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CLARIFICATION AND OPTIMIZATION OF POMEGRANATE (*PUNICA GRANTUM*) JUICE WITH RESPONSE SURFACE METHODOLOGY.

[1*]Anant.C. Gahire,
Dept. Food Technology,
University Institute of Chemical Technology,
North Maharashtra University, Jalgaon (Maharashtra)

Abstract:

Pomegranate (*Punica granatum*) is nutritionally rich fruit contain high amount of ascorbic acid thiamin and other nutrients. Pomegranate variety Ganesh was used for clarification. Physicochemical analysis of juice was carried out. Juice extracted by mixer contains tannin, pectin which are responsible for bitterness and turbidity. Enzyme treatment given to the fruit juice in 0.05% to 0.30%s/v concentrations. Single enzyme shows that pectinase 0.20% gives clarity about 68.75% and protease gives 57.85% clarity at 0.15% and bentonite gives 72.00%concentration at 50^0C for 60 min. Combine enzyme concentration was optimized by Central Composite Design. The optimum combine concentration after application of CCD were found Pectinase (0.10%), Protease (0.25%) at 50^0C for 60 min for pomegranate.. The above concentrations were optimized for temperature (30 to 60^{o}C) and time (30 to 110 min). The optimum clarification parameters were found combine Concentrations of enzymes 0.10% pectinase and 0.25%protease at 50^0C for 80 min which had given maximum transmittance of 77.40%which is optimized with the help of CCD.. Chemical analysis results show that the T.S.S. slightly decreased (13.80 to 13.30) and ascorbic acid (mg/100ml) content decreases (15.18 to 13.20) while pH of the juice increases organoleptic characters and overall acceptability of clarified juice was enhanced compared to non-clarified juice.

Keywords: CV.Ganesh, Pectinase, Protease, Extraction, Clarification, central composite design..

Introduction:

A pomegranate (Punica granatum) is a fruit-bearing deciduous shrub or small tree growing to between five and eight meters tall. The pomegranate is Caucasus since ancient times. LaRue, James H. (1980). Pomegranate is one of the important fruit crops commercially grown in Maharashtra. The area under this crop is 77,716 hectares with the production of 4.76 lakh tones. The varieties that are grown commercially include Ganesh, G-137 and Mridula. (MPGARA report, Pune). The clarification by the combination of protease and pectinase is a novel concept. Earlier research has been carried out on cherry juice to reduce turbidity of the juice. Other fruit such as watermelon, pineapple, sapota, orange and beet root juices was clarified by the single enzyme treatment. Human studies being too preliminary for Food and Drug Administration (FDA) approval of a health claim on product labels, manufacturers and marketers of pomegranate juice have liberally used evolving research results for product promotion, especially for putative antioxidant health benefits. In February 2010, the FDA issued a warning letter to one such manufacturer, POM Wonderful, for using published literature to make illegal claims of unproven antioxidant and anti-disease benefits.(Kulkarni AP, Mahal HS, Kapoor S and Aradhya SM; Feb 21, 2007).

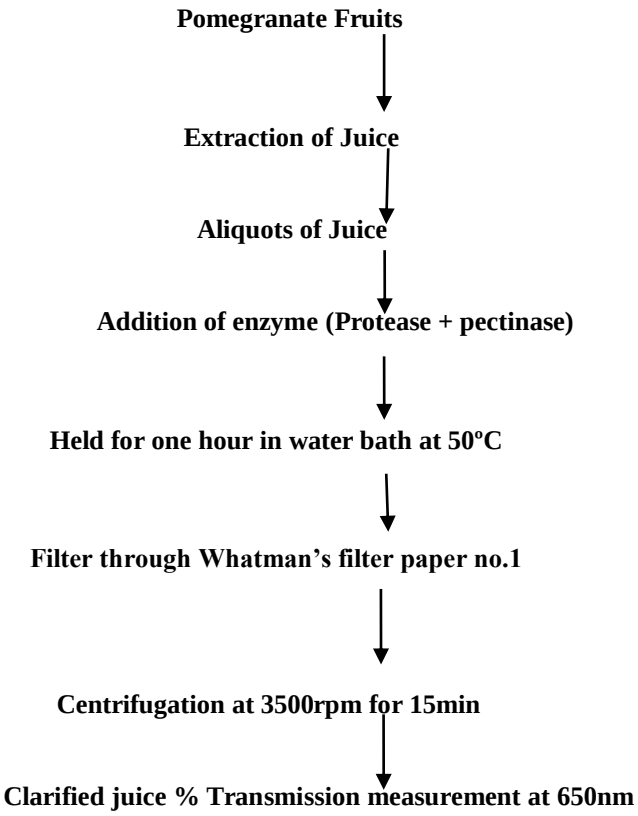
Material and Methods:

Pomegranate fruit procured from Jalgaon local market, clarifying enzymes purchased from enzyme India pvt ltd. Chennai of activity pectinase (30,000U/gm.) and protease ((90,000U/gm.)

