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Fermentation of Litchi (*Litchi chinensis* Sonn.) Fruits into Wine

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ABSTRACT

A wine from litchi (*Litchi chinensis* Sonn. var. *Shahi*) fruits having high nutritional value was prepared by fermentation using wine yeast (*Saccharomyces cerevisiae* var. *bayanus*). The wine was light yellow in colour, acidic in test [titratable acidity (0.59 g tartaric acid/100 ml)], rose-flavoured and with tannin (0.72 mg/100 ml) and low ethanol (6.5%) concentration. The sensory evaluation rated the litchi wine quite acceptable as an alcoholic beverage Principal component analysis (PCA) reduced the eight original analyses (proximate) variables to two independent components (factors, PC1 and PC2), which accounted for 100% of the variation of litchi wine. Similarly, PCA analysis reduced the five original sensory attributes to three independent components (PC1, PC2 and PC3) that accounted for 79.3 % of the variation of litchi wine.

Keywords: principal component analysis, *Saccharomyces cereviseae* var. *bayanus*, sensory evaluation, titratable acidity, total soluble solids, tropical fruits
Abbreviations: PC, principal component; **PCA**, principal component analysis; **SMS**, sodium metabisulphite; **TA**, titratable acidity; **TSS**, total soluble solids

INTRODUCTION

Litchi (*Litchi chinensis* Sonn.) is an evergreen sub-tropical fruit crop belonging to the family *Sapindaceae*, which is indigenous to Southern China and northern Vietnam (Sivakumar *et al*. 2007). This tree is highly specific to climatic requirements and probably due to this reason its cultivation is restricted to a few countries in the world. India and China account for 91% of the world litchi production (Jiang *et al*. 2003). India produced 429,600 metric tonnes of litchi annually from 60,200 ha (National Horticulture Board, New Delhi 2005). The litchi tree is 3-4 m in height, dense, round-topped and slow-growing with evergreen leaves having 6- 9 elliptic, oblong and lanceolate abruptly pointed leaves. The fruits, commonly called a litchi nut, are about (2.5-3.8 cm) in diameter when fresh, and have a red brittle shell with white translucent flesh and a single large seed (Jiang *et al*. 2003). The nutritive value and pleasant rose flavour of litchi fruits make its juice a delicacy. Litchi fruits provide carbohydrates, organic acids (i.e. lactic, acetic, succinic, citric and phosphoric), vitamin C, aroma compounds (β-damascenone, linalool, furaneol, ethyle hexanoate, geraniol, etc.) (Ong and Acrees 1999) and minerals like calcium, magnesium, potassium, phosphorus and iron (Mahajan and Goswami 2004) and consumption of litchi fruit would meet 2-4% of the dietary reference intakes (Wall 2006). Despite these high quality attributes, litchi fruits are perishable (Sivakumar *et al*. 2007) and get spoiled within 4-5 days after harvest which is due to an unidentified physiological disorder (Revathy and Narasimham 1997). Litchi varieties grown in India are highly variable under different climatic conditions. According to Singh (1997), 33 varieties of litchi were grown in India; among these '*Shahi*' is still the most popular variety due to its high sugar content (18–22° Brix) and distinct (rose) aroma. The fruits ripen in May.

Principal component analysis (PCA) is used in all scientific branches (Arvanitoyannis and Tzouros 2005; Mohapatra *et al*. 2007). This method is advantageously applied for the evaluation in food product analyses (Mohapatra *et al*. 2007). PCA is used for the reduction of information on a large number of variables into a small set while loosing only a small number of information (Fievez *et al*. 2003). The major feature of this method is a reduction of the dimensionality in a set of variables by constructing an uncorrelated linear combination of them. The combinations are computed in such a way that the first component accounts for the major part of the variance, that is the major axis of the points in the *P*-dimensional space (Tzouros and Arvanitoyannis 2001). For example, PCA analysis of sweet potato curd reduced the seven original sensory/functionnal attributes (sweetness, colour, texture, flavour, appearance, overall acceptability and purchase orientation) to three independent principal components (PC), i.e. PC1 (35%), PC2 (20%) and PC3 (15%) that together accounted for 70% of the total variation (Mohapatra *et al*. 2007).

