

(Mahantesh, J.S.) (M Vaundhara)

Student Major Advisor

ನೇರಳೆ (ಸೈಜೀಜಿಯಂ ಕ್ಯುಮಿನಿ) ಹಣ್ಣಿನಿಂದ ಸೂಕ್ಷ್ಮಜೀವಿ ನಿರೋಧ ಸಾರವರ್ಧಿತ ವೈನ್ ತಯಾರಿಸುವ ವಿಧಾನ

ಸಾರಾಂಶ

ನೇರಳೆ ಹಣ್ಣಿನಿಂದ ಸೂಕ್ಷ್ಮಜೀವಿ ನಿರೋಧ ಸಾರವರ್ಧಿತ ವೈನ್ ತಯಾರಿಸುವ ವಿಧಾನ ಕುರಿತು ಪ್ರಯೋಗವನ್ನು ತೋಟಗಾರಿಕೆ ವಿಭಾಗ, ಕೃಷಿ ವಿಶ್ವವಿದ್ಯಾನಿಲಯ, ಗಾಂಧಿ ಕೃಷಿ ವಿಜ್ಞಾನ ಕೇಂದ್ರ, ಬೆಂಗಳೂರಿನಲ್ಲಿ 2010 – 2011ರವರೆಗೆ ಕೈಗೊಳ್ಳಲಾಯಿತು. ಈ ಪ್ರಯೋಗವನ್ನು 5 ಉಪಚಾರ ಮತ್ತು 4 ಪ್ರತಿಕೃತಿಗಳಲ್ಲಿ ಕಂಪ್ಲೀಟ್ಲಿ ರ‍್ಯಾಂಡಮೈಸ್ಡ್ ಡಿಸೈನ್ ಬಳಸಿಕೊಂಡು ಮಾಡಲಾಯಿತು.

ಈ ಪ್ರಯೋಗದಲ್ಲಿ ಮೊಟ್ಟಮೊದಲ ಬಾರಿಗೆ ಇಡೀ ನೇರಳೆ ಹಣ್ಣನ್ನು ಉಪಯೋಗಿಸಿ, ಪುನರಾವರ್ತಿತ ಹಿಂಡುವ ವಿಧಾನದಿಂದ, ಮೂರು ಒಬ್ಬೆಗಳಲ್ಲಿ ವೈನನ್ನು ತಯಾರಿಸಲಾಯಿತು. ವೈನ್ ತಯಾರಿಸಲು 24° ಬ್ರಿಕ್ಸ್ ಸಕ್ಕರೆ ಪಾಕ ಮತ್ತು 0.025, 0.05, 0.075 ಮತ್ತು 0.1% ಔಷಧೀಯ ತೊಗಟೆ ನೀಡುವ ಕೊಡಸಿಗೆ (ಹೊಲೆರಿನಾ ಆ್ಯಂಟಿಡಿಸೆಂಟ್ರಿಕಾ) ಪುಡಿಯನ್ನು ಬಳಸಲಾಯಿತು. ಹೀಗೆ ವಿವಿಧ ಒಬ್ಬೆಗಳ ವೈನನ್ನು ಮೂರು ತಿಂಗಳವರೆಗೆ ಶೇಖರಿಸಿ, ಜೀವರಸಾಯನಿಕ ವಿಶ್ಲೇಷಣೆಯನ್ನು ಮಾಡಲಾಯಿತು. ಮೂರು ಒಬ್ಬೆಗಳ ಪೈಕಿ ಮೊದಲನೆ ಒಬ್ಬೆಯು ಹೆಚ್ಚು ಸ್ವೀಕಾರಾರ್ಹವಾಗಿದ್ದು, ಅದರಲ್ಲಿ ಅತ್ಯುತ್ತಮ ರಸಸಾರ (3.19), ಒಟ್ಟು ಕರಗುವ ಘನ ವಸ್ತುಗಳು (8.53° ಬ್ರಿಕ್ಸ್), ಆಂಥೋಸೈನಿನ್ (64 ಮಿ. ಗ್ರಾಂ / 100 ಮಿ.ಲೀ), ಒಟ್ಟು ಸಕ್ಕರೆ ಅಂಶ (5.84%), ರೆಡ್ಯುಸಿಂಗ್ ಸಕ್ಕರೆ ಅಂಶ (6.59%), ಮದ್ಯದ ಅಂಶ (8.04%) ಟೈಟ್ರೇಟೇಬಲ್ ಆ್ಯಸಿಡಿಟಿ (0.75%), ಟ್ಯಾನಿನ್ ಅಂಶ (126 ಮಿ. ಗ್ರಾಂ/100 ಮಿ.ಲೀ) ಒಟ್ಟು ಕಾರ್ಬಾಲಿಕ್ ಆಮ್ಲದ ಅಂಶ (234.5 ಮಿಲಿ. ಗ್ರಾಂ / 100 ಮಿ.ಲೀ) ದಾಖಲಾಗಿದ್ದು, ಈ ವೈನನ್ನು 0.075% ಕೊಡಸಿಗೆ ತೊಗಟೆ ಪುಡಿಯೊಂದಿಗೆ ಸಾರವರ್ಧಿಸಿ ತೆಗೆಯಲಾಯಿತು.

ಕೊಡಸಿಗೆ ತೊಗಟೆಯ ಸೂಕ್ಷ್ಮಜೀವಿ ನಿರೋಧ ಸಾರವರ್ಧನೆಯ ಅಧ್ಯಯನ ಮಾಡಲಾಯಿತು. ಈ ಅಧ್ಯಯನದಲ್ಲಿ ಮೂರು ವಿವಿಧ ಸೂಕ್ಷ್ಮ ಜೀವಿಗಳನ್ನು (*ಇ.ಕೋಲೈ, ಸಾಲ್ಮೋನೆಲ್ಲ ಟೈಫಿ, ಸ್ಟೆಫೈಲೋಕೋಕಸ್ ಆರಿಯಸ್*) ಉಪಯೋಗಿಸಿ ಕೊಡಸಿಗೆ ಪುಡಿಯ ಸೂಕ್ಷ್ಮಜೀವಿ ನಿರೋಧಕ ಅಧ್ಯಯನ ಮಾಡಲಾಯಿತು. ಈ ಅಧ್ಯಯನದಲ್ಲಿ ಕೊಡಸಿಗೆಯ ಭಿನ್ನ ಸ್ವರೂಪದ ಕ್ರಿಯಾಕರ್ಮದಿಂದಾಗಿ ಯಾವುದೇ ರೀತಿಯ ಸೂಕ್ಷ್ಮ ಜೀವಿ ಅವರೋಧ ಕಂಡುಬಂದಿಲ್ಲ.

ಮೇಲ್ಕಂಡ ಫಲಿತಾಂಶದ ಪ್ರಕಾರ, ಕುಟಜ ಪುಡಿಯನ್ನು ಉಪಯೋಗಿಸಿ, ಮೂರೂ ಒಬ್ಬೆಗಳಲ್ಲಿ ತೆಗೆದ ವೈನ್ ಸ್ವೀಕಾರಾರ್ಹವಾಗಿದೆ. ಮೊದಲನೆ ಒಬ್ಬೆಯಲ್ಲಿ 0.075% ಕೊಡಸಿಗೆ ತೊಗಟೆ ಪುಡಿ ಬೆರೆಸಿ ತೆಗೆದ ವೈನ್ ಅತ್ಯುತ್ತಮ ಗುಣಮಟ್ಟದ್ದೆಂದು ಕಂಡು ಬಂದಿದೆ.

ವಿದ್ಯಾರ್ಥಿಯ ಸಹಿ	ಪ್ರಧಾನ ಮಾರ್ಗದರ್ಶಕರ ಸಹಿ
(ಮಹಾಂತೇಶ್. ಜೆ. ಎಸ್.)	(ಎಮ್. ವಸುಂಧರ)

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Introduction

I. INTRODUCTION

Wine is an alcoholic beverage resulting from fermentation by yeast with proper processing and addition. The natural chemical balance of grapes is such that they can ferment without the addition of sugar, acids, enzymes or other nutrients. Yeast utilizes the sugars found in the grapes and converts them into alcohol. Different varieties of grapes and strains of yeasts are used depending on the type of wine being produced (Appendix I). Although other fruits such as apples and berries can also be fermented, the resultant wines are normally named after the fruit from which they are produced (for example, Pomegranate, Jamun and Amla wine) and are generally known as fruit wine or country wine. Fermentation adds the advantage of acid formation and removal of fermentable sugars, which serve to prevent growth of pathogenic microorganisms and to stabilize the products. In addition, fermentation offers the potential for flavor enhancement in the products and makes them even tastier. Wines or fermented beverages not only provide calories but vitamin B complex as well, thus preventing beriberi and pellagra (nutritional deficiencies). They even contain small amounts of proteins and amino acids. Besides the use of herbs for fermented beverages may provide added medicinal or nutraceutical value (Steinkraus, 1983).

Jamun (*Syzgium cumini* L.) is an evergreen tropical tree belonging to family Myrtaceae, native to India and Indonesia. The berries are oblong, ovoid and shinning crimson black (rich in anthocyanin pigment, an anti-oxidant) when fully ripe. Jamun fruits are universally accepted to be very good for medicinal purposes especially for curing diabetes because of its effect on the pancreas (Joshi, 2001). The fruit and its juice and the seed contain a biochemical called ‘jamboline’ which is believed to check the pathological conversion of starch into sugar in case of increased production of glucose. Besides, the Jamun fruit is an effective food remedy for bleeding piles and correcting liver disorders (Joshi, 2001).

Also, Kutaja has several medicinal properties. It belongs to Apocynaceae family and is botanically known as *Holarrhena antidysenterica.* This plant is often confused with another plant of the same family called *Wrightia tinctoria,* which is medicinally inert. Orally Kutaja is effectively used in various maladies. It works well in the treatment of diarrhea and dysentery, associated with bleeding as well. Since centuries, it has been used as a household remedy for the same. The decoction of its skin is an effective medicament for diarrhea with bleeding. Kutaja gives excellent results in colitis and diarrhea. Kutaja is beneficial also in skin diseases, especially of oozing type. It can be used as an adjuvant in the treatment of obesity to get rid of excessive fats, as it scrapes them out. The herb is also useful in gout. The skin of the bark, grated in cow's milk works well in removal of painful urinary stones. Kutaja skin and seed powder is a popular household remedy for intestinal worm infestations in children. The response in *Entamoeba histolytica* cystpassers when treated with Kutaja bark was found to be excellent (Kavitha and Niranjali, 2009).

In traditional Indian medicine, fruits of Jamun are used. Also Jamun is available in plenty during the season (May-August) and marketed on roadside by push cart vendors in most unhygienic conditions. Therefore, it is envisaged to find out hygienic and scientific methods to process the fruits for making alcoholic beverages like wine, for long time storage and consumption. Also attempts can be made for repeated extraction of wine from the same batch of fruits. This helps not only to utilize the excess produce during the season but also ensures sustained development of sustained cottage scale industry in rural areas. Similarly Kutaja is another plant with antidysentric properties. Hence these two are combined to prepare acceptable drink like wine to be used as a potential remedy for dysentery and diarrhea.

Generally fruits are crushed and used for wine preparation. Jamun seeds, along with the pulp, also have medicinal properties (Joshi, 2001). Hence a novel method to utilize the whole fruits for the preparation of wine in batches was attempted to maximize the health benefits of the drink.

Keeping in view of the above facts and in order to explore the possibility of preparing Jamun wine from whole fruits with antimicrobial properties, the present study was undertaken with the following objectives.

(i) Preparation and developing protocol of *Syzigium cumini* wine

(ii) To standardize the concentration of Kutaja (*Holarrhena antidysentrica*) in Jamun wine

(iii) To test the efficacy of Jamun wine with respect to antimicrobial properties and also its sensory evaluation.

Review of Literature

II. REVIEW OF LITERATURE

Fruits are processed for many different reasons. These range from the removal of anti-nutritional factors and increasing the shelf life of the final product to value addition which increase both employment and income generating opportunities. Fermentation is one of the most ancient and most important food processing technologies. Fermentation requires very little sophisticated equipment, either to carry out the fermentation or for subsequent storage of the fermented product. It is a technique that has been employed for generations to preserve food for consumption at a later date and to improve food security. There are reports around the world stating the role of fermented foods in preservation of fruits to enhance food security.

Wine making is an ancient practice in many countries; considerable work has been done on various aspects of wine making from different fruits. The character and quality mainly depends on variety, composition of fruits from which it is made. A comprehensive survey of available literature on utilization of jamun and other related fruit crops for wine making and other processed products by fermentation are reviewed here under.

The aspects covered under the review include:

2.1 SUITABILITY OF JAMUN AND OTHER FRUITS FOR WINE MAKING

2.2 SUITABILITY OF MEDICINAL PLANTS FOR WINE MAKING

2.3 YEAST AND ITS RELEVANCE IN WINE MAKING

2.4 ANTIMICROBIAL PROPERTIES OF MEDICINAL PLANTS

2.5 BIOCHEMICAL TESTS

2.6 ORGANOLEPTIC CHARACTERISTICS OF WINE

2.1 SUITABILITY OF JAMUN AND OTHER FRUITS FOR WINE MAKING

Datta and Biswas (1942) have described processes for making vinegar from fruit juices. The jackfruit juice recovered from the ripe fruits yielded 7 per cent alcohol and 6 per cent acetic acid on fermentation.

Bardiya *et al.* (1974) have standardized the procedure for guava wine preparation. Diluted guava pulp wine had a higher alcohol (11.5 per cent) followed by guava wine (10.8 per cent).

Singh and Manjrekar (1976) reported the varietal differences in the physico-chemical characteristics of different apple cultivars. Lowest alcohol was obtained in 'Ambri', 'Kashmiri' and highest in 'Red Delicious' and 'Granny Smith' cider varieties. 'Golden Delicious' and 'Red Delicious' had the highest organoleptic scores and were considered suitable for cider production.

Singh and Manjrekar (1976) compared cider stored at room temperature and at 30^0 C. It was observed that there was a decrease in total soluble solids at 37^0C, pH rose at both temperatures, alcohol contents continued to increase but, tannins and reducing sugars decreased during storage at both the temperatures.

Kulkarni *et al.* (1980) analysed the chemical composition of 10 commercial table varieties of mango wine. Patel *et al. (*1984) developed the technology for the production of fenny from cashew apple fruits. The fenny prepared was contained 43 per cent alcohol.

Kundu *et al.* (1980) standardized banana wine preparation using both pectin esterase enzyme method and diluted pulp method. There were no significant differences in chemical compositions of wines made by these methods.

Suresh and Ethiraj (1987) studied the effect of maturity of 'Arka Kanchan', 'Thompson', 'Arka Shyam' and 'Arka Hans' could give good quality dry and sweet wines. They also reported that wine from 'Arkavati' had typical Muscat flavour, whereas 'Arka Shyam' had good colour and less of foxy flavour.

Berry and Kalra (1989) reported that jackfruit in general contains high amount of total soluble solids (TSS), proteinacious substances, vitamins and minerals thus making it a suitable medium for growth of wine yeasts.

Joshi *et al.* (1990) prepared the wild apricot wine by dilution of pulp in the ratio of 1: 2 for proper fermentation and reported the mineral composition of wine as satisfactory.

Shukla *et al.* (1991) prepared acceptable dry wines from the three cultivars *viz.*, Pharenda, Jamun and Kathajamun but the wine prepared from Jamun was adjudged best followed by Kathajamun. In sensory evaluation, the wines secured 70.3 and 83.0 per cent marks and all the three cultivars were found suitable for wine making.

Joshi *et al.* (1991) reported that vermouth of commercial acceptability could be prepared from plum. In the vermouth with increased levels of alcohol, total soluble solids, pH, aldehydes, esters, phenols, per cent sediment increased while titratable acidity and vitamin C declined. Herb/spices extract addition increased the total phenols, aldehydes and ester content of vermouth.

Teotia *et al.* (1991) fermented the muskmelon juice recovered from the fruits unfit for table purpose with *Saccharomyces cerevisiae* for 96 hours at 30^0 C. The fermented juice contained 6.5 per cent (w/v) alcohol and exhibited a very good sensory quality.

Bhajipale *et al.* (1998) reported that the over ripened fruits produced a tasty, cherry red colored wine with 8 per cent alcohol and 438 ml of wine yield/kg of fruits.

Jackson and Badrie (2003) reported that fermentation studies carried out to find out the effects of addition of fruit peel (0, 5, 10 and 15 per cent) to banana must on the physico-chemical, microbiological and sensory qualities of wines. A consumer test indicated that a wine from 15 per cent peel was most liked for flavor astringency than other wines.

2.2 SUITABILITY OF MEDICINAL PLANTS FOR WINE MAKING

Generally, fermentation of herbs gives the added advantage of medicinal value of the particular herb to wine. Use of herb for wine making is age old wisdom.

Sapna *et al.* (2002) carried out an investigation to develop herbal and spice beverages. The possibility of making spice wines more approximately called elixirs was examined. The nutritive value in terms of chemical components and mineral composition keeping quality of the product under ambient temperature were worked out

Deta *et al.* (2004) prepared Lime-Aonla spiced beverages from desi variety of Aonla and Kagzi Lime. The ready to serve beverage stored in white and amber coloured bottle for six months at ambient temperature, cool chamber, low temperature, showed a gradual increase in sensory evaluation, acidity, ascorbic acid and tannin contents. Retention of ascorbic acid was more in beverages stored in amber colored bottles under low pressure.

Grieve (1967) described some of the common herbal wines. Cowslip wine, made from its flower is an excellent sedative against nervous debility and paralysis. Rosemary wine if taken in small quantity acts as a quieting cordial to a weak heart subject to palpitation and relieves accompanying dropsy by stimulating the kidneys.

Kroes *et al. (*1993a) reported the immunological properties obtained by fermentation of a decoction of *Azadirachta indica* bark to which sugar and a mixture of plant preparations including flower extracts of *Woodfordia fructiosa* were added.

Kroes *et al.* (1993b) further reported the use of *Woodfordia fruticosa* with the fermented *Nimba arishta. Woodfordia fruticosa* has an effect of immunomodulatory activity and on alchol, gallic acid and sugar content of the arishta.

Anon (1981) reported the production of wines using *Opuntia ficus indica* with *Sacchromyces cervisiae* (Montrachet strain) and 10 mg/l initial sulphur dioxide were acceptable in panel tests.

Diguet (1928) reported the use of *Opuntia streptacantha* Lemm., *O.robusta* Weld., *O.leucotricha* De. And *O.orbiculata* Salm dysk. For preparation of wine which was sweet and alcoholic with red color.

Onkarayya (1985) made an attempt to prepare sweet and dry vermouth from mango. Different types of herb mixtures were used to prepare this in varied proportion. Sweet vermouth with higher herb mixture produced higher alchol content (18%)

2.3 YEAST AND ITS RELEVANCE IN WINE MAKING

Goyal and Spotts (1996) isolated yeast from the surface of pear and apple fruits. The yeast population from the surface of Golden Delicious apple fruits was approximately 8.0 $x10^3$ cfu/cm^2.

Martini *et al.* (1996) indicated that surface of mature grapes are the common habitat of *Kloecker apiculata*, which was isolated from different varieties of grapes. Some of them were round to oval shaped cells (pichia). Apparently members of the high ethanol tolerant species of *Saccharomyces cerevisiae* were unique of the isolates from fruit surface.

Charoenchai *et al.* (1998) studied fermentation temperature, pH and sugar concentration of juice affecting the growth and metabolic activity of the yeast, inhibitory effect of SO_2, CO_2 and ethanol concentration on the specific growth rates and viability of *Saccharomyces cerevisiae*.

Bajaj *et al.* (2001) isolated 22 strains of yeasts from sources like molasses, fruits, soil, honey and fermented foods. Isolates SBS12, and SBS17 and SBS 20 could not grow in more than 8 per cent ethanol, where as SBS13 and SBS 14 were able to grow in 10 per cent ethanol.

