Effect of tannase (*Aspergillus ficcum*) on physicochemical properties of clarified *Jamun* juice

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Abstract: Indian black berry known as *Jamun* is a minor fruit which contains high amount of tannin. Extraction and clarification of juice is quite difficult due to its pulp nature. Tannase a membrane bound enzyme is added to clarify the *Jamun* juice which helps to obtain high yield. The target area of the work is to build up the procedure for streamlining of process factor to obtain *Jamun* juice, utilizing tannase (strain: *Aspergillus ficcum*). Physicochemical parameters (clarity, colour change, polyphenol, turbidity, protein, TSS and yield) were analyzed at a temperature range between (30^oC – 50^oC), with (0.01% – 0.1% w/v) concentration and time orbit of (40-120 min). Coefficient of determination (R^2) value more than 0.9 have been used to measure the prominent differences in the response characteristics. Clarified juice was obtained at 0.05% enzyme concentration at 40^oC for 80 minutes.

Keywords*: Jamun* (Indian black berry), enzymatic extraction, tannase(*Aspergillus ficcum*), box-Behnken design, optimization

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1 Introduction

Among a few tropical fruits in India, *Jamun* (*Syzygium cuminii*) is an indigenous minor fruit with high sanative worth. It is native to East Asian and Indian sub-continents, also grow in regions like Philippines, Florida, Hawaii, Australia, Kenya, and so forth. There is no standard variety of *Jamun*, since it is based on the place. The harvesting time of the fruit is short (30 to 40 days) in the time of monsoon season. The fruit is beefy, dull purple shaded color and ovoid fit with a single hard seed at core. It is utilized for juices, jellies, jam, and the seed with own therapeutic esteem for ailments like diabetics and diarrhea (Baliga et al., 2013). Contrasted with other well-known natural products like sapota, papaya, guava, and, banana *Jamun* have more antioxidant properties. The existence of anthocyanins, antioxidants, tannin make the fruit as cancer prevention agent (Arun et al., 2011; Aqil et al., 2012).

Saltwater soaking or pricking method is used to increase the palatability of the astringent fruits. In India, it is used locally as bottled drink with added sodium benzoate, water, citric acid and sugar by boiling it for five to ten minutes with temperature of 140°F (Chung et al., 2017; Ghosh et al., 2018). A considerable number of anthocyanins, tannins and sugar became waste which are in contact with the pomace during the extraction of the fruit. There is no proper cultivation method for *Jamun* and due to the unorganized cultivation method, per year huge losses incurred. The postharvest management of this fruit is further difficult because of its perishable nature. These can be stored for three weeks at 85%-95% humidity in the perforated bags after precooling (Shahnawaz and Sheikh,

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2011).

To obtain a high amount of juice with all nutritional quality, few methods are available like enzymatic treatment. Several enzymes had been used for the juice clarification to obtain a high yield like tannase (tannin acyl hydrolase, EC Number 232-804-4) which accelerates the process of bond hydrolysis in hydrolysable tannins through liberating glucose and gallic acid (Lekha and Lonsane, 1997). Generally, one of the major sources and application of tannin is in tea and coffee industry. Tannase have significant application in the production of soft drinks flavoured with coffee, beer and fruit juice clarifying process. It also enhances the flavour elevating activity in the grape wine, due to the presence of gallic acid esters.

Recently, Tannase, proved its potential in reducing cloudiness and haze formation in the fluid-based industry. Rout and Banerjee (2006) conveyed that this treatment for juice of pomegranate has shown 25% debasement of tannin. Whereas according to Srivastava and Kar (2009), the treatment for juice of anola with tannin enzyme caused 68.8% evacuation of tannin lowering the astringency. Motoichi et al. (2001) asserts that vegetable and fruit juice can be put away for longer span without demonstrating any turbidity or precipitation when they are treated with carboxylic ester hydrolase, in specific tannase or chlorogenase. Proanthocyanidins was extracted from País grape skin and seeds by using tannase in part with other enzymes (Fernández et al., 2015). Juice of apple-cashew is proofed with tannase enzyme (0.1%) for 1 hour, a temperature of 30^{o}C and passes through membrane filtration which had shown a better physical stability and dipped astringency at long term storage study (Campos et al., 2002). According to the review it has been noticed that a critical gap has been noticed for enzymatic conditions for tannase enzyme of *Jamun* juice extraction.

