Research Article

Effect of Formulation on Quality of Bottle Gourd (*L. Siceraria*) Blend Juice

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Abstract

Bottle gourd (*L. siceraria*) juice was duly blended with Aonla, Lemon and Ginger juices formulating through central composite design using response surface methodology with 30 experimental combinations. The statistical models were generated to analyze the dependent variables with respect to the independent variables. In the optimization process, the specific constraints were applied on the variables to maximize chemical contents to boost quality and over all acceptability to meet criterion of food safety and standards regulations, 2011. The R-squared values for all responses were found more than 0.91 showing good fit of models to the data and second order generic equation was derived. The best solution for quality blend juice was found with the desirability value of 0.89 having chemical contents as ascorbic acid 50.16 mg/100 ml, pH 3.51, TSS 5.13 ^{0}Brix and OAA 6.86 at 87.90 ml of bottle gourd, 23.40 ml of aonla, 5.70 ml of lemon and 6.00 ml of ginger juice during optimization. The t-test in the validation indicated that there was no significant difference between the predicted and observed values of responses; hence the model was significant and fitted to the data perfectly for the formulation of desired quality blend juice.

Keywords: Blend juice, pH, TSS, Ascorbic acid, Overall acceptability

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Introduction

Fruits and vegetables are important sources of chemical contents required for human nutrition in daily diet. Among various fruits and vegetables grown worldwide, bottle gourd (*Langenaria siceraria)* fruit has a high place in daily diet. It belongs to *Cucurbitaceae* family and known as Calabash, Cojombro, Courage bouteille, Gucuzzi, Guiro amargo, Mokwa, Oo Lo Kwa, Trumpet gourd, Upo, Talayag, Zucca melon [1]. Tender bottle gourd fruits are widely used for the preparation of vegetable, sweet and Kofta [2, 3]. Bottle gourd is a good source of vitamins, minerals, cucurbitacins, choline, polyphenols, campesterol and sitosterol and is well known for its antioxidant, antihyperlipidemic, anti-stress, adaptogenic, aphrodisiac, analgesic, anti-inflammatory, antidote to certain poisons, alternative purgative, cardio protective, cardio tonic, cooling, diuretic, hepatoprotective and immunomodulatory properties [4-8].

Bottle gourd fruit have higher edible index, proving its importance for processing. The bottle gourd juice has short self-life and requires further preservation. Also, normally pure bottle gourd juice is not consumed. Current diversified food trend toward healthier diets makes blend juice consumption an important natural food alternative and improves the availability of its nutritive compounds. Great potentiality exists for commercial processing of this underutilized fruit for production of blended juice. To improve the taste, aroma, palatability and nutritive value of the bottle gourd juice, it was blended with highly nutritive fruit juices namely aonla (*Emblicaofficinalis Gaertn*.) and lemon (*Citrus x limon*) with spice ginger (*Zingiber officinale*) extract as flavouring agent [9, 10]. The objective of the present experiment was to find out the effect of formulation on the quality of bottle gourd blend juice to meet food safety and standards regulations, 2011.

Materials and Methods

Bottle gourd fruits (*cv.* ABG-1), aonla fruits (*cv.* Anand-2), lemon fruits and spice ginger rhizome were procured from the Horticultural Farm, Anand Agricultural University and local vegetable market (Anand, India). The fruits and ginger rhizomes were cleaned and washed twice with running tap water. The bottle gourd fruits were sliced in 5 mm and blanched at 100 ^{0}C in water bath. Anola fruits were blanched in boiling water for 6 min. Juice from both fruits was extracted separately using centrifugal juicer. Lemon juice was extracted squeezing the cut lemon while juice from ginger was prepared using a domestic mixer cum grinder. Prepared juices were strained through muslin cloth before blending. Response surface methodology (RSM) using central composite design (CCD) was used to formulate 30 experiments for statistical optimization of the blend juice [11-14].

Chemical content ascorbic acid (mg/100ml) were estimated by visual titration method using 2, 6-dichlorophenol-indophenol as described by Ranganna [15]. TSS (^{0}Brix) of the juice were measured using refractometer-PAL-1(ERMA, Japan) having 0-53 ^{0}Brix range. For sensory quality, evaluating appearance & colour, flavor, taste, consistency and overall acceptability of the blend juice on 9 point hedonic rating as suggested by Ranganna [15] were carried out.