Martinez *et al.* (2001) reported that autolysis of yeast, releases different products in wine with special emphasis on proteolysis and production of nitrogen compounds, polysaccharides, nucleic acids, and lipids upon fermentation and degradation of volatile compounds.

Nahvi *et al.* (2002) isolated 50 strains of yeasts from several sources and identified. They reported that ethanol production from *Saccharomyces cerevisiae* from beet molasses was at 5.62 per cent which was then increased to 6.19 per cent by the addition of ammonium sulphate as nitrogen source.

Rai and Subba (2003) isolated yeasts from 10 different murcha plants and test-fermentation was carried out to assess their brewing value. The final brix, pH and alcohol content of the test wines ranged from 7.5-8.0 per cent, 4.5-5.0 per cent and 3.61-4.41 per cent (v/v) respectively, and at 5 per cent level the variance between each of the above parameters were found to be significant.

2.4 ANTIMICROBIAL PROPERTIES OF MEDICINAL PLANTS

The ethanolic extracts of Chinese medicinal plants like *Melaphis chinensis* revealed tannic acid, propyl gallate, gallic acid and ellagic acid as active constituents identified as gallotannins. Tannic acid and propylgallate were inhibitory as the ester linkage between gallic acid and glucose (to form tannic acid) or propanol (to form Propyl gallate) is important to the antimicrobial potential of these compounds (Chung *et al.*, 1993)

Tharib *et al.* (1983) suggested that several compounds isolated from *Artremesia campestris* is known to possess antimicrobial activity. These compounds were shown to possess microbial inhibition capacity's of 30 µg/ml against number of *bacillus* species at a concentration of 125 µg/ml. Six of the extracted compounds inhibited growth of *Staphylococcus aureus*. Three of the compounds inhibited *E.coli* and two inhibited growth of *Proteus vulgaris*.

Methanolic extracts of *Pelargonium* speices (Geraniaceae) had a strong antibacterial activity for more than 18 bacterial species but there was poor antifungal action. Flavonoids and tannins present in pelargoniums is the main factor for the antibacterial activity (Lis balchin and Deans, 1996)

Hiremath *et al.* (1993) suggested that the invitro evaluation of four successive solvent extracts of *Acalypha indica* showed marked antibacterial and antifungal activity against representative bacteria and fungi. The solvent extracts were petroleum ether, chloroform, ethanol, sulphamethaxole etc

The crude leaf extract of plant *Juniperus communis* (Linn.) showed antimicrobial activity where all gram +ve bacteria tested namely *Staphylococcus aureus, Micrococcus, Bacillus, Streptococcus, E.coli, Vibrio,* and *Klebsiella* were found to be sensitive to a concentration of 250 µg/ml of leaf extract (Chatterjee *et al.*, 1993)

2.5 BIOCHEMICAL TESTS

2.5.1 pH AND ITS RELEVANCE IN WINE

Suresh and Ethiraj (1987) reported that most of the grape cultivars had pH in between 3.70 to 4.11. This is the optimum pH for grape wine making.

Shukla *et al.* (1991) analysed the pH of the wine samples prepared from different varieties of Jambal fruit. The pH of these wines ranged from 3.50 to 3.40. Teotia *et al.* (1991) reported that fermented muskmelon juice had pH of 4.52.

Attri *et al.* (1994) opined that sand pear based wine had a pH of 3.99 where as in vermouth pH decreased to 3.95.

Zoecklein *et al.* (1999) studied the pH of white wine produced from four different yeast strains. Wine produced from strain FB had a higher pH (3.15), followed by PDM and YL1 9 (3.11).

Olasupo and Obayori (2003) prepared palm wine and monitored for changes in pH at 24 hrs intervals during 5 days of fermentation. The pH progressively decreased from 7.3 to 3.5 at the end of fermentation.

2.5.2 T.S.S (˚Brix) AND ITS RELEVANCE IN WINE

The ultimate quality of jamun wine mainly depends on the original total soluble solids of the fruits.

Berry and Kalra (1989) studied the chemical composition of jack fruit in respect of protein, fat, crude fibre, starch, reducing sugars, minerals and vitamins. The ripe fruits were reported to contain glucose, fructose and sucrose which impart sweetness to pulp. The total sugar was reported to be 14.5 per cent and reducing sugars 56.03 per cent.

Joshi *et al.* (1991) studied the physico-chemical characteristics of wild apricot wine and concluded that apricot wine had TSS of 6.80° Brix.

Vyas and Kochhar (1993) assessed the chemical composition of wines made from culled apple fruits. Wines produced from var. golden delicious had 9° Brix where as wine produced from red delicious had 8.9° Brix.

Singh *et al.* (1998) studied the optimum T.S.S of kinnow fruit juice for wine making. The T.S.S of the fruit varied from 20-26° Brix. The maximum ethanol was observed in juice with 24° Brix after five days of kinnow juice fermentation.

Ayoga (1999) determined the T.S.S content of pineapple juice for wine making. The T.S.S content of pineapple juice was between 12- 15° Brix.

Nandini and Dommen (2002) screened 20 different mango cultivars for wine making. The T.S.S content of these cultivars ranged from 14° Brix to 19.65° Brix.

Jackson and Badrie (2003) studied the effects of addition fruit peel to banana must on the physico-chemical, microbiological and sensory quality of wine. Wine with 15 per cent peel addition had significantly higher T.S.S (9.07° Brix) than other wines (7.02- 7.57° Brix).

Deta *et al.* (2004) observed increase in the total soluble solids, reducing sugars due to the conversion of complex sugars to simple sugars in fermentation during storage and non enzymatic browning in spiced beverage.

2.5.3 TOTAL TITRATABLE ACIDITY AND ITS RELEVANCE IN WINE

Kulkarni *et al.* (1980) analyzed wine samples prepared from different mango varieties. The titratable acidity of these wine samples ranged from 0.602 to 2.381 per cent.

Teotia *et al.* (1991) assessed the chemical composition of fermented muskmelon juice. The titratable acidity of fermented muskmelon juice was 0.28 per cent and after 6 months ageing it increased to 0.29 per cent.

Attri *et al.* (1994) estimated the titratable acidity of sand pear base wine and concluded that, it had 0.37 per cent titratable acidity expressed as per cent malic acid.

Zoecklein *et al.* (1999) studied the effect of four strains of *Saccharomyces cerevisiae* on white riesling wine composition. VL1 strain had a lower titratable acidity of 0.72 per cent whereas FB strain had high titratable acidity of 0.75 per cent.

Sapna *et al.* (2002) studied fermentation of different herbs and reported more titratable acidity in thyme wine (1.39) per cent followed by French basil (1.24) per cent and lower in Melissa wine (0.80) per cent after nine weeks of storage of wines. The increase in titratable acidity in fermentation could be due to the production of certain organic acids by yeast cells.

Olasupo and Obayori (2003) monitored palm wine for changes in acidity at 24 hr interval during five days of fermentation. The initial per cent acidity was 0.42 which increased to 0.83 on the fifth day.

2.5.4 RESIDUAL SUGAR AND ITS RELEVANCE IN WINE

Rice *et al.* (1968) estimated residual sugars in New York state wine by paper chromatography. The wine produced from fresh American varieties of grapes contained eleven sugars *viz.,* raffinose, lactose, maltose, sucrose, galactose, glucose, fructose, arabinose, xylose, ribose rhamnose.

'Pulqe' is a national drink of Mexico which was inherited from the Aztecs. It is an alcoholic beverage prepared by the fermentation of agave juice. The beverage had a total soluble solids of 25 to 30 (^{0} Brix), reducing sugar 200 to 500 mg/100 ml, total solids 2 to 3g/100 ml (Gonclaves De Limno, 1975).

Kundu *et al.* (1980) assessed the sugar content of grape wine prepared from different exotic varieties. The sugar content of these wines ranged from 0.16 per cent to 1.00 per cent.

Shukla and Revis (1985) determined the sugar content of wine prepared from different orange cultivars and ranged from 0.08 per cent 0.21 per cent.

Wilder (1992) studied the reducing sugar content of US table wines prepared from 3 different varieties, *viz.*, Seyval blank, Vidal blanc and Catawba. Maximum amount of reducing sugar was found in Catawba (12.1g/l) where as minimum was recorded in seyval blank (1.4g/l).

Bhajipale *et al.* (1998) studied the chemical composition of Karonda wine. The reducing content of wine ranged from 3.12 per cent to 3.15 per cent.

Sapna *et al.* (2002) observed reducing sugar content in French basil wine 1.13 per cent followed by Melissa wine 1.05 per cent after 9 weeks of storage.

Olasupo and Obayori (2003) prepared palm wine and monitored the process for changes in sugar content for 24 hr interval during the 5 days of fermentation. The sugar content decreased significantly from an initial value of 12 to 4 per cent on fifth day.

Tominac *et al.* (2005) studied two autochthonous *Saccharomyces cerevisiae* yeast strains (ZIM 1900 from cabernet sauvignon grapes and ZIM 1899) isolated from Istria and Croatia regions and were tested for their wine production properties. The fermentation profiles were monitored by measuring CO_2 evolution and CO_2 production rate. Reducing sugars, ethanol, total and volatile acids in wines produced by different yeast strains.

2.5.5 ALCOHOL AND ITS RELEVANCE IN WINE MAKING

Vidal-Carou *et al.* (1990) analyzed the alcohol content of Spanish white wine. The alcohol content of these wines ranged from 8.9 per cent to 14.7 percent.

Mora *et al.* (1990) screened three species, for the alcohol production by inoculating to the grape must. Among the three yeast species, *Saccharomyces cerevisiae* strain I produced 12.9 per cent alcohol followed by *Saccharomyces* strain II (12.8 per cent) and *kluyeromyces thermotolerans* (12.7 per cent).

Chikkasubbanna *et al.* (1990) reported that the alcohol percent of the grape wine increased due to a decrease in total soluble sugars due to the activity of yeast during fermentation. Simultaneous saccharification and fermentation of Jerusalem artichoke tubers were conducted batch wise at 30˚C using *Aspergillus niger* No.817 and *Saccharomyces cerevisiae* No.1200. Ethanol content obtained were 10.4 per cent v/v from the ground tubers after 15 hrs. 15.6 per cent from the juice concentrate after 72 hr and 20.1 per cent from the flour after 20 hr was recorded.

Joshi *et al.* (1991) estimated the alcohol content of plume vermouth. It contained 12.2 per cent alcohol (v/v).

Adusule *et al.* (1992) estimated the alcohol content in pomegranate wine and reported that upon incubation alcohol content increased and was observed to be 6.6 per cent.

Chaudhary and Chincholkar (1996) reported that ethanol is toxic to yeasts. The inhibition is negligible at 2 per cent (w/v) and is markedly increased at 11 per cent. They also showed that immobilized yeast strains could perform better in fermentation process.

Srivastava *et al.* (1997) compared ethanol production capacity of three yeast strains by inoculating the guava pulp. The maximum production of ethanol at optimum natural sugar concentration (10per cent) of guava pulp was 5.8 (w/v) by isolate 2, which was slightly more than the quantity of ethanol produced by *Saccharomyces cerevisiae* (5.0 per cent) and isolate-I (5.3 per cent).

Ayoga (1999) reported that the isolate *Saccharomyces cerevisiae* produced a high ethanol yield of 10.2 per cent (v/v) compared with a commercial wine yeast which yielded 7.4 per cent (v/v) ethanol.

Ethanol is not formed immediately after inoculation with *Saccharomyces cerevisiae*. Due to anaerobic conditions, a small fraction of the must sugars are transformed by glycerol pyruvic fermentation to glycerol pyruvate and a low concentration of a range of volatile compounds that make up the fermentation bouquet (Zoecklein *et al.*, 1999).

Nigam (2000) studied ethanol production by immobilized cells of *Saccharomyces cerevisiae* ATCC 24553 from pineapple canary waste. The maximum specific ethanol productivity was 1.2 g ethanol /g dry weight at a dilution rate of 1.5/hr. He also reported that, the volumetric ethanol productivity of immobilized cells was comparatively 11.5 times higher than free cells.

Sapna *et al.* (2002) obtained an alcohol content of 6.57 to 6.75 per cent in Japanese wine, coriander wine had 7.05 to 7.37 per cent in the thirds and after nine weeks of storage. The increase in alcohol content was due to the complete conversion of sugars to alcohol.

2.5.6 TANNIN AND ITS RELEVANCE IN WINE

Bhajipale *et al.* (1998) reported that the wines prepared from the karonda were having tannins of 0.27 per cent at mature green stage, 0.13 per cent at partial ripe stage, 0.11 per cent at ripe stage, 0.09 per cent at over ripe stage.

Reddy and Reddy, (2009) estimated the tannins in wine produced from different mango cultivars *viz.,* Alphonso (0.011%), Raspuri (0.072%), Banganpalli (0.012%), Totapuri (0.012%), Neelam (0.014%), Malgoa (0.065%), Suvarnarekha (0.025%), Rumani (0.027%), Jahangir (0.042%).

Somesh *et al.* (2009) estimated the total phenols (mg/l) in different Strawberry wine among different cultivars like Camarosa Chandler and Doughlas. Highest total phenols were found in wine made from Camarosa cultivar (150.2mg/l).

2.6 ORGANOLEPTIC CHARACTERISTICS OF WINE

Bardiya *et al.* (1974) reported that wines prepared from guava juice were found to be highly acceptable due to low tannin content, colour and flavour.

Organoleptically the wine of variety fazli was scored highest rank of 14.2 followed by Langra, Chausa and Mallika with 12.0, 11.8 and 10.4 respectively out of 20 points (Kulkarni *et al.*, 1980).

Vyas and Kochhar (1993) evaluated the apple varieties of Himachal Pradesh for cider and wine making and reported that, the variety golden delicious was found to be suitable for the production of acceptable quality wines.

Lema *et al.* (1996) compared the performance of *Saccharomyces* populations with non *Saccharomyces* population isolated from must for the production of some components of albarino wine aroma. Predominance of *Saccharomyces cerevisiae* from the first day of fermentation in the musts, favoured the production of some volatile compounds traditionally related to good quality of wine aroma.

The organoleptic evaluation revealed that French basil wine had a highest score for appearance (1.33 out of 2.00), body (0.93 out of 2.00), flavour (1.67 out of 2.00) and general quality (14.00 out of 20). Japanese mint had highest score for color (1.67 out of 2.00), vinegar (1.33 out of 2.00), total acidity (1.47 out of 2.00) and general quality 14.00 out of

20.00). Rosemary wine had highest score for sweetness (1.47 out of 2.00) and body (0.93 out of 2.00) (Sapna *et al.,* 2002).

Nandini and Dommen (2002) reported that varieties 'Neelam', 'Baneshan', 'Suvarnarekha' and 'kalapaddy' were most acceptable ones with a score of 4.0 followed by the varieties 'mulgoa' and 'Chausa' with a score of 3.5 each by organoleptic evaluation the lowest mean score (3.0) was observed for the variety 'panchasara varikka'.

Patel and Shibamoto (2003) identified 53 volatile compounds in wines using gas chromatography and mass spectrometry; the major volatiles found were seven alcohols, seven esters, and four acids. The different *Saccharomyces cecrevisiae* strains were attributed to the characteristics flavours in symphony wines.

Pasti (2004) reported that phenols were responsible for some organoleptic properties of wine, including colour and astringency. The red wine produced from these grapes had unsatisfactory color and flavouring properties, because of the phenols that determined the colour, bitterness astringency "mouth feel" of red wines.

Hidalgo *et al.* (2004) studied the sensory and analytical characteristics of five rose sparkling wines manufactured by the traditional method. Moreover, the changes that were taking place in the volatile fraction of the wines during fermentation and aging of the wine were not clear.

Fia *et al.* (2005) reported that beta-glucosidase activity was involved in the hydrolysis of several important compounds for the development of flavour during fermentation. Of the three *Saccharomyces cerevisiae* strains, one *Hanseniaspora valbyensis* strain and one *Brettanomyces anomalous* strain showed beta-glucosidase activity.

Material and Methods

III. MATERIAL AND METHODS

The present investigation on fermentation of Jamun fruits was conducted at the Department of Plantation Medicinal Aromatic and Spices, Division of Horticulture, University of Agricultural Sciences, Bangalore, during the year 2010-11.

3.1 MATERIALS USED

3.1.1 FRUITS

Jamun (*Syzigium cumini* L.) fruits of good quality of Rajamun variety were obtained from the Sanjeevini Vatika, GKVK in the month of July for the preparation of wine.

3.1.2 HOLARRHENA (KUTAJA)

Kutaja (*Holarrhena antidysentrica*) bark was also obtained from Sanjeevini Vatika, GKVK in the month of July. It was dried in hot air oven for 72 hours until the sample became dry. Samples were then cut into small bits and later powdered (Plate 1).

3.1.3 BOTTLES

The bottles used for the experiment were procured from local market, Bangalore and they were washed twice using mild detergents and rinsed with water, then they were autoclaved at 121°C for twenty minutes and used for storing Jamun wine.

3.1.4 CONICAL FLASKS

Fifteen conical flasks of two litre capacity were used for the preparation of wine and they were used only after sterilization.

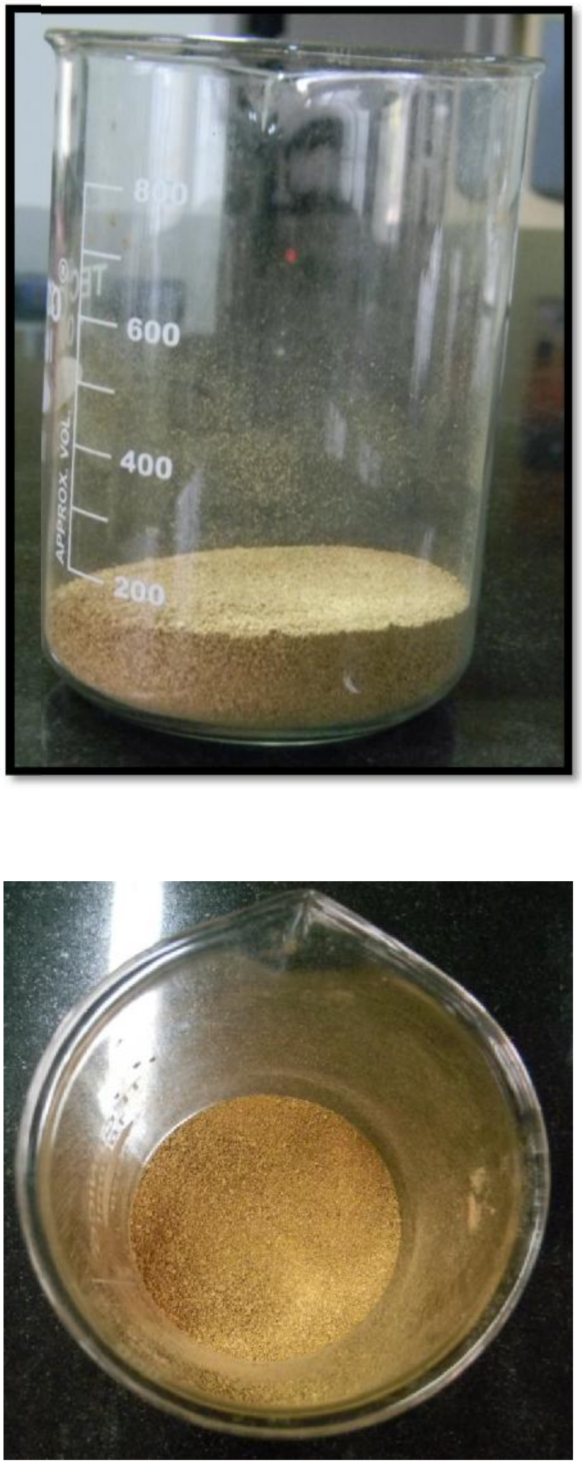


Plate 1 : Kutaja powder

3.1.5 STERILIZATION

The conical flasks, bottles and carbon dioxide discharge tubes were autoclaved at 121°C for twenty minutes and then they were used for the experiment (Figure 1).

3.1.6 CHEMICALS USED

All the chemicals used in this investigation were of analytical grade. They were obtained from Industrial Laboratories and Equipment Ltd., Bangalore.

