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An approach for development of wine from Jamun including optimization of fermentation parameters

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Abstract
Jamun wine production ia an alternative for deployment of highly seasonal (May to June), highly perishable underutilized jamun fruits with short storability (upto 2-3 days under ambient temperature). The exploitation of underutilized fruits of jamun can provide a solution to nutrition, livelihood and economic security of tribals by using available traditional wisdom as well as modern processing technology. Setting up of fruit wineries could result in the economic upliftment of these tribals by generating employment opportunities. Considering this in view an investigation was undertaken for preparation of jamun wine at Faculty of fruit Science, College of Horticulture, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola during 2015-17. Process factors for the production of red wine from jamun fruits were successfully optimized using Optimal Custom design. The design involved two numeric factors; levels of yeast and fermentation duration. All the factors were considered significant by ANOVA using Response Surface Methodology. Quadratic model was developed and validated to explain the fermentation process. Numerical optimization of the process factors was achieved with the target of optimizing level of yeast and fermentation duration. The optimum conditions are; yeast level of 0.211g/l, fermentation duration of 18 days, results in desirable total soluble solids of 4.766 ^{0}Brix, anthocyanin content 49.645, ethanol 9.459% and antioxidant activity 85.125%DPPH with maximum desirability. Considering the findings of investigation and highest net monitory returns (5.86 B:C ration) obtained jamun wine production it could be concluded that, the appropriate combination of levels of yeast and fermentation duration is the needed for quality jamun wine production.

Keywords: ANOVA, optimal custom design, response surface methodology, optimization and jamun

Introduction
The Prime Minister's vision of doubling farmer's income by 2022 is worth serious attention. This laudable objective could not only improve the well being of our farmers but can also be a trigger to boost agri-based manufacturing growth in rural India. The goal should be to use this effort to trigger a much needed boost to agriculture and to agri-related value addition and manufacturing. Now a day's the Indian Agriculture has been diverting towards economical crops. However, diversification to more productive and remunerative crops has become the new milestone to be achieved in Indian Agriculture. A shift in favour of horticultural crops as a more viable and attractive alternative is a part of such diversification drive and strategy. With rapid change in attitude of farmers of the region from subsistence farming to commercial one and with innovation and ideas regarding resource optimization and risk reduction, agricultural land use has been influenced to a predominant scale (Malhotra S.K., 2017) [15].

In India about 88819 thousand tonnes fruits are produced annually (Anon., 2016) [2]. But hardly 1.2% of this is utilised for processing and preservation and about 30-33% of the total production is wasted due to spoilage during handling, transportation and lack of cold storage facility (Baisya, 1980) [3]. Due to high water or juice content, they are perishable. With increased production of particular fruit in a season, there is a glut in the market. In order to minimise postharvest losses and to avoid market glut, fruits are need to be effectively utilised in processing industry (Sahota and Sunil, 2006) [19]. Jamun is one of the underutilized fruit. Jamun fruits are highly seasonal (May to June), highly perishable and short storability (upto 2-3 days under ambient temperature), due to its short availability, minimum storage and less popularity this fruit is considered as an unexploited fruit crop in India. The exploitation of underutilized fruits can provide a solution to nutrition, livelihood and economic security of tribals by using available traditional wisdom as well as modern processing technology.

Setting up of fruit wineries could result in the economic upliftment of these tribals by generating employment opportunities.

The world production of jamun is estimated at 13.5 million tonnes out of which 15.4% is contributed by India (Anon, 2014) [1]. The Eastern Vidarbha, Melghat and Konkan region of Maharashtra and are known to have large number of jamun trees. However, large quantities of jamun are lost either through rainfall or in sorting and grading to meet quality standards for fresh fruit export market, which could otherwise be used for processing. In India alone wastage of Jamun is 0.5 - 2MT. A large quantity of marketable surplus fruit is available in all jamun growing regions which need to be processed and converted into value added products (Patil *et al.*, 2012) like jamun wine. Eastern Vidarbha region *viz*., Gadchiroli district of Maharashtra is a treasure of underutilized jamun fruits. If exploited properly has the potential of transforming the economy of this tribal dominated region. The exploitation of underutilized fruits can provide a solution to nutrition, livelihood and economic security of tribals by using available traditional wisdom as well as modern processing technology.

