International Journal of Advanced Biotechnology and Research(IJBR)
ISSN 0976-2612, Online ISSN 2278–599X,
Vol 6, Issue1, 2015, pp 49-56
http://www.bipublication.com

Multienzymatic Clarification of Blended Pineapple and Mango Pulp Using Response Surface Methodology

Dnyaneshwar B. Jori*, Amesh V. Pawar, Dnyaneshwar C. Kudake and Pankaj G. Kotgirkar

University Institute of Chemical Technology, NMU, Jalgaon-425001
*Corresponding author: dnyaneshwarjori3030@gmail.com Tel: +91 9503282491

[Received 20/12/2014, Accepted-02/01/2015]

ABSTRACT:

Pineapple and mango are major fruits and their products (e.g. juices, concentrates, nectars, etc.) have high acceptance by consumers globally. The study was carried out with an objective of preparing excellent quality clarified blended pineapple and mango juice with high yield by multi enzymes system. Pineapple and mango pulp were added in 1:1 proportion to prepare its blended pulp for enzymatic treatment. Cellulase (0.15-0.75%) and pectinase (0.2-0.6%) were added in varying combination and temperature (35-55ºC) range for treatment period of 1 hr to prepare clarified blended juice. The experimental run for the treatment was designed using design expert (9.0.1) software and the parameters optimized using Response Surface Methodology. The optimized condition achieved for clarified blended pulp with 81.92% clarity and 88.53% yield was treatment of pulp with 0.34% cellulase and 0.5% pectinase enzymes in combination at 45.5ºC for 1 hr.

Keywords: pineapple, mango, pectinase, cellulase, response surface methodology, yield, clarity

[1] INTRODUCTION:

Fruit beverages made from tropical fruits are very popular all around the world. Present scenario suggests huge demand of fruit juices from citrus, seasonal and tropical fruits worldwide. Clarified pulp and juices are always in high demand for the preparation of ready to serve drinks, nutritional carbonated beverages, cordials, jelly, concentrate, nectars, etc. [1].

Mango (*Mangifera indica* L.) is one of the most important tropical fruits with a global production exceeding 31 million tons in 2007. China, India and Mexico are the leading producers. Besides the fresh fruit, processed mango products viz. juices, nectars, concentrates, jams, jelly powders, fruit bars, flakes, and dried fruits have become increasingly popular in Europe and North America [2].

Pineapple (*Ananas comosus*), the second most popular tropical fruit in India after bananas are major source vitamins (A, B_1, B_2, B_6, C) and minerals (calcium, magnesium, potassium, iron, zinc, etc.) [3]. Large consumption of pineapple is done mostly as a canning industry by-product and in the blend composition to obtain new flavors [4] and consumers demands for pineapple juice is high due to its convenient colour, flavour and refreshing properties [5]. Pineapple is commercially considered as consumer accepted fruit but the complete use of the fruit is not fully tapped. This fruit still requires wide research in terms of

utilization of residue and enhanced juice yield with optimum overall acceptability [6].

Fruit juices are clarified by techniques like membrane filtration, sedimentation, stabilization with use of clarifying agents (gelatin, bentonite, chitosan, polyvinyl pyrrolidone) and enzymatic treatment [7]. The limitations of filtration, sedimentation and stabilization are low yield and clarity, due to the presence high molecular weight polysaccharides like cellulose and hemicellulose in the plant cell wall [8, 9]. High viscosity fruit juice after mechanical and physical crushing of fruits rich in pectin and remaining strongly bound with the pulp in the jellified mass like structure which reduces the juice volume and process yield [10].

Recently enzymes have been extensively used in fruit juice clarification because of following advantages: a) Complete degradation of polysaccharides into simple soluble sugars b) Maximum clarification c) Increases juice volume with retention of natural color, aroma and phenolic components [11, 12].

