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Review

Wine production process from guava (*Psidium guajava* L.)

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The potential of wine production from guava is presented in this review. Guava is easy to culture, posseses high nutritive value and its products like juices, beverages, nectars etc. are largely appreciated by the consumers. Guava juice requires 'chaptalization' so as to adjust its Brix and prepare a perfect wine out of it. The chaptalized juice ("must") is treated with pectinase or a combination of enzymes and fermented with traditional yeasts at a temperature range of 22 to 30°C and inoculum size of 6 to 11% (v/v). The addition of N and P improves ethanol production and quality parameters of guava wine. Racking and ageing of guava wine also improves the sensory and organoleptic characteristics of guava wine.

Key words: Guava, *Saccharomyces cerevisiae*, Brix, inoculum size, temperature, DAHP supplementation.

INTRODUCTION

The nutritional role of wine is important since its average contribution to total energy intake is estimated to be 10 to 20% among adult males (Macrae et al., 1993). During the past few decades, grapes have been the main fruit for wine production. Despite that, several studies have investigated the suitability of other fruits as substrates for the purpose of wine production (Okunowo et al., 2005; Joshi and Attri, 2005). Moreover, the seasonal availability and high cost of grapes in the tropical regions has also necessitated the search for alternative fruit sources in tropical countries (Alobo and Offonry, 2009).

There is abundance of tropical fruits in India which includes guava, watermelon, pineapple, plum, orange etc. These fruits are highly perishable, being susceptible to bacterial and fungal contamination, thus leading to their spoilage, mechanical damage and over ripeness (Ihekoroye and Ngoddy, 1985). Hence, these fruits are difficult to keep for long and are utilized either as fresh or processed into juice and speciality products (Oyeleke and Olaniyan, 2007).

High rate wastage of these fruits especially at their peak of production season necessitates the need for alternative preservation and post harvest technologies towards their value addition that can reduce the level of post harvest losses besides increasing diversity of wines (Okoro, 2007; Alobo and Offonry, 2009).

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GUAVA AS A WINE SUBSTRATE

Guava (*Psidium guajava* L.) (Family Myrtaceae) is one of the most important fruits in India. It is one of the exotic fruits prized for its very pleasant, sub acidic and aromatic pulp. Guava, known as the poor man's apple of the tropics, has a long history of traditional use, much of which is being validated by scientific research. It is fourth most important fruit in India after citrus, mango and banana and second in the state of Punjab after kinnow with an annual production of 1.5 lakh MT (Stat. abs. Pb, 2009). It ranks sixth in terms of acerage under different fruits in this country and is grown in the states of Uttar Pradesh, Bihar, Madhya Pradesh and Maharashtra at large scale.

Guava has great potential for extensive commercial use because of its ease of culture, high nutritive value and popularity of processed Guava products. While ripe fruit is usually eaten as a dessert, processed products like juices, nectar, jam, jellies, baby foods, puree, beverage base, syrup and wine are also prepared from guava (Shankar et al., 2006).

Some parts of guava tree have medicinal uses as its leaves are used for curing diarrhea. Leaves can be made into tea and this astringent decocation can cure stomache and act as a vermifuge (Pranee et al., 1999). Fermented guava fruit is also helpful in curing diabetes (Kavimani et al., 1997). Besides, Guava is a good source of vitamin C, carbohydrates, proteins, minerals, pectin, calcium and phosphorus (Garg et al., 2007). The fruit contains high concentration of vitamin A (200 to 400 IU), ascorbic acid (88.2 to 250.8 mg/100 g), lycopene (45.3 µg/g FW), total sugars (10 to 15.3%), reducing sugars (2.05 to 6.08%), acids (10 to 15.3%), pectins (0.62%) and phenols (170 to 345 GAE/g FW) (Kaur et al., 2009).

CHEMICAL CHARACTERISTICS OF GUAVA

Total soluble solids

Soluble solids are the basic requirement for the function of guava or any other fruit for wine production. It has been reported that in white and pink seedling varieties of guava, the chief sugar components are fructose and sucrose (Mowlah and Itoo, 1982). The TSS content in different varieties ('Hafsi', 'Apple color', 'Allahabad Safeda', 'Sardar', 'Red fleshed guava', 'L-49') varies from 8.0 to 15.0% (Chatterjee et al., 1992; Singh, 1998; Pandey and Singh, 1998). Sharma et al. (2010) reported that the total soluble solids ranged from 9.4 to 13.5°B in 22 genotypes of guava.