Very little information is available on the processing of litchi fruits except for juice preparation. Wine is prepared from litchi fruits in China in a traditional way (http://www.fao.org), but no scientific procedure is followed. In view of many medicinal and aromatic properties of litchi fruits and because of its short availability period and difficulty in storage conditions (Revathy and Narasimham 1997; Sivakumar *et al*. 2007), we made an attempt in this study to prepare wine from litchi fruits using the wine yeast, *Saccharomyces cerevisiae* var. *bayanus*, previously used for fermenting cashew (Mohanty *et al.* 2006) and the sensory attributes (taste, aroma, flavour, colour/ appearance and aftertaste) were evaluated. For the evaluation of the results of biochemical composition and consumers' acceptability (sensory analyses) of litchi wine, multivariate statistical methods (correlation analysis and PCA) were employed.

MATERIALS AND METHODS

Litchi

Fully ripened litchi fruits (var. *Shahi*) were collected from the experimental plots of the National Research Centre for Litchi, Mu-

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Table 1 Chemical composition of litchi fruits (var. Sahi).

Components	Pulp (g/100 g)	Juice (g/100 ml)
Weight	69.00 ± 4.50	N.D.
Moisture	80.70 ± 5.20	N.D.
Total sugar	38.00 ± 3.10	31.60 ± 4.10
Reducing sugar	31.40 ± 4.30	26.90 ± 3.30
Ascorbic acid (Vitamin C)	7.80 ± 0.77	6.84 ± 0.52
Phenol	1.80 ± 0.05	0.22 ± 0.06
Tannin	1.30 ± 0.03	0.85 ± 0.07
Total nitrogen	0.13 ± 0.00	N.D.
Ash	0.32 ± 0.05	N.D.

All values ± standard error.

zaffarpur, Bihar, India in May 2006 (day temperature, 38 ± 2°C, night temperature, 30 ± 2°C). The litchi fruits were brought fresh to the laboratory and immediately processed. The composition of litchi fruits is given in **Table 1**.

Wine yeast

The wine yeast *S. cerevisiae* var. *bayanus* was maintained on potato dextrose agar slants at 10°C in a refrigerator. The yeast culture was a gift from Dr. E. Suresh, Principal Scientist (Microbiology), Indian Institute of Horticultural Research, Bangalore, India and is frequently used in wine making (Mohanty *et al*. 2006; Chowdhury and Ray 2007).

Fermentation process

One kilogram of litchi fruits were peeled by hand; skin and seeds were separated from the pulp. Then litchi pulp was crushed in a mixture/grinder (TTK Prestige Ltd., Bangalore, India) and the juice was extracted by using a juice squeezer. The juice (must) filtered through cheese cotton cloth had 17° Brix and was treated with sodium metabisulphite (SMS) (100 µg/ml) to inhibit the growth of undesirable microorganisms such as acetic acid bacteria, wild yeasts and moulds. The pH of the juice was adjusted to 3.8 with 1 N tartaric acid and then inoculated with 2% (v/v) starter culture (prepared with grape juice) of *S. cerevisiae* var. *bayanus*.

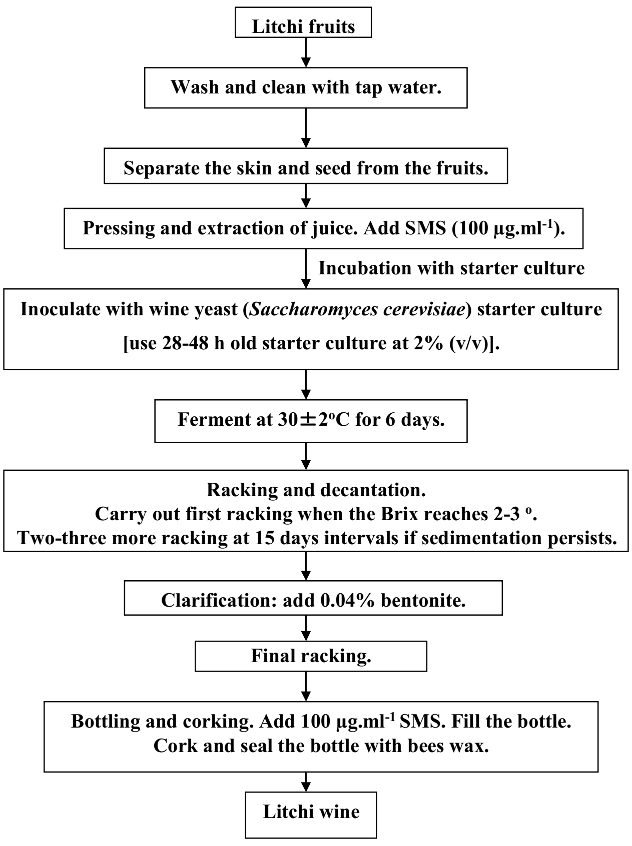

Fig. 1 A flow chart for making litchi wine.