Clarification of juice:

Domestic mixer of will be used for juice extraction. The juice extracted from mixer at low level for 30 sec. The extracted pomegranate juice was used for clarification by using commercially available pectolytic enzyme, proteolytic enzyme, and bentonite. Fruit juice was treated with different enzyme concentrations. Then subjected to the incubation for one hour at 50ºC and filtered through whatman filter paper no.1. After centrifugation, it was stored at 4ºC and % transmission measured at 650nm on UV spectrophotometer Chemical analysis of juice carried out according to the S. Ranganna (1983) analysis of fruits and vegetable products.

Method:



Result and Discussion:

Single enzyme and fining agent treatment was given to the Ganesh pomegranate at various concentration from 0.05% to 0.30% s/v at 50ºC temperature for one hour. Following results are obtained.

Table no.1Effect of pectinase:

Sr.No.	Concentration (%)	% Transmission
1	0.05	61.50
2	0.10	62.50
3	0.15	63.35
4	0.20	68.75
5	0.25	64.50
6	0.30	60.60

Optical density measured at 650 nm by U V spectrophotometer

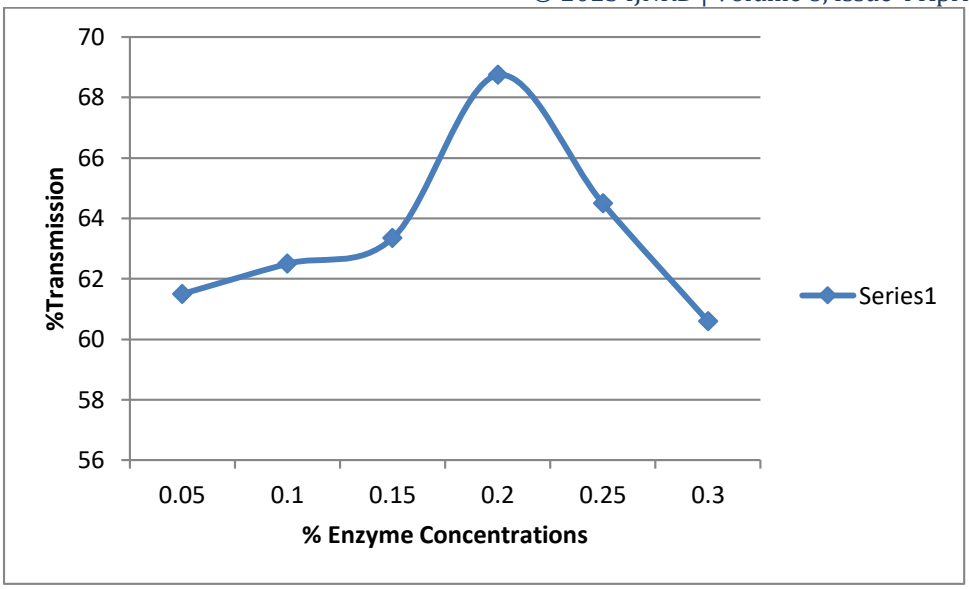


fig.no.1

Table no.1 and fig no.1 indicates that, the extracted juice sample from pomegranate variety Ganesh was clarified by different concentrations of pectinase enzyme at 50^0C for 50 min we found that pectinase at 0.20 % concentration had given maximum clarity (68.75%).

Table no.2 Effect of protease:

Sr.No.	Concentration (%)	% Transmission
1	0.05	52.75
2	0.10	54.80
3	0.15	57.85
4	0.20	56.10
5	0.25	51.85
6	0.30	49.70