Individual sugar concentration has been found to increase gradually with fruit growth and development except during the end of the growth period (Rodriguez et al., 1971). The maximum level is found to be vary from 5.64 to 7.67, 1.90 to 8.00 and 6.20 to 7.78 mg/ 100 ml of juice for fructose, glucose and sucrose, respectively, in all the cultivars studied by Rashida et al.(1997). In 126 days old cultivars of 'Shambati', 'Pakistani', 'Shendi' and 'Ganib', fructose represented 20, 38, 37 and 41%, glucose represented around 59, 23, 25 and 14% and sucrose represented around 21, 39, 37 and 45% of the total sugar for the cultivars, respectively (Sharma et al., 2010). We found reducing sugars content of 3.40, 3.29 and 3.68% in Punjab pink, Arka Amulya and Lucknow-49, respectively (Pooja, 2011). The TSS and fermentable sugars present in different cultivars of guava available in literature, thus indicate that it requires "chaptalization" (supplementation of sugars) before it is used for wine production.

Titrable acidity

Titrable acidity is an asset for the fruits destined for wine production as it provides a conducive environment to the yeasts. Guava is a sub acidic fruit as its soluble solids are composed mainly of organic acids and sugars. The guava pulp possesses a total acidity of 0.3 to 0.8% (w/v) in general with interspecies differences. In guava cultivars ('Spear Acid', 'Hisar Safeda', 'Lucknow-49', 'Patillo', 'Punjab pink, 'Arka Amulya') acidity has been found to be in the range of 0.37 to 0.96% (Jain and Nema, 2007; Sharma et al., 2010; Pooja, 2011). Aulakh (2004) found that the amount of titrable acidity increases continuously from 30 days after fruit set to 135 days after fruit set in winter season (0.31 to 0.62%). Similarly, during rainy season it was found to increase up to 0.58 from 0.28% at 110 days after the fruit set. The organic acids (0.3 to 0.8%) contributing to acidity in Guava are mainly citric, malic, glycolic, tartaric and lactic acids (Hui, 2006). Further, acidity was found to be season dependent with 'Allahabad Safeda' and 'Sardar' guava cultivars showing 0.25 and 0.19% during rainy season and 0.31 and 0.33% during winter season crop.

The major organic acids in guava pulp have been found to be citric, malic, glycolic, acetic and lactic acids. Archana and Siddiqui (2004) found that acetic acid ranged from 55.40 to 122.13 µ mol kg^{-1}. Quantitative determinations using succinic acid as an internal standard showed that citric and malic acid are chief organic acids with lactic acid in smaller quantities among cultivated guavas. However, in wild guavas, citric acid was the predominant acid, with lesser amounts of malic and lactic acids (Chan et al., 1971). Fifty one acids have been identified in guava (*P. guajava* L.) with (E)-cinnamoic acid (0.4 mg/kg) and (Z)-3-hexenoic acid (0.2 mg/kg) as major constituents (Idstein et al., 1985).

Ascorbic acid

Vitamin C (L-ascorbic acid) is the lactone 2, 3-dienol-L-gluconic acid and it belongs to the water soluble class of vitamins. Humans need a daily intake of 30 to 45 mg per day while deficiency of this vitamin leads to scurvy. Though, oranges are well known as fruits rich in vitamin C but guava is far superior with vitamin C content three to six times higher than that in orange.

Also, White fleshed guava is reported to be a better source of vitamin C (142.6 mg/100 g) than pink fleshed guava and is also rich in other antioxidants such as phenolics and ß-carotenes. The vitamin C content varies widely depending on the cultivar as ascorbic acid content of guava cultivars ranges from 149.0 to 250 mg per 100 g of pulp (Pandey and Singh, 1998; Bal and Dhaliwal, 2004; Thaipong and Boonprakob, 2005). Thaipong et al. (2005) observed highest vitamin C content in 'Fan Retief' (397 mg/100 g FW). In our studies, ascorbic acid content ranged from 169.7 to 229.6 mg/100 g DW in three varieties of guava (Pooja, 2011).