Cellulases cleaves β-1,4-D-glucan linkages of cellulose to yield glucose, cellobiose and oligosaccharides [13, 14] while Pectinases hydrolyzes α-1, 4-glycosidic linkages of pectins to produce polygalacturonic acid monomers [15]. The use of cellulolytic and pectinolytic enzymes in combination for clarification enhances the juice yield and clarity due to simultaneous degradation of polysaccharides [16].

Response Surface Methodology (RSM) is widely used method for the design of several experiments as it decreases the number of experimental trials as it reduces the required time and is less laborious than other approaches. RSM has been constantly and effectively demonstrated for optimization multiple variable process parameters in several sectors of food and bioprocessing industries [17].

In the present study the optimization of combined treatment of pectinase and cellulase was investigated with respect to temperature and maintaining constant enzyme treatment or incubation for clarification of blended pineapple and mango pulp by using RSM.

[II] MATERIALS AND METHODS

The local variety of pineapple fruits "*JEW* "and mango fruits "*BADAM*" was purchased from the local market, Jalgaon, Maharashtra for the said study. Cellulase (activity 800-1100 U/g) and pectinase (activity 800-1200 U/g) were procured from Sigma –Aldrich Pvt. Ltd. All other reagents used in the work were of analytical reagent grade and procured from reliable sources.

2.1 Experimental Design

Design-Expert (9.0.1) software with Central Composite Rotatable Design (CCRD) was selected to analyze the effects of three independent variables pectinase, cellulase and temperature on juice yield and clarity. The range and levels of the pectinase to be added in blended pulp were chosen based on research work done by Pal and Khanum [16] and the range of temperature and cellulase was selected on the basis of finding of research work carried out by [18]. Table 1 is showing the designing domain for designing the optimum experimental runs for the work.

Table: 1. The experimental domain

Independent Variable	Coded Variables				
	-α	**-1**	**0**	**+1**	**+ α**
Cellulase (% w/w)	0.15	0.30	0.45	0.60	0.75
Pectinase (% w/w)	0.2	0.30	0.40	0.50	0.60
Temperature (°C)	35	40	45	50	55

The said domain of various parameters was feed in software which resulted designing runs mentioned in Table 2.

In the design of the experimental runs following test factors were considered and coded according to the following equation

$$x_i = X_i - X_0/\delta X_i$$

Where, x*i* is the dimensionless coded value of the i^{th} independent variable; X*i* is the natural value of the i^{th} independent variable; X_0 is the natural value of the i^{th} independent variable at the center point

and δXi is the step change value, experimental results of the runs were fitted with a 2nd order polynomial equation as follows

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{11}X^2_1 + b_{22}X^2_2 + b_{33}X^2_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3$$

Where, Y is the predicted response; b_0 is the intercept; b_1, b_2, b_3 are the linear coefficient; b_{11}, b_{22}, b_{33} are the squared co-efficient and b_{12}, b_{13}, b_{23} are the interaction co-efficients.

Table: 2. Experimental designs and results of blended juice

	Coded Level			Observed Responses	
Run	**A**	**B**	**C**	**T (%)**	**Yield (%)**
1	-1	-1	-1	74.69	83.6
2	1	-1	-1	78.23	85.3
3	-1	1	-1	78.23	86.2
4	1	1	-1	81.49	87.1
5	-1	-1	1	72.85	83.3
6	1	-1	1	75.69	84.3
7	-1	1	1	75.22	84.1
8	1	1	1	82.13	88.6
9	-2	0	0	72.97	80.2
10	2	0	0	81.6	87.2
11	0	-2	0	70.2	78.1
12	0	2	0	84.47	90.1
13	0	0	-2	66.3	75.2
14	0	0	2	73.47	82.6
15	0	0	0	83.1	89
16	0	0	0	83.2	89.4
17	0	0	0	83.19	89.4
18	0	0	0	83.16	89.3
19	0	0	0	83.23	89.45
20	0	0	0	83.2	89.3

2.2 METHODOLOGY

Pulp preparation: Pineapple fruit was washed with water to remove dirt and dust particles, crown removed and fruit was peeled. The flesh with core was sliced and then crushed in mixer for 4-5 minutes. Same procedure was done for mango fruits. Addition of both fruit pulp was done in equal ratio for preparation of blended pulp. The pH of pulp was kept natural which was around 3.9±0.1 and used for further experiments.

