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Process optimization of pectinase enzyme in Palmyrah fruit pulp for clarification

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Abstract
Palmyrah fruit (*Borassus flabellifer* L) pulp is rich in soluble sugars, provitamin A, vitamin C, minerals and lycopene. Therefore, it can be considered as a potential source of raw material for the development of industrial viable products. As Palmyrah fruit pulp contains 6.7% of pectin, clarification is an important step in juice processing. The main objective of this research is to obtain quality Palmyrah fruit juice by using pectinase treatment. This research was conducted at different enzyme concentrations (0.5 %, 1.0%, 1.5 %, 2.0 %), time durations (1 hour, 2 hours,3 hours), and temperatures (40 ºC, 50 ºC). The ratio between Total soluble solids (TSS) and Titratable Acidity (TA) was measured. Optimum condition was found out by general full factorial design in Minitab 17 using the ratio of TSS/TA. Pectinase enzyme degrades the pectin. As a result, extraction yield, reducing sugars, soluble dry matter content, galacturonic acid content and titrable acidity of the products were increased. The recommended combinations of treatment to clarify the Palmyrah fruit pulp using pectinase enzyme is 1% pectinase enzyme, at 40 ºC incubation temperature and 1 hour incubation time and "2 % pectinase enzyme, 40 ºC incubation temperature, 1 hour incubation time.

Keywords: palmyrah, pectinase enzyme, clarification, process optimization

1. Introduction
Borassus flabellifer L, belongs to the family Arecaceae, commonly known as Palmyrah palm [1]. It is available in the arid tropic country like India, Sri Lanka and South East Asia, East Africa and South America [2]. Various studies show that pulp is rich in soluble sugars, provitamin A, vitamin C, minerals and lycopene. Lycopene is an antioxidant beneficial for cardiovascular ailments and cancer [3]. Therefore, it can be considered as a potential source of raw material for the development of industrial viable products through value addition.
As Palmyrah fruit pulp contains 6.7% of pectin [2]. This leads to turbidity during the processing of clear fruit juices and also leads to many difficulties in filtering the juice to an acceptable clarity [5]. During the production of wine from pulp, methyl groups associated with pectin are released as methanol which is poisonous. Thus, removal of pectin is an essential step in pulp based products [4, 12].
Pectins are categorized as either soluble or insoluble fibre. These cannot be absorbed by the human digestive system. But, enzymes degrade them to short polysaccharide fragments that may be absorbed by body [5].
As the clarity of the juice is a determinant factor for consumers, nowadays pectinase enzyme is used in fruit industries for juice extraction and clarification. Pectinase enzyme degrade the pectin. As a result extraction yield, reducing sugars, soluble dry matter content, galacturonic acid content and titrable acidity of the products are increased. The enzyme treated pulp has a lower viscosity and the quantity of waste pomace is also reduced [5].
The ratio of TSS/TA was considered as the factor indicating the balance between the acid and sweet taste of grape juice. And this ratio affects the quality of the juice [7]. Mechanism of enzyme activity based on three factors namely enzyme concentration, incubation temperature and incubation time [6].

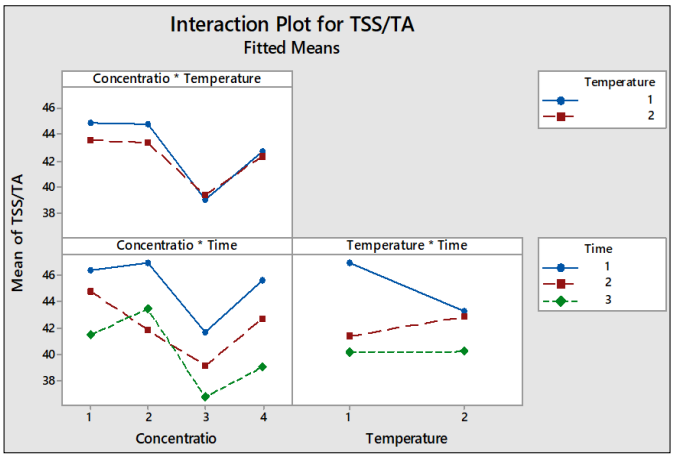

Fig 1

2. Materials and methods
2.1 Sample collection
Same types of fresh Palmyrah fruits which were free from physical and insect damage were collected from Navatkuli area.