Pectin

Guava contains high content of pectin (a structural heteropolysaccharide contained in the primary cell walls

of terrestrial plants), cellulose and hemicellulose. Pectin content ranges between 0.47 to 1·00% in different varieties (‘Allahabad safeda’, ‘Banarsi surkh’, ‘Lucknow-49’, ‘Shambati’ and ‘Shendi’) of guava (Rodriguez et al., 1971, 1997; Panda et al., 2009). Further, total pectin for different cultivars significantly increases with fruit growth and development. In ‘Pakistani’ and ‘Ganib’ cultivars, it was found to reach its maximum when the fruits were 106 days old. High content of pectin causes problems in juice extraction which results in unclarified juice leading to haziness in produced wine. Hence, to increase the juice extraction and for production of clear wine, a pectinase enzyme pre-treatment is necessary.

PREPARATION OF WINE FROM GUAVA

Pre-fermentation treatment

Commercially available enzymes have been widely used in the oenological industry in wine- producing countries to improve important characteristics of wines, such as aroma and colour. They are used to increase the grape “must” yield during pressing, facilitate the settling of “musts”, and improve clarification and filtration. The use of pectolytic enzymes has been shown to be suitable to improve the extraction of colour in red wines (Revilla and González, 2003; Bautista-Ortín et al., 2005), aroma compounds (Cabaroglu et al., 2003) and soluble polysaccharides (Doco et al., 2007) from the skins and pulp of the grapes. One of the most studied and widely used commercial pectinases is polygalacturonase.

Commercial preparations containing pectinases, arabinase and cellulase have also been employed for guava juice extraction (Kashyap et al., 2001). Besides, pectinases have also been used to clear the haze in finished wines and hence clear the wine.

Ahmad et al. (2009) reported that maximum yield (85.1%) and clarity (93.5%) were obtained using 0.245% pectinase, 0.135% cellulase and 0.13% hemicellulase at 50°C for 9.25 h incubation. A similar effect was also reported by Diwan and Shukla (2005) in enzymically hydrolyzed Guava pulp under different set of conditions. Kaur et al. (2009) recommended optimized enzymatic treatment conditions as enzyme concentration 0.70 mg/100g Guava pulp, incubation time (7.27 h) and incubation temperature of 43.3°C.

Pectinases have also been shown to be affected by temperature and time of enzyme treatment as increasing exposure time elevates yield but also causes a reduction in ascorbic acid content of the juice due to its oxidation (Imungi et al., 1980). Thus, pectinase has been shown to affect the oenological properties of wines. However, the effect of pectolytic enzymes using “must” from sun-dried grapes of the Pedro Ximenez variety on oenological parameters before and after enzymatic treatments with pre-fermentative maceration at room temperature for three hours revealed that the enzyme treatment had no effect on total polyphenols and other chemical characteristics (Espejo and. Armada 2010). In guava, we have seen that a temperature of 45°C, for 6 h is sufficient to yield a 47% clarity in juices of different varieties of guava studied (Pooja, 2011).

Selection of yeast

Yeast as a group of microorganisms has been quantitatively and commercially exploited as a fermentative species to carry out alcoholic fermentation and this has urged many scientists to study the factors governing its growth, survival and biological activities in different fruit ecosystems (Heard and Fleet, 1985). Yeasts play a prominent role in wine fermentations, which can strongly affect the quality and flavour of the final product (Querol and Fleet, 2006). Yeast owes its inverting and fermentative property to the various enzymes present in it like sucrase, zymase, maltase, lactase, reductase, carboxylase etc. But yeast of different species do not contain the same enzymes and hence different yeast species behave differently towards the various sugars. Among several yeasts, *Saccharomyces cerevisiae* and *S. bayanus* var. *uvarum* are the most important species present during the fermentation process (Pretorius, 2000; Querol and Fleet, 2006). It has been established that the growth of yeast during fermentation depends on the media composition (substrate), the initial levels of pH, temperature and dissolved oxygen (Ruiz et al., 2004).

The selection of a good yeast strain having desirable properties is a prerequisite for the quality wine production (Degree, 1993). Enological traits of *S. cerevisiae* have been divided into two groups, that is, technological and qualitative, and both groups have to be considered in the selection of wine yeasts. The technological ones influence the fermentation efficiency, and the qualitative ones determine the chemical composition and sensorial characteristics of wines. In traditional winemaking, natural fermentation of grape juice is carried out by a sequence of different yeast species. The early stages of the alcoholic fermentation are dominated by the growth of non-*Saccharomyces* yeasts, characterized by a low fermentative power. Of these, *Hanseniaspora* (*Kloeckera*) and *Candida (*e.g. *Candida stellata* and *Candida pulcherrima*) are more frequently the principal yeasts observed both in spontaneous and inoculated fermentations (Heard and Fleet, 1986). This is followed by appearance of more ethanol tolerant *Saccharomyces* sp. Various strains of *S. cerevisiae* are available as starter cultures to supply distinctive sensory attributes to wine (Cavazza et al., 1989). Winemakers rely on rapid fermentations and they produce predictable flavor and aroma characteristics in the finished wine. This makes research on genetics and physiology of wine yeast, of

paramount importance. The desired *S. cerevisiae* characteristics for winemaking include: Osmotolerance, relative insensitivity to high acidity, and acceptance of low oxygen concentrations.

