

Optimization of alcoholic fermentation of dragon fruit juice using response surface methodology

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Abstract

In recent years, dragon fruit has been applied in food processing with various products. The purpose of this study aimed to valorize a fermented fruit drink from a Vietnamese local dragon fruit by alcoholic fermentation of the juice using *Saccharomyces cerevisiae*. Response surface methodology was applied to find the optimal condition of varied factors including total soluble solids content, inoculum size of the yeast and fermentation time. The experiments were carried out according to the central composite design with the sugar content ranged from 16 to 20°Brix, the inoculum size from 1 to 3% yeast and the fermentation time from 40 to 48 hrs. A quadratic model was respectively developed to correlate the investigated variables to the remained sugar content and ethanol yield of the product. The optimized conditions for fermentation were selected as 18°Brix of initial fruit juice; 2% v/v of added yeast inoculation rate and 44-hours fermentation time. Under these conditions, final fermented dragon fruit juice achieved alcohol content of 3.54% v/v with remaining sugar content of 14.6°Brix and a level of 'moderately like' obtained by sensorial evaluation.

1. Introduction

Dragon fruit (*Hylocereus* spp.) is a tropical fruit widely cultivated in tropical and subtropical countries, especially in Vietnam. Beside the attractive appearance, the pulp is juicy and contains numerous small black seeds. It is also considered as a potential source of micronutrients and antioxidants (Mahattanatawee *et al.*, 2006; Lim *et al.*, 2007; Lim *et al.*, 2010).

Fermented fruit juice can be used as a functional fermented food with many health benefits and becomes a popular drink being enjoyed worldwide. The vitamins, minerals and nitrogenous compounds in the fruit juice are essential to yeast growth and fermentation. In general, fermented juices of other fruits are processed in the same way as wine made from grapes. During fermentation, significant compositional changes take part as yeast metabolites sugars in the juice to convert into ethanol and carbon dioxide as a by-product. In addition to fermenting, yeast influences the character and flavor of products (Marquez *et al.*, 2012). Brewer's yeast, *Saccharomyces cerevisiae*, is very common in the brewery and wine industry. It was reported that *Saccharomyces cerevisiae* has been successfully used for alcoholic fermentation of juices collected from mango, lychee and papaya (Lee *et al.*, 2010; Reddy and Reddy,

2011; Chen and Liu, 2016). Effects of *Saccharomyces cerevisiae* Lalvin EC-1118 on physicochemical and oenological properties of red dragon fruit wine has been recently reported by Jiang *et al.* (2020). Choo *et al.* (2018) also studied the optimization of fermentation of Malaysian red dragon fruit for betalains concentration.

Recently in Vietnam, there has been a tendency for research to focus on adding value to local plant derivatives (Huan and Hong, 2016; Le *et al.*, 2017). In many studies, response surface methodology (RSM) has been widely applied to optimize the processing because it can effectively reduce the number of experimental trials (Živković *et al.*, 2018; Rahmawati *et al.*, 2019). The aim of this work was using RSM to optimize the alcoholic fermentation process of dragon fruit juice by *Saccharomyces cerevisiae* in order to valorize a Vietnamese local fruit by producing a fermented drink.

2. Materials and methods

2.1 Materials

2.1.1 Chemicals

Pectinex Ultra SP-L was obtained from Novo Nordisk Ferment (Dittingen, Switzerland). Folin-Ciocalteu reagent ($\geq 99.8\%$), potassium metabisulfite (\geq

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98.8%) and standard gallic acid (GA) ($\geq 99.9\%$) were supplied by Merck (Darmstadt, Germany). All other chemicals were analytical grade.

Raw material: White-flesh dragon fruits (*Hylocereus undatus*) were collected with the similarity in terms of maturity, weight and without any defects or crushes, from farmer households at Ham Thuan Nam district, Binh Thuan province, Viet Nam. They were washed and peeled before collecting fruit flesh. The flesh was stored by direct frozen -18°C before usage. The pulp was crushed directly and added water at a ratio of 1:2 (w/v) to get the dragon fruit juice (Wichienchot *et al.*, 2010). The juice was treated with pectinase (Pectinex Ultra SP-L) at 40°C in 120 mins (Truong and Dang, 2016), then filtered through a filter cloth to remove the solid parts.

2.1.2 Yeast

Saccharomyces cerevisiae 135 strain was obtained from the Yeast Collection of the Department of Viticulture and Enology, UC Davis (USA).