3.1.7 YEAST

A pure culture of *Saccharomyces cerevisiae* var. *ellipsoideus* (USD 552) was procured from the Indian Institute of Science, Bangalore. It was kept refrigerated at 0 to 5^{0}C until its further use for inoculum preparation.

3.1.8 MICROBIAL CULTURE

Mother cultures of *Escherichia coli, Staphylococcus aureus* and *Salmonella typhi* were obtained from the Department of Microbiology, University of Agricultural Sciences, Bangalore. They were later sub-cultured and stored at refrigerated temperature until their further use for broth preparation.

3.2 EXPERIMENTAL DETAILS

Design: Completely Randomized Design

Number of replication: 4

Number of treatments: 5

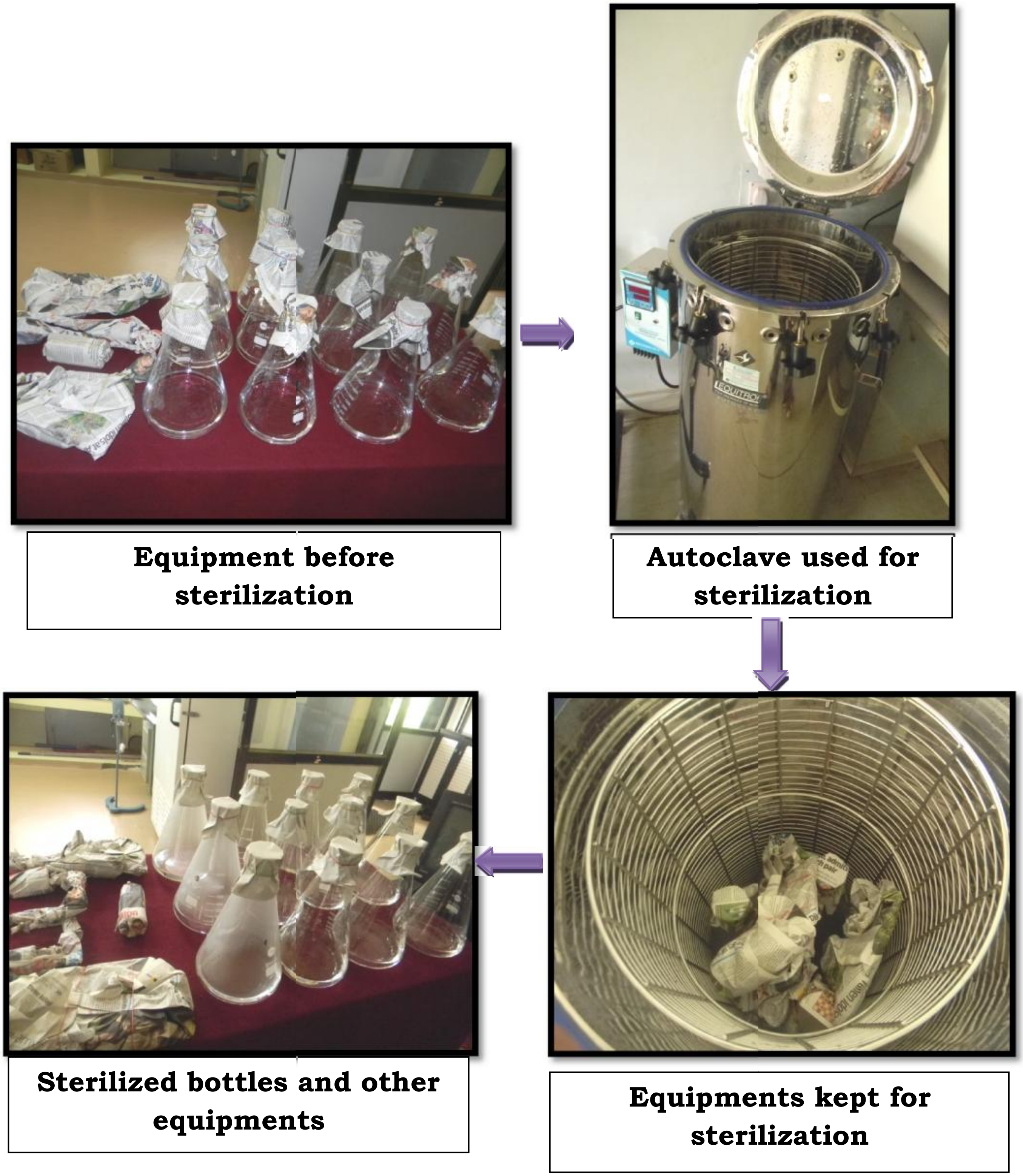


Fig. 1. Sterilization of equipments required for wine preparation

3.2.1 TREATMENT DETAILS

Treatment 1 : Control(100% Jamun wine)

Treatment 2 : Jamun wine with 0.025% Kutaja powder

Treatment 3 : Jamun wine with 0.05% Kutaja powder

Treatment 4 : Jamun wine with 0.075% Kutaja powder

Treatment 5 : Jamun wine with 0.1% Kutaja powder

3.3 METHODS

3.3.1 INOCULUM PREPARATION

200g of seedless grapes was taken. The stalk was removed and washed thoroughly with running water to remove the pesticide residue. Cleaned grapes were put in blender and thoroughly blended. 200ml of water was added to the blender. It was boiled for five minutes after transferring it into a conical flask. Then 200ml of juice was transferred into 500ml conical flask by using clean funnel. The juice was transferred when it was still boiling and the cap was closed. It was kept for cooling at room temperature. Then 100g of wine yeast powder was added and gently stirred after closing the cap. It started fermenting in 24 hours. It was allowed to ferment for three days. It was stirred daily and the gas was released.

3.3.2 PREPARATION OF JAMUN FRUITS

One kilogram of matured, disease free Jamun fruits were taken. They were washed with two grams of Pottasium metabisulphite dissolved in one litre of water and put in a basket to drain excess water. They were again washed with running water and the water was drained completely (Figure 2).

Figure 2 : Preparation of fruits for wine making

3.3.3 PREPARATION OF SUGAR SYRUP

24°Brix sugar syrup was prepared by dissolving 320 grams of sugar in one litre of water (Figure 3).

3.3.4.1 PREPARATION OF JAMUN WINE FOR FIRST BATCH.

The sugar syrup prepared was boiled for five minutes and the boiling syrup was poured over Jamun fruits in such a way that it covered the Jamun fruits completely. It was closed and allowed to cool. The desired concentration of the Kutaja powder was added according to the treatment details. After it came to room temperature, 5 ml of inoculum was added per kilogram of fruit. It was closed and watched for fermentation within 24 hours. After 5 days the fermenting liquid was poured into two liter bottles (only the Jamun fruits remained in the conical flask). After 5th day it was allowed to ferment under airtight condition preferably in absence of light. It took about 10 days. Gas was released every 2-3 days (Figure 3 and plate 2).

3.3.4.2 PREPARATION OF JAMUN WINE FOR SECOND BATCH.

Fruits from batch one were reused for the second batch. The methodology was repeated as per the first batch preparation. After 5 days the fermenting liquid of second batch was poured into two liter bottles (only the Jamun fruits remained in the conical flask). After 5th day it was allowed to ferment under airtight condition preferably in absence of light. It took about 10 days. Gas was released every 2-3 days.

3.3.4.3 PREPARATION OF JAMUN WINE FOR THIRD BATCH.

Fruits from batch two were again reused for the third batch. The methodology of preparation was repeated for the third batch also. After 5 days the fermenting liquid of third batch was poured into two liter bottles (only the Jamun fruits remained in the conical flask). After 5th day it was

Preparation of sugar syrup

Pouring the measured hot sugar syrup on Jamun fruits in the sterilized conical flask

Addition of inoculum after cooling in sterilized condition

Filled conical flasks kept for cooling

Figure 3 : Preparation of wine for primary fermentation

allowed to ferment under airtight condition preferably in absence of light. It took about 10 days. Gas was released every 2-3 days.

3.3.5 BIOCHEMICAL PARAMETERS

The prepared wine was analyzed for several of the parameters like pH, T.S.S., acidity, reducing, non-reducing, total sugars, alcohol, tannins, anthocyanin and phenols after 90 days of storage.

3.3.5.1 pH

The pH of the Jamun wine was measured by using digital pH meter. The temperature was kept constant while taking observations for all the samples.

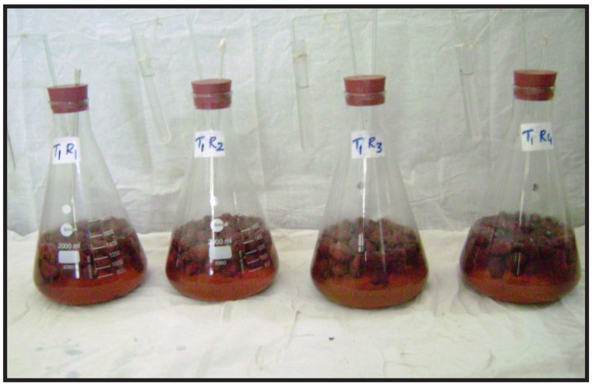
3.3.5.2 TOTAL SOLUBLE SOLIDS (TSS)

The content of total soluble solids (T.S.S.) in the wine was determined with the help of digital hand refractometer and expressed as Degree Brix (°B) and readings are corrected and adjusted at 22^{0}C. Care was taken that the prism of the refractometer was washed with distilled water and wiped dry before every reading.

3.3.5.3 TITRATABLE ACIDITY

Acidity of the wine was determined by titration with 0.1 N sodium hydroxide (NaOH) (Ranganna, 1977). 5 ml of wine was taken in a 50 ml conical flask. One drop of phenolphthalein indicator was added. The solution was homogenized and titrated against 0.1 N NaOH taken in a burette. The titre value was noted down. The percentage of acidity was expressed in terms of milligrams (mg) of anhydrous citric acid present per 100 ml of sample.

$$\text{Total Acid (\%)} = \frac{1 \text{ X Eq. Wt. of acid X Normality of NaOH X Titer X } 100}{\text{Weight of Sample}}$$



Control(100% jamun wine)

Jamun wine with 0.025% kutaja extract

Jamun wine with 0.05% kutaja extract

Jamun wine with 0.075% kutaja extract

Jamun wine with 0.1% kutaja extract

Plate 2. Jamun wine with different concentration of Kutaja

3.3.5.4 ALCOHOL

Alcohol estimation were made as per the procedure cited in Hand book of analysis and quality control (Ranganna, 1977)

REAGENTS:

a. Potassium dichromate solution: 32.5g of Potassium dichromate was taken in 350ml of water. It was dissolved properly and to this 350ml of sulphuric acid (H_2SO_4) was added slowly. When the solution got cooled, it was made up to 1000ml.

PREPARATION OF STANDARD CURVE:

Standard ethanol solution in water was prepared ranging from 1 to 10%. 5ml of standard ethanol solution of 1% was taken in 45ml of distilled water. From this 10ml of diluted sample was taken and 30ml of distilled water was added in distillation flask. 25ml of potassium dichromate solution was taken in 50ml conical flask and kept near a receiving funnel to collect about 20ml of the distillate in a volumetric flask containing 25ml of chromic acid so that the total volume was 40-45ml. Later it was incubated at 60^0C for 20 minutes and cooled to room temperature. Volume was made up to 50ml and the intensity of colour was measured on spectrophoto-meter at 600nm. The same procedure was repeated using 2%, 3%, 4% up to 10% of standard ethyl alcohol and the standard curve was plotted by plotting optical density against the concentration of alcohol.

ALCOHOL IN SAMPLE

One milliliter of wine sample was taken in a beaker along with 10ml of distilled water. 30 milliliter of distilled water was added and kept in a distillation flask. 25ml of potassium dichromate solution was taken in 50ml conical flask and kept near a receiving funnel to collect about

20ml of the distillate in a volumetric flask containing 25ml of chromic acid so that the total volume was 40-45ml. Later it was incubated at 60^0C for 20 minutes and cooled to room temperature. Volume was made up to 50ml and the intensity of colour was measured on spectrophotometer at 600nm. The alcohol content was found out by comparing with standard curve.

3.3.5.5 ANTHOCYANIN

Anthocyanin estimation was made as per the procedure cited in Hand book of analysis and quality control (Ranganna, 1977)

REAGENTS

0.1 N Hydro-chloric acid(HCl)

PROCEDURE:

Five milliliter of wine sample was taken in 100ml conical flask and 50ml of 0.1N HCl was added. It was shaken well for 10 minutes in mechanical shaker and kept in dark place for one hour. The absorbance was measured at 480nm against blank. A standard curve was plotted on graph showing absorbance against standard.

3.3.5.6 TANNINS

Tannin estimation was made as per the procedure cited in Hand book of analysis and quality control (Ranganna, 1977)

REAGENTS:

a. **Folin denins reagent:** 50gm of sodium tungstate, 10g phosphomolybidic acid and 25 ml of phosphoric acid was added to 750ml of water. It was then cooled and diluted to 1000ml with water.

b. Saturated sodium carbonate: 25gm anhydrous sodium carbonate was added to 100ml water and heated to 20-30^{0}C to dissolve properly. It was then cooled overnight and filtered after a few crystals of anhydrous sodium carbonate were observed.

C. Standard tannic acid: 100mg tannic acid was dissolved in one liter of water.

PREPARATION OF STANDARD CURVE:

0, 1, 2, 3, 4upto 10ml standard tannic acid solution was pipetted out into 100ml volumetric flask containing 75 ml water. 5ml of Folin-dennis reagent and 10ml sodium carbonate solution was added to this. The volume was made up to 100ml. it was shaken well and optical density (absorbance) was recorded at 760nm wavelength. A standard curve was plotted on graph showing absorbance against tannic acid (mg per 100ml).

PROCEDURE:

The absorbance in each case was recorded using 1ml filtered sample and the tannic acid present in the sample was found out by comparing the value with standard curve. If the value of absorbance was too high, proper dilution was used.

3.3.5.7 PHENOLS

Phenol estimation was made as per the procedure cited in Hand book of analysis and quality control (Ranganna, 1977)

REAGENTS:

a. Folin ciocalteau reagent: 50gm of sodium tungstate, 10g phosphomolybidic acid and 25 ml of phosphoric acid was added to 750ml of water. It was then cooled and diluted to 1000ml with water.

b. **Sodium carbonate (20%):** 25gm anhydrous sodium carbonate was added to 100ml water and heated to 20-30^{0}C to dissolve properly. It was then cooled overnight and filtered after a few crystals of anhydrous sodium carbonate were observed.

c. **Standard catechol reagent:** 100mg catechol was dissolved in 100 ml water.

PREPARATION OF STANDARD CURVE:

0.2, 0.4, 0.6, 0.8, 1.0 up to 2 milliliter standard catechol reagent solution was pipetted out in a test tube and the volume was made up to 3 ml with distilled water. 2 ml Folin-catechol reagent was added and after three minutes 2 ml of sodium carbonate solution was added to the test tube. It was mixed thoroughly and the test tube was placed in boiling water bath for one minute. It was cooled and the absorbance was measured at 650nm against reagent blank. A standard curve was plotted on graph showing absorbance against catechol reagent (mg of phenols per ml of sample).

PROCEDURE:

The absorbance in each case was recorded using 1ml filtered sample and the phenols present in the sample were found out by comparing the value with standard curve. If the value of absorbance was too high, proper dilution was used.

3.3.5.8 SUGAR CONTENT OF THE PREPARED PRODUCTS.

3.3.5.8.1 REDUCING SUGARS

Lane and Eyon method was followed as suggested by Ranganna, 1977. Five (ml) of the wine was taken in 250 ml volumetric flask containing 100 ml of distilled water. One drop of phenolphthalein indicator was added and neutralized with 1 N NaOH. Two ml of lead acetate (45%) was added to it and kept undisturbed for 20 minutes, followed by addition of two ml of potassium oxalate (22%). The solution was filtered using Whatman filter paper. The volume was made up to 250 ml and the filtrate was titrated against 10 ml of Fehling mixture. It was repeatedly tireated till brick red colour was obtained. The titer value was recorded and used for calculations of reducing sugars (%).

$$\text{Reducing sugars (\%)} = \frac{\text{Factor (0.052) X Dilution X 100}}{\text{Titer X Weight of Sample}}$$

3.3.5.8.2 TOTAL SUGARS

Five ml of filtrate was hydrolyzed by mixing with 10g of citric acid and kept overnight. Then, it was neutralized with sodium hydroxide using phenolphthalein as indicator. The volume was made up to 250 ml and the filtrate was titrated against 10 ml of Fehling mixture. The titer value was used for calculations of total sugars (%).

$$\text{Total sugars (\%)} = \frac{\text{Factor (0.052) X Dilution X 100}}{\text{Titer X Weight of Sample}}$$

3.3.5.8.3 NON- REDUCING SUGARS

The content of non- reducing sugars was calculated using the following formula.

Non-reducing sugars (%) = Total sugars (%) – Reducing sugars (%)

3.3 ORGANOLEPTIC EVALUATION OF JAMUN WINE

Jamun wine was tested for sensory attributes such as body**,** flavor, astringency appearance, general quality and total score by a panel of 10 judges by following numerical scoring method (Amerine *et al.,* 1972). The chart used for evaluating the products where samples were ranked for quality parameters from higher to lower, 5 to1 (Appendix-II) in descending order of acceptability is given (Appendix-III)

3.4 STATISTICAL ANALYSIS

CRD (Complete Randomized Design) was used for conducting the experiment and results were analyzed as per the guide lines suggested by Panse and Sukhatme, (1978)

3.5 MICROBIAL ANALYSIS

3.5.1 INHIBITION ZONE TECHNIQUE

This method is also known as filter paper disc agar diffusion method. Whatman No 1 filter papers were cut into discs of 6 mm diameter. Nutrient agar/Potato dextrose agar medium was sterilized at 15 pounds pressure and 121°C for 20 minutes. Borosil petriplates of nine centimeter diameter were also sterilized. 20 ml of molten agar medium was poured into each petriplates under aseptic condition inside the laminar airflow chamber, it was allowed for solidifaction.

Bacterial cultures in respective broth were used as inoculums. 0.5 ml of inoculums was pipetted out in sterilized micropipette. This was poured on to the well set media. On this the inoculums was spread using a sterilized glass spreader

Filter paper discs were arranged on the aluminum foil. These were moistened by 1000µl wine of different treatment (with varying concentrations of *Holarrhena*) taken in a micropippete. Three filter paper discs were used as replicate for one treatment. These were placed on the seeded agar in petriplates at equidistance in three locations with the help of a sterilized forceps. Similarly filter paper discs moistened with wine of different treatments were placed on the seeded agar medium in different petriplates. Each petriplate was then sealed using klin wrap and placed in inverted position. Inoculated petriplates were incubated at room temperature for 48 hours.

3.5.2 OBSERVATION

The diameter (mm) of inhibition zone produced due to the antibacterial activity of wine around each filter paper disc was measured in four directions. The average of these four readings was taken after subtracting the values with the diameter of filter paper disc. Similarly, inhibition zone in control was also recorded.

3.6 BENEFIT COST RATIO OF THE BEST ACCEPTED WINE

Cost economics of the prepared wine was studied for the best treatment (APPENDIX III). The net income was calculated by the following formula:

Net income = Total income – total cost

Since benefit cost ratio is an effective indicator of the commercial feasibility of the preparation of these products, it was also calculated using formula:

Benefit cost ratio = Total income: Total cost

Experimental Results

IV. EXPERIMENTAL RESULTS

The experiment results on the study of "Preparation of Jamun wine with antimicrobial properties" are presented in this chapter.

4.1 pH

Data on pH of wine as influenced by different treatments in three batches after 90 days of storage are presented in Table 1 and Figure 4.

The difference in pH was found non-significant between the treatments. However an increasing trend in pH in all the treatments was observed from first batch to third batch.

4.2 TOTAL SOLUBLE SOLIDS

Data on TSS of wine as influenced by different treatments in three batches after 90 days of storage are presented in Table 2 and Figure 5.