In some cases, wine produced with yeast mono-cultures lacks flavor complexity that may originate from good indigenous fermentations. The potential of non-*Saccharomyces* yeasts to enhance wine aroma intensity and flavor complexity is also found to be considerable. Some of these strains such as *Kloeckera apiculata*, *Pichia fermentans, C. stellata* and other species have been studied for their interesting organoleptic contributions (Clemente et al., 2005; Ugliano and Henschke, 2009). Moreover, the wine yeast, *S. cerevisiae* also plays a central role in the production of volatile sulfur compounds. Through the sulfate reduction sequence pathway, the hydrogen sulphate (HS) is formed, which can lead to the formation of hydrogen sulfide and various mercaptan compounds, thus adding bouquet to the wine (Swiegers and Pretorius, 2007).

Guava 'must' fermentation

The chief sugars of guava juice are glucose and fructose that are fermented by *S. cerevisae* to produce ethanol. A number of factors affect yeast fermentation performance like the nature of yeast strain, fermentation temperature, media composition, pH, substrate concentration, mode of substrate feeding, ethanol concentration etc (D'Amore, 1992). Hence, these factors must be studied in detail, especially the interactions between them and their influence on fermentating microorganisms. The effect of important fermentation parameters as fermentation of guava is described subsequenty:

Effect of temperature

Temperature can affect the sensitivity of yeasts to alcohol concentration, growth rate, rate of fermentation, viability, length of lag phase, enzyme and membrane function, etc. Because yeast strains differ in response to temperature, the optimum temperature for vinification can vary widely. Torija et al. (2001) observed a mixed response to fermentation temperature (15 to 35°C) on mixed strain population of *S. cerevisiae*. Alcohol yield was higher at higher temperature while at lower temperature secondary metabolites such as volatiles, esters, glycerol etc. increased (Roehr, 2001; Torija et al., 2003; Pramanik, 2003; Robinson, 2006). Fermentations conducted under low temperatures enable a rise in production and aroma retention, which may favor an improvement in the aromatic profile of the wine (Torija et al., 2003). This is because fermentations alter yeast nitrogen transport and metabolism thus hampering the coordination between carbon and nitrogen metabolisms at low temperatures (Beltran et al., 2007). On the other hand, high temperature may disrupt enzyme and membrane functions, resulting in stuck fermentation (Sener et al., 2007). Fermentation at higher temperatures may have adverse effect on the wine in stunning the yeast to inactivity and even "boiling off" some of the flavors of the wines.

Among the different reports available in literature on guava fermentation, a temperature range of 25 to 30°C has been reported for different varieties (Srivastava, 1997; Shankar et al., 2006; Yu and Zhang, 2008; Sevda and Rodrigues, 2011; Pooja, 2011). Sevda and Rodrigues (2011) reported that for *S. cervisiae* NCIM 3095, the maximum ethanol production is 7.784 % (v/v) at 25°C and for NCIM 3287, the maximum ethanol production is 8.396% (v/v) at 25°C.

High temperature shift (Heat shock) has been used for increasing glycerol production by yeast which contributes towards smooth mouth feel in guava wine production (Sevda and Rodrigues, 2011). Among the temperature range (15 to 35°C) studied by us, the results revealed the maximum ethanol production of 11.1, 11 and 11% at 25°C in three guava varieties viz. Punjab pink, Arka amulya and Lucknow-49, respectively (Pooja, 2011).

Effect of pH

The pH of the growth medium is another important parameter for the successful progress of fermentation because it influences yeast growth as well as ethanol formation besides sensory quality of wine. It is known that a wine with a pH of less than 3.4 presents a notable resistance to bacterial attack. However, in a wine with a pH more than 3.6, the development of harmful microbial flora may occur. On the other hand, fermentations conducted in excessively acidic media become too slow due to the low growth rate of the yeast (Ough, 1991). The pH of the medium may change in response to metabolic activities of microorganisms. Also, during the course of fermentation, nitrogen source can significantly affect the pH and therefore initial pH of the medium must be adjusted carefully.

