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Process Optimization of Pectinase Enzyme Aided Clarification of Israel Blue (*Vitis Vinifera* L.) Grape Juice

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Abstract: Israel blue grapes **(*Vitis vinifera* L.)** consist of thick pulp with high viscosity. The thick pulp, haze and sediment formation creates difficulty in making clear Israel blue grape juice with high juice quality. Hence, there is a need for extraction of juice with high degree of clarification which leads to elevated juice quality. The objective of this research study is to obtain high quality clarified grape juice with the help of Pectinase enzyme. The study was conducted at different enzyme concentrations (0.05 %, 0.15 %, 0.5 %, 1.5 %, 2 %), time durations (1 hour, 2 hours), and temperatures (40 ^{0}C, 50 ^{0}C). The ratio between Total Soluble solids (TSS) and Titratable Acidity (TA) was measured as the dependent variable to assess the juice quality. Process optimization has been done using General full factorial design. Optimization has been done for maximum ratios of TSS and TA. The recommended parameters for extraction of juice were significant at ($p < 0.05$) as 2% enzyme concentration at 40 °C incubation temperature for 2 hours incubation time with TSS/TA ratio of 26.46 and 1.5 % pectinase enzyme concentration at 40 ^{0}C incubation temperature for 2 hours incubation time with TSS/TA ratio of 24.07.

Key words: Israel blue; Grape juice clarification; Pectinase enzyme; Process optimization; General full factorial design

Introduction

Grapes are one of the most important fruit crops in the world. Most of the grape varieties which belong to the family Vitaceae are primarily distributed in tropical regions [1]. They are consumed as fresh fruits as well as wine, juice and other processed products. Grapes are small, round or oval berries that feature semi-translucent flesh encased by a smooth skin. Some grapes contain edible seeds while some are seedless. Fresh grape juice is responsible for an increased acidity due to the existence of tartaric, malic and citric acids, resulting a low pH value and equilibrium between TSS/TA ratios [2]. The combination of unique texture, sweet and tart flavor has made grapes a popular fruit. The sensory characteristics of grape juice are mostly reliant on the balance among the sugars, acids, natural flavors, phenolic compounds and various color components [3]. Consumers are not keen to compromise either sensory quality or convenience when opting for health benefits in food products [4]. Hence, in the selection of grape cultivars for functional grape juice products, their physicochemical parameters, which contribute to sensory qualities, are of great concern. Apart from the physicochemical characteristics, fresh grape juice quality is also related to the amount of phenolic compounds that directly influence the taste of the juice and the nutritional quality. High content of phenolic compounds results in high astringency flavor. Grape juice owns nutritional and bioactive properties and it is beneficial to maintain good health, preventing diseases such as cancer [5],[6]. Poly Phenol Oxidase (PPO) browning, haze and sediment formation and microbial spoilage are the major quality problems encountered in the production of the juice. Enzymatic treatment highly facilitates the reduction of these issues with regards to grape juice. Cloudiness of the grape juice is mainly related to the presence of pectin, which is hard to remove except by enzymatic treatment using pectinases [7],[8].

Optimization of enzymatic pretreatment to clarify grape juice have been reported to prevent cloudiness [9],[10],[11]. Pectin degradation enables to reduce membrane fouling caused by the colloidal components of the filtered juice, resulting in a subsequent drop on the flux in filtration processes [12],[13]. Enzymes are currently used in grape juice production, for mash treatment to obtain a partial or total liquefaction of the fruit flesh for the production of fruit pulps and nectars, to increase juice yield and enrich extraction of other fruit components such as color and flavor; for juice treatment to reduce its viscosity and enable concentration, for better clarification, filtration, stabilization etc. One of the essential mechanisms underlying enzyme application is the mechanism of enzyme activity, which is based on the interaction of three parameters namely enzyme concentration, incubation temperature and incubation time [14]. Enzymatic treatment using pectinase enzyme is one of the best ways to diminish pectin, the main cause of sedimentation in fruit juices by hydrolyzing pectin and triggering pectin–protein complexes to flocculate [15].

Materials and methods

Plant Materials

Fresh, ripen grapes without any visual blemishes, from the *Vitis vinifera* L. variety Israel blue, cultivated in the vicinity of Jaffna, North region of Sri Lanka were purchased from a generous farmer for the preparation of grape juices. The cultivar was grown on Pandhal trellis system in the vine yard.