There was a significant difference in total soluble solid content of Jamun wine between the treatments. In first batch, the highest TSS was recorded in control (8.79˚B), whereas the lowest content was observed in Jamun wine with 0.1% Kutaja powder (8.51˚B).

The highest TSS content in second batch was observed in control (10.65˚B) while the lowest was observed in Jamun wine with 0.1% Kutaja powder (10.49˚B) which differed significantly.

Control had highest TSS content (12.57˚B) in third batch which was on par with Jamun wine with 0.025% Kutaja powder (12.50˚B) and the lowest was observed in Jamun wine with 0.1% Kutaja powder (12.41˚B), which differed significantly.

Table 1. Effect of different treatments on pH content of Jamun wine at 90 days of storage

Treatments	pH		
	BATCH-1	BATCH-2	BATCH-3
Control (100% Jamun wine)	3.23	3.45	3.70
Jamun wine + 0.025% Kutaja powder	3.22	3.43	3.69
Jamun wine + 0.05% Kutaja powder	3.20	3.42	3.68
Jamun wine + 0.075% Kutaja powder	3.19	3.41	3.66
Jamun wine + 0.1% Kutaja powder	3.18	3.40	3.65
F-test	NS	NS	NS

NS- Non Significant

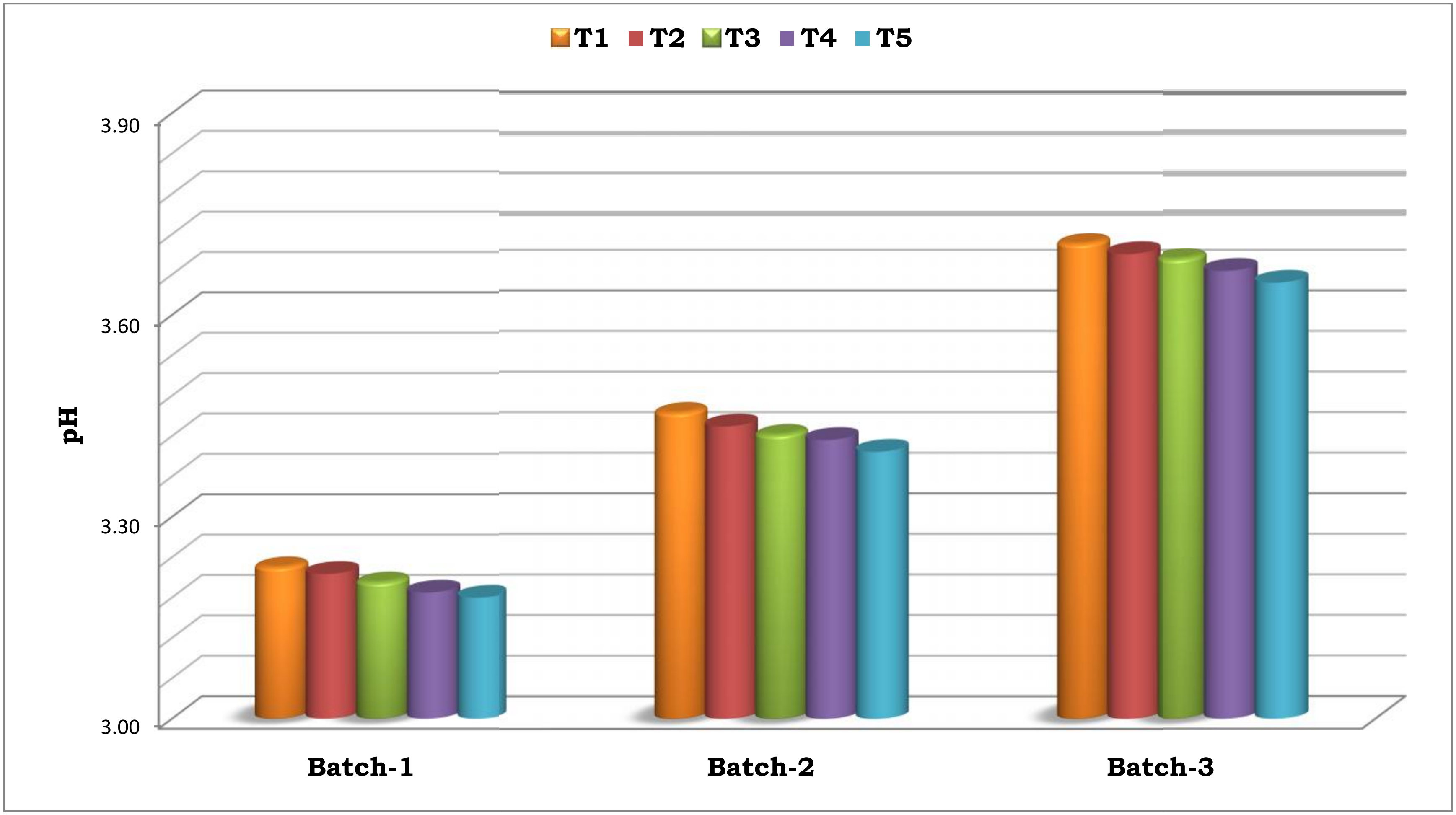


Figure 4. Effect of different treatments on pH content of Jamun wine at 90 days of storage

T1 : Control(100% jamun wine)
T2 : Jamun wine with 0.025% Kutaja powder
T3 : Jamun wine with 0.05% Kutaja powder
T4 : Jamun wine with 0.075% Kutaja powder
T5 : Jamun wine with 0.1% Kutaja powder

Table 2. Effect of different treatments on Total Soluble Solids (TSS) content of Jamun wine at 90 days of storage

Treatments	TSS(^{0}B)		
	BATCH-1	BATCH-2	BATCH-3
Control (100% Jamun wine)	8.79	10.65	12.57
Jamun wine + 0.025% Kutaja powder	8.67	10.60	12.50
Jamun wine + 0.05% Kutaja powder	8.59	10.57	12.47
Jamun wine + 0.075% Kutaja powder	8.53	10.52	12.44
Jamun wine + 0.1% Kutaja powder	8.51	10.49	12.41
F-test	*	*	*
S.Em±	0.02	0.01	0.02
CD at 1%	0.07	0.03	0.07

*- Significant at 1%

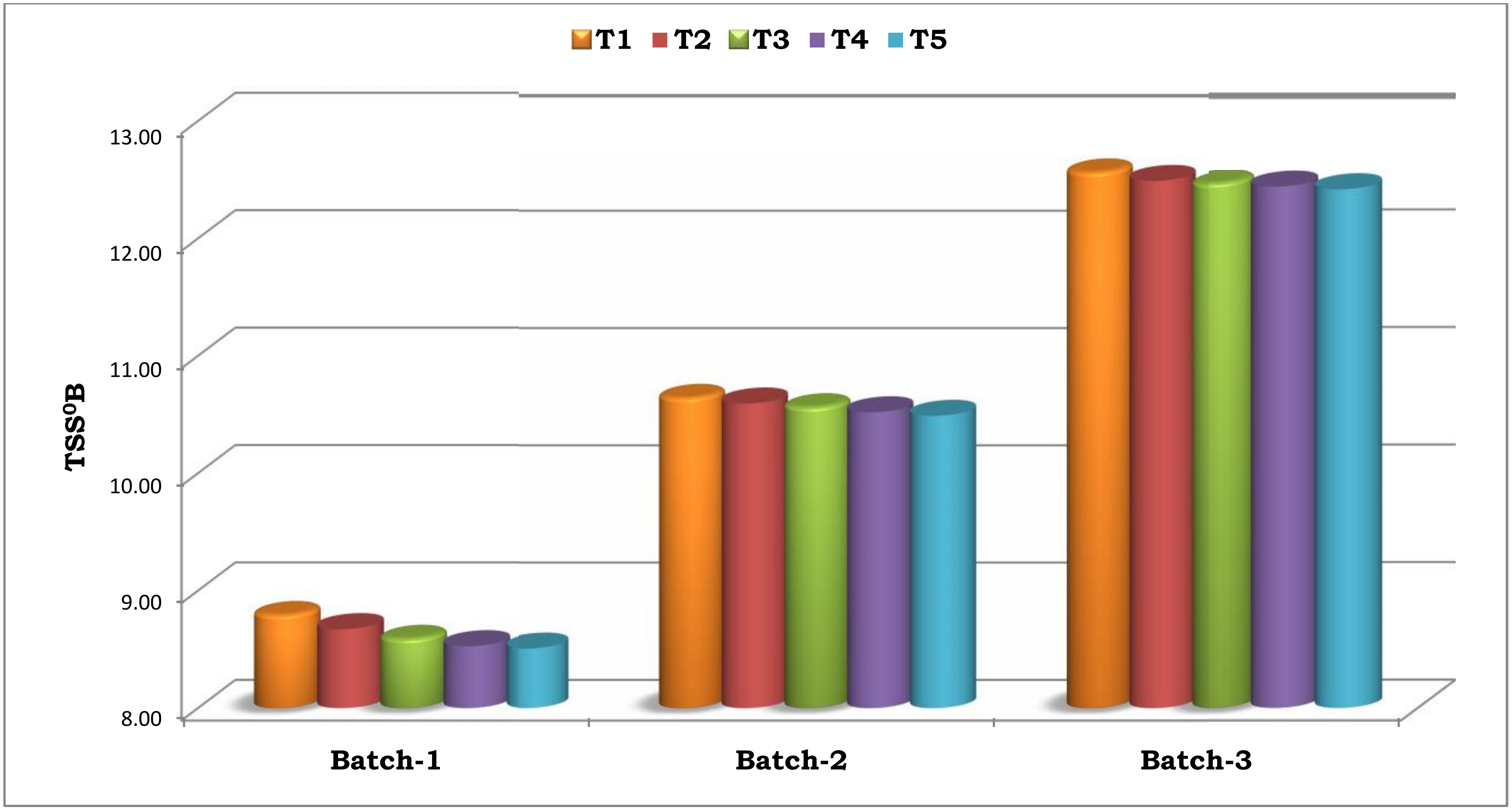


Figure 5. Effect of different treatments on Total Soluble Solids (TSS⁰B) content of Jamun wine at 90 days of storage

T1 : Control(100% jamun wine)
T2 : Jamun wine with 0.025% Kutaja powder
T3 : Jamun wine with 0.05% Kutaja powder
T4 : Jamun wine with 0.075% Kutaja powder
T5 : Jamun wine with 0.1% Kutaja powder

A decreasing trend was observed in TSS content in between the treatments. However TSS content had an increasing trend from first batch to third batch.

4.3 TITRATABLE ACIDITY

Data on acidity of wine as influenced by different treatments in three batches after 90 days of storage are presented in Table 3 and Figure 6.

The titratable acidity differed significantly between the treatments.

Highest was recorded in Jamun wine with 0.1% Kutaja (0.7%) which was on par with Jamun wine with 0.075% Kutaja powder (0.67%) and Jamun wine with 0.05% Kutaja powder (0.67%), whereas the lowest was recorded in control (0.61%) in the first batch.

In second batch it was highest in Jamun wine with 0.1% Kutaja powder (0.51%) which was on par with Jamun wine with 0.075% Kutaja powder (0.49%) and Jamun wine with 0.05% Kutaja powder (0.48%), while the lowest was recorded in control (0.44%).

Similarly in third batch, highest was in Jamun wine with 0.1% Kutaja powder (0.40%) which was on par with Jamun wine with 0.075% Kutaja powder (0.39%) and Jamun wine with 0.05% Kutaja powder (0.37%), while the least was recorded in control (0.34%).

The trend was similar in all the three batches between the treatments, however a decreasing trend was observed in titratable acidity between the batches from first batch to the third batch.

Table 3. Effect of different treatments on total titratable acidity of Jamun wine at 90 days of storage

Treatments	Total titratable acidity (%)		
	BATCH-1	BATCH-2	BATCH-3
Control (100% Jamun wine)	0.61	0.44	0.34
Jamun wine + 0.025% Kutaja powder	0.66	0.46	0.36
Jamun wine + 0.05% Kutaja powder	0.67	0.48	0.37
Jamun wine + 0.075% Kutaja powder	0.67	0.49	0.39
Jamun wine + 0.1% Kutaja powder	0.70	0.51	0.40
F-test	*	*	*
S.Em±	0.01	0.01	0.01
CD at 1%	0.03	0.03	0.03

*- Significant at 1%

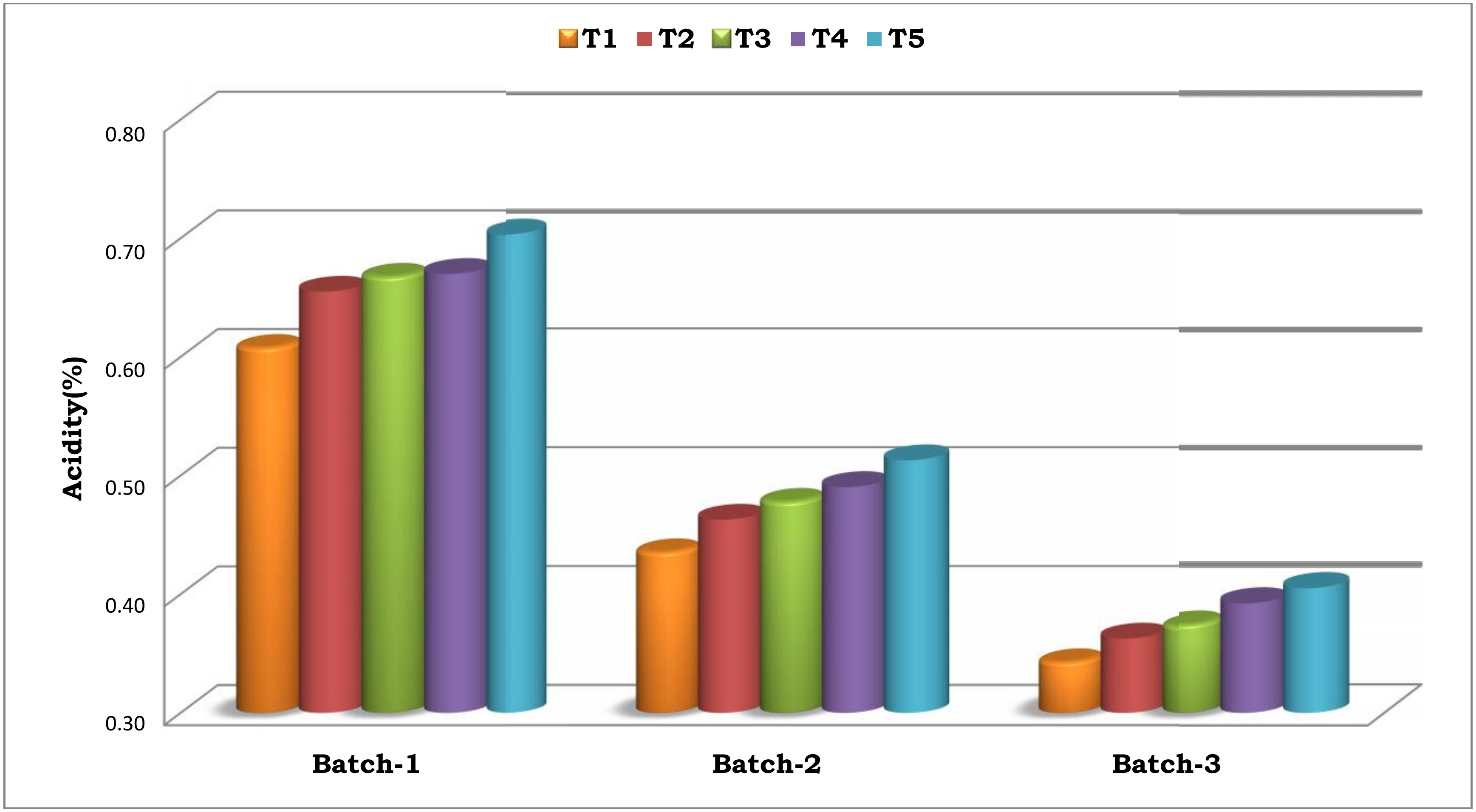


Figure 6. Effect of different treatments on acidity (%) content of Jamun wine at 90 days of storage

T1 : Control(100% jamun wine)
T2 : Jamun wine with 0.025% Kutaja powder
T3 : Jamun wine with 0.05% Kutaja powder
T4 : Jamun wine with 0.075% Kutaja powder
T5 : Jamun wine with 0.1% Kutaja powder

4.4 ALCOHOL

Data on alcohol content of wine as influenced by different treatments in three batches after 90 days of storage are presented in Table 4 and Figure 7.

The alcohol content of the wine varied significantly among the treatments. Jamun wine with 0.1% Kutaja powder had higher alcohol content (8.06%) which was on par with Jamun wine with 0.075% Kutaja powder (8.04%) in the first batch. It was less in control (7.90%).

In second batch, the highest alcohol content was recorded in Jamun wine with 0.1% Kutaja powder (7.02%) which was on par with Jamun wine with 0.075% Kutaja powder (7.01%) while the lowest was recorded in control (6.94%).

The highest was recorded in Jamun wine with 0.1% Kutaja powder (6.02%) which was on par with all the treatments except control which had the least alcohol content (5.94%) in third batch.

The alcohol content decreased from first batch to the third batch.

4.5 ANTHOCYANIN

Data on anthocyanin content of wine as influenced by different treatments in three batches after 90 days of storage are presented in Table 5 and Figure 8.

The anthocyanin content was found non-significant between the treatments in all the batches. However anthocyanin content decreased from the first batch to third batch .