The fruit, its juice and the seed contain an alkaloid, jambosine and a glycoside called 'jamboline' or 'antimellin' (Chowdhury and Ray, 2007) [5]. The glycoside Jamboline is the main compound which helps in controlling the blood sugar level by switching off the mechanism of starch converting to sugar when there is optimum amount of sugar already present in the blood. There is a very high anthocyanin content in jamun fruits which attributes to its antioxidant and free radical scavenging activity. The fruit pulp having anti cancerous, antidiabetic, antioxidant, antiviral and antihyperlipidemic properties cannot be achieved from any other fruit (Chaudhary and Mukhopadhyay, 2012).

Indian wine is set for record production levels of about 18 million liters in 2016, but it is the domestic rather than export market that is really driving sales. India's domestic sales rose by 20% in 2015. With the increasing demand of wine industry, these underutilized, forest grown jamun fruits can be utilized for the production of jamun wine which will not only help in enhancing the income of poor tribal people with their traditional knowledge but also help in enhancing the farmer's income and national income as well. The present study is an attempt to focus on the qualitative parameters and it also comprehensively examines the cost and returns in the production of wine from this underutilized jamun fruit.

Material and Methods

The experiment consisted of levels of yeast (*Saccharomyces cerevisiae var.ellipsoideus* inoculated at 0.20 g/l, 0.25 g/l and 0.30 g/l) and fermentation duration (7, 14 and 21 days) which were replicated thrice. Wine is an alcoholic product obtained by fermentation of fruit juices with yeast. The procedure was followed for the preparation of jamun wine as outlined by (Joshi (1995) [8] at Faculty of fruit Science, College of Horticulture, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola during 2015-17. The observations regarding the total soluble solids, ethanol, antioxidant activity and anthocyanin content of jamun wine was taken at interval of 3 months from fresh jamun wine till 9 months aged jamun wine. The Total Soluble Solids (TSS) were determined using Atago make RX 1000 digital refractometer. Ethyl alcohol content of wine was estimated by using specific gravity (Ranganna, 1979) [17]. The anthocyanins in wines were measured by slight modification in method suggested by Joshi *et al*., 2012 [10, 12]. Anthocyanin content was expressed in terms of absorbance units at 530 nm (A-530 nm) per ml of wine. The antioxidant Activity in Jamun wine was estimated by Diphenyl-p-picrylhydrazyl (DPPH) method described by Manzocco *et al.* (1998) [16]. The experimental work was strictly carried out based on the Optimal Custom design (OCD) matrix as depicted in Table 2. The cost benefit ratio was calculated by following the procedure given by David *et al.*, 2013 [16].

Statistical Optimization of screened parameters by Response Surface Methodology (RSM)

Following the selection of critical variables by Optimal Custom design, RSM was employed to optimize yeast levels and fermentation duration for wine production. Experimental designs were generated and analyzed by using the statistical software package Design-Expert. 11, Stat-Ease Inc., Minneapolis, MN, USA. Response surface methodology (RSM) with Optimal Custom design was employed to optimize the different parameters, *viz.* total soluble solids, ethanol, anthocyanin content and total antioxidant capacity. The maximum and minimum range of two independent variables is shown in Table 1.

Table 1: Factors and Levels for Optimal Custom Design

Factors	Name	Units	Type	Minimum	Maximum
A	Level of yeast	g/l	Numeric	0.20	0.30
B	Days of fermentation	Days	Numeric	7	21

Results and Discussion

A) Optimization using OCD (Optimal Custom Design)

The optimization of process factors for the production of jamun wine was achieved using Optimal Custom Design which is a type of response surface methodology (RSM). The OCD had full factorial cores encompassing two numeric factors. The numeric factors had low and high factorial levels, low and high axial levels and center points. The factors and levels used for the OCD are shown on the Table 1, while the design matrix with the response data is shown on Table 2.

B) Data analysis

The design of experiments, analysis of the results and prediction of the responses were carried out using Design-Expert software (Version 11). Comparisons of means were performed by one-way ANOVA (analysis of variance), (p-value < 0.05).