The calculated amount of enzymes were added in the prepared pulp and mixed well. All the designed experimental runs were conducted for one hour at different temperature of pulp (35, 40, 45 50 and 55°C). The temperatures of pulp for the runs were maintained on water bath. At the end of treatment period the pulp was blanched at 90±1°C for 5 minutes in water bath to inactivate enzymes, cooled followed by centrifugation at 4,000 rpm for 30 minutes. The supernatant was collected and analyzed for juice yield and clarity.

2.3 Response Analysis

The volume of filtrate was measured and yield was expressed as % yield (v/w). Juice clarity was measured by transmittance (%T) at 650 nm using UV-vis Spectrophotometer (Systronics, model 2205).

2.4 Data Analysis

The results of analysis were statistically analyzed using design expert (9.0.1) software. The quality of fit of the polynomial model equation was expressed by the coefficient of determination, R^2 and its statistical significance was checked by Fishers F-test. The level of significance was given as p-value.

[III] RESULTS AND DISCUSSION

3.1 Experimental Interpretation

The experimental values for juice yield and clarity under different treatment conditions are enlisted in Table 2. Significantly lower yield (75.2%) and clarity (66.3) was observed in the control (without treatment with enzymes) as compared to the enzyme treated samples. Comparatively minimum yield (75.2 %) and clarity (66.3%) was observed in run number 13 (pectinase, cellulase at center level and temperature at negative α level), while the maximum yield (90.1%) and clarity (84.47%) was recorded in run number 12 (where cellulase and temperature was at central level and pectinase at + α level). Comparison of run between 5 and 6, 7 and 8, 9 and 10, 11 and 12 revealed that increase in concentrations of all the enzymes from their negative α to positive α level and temperature from negative α to positive 1 increased the response variables substantially.

The effects of change in variables on process response are explained in terms of their statistical coefficients (Table 3). The variables were analyzed for their linear, quadratic and interactive effects which gave the following equations (in terms of

coded unit) to predict the juice yield and clarity within the experimental domain.

Juice clarity = 83.25 + 2.11 (cellulase) + 2.76 (pectinase) + 0.47 (temperature) + 0.47 (cellulase × pectinase) + 0.37 (cellulase × temperature) + 0.25 (pectinase × temperature) - 1.44 (cellulase^2) - 1.43 (pectinase ^{2}) – 3.29 (temperature ^{2})

Juice yield = 89.55 + 1.38 (cellulase) + 2.09 (pectinase) + 0.81 (temperature) + 0.34 (cellulase × pectinase) + 0.36 (cellulase × temperature) + 0.08 (pectinase + temperature) - 1.28 (cellulase^2) - 1.18 (pectinase ^{2}) – 2.48 (temperature^2)

The juice clarity, measured in terms of transmittance (%T) at 650 nm, was significantly affected by all the independent variables at their quadratic level. The juices obtained after enzymatic treatment had more yield and corresponding clarity compared to the untreated one (i.e. control) because of the hydrolysis of pectic and cellulosic compounds.

3.2 ANOVA Analysis

The acceptability of model was verified by employing Fisher's test as mentioned in Table 4. As per software, experiment is significant when the F value is several times larger than p-value. As in the stated result the F value obtained was 7.56 for yield and 13.33 for clarity respectively and thus passes the Fisher's test and confirms significance of experimental results. The same can also be verified from very low probability value (p model <0.002) for both juice yield and clarity. There is a quadratic relationship between the independent variables and response. The goodness of fit of the model was examined by determination coefficients [R^2 (yield) = 0.8718, R^2 (clarity) = 0.9230]. The closer value of R^2 to unity indicates the empirical model fits the actual data. It also implies that the sample variations of more than 89% was attributed to the variables and only less than 11% of the total variation could not be explained by the model. A lower value of coefficients of variation (Yield = 2.41% and Clarity = 2.60%) showed the experiments conducted were precise and reliable [19].