2.1.3 Media culture

Hansen medium (HiMedia, Mumbai, India) was used for stock culture and inoculum preparation. One liter of the medium composed of the following components: 10 g peptone, 50 g glucose, 3 g KH_2PO_4 , 2.5 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$. The medium was sterilized at 121°C for 15 mins before inoculating yeast.

2.2 Methods

2.2.1 Fermentation of dragon fruit juice

The dragon fruit juice was mixed with saccharose to achieve the investigated total soluble solids content (TSS). Potassium metabisulfite was added in the juice at a concentration of 100 mg/L to inhibit bacterial growth (Santos *et al.*, 2013). After that, yeast was added into the juice at a density of 1.2×10^7 (cells/mL). Fermentation was conducted at anaerobic conditions and room temperature of $30\text{--}32^{\circ}\text{C}$. With intended short fermentation time, final fermented juice contained low alcohol content and retained sweet characteristic of fruit juice as in the range of a cider.

2.2.2 Optimization of processing parameters for fermentation

A central composite design (CCD) was employed for the experimental data (Table 1). Three independent process variables were initial TSS ($^{\circ}\text{Brix}$; X_1), yeast inoculation rate (% v/v; X_2) and fermentation time (hours; X_3). The selected response variables were TSS of the product ($^{\circ}\text{Brix}$; Y_1) and alcohol content (v/v; Y_2). JMP version 10 was used to fit the quadratic response surface model to the experimental data.

Table 1. Variable values of the parameters

Parameters (Factors)	Variable values		
X_1 : Initial TSS ($^{\circ}\text{Brix}$)	16	18	20
X_2 : Yeast inoculation rate (% v/v)	1	2	3
X_3 : Fermentation time (hours)	40	44	48

2.3 Analytical methods

2.3.1 Determination of total soluble solids content

Hand-held refractometer (Atago, Japan) with detection level range of $0 - 32^{\circ}\text{Brix}$ was used to determine the sugar content, expressed as total soluble solids (TSS) content or Brix level of a sample. Samples were equilibrated to room temperature before used. The refractometer was first calibrated with distilled water and a reading of 0°Brix obtained.

2.3.2 Determination of pH

The pH of samples was measured using pH meter (Hanna pH211, USA). Calibration was done using the buffer solutions of pH 7 and pH 4 prior to measurement.

2.3.3 Determination of vitamin C content

Vitamin C was determined by using the iodine titration method (Suntornsuk *et al.*, 2002) with slight modification. Starch indicator solution was prepared by mixing 1 g of starch with 200 mL of boiling water. The solution must immediately remove from heat and left for cool. Each 25 mL of fresh juice sample was transferred into a 250 mL Erlenmeyer flask. About 25 mL of 2N sulfuric acid was added, mixed, diluted with water (50 mL) and starch indicator solution (3 mL) was added. The solution was directly titrated with 0.001N standardized iodine solution. A blank titration was performed prior to titration of each sample. One mL of 0.001N iodine was equivalent to 88.06 μg ascorbic acid.

2.3.4 Determination of total phenolic content

The total phenolic concentration was determined spectrophotometrically by using the Folin-Ciocalteu colorimetric method (Musa *et al.*, 2011). Samples of 1 mL fresh fruit juices were diluted with 4 mL distilled water. Then, 0.2 mL an aliquot of diluted juice samples and 1 mL of Folin-Ciocalteu reagent were mixed into a 20-mL calibrated flask. After 1 min, 4 mL of sodium carbonate (20% v/v) was added and the volume was made to 20 mL with distilled water. Finally, the mixture was allowed to stand at room temperature in darkness for 30 min and the absorbance of the solution at 765 nm was measured with a UV-VIS 2502 spectrometer (LaboMed Inc, USA). The total polyphenolic concentration was calculated from a calibration curve using gallic acid as a standard (50–500 mg/100 g). The result was recorded in

terms of mg of GA equivalents per 100 g of sample (mg GA/100 g).

2.3.5 Determination of alcohol content

Alcoholic volatile compounds in samples were separated by distillation, and the gravity of the distillate was measured by hydrometer. The alcohol content was then converted (AOAC, 1990).