In fact, combined treatment of the "must" by pH adjustment and sulfur dioxide addition has been considered as an appropriate technique to prevent microbial spoilage in wine fermentation because low pH can improve the pasteurization effect of sulfur dioxide and give the winemaker important information about how much sulfur dioxide is needed to control microbes effectively. This is significant because high sulfur dioxide content in the "must" negatively affects human health (Robinson, 2003).

pH has been found to affect malic acid (an important volatile compound affects titrable acidity of wine). It has been reported that malolactic fermentation (MLF) rates are directly proportional to initial pH values and MLF is

associated with greater diacetyl, acetoin, and volatile acidity production (Bousbouras and Kunkee, 2007; Liu and Gallander, 1983). Guava 'must' had a pH range between 4.2 to 4.4 in three different varieties studied by us (Pooja, 2011) which was suitable for its fermentation. A pH level of 4.0 to 5.0 was found to be optimum for guava "must" fermentation in other studies too (Yu and Zhang, 2008; Shankar et al., 2006; Sevda and Rodrigues, 2011).

Effect of initial sugar level

The concentration of initial sugars is an important parameter in the final ethanol production and its sensory quality. It has been observed that initial sugar level of juice greatly affects the rate of fermentation. Use of concentrated sugar substrate is one of the ways to obtain high ethanol yield during fermentation. However, high substrate concentrations are inhibitory to fermentation due to osmotic stress (Jones et al., 1981). There are reports that high sugar sensitivity (osmotolerance) in *S. cerevisae* is due to higher intracellular accumulation of ethanol (Strchaiano and Goma, 1983). Different strains present variable levels of tolerance to this accumulating ethanol and hence their requirement of different initial sugar concentration for fermentation. In some strains, this level is just 10 to 15% (Praminik, 2003; Asli, 2010) and this has been observed in guava fermentation also (Cheema, 1989; Srivastava et al., 1997). However, initial sugars as high as 25 to 30% have also been employed successfully for ethanolic fermentation (Bertolini et al., 1991). In guava, we optimized 25% (w/v), Sevda and Rodriguez (2011) optimized 20 to 22% (w/v) and Yu and Zhang (2008) optimized 20% (w/v) as initial sugar concentration for guava wine production.

The effect of initial sugar concentration on time of fermentation has also been observed as higher sugars tend to prolong fermentation (Borzani et al., 1993). Higher initial sugars also possess better retention of ascorbic acid, increase in concentration of total esters and phenols thus improving the wine quality (Attri, 2009).

Effect of inoculum size

The standardization of inoculum size is important as sugar consumption is a balance between biomass development and ethanol production and a high inoculum size will thus be a compromise on amount of ethanol produced. We have observed that ethanol production increases with increase in inoculum concentration up to 9% (v/v) and decreases significantly beyond inoculum level of 9% (v/v) in case of three varieties of guava viz; Punjab pink, Arka amulya and Lucknow-49 (Pooja, 2011). Similar trends have also been reported by Singh and Kaur (2009) where they observed 10% (v/v) as optimized inoculum level for litchi wine production.

In guava, Srivastava et al. (1997) reported that 10% inoculum size added in non chaptalized guava pulp led to the production of 5.8% ethanol (w/v) by *S. cerevisae*. An optimized inoculum level of 10% v/v for alcoholic fermentation of jamun, plum, apple, pear juice, guava and 7.5% inoculum size for kinnow wine production has been observed in other research reports (Tewari et al., 1987; Cheema, 1989; Panesar et al., 2009).

Effect of nitrogen and phosphorus sources

The intrinsic importance of assimilable nitrogen to yeast growth and metabolism is a well known recognized fact in wine making. Factors such as the juice composition and the kind of the yeast strain can affect the assimilation of the nitrogenous compounds (Colombié et al., 2007; Manginot et al., 1998) as the metabolism of amino acids can affect the efficiency of the alcoholic fermentation and the quality of the product (Berthels et al., 2004). Imbalances and in particular deficiencies in the supply of assimiable nitrogen compounds has remained as the most common causes of stuck/sluggish fermentation. Presence of sufficient amount of nitrogen in the fruit juice is reported to enhance the yeast growth and sugar catabolic rate. However, addition of excess N and P source can inhibit the fermentation process. Hence addition of N and P sources in an optimum quantity is an important fermentation parameter.