3.3 Graphical Interpretation

Clarity

Figure 1.a represents the 3-D surface graphics showing the interactive effects between variables cellulase and pectinase where the variable kept constant are temperature with concentration values 45°C respectively. Figure 1.b shows interactive effect between cellulase and temperature where other variable i.e. pectinase is kept constant at 0.4% respectively. Similarly figure 1.c shows the collaborative effect in between pectinase and temperature whose values are kept changing and the value kept constant of cellulase was 0.45% each. From figure 1 (a, b and c) variations in fruit juice were observed from 35°C to 55°C. Lowest clarity was observed at 35 and 55^{0}C i.e.66.3 and 73.47% respectively. This was because enzymes show higher activity at the optimum temperature.

Yield

All the independent variables with respect to their importance were checked by keeping values changing of two independent variables and another two were kept constant.

Figure 2.a represents the 3-D graphics showing the interactive effects between variables cellulase and pectinase with the variables kept constant are temperature at 45°C respectively. Figure 2.b shows interactive effect between temperature and cellulase where other variable i.e. pectinase was kept constant at 0.4% respectively. Similarly figure 2.c shows the collaborative effect in between pectinase and temperature and whose values are kept changing and the value kept constant of cellulase was 0.45% each. From figure 2 (a, b, c) it was observed that clarity changes with varying enzyme concentration from its low level to high level i.e. from 0.15% to 0.75% respectively. Highest yield was obtained at the high enzymes concentration at constant temperature of 45°C for 1 hour. Significantly superior enzyme activity was obtained in temperature range 40°C to 50°C. Thus higher yield achieved can be seen in through the 3-D graphs.

Table: 3. Coefficient of the regression equation for blended juice

Term	Yield		Clarity	
	Coefficient	**p-value**	**Coefficient**	**p-value**
Constant	89.54	0.0020	83.25	0.0002
A-Cellulase	1.38	0.0233	2.11	0.0019
B-Pectinase	2.09	0.0023	2.75	0.0002
C-Temperature	0.80	0.1423	0.47	0.3727
AB	0.33	0.6576	0.47	0.5246
AC	0.36	0.6332	0.36	0.6198
BC	0.087	0.9034	0.25	0.7334
A^2	-1.28	0.0124	-1.43	0.0051
B^2	-1.18	0.0165	-1.42	0.0053
C^2	-2.48	0.0001	-3.28	1.0459