Optical density measured at 650 nm by U V spectrophotometer

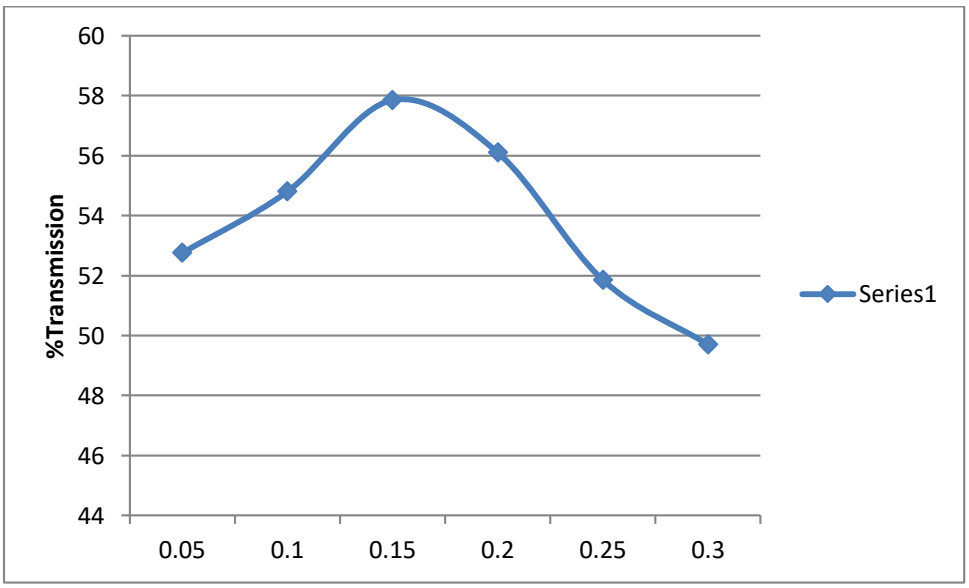


fig.no.2

Table no.2 and fig.no.2 indicates that, the extracted juice sample from pomegranate variety Ganesh was clarified by different concentrations of protiase enzyme at 50^0C for 50 min we found that protease at 0.15 % concentration had given maximum clarity (57.85%).

Table no.3 Effect of bentonite:-

Sr.No.	Concentration (%)	% Transmission
1	0.05	66.80
2	0.10	72.00
3	0.15	69.70
4	0.20	67.70
5	0.25	62.70
6	0.30	61.70

Optical density measured at 650 nm by U V spectrophotometer.

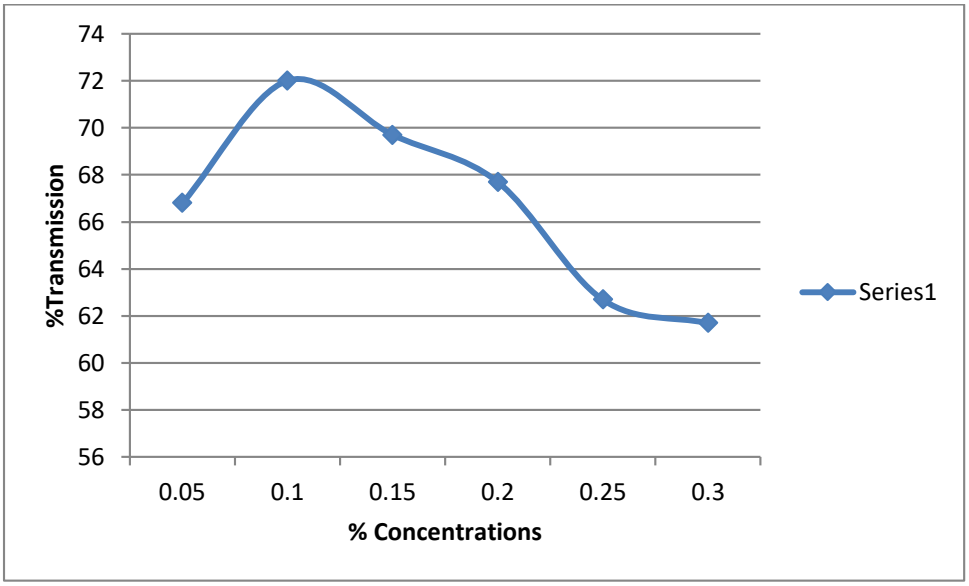


fig.no.3

table no.3 and fig no.3 indicates that, the extracted juice sample from pomegranate variety Ganesh was clarified by different concentrations of bentonite at 50ºC for 50 min we found that pectinase at 0.10 % concentration had given maximum clarity (72.00%.)

Experimental Design:

Single enzyme treatment firstly given to the juice which shows that pectinase 0.20% gives clarity about 68.75% and protease gives 57.85% clarity at 0.15% concentration at 50^0C for 60 min. The combine enzyme concentration was optimized by Central Composite Design software of Quadratic Model.

Table no.4

		Factor 1	**Factor 2**	**Response 1**
Std	Run	A:pectinase %	B:protease %	R1 % Transmission
7	1	0.1	0.179289322	63.9
9	2	0.1	0.25	72
1	3	0.05	0.2	66.02
6	4	0.170710678	0.25	68.3
10	5	0.1	0.25	73
2	6	0.15	0.2	63.05
11	7	0.1	0.25	73
12	8	0.1	0.25	73
3	9	0.05	0.3	65.36
13	10	0.1	0.25	73
5	11	0.029289322	0.25	64.84

4	12	0.15	0.3	72.8
8	13	0.1	0.320710678	70.19

Design Summary:

Table no.5

Design Summary

Study Type	Response Surface	Runs	13
Design Type	Central Composite	Blocks	No Blocks
Design Model	Quadratic	Build Time (ms)	120.274

Factor	Name	Units	Type	Subtype	Minimum	Maximum	-1 Actual	+1 Actual	Mean	Std. Dev.	
A	pectinase	%	Numeric	Continuous	0.029289	0.170711	0.05	0.15	0.1	0.039223	
B	protease	%	Numeric	Continuous	0.179289	0.320711	0.2	0.3	0.25	0.039223	
Response	Name	Units	Obs	Analysis	Minimum	Maximum	Mean	Std. Dev.	Ratio	Trans	Model
Y1	R1 % Transmission		13	Polynomial	63.05	73	69.11231	3.979429	1.157811	None	Quadratic

Table no.5 indicate that ,the 2 Factors: A, B. Design Matrix Evaluation for Response Surface Quadratic Model. No aliases found for Quadratic Model:

Degrees of Freedom for Evaluation:

Model: 5

Residual: 7

Lack of fir: 3

Pure error: 4

Core total: 12

A recommendation is a minimum of 3 lack of fit df and 4 df for pure error. This ensures a valid lack of fit test. Fewer df will lead to a test that may not detect lack of fit. Power at 5 % alpha level to detect signal/noise ratios of:

Table no.6

Term	StdErr**	VIF	Ri-Squared	0.5 Std. Dev.	1 Std. Dev.	2 Std. Dev.
A	0.353553	1	0	9.4 %	23.2 %	68.1 %
B	0.353553	1	0	9.4 %	23.2 %	68.1 %
AB	0.5	1	0	7.2 %	14.0 %	40.8 %
A^2	0.379144	1.017308	0.017013	20.8 %	62.1 %	99.4 %
B^2	0.379144	1.017308	0.017013	20.8 %	62.1 %	99.4 %

****Basis Std. Dev. = 1.0**

Measures Derived From the (X'X)^-1 Matrix

Std	Leverage	Point Type
1	0.625	Factorial
2	0.625	Factorial
3	0.625	Factorial
4	0.625	Factorial
5	0.625	Axial
6	0.625	Axial
7	0.625	Axial
8	0.625	Axial
9	0.2	Center
10	0.2	Center
11	0.2	Center
12	0.2	Center
13	0.2	Center
Average =	0.461538	

TRANSMISSION:-

Table no.7

Response	1	R1 % Transmissoin	Transform:	None

***** WARNING: The Cubic Model and higher are Aliased! *****

Summary (detailed tables shown below)

Source	Sequential p-value	Lack of Fit p-value	Adjusted R-Squared	Predicted R-Squared	
Linear	0.2067	0.0002	0.124536	-0.23614	
2FI	0.1734	0.0002	0.217351	-0.29956	
Quadratic	< 0.0001	0.9855	0.992538	0.992407	Suggested
Cubic	0.9200	1.0000	0.989896	0.993422	Aliased

Sequential Model Sum of Squares [Type I]

	Sum of	Mean	F	p-value

Source	Squares	df	Square	Value	Prob > F	
Mean vs Total	62094.64	1	62094.64			
Linear vs Mean	51.39298	2	25.69649	1.853506	0.2067	
2FI vs Linear	27.09203	1	27.09203	2.185914	0.1734	
Quadratic vs 2FI	110.7181	2	55.35905	468.5101	< 0.0001	Suggested
Cubic vs Quadratic	0.027119	2	0.013559	0.084745	0.9200	Aliased
Residual	0.8	5	0.16			
Total	62284.67	13	4791.129			

I+"Sequential Model Sum of Squares [Type I]"0+: the highest order polynomial selected where the additional terms are significant and the model is not aliased.

Lack of Fit Tests

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Linear	137.8372	6	22.97287	114.8644	0.0002	
2FI	110.7452	5	22.14904	110.7452	0.0002	
Quadratic	0.027119	3	0.00904	0.045198	0.9855	Suggested
Cubic	0	1	0	0	1.0000	Aliased
Pure Error	0.8	4	0.2			

I+"Lack of Fit Tests"0+: the selected model to have insignificant lack-of-fit.

Model Summary Statistics

Source	Std. Dev.	R-Squared	Adjusted R-Squared	Predicted R-Squared	PRESS	
Linear	3.723402	0.270446	0.124536	-0.23614	234.9043	
2FI	3.520499	0.413013	0.217351	-0.29956	246.9566	
Quadratic	0.343744	0.995647	0.992538	0.992407	1.442843	Suggested
Cubic	0.4	0.99579	0.989896	0.993422	1.25	Aliased

I+"Model Summary Statistics"0+: Focus on the model maximizing the "Adjusted R-Squared" and the "Predicted R-Squared".