2.3.6 Sensory evaluation

The sensory analysis was performed with non-trained panelists. The fermented dragon fruit juice was evaluated by 50 panelists of both sexes ranging from 18 to 28 years of age. Samples of 10 mL were served in clear glasses with a capacity of 25 mL. The evaluation sessions took place between 9 and 10 am at room temperature (30 - 32°C) under white light. The samples were evaluated for taste, aroma, appearance and overall impression according to the hedonic scale of nine categories: extremely dislike = 1; very much dislike = 2; moderately dislike = 3; slightly dislike = 4; neither like nor dislike = 5; slightly like = 6; moderately like = 7; very much like = 8 and extremely like = 9 (Ihekoronye and Ngoddy, 1985).

2.4 Statistical analysis

Experiments were carried out in triplicate and average values with standard errors were reported. Mean values of various parameters were computed and compared using analysis of variance (ANOVA) with the JMP software (version 10.0). Means and standard errors were calculated. The significant difference was defined at $p < 0.05$.

3. Results and discussion

3.1 Physical and chemical properties of local dragon fruit juice

The raw material is one of the important factors affecting the quality of fermented juice. The chemical compositions of the local dragon fruit juice were given in Table 2. Comparing these results with those obtained by Liaotrakoon (2013), it was observed that pH, total phenolic content and soluble solids of the investigated dragon fruit juice were similar (pH 4.5, 18.75 mg GA/100 g and 11.7°Brix, respectively). Similar results were also found in the production of dragon fruit juice and dragon fruit wine studied by Ma *et al.* (2010) and Foong *et al.* (2012) respectively. While the vitamin C content (16.66±0.17 mg/100 mL) of the juice found in this study is higher than the one reported by Liaotrakoon (2013) (7.32±0.21 mg/100 mL). The difference in vitamin C content was most likely due to differences in the determination of vitamin C method, maturation stage

and environmental growth variation of the fruit.

Table 2. Physical and chemical properties of dragon fruit juice

Parameters	Results
TSS (°Brix)	11.8±0.2
pH	4.5±0.03
Vitamin C (mg/100 mL)	16.66±0.17
TTA (g citric acid/100 mL)	0.58±0.02
Total phenolic content (mg GA/100 g)	17.04±0.14

3.2 Evaluation of key variables affecting fermentation of dragon fruit juice

The fermentation process was affected by different factors, especially the initial sugar content (°Brix), yeast inoculation rate (% v/v) and fermentation time (hours). Results of remained sugar content (°Brix) as well as the alcohol content (% v/v) of final fermented dragon fruit juices at different treatment levels were shown in Table 3. The analysis of variance (ANOVA) was performed for each response and optimal models.

3.2.1 Effect of processing factors on sugar content of fermented product

The responses of remaining sugar content left behind the alcoholic fermentation of dragon fruit juice can be obtained from the response surface design method. Statistical significance of the model term coefficients was determined by student's t-test and p test values as illustrated in Table 4.

It was shown that initial sugar content, yeast rate and fermentation time all significantly affected the remaining sugar content of the fermented product ($p < 0.05$). Quadratic equation showing the effect of factors on the Brix level of the final product was obtained as in Equation 1.

$$Y_1 = 14.51 + 1.56X_1 - 0.98X_2 - 0.42X_3 - 0.325X_1X_3 + 1.634 X_1^2 \quad (1)$$

In which, Y_1 is TSS of fermented product (°Brix); X_1 is initial TSS (°Brix); X_2 is yeast rate (% v/v); X_3 is fermentation time (hours).

The correlation between the initial Brix level and the Brix level of final fermented dragon juice was positively correlated. It showed that the higher the Brix level before fermentation was, the more Brix level after fermentation was obtained. The yeast inoculation rate and fermentation time-correlated negatively with the Brix level of product. Because more yeasts would utilize more sugar to grow and produce alcohol, the longer fermentation time lasted, the more sugar was consumed by yeast for fermentation, which led to a reduction of Brix level in the fermented product. The amount of yeast added to fermentation affected on criteria of final products as high or low Brix level. In another study,

Table 3. Changes in total soluble solids content (TSS) and alcohol content of product at different treatment levels

Exp.	Code	Initial TSS, X ₁ (°Brix)	Yeast rate, X ₂ (% v/v)	Fermentation time, X ₃ (hours)	TSS of product, Y ₁ (°Brix)	Alcohol content, Y ₂ (% v/v)
1	---	16	1	40	15.4	0.62
2	---+	16	1	48	15.2	0.75
3	a00	16	2	44	14.6	2.54
4	---+	16	3	40	14.8	2.27
5	---++	16	3	48	13.2	3.52
6	0a0	18	1	44	15.9	2.04
7	00a	18	2	40	14.9	3.12
8	0	18	2	44	14.6	3.54
9	0	18	2	44	14.6	3.54
10	00A	18	2	48	13.3	4.22
11	0A0	18	3	44	13.9	4.54
12	+++	20	1	40	19.2	1.03
13	+++	20	1	48	19	1.42
14	A00	20	2	44	17.6	2.05
15	+++	20	3	40	16.8	3.76
16	+++	20	3	48	16.2	4.82