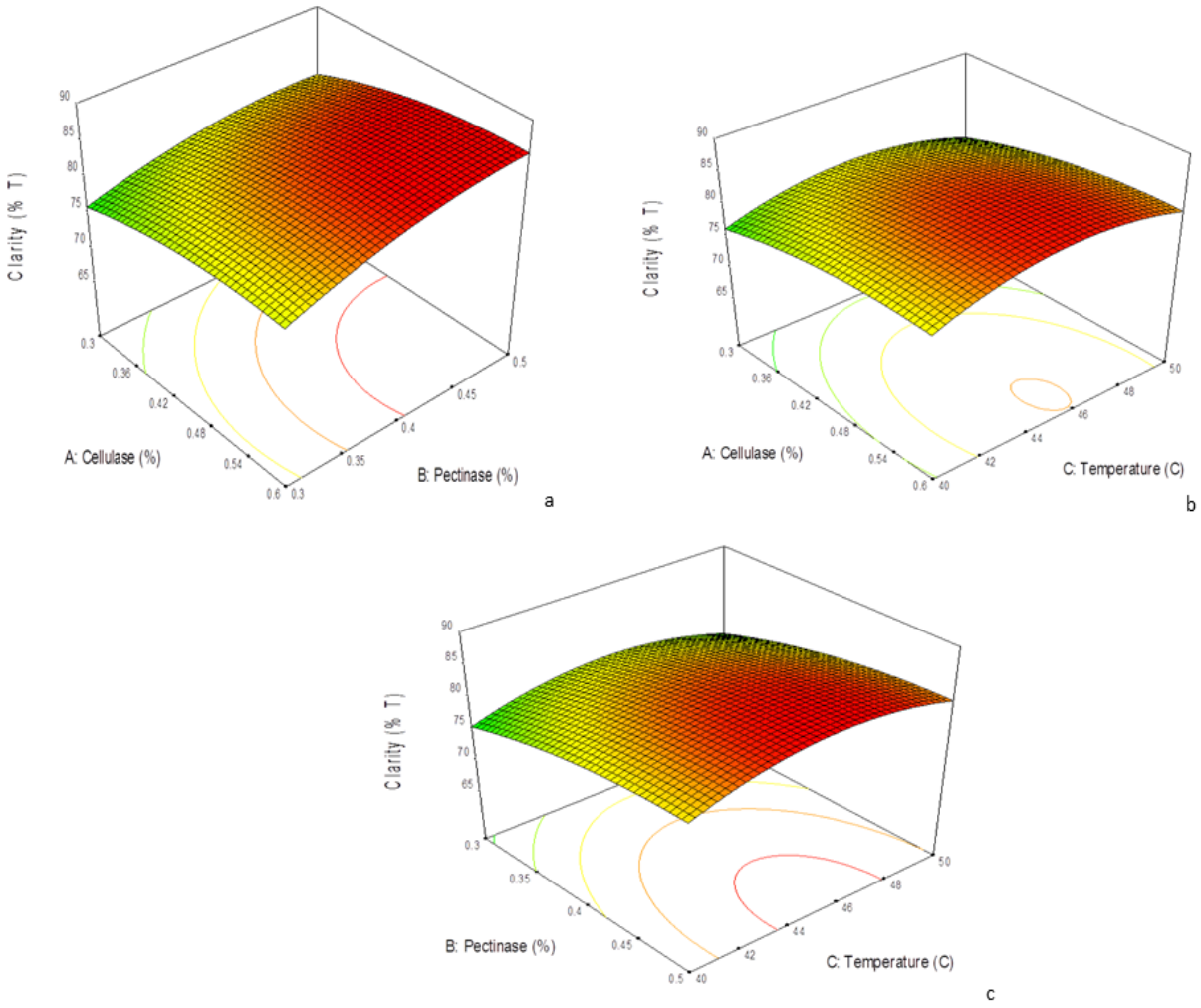

Fig 1 (a, b and c): Effect of enzymes on clarity of blended juice by varying one enzyme and temperature keeping other two constant

Table: 4. Regression analysis (ANOVA) for process response of pineapple juice

Response	Source	Sum of square	Degree of freedom	Mean square	F- value	P-value
Clarity	Model	495.73	9	55.08	13.33	0.0002
	Residual	41.3	10	4.13		
	Total	537.07	19			
	C.V	2.60	R^2=0.9230			
Yield	Model	290.27	9	32.25	7.56	0.0020
	Residual	242.68	10	4.27		
	Total	332.95	19			
	C.V	2.41	R^2=0.8718			