ANOVA:-

Table no.8 ANOVA for Response Surface Quadratic Model

Analysis of variance table [Partial sum of squares - Type III]

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	189.2031	5	37.84062	320.2496	< 0.0001	significant
A-pectinase	10.95864	1	10.95864	92.74424	< 0.0001	
B-protease	40.43434	1	40.43434	342.2005	< 0.0001	
AB	27.09203	1	27.09203	229.2829	< 0.0001	

A^2	67.5007		1	67.5007	571.2662	< 0.0001
B^2	57.60004		1	57.60004	487.4758	< 0.0001
Residual	0.827119		7	0.11816		
Lack of Fit	0.027119	3	0.00904	0.045198	0.9855	not significant
Pure Error	0.8	4	0.2			
Cor Total	190.0302	12				

Table no.8 The Model F-value of 320.25 implies the model is significant. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A, B, AB, A++2+-, B++2+- are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model. The "Lack of Fit F-value" of 0.05 implies the Lack of Fit is not significant relative to the pure error. There is a 98.55% chance that a "Lack of Fit F-value" this large could occur due to noise. Non-significant lack of fit is good -- we want the model to fit.

Table no.9

Std. Dev.	**0.343744**	**R-Squared**	**0.995647**
Mean	69.11231	Adj R-Squared	0.992538
C.V. %	0.49737	Pred R-Squared	0.992407
PRESS	1.442843	Adeq Precision	41.54257

From the table no.9. the "Pred R-Squared" of 0.9924 is in reasonable agreement with the "Adj R-Squared" of 0.9925."Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 41.543 indicates an adequate signal. This model can be used to navigate the design space.

Table no.10

	Coefficient		**Standard**	**95% CI**	**95% CI**	
Factor	Estimate	df	Error	Low	High	VIF
Intercept	72.8	1	0.153727	72.43649	73.16351	
A-pectinase	1.170397	1	0.121532	0.88302	1.457774	1
B-protease	2.248175	1	0.121532	1.960798	2.535552	1
AB	2.6025	1	0.171872	2.196088	3.008912	1
A^2	-3.115	1	0.130328	-3.42318	-2.80682	1.017308
B^2	-2.8775	1	0.130328	-3.18568	-2.56932	1.017308

Table no.11

Final Equation in Terms of Coded Factors:	
R1 % Transmission	=
72.8	
1.170397	* A
2.248175	* B
2.6025	* A * B
-3.115	* A^2

-2.8775	* B^2

Final Equation in Terms of Actual Factors:

R1 % Transmission	=
0.845828	
12.35795	* pectinase
516.3635	* protease
1041	* pectinase * protease
-1246	* pectinase^2
-1151	* protease^2

From the table no.11 the Diagnostics Case Statistics Report has been moved to the Diagnostics Node. In the Diagnostics Node, Select Case Statistics from the View Menu.

Proceed to Diagnostic Plots (the next icon in progression):

1) Normal probability plot of the Standardized residuals to check for normality of residuals.
2) Standardized residuals versus predicted values to check for constant error.
3) Externally Standardized Residuals to look for outliers, i.e., influential values.
4) Box-Cox plot for power transformations.