Further, fermentation process not only depends upon the quantity of N and P added, but also on the quality of these supplements. Various nitrogen and phosphorus sources like ammonium sulphate, diammonium phosphate, potassium dihyrogen phosphate, di potassium hygrogen phosphate, amino acids, etc. have been used to carry out fermentation efficiently and rapidly in different fruit wine fermentations (Ough, 1991; Shankar et al., 2006; Soni et al., 2009; Asli, 2010; Pooja, 2011).

It has been found that di-ammonium hydrogen ortho phosphate (DAHP) supplementation improves the wine colour, total acids, bouquet, taste, aroma and overall sensory quality. Shankar et al. (2006) reported that wine produced from Guava pulp (1:4 dilution with water) with 0.1% DAHP supplemention was better in its sensory attributes and had the high percentage ethanol than the non supplemented one. Patil and Patil (2006) had similar observations with pineapple wine fermentation. The effect of DAHP supplementation at the rate of 0.3% (w/v) produced significantly higher ethanol percentage of 13.6±0.2 with a fermentation efficiency of 93.8 ± 0.8% in three different varieties of Guava (Pooja, 2011). A scheme for preparation of guava wine is presented in Figure 1.

POST FERMENTATIVE TREATMENTS

Consumer acceptability is the final goal of wine

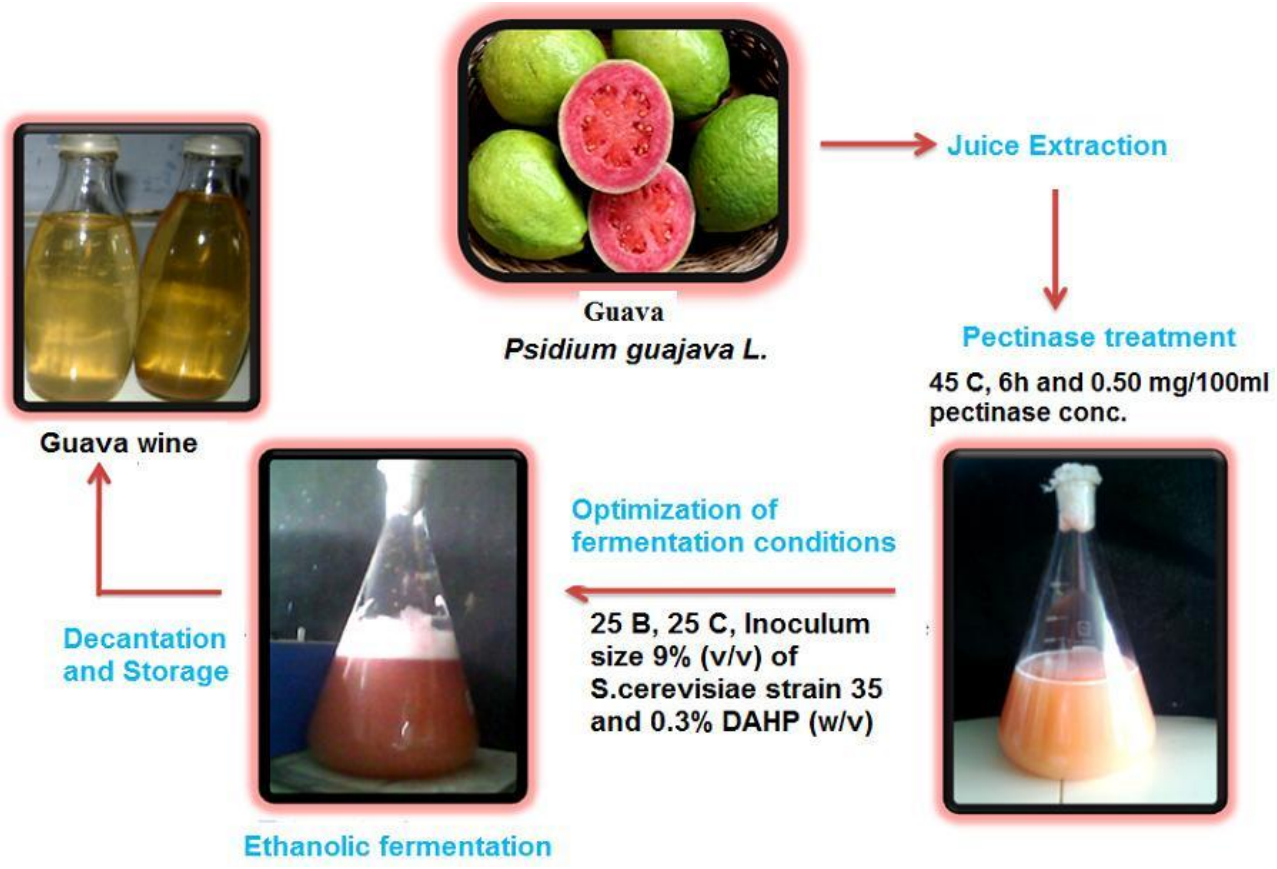

Figure 1. Scheme for preparation of Guava wine.