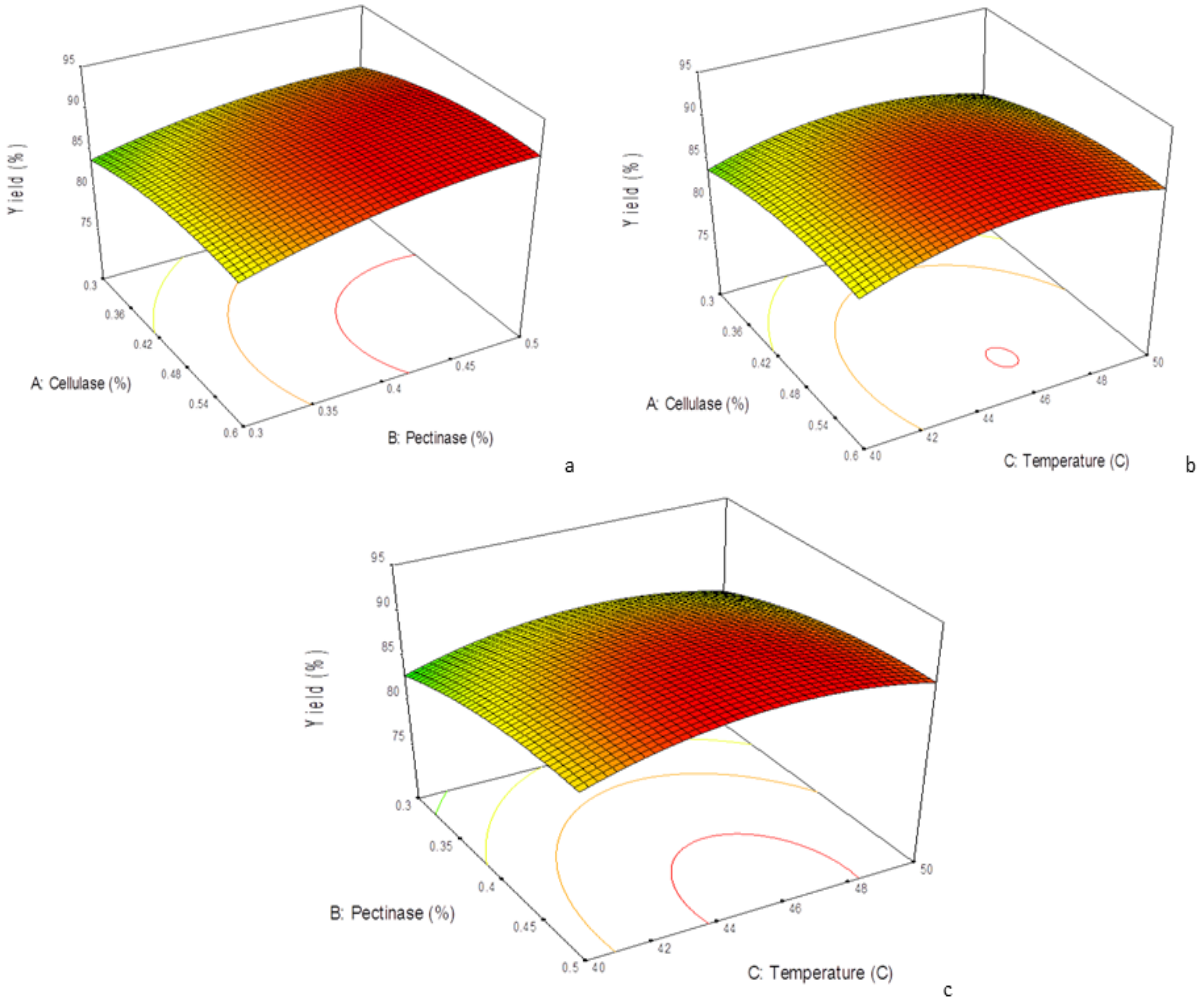

Fig 2 (a, b and c): Effect of enzymes on yield of blended juice by varying one enzyme and temperature keeping other two constant

3.4 Perturbation Interpretation

The simultaneous effect of variations in levels of all the independent factors on process response can be seen in the perturbation graph (Fig. 3). The plot reveals that the juice yield and clarity were most sensitive to cellulase and temperature while least sensitive to pectinase treatment. This is due to the presence of abundant amount cellulosic compounds in the cell wall of pineapple and mango fruit cells which affects the yield and clarity of blended fruit juice. Thus more the cellulase concentration the more rapid will be cellulose degradation [16]. With respect to temperature it was observed that increasing temperature beyond optimum level decreases enzyme activity due to heat inactivation of enzymes. During process optimization synergistic effect of process variables were considered. Condition for optimization of variables was decided on the basis of perturbation graph suggesting 45°C temperature to be optimal.