Fermentation was carried out at a temperature of 30 ± 2°C for 6 days. Racking of wine was carried out when total soluble solids (TSS) reached 4-6° Brix. Two or three more rackings were done at 15 days intervals to remove any sediment deposited in wine. After racking, the wine was clarified with the addition of 0.04% bentonite. Another dose of SMS (100 µg/ml) was added as preservative before bottling. This experiment consisted of two replicates. The flow chart for making litchi wine is shown in **Fig. 1**.

Biochemical analysis

The chemical compositions of litchi fruits were determined as follows: moisture was determined by vacuum oven, weight of pulp was determined by an electronic balance and values were expressed as g/100 g (Analytical methods for Tuber Crops 2000). Reducing sugar, total sugar and ascorbic acid were quantified by Nelson's (Nelson 1944; Mahadevan and Sridhar 1993) methods, respectively, and were expressed as g/100 g. The phenol and tannin content were estimated by the method described by Amerine and Ough (1984) and expressed as g/100 g.

The chemical composition of litchi must and wine was determined as follows. Total titratable acidity (TA), tannin, lactic acid and ethanol content of wine were determined by the methods described by Amerine and Ough (1984). Reducing sugar in must/wine was quantified by Nelson's method (Nelson 1944). Total phenolic content in must/wine was quantified by the Folin-Ciocalteu method (Bray and Thorpe 1954). The pH of the must wine were measured using a pH meter (Systronics Ltd. Ahmadabad, India). TSS of must and wine were determined by using a hand refractometer (Sipcon, Jallandhar, India).

Sensory evaluation assay

Sensory attributes (taste, aroma, flavour, colour/appearance and aftertaste) were evaluated using a 5-point Hedonic scale (where 1 = dislike extremely and 5 = like extremely) by 16 panelists (8 men and 8 women, aged 20-35 years) selected from post-graduate students, staff and faculty of several horticultural-related departments who were familiar with wine consumption. Samples were served in clean transparent glasses (tumblers), which had been labeled with 3 digit random numbers. Questionnaires and water for mouth ranging between each tasting were provided. Prior to evaluation, a session was held to familiarize panelists with the product. The panelists were asked to read through the questionnaires, and the meaning of each attribute (taste, aroma, flavour, colour/appearance and aftertaste) was explained to the panelists to avoid any misinterpretation (Kilcast and Subramanian 2000). Tasters were not allowed to discuss their scores with one another during the evaluation session.

The litchi wine produced was presented to the trained panel of sensory analysts. Another set of litchi wine sample was evaluated as a second replication the following day. The sensory evaluation data were presented as the means of the panelist's score. A standard *t*-test was used to test for the statistical significance of the differences observed between the scores of the two sets (Cass 1980).

Statistical methods

For the evolution of the sensory and analytical results, the multivariate statistical methods, correlation analysis and PCA, were applied. The data matrix of the analytical and sensory results was analyzed by SPSS (SPSS Software for Windows release 10.0; SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Many tropical fruits such as mango, jackfruit, litchi, banana and cashew apple have been shown to be suitable for fermentation, mainly because of their appropriate taste, flavour, availability, high sugar and water content and overall chemical composition (Muniz *et al*. 2008).

The composition of must and wine prepared from litchi is presented in **Table 2**. Must is defined as the expressed juice of fruit along with some amount of skin and particles of flesh before and during fermentation whereas wine is the

Table 2 Composition of litchi must and wine.

Parameters	Litchi wine	
	Must	Wine
TSS (° Brix)	17.00 ± 0.00*	2.80 ± 0.00
Reducing sugar (g/100 ml)	26.90 ± 0.02	8.00 ± 0.11
Titratable acidity (g tartaric acid/100 ml)	0.43 ± 0.03	0.59 ± 0.02
pH	4.88 ± 0.01	3.92 ± 0.12
Phenol (g/100 ml)	0.22 ± 0.12	0.22 ± 0.03
Tannin (mg/100 ml)	0.85 ± 0.15	0.72 ± 0.01
Lactic acid (mg/100 ml)	0.16 ± 0.00	0.38 ± 0.02
Ethanol (%)	N.D.	6.50 ± 0.25

All values ± standard error; ND = not detected.