Table 4. Effect of different treatments on alcohol content of Jamun wine at 90 days of storage

Treatments	Alcohol (%)		
	BATCH-1	BATCH-2	BATCH-3
Control (100% Jamun wine)	7.90	6.94	5.94
Jamun wine + 0.025% Kutaja powder	7.97	6.97	5.98
Jamun wine + 0.05% Kutaja powder	8.00	6.98	5.99
Jamun wine + 0.075% Kutaja powder	8.04	7.01	6.01
Jamun wine + 0.1% Kutaja powder	8.06	7.02	6.02
F-test	*	*	*
S.Em±	0.01	0.01	0.01
CD at 1%	0.04	0.03	0.04

*- Significant at 1%

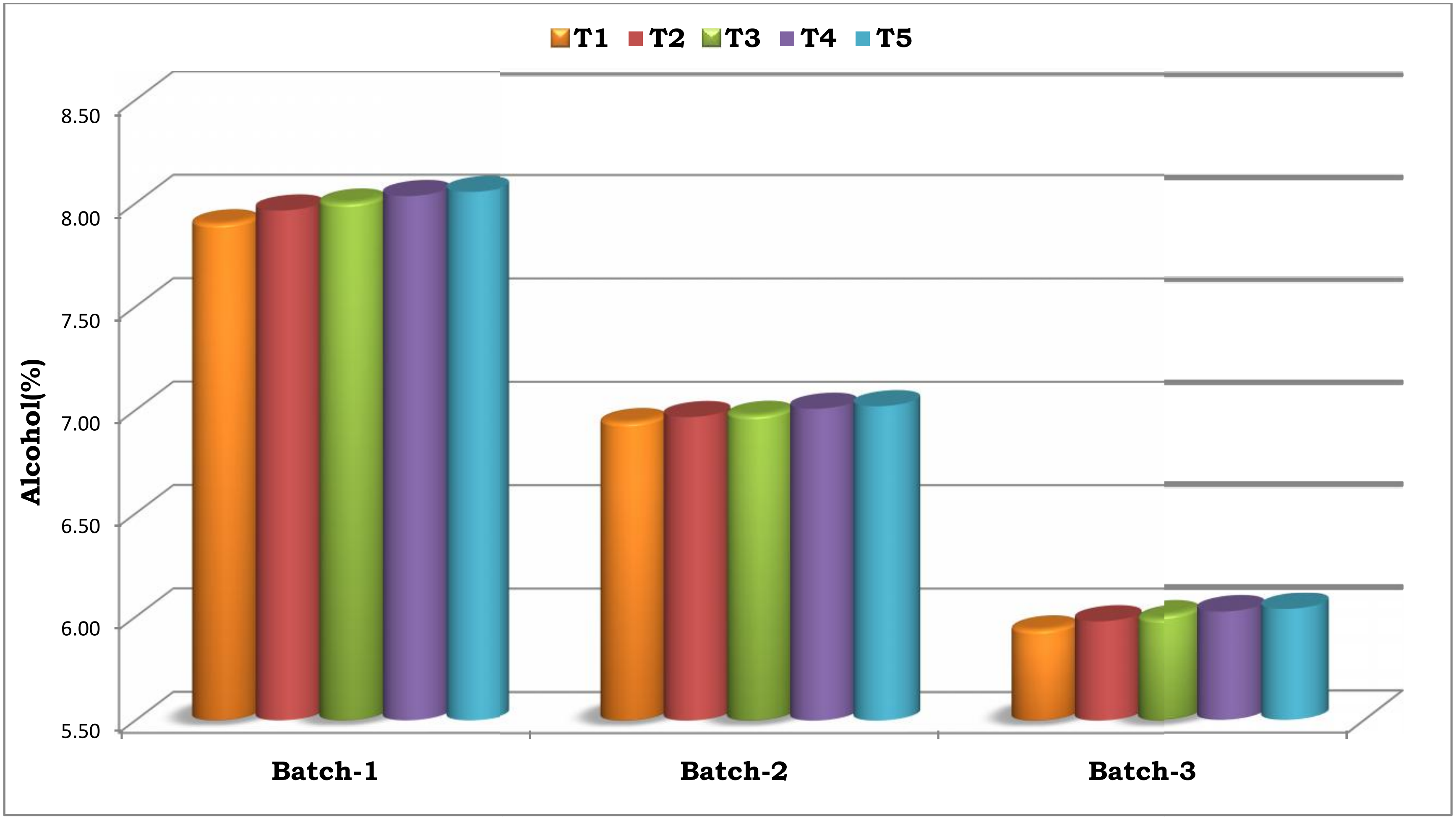


Figure 7. Effect of different treatments on alcohol content (%) of Jamun wine at 90 days of storage

T1 : Control(100% jamun wine)
T2 : Jamun wine with 0.025% Kutaja powder
T3 : Jamun wine with 0.05% Kutaja powder
T4 : Jamun wine with 0.075% Kutaja powder
T5 : Jamun wine with 0.1% Kutaja powder

Table 5. Effect of different treatments on anthocyanin content of Jamun wine at 90 days of storage

Treatments	Anthocyanin (mg per 100ml)		
	BATCH-1	BATCH-2	BATCH-3
Control (100% Jamun wine)	67.25	53.50	43.25
Jamun wine + 0.025% Kutaja powder	64.75	52.75	42.50
Jamun wine + 0.05% Kutaja powder	64.25	52.00	41.50
Jamun wine + 0.075% Kutaja powder	64.00	51.25	39.75
Jamun wine + 0.1% Kutaja powder	63.75	49.50	36.00
F-test	NS	NS	NS

NS- Non Significant

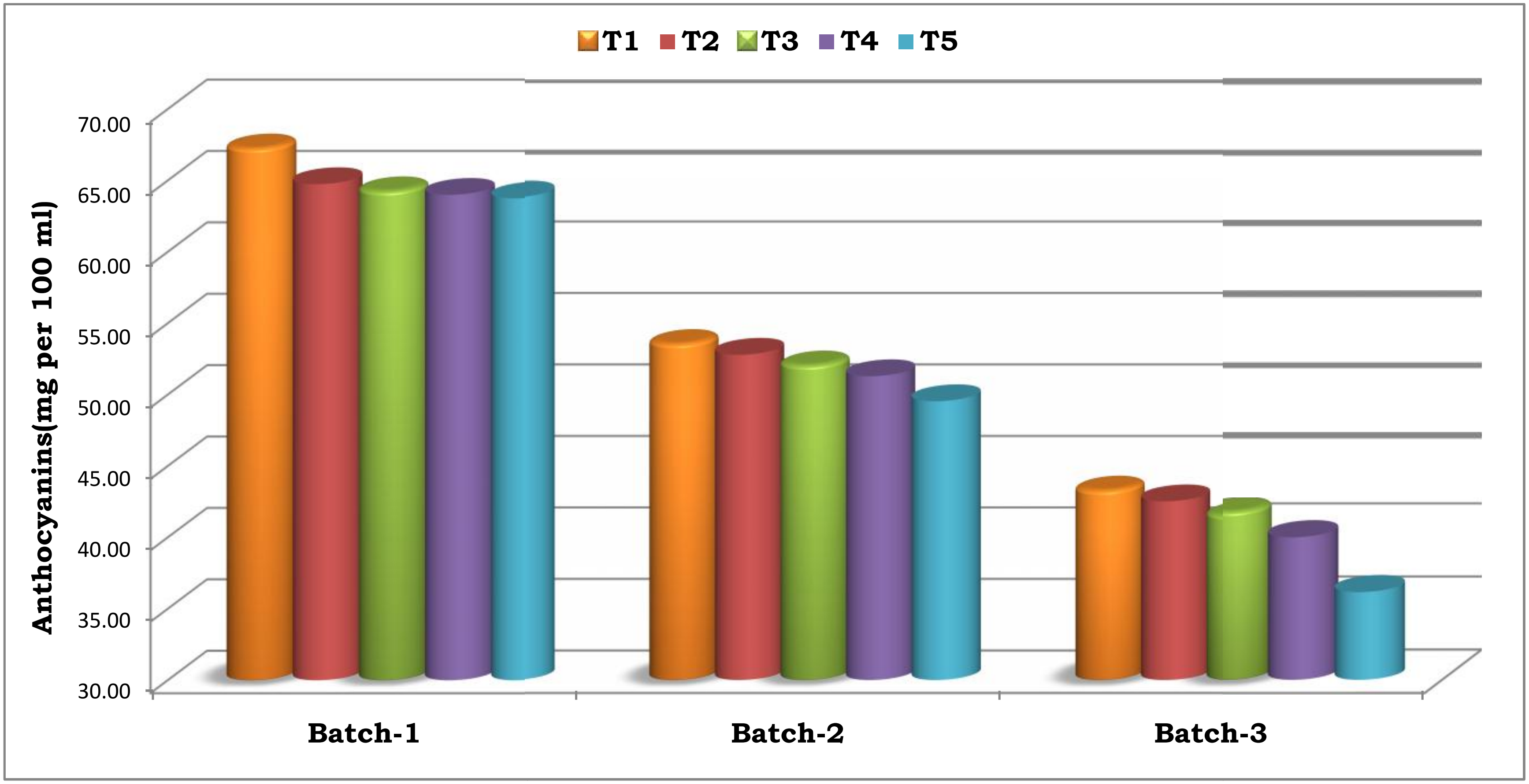


Figure 8. Effect of different treatments on anthocyanin (mg per 100ml) content of Jamun wine at 90 days of storage

T1 : Control(100% jamun wine)
T2 : Jamun wine with 0.025% Kutaja powder
T3 : Jamun wine with 0.05% Kutaja powder
T4 : Jamun wine with 0.075% Kutaja powder
T5 : Jamun wine with 0.1% Kutaja powder

4.6 TANNINS

Data on tannin content of wine as influenced by different treatments in three batches after 90 days of storage are presented in Table 6 and Figure 9.

In first batch, highest tannin content was recorded in Jamun wine with 0.1% Kutaja powder (127.25mg/100 ml) which was on par with Jamun wine with 0.075% Kutaja powder (126 mg/100 ml) and Jamun wine with 0.05% Kutaja powder (125.50 mg/100 ml), while the lowest was observed in control (121.25 mg/100 ml).

In second batch, the tannin content was recorded highest in Jamun wine with 0.1% Kutaja powder (96.50 mg/100 ml) which was on par with Jamun wine with 0.075% Kutaja powder (95.00 mg/100 ml) while the lowest was recorded in control (89.50 mg/100 ml).

Similar to the second batch, in third batch also the highest tannin content was observed in Jamun wine with 0.1% Kutaja powder (80.25 mg/100 ml) which was on par with Jamun wine with 0.075% Kutaja powder (78.25 mg/100 ml) and control had the lowest tannin content (73.25 mg/100 ml).

There was an increase in tannin content between the treatments from control to Jamun wine with 0.1% Kutaja powder. However the tannin content decreased from first batch to the third batch.

4.7 PHENOLS

Data on phenol content of wine as influenced by different treatments in three batches after 90 days of storage are presented in Table 7 and Figure 10.

Table 6. Effect of different treatments on tannin content of Jamun wine at 90 days of storage

Treatments	Tannin (mg per 100 ml)		
	BATCH-1	BATCH-2	BATCH-3
Control (100% Jamun wine)	121.25	89.50	73.25
Jamun wine + 0.025% Kutaja powder	123.00	91.75	75.25
Jamun wine + 0.05% Kutaja powder	125.50	92.75	76.50
Jamun wine + 0.075% Kutaja powder	126.00	95.00	78.25
Jamun wine + 0.1% Kutaja powder	127.25	96.50	80.25
F-test	*	*	*
S.Em±	0.58	0.40	0.86
CD at 1%	2.44	1.66	3.57

*- Significant at 1%

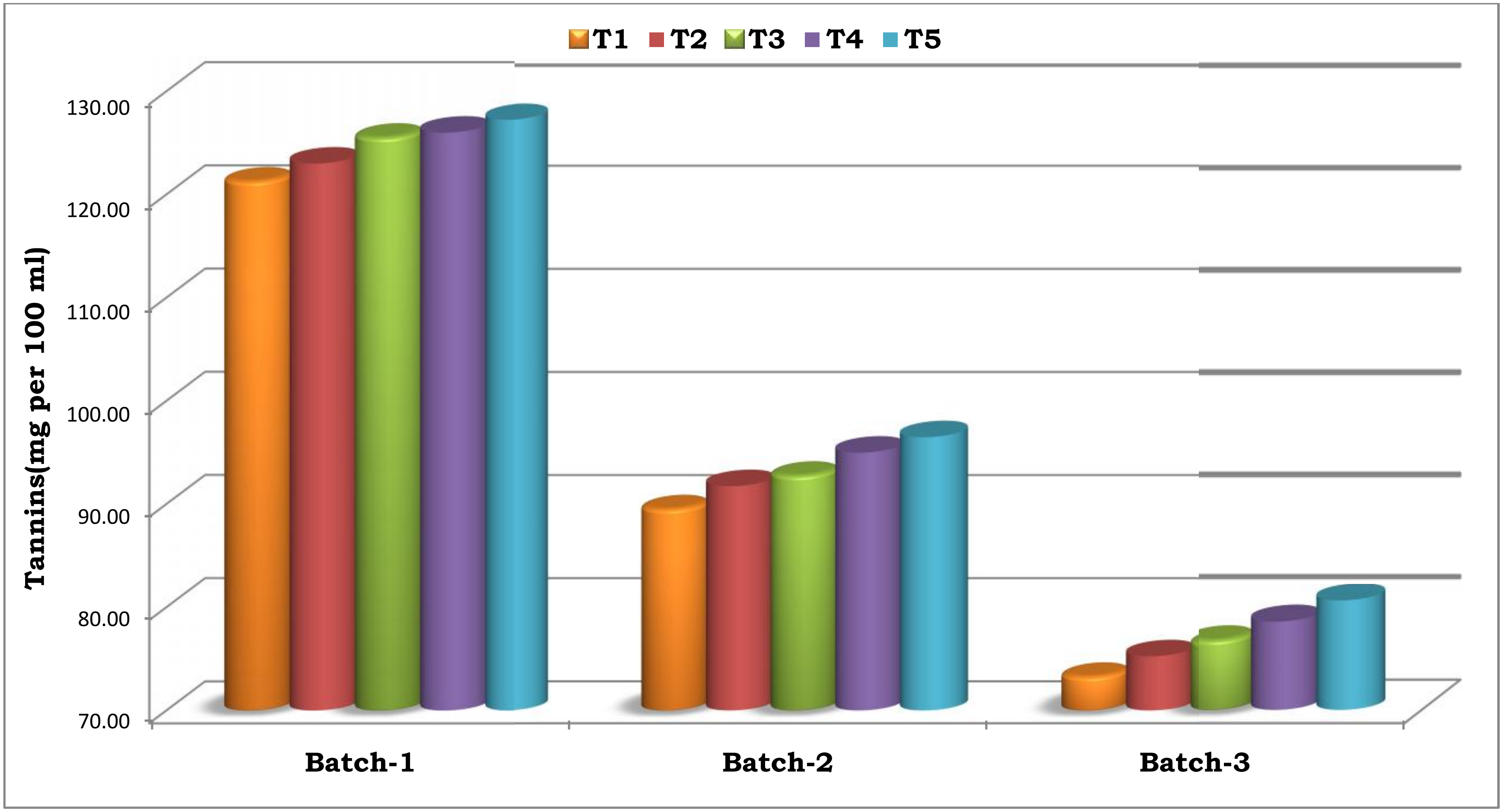


Figure 9. Effect of different treatments on tannin (mg per 100ml) content of Jamun wine at 90 days of storage

T1 : Control(100% jamun wine)
T2 : Jamun wine with 0.025% Kutaja powder
T3 : Jamun wine with 0.05% Kutaja powder
T4 : Jamun wine with 0.075% Kutaja powder
T5 : Jamun wine with 0.1% Kutaja powder

Table 7. Effect of different treatments on phenol content of Jamun wine at 90 days of storage

Treatments	Phenol (mg per 100 ml)		
	BATCH-1	BATCH-2	BATCH-3
Control (100% Jamun wine)	228.50	221.25	215.00
Jamun wine + 0.025% Kutaja powder	230.75	223.00	217.25
Jamun wine + 0.05% Kutaja powder	232.50	226.25	220.50
Jamun wine + 0.075% Kutaja powder	234.50	227.75	222.50
Jamun wine + 0.1% Kutaja powder	236.50	230.00	225.00
F-test	*	*	*
S.Em±	1.02	0.76	0.82
CD at 1%	4.26	3.15	3.43

*- Significant at 1%

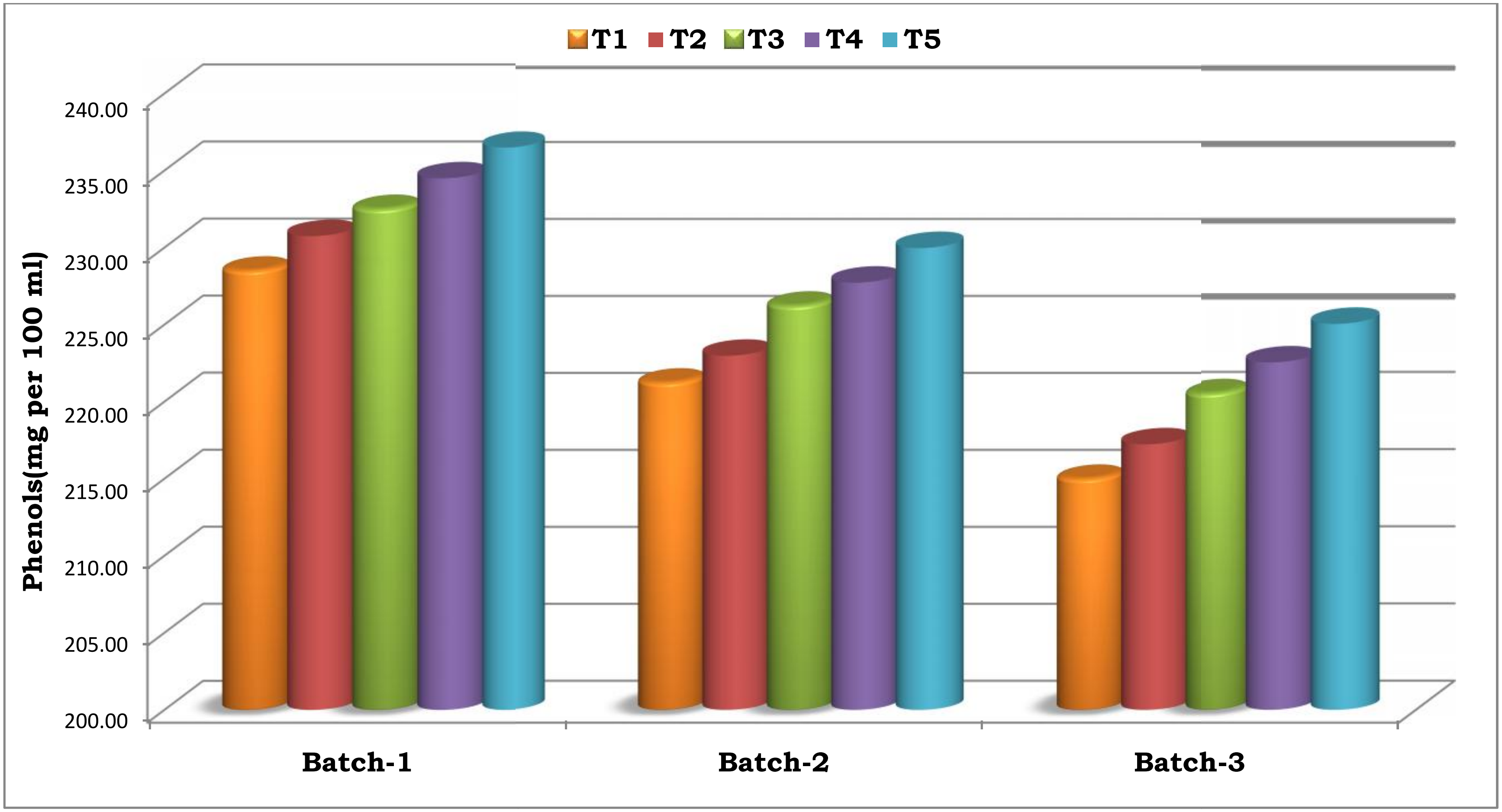


Figure 10. Effect of different treatments on phenol (mg per 100ml) content of Jamun wine at 90 days of storage

T1 : Control(100% jamun wine)
T2 : Jamun wine with 0.025% Kutaja powder
T3 : Jamun wine with 0.05% Kutaja powder
T4 : Jamun wine with 0.075% Kutaja powder
T5 : Jamun wine with 0.1% Kutaja powder

In first batch, highest phenol content was recorded in Jamun wine with 0.1% Kutaja powder (236.50 mg/100 ml) which was on par with Jamun wine with 0.075% Kutaja powder (234.50 mg/100 ml) and Jamun wine with 0.05% Kutaja powder (232.50 mg/100 ml). The lowest was observed in control (228.50 mg/100 ml).

Highest phenol content was observed in Jamun wine with 0.1% Kutaja powder (230 mg/100 ml) in second batch which was on par with Jamun wine with 0.075% Kutaja powder (227.75 mg/100 ml) whereas the lowest was observed in control (221.25 mg/100 ml).

Highest phenol content was observed in Jamun wine with 0.1% Kutaja powder (225 mg/100 ml) in third batch which was on par with Jamun wine with 0.075% Kutaja powder (222.50 mg/100 ml) whereas the lowest was observed in control (215 mg/100 ml).

The phenolic content increased between the treatments but decreased from first batch to third batch.

4.8 TOTAL SUGARS

Data on total sugar content of wine as influenced by different treatments in three batches after 90 days of storage are presented in Table 8 and Figure 11.

There was a significant difference in Total Sugar content of Jamun wine between the treatments. In first batch, the highest total sugar was recorded in control (8.79%), whereas the lowest content was observed in Jamun wine with 0.1% Kutaja powder (8.51%).

The highest total sugar content in second batch was observed in control (9.05%) while the lowest was observed in Jamun wine with 0.1% Kutaja powder (8.89%) which differed significantly.