Table 2: Design matrix for Optimal Custom Design (OCD)

Run	Yeast levels (g/l)	Fermentation Duration (In Days)	Total Soluble Solids (^{0}Brix)	Ethanol (%)	Anthocyanin (mg/100ml)	Antioxidant Activity (%DPPH)
1	0.25	14	5.28	9.43	32.86	83.22
2	0.2	21	4.29	9.23	67.03	86.32
3	0.3	7	5.34	9.4	51.71	81.69
4	0.25	14	5.62	9.5	52.86	83.22
5	0.3	7	5.34	8.61	41.71	81.69
6	0.25	14	5.62	9.43	31.86	85.22
7	0.3	14	6.25	9.41	54.27	81.09
8	0.2	14	5.28	9.24	34.15	82.31
9	0.2	7	6.09	7.28	47.14	79.69
10	0.25	21	4.98	9.53	60.12	86.34
11	0.3	7	5.34	8.61	41.17	81.69
12	0.2	14	5.28	9.24	34.15	82.31
13	0.25	21	4.98	9.53	59.12	86.34
14	0.25	14	5.62	9.43	32.86	83.22
15	0.3	21	5.9	9.54	58.46	85.05
16	0.25	7	5.56	8.86	52.25	81.4

C) Model equations

The model equation was presented on coded form. The coded form can only be used for response prediction because it removes the factor's unit of measures. The actual form cannot be used because it has been scaled to accommodate their different units of measures. The model equation is good enough to help one move in the proper direction, but not to make exact prediction particularly outside the actual experimental region.

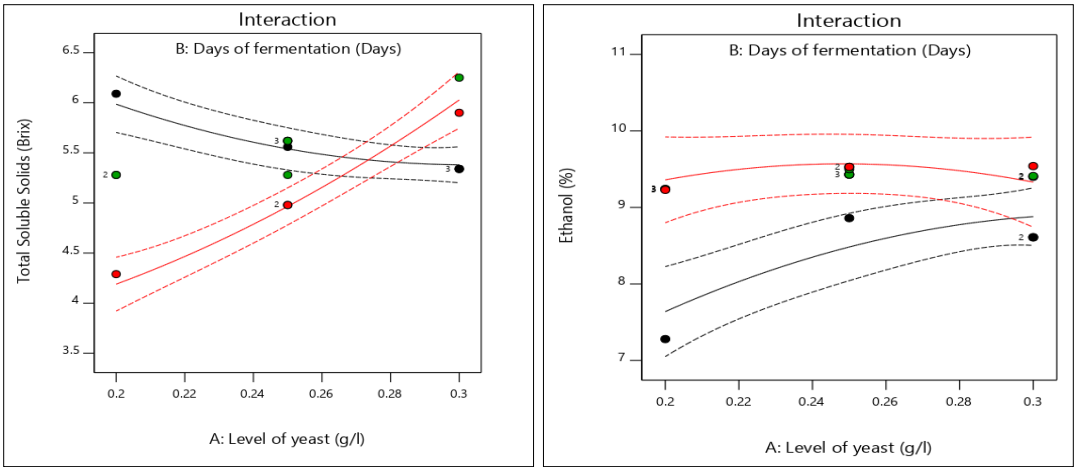
Table 3: The fitted quadratic model in terms of coded variables for different responses

Response	Coded equation	Regression (p- value)	R^2	R^2 Adjusted	Lack of fit	Sum of squares	F-value
Total Soluble Solids	$5.55+0.3082A-0.2872B+0.6106AB+0.1432A^2-0.2938B^2$	0.0810	0.9340	0.9010	3.44	3.25	28.32
Ethanol (%)	$9.52+0.3027A+0.5433B-0.3177AB-0.2239\ A^2-0.4959B^2$	0.1145	0.8176	0.7264	2.85	5.11	8.96
Anthocyanin (mg/100ml)	$38.77+1.28A+7.61B-0.0968AB+0.9724A^2+14.31\ B^2$	0.1766	0.6135	0.4472	2.19	2009.8	3.43
Antioxidant Activity (%DPPH)	$83.46-0.0298+2.38B-0.9061AB-1.22\ A^2+0.7810\ B^2$	0.2318	0.9184	0.8776	1.82	65.38	22.51

*A- Level of Yeast, *B- Fermentation duration

D) Effect of yeast levels and fermentation duration on total soluble solids, ethanol, Anthocyanin content and antioxidant activity of jamun wine