Chemicals

Analytical grade sodium hydroxide and sodium hypochlorite solution were purchased from Sigma-Aldrich (Sigma-Aldrich chemicals PVT LTD. Sri Lanka). Pectinase enzyme under the brand name PEC600, with enzyme activity of 6,000,000 U/ml was purchased from Sunson Industry Group Co., LTD. China to treat the Israel blue grape pulp.

Sample Preparation

Grape juice was subjected to an enzymatic treatment for further clarification according to a standard method with further modifications [16]. Fresh ripen grapes were detached from the stems, sorted and cleaned by

washing three times with potable water, with 150 ppm chlorine solution and again three times with potable water. After draining, grapes were weighed and blended in a Philips 3 Jar 550 W Supreme Mixer Grinder – HL1618 and converted into a pulp. The pulp was blanched by heating at 60-65 ^{0}C for 15 minutes and allowed to cool under room temperature (25 ^{0}C). The pulp was weighed and calculated quantities of pectinase enzyme were added to the pulp. The pulp was poured to a clean jar, mixed well, capped and incubated. Concentration of the pectinase enzyme, incubation temperature and incubation time were used as three independent variables to treat the grape pulp and clarify the juice as shown in Table 1 [17]. To study the effective treatment combinations, grape juice samples were prepared in duplicates. After incubation, the pulp was manually pressed using a clean nylon cloth bag and the juice obtained was heated under 90 ^{0}C for 5 minutes to inactivate the enzyme. The heated juice was filtered through a Whatman™ 1001-090 Grade 1 qualitative filter paper with diameter of 9 mm and pore size of 11µm. The filtered juice was finally pasteurized under 90 ^{0}C for 5 minutes. Soon after pasteurization, juice was removed from the flame, filled into clean, previously washed and dried PET bottles and capped. The sealed bottles were dipped in a water bath at room temperature, allowed to cool and labelled. The TSS, Titratable Acidity (TA) and TSS/TA ratio in grape juice samples obtained from the above mentioned treatment combinations, were measured. The TSS/TA ratio was calculated as the ratio of TSS in g/100 g juice to TA in g/100 mL juice.

Table 1: Different processing conditions for enzymatic treatment of Israel Blue grape pulp

Pectinase enzyme Concentration (%)	Incubation temperature (^{0}C)	Incubation time (hours)
0.05	40	1
0.05	40	2
0.05	50	1
0.05	50	2
0.15	40	1
0.15	40	2
0.15	50	1
0.15	50	2
0.50	40	1
0.50	40	2
0.50	50	1
0.50	50	2
1.50	40	1
1.50	40	2
1.50	50	1
1.50	50	2
2.00	40	1
2.00	40	2
2.00	50	1
2.00	50	2

Analysis of Total Soluble Solids and Titratable Acidity of Juice

Total Soluble Solids (TSS)

The brix values (TSS) of grape juice samples were measured using a portable hand held refractometer at room temperature.

Tiitratable Acidity (TA)

Titratable acidity was determined according to the AOAC method [18]. A standard solution of 0.1N NaOH was prepared. Simultaneously 5 mL of grape juice was diluted up to 50 mL with distilled water and with few drops (nearly 1ml) of phenolphthalein indicator, it was titrated against the standard NaOH solution to obtain an end point at pH 8.2 that gives a consistent pink color for 30 seconds. The following equation was used to determine the Titratable Acidity (TA) as tartaric acid g/100 mL [19].

$$\text{Titatable Acidity} = \frac{V_1 \times N \times 75 \times 100}{1000 \times V_2}$$

V_1 - Volume in ml of standard NaOH required for titration

N - Normality of the standard NaOH

V_2 - Volume in ml of the grape juice sample taken for the test

Optimization study using a statistical design to determine the best two levels of treatment combinations

Appropriate factors and levels to establish the statistical model are shown in Table 2. A general full factorial design was fabricated using the software MINITAB® 17 to predict the treatment combinations that give the highest output, TSS/TA ratio. Using the general full factorial design the best two levels of treatment combinations were determined [20].

Table 2: Factors and levels used to establish the statistical model using general full factorial Design

Factor	Levels
Pectinase Enzyme Concentration	0.05 %
	0.15 %
	0.50 %
	1.50 %
	2.00 %
Incubation Temperature	40 ^{0}C
	50 ^{0}C
Incubation Time	1 hour
	2 hours

Results and discussion

Enzymatic hydrolysis of the cell walls increases the extraction yield, reducing sugars, soluble dry matter content, Galacturonic acid content and titrable acidity of the products [21]. It is presumed that the TSS/TA ratio is one of the major analytical measurements for quality in juices. TSS/TA ratio describes juice quality more precisely than when the two parameters are individually considered [22].