Table 3 Sensory evaluation of the litchi fruit wine*

Attributes**	Litchi wine
Taste	4.5
Aroma	3.0
Flavour	3.9
Colour/appearance	3.2
Aftertaste	3.5

n = 32; * Values are means of the panelists' scores; ** 1 = dislike extremely, 2 = like moderately; 3 = like much; 4 = like very much; 5 = like extremely

undistilled alcoholic beverage made out of must. The titratable acidity increased from 0.43 (g tartaric acid/100 ml) in must to 0.59 (g tartaric acid/100 ml) in the finished wine. The increase in TA was concomitant with a decrease in pH from 4.88 in must to 3.92 in wine. As expected, the reducing sugar (g/100 ml) content decreased from an initial value of 26.9 in must to 8.0 in wine. However, the phenolic content remained unchanged in wine samples. Litchi wine obtained from our study was a light yellow (in appearance) beverage with alcohol (ethanol) content of 6.5%. It was slightly acidic [lactic acid, 0.38 (g/100 ml); titratable acidity, 0.59 (g/100 ml)], which together with tannin content (0.72 mg/100 ml) imparted the characteristic litchi flavour (rose flavour).

There are very few studies on alcoholic beverage produced from litchi fruits. In China, litchi wine is prepared traditionally (http://www.fao.org). The process involves crushing the fruits with previously fermented litchi juice. The fermentation of litchi juice can take up to seven months for completion. However, no scientific study has been made with Chinese litchi wine.

There are only a few studies, in general, on fermented beverages produced from tropical fruits. Onwuka and Awan (2001) produced a sparkling wine from over-ripe banana and plantain with an alcohol content of 11.3%, pH of 4.0, 1.02 g/l tartaric acid for TA, and 8.0° Brix for TSS. Akubor *et al.* (2003) obtained different compositions of banana wine [pH, 3.3; TA (g/l tartaric acid), 0.85; alcohol, 5%, TSS (° Brix), 4.8 and residual sugar, 0.04 g/ 100 ml]. Wine from mango (*Mangifera indica* L.) pulp was prepared by Srisamatthakarn *et al.* (2003) using the wine yeast, *S. cerevisiae* var. *burgundy*. The wine had a TSS (°Brix) of 8.2, TA (g tartaric acid/100 ml) of 0.82-0.88, pH of 3.65-3.68 and alcohol content of 8.8-10%. Similarly, a red wine made out of jamun (*Syzgium cumini* L.) fruits using *S. cerevisiae* var. *bayanus* had a TA (g tartaric acid/100 ml) of 1.11, TSS (°Brix) of 2.8, pH of 3.3, high tannin (1.7 mg/100 ml), high anthocyanin (60 mg/100 ml) and alcohol content of 6% (Chowdhury and Ray 2007). Other tropical fruits from which wine preparation reported are carambola (*Averrhoa carambola* L.) (Bridgebassie and Badrie 2004), papaya (*Carica papaya* L.) (Byakweli *et al.* 1994), black mulberry (*Morus nigra* L.) (Darias-Marin *et al.* 2003), melon (*Cucumis melo* L.) (Hernández-Gómez *et al.* 2005), marula (*Sclerocarya birrea* subsp. *Caffra*) (Fundira *et al.* 2002), yellow mombin (*Spondias purpurea* L.) (Dias *et al.* 2003), custard apple (*Annona squamosa* L.) (Muniz *et al.* 2002), mangaba (*Hancornia speciosa* Gomes) (Muniz *et al.* 2002), etc. In a recent study, Mohanty *et al.* (2006) prepared wine from cashew apple using *S. cerevisiae* var. *bayanus* as the starter culture.

Sensory evaluation analysis (**Table 3**) shows that litchi wine having a unique 'rose' flavour was 'liked much' by the panelists and there was no significant difference between the two sets ($p<0.05$) in most sensory parameters.

Statistical evaluation of proximate data

Correlation analysis was used for the measurement of the linear association between variables. Pearson's correlation co-efficient (r^2) among the analytical variables for litchi wine is presented in **Table 4**. Higher significant correlations found were TSS-TA (–0.866), TA-pH (0.925), TA-phenol (0. 866), phenol-tannin (0.786), etc.

PCA is used for the reduction of information on a large number of variables into a small set while losing only a small amount of information (Fievez *et al.* 2003). Using PCA, the original eight analytical variables of litchi wine were reduced to two principal components (PC1 and PC2), which had Eigen values larger than one and retained for rotation. PC1 accounted for 70% and PC2 accounted for 30% of the total variations (**Table 5**). To assist the interpretation of dimensions, the factor pattern was rotated using the Varimax method. Based on the guidelines provided by Stevens (2002), an attribute was correlated to load heavily on a given component if the factor loading was greater than 0.72. A total of eight proximate attributes loaded heavily on two dimensions. Five analytical variables, i.e. reducing sugar (+ve), TA (+ve), pH (+ve), phenol (+ve) and LA (–ve) were loaded on PC1 and TSS (+ve), tannin (+ve) and etha-

Table 5 Percentage variance and variable loadings for two analytical principal components of litchi wine (PC1 and PC2) using varimax rotation.