(+) Upper limit value, (-) Lower limit value, (a) High axis value, (A) Low axis value, (0) Value at midpoint

Table 4. Effect of factors on remaining sugar content of the final fermented product

Term	Estimate	Std Error	t Ratio	Prob> t
Intercept	14.510345	0.140061	103.6	<.0001*
TSS (16,20)	1.56	0.093554	16.67	<.0001*
Yeast rate (1,3)	-0.98	0.093554	-10.48	<.0001*
Time (40,48)	-0.42	0.093554	-4.49	0.0042*
TSS*Yeast rate	-0.325	0.104596	-3.11	0.0209*
TSS*Time	0.125	0.104596	1.2	0.2771
Yeast rate*Time	-0.225	0.104596	-2.15	0.075
TSS*TSS	1.6344828	0.182204	8.97	0.0001*
Yeast rate*Yeast rate	0.4344828	0.182204	2.38	0.0544
Time*Time	-0.365517	0.182204	-2.01	0.0917

glucose consumption profile was reported during grape juice fermentation by *Saccharomyces cerevisiae* (Tronchoni et al., 2009).

3.2.2 Effect of factors on alcohol content of fermented product

Effect of processing parameters on the alcohol content of the final product can be observed through the student's t-test and p test values in Table 5. In particular, only two factors including initial Brix level and yeast rate were positively correlated to the alcohol content of the fermented product. Fermentation time did not show a significant effect due to a short-investigated time in the range of 40 - 48 hrs. Hence there was not any significant difference among alcohol contents of fermented juice products.

Quadratic equation showing the effects of processing factors on alcohol content was obtained in Equation 2.

$$Y_2 = 3.513 + 0.338X_1 + 1.305X_2 + 0.214X_1X_2 - 1.204X_1^2 \quad (2)$$

In which, Y₂ is alcohol content of fermented product (% v/v); X₁ is initial TSS (°Brix); X₂ is yeast rate (% v/v).

In addition, it can be also observed that two factors including the Brix level of the juice and yeast inoculation rate had an interaction during fermentation. This meant that when the initial sugar level was too low, the yeast would not have enough nutrients to increase biomass, which led to producing low alcohol content. Too much sugar added would inhibit yeast activity due to large osmotic pressure difference, resulting in the reduction of performance yeast. However, Brix level in the range of 16-20°Brix was still at the permitted range of Brix level (10-25°Brix) (Ough, 1966) thus fermentation activity still occurred normally. Turhan et al. (2010) reported a significant effect of inoculum size of *Saccharomyces cerevisiae* on the ethanol production from carob extract. It was also reported that *Saccharomyces cerevisiae* can

Table 5. Effect of factors on the alcohol content of the fermented product

Term	Estimate	Std Error	t Ratio	Prob> t
Intercept	3.5127586	0.188077	18.68	<.0001*
TSS (16,20)	0.338	0.125626	2.69	0.0360*
Yeast rate (1,3)	1.305	0.125626	10.39	<.0001*
Time (40,48)	0.393	0.125626	3.13	0.0240
TSS*Yeast rate	0.21375	0.140454	1.52	0.0189*
TSS*Time	0.00875	0.140454	0.06	0.9523
Yeast rate*Time	0.22375	0.140454	1.59	0.1623
TSS*TSS	-1.204138	0.244667	-4.92	0.0027*
Yeast rate*Yeast rate	-0.209138	0.244667	-0.85	0.4255
Time*Time	0.1708621	0.244667	0.70	0.5111

convert the sugar in the fruit juices into alcohol and organic acids, that later react to form aldehydes, esters, and other chemical compounds (Fleet, 2003; Duarte *et al.*, 2010).

3.3 Optimization of processing parameters for fermentation

A three-dimensional response surface for the impact of the investigated processing factors on the Brix level and alcohol content of the fermented product was established (Figure 1).