The objectives of the present work are to determine and optimize the temperature, incubation time, and concentration for *Jamun* juice clarification for a high yield with tannase and to check the deviations in physicochemical characteristics (clarity, color change, polyphenol, turbidity, protein, yield and along with Total Soluble Solid (TSS) in the fresh and treated samples.

2 Materials and methods

2.1 Sample

Jamun fruits are highly putrescible. Thus, ripe, fresh and clean *Jamun* (*Ram Jamun*) were collected from Rourkela (Sundergadh Dist.), Odisha, India (84.54E longitude; 22.12N latitude). They were further cleaned properly, washed, sealed in polythene bags (perforated), and stored at temperature of -20^{o}C±1^{o}C for future use (Benherlal and Arumughan, 2010). Before experiments, samples were thawed for 3 h at room temperature. Tannase (Commercial Name: Tannase; activity ≥150 U g^{-1}) from an *Aspergillus ficcum* was purchased from the Sigma-Aldrich, Bangalore, India. All the compounds utilized in the experiment were analytically rated and obtained at Sisco Research Pvt. Ltd, Mumbai, India. The glass apparatus utilized in performing the experiment were obtained from Borosil Glass Works Ltd., Mumbai, India.

2.2 Juice extraction method

Jamun picked out of the freezer were properly thawed for about a period of 3 hours. Prior to extraction, the seeds were removed out manually. Then the thick pulp was mixed with (Bajaj Mixer GX-1 500-watt mixer grinder) for about five minutes to its high rpm. Afterwards, the enzyme and pulp were added into beakers in accordance with convenient temperature and time as per the experiment design. Juice extraction was carried out according to the experimental design.

2.3 Enzymatic treatment

The experimental design showing coded and real variables is presented in Table 1. 100g of weighed homogenous pulp was used for each experimental run. Independent variables include enzyme incubation temperature, X_1(30^{o}C–50^{o}C), concentration, X_2 (0.01-0.1 w/v percent), and incubation time, X_3 (40–120 min). The mixture was subjected to vigorous mixing in the incubated orbital shaker (REMI CIS24 PLUS) at a speed of 120 rpm. At the end, the enzyme has been inactivated at - 2^{o}C for 5

m (Kashyap et al., 2001; Molinari and Silva, 1997; Sandri et al., 2014). It was then allowed to cool further to room temperature and sifted using the muslin cloth and the filtrate were further analyzed for various chemical-physical properties.

2.4 Analysis of juice

Physical and chemical attributes (clarity, colour change, polyphenol, turbidity, protein, TSS and yield) were response attributes which were optimized. The slim viscometer, which is U-tube shaped attained from the Zenith Glassware, Kolkata, India, was used in the determination of the viscosity of the juice at room temperature ($28^oC \pm 1^oC$). Digital Turbidity-meter (Model 335, Deluxe Company, India) was used to determine the turbidity of the juice in Nephelometric Turbidity Units (NTU) (Sin et al., 2006). Yield was estimated conferring to Shahnawaz and Sheik (2011). Clarity of the concentrate has been measured at 660 nm (%T) by spectrophotometric method (Rai et al., 2004). Colour measurements were carried out using a Hunter colorimeter (Colorflex EZ, USA). The color changes were restrained according to Duangmal et al. (2004). Bovine Serum Albumin (BSA) was used for standard curve preparation for protein estimation. Lowry's dye binding method has been used for the estimation of protein (Lowry et al., 1951). Concentration of polyphenols was detected by employing spectrophotometer (Model: AU 2701, Systronics India Ltd) at the 650nm subsequently to Folin-Ciocalteu method depicted in Singleton et al., 1999. The result has been demonstrated as the (mg) of Gallic acid equivalent/gram. The Abbe-type Refractometer was employed for demonstration of (TSS) and expressed as degree Brix (^oB) of the sample.

2.5 Experimental design

RSM (Box-Behnken Design) was used to determine the idyllic conditions of sample juice using Design Expert Software (Version 8.0.7.1) which enables quantifiable examination and exploratory configuration.