Experimentation values were analyzed by the Design-Expert software, (Design expert® 8.0.7.1, Statease Inc., Minneapolis, USA) and the statistical software SAS® version 9.3 (SAS Institute Inc., Statistical Analysis System, Cary, NC). The T-test was used to analyze responses of optimized blend juice as compared to desired at $P < 0.05$.

Results and Discussion

To evaluate the effects of different formulated blend juice samples, the experiments were conducted taking pre-treated bottle gourd and aonla and, lemon and ginger juices at the actual range of levels 70-100, 01-30, 01-20 and 01-06 ml, respectively as independent variables. The ascorbic acid, pH, TSS and overall acceptability scores were their responses as dependent variables. The treatments consists 4 numeric factors as independent variables, varied over 5 levels in terms of plus and minus alpha. Numerical factors decided using the coded values of -1, -0.5, to +0.5, +1 through 0 was the centre point. The independent variables and its coded levels considered in experiments were showed in **Table 1**.

Table 1 Levels and experimental ranges of independent variables used in RSM in terms of coded factors for blend juices

Independent variables		Range of levels (Coded)				
		-1	**-0.5**	**0**	**+0.5**	**+1**
A	Bottle gourd juice (ml)	70.00	77.50	85.00	92.50	100.00
B	Aonla juice (ml)	01.00	08.25	15.50	22.75	30.00
C	Lemon juice (ml)	01.00	05.75	10.50	15.25	20.00
D	Ginger juice (ml)	01.00	02.25	03.50	04.75	06.00

Effect of formulation on ascorbic acid content of blend juice

The perturbation graph at actual factors showed the effect of independent variables on the responses revealed that the bottle gourd and ginger juice level lowering the effect of ascorbic acid while aonla juice level elevated ascorbic acid in the blend juice (**Figure 1**). ANOVA indicated that bottle gourd and aonla juices, and their interaction had highly significant ($p<0.01$) effect on ascorbic acid content of blend juice while ginger juice, interaction of anola and lemon juices, and the quadratic term A^2 and B^2 was significant only at 5% probability (**Table 2**). Non- significant effect was also observed for lemon juice, rest of the interactions and quadratic term C^2 and D^2 on ascorbic acid content of blend juice. The regression equation describing the effect of process variables on ascorbic acid content of blend juice in terms of actual levels of variables was derived as:

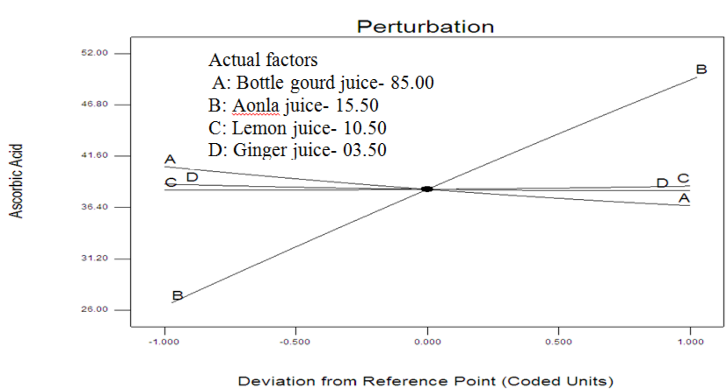

Figure 1 Effect of independent variables on ascorbic acid of blend juice

$$\textbf{Ascorbic acid} = +66.2391 - 1.12074 * A + 3.05351 * B - 0.015184 * C - 2.51151 * D - 0.0125 * AB + 0.0015 * AC + 0.016752 * AD - 0.01613 * BC - 0.00676 * BD + 0.0038 * CD + 0.00573 * A^2 - 0.00711 * B^2 + 0.00623 * C^2 + 0.12571 * D^2$$

Table 2 ANOVA for effect of different raw juices on ascorbic acid content of blend juice

