RESEARCH ARTICLE

Optimization of Process Parameters for Wine Production from Sapota Fruits

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ABSTRACT

Sapota is a long-lived evergreen tree belonging to the family Sapotaceae. These fruits are susceptible to low temperatures and hence cannot be stored in cold storage for an extended period due to their high perishability. Fermentation is a vital method in developing value-developing value-added products from fruits with improved physicochemical and sensory quality attributes, especially flavor and nutritional constituents. The present study aimed to optimize the process parameters necessary for producing quality sapota wine for product diversification and to cater the needs of medium scale industries and sapota growers. The pressed and pulped juice, along with three different strains of yeast cultures (NCIM 3304, NCIM 3215, NCIM 3281 and natural strain), was fermented for a period of 25 days with an aging period of 2 months under room temperature (28±2 °C) and refrigerated temperature (5±2 °C). The optimized process parameters for the preparation of sapota wine were obtained from pressed juice using NCIM 3304 yeast strain with a fermentation period of 25 days and an aging period of 60 days at refrigerated conditions and recorded good physicochemical and organoleptic properties.

Keywords: *Sapota; Fermentation; Optimization; Refrigeration; Aging*

INTRODUCTION

Fruits are essential sources of natural sugars, antioxidants, vitamins, and minerals and generally have industrial and therapeutic applications. Sapota (*Achras sapota Linn*.) is one of the leading tropical fruits belonging to the family Sapotaceae. The pulp is yellowish, tender, granular, sweet, juicy, and scented and features a high latex content. Matured sapota fruit is a good source of carbohydrates (21.4 g/100 g), dietary fibers (10.9 g/100g), tannin (3.16– 3.45%), ascorbic acid, and minerals like calcium (28 mg/100 g) and phosphorus (27 mg/100g) (Ramulu and Rao, 2003). The sapota pulp is also rich in iron, which is required for developing hemoglobin and vitamin A. The amino acids in the fruits are glutamic acid, glycine, alanine, methionine, phenylalanine, proline, hydroxyproline, threonine, taurine, tyrosine, serine, valine, and phosphoethanolamine along with urea (Gurusharansingh, 2001). India is the largest producer of Sapota in the world, with an annual production of 12 lakh tonnes occupying a total area of 1.01 lakh hectares (National Horticulture Board, 2019). Next to China, India is the second-largest producer of fruits and vegetables and accounts for about 15% of the world's production. India loses about 35-40% of its product due to improper post-harvest management. After harvesting, the fruit ripens within 8-10 days and hence has a shorter shelf-life, so the postharvest loss of this fruit is one of the most demanding problems in tropical countries like India (Morais *et al.,* 2006). Further, during glut periods, surplus production of fruits needs to be utilized for processing and preservation into value-added food products such as wine (Madan and Ullasa, 1993). Wine is an alcoholic and fermented beverage prepared from fruit juices with suitable processing methods. Alcoholic fermentation is a widely adopted technique for manufacturing beverages from fruits, particularly wines. Wine is primarily produced from grapes, apples, and other acidic fruits.

Large quantities of ripened Sapota rapidly deteriorate and are usually wasted due to poor handling and inadequate storage facilities, with post-harvest losses ranging from 25%–30% (Nitesh, 2013). Hence, an appropriate value-addition technique not only reduces the post-harvest losses but also aid in generating more profits and stimulate the sustainable use of biomass (Duarte *et al.,* 2010). In this regard, fermentation seems to be an efficient means of preserving the sapota fruits during a glut in the market, which has been employed for generations to preserve fruit in the form of drinks for consumption later and improve food security. Being rich in sugars, Sapota is also a suitable substrate for the alcohol and liquor industry (Chundawat, 1998). Sapota fruit serves as a suitable raw material for fermentation mainly because of its appropriate taste, flavor, availability, high sugar and water content with a high flesh-to-seed ratio (80:9) and overall chemical composition (Ray and Ward, 2006). Being fruit-based fermented and undistilled products, wine contains most nutrients are present in the original fruit juice. The nutritive value of the wine is increased due to the release of amino acids and other nutrients from yeast during fermentation (Pawar, 2009). The production of wine from Sapota can reduce the level of post-harvest losses, besides increasing the diversity of wines in India. Usually, wine is prepared from clear juices by removing the fibrous material during filtration. Hence, the present study aims to optimize the process parameters involved in the fermentation of wine from sapota juice and sapota pulp, and also the physicochemical characteristics during fermentation have also been observed.