The Design consists of three independent variables be X_1, X_2, X_3, of enzyme. Temperature range (30^oC–50^oC, with (0.01 up to the 0.1 percent w/v of concentration) and the orbit of time being (40-120 minutes) are as presented in Table 1. The scientific expression used to point the variables in form of linear, interaction and quadratic positions:

$$Y = b_0 + \sum b_i X_i + \sum b_{ii} X_i^2 + \sum b_{ij} X_i X_j \quad (1)$$

Where, Y denotes experimental responses, X_i X_j indicates the variable levels.

b_0 is a constant, b_i is a linear coefficient, b_{ii} is the quadratic term and b_{ij} is the interaction term's coefficient.

Table 1 Experimental sketch pointed coded and realtime values of autonomous variables

Experiment. No	Coded Variables			Real Variables		
	x_1	x_2	x_3	X_1 (^oC)	X_2 (%)	X_3 (min)
	-1	-1	0	30	0.01	80
	1	-1	0	50	0.01	80
	-1	1	0	30	0.10	80
	1	1	0	50	0.10	80
	-1	0	-1	30	0.06	40
	1	0	-1	50	0.06	40
	-1	0	1	30	0.06	120
	1	0	1	50	0.06	120
	0	-1	-1	40	0.01	40
	0	1	-1	40	0.10	40
	0	-1	1	40	0.01	120
	0	1	1	40	0.10	120
	0	0	0	40	0.06	80
	0	0	0	40	0.06	80
	0	0	0	40	0.06	80
	0	0	0	40	0.06	80
	0	0	0	40	0.06	80

ANOVA was used for model validation; 3D plots were generated based on 1 variable constant to be center point and other 2 varying variables within the experimental range. Seven response attributes (both physical and chemical together) were selected and model equations representing relationships between independent and dependent variables were generated.

All experiments are done repetitive times and average values of various quality parameters were reported. Coefficient of determination (R^2) value was used for validation.

3 Result and discussions

Fresh *Jamun* juice has 65% yield, 116 NTU turbidity and -78.3%T clarity. The colour value offormer is, a*(2.65) and b*(-1.68), L*(5.76). The juice has nutritional composition of 89.08 mg GAE g^{-1} of polyphenol with 13°B, 132.70 mg g^{-1} protein. The model was satisfactory with agreeable approximations of R^2.

Table 2 R^2 value for independent variables for clarified enzymatic *Jamun* Juice

Regression Coefficient	Yield (%)	Turbidity (NTU)	Clarity (%T)	ΔE	Protein (mg g^{-1})	Polyphenol (mgGAE g^{-1})	TSS (°B)
b_0	78.20	37.26	42.34	36.23	718.66	128.16	16.12
A (Temp.)	0.38	24.52	-11.04	0.39	158.47	-3.75	1.19
B (Conc.)	- 0.5	2.9	-1.07	-0.043	11.02	-4.8	0.038
C (Time)	- 0.63	-.5	-1.60	-0.036	2.87	-6.07	0.25
A^2	- 0.85	15.29	-6.03	-0.43	-186.53	10.8	-1.34
B^2	-1.10	-5.03	1.40	-0.035	-59.67	-7.01	0.11
C^2	-2.35	0.04	-2.67	0.073	34.40	-8.18	-0.11
AB	1.25	-2.18	-0.55	$-1.5*10^{-3}$	-9.55	-13.36	-0.10
AC	-1.01	-2.47	0.74	0.022	15.26	-0.01	-0.18
BC	0.25	-0.38	0.75	-0.042	40.16	-2.64	-0.38
R^2	0.87	0.98	0.99	0.96	0.94	0.82	0.97

3.1 Effects of variables on yield

The yield ranges from 74-79 mL, as mentioned in Table 2, (A) ($p<0.0001$) being Temperature posed linear positive (+) effect however (B) ($p<0.0001$) being Concentration and (C) ($p<0.0001$) being Time, posed linear negative (-) effect. All quadratic structures pose a negative effect ($p<0.01$). Coded variables, for the in terms of juice yield is opted for regression analysis in the model is given below:

$$Yield\ (\%) = 78.20 + 0.38A - 0.5B - 0.63C - 0.85A^2 - 1.10B^2 - 2.35C^2 + 1.25AB - 1.01AC + 0.25BC \quad (2)$$

Where, A= Temperature, B=Concentration, C= Time.

The fit model gave a coefficient of determination (R^2) of 87.84%. From the above equation and in Figure 1(r) it was detected that with the steady surge in the enzyme dosage shown, the yield decreased as it could not reduce the adherence of protein - pectinase bonding. The interaction effect showed a positive result. Figure 1(s) shows that time - temperature had linear effect on the juice yield. Figure 1(t) showed that juice yield was the maximum at 80 min. A similar trend of observations was reported by Campos et al. (2002).