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F
Model	3257.69	14	232.69	932.81	< 0.0001
A	96.07	1	96.07	385.13	< 0.0001
B	3135.86	1	3135.86	12570.92	< 0.0001
C	0.74	1	0.74	2.95	0.1066
D	2.78	1	2.78	11.16	0.0045
AB	7.41	1	7.41	29.69	< 0.0001
AC	0.046	1	0.046	0.18	0.6745
AD	0.39	1	0.39	1.58	0.2277
BC	4.94	1	4.94	19.78	0.0005
BD	0.06	1	0.06	0.24	0.631
CD	0.00814	1	0.00814	0.033	0.859
A^2	2.85	1	2.85	11.41	0.0041
B^2	3.83	1	3.83	15.36	0.0014
C^2	0.54	1	0.54	2.17	0.1614
D^2	1.06	1	1.06	4.24	0.0572
Residual	3.74	15	0.25		
Lack of Fit	1.55	10	0.16	0.36	0.9231
Pure Error	2.19	5	0.44		
Cor Total	3261.44	29			
R-Squared	0.9989				
Adj R-Squared	0.9978				
Adeq Precision	129.465				
C.V. %	1.29				
Std. Dev.	0.5				

Effect of formulation on pH of blend juice

The pH of the blend juice was increased significantly with increase in the quantities of bottle gourd and ginger juices (**Figure 2**). A positive correlation was observed between bottle gourd juice and pH of blended juice. Ginger juice also had direct variation with pH of blend juice but effect was lower than that of the bottle gourd juice as its proportion was very low. The addition of aonla and lemon juices improved the ascorbic acid content of the blended juice along with increase in acidity. The finding is in conformity with the result reported by Majumdar et al. (2011) [12]. The bottle gourd, aonla, lemon, ginger, interaction of aonla and lemon juice, and the quadratic term B^2 and C^2 had highly significant (p<0.01) effect on pH of the blend juice while the interaction of bottle gourd and aonla, bottle gourd and lemon, and the quadratic term A^2 and D^2 was significant only at 5% probability (**Table 3**). The effect of the process variables on pH analysis of the blend juice in terms of actual levels of variables was described in terms of regression equation as:

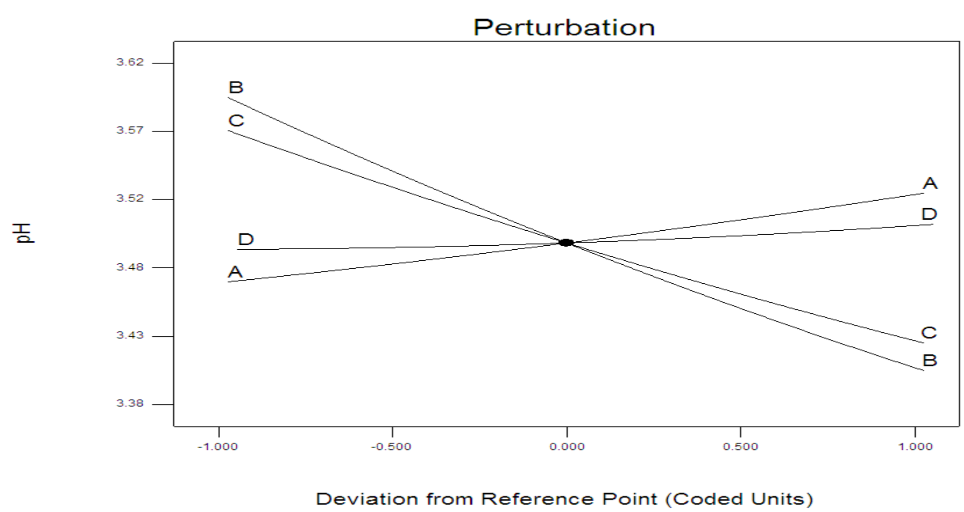

Figure 2 Effect of independent variables on pH of blend juice

$$pH = +4.26398 - 0.00822 * A - 0.02651 * B - 0.04213 * C - 0.00217 * D + 0.0000721 * AB + 0.000203 * AC - 0.000022 * AD + 0.000270 * BC - 0.00025 * BD - 0.000243 * CD + 0.0000544 * A^2 + 0.000168 * B^2 + 0.000282 * C^2 + 0.00249 * D^2$$