Table 8. Effect of different treatments on Total sugar content of Jamun wine at 90 days of storage

Treatments	Total sugar (%)		
	BATCH-1	BATCH-2	BATCH-3
Control (100% Jamun wine)	7.22	9.05	10.97
Jamun wine + 0.025% Kutaja powder	7.04	9.00	10.90
Jamun wine + 0.05% Kutaja powder	7.00	8.97	10.87
Jamun wine + 0.075% Kutaja powder	6.95	8.92	10.84
Jamun wine + 0.1% Kutaja powder	6.91	8.89	10.81
F-test	*	*	*
S.Em±	0.02	0.01	0.02
CD at 1%	0.07	0.03	0.07

*- Significant at 1%

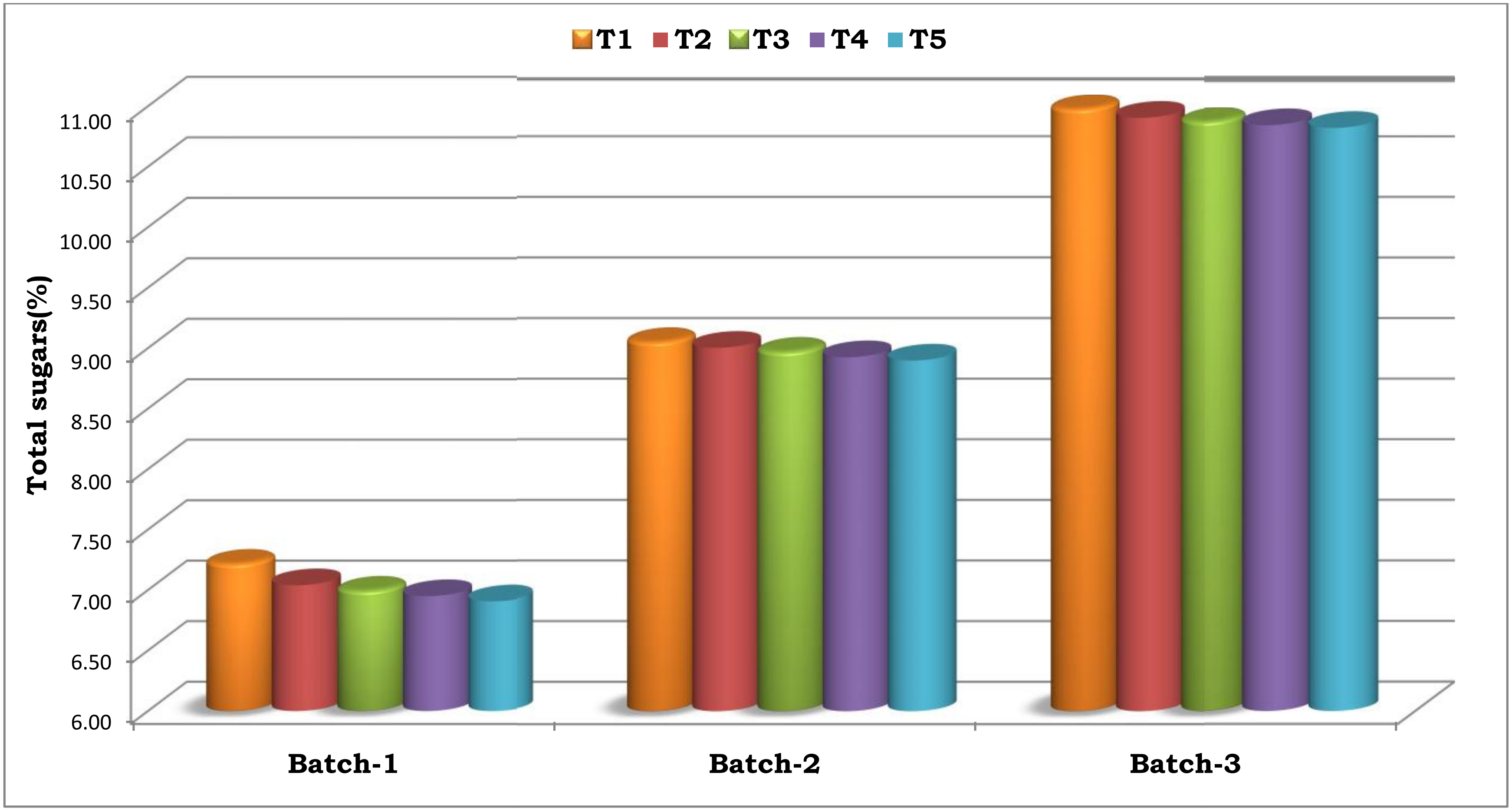


Figure 11. Effect of different treatments on total sugars (%) content of Jamun wine at 90 days of storage

T1 : Control(100% jamun wine)
T2 : Jamun wine with 0.025% Kutaja powder
T3 : Jamun wine with 0.05% Kutaja powder
T4 : Jamun wine with 0.075% Kutaja powder
T5 : Jamun wine with 0.1% Kutaja powder

Control had highest total sugar content (10.97%) in third batch which was on par with Jamun wine with 0.025% Kutaja powder (10.9%) and the lowest was observed in Jamun wine with 0.1% Kutaja powder (10.81%), values differed significantly.

4.9 REDUCING SUGAR

Data on reducing sugar content of wine as influenced by different treatments in three batches after 90 days of storage are presented in Table 9 and Figure 12.

There was a significant difference in reducing sugar content of Jamun wine between the treatments. In first batch, the highest reducing sugar was recorded in control (6.15%), whereas the lowest content was observed in Jamun wine with 0.1% Kutaja powder (5.81%).

In second batch, highest reducing sugar was recorded in control (7.94%) which was on par with Jamun wine with 0.025% Kutaja powder (7.90%) and Jamun wine with 0.05% Kutaja powder (7.88%), while the lowest was observed in Jamun wine with 0.1% Kutaja powder (7.80%).

Control of third batch had highest reducing sugar content (9.89%) which was on par with Jamun wine with 0.025% Kutaja powder (9.83%) and the lowest was observed in Jamun wine with 0.1% Kutaja powder (9.71%), which differed significantly.

The reducing sugars had an increasing trend from first batch to the third batch.

4.10 MICROBIAL ANALYSIS

There was no inhibition zone in all the three microorganisms studied for the effect of Kutaja in all the treatments in all the three batches.

Table 9. Effect of different treatments on Reducing sugar content of Jamun wine at 90 days of storage

Treatments	Reducing sugar (%)		
	BATCH-1	BATCH-2	BATCH-3
Control (100% Jamun wine)	6.15	7.94	9.89
Jamun wine + 0.025% Kutaja powder	5.94	7.90	9.83
Jamun wine + 0.05% Kutaja powder	5.91	7.88	9.78
Jamun wine + 0.075% Kutaja powder	5.84	7.81	9.75
Jamun wine + 0.1% Kutaja powder	5.81	7.80	9.71
F-test	*	*	*
S.Em±	0.02	0.02	0.02
CD at 1%	0.08	0.08	0.08

*- Significant at 1%

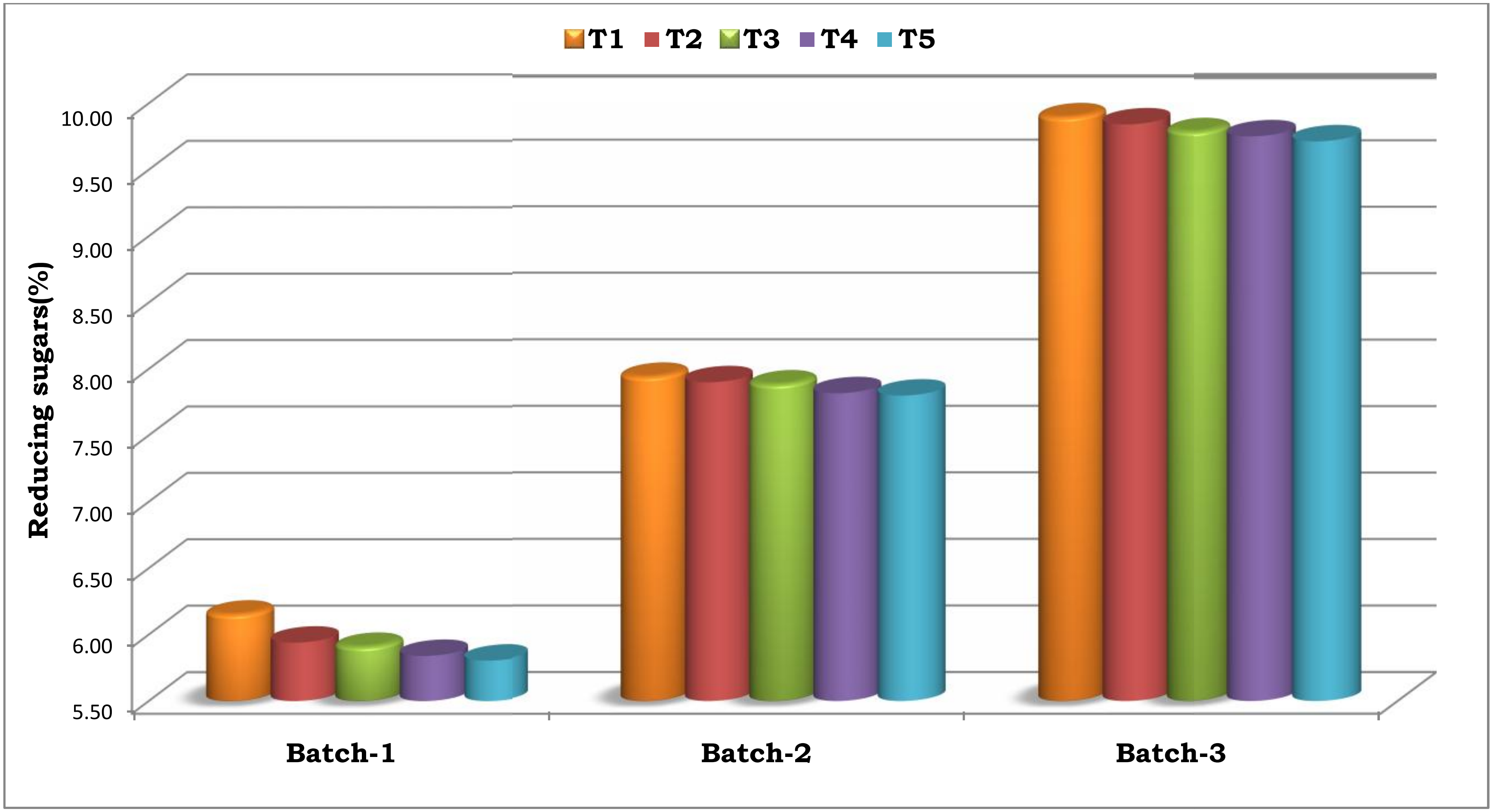


Figure 12. Effect of different treatments on reducing sugars (%) content of Jamun wine at 90 days of storage

T1 : Control(100% jamun wine)
T2 : Jamun wine with 0.025% Kutaja powder
T3 : Jamun wine with 0.05% Kutaja powder
T4 : Jamun wine with 0.075% Kutaja powder
T5 : Jamun wine with 0.1% Kutaja powder

4.11 ORGANOLEPTIC EVALUATION

The quality of Jamun wine samples was assessed by organoleptic or sensory evaluation by panel of judges. The scores obtained through sensory evaluation are presented in Table 10.

4.11.1 APPEARANCE

The highest score for appearance was recorded in Jamun wine with 0.1% Kutaja powder (4.5) from the first batch. This treatment was very much attractive and it had a clear and good appearance. When compared to all other treatments this was found superior with respect to appearance. The wine with 0.1% Kutaja powder (2) from the third batch got least sensory score with respect to appearance.

4.11.2 COLOUR

Control (4.5) from second batch recorded highest sensory score with respect to colour. It had a rosy bright colour. The wine was found to be attractive and acceptable. Jamun wine with 0.1% Kutaja powder (2.5) of the third batch recorded least sensory score with respect to colour.

4.11.3 SWEETNESS

Data recorded on sweetness of wine as influenced by different concentrations of Kutaja are tabulated in Table 10. The maximum score for sweetness was recorded in Jamun wine treated with 0.1% Kutaja powder (4.0) from the first batch, which had low sweetness and least sensory score was observed in the treatment with 0.025% Kutaja powder in the Jamun wine from the third batch, which had moderately high sweetness. The wine was found attractive and acceptable.

Table 10.1. Sensory evaluation of first batch of Jamun wine at 90 days of storage.

Treatments	Organoleptic scores							
	Appearance	colour	Aroma/ bouquet	acidity	sweetness	body	astringency	overall acceptability
Control (100% Jamun wine)	3.5	3	7.5	2.5	3.6	3.5	2.31	29.61
Jamun wine + 0.025% kutaja powder	3.5	3.45	8	2.7	3.4	3.4	2.44	30.89
Jamun wine + 0.05% kutaja powder	3.5	3.25	6.5	2.9	3.2	3.2	2.31	32.11
Jamun wine + 0.075% kutaja powder	4.2	4	8	3.5	3.4	3.2	1.5	36.5
Jamun wine + 0.1% kutaja powder	4.5	4.13	7	3.2	4	3.8	1.37	36.25

Table 10.2. Sensory evaluation of second batch of Jamun wine at 90 days of storage

Treatments	Organoleptic scores							
	Appearance	**colour**	**Aroma/ bouquet**	**Acidity**	**sweetness**	**body**	**astringency**	**overall acceptability**
Control (100% Jamun wine)	3.2	4.5	6.5	3.3	2.5	3.2	3.5	33.2
Jamun wine + 0.025% kutaja powder	3.4	4.2	6.2	3	2.4	4	3.4	33.2
Jamun wine + 0.05% kutaja powder	3.3	3.8	6.3	3.4	2	3.5	3.4	32.3
Jamun wine + 0.075% kutaja powder	3.5	4.1	6.2	3.3	2.5	3.7	3.2	33.7
Jamun wine + 0.1% kutaja powder	3.2	3.5	5.8	3.2	2.3	3.3	3.4	31.2

Table 10.3. Sensory evaluation of third batch of Jamun wine at 90 days of storage

Treatments	Organoleptic scores							
	Appearance	colour	Aroma/ bouquet	acidity	sweetness	body	astringency	overall acceptability
Control (100% Jamun wine)	2.3	3.2	5	4	1.6	3.2	2.4	28.4
Jamun wine + 0.025% kutaja powder	2.8	2.8	4.5	3.8	1.2	3.6	2.6	27.6
Jamun wine + 0.05% kutaja powder	2.7	3	5.2	3.9	2	3	2.7	29
Jamun wine + 0.075% kutaja powder	2.5	2.7	5.4	4.2	1.5	2.8	2.5	29.2
Jamun wine + 0.1% kutaja powder	2	2.5	3.5	3.7	1.3	2.2	2.1	23

4.11.4 FLAVOUR

Data recorded on flavour of Jamun wine is tabulated in Table 10. The highest score for the flavour was recorded in first batch of Jamun wine with 0.075% Kutaja powder (4.5) which had a fruity flavor. Least sensory score recorded in first batch of Jamun wine with 0.025% Kutaja (1.5) powder which had an off-eggy flavor.

4.11.5 ASTRINGENCY

Data recorded on astringency of Jamun wine is presented in Table 10. The maximum score for astringency was recorded in second batch of control (3.5) which had moderate astringency however the least score was recorded in Jamun wine with 0.075% Kutaja powder from the first batch (1.5). The wine was found acceptable with respect to astringency.

4.11.6 OVERALL ACCEPTABILITY

Data from Table 10 showed that the highest sensory score was obtained from first batch of Jamun wine prepared with 0.075% Kutaja powder (73%) while the lowest was obtained in third batch of Jamun wine prepared with 0.1% Kutaja powder (46%).

Discussion

V. DISCUSSION

The results on the study of "Preparation of Jamun wine with antimicrobial properties" presented in the previous chapter are discussed here in light of relevant literature.

5.1 FIRST BATCH

5.1.1 pH

The difference between the pH was found non-significant between the treatments. In subsequent batches there was increase in pH

5.1.2 TOTAL SOLUBLE SOLIDS

The highest TSS was recorded in control (8.79˚B) and the lowest content was observed in Jamun wine with 0.1% Kutaja powder (8.51˚B). There was a fall in TSS during the period of storage this may be due to the conversion of sugars to alcohol. Also the increase in Kutaja reduced the TSS content of the wine due to its masking effect, due to the presence of bitter principle. Similar results were obtained by Kroes *et al* (1993), who reported the use of *Woodfordia fruticosa* with the fermented *Nimba arishta.*

5.1.3 TITRABLE ACIDITY

The titrable acidity of the wine sample increased after fermentation. This may be possibly due to the production of certain organic acids during the process of fermentation, by yeast cells. Significantly maximum value of titrable acidity was recorded in Jamun wine with 0.1% Kutaja (0.7%) and the lowest was recorded in control (0.61%). These findings are comparable with the investigations of Shukla and Revis (1985), where the total acidity in wines of different orange cultivars ranged from 0.68 to 0.96 per cent.

5.1.4 ALCOHOL

The observations on alcohol content showed that Jamun wine with 0.1% Kutaja powder had higher alcohol content (8.06%) and was less in control (7.90%). The increase in alcohol content was due to the conversion of sugars into alcohol. The results obtained in this study are in conformity to that reported by Chikkasubbanna *et al.* (1990). They reported that the alcohol per cent of the grape wine increased due to the decrease in total soluble sugars as well as yeast activity during fermentation. This alcohol content is somewhat low and methods to bring this up to 12-13 per cent need to be evolved.

5.1.5 ANTHOCYANIN

The anthocyanin content was found non-significant between the treatments. But it decreased in subsequent batches. The method of extraction followed, the addition of Kutaja does not clearly influence the color extraction under experimental conditions deployed.

5.1.6 TANNINS

Tannins contribute to the quality by improving the body, helps in stabilizing the color and aids during the clarification of wines. Highest tannin was recorded in Jamun wine with 0.1% Kutaja powder (127.25 mg/100 ml) while the lowest was observed in control (121.25 mg/100 ml). Similar results were obtained by Shukla and Revis (1985), who examined the ecological properties of some orange cultivars grown in India and concluded that tannin content ranged from 37 to 157 mg per 100 ml in wines. The addition of Kutaja does not show up significantly over background tannin arising from the fruits.

5.1.7 PHENOLS

The results revealed that phenol content was highest in Jamun wine with 0.1% Kutaja powder (236.50 mg/100 ml) and the lowest was observed in control (228.50 mg/100 ml). Phenolic compounds influence the taste of wine. The presence of small quantities of phenolic compounds is desirable in wines. However higher concentration of phenolic compounds contributes to the bitter taste of the wine. Similar analysis was conducted by Joshi *et al* (1990), who reported 240 mg total phenols per 100 ml of wine. The addition of Kutaja clearly increased the phenol content of the wine.

5.1.8 TOTAL SUGARS

The results showed that there was a significant difference in total sugar content of Jamun wine between the treatments. The highest total sugar was recorded in control (8.79%), whereas the lowest content was observed in Jamun wine with 0.1% Kutaja powder (8.51%). The decrease in sugar content of wine indicates the utilization of sugar during fermentation. The extent of sugars lost and converted to alcohol reduces with the number of extracts and reason for this is not obvious from the data available and needs further research efforts to explain it.

5.1.9 REDUCING SUGARS

The investigations showed that significantly higher extracts of reducing sugars were found in control (6.15%), whereas the lowest content was observed in Jamun wine with 0.1% Kutaja powder (5.81%). These results are in accordance with the results obtained by Bardiya *et al.* (1974), who reported that 7.2% of reducing sugars were present in guava wine. This observation makes the production of dry wine in this process with Jamun difficult. Alternatives to this method need to be developed because one of the primary/potential users for jamun wine would be diabetic, where sugar in the wine is not desirable.

5.2 SECOND BATCH

5.2.1 pH

The difference between the pH was found non-significant between the treatments.

5.2.2 TOTAL SOLUBLE SOLIDS

As the Kutaja concentration in the wine increased there was decrease in the TSS content. The highest TSS content in second batch was observed in control (10.65˚B) while the lowest was observed in Jamun wine with 0.1% Kutaja powder (10.49˚B) which differed significantly. In storage, the wine TSS decreased as the storage period advanced in all the treatments. Similar results are obtained in sapota by Gautham and Chundawat (1998) where wine contained 9˚B total soluble solids in clarified juice. The TSS is dominated by reducing sugars in other words the added sugar itself. Only about two per cent TSS can be explained by solutes and sugars (non fermentable) extracted from the fruit.