3.2 Effects of variables on clarity

(22.65%-49.50%) (%T) depict the significant difference with the clarity of juice. Clarity is vital parameter in distinguishing the consumer acceptability and satisfaction. From Table 2, observed all variables have shown a negative (-) effect with ($p<0.0001$). All the negative (-) effects usually shown up naturally, but the interaction terms have the negative (-) effects too.

The actual value of the effects of variables of juice extracts on clarity is:

$$Clarity\ (\%T) = 42.34 - 11.04A - 1.07B - 1.60C - 6.03A^2 + 1.40B^2 - 2.67C^2 - 0.55AB + 0.74AC + 0.75BC \quad (3)$$

Coefficient of the determination (R^2) for equivalence is 0.99, which signposts that the model has ability to elucidate 99% of data-variability. From Figure 1(u) and (v) the graphs follow the same trend where changes in the interaction effect of variables combinations shows that clarity remains constant with respect to concentration and time but with temperature change, clarity declines with upsurge in temperature. Figure 1 (w) showed that time concentration interaction first increased the clarity then decreased. The high dosage of enzyme and time has a reverse impact on the clarity. Clarity was maximum, at a

higher enzyme dosage and minimum temperature. Similar tendency has been perceived in clarification process of banana and litchi juice (Lee et al., 2006; Shah and Nath, 2007).

3.3 Effects of variables on turbidity

Turbidity ranges from 20.58-74NTU. Turbidity is considered to be one of the crucial picked points in the clarification process. The acceptability was higher, with lower turbidity value. From the Table 2 it was detected that (A) ($p<0.0001$), (B) ($p<0.0001$) had a linear constructive (+) effect, but (C) ($p<0.0001$) had linear destructive (-) effect. In event of quadratic standings except concentration, temperature, time has constructive (+) effect was significant ($p<0.001$). The Regression model to the turbidity in lieu the effect independent variables was

$$Turbidity\ (\%NTU) = 37.26 + 24.52A + 2.9B - 0.50C + 15.29A^2 - 5.03B^2 + 0.048C^2 - 2.18AB - 2.47AC - 0.38BC \quad (4)$$

The interaction term has no noteworthy value. The coefficient of determination (R^2) for former was 0.98. From the Figure 1 (x) experiential interaction effect of concentration & temperature showed a positive slope. With the temperature, turbidity - also upsurges. In event of Figure. 1 (z) interaction effect of the time - concentration had varied drift. Turbidity was almost constant with time but as turbidity value increased with concentration of enzyme but, after the firm period it started declining. Pinelo et al. (2010) showed the similar trend with cherry juice. Results colliding is in case of both clarity and turbidity.

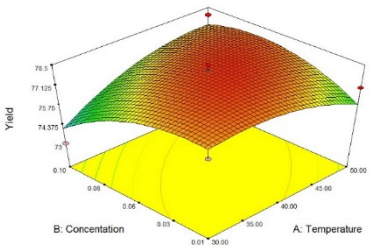
3.4 Variable's effects on the protein

Protein – pectin bond denaturation, is a main mechanism in clarification. Increased protein concentration was the sign of proper clarification. Protein content was in the range of 219 mg g^{-1}-765 mg g^{-1}. Table 2 signifies that all the factors (A) ($p<0.0001$) temperature, (C) time and the (B) ($p<0.0001$) enzyme concentration has linear forward slope. The quadratic terms ($p<0.001$) also have negative impact with respect to time.

The regression model for protein in lieu of the effect of variables on juice extracts in terms of the actual note is

$$Protein\ \left(\frac{mg}{g}\right) = 718.66 + 158.47A + 11.02B + 2.87C - 186.53A^2 - 59.67B^2 + 34.4C^2 - 9.55AB + 15.26AC + 40.16BC \quad (5)$$