2.2 Preparation of Palmyrah fruit pulp
The pulp was extracted manually using water in the ratio (v/v) of 1:1. Then it was filtered through muslin cloth [2].

2.3 Pectinase treatment

About 50ml of Palmyrah fruit pulp taken into beakers. Calculated quantities of pectinase enzyme purchased from Sunson Industry Group Co LTD. China, under the brand name PEC600, with an enzyme activity of 6,000,000U/ml were added to the pulp. Concentration of 0.5 %, 1.0%, 1.5 %, 2 %, incubation time of 1 hour, 2 hours, 3 hours and incubation temperature of 40 ºC, 50 ºC were taken as the variables to treat the Palmyrah fruit pulp. The ratio between Total Soluble solids (TSS) and Titratable Acidity (TA) was measured [6].

2.4 Titratable acidity

Titratable acidity was determined according to the AOAC method. Palmyrah pulp of 5ml was diluted up to 50 mL with distilled water. Then 1 mL of phenolphthalein indicator was added and titrated with standardized 0.1 N NaOH until the solution turned to light pink colour and the volume of NaOH needed for the titration was recorded. The following equation was used to determine the Titratable Acidity (TA) as tartaric acid g/100 mL [6, 9].

Calculations

Titratable acidity as tartaric acid (g/ 100 mL) =

$$(V1 \times N \times 75 \times 100)/ (V2*1000)$$

V1 = Volume in mL of NaOH used in titration
V2 = Volume in mL of the sample
N = Normality of the standard NaOH

2.5 Total soluble solids

Initially a drop of distilled water was placed on the lense of the brix meter and the refractometer reading was taken by looking through the eye piece. Then the lense was cleaned using a tissue paper and one to two drops of palmyrah fruit pulp was placed on the lense and the readings were taken [8].

2.6 Statistical design used to find out the best combination of treatment

To find out the optimum condition of the pectinase treatment, general full factorial design in Minitab 17 statistical package was used. So TSS/TA was used as the output response to find out the best two treatment combinations [6].

Table 1: Factors and levels used to establish the statistical model using general full factorial Design

Factors	Levels
Concentration	0.5%
	1.0%
	1.5%
	2.0%
Temperature	40^{o}C
	50^{o}C
Incubation time	1 hr
	2 hr
	3 hr

3. Results and Discussion

Pectinase treatment increases extraction yield, reducing sugars, soluble dry matter content, and titrable acidity of the juice [5]. Pectinase enzyme hydrolyses pectic chains and help for the draining of juice from the pomace with an increase yield with a lower viscosity. The addition of this enzyme lowers viscosity and causes cloud particles to aggregate into larger units, which settles as sediment and help for the clarification process. According to the recent studies conducted in the physicochemical analysis of grape juice samples after the treatment of pectinase enzyme, the ratio of TSS/TA was considered as the factor indicating the balance between the acid and sweet taste of grape juice [6]. And this ratio affects the quality of the juice [7]. So TSS/TA was used as factor to find out the best two treatment combinations.

Table 2: Optimization of Pectinase enzyme

Pectinase concentration (%)	Temperature (ºC)	Incubation time (hr)	Brix	TA	TSS/TA
0.5	40	1	10.86	0.23	47.22
0.5	40	1	10.85	0.24	45.21
0.5	40	2	10.91	0.25	43.64
0.5	40	2	10.91	0.24	45.46
0.5	40	3	11.00	0.26	42.31
0.5	40	3	10.89	0.24	45.38
0.5	50	1	10.90	0.24	45.42
0.5	50	1	10.92	0.23	47.48
0.5	50	2	11.02	0.24	45.92
0.5	50	2	11.02	0.25	44.08
0.5	50	3	11.08	0.26	42.62
0.5	50	3	11.05	0.25	35.76
1.0	40	1	11.09	0.22	50.41
1.0	40	1	11.13	0.21	53.00
1.0	40	2	11.34	0.27	42.00
1.0	40	2	11.33	0.29	39.07
1.0	40	3	11.55	0.27	42.78
1.0	40	3	11.60	0.28	41.43
1.0	50	1	11.17	0.27	41.37
1.0	50	1	11.15	0.26	42.88
1.0	50	2	11.87	0.27	43.96
1.0	50	2	11.86	0.28	42.36
1.0	50	3	12.51	0.27	46.33