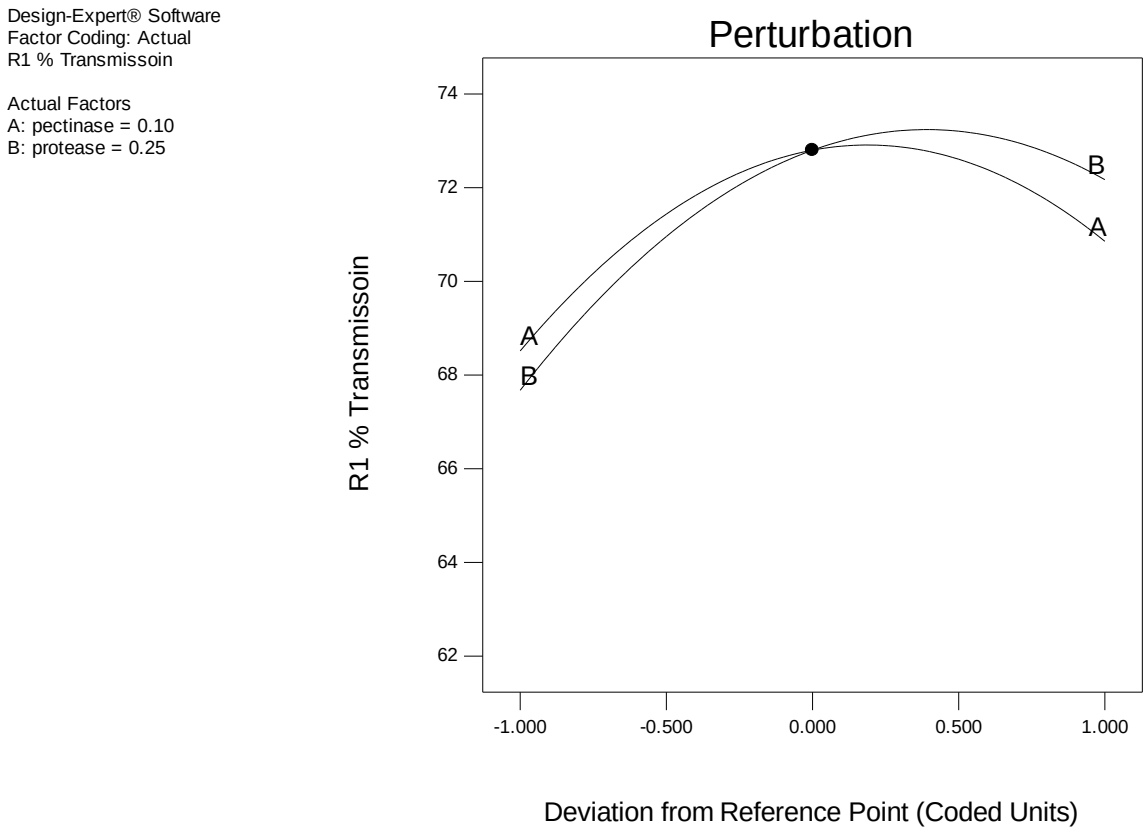


Fig no.4

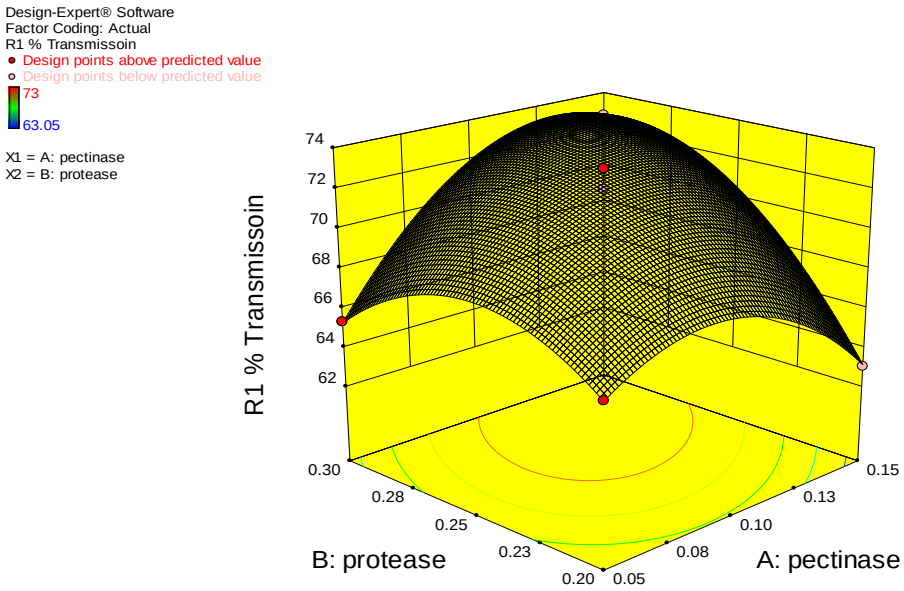


Fig.no.5

Table no.12

OPTIMIZATION:-

Constraints						
Name	Goal	Lower Limit	Upper Limit	Lower Weight	Upper Weight	Importance
A:pectinase	is in range	0.05	0.15	1	1	3
B:protease	is in range	0.2	0.3	1	1	3
R1 % Transmission	is in range	63.05	73	1	1	3

Solutions Number	pectinase	Protease	R1 % Transmission	Desirability	
1	0.10	0.25	72.8	1	Selected
2	0.05	0.30	65.2828	1	
3	0.15	0.30	72.8286	1	
4	0.15	0.20	63.1272	1	
5	0.05	0.20	65.9914	1	
6	0.15	0.25	71.2406	1	
7	0.14	0.21	66.9633	1	
8	0.09	0.27	72.4394	1	
9	0.05	0.29	67.0998	1	
10	0.06	0.22	69.1321	1	
11	0.12	0.23	70.9878	1	
12	0.06	0.29	67.5286	1	
13	0.15	0.26	72.1459	1	
14	0.14	0.26	72.752	1	