The (R^2) value for a fit model has been observed to be equals to 0.94, which shows that regression model has the ability to illustrate 94% variability of data present. Figure 2(r) shows that with the increase in the temperature, the amount of protein increases but with increase in the enzymatic dosage, protein content goes up. Degradation of tannin and protein bonds from the cell wall caused by enzyme at high temperature. Figure 2 (t) shows that interaction effect of time and concentration follows hyperbolic curve. So, this can be reason out that along with the concentration of the enzyme, the time also plays a role in showing up the maximum consequence on amount protein. At lowest temperature 40°C with concentration of 0.06% and with the 80-minute treatment time there is maximum retention of the protein content 765 mg g^{-1}. Motoichi et al. (2001) observed the same trend of high amount of protein content in fruit juice treatment.



igure1(r)

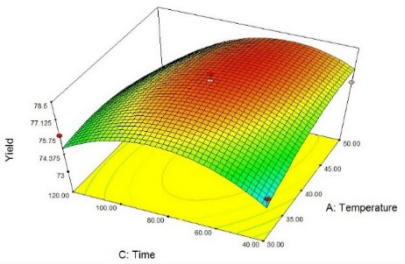


Figure 1(s)

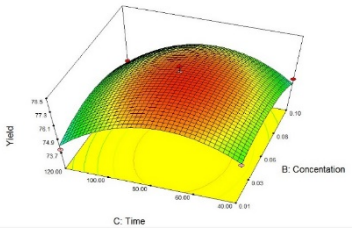


Figure1(t)

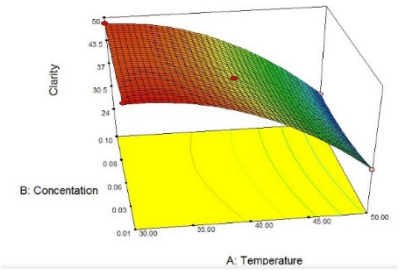


Figure1(u)

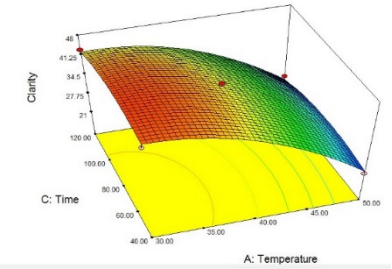


Figure1(v)

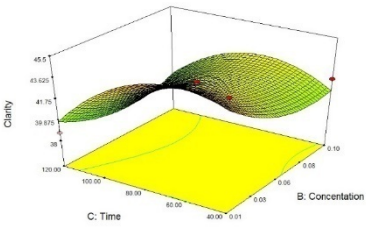


Figure1(w)

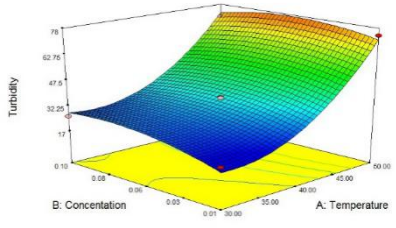


Figure1(x)

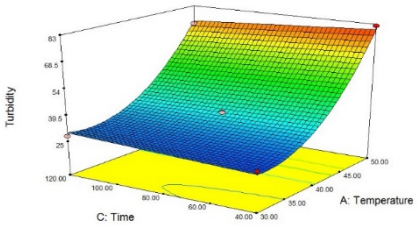


Figure1(y)

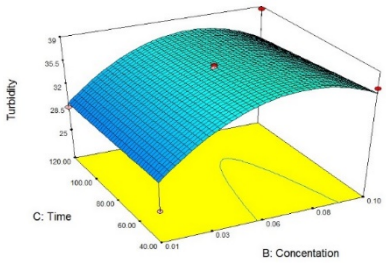


Figure 1(z)

Figure 1 (Yield, clarity and turbidity)'s surfaces responses to be the function of (r, u, x) a temperature & enzyme concentration, (s, v, y) a temperature & time, (t, w, z) a enzyme concentration and time.

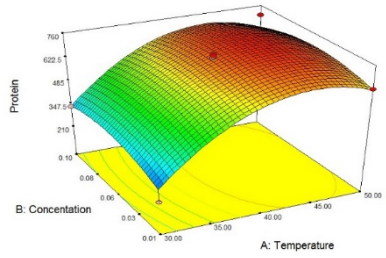
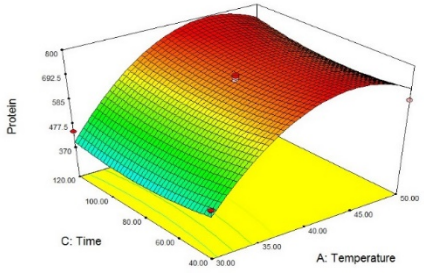