Each point on the three-dimensional surface in Figure 1 illustrated a value of Brix level or alcohol content of the final product which was affected by three investigated processing factors. According to response surface, levels and trend of influence of all three investigated factors on the monitored results can be observed. As results, the lowest or highest alcohol content and Brix level of fermented product could be observed. After being analyzed by the developed model, parameters were optimized and showed in Figure 2.

Table 6 shows the value of Brix level and alcohol content of the product which was predicted at the

optimum conditions of process parameters in comparison with the actual value obtained by carrying out the experiment. It can be seen that at the optimum levels of processing parameters, the experimental value of Brix level of 14.6°Brix and alcohol content of 3.54% (v/v) were obtained which were very close to its predicted value by the quadratic model. Thus, the processing parameters consisting of 18°Brix; 2% (v/v) yeast rate and fermentation time of 44 hrs were finally selected for the dragon fruit juice fermentation by *S. cerevisiae* 135. The optimized initial sugar content obtained in this study is in the same range with those reported in alcoholic fermentation by *Saccharomyces cerevisiae* of guava and cashew apple juices (Attri, 2009; Sevdá and Rodrigues, 2011).

Table 6. Predicted and actual values of the optimum experimental conditions

Parameters	Predicted value	Actual value
Initial TSS (°Brix)	18	18
Yeast rate (% v/v)	2	2
Fermented time (hours)	44	44
TSS of product (°Brix)	14.5	14.6
Alcohol content of product (% v/v)	3.51	3.54

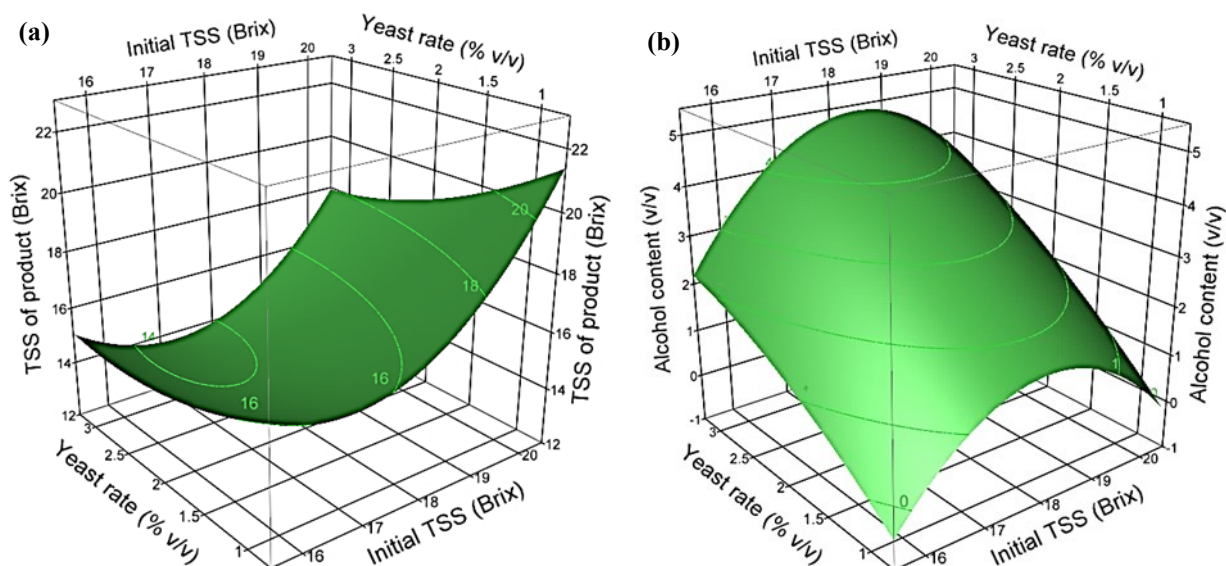


Figure 1. Response surface of Brix level (a) and alcohol content (b) of fermented product