Parameters	Litchi wine	
	PC1	PC2
Percentage of variance explained Loadings	69.874	30.124
TSS	-0.676	0.737
Reducing sugar	0.983	0.183
Titratable acidity	0.954	-0.300
pH	0.996	0.008
Phenol	0.976	0.217
Tannin	0.633	0.774
Lactic acid	-0.954	0.300
Ethanol	-0.002	1.000

Table 4 Correlation coefficients for analytical variables for litchi wine.

TSS	Reducing sugar	Titratable acidity	pH	Phenol	Tannin	Lactic acid	Ethanol
Litchi wine							
1.000	-0.529	-0.866*	-0.611	-0.500	0.143	0.866**	0.756*
	1.000	0.882**	0.995**	0.999**	0.764*	-0.882**	0.156
		1.000	0.925**	0.866**	0.371	-1.000**	-0.327
			1.000	0.991**	0.696*	-0.925**	0.056
				1.000	0.786*	-0.866**	0.189
					1.000	-0.371	0.756*
						1.000	0.327
							1.000

* Correlation is significant at the 0.05 level (2-tailed).
** Correlation is significant at the 0.01 level (2-tailed).

Table 6 Correlation coefficients for sensory acceptability for litchi wine.

Taste	Aroma	Flavour	Colour	After taste
		Litchi wine		
1.000	-0.334	-0.290	0.076	-0.013
	1.000	-0.147	0.290	-0.089
		1.000	-0.107	0.011
			1.000	-0.376*
				1.000

* Correlation is significant at the 0.05 level (2-tailed).

Table 7 Percentage variance and variable loadings for sensory principal components using varimax rotation.

Parameters	Litchi wine		
	PC1	PC 2	PC 3
Percentage of variance explained Loadings	31.278	27.288	20.699
Taste	0.124	-0.726	0.523
Aroma	0.190	0.872	0.230
Flavour	-0.003	-0.505	-0.919
Colour	0.791	0.186	0.201
After taste	-0.854	0.059	0.129

nol (+ve) were loaded on PC2, indicating a strong correlation among these attributes.

Statistical evaluation of sensory data

The correlation coefficients among consumers' acceptability of sensory attributes of litchi wine are presented in **Table 6**. A significant correlation was only found between colour and after taste (–0.376). PCA reduced the original five variables (taste, aroma, flavour, colour and after taste) into three principal components (PC1, PC2 and PC3, which accounted for 79% of the total variance (**Table 7**). The most notable variables were colour (loading 0.791) and after taste (loading –0.854) on PC1 and taste (–0.726) and aroma (loading 0.872) on PC2 and flavour (loading –0.919) on PC3. All the variables meet Steven's guidelines (< 0.72).

There are several studies where PCA was applied for the evaluation in food product analysis. This has been extensively reviewed by Tzouros and Arvanitoyannis (2001). Destefannis *et al*. (2000) used PCA for the study of the relationship between chemical, physical and sensory variables (eighteen) measured on various beef meat specimens. The first three components accounted for 63% of the total variance (PC1 34%, PC2 20.6% and PC3 38%). Soria *et al*. (1999) applied PCA for the evaluation of apples that were cleaned up after collection by different methods. The authors also evaluated the qualitative attributes of apples as well as ethylene production. PCA was used for the study of relationship between proximate and sensory variables regarding β-carotene and anthocyanin rich sweet potato curd (Panda *et al*. 2006; Mohapatra *et al*. 2007). Panda *et al*. (2007) applied PCA for the evaluation of lacto-pickles prepared from β-carotene rich sweet potato which reduced the six original analytical variables into two independent components, which accounted for 92% of the total variations. Panda and Ray (2007) also applied PCA for the evaluation of both proximate and sensory variables of β-carotene rich sweet potato lacto-juice. The eight original proximate variables were reduced to three independent components, which accounted for 99.9% of the total variation. Similarly, five original sensory variables were reduced to two independent components accounting for 83.1% of total variations.

Many tropical fruits, although having excellent taste and flavour, are available for a short period (2-3 months) in a year and are highly perishable due to their high sugar and moisture content (Ward and Ray 2006; Muniz *et al*. 2008). Litchi fruits are seasonally available in India in huge quantities during the latter part of summer (May-June) but they find little post harvest application at present except in the fresh juice industry. Litchi wine will be a viable technology for processing litchi fruits and will have a significant domestic and export market.

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