Figure2 (r)



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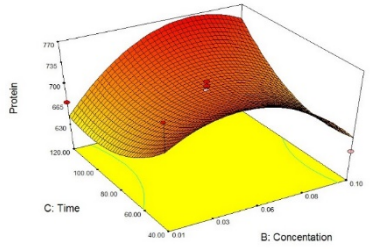

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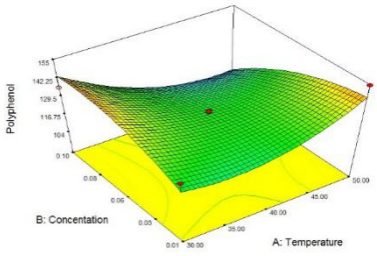
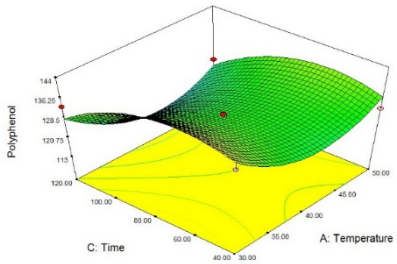


Figure2(u)



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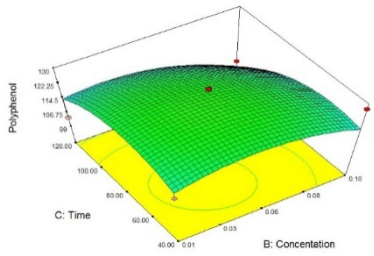

Figure2(w)

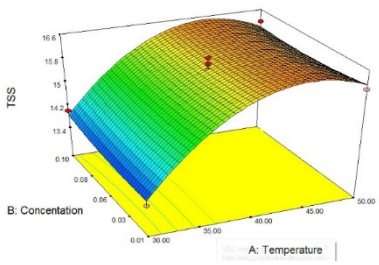

Figure2(x)

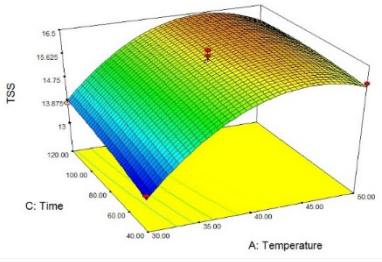

Figure2(y)

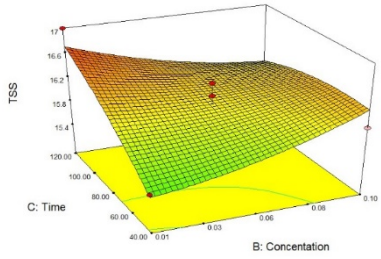

Figure 2(z)

Figure 2 . Response surfaces to protein, polyphenol & TSS to be function of (r, u, x) temperature, enzyme concentration (s, v, y) temperature, time, (t, w, z) enzyme concentration & time.

3.5 Effects of variables on polyphenols

Being one of crucial component in the *Jamun* fruit the phenol, its extraction content increases with the effect of variables. Polyphenol content ranges 104-154 mgGAE g^{-1} as the process of clarification depends on various parameters. In the event of polyphenols, all the autonomous parameters have the negative linear and composed effect for (B) and (C) variables. It also shown up negative ($p<0.05$). The model of this can be instituted as:

$$Polyphenol = 128.16 - 3.75A - 4.84B - 6.07C + 10.8A^2 - 7.01B^2 - 8.18C^2 - 13.36AB - 0.01AC - 2.64BC \quad (6)$$

If the (R^2) value was equals to 0.82 it shows that regression archetypal has the ability to illustrate the 82% polyphenolic variability in the juice. Figure 2 (u) shows polyphenols value goes up with the concentration and temperature but combined interaction, increases up to a level and after a while move downwards. From Figure 2 (v) polyphenols value goes up with the time and temperature and the polyphenol content decrease with respect to interaction effect. Production of gallic acid esters increase the amount of polyphenol content in the juice. Figure 2 (w) also shows down trend at the interaction effect of time and concentration. So, to conclude, that in case of polyphenol activities time temperature has its effect. At 30^{o}C with concentration 0.1% and 120-minute incubation time, more amount of polyphenol content can be obtained.