MATERIAL AND METHODS

Extraction of juice and pulp from sapota fruits

Matured Sapota fruits were procured from the College Orchard, Tamil Nadu Agricultural University, Coimbatore, and they were ripened naturally by covering them in paper and storing them in wooden boxes for up to 4 days at room temperature (28±2 °C). The pulp from ripened sapota fruits were obtained by pressing. One-half of the pulp was incubated for five h with the action of 0.05% pectinase enzyme at the ambient condition to get clear juice. Then the sapota juice and pulp were pasteurized at 72 ^{o}C for 15 minutes to inactivate the enzymes and spoilage microflora. The pasteurized juice and pulp extracted from sapota fruits were used for making wine.

Preparation of starter culture

The yeast cultures NCIM 3304, NCIM 3215, and NCIM 3281, purchased from the National Chemical Laboratory, Pune, India, were selected and grown in yeast extract glucose broth under ambient conditions. An appropriate quantity of nutrients was dissolved one after another in 100 mL of distilled water, and the broth was sterilized by autoclaving at 121 ^{o}C for 15 minutes. The yeast strains were inoculated aseptically and incubated for 48 h. The yeast cultures with a population of 108/109 CFU/mL of the yeast extract glucose broth were used as a starter culture.

Fermentation of sapota juice and pulp

Five liters of pasteurized juice and pulp were taken separately and allowed to ferment in the fermenter vessel, and they were inoculated with the starter cultures (5%, v/v) and incubated at 28±2 ^{o}C for 25 days. Fermentation was carried out with a stirrer speed of 100 rpm and aeration rate of 1-5 lpm to maintain the dissolved oxygen level from 1-20 mg/L. The wine is subjected to continuous agitation, and aeration leads to oxidation, so agitation is given at periodic intervals. At regular intervals, the samples were taken, and the physicochemical characteristics were analyzed and compared between juice and pulp. After 25 days of fermentation, samples were withdrawn and the best ones were subjected to aging. The wine was prepared separately from the juice and pulp and compared to the quality characteristics.

Aging of fermented wine

Aging improves the quality of wine and imparts flavor to the wine. After settling, wines were subjected to an aging process for two months in air-tight narrow-mouth glass bottles at room temperature (28±2 ^{o}C) and refrigerated temperature (5±2 ^{o}C). The physicochemical characteristics were analysed at 0, 20, 40 and 60 days.

Quality assessment of wine

Physicochemical analysis of wine

The pH and temperature were determined using a pH meter (Hanna H12216-02) and an analytical thermometer. Total dissolved solids were determined using a hand-held refractometer. The final alcohol content of both wines was determined using the density method, while the total acidity of the wines was determined by titration as described by AOAC (2005) and Thimmaiah (2012). The color L, a, b values of the samples were measured using a Hunter Lab Colorimeter (Model: $45^{o}/0^{o}$, M/s Hunter Lab, Reston, Virginia, USA).

Sensory evaluation

A panel of 30 students, staff, and faculty members of Tamil Nadu Agricultural University, India, was involved in the evaluation process. They assessed the color, flavor, taste, and overall acceptability of the mango wines on a 9-point hedonic scale where 1 indicated dislike extremely and 9 indicated like extremely. The panelists are familiar with all the attributes and quality of the wine.

Statistical analysis

The results of the various analyses were expressed as mean ± standard deviation. The data obtained for the various analyses were expressed as mean ± standard deviation. The analysis of variance using a Completely Randomized Design (CRD) as described by Arankacami and Rangaswamy (1995) was conducted to find out the impact of storage temperature and days of aging on the physicochemical characteristics in sapota wines during the aging process.