development. The crude wine obtained after fermentation contains yeast, protein hazes, residual sugars and does not have complete quality (sensory) attributes. Hence, it requires post fermentative treatments (finishing) to make a wine potable. In fact, there are five goals of "finishing" a wine: clarity, stability, compositional adjustment, possible blending and packaging. It is important, especially in white wines like guava wine, that the wine at the point of consumption should not be cloudy or contain any haze or precipitate. It is also important to prevent unwanted microbial growth from occurring in the wine after the primary fermentation is complete as exposure to air (after CO_2 blanket has dissipated) will affect the flavor and aroma profile (Robinson, 2003).

Racking

Racking is the process of siphoning the wine into a new, clean barrel. Racking allows clarification and aids in stabilization. Wine that is allowed to age on the lees often develops "off-tastes". A racking hose or tubing is used and can be attached to a racking cone to make this task easier. The racking process is repeated several times during the aging of wine. Repeated racking produces the clarity required in wine, especially if it is aged in a barrel (Robinson, 2003). Besides clarification, racking also provides suitable conditions for oxygen to dissolve in the wine, at a rate varying from 2.5 to 5mg/L. Oxygen eliminates certain unpleasant reduction odors (H_2S), as well as iron (ferric casse) and is also responsible for intensifying color of wine (Ribereau-Gayon et al., 2000). We observed that during the storage period (up to 90 days) of guava wine, various parameters (pH, ascorbic acid, total phenols, percentage ethanol) decreased significantly. Inspite of differences in initial ethanol levels the final percentage ethanol (at 90 days) was constant at 12.6 to 12.8 which are reasonably good for wine (Pooja, 2011). Yu and Zhang (2008) reported filtration and pasteurization of young guava wine to prepare a clear guava wine.

Effect of fining agents

Fining is the non-mechanical removal of unwanted and/or unstable elements in juice or wine by the addition of inert and adsorptive substances. It generally involves the formation of an insoluble deposit which is separated from the liquid by either filtration or racking. Interactions may

Table 1. Effect of storage time on microbiological and physicochemical properties of wine var. Punjab pink, Arka amulya and Lucknow-49.

Storage time (days)	Parameters														
	Percentage ethanol (v/v)			pH			Ascorbic acid (mg/100ml)			Total phenols (mg/100ml)			Total yeast count (cfu/ml)		
	Punjab pink	Arka amulya	L-49	Punjab pink	Arka amulya	L-49	Punjab pink	Arka amulya	L-49	Punjab pink	Arka amulya	L-49	Punjab pink	Arka amulya	L-49
0	13.8	13.5	13.5	3.4	3.6	3.5	83.6	76.0	91.2	337.0	268.0	246.0	$7.3X10^6$	$6.2X10^6$	$7.0 X 10^6$
15	13.7	13.3	13.5	3.4	3.6	3.5	80.3	74.3	88.7	326.0	257.0	232.0	$2.1X10^1$	$1.5X10^1$	$1.1 X10^1$
30	13.5	13.2	13.3	3.4	3.5	3.4	77.6	70.6	86.0	318.0	242.0	229.0	$0.3X10^1$	$0.6X10^1$	$0.4X10^1$
45	13.5	13.2	13.1	3.3	3.5	3.4	75.2	68.4	84.1	315.0	231.0	201.0	0.0	0.0	0.0
60	13.3	13.0	12.8	3.2	3.5	3.4	71.9	67.2	80.3	302.0	229.0	194.0	0.0	0.0	0.0
75	12.9	13.0	12.7	3.3	3.4	3.4	67.0	65.1	78.9	287.0	215.0	189.0	0.0	0.0	0.0
90	12.8	12.8	12.6	3.3	3.4	3.4	63.0	64.2	76.0	281.0	211.0	180.0	0.0	0.0	0.0
CD (5%)		0.291			NS			0.754			0.984			-	

Residual sugars in all the varieties were not detected even at 0 day of storage.

include electrostatic charges, hydrogen bonds, ion exchange and hydrophobic reactions. Fining agents are used to achieve clarity and to improve color, flavor and physical stability e.g., Earths (bentonite), proteins (gelatin, isinglass, casein, albumen), polysaccharides (agars), carbons, synthetic polymers (PVPP), silicon dioxide (kieselsol) and others (including chelators and enzymes).