15	0.08	0.25	71.8218	1
16	0.08	0.26	72.0029	1
17	0.09	0.26	72.5641	1
18	0.15	0.21	65.6714	1
19	0.15	0.27	72.6415	1
20	0.10	0.23	71.3153	1
21	0.09	0.25	72.5927	1
22	0.09	0.30	71.3519	1
23	0.12	0.22	69.9697	1
24	0.08	0.28	71.5089	1
25	0.05	0.21	66.9704	1
26	0.14	0.26	72.3771	1
27	0.08	0.28	71.5159	1
28	0.10	0.22	70.53	1
29	0.12	0.20	67.614	1
30	0.06	0.27	68.6419	1
31	0.09	0.28	72.8982	1
32	0.09	0.21	69.0375	1
33	0.08	0.23	71.1735	1
34	0.12	0.23	70.4577	1
35	0.10	0.29	72.6111	1

35 Solutions found

Number of Starting Points: 35

pectinase	protease
0.1	0.25
0.05	0.3
0.15	0.3
0.15	0.2
0.05	0.2
0.125129	0.27416
0.138293	0.211881
0.086883	0.270328
0.052746	0.287882
0.061472	0.223813
0.115637	0.228276
0.056054	0.289605
0.143595	0.282118
0.137678	0.2619
0.079327	0.251787
0.080687	0.257255
0.088268	0.259784
0.147396	0.210943
0.148761	0.274714
0.09525	0.22903

0.093839	0.249596
0.089778	0.298961
0.122114	0.222511
0.078145	0.275564
0.050631	0.20939
0.129352	0.277584
0.079402	0.278705
0.104216	0.221456
0.11528	0.203897
0.055037	0.271789
0.094996	0.276436
0.088819	0.208658
0.080498	0.233889
0.120498	0.225636
0.095016	0.285565
0.146283	0.254366

POINT PREDECTION:-

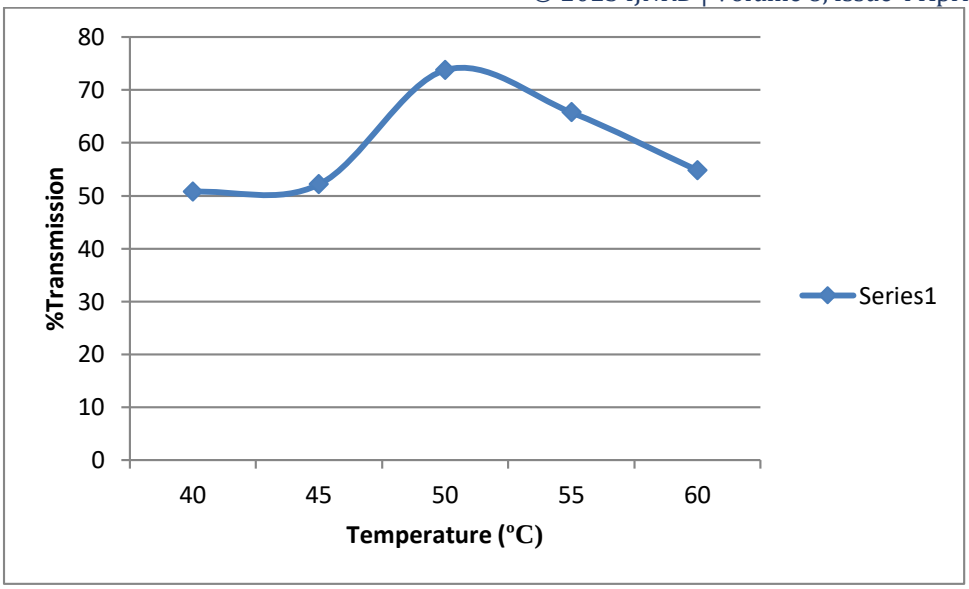
Table no.13

Factor	**Name**	**Level**	**Low Level**	**High Level**	**Std. Dev.**	**Coding**			
A	pectinase	0.1	0.05	0.15	0	Actual			
B	protease	0.25	0.2	0.3	0	Actual			
Response	Prediction	SE Mean	95% CI low	95% CI high	SE Pred	95% PI low	95% PI high	99% of Population TI low	95% TI high
R1 % Transmissoin	72.8	0.153727	72.43649	73.16351	0.376552	71.90959	73.69041	70.91751	74.68249

Table no.14 Effect of temperature on clarity of juice

Sr.no	**Temperature(ºC)**	**% Transmission**
1	40	50.75
2	45	52.15
3	50	73.75
4	55	65.75
5	60	54.80

A extracted juice sample from pomegranate variety Ganesh was clarified by combine concentrations of pectinase(0.10%) and protease (0.25%) enzymes at 50ºC for 50 min we found these concentrations had given maximum clarity(73.75%).



A extracted juice sample from pomegranate variety Ganesh was clarified by combine concentrations of pectinase(0.10%) and protease (0.25%) enzymes at 50ºC for 50 min we found these concentrations had given maximum clarity(73.75%).