5.2.3 TITRABLE ACIDITY

As the Kutaja concentration in the wine increased there was increase in the acidity content in all the treatments. It was highest in Jamun wine with 0.1% Kutaja powder (0.51%) while the lowest was recorded in control (0.44%). In storage, as the period increased the titrable acidity increased, this may be due to the production of organic acids by yeast cells. Similar results were reported by Kulkarni *et al* (1980) in mango wine and Shukla and Revis (1985) in oranges, that production of certain organic acids like malic acid, lactic acid, and succinic acid was the possible reasons. While a larger extent of titrable acidity leaves a sour taste. Acidity and low pH are required to prevent bacterial fermentation and for a fruity taste. This wine had appropriate extent of titrable acidity.

5.2.4 ALCOHOL

Gautham and Chundawat (1998) reported that the increase in alcohol content might be due to higher levels of reducing sugars which are more readily fermentable giving rise to higher levels of alcohol. As the storage days increased there was increase in extent of alcohol. The highest alcohol content was recorded in Jamun wine with 0.1% Kutaja powder (7.02%) while the lowest was recorded in control (6.94%). It appears that Kutaja addition improves fermentation and alcohol content, although the reasons are not discernible from available data and requires further research effort and analysis.

5.2.5 ANTHOCYANIN

The anthocyanin content was found non-significant between the treatments. The method of extraction followed, the addition of Kutaja does not clearly influence the color extraction under experimental conditions deployed.

5.2.6 TANNINS

Tannin content of the wine increased with the increase in Kutaja concentration. This is due to the addition of tannin from Kutaja itself. The tannin content was recorded highest in Jamun wine with 0.1% Kutaja powder (96.50 mg/100 ml) and the lowest was recorded in control (89.50 mg/100 ml). The tannin content was found to decrease as the storage period advances. This result was supported by the study of Attri *et al.* (1994), they estimated the total phenol content in cashew apple wine. The total phenol content of 152.08 mg per liter was found in the cashew apple wine after 10 days of fermentation. Kutaja addition at 100 mg per liter or 10 mg per 100 ml is clearly the main reason for increase in tannic material but it did not commensurate with the concentration added.

5.2.7 PHENOLS

As the concentration of Kutaja in the wine increased the phenolic content also increased. The highest phenolic content was recorded in Jamun wine with 0.1% Kutaja powder (230 mg/100 ml) in second batch whereas the lowest was observed in control (221.25 mg/100 ml). In storage, as the storage period advanced, the phenolic content also decreased. The results are on par with the results of Augustin (1987) where he found higher phenolic content in cashew apple wine. This might be due to high rate of fermentation releasing higher amounts of phenols and tannins. Addition of 10 mg per 100 ml of Kutaja increased the phenolic content marginally. Much of the phenols therefore comes from the fruit extraction.

5.2.8 TOTAL SUGARS

Gautham and Chundawat (1998) found increased levels of reducing sugar and total sugars in wine from non clarified juice which was due to slow rate of fermentation As the Kutaja concentration in the wine increased there was decrease in the total sugar content. The treatment of Jamun wine with 0.1% Kutaja powder recorded lower total sugar (8.89%). The control recorded higher total sugar content (9.05%). In storage as the period advanced the total sugar content also decreased.

5.2.9 REDUCING SUGARS

In storage, as the period advanced, the reducing sugar content decreased. Highest reducing sugar was recorded in control (7.94%) and the lowest was observed in Jamun wine with 0.1% Kutaja powder

(7.80%). These results are agreement with the results of Subba Rao (1973) with cashew apple wine and Bardiya *et al.* (1974) in Guava wine. Reducing sugars (glucose and fructose) add sweetness to the wine and is not acceptable to diabetic wine consumers to whom the product is devised.

5.3 THIRD BATCH

5.3.1 pH

The difference between the pH was found non-significant between the treatments.

5.3.2 TOTAL SOLUBLE SOLIDS

The highest TSS was recorded in control (12.57˚B) and the lowest content was observed in Jamun wine with 0.1% Kutaja powder (12.41˚B). There was a fall in TSS during the period of storage this may be due to the conversion of sugars to alcohol. Similar results were obtained by Vyas and Kochhar (1993). They assessed the chemical composition of wines made from apple fruits where wines produced from var. Golden Delicious had 9˚B and those from produced from Red Delicious had 8.9˚B. This suggests that nearly four per cent of TSS is not accounted as reducing sugars, potentially pectin etc. This gave a better body to the wine.

5.3.3 TITRABLE ACIDITY

It is important to have the perfect balance of sugar, acidity and pH in the must for quality wine production. Titrable acidity of wine depends upon the total organic acids content of the wine. Highest acidity was noticed in Jamun wine with 0.1% Kutaja powder (0.40%), while the least was recorded in control (0.34%). The increase in the acidity after fermentation and during ageing might be due possible production of certain organic acids. During ageing, acetic acid (volatile acidity) can result from the coupled oxidation of wine phenolics to yield peroxide which in turn oxidizes ethanol to acetaldehyde and subsequently to acetic acid (Zoecklein *et al.,* 1999). The acceptable range for total acidity in most wines is between 5.5 and 8.5 mg/litre (0.55 to 0.85%). White wines are generally preferred at higher end of the scale whereas red wines are more appreciated at lower end (Jackson and Badrie, 2003). It is not clear how addition of Kutaja increased acidity and needs to be investigated.

5.3.4 ALCOHOL

The observations on alcohol content showed that Jamun wine with 0.1% Kutaja powder had higher alcohol content (6.02%) and was less in control (5.94%). The increase in alcohol content was due to the conversion of sugars into alcohol. The results obtained in this study are in conformity to that reported by Kotecha (2010) who recorded a similar range of alcohol content in wines of banana and ber (7.6%), Jamun (8.4 %), sapota 7.2% and strawberry (7.0%). 6-9 per cent alcohol in wine gives a lower keeping quality than Grape wines, 12 per cent alcohol necessitating finding new methods to increase alcohol concentration to 12 per cent

5.3.5 ANTHOCYANIN

The anthocyanin content was found non-significant between the treatments

5.3.6 TANNINS

Tannins contribute to the quality by improving the body, helps in stabilizing the color and aids during the clarification of wines. Tannic acid had no effect on browning or ageing but increased color intensity. Highest tannin was recorded in Jamun wine with 0.1% Kutaja powder

(80.25 mg/100 ml) while the lowest was observed in control (73.25 mg/100 ml). Similar result was obtained by Bardiya *et al* (1974) who studied the chemical composition of guava wine and recorded tannin content of 120mg per 100 ml.

5.3.7 PHENOLS

The results revealed that phenol content was highest in Jamun wine with 0.1% Kutaja powder (225mg/100 ml) and the lowest was observed in control (222.50 mg/100 ml). Phenolic compounds influenced the taste of wine. The presence of small quantities of phenolic compounds was desirable in wines. However higher concentration of phenolic compounds contributed to the bitter taste of the wine. Somesh *et al.* (2009) estimated the total phenols (mg/l) in different Strawberry wine among different cultivars like Camarosa, Chandler and Doughlas. Highest total phenols were found in Camarosa cultivar (150.2mg/l).

5.3.8 TOTAL SUGARS

Singh *et al.* (1998) studied on the production of litchi wine. The total sugar content decreased considerably from the initial value. As the Kutaja concentration in the wine increased there was decrease in the total sugar content. The treatment of Jamun wine with 0.1% Kutaja powder recorded lower total sugar (10.97%). The control recorded higher total sugar content (10.81%). In storage, as the period advanced, the total sugar content decreased.

5.3.9 REDUCING SUGARS

The investigation showed that control had highest reducing sugar content (9.89%) and the lowest was observed in Jamun wine with 0.1% Kutaja powder (9.71%). These results were in accordance with the results obtained by Karni and Shant (1984) for apple ciders.

5.4 MICROBIAL ANALYSIS

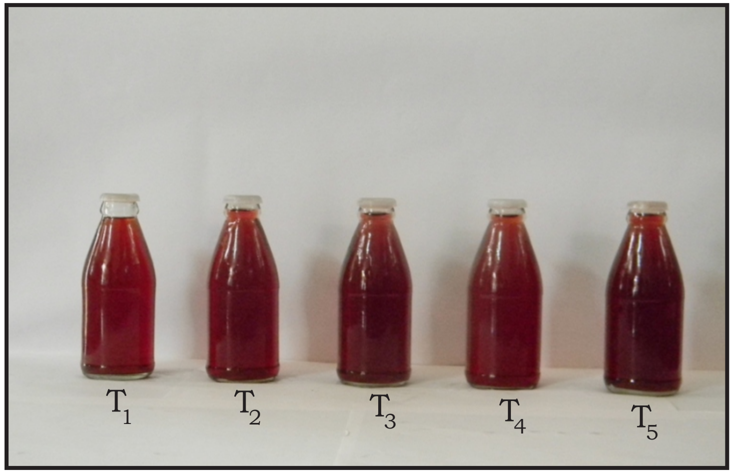
The presence of antibacterial substances in the higher plants is well established (Srinivasan *et al.,* 2001). Plants have provided a source of inspiration for novel drug compounds as plant derived medicines have made significant contribution towards human health. Hence the Jamun wine with *Holarrhena antidysentrica* was studied for antimicrobial properties. But none of the test organism used produced zones of inhibition (Plate 4). This may be due to the fact that the Gram negative bacteria cell wall is a multilayered structure (Yao and Moellering, 1995). The passage of the compound through the cell wall may be inhibited.

Also the mode of action of *Holarrhena* as reported by Kavitha and Niranjali (2009) is to reduce initial bacterial adhesion to intact epithelial cells and it may exert an antiadherence effect against the pathogenesis of EPEC in host epithelial cells.

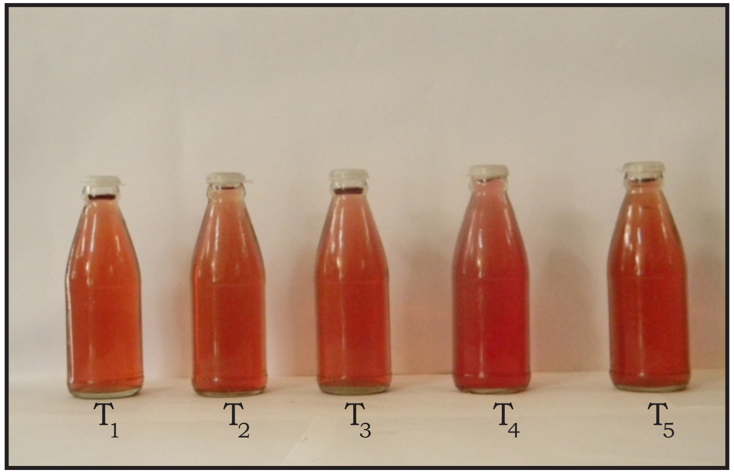
Though there was no antimicrobial effect, Jamun wine with Kutaja powder had potential benefit as the wine itself had certain medicinal properties along with Kutaja which has a long history in alternative systems of medicine. Hence it could be a recreational drink as well as a potential medicine and protectant against the problems like dysentery, diabetes, diarrhea etc

5.5 ORGANOLEPTIC EVALUATION

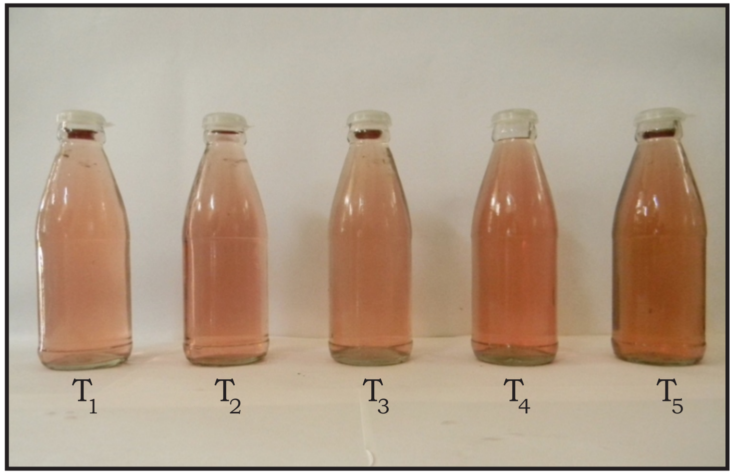
The qualities of Jamun wine samples (Plate 3) were assessed by organoleptic or sensory evaluation. The wine was evaluated based on the appearance, for its overall quality. The score obtained by sensory evaluation are discussed here under.



FIRST BATCH

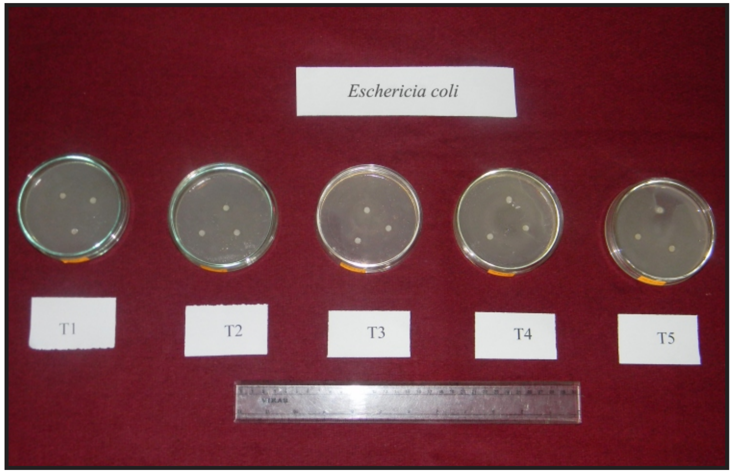


SECOND BATCH



THIRD BATCH

Plate 3. Different batches of Jamun wine with Kutaja



Eschericia coli

Salmonella typhi

Staphylococcus aureus

Plate 4. Microbial inhibition of *Eschericia coli*, *Salmonella typhi* and *Staphylococcus aureus* in different Jamun wine treatments by disc diffusion method

5.5.1 APPEARANCE

The highest score for appearance was recorded in Jamun wine with 0.1% Kutaja powder. This treatment was very much attractive and it was acceptable. These results are also in agreement with Bardiya *et al.* (1974) and Kundu *et al.* (1976). This may be due to low tannin content, color and flavor.

5.5.2 COLOR

Color was very much attractive in control with a sensory score 4.5, these results are similar to the findings of Singh and Manjrekar (1976).

5.5.3 SWEETNESS

Sufficient sugar was found in Jamun wine with 0.1% Kutaja powder and it was acceptable. This may be due lower sugar concentration in this treatment. These results are on par with the results of Kulkarni *et al.* (1980).

5.5.4 FLAVOUR

Pleasant flavour was felt in the treatment of Jamun wine with 0.075% Kutaja powder and wine was very acceptable and attractive. Similar results were obtained by Kulkarni *et al.* (1980). Higher levels of sugar may be due to abrupt arresting of fermentation.

5.5.5 ASTRINGENCY

Least astringency was found in control, this might be due to the absence of Kutaja powder. Similar results were obtained by Gautham and Chundawat (1998). The moderate astringency may be due to the presence of less tannins and phenols. But the nature and type of astringency provided by Kutaja was more desirable to the palate.

5.5.6 OVERALL ACCEPTABILITY

Overall acceptability was good in first batch of Jamun wine with 0.075% Kutaja powder. This may be due to the presence of sufficient sweetness, pleasant flavor, moderate astringency and acidity, acceptable flavor, colour and appearance. These results were on par with the findings of Gautham and Chundawat (1998), Kulkarni *et al.* (1980), Bardiya *et al*. (1974) and Kundu *et al*. (1976).

5.6 BENEFIT COST RATIO OF WINE

Due to repeated extractions from the same fruits the wine recovery tripled. Hence the benefit cost ratio was also higher (2.02:1).Therefore the wine preparation from this method was found highly feasible (APPENDIX IV).

Summary

VI. SUMMARY

A study on “The preparation of Jamun wine with antimicrobial properties” was carried out at the Division of Horticulture, University of Agricultural Sciences, GKVK, Bangalore during the year 2010-2011.

Whole fruits of Jamun were used for the preparation of wine. Generally wine (Grapes) is prepared by crushing them thoroughly and is used for only one extraction. In the present investigation efforts were made to utilize the fruits as many times as possible. Hence whole fruits along with the seeds which are known to have high therapeutic qualities were made use of. Also Kutaja, a highly acclaimed medicinal bark, known for its antimicrobial quality was used. Kutaja’s inhibiting action against the microbes *viz., Escherichia coli, Staphylococcus aureus* and *Salmonella typhi* was studied. It was realized that the fruits along with the seed could be conveniently utilized to prepare three batches of value added Jamun wine which were acceptable organoleptically.

Following are the salient findings from the present investigation.

The difference between the pH was found non-significant between the treatments. However an increasing trend in pH in all the treatments was observed from first batch to third batch. It varied from 3.18 to 3.65 across the three batches.

Chemical composition of the wine revealed that total soluble solids were highest in the treatment without Kutaja powder. The TSS content was maximum in control in all the three batches (8.79, 10.65 and 12.57˚B in first, second and third batch respectively) while the minimum was recorded in Jamun wine with 0.1% Kutaja powder in all the three batches (8.51, 10.49 and 12.41˚B in first, second and third batch respectively). A decreasing trend was observed in TSS content in all the treatments. However TSS content had a increasing trend from first batch to third batch.

Data on titratable acidity showed that Jamun wine with 0.1% Kutaja powder had the highest value in all the three batches (0.70, 0.51 and 0.40% in first, second and third batch respectively) while the lowest was recorded in control (0.61, 0.44 and 0.34% in first, second and third batch respectively). The trend was similar in all the three batches between the treatments, however a decreasing trend was observed in titratable acidity between the batches from first batch to the third batch.

The observations regarding alcohol content indicated that maximum value was in Jamun wine with 0.1% Kutaja powder in all the three batches (8.06, 7.02 and 6.02% in first, second and third batch respectively) whereas the minimum value was in control in all the batches (7.90, 6.94 and 5.94% in first, second and third batch respectively). The alcohol content decreased from first batch to the third batch.

The anthocyanin content was found non-significant between the treatments in all the batches. However anthocyanin content decreased from the first batch to third batch. It varied from 36 to 67.25mg per 100ml across the three batches.

Tannin content was highest in Jamun wine with 0.1% Kutaja powder in all the batches (127.25, 96.50 and 80.25mg/100 ml in first, second and third batch respectively) whereas the lowest was in control (121.25, 89.50 and 73.25mg/100 ml in first, second and third batch respectively). There was an increase in tannin content between the treatments from control to Jamun wine with 0.1% Kutaja powder. However the tannin content decreased from first batch to the third batch.

The data on phenol content showed that Jamun wine with 0.1% Kutaja powder had significantly higher value in all the batches (236.50, 230.00 and 225.00mg/100 ml in first, second and third batch respectively) while lower values were obtained in control in all the three batches (228.50, 221.25 and 215.00mg/100 ml in first, second and third batch respectively). The phenolic content increased between the treatments but decreased from first batch to third batch.

Total sugar content was significantly high in control in all the three batches (7.22, 9.05 and 10.97% in first, second and third batch respectively) whereas was low in Jamun wine with 0.1% Kutaja powder in all the batches (6.91, 8.89 and 10.81% in first, second and third batch respectively). The total sugar content decreased between the treatments but increased from first batch to third batch. The fermentability gradually fell in consecutive batches.

Data on reducing sugars revealed that control had the highest content in all the batches (6.15, 7.94 and 9.89% in first, second and third batch respectively). The lowest was observed in Jamun wine with 0.1% Kutaja powder in all the batches (5.81, 7.80 and 9.71% in first, second and third batch respectively). The reducing sugars had an increasing trend from first batch to the third batch.

Organoleptic evaluation of the wine revealed that Jamun wine with 0.075% Kutaja powder had the maximum score for general quality (36.50 out of 50) while the lowest was obtained for Jamun wine with 0.1% Kutaja powder (23 out of 50). Also all the three batches of wine were found acceptable.