1.0	50	3	12.52	0.29	43.17
1.5	40	1	11.75	0.28	41.96
1.5	40	1	11.74	0.28	41.93
1.5	40	2	11.89	0.33	36.03
1.5	40	2	11.86	0.33	35.94
1.5	40	3	12.14	0.30	40.47
1.5	40	3	12.11	0.32	37.84
1.5	50	1	11.79	0.29	40.66
1.5	50	1	11.78	0.28	42.07
1.5	50	2	11.61	0.27	43.00
1.5	50	2	11.64	0.28	41.57
1.5	50	3	12.03	0.34	35.38
1.5	50	3	12.03	0.36	33.42
2.0	40	1	11.75	0.24	48.96
2.0	40	1	11.73	0.25	46.92
2.0	40	2	12.20	0.27	45.19
2.0	40	2	12.27	0.28	43.82
2.0	40	3	11.74	0.33	35.58
2.0	40	3	11.70	0.33	35.45
2.0	50	1	12.05	0.27	44.63
2.0	50	1	12.16	0.29	41.93
2.0	50	2	12.71	0.31	41.00
2.0	50	2	12.72	0.31	41.03
2.0	50	3	11.74	0.28	41.93
2.0	50	3	11.70	0.27	43.33

A, B, C factors interpret pectinase enzyme concentration, incubation temperature and incubation time respectively.

Table 3: Summary table based on ANOVA for the significant factors affecting the output response TSS/TA

Factors	p values
A	0.000
B	0.164
C	0.000
A*B	0.072
A*C	0.534
B*C	0.001
A*B*C	0.000

As shown in table 3, the main effects due to the factor A, C and the interaction effects due to factors B*C and A*B*C are highly significant at($p<0.05$) level. Researchers conducted in the optimization of pectinase enzyme in water melon juice also proved that, interaction effect between incubation time and incubation temperature was significant ($p<0.05$). Incubation temperature and incubation time also increase enzyme activity by increasing the kinetic energy within molecules and makes the reaction faster [9].

Fig.1 shows 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 [6]. According to the fig.1, Concentration*Temperature interaction plot shows the pectinase enzyme concentration of 0.5% and the incubation temperature of 40 ºC give the highest TSS/TA ratio (44.86). Pectinase enzyme concentration of 1.0 % and the incubation temperature of 40 ºC give the second highest TSS/TA ratio (44.78). Pectinase enzyme concentration of 1.5% and the incubation temperature being 40 ºC give the lowest TSS/TA ratio (39.03). The interaction plot Concentration * Time shows the pectinase enzyme concentration of 1 % and the incubation time being 1 hour give the highest TSS/TA ratio (46.91) and pectinase enzyme concentration of 0.5 % and the incubation time of 2 hours give the second highest TSS/TA ratio (46.33). Pectinase enzyme concentration of 1.5% and the incubation time of 3 hours give the lowest TSS/TA ratio (36.77). The interaction plot Temperature * Time shows the incubation temperature of 40 ºC and the incubation time of 1hour give the highest TSS/TA ratio (46.95). Incubation temperature of 50 ºC and the incubation time of 3 hours give the lowest TSS/TA ratio (40.15) [6].

Fig.1 Interaction plot of enzyme concentration (A), Incubation temperature (B) and Incubation time(C) on means of TSS/TA

The two levels of treatment combinations "1% pectinase enzyme, 40 ºC incubation temperature, 1 hour incubation time" and "2 % pectinase enzyme, 40 ºC incubation temperature, 1 hour incubation time" were determined as the best two levels of treatment combinations, respectively have a highest TSS/TA ratio value in Palmyrah fruit juice. Based on the previous researches on pectinase treatment in different fruits, optimum conditions were also near to these values. According to the previous studies on grape juice, the best two combinations of treatment that results in highest values of TSS/TA ratio are "2 % pectinase enzyme, 40 ºC incubation temperatures, 2 hours incubation time" and "1.5 % pectinase enzyme, 40 ºC incubation temperature, 2 hours incubation time"[6]. The suggested parameters for extraction of Jamun juice were at 0.05% enzyme concentration at 44 ºC for 80 minutes [11].

4. Conclusion

The recommended combinations of treatment to clarify the Palmyrah fruit pulp using pectinase enzyme is 1% pectinase enzyme, 40 ºC incubation temperatures and 1 hour incubation time and 2 % pectinase enzyme, 40 ºC incubation temperatures, 1 hour incubation time.

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