Table no.15 Effect of time on clarity of juice:-

Sr. no.	Time in minute	% Transmission
1	30	62.80
2	40	63.85
3	50	68.65
4	60	73.00
5	70	74.22
6	80	77.40
7	90	41.65
8	100	41.55
9	110	40.75

Fig no. Effect of time on clarity of juice:-

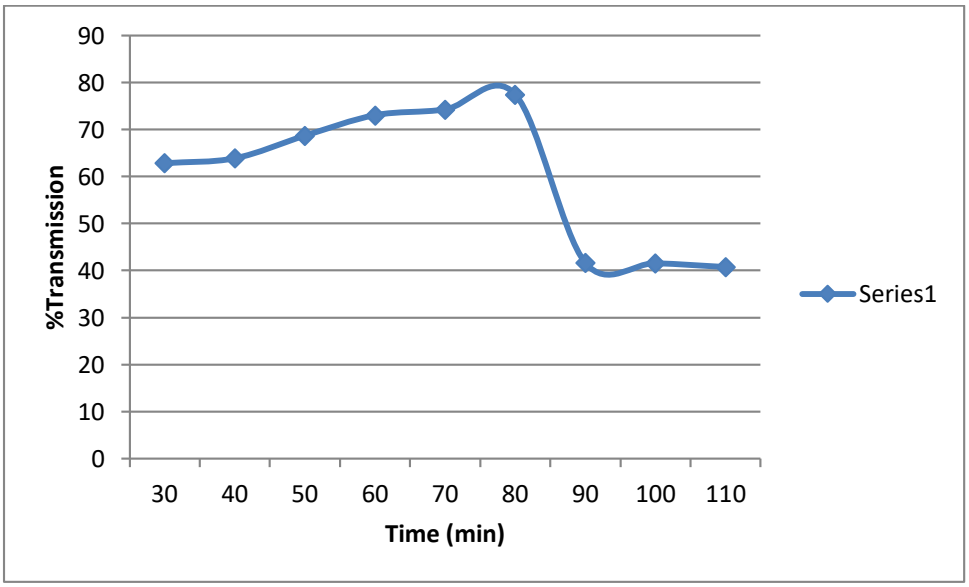


Fig.no.6

Table no.15 and fig no.6 indicate that, extracted juice sample from pomegranate variety Ganesh was clarified by combine concentrations of pectinase(0.10%) and protease (0.25%) enzymes at 50ºC for 80 min we found these concentrations had given maximum clarity(77.40%).

Non clarified Ganesh juice

Clarified Ganesh juice

Fig.no.7

Chemical characteristics of pomegranate fruit (Ganesh) Juice:-

Table no.16

Sr.no.	Characteristics	Before clarification	After clarification
1	Total soluble solids (percent)	13.80	13.30
2	Acidity(percent unhydrous citric acids)	0.285	0.22
3	PH	3.42	3.68
4	Tannin(percent tannic acid)	0.2839	0.0340
5	Reducing sugars gm/100ml	13.24	12.59
6	Non Reducing sugars gm/100ml	1.216	1.1495
7	Total sugar gm/100ml	14.52	13.80
8	Ascorbic acid (mg/ml)	15.18	13.20
9	Pectin(% calcium pectate)	1.8	0.542

SUMMARY AND CONCLUSION

The investigations were carried out for extraction and clarification of pomegranate juice. The freshly harvested pomegranate Ganesh fruits of uniform size, cleaned were taken. The physic-chemical characteristics of juice were determined. The manually separated pomegranate arils were used for juice extraction by using domestic mixer. The freshly extracted juice was clarified by using different fining agents (pectinase, protease, bentonite) from 0.05 to 0.30% s/v. Single enzyme treatment firstly given to the juice which shows that pectinase 0.20% gives clarity about 68.75% and protease gives 57.85% clarity at 0.15% and bentonite gives 72.00%concentration at 50^0C for 60 min. The optimum combine concentration after application of CCD were found Pectinase (0.10%) and Protease (0.25%) enzymes at 50^0C for 60 min for Ganesh pomegranate. Temperature (40 to 60ºC) and time(30TO 110 min) are optimized which results into temperature 50ºC and time 80 min gives maximum clarity of 77.40%.After the clarification

chemical analysis shows that there is significant decrease in pectin content 1.8 % to 0.542%,tannin content of juice also decreases 0.2839% to 0.0340% which helps to improve clarity and minimize bitterness. Chemical analysis results show that the T.S.S. slightly decreases (13.80 to 13.30) and ascorbic acid (mg/100ml) content decreases (15.18 to 13).whereas pH of the juice increases. Organoleptic evaluation of the juice shows that clarified juice has more overall acceptability than non-clarified juice.

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