Hence TSS/TA ratio was used as the output response to determine the best two treatment combinations that produce a grape juice with high quality. Results obtained for TSS, TA, and TSS/TA ratio of grape juices based on different factors and levels used in the experiment are shown in Table 3. TSS/TA ratio equivalent to 20 has been defined as the lowest value for consumer preference [23].

Table 3: Effects of different enzymatic treatments on TSS, TA, TSS: TA and pH values of Grape juice

Sample	Pectinase concentration (%)	Temperature (^{0}C)	Incubation time (hour)	TSS (%)	TA (%)	pH	TSS:TA
1	0.05	40	2	12.5	0.56	4.1	22.32
2	0.05	40	2	12.5	0.58	4.05	21.55
3	0.05	40	1	12	0.87	3.45	13.79
4	0.05	40	1	12	0.81	3.36	14.81
5	0.05	50	1	11.5	0.78	3.62	14.74
6	0.05	50	1	11.5	0.77	3.71	14.94
7	0.05	50	2	13	0.87	3.54	14.94
8	0.05	50	2	13	0.82	3.41	15.85
9	0.15	40	2	14.5	0.77	3.9	18.83
10	0.15	40	2	14	0.75	3.85	18.72
11	0.15	40	1	13.5	0.78	3.89	17.31
12	0.15	40	1	13.5	0.78	3.88	17.38
13	0.15	50	2	15	0.79	3.72	18.99
14	0.15	50	2	14.5	0.86	3.74	16.82
15	0.15	50	1	14	0.81	3.67	17.28
16	0.15	50	1	14	0.93	3.65	15.12
17	0.5	40	2	15.5	0.68	3.71	22.68
18	0.5	40	2	15.5	0.68	3.68	22.79
19	0.5	40	1	13	0.65	3.8	19.96
20	0.5	40	1	13.5	0.68	3.76	19.97
21	0.5	50	2	16	0.81	3.64	19.76
22	0.5	50	2	16	0.81	3.8	19.82
23	0.5	50	1	12.5	0.78	3.66	16.03
24	0.5	50	1	13	0.81	3.62	16.06
25	1.5	40	2	15.5	0.65	3.7	23.77
26	1.5	40	2	16	0.66	3.6	24.36
27	1.5	40	1	14.5	0.66	3.71	21.83
28	1.5	40	1	14.5	0.67	3.68	21.68
29	1.5	50	2	16	0.79	3.54	20.25
30	1.5	50	2	16.5	0.79	3.58	20.76
31	1.5	50	1	13.5	0.81	3.62	16.67
32	1.5	50	1	14	0.73	3.67	19.07
33	2	40	2	18.5	0.70	3.68	26.53
34	2	40	2	19	0.72	3.64	26.39
35	2	40	1	17	0.71	3.72	23.79
36	2	40	1	17	0.72	3.77	23.77
37	2	50	2	18	0.81	3.83	22.15
38	2	50	2	18.5	0.83	3.88	22.19
39	2	50	1	17.5	0.83	3.71	21.18
40	2	50	1	17.5	0.83	3.69	21.21

Identification of important effects

A, B, C factors interpret pectinase enzyme concentration, incubation temperature and incubation time, respectively. As shown in Table 4, the main effects due to factors A, B, C and the interaction effects due to

factors A*B, A*C, B*C, A*B*C are highly significant at ($p < 0.05$) level.

Table 4: Summary table based on ANOVA for the significant factors affecting the output response TSS: TA

Factors	p values
A	0.000
B	0.000
C	0.000
A*B	0.006
A*C	0.010
B*C	0.004
A*B*C	0.000