RESULTS AND DISCUSSION

Effect of Fermentation on Physicochemical Characteristics of Wine prepared from juice and pulp

The fermentation process was enhanced by providing aeration (1.5 lpm) and agitation (200 rpm) for 15 minutes initially at a constant temperature of 28±2 °C. From the preliminary trials, the NCIM 3304 yeast strain used for wine-making recorded higher alcohol content, less acidic, and high pH after fermentation. The changes in physicochemical parameters during the fermentation period of 25 days are presented in Fig.1. The pH for pressed juice and pulped juice was 4.35 and 4.30, respectively. The pH decreased rapidly and stabilized at 3.89 and 3.87 for pressed and pulped juice, respectively (Lokesh *et al.,* 2014). The decrease in pH may be due to the production of fermentation metabolites such as acetic acid, phenolic compounds, esters, and CO_2. This shows an inverse relation between pH and the duration of the fermentation of sapota juice and pulp (Singh and Kaur, 2009).

From Figure 1, it was evident that TSS reduced with an increase in the duration of fermentation. The initial TSS of pressed and pulped juice was 25°Brix and 26.5°Brix, whereas the final TSS of 13.5 and 15°Brix were obtained after a fermentation period of 25 days. A similar study for palm wine has been reported by Ogodo *et al.* (2015) and Kamassah *et al.* (2013) for Mango wine. The titratable acidity increased initially and stabilized during fermentation from 0.15 to 0.30% for pressed juice and 0.167 to 0.306% for pulped juice. Similar values on Mango wine revealed that the titratable acidity increased with increasing fermentation time and ranged from 0.21 to 0.59% in wine A and from 0.21 to 0.63% in wine B as described by Ogodo *et al.* (2015). Among the pressed juice and pulped juice, the dissolved oxygen was higher in pulped (2.80 mg/L) than in pressed juice (2.55 mg/L) after 25 days of fermentation. The above findings show that the consumption of oxygen by the yeast cells during fermentation reduces the dissolved oxygen in the juice. The alcohol content of the pressed and pulped juice increased from 7 to 10 (% v/v) and 8 to 13.2 (% v/v) at the end of 25 days of fermentation. This might be due to cellulosic interactions with yeast during the fermentation process. The increase in alcohol may be due to the conversion of sugars into ethanol during fermentation. The results obtained were in confirmation with Ranjitha *et al.* (2015) and Jadhav (2018). At the end of fermentation, the wine made from pressed juice using NCIM 3304 contained a pH, TSS, TA, DO and Alcohol content of 3.87, 15.0°brix, 0.306(%), 2.80(mg/L) and 13.2(%v/v), respectively were used for the aging process. The wine made from clear juice (Pressed juice) had a superior quality which was used for the further aging process.

Effect of Aging on Physicochemical Characteristics of Wine

The changes in the physicochemical characteristics of wine during aging in a dark place are given in Table 2. The titratable acidity and pH of freshly fermented sapota wine ranged from 0.150 to 0.300 per cent and from 4.35 to 3.89 respectively. During aging, a gradual decrease in pH from 3.89±0.05 to 3.21± 0.07 at room temperature and from 3.89±0.06 to 3.43±0.02 at refrigerated temperature, respectively. An increasing titratable acidity was observed in both wines stored at room and refrigerated temperatures from 0.35±0.01 to 0.41±0.02 and 0.35±0.01 to 0.40±0.01 (Kotecha *et al.,* 1994). This might be due to the oxidation of sugar molecules into organic acid, which increased the titratable acidity and decreased pH content of the fruit wines during aging. The acidic nature of the wine was probably due to the accretion of organic acids such as lactic and ascorbic acids, which diminishes the influence of spoilage bacteria. The titratable acidity of any fruit wine is a critical characteristic varying between 0.5 to 1.0%. The acidic nature of the wine was most likely due to the accretion of organic acids like lactic acid and ascorbic acid, which reduce the impact of pathogenic and spoilage bacteria (Liu, 2003). The difference among the wines stored at different temperature conditions and storage (aging) days on pH and titaratable acidity were insignificant at $p<0.05$. A similar view was also reported by Pawar (2009) in sapota wine and Kocher and Pooja (2011) in guava wine.