Table 3 ANOVA for effect of different raw juices on pH of blend juice

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F
Model	0.38	14	0.027	838.92	< 0.0001
A	0.024	1	0.024	727	< 0.0001
B	0.22	1	0.22	6734.88	< 0.0001
C	0.13	1	0.13	4057.5	< 0.0001
D	0.0018	1	0.0018	55.02	< 0.0001
AB	0.000246	1	0.000246	7.51	0.0152
AC	0.000834	1	0.000834	25.47	0.0001
AD	0.000000681	1	0.000000681	0.021	0.8873
BC	0.00138	1	0.00138	42.11	< 0.0001
BD	0.0000824	1	0.0000824	2.52	0.1335
CD	0.0000334	1	0.0000334	1.02	0.3288
A^2	0.000257	1	0.000257	7.84	0.0134
B^2	0.00214	1	0.00214	65.4	< 0.0001
C^2	0.00111	1	0.00111	33.89	< 0.0001
D^2	0.000414	1	0.000414	12.64	0.0029
Residual	0.000491	15	0.0000327		
Lack of Fit	0.000324	10	0.0000324	0.97	0.5499
Pure Error	0.000167	5	0.0000334		
Cor Total	0.38	29			
R-Squared	0.9987				
Adj R-Squared	0.9975				
Adeq Precision	104.007				
C.V. %	0.16				
Std. Dev.	0.00572				

Effect of formulation on total soluble solids (TSS) of blend juice

The TSS of blend juice had direct relationship with the amount of aonla and lemon juices in the blend and had inverse relationship with the quantity of bottle gourd and ginger juices (**Figure 3**). The TSS of the blend juice decreased significantly with the increase in quantities of bottle gourd and ginger juices. From ANOVA, it can be observed that the addition of bottle gourd, aonla and lemon juice had highly significant effect at 1% probability while ginger juice had significant ($p<0.05$) effect on TSS of blended juice (**Table 4**). Interaction of bottle gourd and lemon juice had significant ($p<0.05$) effect while no significant effects of other interactions were found. The quadratic term A^2 affected significantly at $p<0.05$, whereas no significant effect was found for B^2, C^2 and D^2 on TSS of blend juice. The regression equation describing the effects of process variables on TSS of blend juice in terms of actual levels of variables was derived as:

$$TSS = +8.15977 - 0.08689 * A + 0.10395 * B - 0.02532 * C - 0.16183 * D - 0.00042 * AB + 0.00076 * AC + 0.00156 * AD - 0.000665 * BC - 0.00115\ BD - 0.000351 * CD + 0.000389 * A^2 + 0.000178 * B^2 - 0.000139 * C^2 + 0.00333 * D^2$$