Effect of Experimental Variables on TSS/TA ratio of grape juice

Figure 1 illustrates the main effects plots. Main effect arises when the mean response changes across the levels of a factor. Factor A (pectinase enzyme concentration) and factor C (incubation time) have positive effects while factor B (incubation temperature) has a negative effect on the output TSS/TA ratio. High levels of incubation temperature can lead to denaturation of pectinase enzyme which can result in diminution of the enzyme activity and give a low value of TSS/TA ratio. Figure 2 illustrates interaction plots of average output TSS/TA ratio for each level of the factor with the level of the second factor which is held constant. Interaction plot A*B illustrates the output TSS/TA ratio for each level of factor B with the level of factor A which is held constant. Interaction plot A*C illustrates the output TSS/TA ratio for each level of factor C with the level of factor A which is held constant. Interaction plot B*C illustrates the output TSS/TA ratio for each level of factor C with the level of factor B which is held constant. Since the interaction effect could amplify or reduce the main effects of the parameters, evaluating interactions is essential [24]. As shown in Figure 2, all three interaction plots illustrate a synergic interaction between particular two experimental factors. Lack of parallelism of the lines reveal a significant interaction between A*B, A*C and B*C. The A*B interaction plot shows the pectinase enzyme concentration being 2 % and the incubation temperature being 40 ^{0}C give the highest TSS/TA ratio (25.12). Pectinase enzyme concentration being 1.50 % and the incubation temperature being 40 ^{0}C give the second highest TSS/TA ratio (22.91). Pectinase enzyme concentration being 0.05% and the incubation temperature being 50 ^{0}C give the lowest TSS/TA ratio (15.12). The A*C interaction plot shows the pectinase enzyme concentration being 2 % and the incubation time being 2 hours give the highest TSS/TA ratio (24.31) and pectinase enzyme concentration being 1.5 % and the incubation time being 2 hours give the second highest TSS/TA ratio (22.29). Pectinase enzyme concentration being 0.05% and the incubation time being 1 hour give the lowest TSS/TA ratio (14.57). The B*C interaction plot shows the incubation temperature being 40^{0}C and the incubation time being 2 hours give the highest TSS/TA ratio (22.79). Incubation temperature being 50^{0}C and the incubation time being 1 hour give the lowest TSS/TA ratio (17.23).

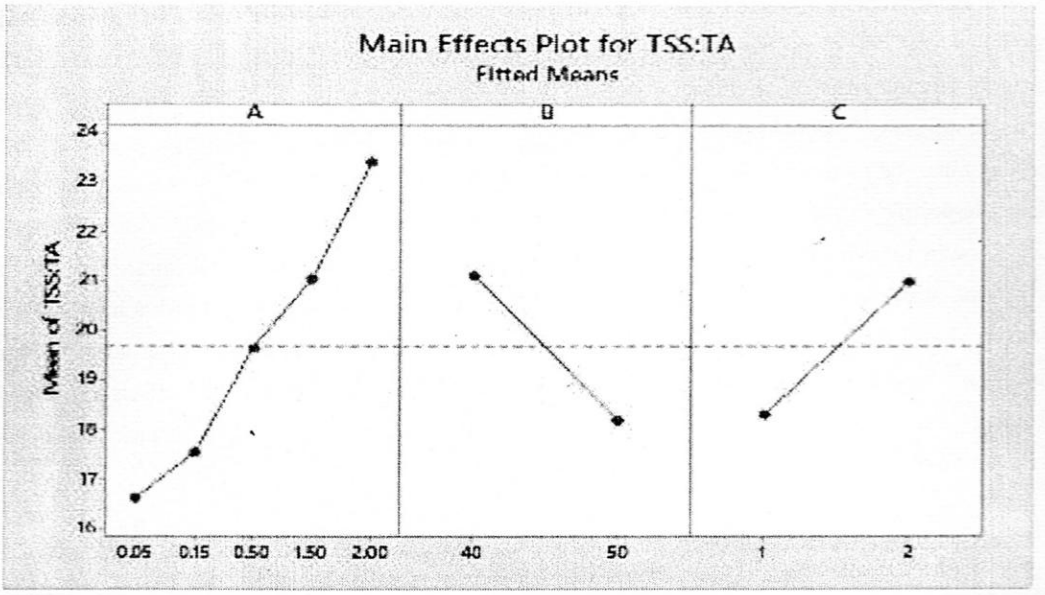

Figure 1: Effects of Enzyme concentration (A), Incubation temperature (B) and Incubation time (C) on mean TSS: TA.