During the aging of sapota wine, the TSS content of the fruit decreased gradually from 13.7±0.17 to 9.4±0.03°brix at room temperature and whereas it was from 13.7±0.37 to 10.8±0.33°brix at refrigerated condition. The TSS content of wines was decreased with advancement in aging. The drop in TSS in wine was indicative of the consumption of sugar sources by the wine yeast for ethanol production. The decreasing TSS content was lesser in wine kept at refrigerated temperature. The decreasing trend in TSS content is in line with the results of Pawar (2009), who reported a decrease in TSS content of sapota wine during aging. Maragatham and Panneerselvam (2011) reported that the TSS content of papaya wine was decreased from 12.14 to 9.36°brix with progression in the aging process. The storage temperature, storage periods and their interaction effects had a highly significant difference on TSS of sapota wine. At the final stage of aging (60 days), the maximum increase of 13.0±0.36 to 13.5±0.16% in alcohol per cent was observed in wine at room temperature and minimum of 13.0±0.09 to 13.3±0.06% in wine at refrigerated temperature. This increase in alcohol content is due to the increase in acidity of the wines during aging. These results, coupled with earlier studies conducted by Bons *et al.* (2020) in sapota wine and by Sibounnavong *et al.* (2010) in star gooseberry and carambola.

The color values (L, a and b) of sapota wine showed a significant change during the aging process. The L value ranged from 18.47±0.50 to 13.67±0.31 and 18.47±0.37 to 14.01±0.35, a value increased from 1.40±0.01 to 3.17±0.023 and 1.40±0.02 to 2.98±0.09 and b value decreased from 10.64±0.11 to 4.98±0.02 and 10.64±0.11 to 5.21±0.08 at room and refrigerated conditions respectively. This increase in a value is by the change in color of wine to dark brown at the end of the aging process. The effect of temperature and storage days on color values was highly significant at 5% level. The sapota wine scored an overall acceptability value of 8.0 and 8.5 for the samples stored at room and refrigerated temperatures (Fig.2). Appearance, color, aroma, taste and subtle taste factors such as flavor of wine constitute the quality (Sharma, 2000 and Joshi *et al.,* 2006) reported that aroma and taste of wines is very complex and depend on several factors such as cultivars, agricultural land, vinification practices, fermentation and maturation. Sandeep *et al.* (2014) reported that sapota wine have a unique flavor with a robust exotic taste. Pawar (2009) used a 20-point score card to evaluate the sensory qualities of sapota wine and perceived that the ripe sapota fruits had a good sweet wine with an overall score of 15, compared to grape wine with an overall score of 16, which was used as control. During maturation, the complex chemical reactions involving sugar, acid and phenolic compounds in wines can alter the aroma, color, mouth feel and taste of the wine in a way that is more pleasing to the taste (Pawar, 2010)

CONCLUSION

This study has revealed that acceptable fruit wine can be manufactured from Sapota using yeast strain. It also suggests an avenue to ferment these fruits into value-added products such as wine to preserve their nutrients, minerals, aroma and taste and make them available to consumers all year round. Hence, large-scale production of sapota wine is a substitute to extend the shelf-life of sapota fruit and reduce the post-harvest losses of fruit during glut in the market.

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CONSENT FOR PUBLICATION

All the authors agreed to publish the content.

Ethics Statement

Specific permits were not required for the above studies because no human or animal subjects were involved in this research.

Originality and plagiarism

The authors declare that the work carried out in this research paper is the original work carried out and has not been published earlier or sent for publication to other research journals.

Competing interests

The authors declare that they have no competing interests.

Data Availability

All the data of this manuscript are included in the manuscript. No separate external data source is required.