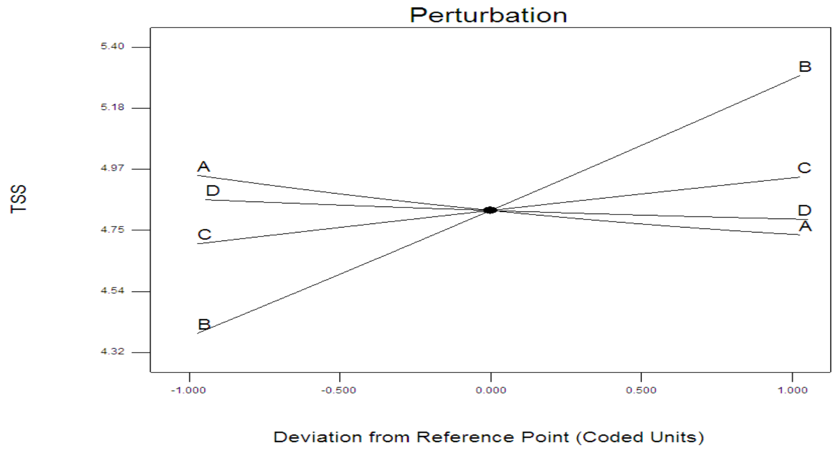

Figure 3 Effect of independent variables on TSS of blend juice

Table 4 ANOVA for effect of different raw juices on TSS of blend juice

Source	**Sum of Squares**	**df**	**Mean Square**	**F Value**	**p-value Prob > F**
Model	5.62	14	0.4	197.8	< 0.0001
A	0.26	1	0.26	128.23	< 0.0001
B	4.95	1	4.95	2437.59	< 0.0001
C	0.33	1	0.33	164.7	< 0.0001
D	0.029	1	0.029	14.25	0.0018
AB	0.00840	1	0.00840	4.14	0.06
AC	0.012	1	0.012	5.78	0.0296
AD	0.00340	1	0.00340	1.68	0.2151
BC	0.00840	1	0.00840	4.14	0.06
BD	0.00174	1	0.00174	0.85	0.3698
CD	0.0000694	1	0.0000694	0.034	0.8558
A^2	0.013	1	0.013	6.46	0.0225
B^2	0.00241	1	0.00241	1.19	0.2931
C^2	0.000268	1	0.000268	0.13	0.7215
D^2	0.000744	1	0.000744	0.37	0.554
Residual	0.03	15	0.00203		
Lack of Fit	0.013	10	0.00127	0.36	0.9223
Pure Error	0.018	5	0.00356		
Cor Total	5.65	29			
R-Squared	0.9946				
Adj R-Squared	0.9896				
Adeq Precision	57.010				
C.V. %	0.93				
Std. Dev.	0.045				

Effect of formulation on overall acceptability (OAA) of blend juice

The OAA scores increased with the increase in aonla juice in the blend and decreased with the increase in lemon juice in the product (**Figure 4**). An inverse relationship was observed between addition of ginger juice and OAA of the blend juice. The OAA scores increased with increase in bottle gourd juice at certain level but further increase of bottle gourd juice decreased the OAA of blend. The decrease in levels of lemon, aonla and bottle gourd juices, the OAA of blend juice also decreased might be due to the complex effect of various responses. Hence, to increase OAA score of blend juice, concentration of one juice has to be increased or decreased with subsequent to other variables. ANOVA showed that the addition of aonla and the quadratic term B^2 and C^2 had highly significant effect on OAA ($p<0.01$) of blend juice (**Table 5**). There was no significant effect of all the interactions and the quadratic terms D^2 on the OAA scores. The regression equation describing the effect of process variables on OAA scores of blend juice in terms of actual levels was described as:

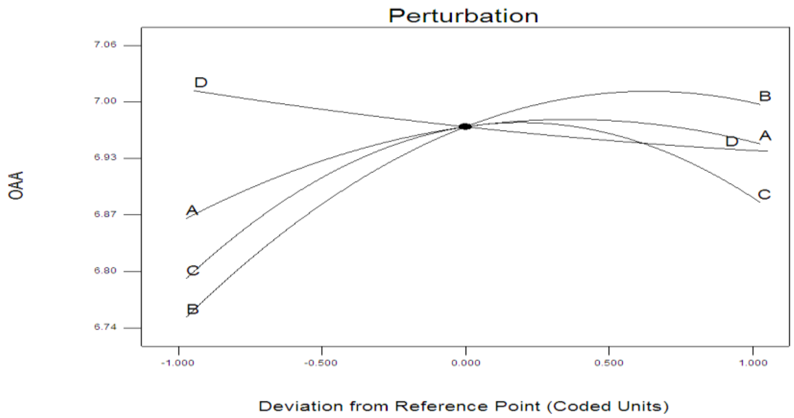

Figure 4 Effect of independent variables on overall acceptability of blend juice

$$\mathbf{OAA} - 3.3849 + 0.20436 * A + 0.069434 * B + 0.13969 * C + 0.020714 * D - 0.000125 * AB - 0.000107 * AC - 0.00167 * AD + 0.00015 * BC + 0.0042 * BD - 0.000798 * CD - 0.00112 * A^2 - 0.00188 * B^2 - 0.00577 * C^2 + 0.00537 * D^2$$

Table 5 ANOVA for effect of different raw juices on OAA of blend juice

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F
Model	1.23	14	0.088	11.94	< 0.0001
A	0.04	1	0.04	5.52	0.0329
B	0.36	1	0.36	49.13	< 0.0001
C	0.044	1	0.044	6	0.0271
D	0.028	1	0.028	3.75	0.0719
AB	0.000745	1	0.000745	0.1	0.7544
AC	0.000231	1	0.000231	0.032	0.8614
AD	0.00393	1	0.00393	0.54	0.4753
BC	0.000425	1	0.000425	0.058	0.8129
BD	0.023	1	0.023	3.16	0.0956
CD	0.000359	1	0.000359	0.049	0.8278
A^2	0.11	1	0.11	14.78	0.0016
B^2	0.27	1	0.27	36.35	< 0.0001
C^2	0.46	1	0.46	63.35	< 0.0001
D^2	0.00193	1	0.00193	0.26	0.6152
Residual	0.11	15	0.00733		
Lack of Fit	0.095	10	0.00945	3.06	0.1146
Pure Error	0.015	5	0.00309		
Cor Total	1.34	29			
R-Squared	0.9177				
Adj R-Squared	0.8408				
Adeq Precision	12.322				
C.V. %	1.27				
Std. Dev.	0.086				