3.6 Effects of variables on TSS

Another important nutritional attribute in the clarification process is the total soluble solids (TSS). Table 2 except concentration, clearly depicts that variables has a linear positive effect with a quadratic negative effect. The interaction effects are negative ($p<0.05$) at evidential stage. The regression model of this can be instituted as:

$$TSS = 16.12 + 1.19A + 0.38B + 0.25C - 1.34A^2 + 0.11B^2 - 0.11C^2 - 0.10AB - 0.18AC - 0.38 \quad (7)$$

The value for (R^2) is 0.97 which points out that the model explains 97% TSS variability of juice. Figure 2 (x) and (y) shows that increase in temperature increases the TSS value but with concentration it has no change. From Figure 2 (z), it can conclude, interaction effect of time and concentration increases up to certain level then decreases. At a specific temperature of 40^{o}C with concentration 0.01%, the TSS value is maximum.

3.7 Effects of variables on colour change

Colour is broke down into the three parameters where, L* value - darkness and lightness of juice, Positive = (a* value) – redness, negative = (b* value) - blueness. The change in colour was quite important. If the value of ΔE is more than 3 then it implies significant difference. Here in this case the ΔE value varies from 35-37. From the Tabular form: 2 in case of the colour change, temperature (A) ($p<0.0001$) had linear positive effect but concentration (B) ($p<0.0001$) and time (C) ($p<0.0001$) had a linear negative effect. Except time ($p<0.001$), the quadratic values possess negative value. The interaction terms are ($p<0.05$), considered to be negative (-).The regression of this can be instituted as:

$$\Delta E = 36.23 + 0.39A - 0.043B - 0.036C - 0.43A^2 - 0.035B^2 + 0.073C^2 - 1.5 * 10^{-3}AB + 0.022AC - 0.042BC \quad (8)$$

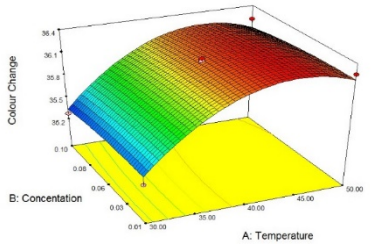

Figure.3 (t)

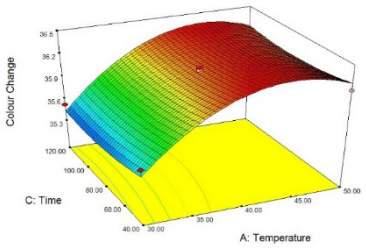

Figure.3 (u)

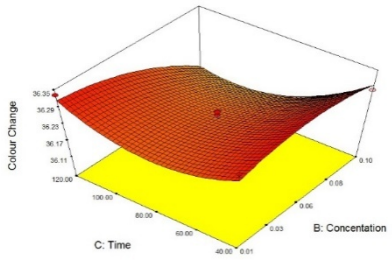

Figure.3 (v)

Figure 3 Response surfaces for colour change to be function of (t) to be temperature- enzyme concentration, (u) to be temperature- time and (v) to be enzyme concentration –time

The coefficient of determination for ΔE value was 0.96 which depicts that experiment is 96% of the variability of the data. From Figure 3(t) with upsurge in concentration, there were no noticeable changes occurs then with change in temperature, the colour varies. The same was observed from the Figure 3 (u) where at the end there was a saturation point obtained at the highest temperature. Figure 3(v) shows a very different trend with the interaction effect of (A) and (C). This result can be vindicated with the results attained from clarity values. The maximum changes colour obtained at high temperature and lower concentration.

4 Conclusion

RSM utilizing Box-Behnken sketch Design had been taken as experimental learning to hone the effects of independent variables and its effect on physicochemical characteristics of the juice. The quality of the extractions was assessed on the basis yield percent, protein and TSS, polyphenol content turbidity, clarity, colour change. According to the dependent parameters the augmented independent variables were temperature (40^{o}C) with 80 minute of time period and concentration of enzyme 0.05 percent (weight/vol). In these conditions, the key properties were considered to be clarity: 42.39% T; polyphenol: 128.31 mg GAE gram^{-1}; protein: 718.66 mg gram^{-1}; yield: 79%; TSS: 16.2^{o}B; Color change: 35.12; turbidity: 37.21NTU. The response surface and the graphical 3D plot silhouette had given good outlook of the clarification process. The response surface sketch would be supportive aimed at the forthcoming scientific learning.

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