Author's contribution

Dr.J.Deepa carried out the experiment and wrote the manuscript. Dr.P.Rajkumar performed the idea of this article and encouraged to investigate and supervised the findings of this work. Dr.P. Preetha verified the analytical methods and contributed to the interpretation of the results.

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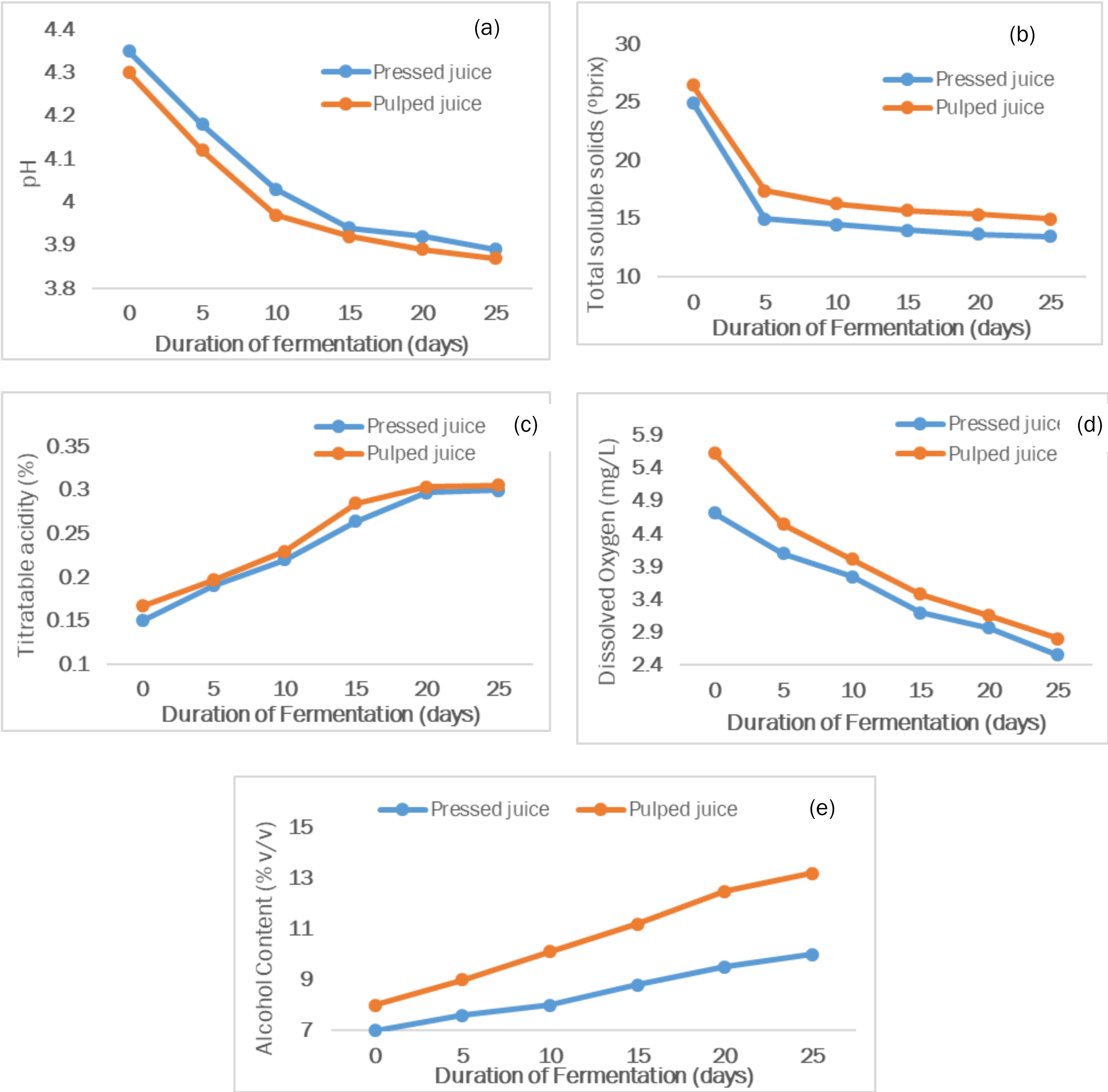