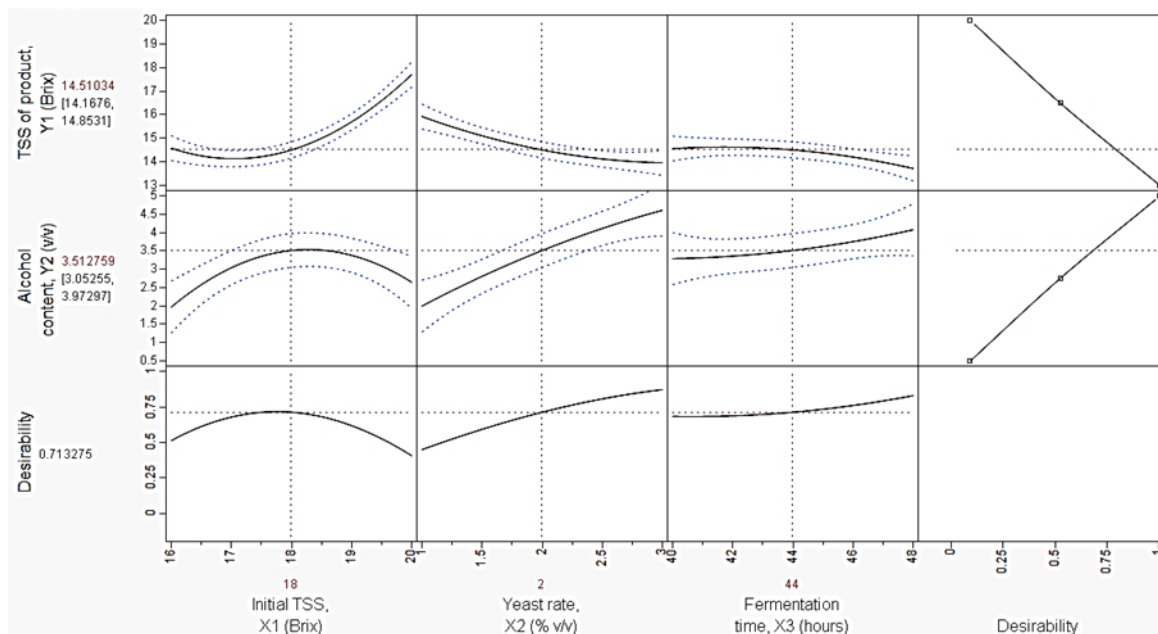


Figure 2. Prediction profile analysis of alcoholic fermentation

3.4 Sensory evaluation of fermented dragon fruit juice

In addition, to evaluate the quality of products, a sensory test of fermented dragon fruit juice by consumer acceptance test was performed. Table 7 presents the frequency of acceptance attributed to fermented juice by 50 untrained tasters, designated based upon a nine point hedonic scale. Similar averages were recorded for the four evaluated attributes, where the appearance achieved the highest value, followed by the general aroma, taste with scores of 7.8, 7.3 and 7.1. The appearance attribute had the highest frequency of the maximum value in the hedonic scale (13 votes—26% of the tasters). The taste of the fermented juice reached an average of 7.1, indicating that the tasters ‘moderately liked’ the taste of the fermented dragon fruit juice. The overall attribute which corresponds to the total acceptance of the fermented juice by the tasters reached the highest frequency (23 tasters, 46% of the total) in point 8 on the hedonic scale, meaning that the tasters ‘liked very much’ the fermented juice. Therefore, fermented dragon fruit juice by *Saccharomyces cerevisiae* 135 was accepted by the customer, hence it can be prompted as potential products for the beverage industry as well as the beverage market.

Table 7. Frequency and average notes for the attributes of sensory analysis

Attribute	Frequency and average notes									
	1	2	3	4	5	6	7	8	9	Mean
Appearance	1	-	1	-	-	4	4	27	13	7.8
Aroma	-	-	-	1	1	10	12	20	6	7.3
Taste	-	-	-	2	4	9	12	18	5	7.1
Overall	-	-	-	1	-	4	16	23	6	7.6

Extremely dislike = 1, very much dislike = 2, moderately dislike = 3, slightly dislike = 4, neither like nor dislike = 5, slightly like = 6, moderately like = 7, very much like = 8 and extremely like = 9.

4. Conclusion

Fermented dragon fruit juice by *Saccharomyces cerevisiae* 135 with total soluble solid content of 11.8° Brix, pH 4.5, vitamin C concentration of 16.66 mg/100 mL, total acidity of 0.58 g of citric acid/100 mL and total phenolic content of 17.04 mg GA/100 g was successfully produced. The selected yeast strain has a good impact on sugar consumption and alcohol production during the primary fermentation process. The optimized conditions for fermentation were established as 18°Brix of fruit juice; 2% added yeast inoculation rate and 44-hour fermentation time. Under the optimal fermentation conditions, the final product achieved alcohol content of 3.54% (v/v) with remaining sugar content of 3.54°Brix. The sensorial quality of the fermented juice was accepted according to the results of the consumer acceptance test.

Conflict of interest

The authors declare no conflict of interest.

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