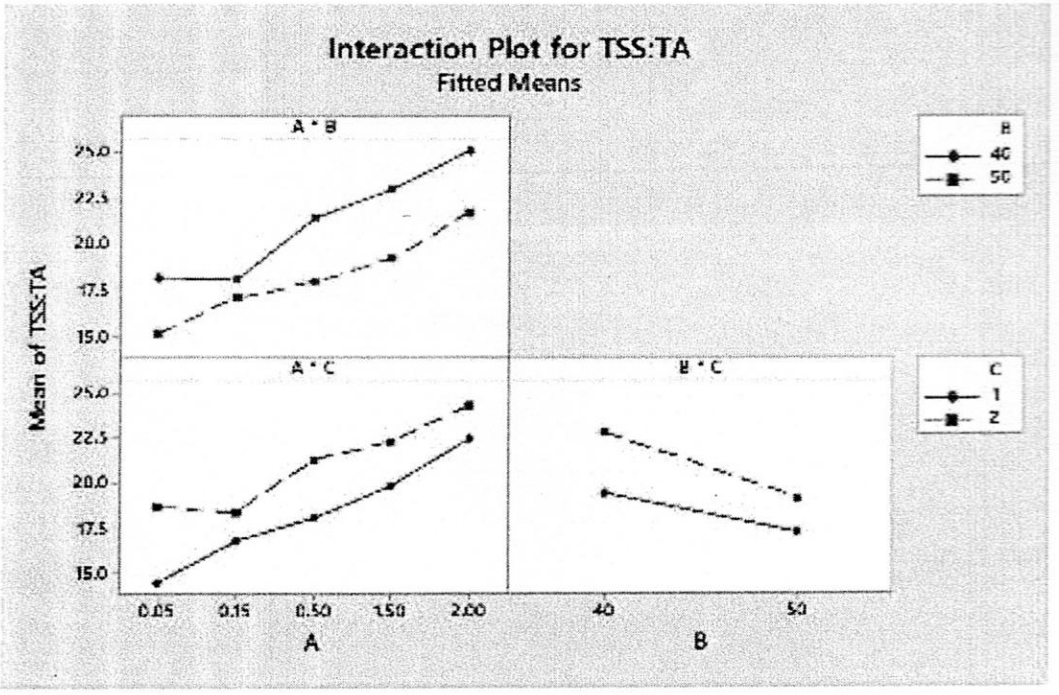

Figure 2: Effects of interaction among Enzyme concentration (A), Incubation temperature (B) and Incubation time (C) on mean TSS: TA.

Response predictions based on results obtained by the experimental model

Based on the ANOVA, main effects plot and interaction plot, the two levels of treatment combinations (2 % pectinase enzyme, 40 ^{0}C incubation temperature, 2 hours incubation time) and (1.5 % pectinase enzyme, 40 ^{0}C incubation temperature, 2 hours incubation time) were determined as the best two levels of treatment combinations, respectively that contributed to gain a highest TSS/TA ratio value in grape juices. The response TSS/TA ratio was predicted using these two treatment combinations as shown in Table 5. A reliable statistical model based on general full factorial design was developed which can be used for the optimization of output TSS/TA ratio of Israel blue grape juice. The model comprises of 97.95% R-squared which is the proportion of total variability explained by the model. Adjusted R-squared is calculated as 95.79%, which is a statistic that is adjusted for the size of the model and prediction R-squared statistic is computed to be 90.90% which indicates that the model is projected to explain about 90.90% of the variability in new data (Table 6). Hence, the experimental design to determine the best two treatment combinations that give a highest TSS/TA ratio in grape juices, not only contribute to estimate the magnitude and direction of the effects of change in factors but also predicts the effects of their mutual interactions [19].

Table 5: Prediction for the response TSS: TA

Variable setting			Fitted	95% Confidence
Enzyme concentration (%)	Incubation temperature (^{0}C)	Incubation time (hours)	value	interval
2.00	40	2	26.46	25.43, 27.49
1.50	40	2	24.06	22.28, 25.84

Table 6: Model summary of the general full factorial design

R-sq	R-sq (adj)	R-sq (pred)
97.95%	95.79%	90.90%

Conclusion

Present study concluded that pectinase enzyme treatment was capable of increasing juice quality of Jaffna

grown Israel blue grape juice. The best two levels of treatment combinations that results in highest values of TSS/TA ratio in Israel blue grape juice can be determined as "2 % pectinase enzyme, 40 ^{0}C incubation temperature, 2 hours incubation time" and "1.5 % pectinase enzyme, 40 ^{0}C incubation temperature, 2 hours incubation time". These two levels of treatment combination can be used to produce high quality grape juice by Israel blue grape cultivar.

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