Level of yeast and fermentation duration was considered significant by ANOVA. The concentration of yeast was studied at low level of 0.20 g/l and high level of 0.30 g/l whereas fermentation duration was studied at minimum 7 days and maximum 21 days of fermenatation. The effect of yeast levels and fermentation duration on total soluble solids, ethanol, anthocyanin content and antioxidant activity of jamun wine was observed in Fig. 1(a, b, c and d) respectively. Increase in the ethanol and antioxidant activity was observed with the increase in the level of yeast from 0.20g/l to 0.30 g/l. In present investigation it was observed that, the Total Soluble Solids was significantly influenced by different yeast levels and fermentation duration. Precipitation of soluble solids during interaction of various components might have resulted in a decreased TSS during maturation (Joshi *et al.*, 1999; Sharma and Joshi, 2003; Sharma *et al.*, 2009, Joshi *et al.*, 2014) [11, 21, 20]. The decrease in the anthocyanin contents by application of *Saccharomyces cerevisiae var. ellipsoideus* inoculated at 0.20 g/l @ 21 days of fermentation could be attributed to the combined effect of thermal degradation, oxidative degradation and condensation reactions with other phenolic compounds such as flavanols and hydroxyl cinnamates (Timberlake and Bridle, 1976; Somers and Pocock, 1990; Ribereau-Gayon *et al.*, 2000; Beer *et al*., 2005) [23, 22, 18, 4].



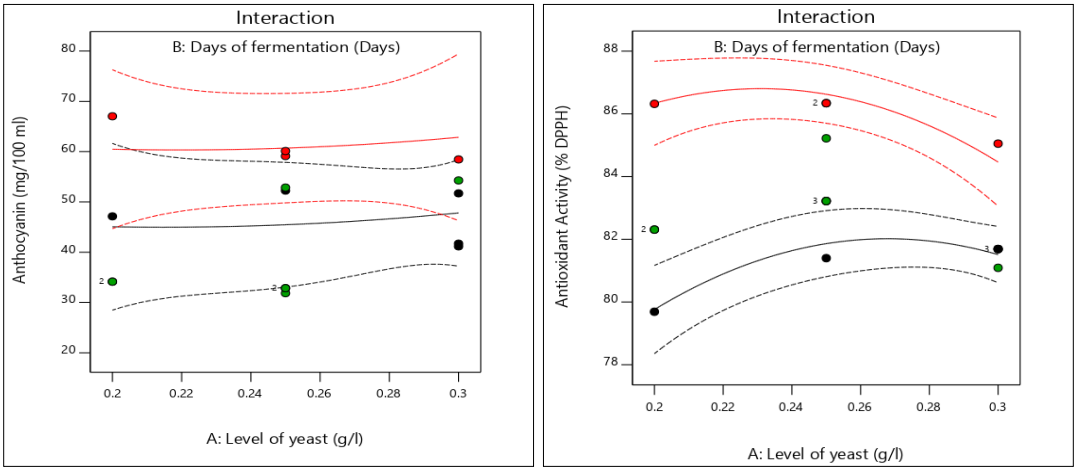


Fig 1: Effect of yeast levels and fermentation duration on (a) Total soluble solids, (b) Ethanol, (c) Anthocyanin content and (d) Antioxidant activity of jamun wine

E) Process optimization

The numerical type of optimization was used to evaluate the response in order to obtain the optimum level of yeast and fermentation duration. The optimum conditions with highest desirability were selected. The optimum conditions are; yeast level of 0.211g/l, fermentation duration of 18 days, results in desirable total soluble solids of 4.766 ^{0}Brix, anthocyanin content 49.645, ethanol 9.459% and antioxidant activity 85.125%DPPH with maximum desirability.

F) Cost Analysis

The cost benefit ratio is an important tool to assess economics of farming. The cost benefit framework is essential a modular partial equilibrium model, with demand and supply relationships that can be calibrated to empirical data and allowing the calculation of economic welfare effects. The table 4 shows net return and cost benefit ratio of wine industry. Per year average 3,00,000 liters of processed wine and the average wholesale market price of per liter is Rs. 600/-. For computing the total returns from wine is per bottle Rs. 450/-, per liter Rs. 600/- and wine industry is Rs. 18,00,00,000/-. By deducting the total cost of per bottle Rs. 296.15/-, per liter Rs. 394.87/- and average cost of wine industry is Rs. 11,84,62,269/-, the net profit comes to about per bottle Rs. 161.53/-, per liter Rs. 215.36/- and wine unit is Rs. 6,15,37,731/-. The cost benefit ratio of wine is 1: 5.86. It is definitely an encouraging return to the wine producer.

Table 4: Net profit and Cost benefit ration for Jamun wine production

Items of Marketing Cost	Cost per Bottle (in Rs)	Cost per liter (in Rs)
Production cost (A+B)	69.079	92.10
Capital recovery cost (C)	7.67	10.23
Total production cost D = (A+B+C)	76.75	102.34
Marketing Cost	219.39	292.53
Total Cost	296.15	394.87
Gross Returns (E)	450.00	600.00
Net Profit	161.53	215.36
CBR (F=E/D)	1:5.86	1:5.86

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