Pectin content is major component in blended pulp contributing to turbidity therefore the level of pectinase was fixed to 0.5% for its maximum activity while cellulose content is less in blended pulp so the cellulose was fixed to the lower level (0.34%) for designing (numerical) the optimization of process through software. The Table 5 shows the said design parameter for optimization with predicted response.

The software predicted response 81.92% for clarity and 88.53% for yield with 90.4% desirability. The predicted response for the optimized condition was then verified by actual run. The observed response for the said optimized condition was 80.7% clarity and 87.5% yield indicating the optimization design by the software was appropriate.

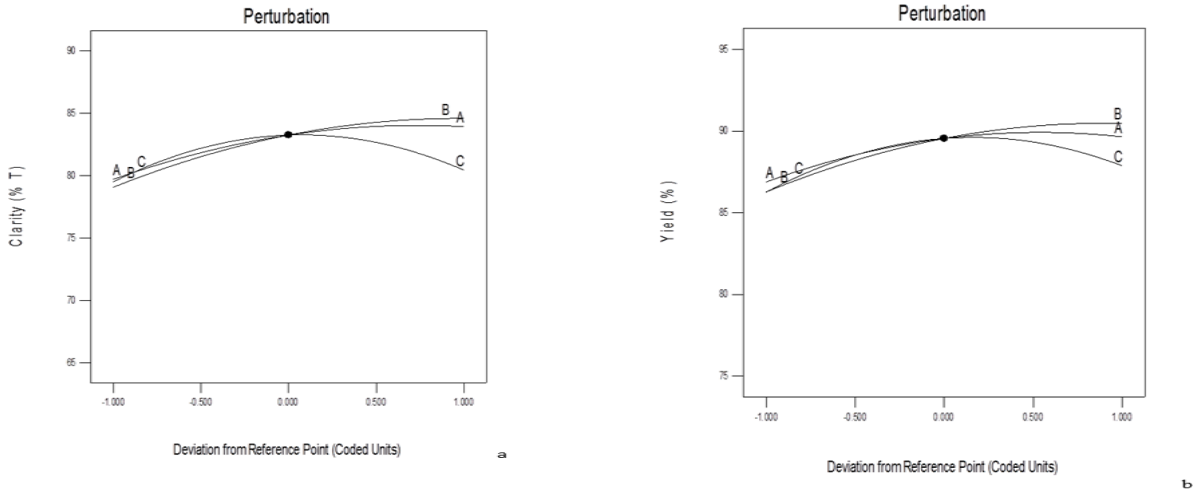

Fig 3: Perturbation graph showing the effect of independent variables on a) clarity and b) yield of blended juice (A-pectinase, B-cellulase, D=Temperature)

TABLE: 5. Constraints, criteria for optimization, solution, along with predicted and observed response value of blended juice

Constraints	Goal	Importance	Predicted Response	Desirability	Observed Responses
A:Cellulase	Minimize	3	0.34	90.4%	-
B:Pectinase	Maximize	3	0.50		-
C:Temperature	is in range	3	45.50		-
Clarity	Maximize	3	81.92		80.7
Yield	Maximize	3	88.53		87.5

[IV] CONCLUSION

From the above study it is concluded that the combine treatment of cellulase and pectinase enzyme is beneficial in the clarification of blended pineapple and mango pulp. Response surface method is relevant for designing optimization parameters for clarification. The optimized condition to obtain a good quality of clarified juice blended pulp of pineapple and mango with 81.92% clarity and 88.53% yield is treatment of pulp (at its natural pH 3.9±0.1) with 0.34 cellulase and 0.5% pectinase enzymes in combination at 45.50°C for 1 hr. with 90.4% desirability.