Fig. 1 Changes in physicochemical characteristics (a) pH (b) Total soluble solids (c) Titratable acidity (d) Dissolved Oxygen (e) Alcohol content of wine from pressed and pulped juice during fermentation period

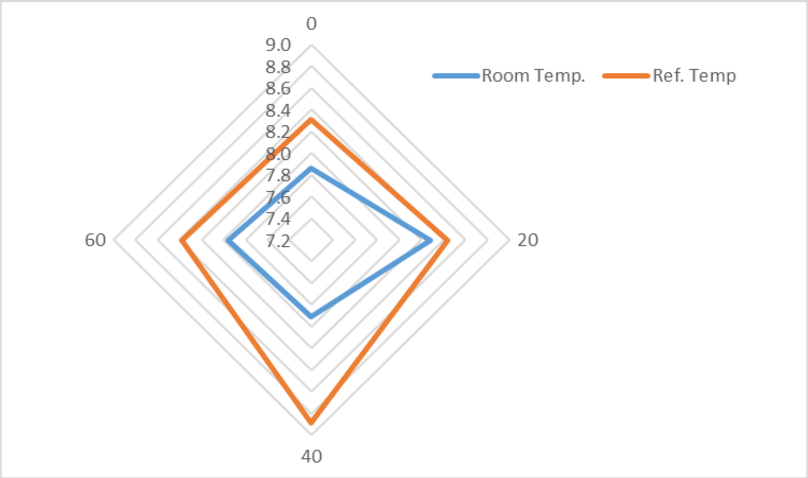

Fig. 2 Changes in sensory characteristics of wine during aging process stored at room temperature and refrigerated temperature

Table 1. Changes in physicochemical characteristics of wine during aging process

Parameters	Aging Duration (days)								CD at 0.05%
	Room Temperature				Refrigerated temperature				
	0	20	40	60	0	20	40	60	
pH	3.89* ± 0.05$	3.67 ±0.08	3.47 ±0.07	3.21 ±0.07	3.89± 0.06	3.79 ±0.07	3.61 ±0.04	3.43 ± 0.02	T =0.018 A =0.026 I = NS
TSS (°brix)	13.7 ± 0.17	12.0 ±0.30	10.9 ±0.34	9.4 ±0.03	13.7 ±0.37	12.5 ±0.14	11.3 ±0.13	10.8 ± 0.33	T = 0.071 A = 0.142 I = 0.116
TA (%)	0.35 ± 0.01	0.37 ±0.01	0.39 ±0.01	0.41 ±0.02	0.35 ±0.01	0.36 ±0.01	0.39 ±0.01	0.40 ± 0.01	T = 0.009 A = 0.017 I = NS
Alcohol content (% v/v)	13.0 ± 0.36	13.1 ±0.27	13.3 ±0.43	13.5 ±0.16	13.0 ±0.19	13.1 ±0.09	13.2 ±0.25	13.3 ± 0.06	T = 0.007 A = 0.001 I = NS
L	18.47 ±0.50	15.23 ±0.30	14.87 ±0.47	13.67 ±0.31	18.47 ±0.37	15.50 ±0.47	14.90 ±0.36	14.01 ±0.35	T = 0.301 A = 0.543 I = 0.502
A	1.40 ± 0.01	1.56 ±0.01	2.94 ±0.07	3.17 ±0.03	1.40 ±0.02	1.50 ±0.03	2.41 ±0.01	2.98 ± 0.09	T = 0.324 A = 0.512 I = 0.492
B	10.64 ±0.11	7.73 ±0.22	5.47 ±0.01	4.98 ±0.02	10.64 ±0.11	7.91 ±0.02	6.19 ±0.11	5.21 ± 0.08	T = 0.336 A = 0.539 I = 0.510

* denotes mean value; $ denotes standard deviation; T – Storage temperature; A = Aging duration; I = Interaction