Microbial analysis did not produce any zone of inhibition with respect to the microorganisms tested.

This study showed a new method to make fruit wine wherein the same fruit could be used at least thrice for wine making. The addition of a medicinal plant powder, Kutaja, to Jamun wine could meet two objectives i.e., a potential health drink for diabetics, which also serves as a mild protectant against diarrhea and amoebic dysentery. While the wine made passed a large number of taste and quality criteria, the presence of residual and unfermented reducing sugars is not very desirable for the purpose for which this wine was envisaged. Additional research efforts are necessary to overcome this. On the other hand this method vastly improves potential livelihoods in villages and creates value to a hitherto neglected fruit and provides its medicinal properties.

FUTURE LINE OF WORK

1. To prepare blends of Jamun fruits with various other medicinal plants.
2. To study the nutraceutical value of the above herbal wine
3. To study the efficacy of herbal wine against other microorganisms and on disease condition.

References

VII. REFERENCES

*ADUSULE, R.N., KOTECHA, P.M. AND KADAM, S.S., 1992, Preparation of wine from pomegranate. *Beverage and Food World.* **18**: 13-14.

AMERINE, M.A., BERG, H.W. AND CRUESS, W.V., 1972, *The technology of wine making.* 3rd Ed. AVI Co. West Port, Connecticut, Pub. pp: 126-132.

ANONYMOUS, 1981, Alcholic beverages from Chilean *Opuntia ficus indica., American J. Enol. Vitic,* **32**(13) : 228-229.

ATTRI, B.L., LAL B.B. AND JOSHI, V.K., 1994, Technology for preparation of sand pear's vermouth. *Indian Food Packer*, **48**: 39-43.

AUGUSTIN, A., 1987, Cashew liquor and wine. *Indian Cashew*, **18** (2): 19-21.

*AYOGA, T.E., 1999, Evaluation and performance of yeast isolated from Nigerian palm wine production from pineapple fruits. *Biores. Technol.*, **69**: 189-190.

BAJAJ, K.B., YOUSUF, S. AND THAKUR, R.L., 2001, Selection and characterization of yeasts for desirable fermentation characterization. *Indian J. Microbiol.*, **44**: 107-110.

BARDIYA, M.C., KUNDU, B.S. AND TAURO, P., 1974, Studies of fruit wine-I. Guava wine. *Haryana J. Hort. Sci.,* **3**: 140-146.

BERRY, S.K., AND KALRA, C.L., 1989, Chemistry and technology of jackfruit (*Artocarpus heterophyllus* L.) – A review. *Indian Food Packer*, **4**: 63-73.

BHAJIPALE, B.J., GUPTA D.N. AND MEHTA, 1998, Effect of different stages of ripening of fruit of Karonda wine. *Indian Food Packer*, **54**: 27-30.

CHAROENICHAI, C., FLEET, G. AND HENSCKE, P.A., 1998, Effect of temperature, pH and sugar concentration on the growth rates and cell biomass of wine yeasts. *American J. Enol. Vitic.*, **47**: 363-368.

CHAUDARI, A.B. AND CHINCHOLKAR, S.B., 1996, New osmotolerant *Schizosaccharomyces* for ethanol production. *J. Food. Sci. Technol.*, **36**: 166-169.

CHATTERJEE, T.K., GHOSH, C.M., MUKHERJEE, K. AND ACHARY, P.M.R., 1993, Antibacterial activity of *Juniperus communis (*Linn) leaf extract in vitro. *Indian J. Microbiol.,* **33**: 272-275.

CHIKKASUBBANNA, V., CHADHA, K.L. AND ETHIRAJ, S., 1990, Influence of maturity of Thompson seedless grapes on the wine composition and quality. *Indian J. Hort.,* **47**: 12-17.

CHUNG, K.T., STEVENS, S.E., LIN, W.F. AND WEI, C.T. 1993. Groeth inhibition of selected food borne bacteria by tannic acid, propyl gallate and related compounds. *L. Appl. Microbiol.* **17** : 29-32.

DATTA, S.C. AND BISWAS, S.C., 1942, Vinegar from sugarcane and other fruit juices. *Indian Farming.* **10**: 527-530.

DETA, B.C., VIJAY, S., POONAM, S. AND SRINIVASTAVA, V.K., 2004, Physico-chemical changes of lime-aonla spiced beverage during storage. *J. Food Sci. Technol.*, **41**: 329- 332.

DIGUET, L., 1928, *Les cactacias utiles du Mexique Archives D'Historic Naturelle, Societe Nationale D'acclimatation de France,* Paris, pp. 139-142.

FIA, G., GIOVANI, G. AND ROSI, I., 2005, Study of beta-glucosidase production by wine related yeasts during alcoholic fermentation. A new rapid fluorimetric method to determine enzymatic activity. *J.Appl. Microbiol.*, **99**: 509-517.

GAUTAM, S.K. AND CHUNDAWAT, B.S., 1998, Standardization of technology for sapota wine making. *Indian Food Packer,* **52** (1): 17-21.

*GONACLAVES, D.L., 1975, Pulque, balche e pajuara universidade federal de pernambuco. *Recife*, Brasil.

GOYAL, C. AND SPOTTS, R.A., 1996, Enumeration of bacterial and yeast colonies of apple fruits and identification of epiphytic yeasts on pear fruits in the specific North West United States. *Microbiol Res.,* **151**: 427-432.

GRIEVE, M., 1967, *A modern herbal.* Hafner Publishing Co., New York and London, Pp 202, 605

HIDALGO, P., PUEYO P., POZO, M.A., MARTINEZ, J. AND POLO, M.C., 2004, Sensory and analytical studies of rose sparkling wines manufactured by secondary fermentation in the bottle. *J. Agric. Food Chem.*, **52**: 6640-6645.

HIREMATH, S.P., BADAMI, S., SWAMY, H.K.S, AND BIRADAR, J.S., 1993, Antibacterial activity of various extracts of *Acalypha indica. Indian J. Microbiol.,* **33**: 75-77

JACKSON, T. AND BADRIE, N., 2003, Utilization of banana peel in wine produced in the Caribbean effects on physico-chemical microbiological and sensory quality of wines. *J. Food. Sci. Tecnol.*, **40**: 153-156.

JOSHI, V.K., ATTRI, B.L AND BAHAJAN, B.V.C., 1991, Studies on the preparation of vermouth from plum. *J. Food Sci. Technol.*, **28**: 138-141.

JOSHI, V.K., BHUTANI, V.P., LAL, B.B. AND SHARMA, R., 1990, A method for preparation of wild apricot (Chulli) wine. *Indian Food Packer,* **44**: 50-53.

JOSHI, S.G., 2001, *Medicinal plants*. New Delhi: Oxford and IBH Publishing Co.

KARNI, P.N. AND SHANT, P.S., 1984, Commercial Kashmir apples quality cider, *Indian Food Packer,* **38** (1-6): 79-82.

KAVITHA, D. AND NIRANJALI, S., 2009, Inhibition of enteropathogenic *Escherichia coli* adhesion on host epithelial cells by *Holarrhena antidysenterica* (L.) wall. *Phytother. Res.,* **12**: 24-27

KOTECHA, P.M., 2010, Possibilities of exploitation of other fruits for wine making, Compendium of winter school on value addition of grapes with special reference to wine making at Rahuri (*Maharshtra*), 5-25 feb 10, pp. 1-198.

KROES, B.H., BERG, A.V.D., LABADIC, .P., ABEYSEKERA, A.M. AND SILVA, K.T.D., 1993, Impact of preparation process on immunomodulatory activities of the ayurvedic drug nimbaaristha. *Phytother. Res.,* **7**(1) : 35-40

KROES, B.H., BERG, A.V.D, LABADIC, R.P., ABEYSEKERA, A.M. AND SILVA, K.T.D., 1993, Fermentation in traditional medicine – the impact of *Woodfordia* flowers on the immunomodulatory activity and the alchol and sugar content of nimbaarishta. *J. Ethnopharmocol.,* **40**(2) : 117-125.

KULKARNI, J.H., SINGH, H. AND CHADHA, K.L., 1980, Preliminary screening of mango variety for wine making. *J. Food. Sci. Technol.*, **17**: 218-220.

KUNDU, B.S., BARDIYA M.C. AND TAURO, P., 1976, Studies on fruit wines : II Banana wine. *Haryana J. Hort. Sci.,* **5** (3 and 4): 160-163.

KUNDU, B.S., BARDIYA, M.C., DAULTA, B.S. AND TAURO, P., 1980, Evaluation of exotic grapes grown in Haryana for white table wines. *J. Food. Sci. Technol.*, **17**: 221-224.

LEMA, C., JARES, C.G., ORRIOLS, I. AND ANGULO, L., 1996, Contribution of *Saccharomyces* and non *Saccharomyces* populations to the production of some components of Albarino wine aroma. *American J. Enol. Vitic.*, **47**: 207-215.

LIS-BALCHIN, M. AND DEANS, S.G., 1996, Antimicrobial activity of Pelargonium speices., *L. Appl. Microbiol.,* **23** : 205-207

MARTINEZ, A.J., POLO M.C. AND CARRASCOSA, A.V., 2001. Structural and ultrastructural changes in yeast cells during autolysis in a model wine system and in sparkling wine. *Int. J. Food. Microbiol.*, **71**: 45-51.

MARTINI, A., MAURIZIO, C. AND GLORIA, S., 1996, Direct enumeration and isolation of wine yeasts from grape surfaces. *American J. Enol. Vitic,* **47**: 435-439.

MORA, J., BARBAS, J.J. AND MULET, A., 1990, Growth of yeast species during fermentation of must inoculated with *Kluyueromyces thermotolerans* and *Saccharomyces cerevisiae. American J. Enol. Vitic.,* **41**: 156-159.

*NAHVI, I., GITI, E. AND LILA, A., 2002, Isolation of a flocculating *Saccharomyces cerevisiae* and investigation of its performance in the fermentation of beet molasses to ethanol. *Biomass Bioenergy*, **23**: 481-486.

NANDINI, P.V. AND DOMMEN, B.E., 2002, Quality parameters of selected mango cultivars. *J. Food Sci. Technol.*, **39**: 379-383.

NIGAM, J.N., 2000, Continuous ethanol production from pineapple cannery waste using immobilized yeast cells. *J. Biotechnol.*, **80**: 189-193.

OLASUPO, N.A. AND OBAYORI, O.S., 2003, Utilization of palm wine (*Elaeis guinensis)* for the improved production of Nigerian indigenous alcoholic drink Ogogoro. *J. Food Pro. Preserv.,* **27**: 365-372.

ONKARAYYA, H., 1985, Mango Vermouth – a new alcholic beverage. *Indian Food Packer,* **39**(1) : 40-45

ONKARAYYA, H., 1986, A rapid modernization process to improve mango dessert wines. *J. Food Sci. Technol.*, **23**: 175-177.

PANSE AND SUKHATME, 1978, Statistical methods for agricultural workers. Indian Council Of Agricultural Research, New Delhi.

*PASTI, G., 2004, Analysis of the relationship between processing technologies for blue grapes and the quality of red wine production. *Elelmezesi - Ipar.*, **58**: 118-123.

*PATEL, J.D., VENKATRAMU, K., PRASAD, M.S. AND VENKATNARAYANA, 1984, Production of feni. *Cashew Causerie.*, **6**(3) : 8-9

PATEL, S. AND SHIBAMOTO, T., 2003, Effect of twenty different yeast strains on the production of volatile components in symphony wine. *J. Food Comp. Anal.,* **16**: 469-476.

RAI, B. AND SUBBA, D., 2003, Screening of fermentative yeasts from murcha plants and assessment of their brewing value. *J. Food Sci. Technol.*, **40**: 382-385.

RANGANNA, S. (1977) *Handbook of analysis and quality control*

REDDY, L.V.A AND REDDY, O.V.S, 2009, Production, optimization and characterization of wine from mango (*Mangifera indica* L.). *Natural Product Radiance*, **8**(4): 426-435.

RICE, A.C., FEFGUSON, J.W. AND BELSCHER, R.S., 1968, Residual sugars in New York state wines. *American J. Enol. Vitic.*, **19**: 1-5.

SAPNA, V., VASUNDHARA, M. AND ANNAPURNA, M.L., 2002, Fermented beverages from spices- a nutrceutical drink. *J. Spices Aromatic Crops,* **11**: 106-111.

SHUKLA, K.G. AND REVIS, B., 1985, Enological qualities of some orange cultivars grown in Garwal hills. *J. Food Sci. Technol.,* **22**: 72-73.

SHUKLA, K.G., JOSHI, M.C., YADAV, S. AND BISHT, N.S., 1991, Jambal wine making: standardization of a methodology and screening of cultivars. *J. Food. Sci.Technol.*, **28**: 142-144.

SINGH, H.P.P. AND MANJREKAR, S.P., 1976, Studies in the preparation of cider from north Indian apples-Varietal differences in the physico chemical characteristics. *Indian Food Packer,* **29**: 11-14.

SINGH, M., PANESAR, P.S. AND MARWAHA, S.S., 1998, Studies on the suitability of kinnow fruits for the production of wine. *J. Food Sci. Technol.,* **35**: 455-457.

SOMESH, S., JOSHI, V.K. AND GHANSHYAM, A., 2009, An overview on strawberry wine production technology, composition, maturation and quality evaluation. *Natural Product Radiance,* **8**(4): 356-365.

SRINIVASAN, D., NATHAN, S., SURESH, T., PERUMALASAMY, O., 2001, Antimicrobial activity of certain Indian medicinal plants used in the folklore medicine. *J. Ethnophamocol.,* **72**: 217-220

SRIVASTAVA, S., MODI, D. R. AND GARG, S.K., 1997, Production of ethanol from guava pulp by yeast strains. *Biores. Technol.*, **60**: 263-265.

STEINKRAUS, K.H., 1983, *Handbook of Indigenous Fermented Foods*. Marcel Dekker, Inc., Pub., New York, Pp. 305.

SUBBA RAO, M.S., 1973, Scope for development of alcoholic beverage from cashew apple. *CFTRI, Mysore,* 161-164.

SURESH, E.R. AND ETHIRAJ, S., 1987, Effect of grape maturity on the composition and quality of wines in India. *American J. Enol. Vitic.*, **38**: 329-331.

TEOTIA, M.S. MANAN, T.K., BERRY, S.K. AND SEHGAL, R.C., 1991, Beverages developement from fermented (*Saccharomyces cerevisiae*) musk melon (*Cucumis melo*) juice. *Indian Food Packer,* **45**: 49-55.

THARIB, S.M., GNAN, S.O. AND BRYAN, G. 1983 Antimicrobial activity of compounds from *Artemisia campestris. J. Food Prot.,* **46** : 185-186

*TOMINAC, V.P., BLAGOJEVIC, K., NOVAK, S., ZECHNER, K. AND MARIC, V., 2005, Fermentative properties of *Saccharomyces cerevisiae* wine yeasts isolated from the Istria region. *Periodicum-Biologorum*, **107**: 59-65.

VIDALCAROU, M.C., AMBATILE, E. A., ULLA, M.C. AND MARINE, F. A., 1990, Histamine and tyramine in Spanish wines: their formation during wine making process. *American J. Enol. Vitic.*, **41**: 150-167.

VYAS, K.K. AND KOCHHAR, A.P.S., 1993, Studies on cider and wine from culled apple fruit available in Himachal Pradesh. *Indian Food Packer*, **47**: 15-21.

WILDER, K.L., 1992, Hydrolysis of sucrose in Eastern U.S. table wines. *American J. Enol. Vitic.*, **43**: 381-383.

YAO, J. AND MOELLERING, R., 1995, Antibacterial agents. In: *Manual of Clinical Microbiology*. Ed by Murray. P., Baron. E., Pfaller. M., Tenover. F., Yolken. R., Pub. ASM, Washington DC, pp.1281-1290.

ZOECKLEIN, B.W., HACKNEY, C.H., DUNCAN, S.F. AND MARCY, J.E., 1999, Effect of fermentation, ageing and the thermal storage on total glycosides, phenol free glycosides and volatile compounds of white Riesling (*Vitis vinifera*) wines. *J. Indian Microbiol. Biotechnol.,* **22**: 100-107.

* Originals not seen

Appendices

APPENDIX - I

GENERAL COMPOSITION OF RED AND WHITE WINE

PARAMETER	RED WINE	WHITE WINE
pH	3.4	3.4
Alcohol level % (v/v)	11.7	11.5
Total SO_2 (mg/l)	106	126
Frees O_2 (mg/l)	49	32
Total acidity (g/L)	5.9	4.5
volatile acidity (g/L)	0.4	0.4
Dissolved oxygen (mg/L)	8.6	6.2
Total phenol content (mg/L)	2990	320
Absorbance at 420nm	0.230	0.012

APPENDIX-II

Guidelines for sensory evaluation of Jamun wine

1. APPEARANCE

Cloudy	.... 1 : Clearly visible suspension material
Clear	.... 3 : A very light haziness
Brilliant	.... 5 : Free from all visible colloidal suspended materials when viewed closely

2. COLOUR

Distinctly off	.... 1 : Undesirable colour not attractive
Normal and correct	.... 3 : Light rose red to dark pink colour
Slightly attractive	.... 5 : Dark pinkish red to blood red colour

3. AROMA AND BOUQUET

Aroma	.... 1 : Odour developed due to Jamun fruits
Bouquet	.... 5 : Odour developed due to processing and ageing (Matured and immature wine)

4. ACIDITY

Too high	.... 1 : Too much acidic
Slightly low or high	.... 3 : Medium acid taste
Distinctly low or high	.... 5 : Low acid wine or flat

5. SWEETNESS

Dry wine .... 1 : Very less sugar(>2^{0}B)

Medium wine .... 3 : Meduim sugar (>6^{0}B)

Sweet .... 5 : high sugar (>10^{0}B)

6. BODY

Measure of alcoholicity and expressed as degree of wateriness or viscosity of wine

Normal .... 1 : Low alcoholic wine should be watery or highly viscous

Medium .... 3 : Moderately alcoholic and watery and less viscous

Low .... 5 : High alcoholic and less watery and low or without viscous

7. ASTRINGENCY

High .... 5 : Bitterness is too high

Medium .... 3 : Moderately bitter or acceptable

Normal .... 1 : Free from bitterness

8. OVERALL ACCEPTABILITY

After taste and overall impression of the wine (like very much, like moderately, like slightly are positive characters. Dislike slightly, dislike moderately and dislike very much are negative characters)

Lacking 1

Slightly 3

Impressive 5

APPENDIX-III

Sensory Evaluation of Jamun wine

Name: -----------------------------   Date: ------------------------

Sl No	Sample code	Appearance	Colour	Aroma & Bouquet	Acidity	Sweetness	Body	Flavour	Astringency	General Quality	Total Score
1											
2											
3											
4											
5											
6											
7											
8											
9											
10											

11											
12											
13											
14											
15											
16											
17											
18											
19											
20											

Appearance-5, Colour-5, Aroma & Bouquet-10, Total acidity-5, Sweetness-5, Body-5, Flavour-5, Astringency-5, General quality-5

Signature

APPENDIX- IV

Benefit Cost ratio of best accepted Jamun wine from three batches (Jamun wine with 0.075% Kutaja powder).

Sl no	Raw materials	Quantity (for three batches)	Amount (Rs) for three litres of wine
1	Jamun	1000 (g)	110=00
2	Sugar	333×3=999 (g)	30=00
3	Kutaja	0.75g×3=2.25 (g)	3=00
4	Bottle and cork	2(No)×3=6	30=00
5	Miscellaneous	1×3=3	30=00
6	Man power	1(30 minutes)×3	45=00
		Total	248

Amount of wine recovery = 3.0 liter from 1 kilo gram of jamun fruits.

Total revenue

Cost of each bottle(500 ml) X Total number of bottles

= 125 X 6

= 750(Rs.)

Net Income

Total revenue – Cost of preparation

= 750 – 248

= 502(Rs.)

Benefit: Cost ratio = 2.02: 1