Certain gelatins can significantly reduce acetic acid bacteria and yeast populations, compared with samples that have been racked but not fined (Murat and Dumeau, 2003). Clarification of apple juice by flocculation and precipitation with bentonite and gelatin was determined by turbidity and zeta-potential which was treated with Polyvinylpolypyrrolidone (PVPP) to remove total polyphenol. Results indicated that risk of haze by free gelatin in juice required at least ten times more gelatin than the optimum dosage for clarification (Benitez and Lozano, 2007).

The effects of different fining agents, used at different concentrations, on the antioxidant status of fined wines were studied by Yildirim (2011). The results demonstrated that the use of a combination of gelatin and Kieselsol led to the highest total phenol value (3,491 mg/L GAE) and antioxidant activities (29%) among the tested fining agents. The results of the grouping of analyzed parameters in n-dimensional space, with different fining agents at different concentrations, demonstrated the importance of a low concentration of fining agents for high antioxidant activity and total phenols. In literature, however we did not came across any reference of fining the guava wine.

Ageing

The ageing of wine and its ability to potentially improve wine quality for its consumption, is one of the most important step after wine production (Robinson, 2006). The ratio of sugars, acids and phenolics to water is a key determination of how well a wine can age. Higher temperatures accelerate the aging process dramatically (e.g, storing wine at 59 degrees ages 50% faster than 55 degrees). Faster aging increases the rate of undesirable chemical reactions which can produce compounds with foul odors and off tastes (Robinson, 2006). Ageing in wooden barrels improves the wine quality by adding desirable components including ethyl acetate, phenolics etc. and leads to reduction in undesirable components such as n-proponal, n-butanol, iso-butanol, isoamyl alcohols (Soni et al., 2009). Soni et al. (2009) also reported that storage of amla wine in oak wooden barrels for a month improves the quality and sensory attributes than the wine stored in glass bottles. We stored our guava at 15°C and recorded significant decrease in phenols, ascorbic acid, ethanol and viable count. The final wine at

the end of 3 months had 12.6±0.2% ethanol (v/v), 67.73±7.18 ascorbic acid (mg/100ml), and 224.0±51.7 total phenols (mg/100 ml) and was free of viable yeast cells (Table 1).

Sensory evaluation

The sensory analysis of wine is an important parameter in determining the quality of wines. It revolves around the taste, feel, aroma and bouquet of the aged wine. A number of methods in the form of hedonic scales and analytical techniques like GCMS have been developed (Amerine and Roessler 1976; Reynolds, 2010). So much is the importance of sensory evaluation that capturing consumers' mind and attitudes towards wines is a flourishing business (Lesschaeve, 2007). Shankar et al. (2006) highlighted the increase in aroma and flavor of guava wine with supplementation of N and P in the "must". We also found that guava wine from three varieties that is, Punjab pink, Arka amulya and Lucknow-49 had enhanced taste, aroma and flavor with ageing of three months (Pooja, 2011).

CONCLUSIONS AND FUTURE PERSPECTIVES

Wine is one of the functional fermented foods having many health benefits like anti-ageing effects, improvement of lung function (from antioxidants in white wine), reduction in coronary heart disease, development of healthier blood vessels and reduction in ulcer-causing bacteria. Many wines are made from fruits having medicinal value (Tapsell et al., 2006). In India, wine industry is grape based and is still in its infancy (Joshi and Attri, 2005) with wineries restricted to Maharashtra. Out of the total 60 wineries in the country, 57 are located in Maharashtra, 2 in Karanataka and 1 in Goa (Patil, 2008). As far as Punjab is concerned, inspite of 61,618 ha under fruits and a production of 10,55,408 MT annually production with guava contributing 1.5 lakh MT (Gill et al., 2009), no winery has been set up here yet. So, developing technology for production of guava wine in India can found to be of great benefit. Further, Guava wine may prove to be a quality wine with alcohol (stimulant) and high contents of phenols and ascorbic acid (antioxidants) besides increasing the economic status of Indian farmers especially during period of glut.

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