REFERENCES

1. Brito, B., Rodriguez, M., Samaniego, I., Jaramillo, 1. Brito, B., Rodriguez, M., Samaniego, I., Jaramillo, M., Andvaillan, F. 2008. Characterizing polysaccharides in pure form and their enzymatic liquefaction. Food Research Technology. 355-361.
2. Juan, B., Heliodoro G., Vrani, I, G., and Cristóbal, N, A. 2010. Juice Extraction from Mango Pulp Using an Enzymatic Complex of Trichoderma sp. produced by Solid-state Fermentation. Food Sci. Biotechnol. 19 (5), 1387-1390.
3. Mohammad, G, A., Andres, D, H., and Klaus, D, K. 2010. Isolation of polysaccharides from pineapple fruit pulp and their enzymatic liquefaction. International Food Research Journal. 193-203.
4. Arthey, D.1995. Food Industries Manual. Fruit and Vegetable Product. 151-175. Blackie Academic and Professional, London.
5. Jori, D., Ladole, M., Gore, A., and Bhand V. 2013. Study on effect of carbonation on storage stability of pineapple fruit juice. International journal of engineering research and technology. Vol. 2 (12), 1841-1147.
6. Sreenath,H, K., Sudarshanakrishna, K, R., and Santhanam, K.1994. Improvement of juice recovery from pineapple pulp/residue using cellulases and pectinases. J Ferment Bioeng. Vol.78, 486–488.
7. Gainvors, A., Karam, N., Lequart, C., and Belarbi, A.1994. Use of Saccharomyces cerevisiae for the clarification of fruit juices. Biotechnol. Lett. Vol. 16, 1329-1334.
8. Uhlig, H. 1998 .Industrial Enzymes and Their Applications. John Wiley & Sons, New York, NY.435.
9. Shallom, D., and Shoham, Y. 2003. Microbial hemicellulases. Curr Opin Microbiology. Vol. 6, 219.
10. Ladole, M. R., Muley, A. B., Patil, I. D., Talib, M. I., Parate, V. R. 2014. Immobilization of tropizyme-P on amino-functionalized magnetic nanoparticles for fruit juice clarification. J Biochem Tech. 5 (4), 838-845.
11. Gerhartz, W.1990. Industrial uses of enzymes. In: Gerhartz W (ed) Enzyme in industry production and application. 77-92. VCH
12. Buchert, J., Koponen, J, M., Suutarinen, M., Mustranta, A., Lille, M., Törrönen, R., and Poutanen, K., 2005. Effect of enzyme aided pressing on anthocyanin yield and profiles in bilberry and blackcurrant juices. Journal of the Science of Food and Agriculture. Vol. 85, 2548-2556.
13. Béguin, P., and Daubert, J, P. 1994. The biological degradation of cellulose. FEMS Microbiol Rev. Vol.13, 25-58.
14. Henrissat, B. 1994. Cellulases and their interaction with cellulose. In Cellulose .Vol. 1,169-196.
15. Grassin, C., and Fauquembergue, P. 1996. Fruit juices. Industrial Enzymology. pp. 226-264. Godfrey T, West S (eds). MacMillan Press, London, UK.
16. Pal, A., and Khanum, F.2011. Efficacy of xylanase purified from *Aspergillus niger* DFR-5 alone and in combination with pectinase and cellulase to improve yield and clarity of pineapple juice. J Food Sci Technol. 560-568.
17. Gallagher, E., O'brien, C, M., Scannell, A, G., and Arendt, E, K. 2003. Use of response surface methodology to produce functional short dough biscuits. J Food Eng. Vol 56. 269-271.
18. Bahramian, S. 2011.Optimization of Enzymatic Extraction of Sugars from Kabkab Date Fruit. Middle-East Journal of Scientific Research,Vol.7 (2), 211-216.
19. Grassin, C., and Fauquembergue, P. 1996. Application of pectinase in beverages. Progress in Biotechnology. Vol.14. 453-462.