Fitment of model and equation

The Prob>F Values less than 0.0001 showed models were highly significant. R-Squared values for all responses were high, showed agreement with each other. The model F Values of 932.81, 838.92, 197.8 and 11.94 for bottle gourd, aonla, lemon and ginger juices showed that the polynomial model was significant fit. The Lack of Fit test were found to be non significant for each responses indicated the developed models were adequate for predicting the responses (Tables 2-5). The R-Squared values > 0.80 is statistically adequate for developing a model or equations [16]. The R-

Squared values for all responses were more than 0.91 showed good fit of models to the data and highly suitable for predicting response through the second order generic equation which could be derived as:

$$y = \beta_0 + \sum_{i=1}^{4} \beta_i X_i + \sum_{i=1}^{4} \beta_{ii} X_i^2 + \sum_{i=j=1}^{4} \beta_{ij} X_i X_j , \qquad i < j$$

Where, $\beta_0, \beta_i, \beta_{ii}, \beta_{ij}$ were regration co-efficient of linear, quadratic and interaction effect respectively and y denoted the number of depended variables.

***Optimization** of model*

During formulation conditions during optimization of blend juice in RSM, the specific constraints were applied on the variables. The optimization criteria were to maximize ascorbic acid and OAA sensory score and, targeted pH and TSS of blend juice (**Table 6**). The best solution for blend juice was found with the desirability value of 0.89 having ascorbic acid 50.16 mg/100 ml, pH 3.51, TSS 5.13 ^{0}Brix and OAA 6.86 at 87.90 ml of bottle gourd, 23.40 ml of aonla, 5.70 ml of lemon and 6.00 ml of ginger juice.

Table 6 Formulation conditions during optimization of blend juice in RSM

Constraints	Goal	Optimization	
		Lower limit	Upper limit
Factor			
A- Bottle gourd juice, ml	in range	70	100
B-Aonla juice, ml	in range	1	30
C-Lemon juice, ml	in range	1	20
D-Ginger juice, ml	in range	1	6
Response			
Ascorbic acid, mg/100 ml	maximize	13.51	60.12
pH	is target = 3.51	3.31	3.72
TSS, ^{O}Brix	is target = 5.20	3.97	5.73
OAA	maximize	6.26	7.04

Validation of model

The validation of the optimum solution was done by conducting the experiment at above levels of variables. The actual values for the responses of the optimized blend juice were compared with the predicted values using t-test (**Table 7**). The t-test indicated that there was no significant difference between the predicted and observed values of responses; hence the model was significant and fitted to the data perfectly.

Table 7 Responses of optimized blend juice as compared to desired

Attributes	Desired score*	Observed score$^@$	t-value$^\#$
Ascorbic acid (mg/100 ml)	50.16	48.27^{NS} ± 1.95	1.67
pH	3.51	3.51^{NS} ± 0.01	0.50
TSS (^{0}Brix)	5.13	5.17^{NS} ± 0.15	0.42
OAA	6.86	6.93^{NS} ± 0.21	0.59

* Predicted values of Design Expert 8.0.7.1 package
$^@$ Actual values (triplicate trials) of optimized product (mean ± std. deviation)
t-values found non-significant (P < 0.05), t-critical= 4.303(P < 0.05)

Conclusions

Desire quality bottle gourd blend juice could be formulated using response surface methodology analyzing the dependent variables with respect to the independent variables. The ascorbic acid was kept maximize to boost quality (vitamin C) of blend juice. The blend juice pH was kept targeted at 3.51 to produce acidic juice to reduce further thermal process, prevent the growth of microorganisms and to increase overall acceptability [17-19]. The TSS was kept targeted at 5.20 to meet a criterion of food safety and standards regulations (FSRRs), 2011[20]. Considering the optimized levels of variables, desired quality blend juice was formulated for further minimal thermal process.

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