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Optimisation of wood apple (*Limonia acidissima* L.) juice extraction for improved yield and quality using commercial pectinase

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Abstract
In this study, the optimization of wood apple juice extraction was achieved through the treatment of the wood apple pulp with three different concentrations of pectinase (0.25%, 0.50% and 0.75%) and incubation at varying temperature and time combinations (40 °C, 50 °C, and 60 °C for 60, 90, and 120 minutes). The resulting juices were assessed for key parameters, including juice recovery, TSS, pH, titratable acidity, ascorbic acid content, total antioxidant activity, reducing sugar, and total sugar content. The most favourable results were obtained when treating the pulp with 0.75% pectinase and incubating it at 50 °C for 120 minutes, resulting in the highest juice recovery (81.70%), TSS (6.11%), acidity (2.77%), ascorbic acid content (5.47 mg/100 ml), reducing sugar (2.83%), total sugar (4.87%), and total antioxidant activity (59.10 mg AEAC per 100 ml).

Keywords: Wood apple, pectinase, incubation

1. Introduction
Wood apple (*Limonia acidissima* L.), a tropical fruit and underutilized fruit, which belongs to Rutaceae family and is traditionally known as a poor man's food because of its medicinal and nutritional value. The fruit is rich in various phytochemicals, such as polyphenols, coumarins, phytosterols, saponins, tannins, flavonoids, flavonols, as well as essential vitamins (such as thiamine, riboflavin, niacin and vitamin C), minerals and amino acids (Vidhya and Narain, 2011) [21]. These components contribute to the fruit's nutritional, therapeutic and healing properties, which encompass antioxidative, antifungal, hypoglycemic, hypolipidemic and hepatoprotective properties (Singhania *et al*., 2020) [20].
Preserving wood apple fruits is essential to utilize them efficiently, prevent wastage and make use of surplus fruit during the off-season. This fruit can be preserved by converting it into various products like jam, jelly, fruit bars, juice, pickles and murabba, extending their shelf life. However, juice extraction is one of the easiest preservation method. The challenge arises from the fact that wood apple fruit cells contain crystalline biochemical components like pectin and hemicellulose, which hinder the filtration of fruit juices, leading to cloudiness. Conventional juice extraction techniques fail to break down these biochemical components, resulting in the accumulation of solid biomass on the filter membrane, a phenomenon known as "fouling." This issue can be effectively resolved through enzymatic treatment of fruit juices. Enzymes in general and pectinase in specific play a significant role in degrading pectin and other polysaccharides, preventing fouling and improving juice yield, recovery and the overall organoleptic properties of the final product.

2. Material and Methods
2.1 Wood apple fruits and pectinase enzyme
Fully ripe wood apple fruits of uniform maturity, size, shape and without any physical injuries were procured from K. R. Market Bengaluru, Karnataka. enzyme procured from Kaypeeyes Biotech Pvt. Ltd, Mysuru.

2.2 Methodology for extraction of wood apple juice
Fully ripe wood apple fruits were opened by breaking against the hard surface. The pulp was scooped out with the help of stainless steel spoon from the hard shell. The pulp was mixed with water in the ratio of 1:1 and left undisturbed for 1 hour.

Seed and fibre were separated by passing through the strainer. The pulp obtained was treated with pectinase enzyme at three different concentrations *i.e.*, 0.25, 0.50 and 0.75 per cent respectively. Treated pulp was subjected to incubation by placing in hot water bath at different time and temperature combination *i.e.*, 40, 50 and 60 °C for 60, 90 and 120 minutes. The treated pulp was extracted for juice by passing through muslin cloth. The recovered juice was pasteurized at 75 °C for 5 min for the inactivation of pectinase enzyme. The recovered juice was filled in vials were centrifuged at 5000 rpm/ 5min. juice was then transferred to clean sterilized glass bottles for estimation of physico-chemical parameters.

2.3 Juice recovery (%)

The juice recovery percentage was calculated by using the following formula

$$\text{Juice recovery (\%)} = \frac{\text{Weight of flesh taken for pulp extraction (g)} - \text{Left out pulp after extraction (g)}}{\text{Weight of flesh taken for pulp extraction (g)}} \times 100$$

2.4 Total soluble solids (°B)

The wood apple juice was analysed for total soluble solids using digital hand refractometer (Make: Erma Optical Work Ltd., Tokyo, Japan, 0-32 °B range) and expressed as degree Brix (°B). Refractometer was calibrated before recording the reading and care was taken that the prism of the refractometer was washed with distilled water and wiped dry before every reading (Anon, 1984) [2].

2.5 pH

Digital pH meter was used to measure the pH of the recovered juice. The pH meter was calibrated with the standard buffer solution of pH 4 and pH 9. The meter's electrode was directly immersed in the wood apple juice and readings were recorded on the pH meter's screen (Jackson, 1973) [23].

2.5.1 Titratable acidity (%)

The total titratable acidity of wood apple juice was determined by visual titration method as explained by Cohen (1971) [7]. One ml of juice was taken in a pipette and added to volumetric flask then the volume was made up to 25 ml with distilled water and titrated against 0.1N NaOH solution using 1-2 drops of phenolphthalein indicator. Formation of pink colour was considered as the end point of titration. Then, the acidity was calculated as follows:

$$\text{Titratable acidity (\%)} = \frac{\text{Titre value} \times \text{N of alkali} \times \text{Vol. made up} \times \text{Eq. wt. of citric acid} \times 100}{\text{Vol. of sample taken for estimation} \times \text{Wt. or vol. of sample (ml)} \times 1000}$$

2.5.2 Ascorbic acid (mg 100 ml^{-1})

The ascorbic acid of wood apple juice was determined by titrimetric method explained in Association of official analytical chemists (AOAC, 2006) [3]. One ml of juice sample was mixed thoroughly with 4 per cent oxalic acid solution and volume was made up to 25 ml and form that 5 ml was taken and volume made up to 25 ml. Ascorbic acid content present in the solution was estimated by titrating a 25 ml quantity of extract against DCPIP. Ascorbic acid content was calculated as mg of ascorbic acid equivalent per 100 ml of juice using a standard curve of L-Ascorbic acid.

$$\text{Ascorbic acid (mg } 100\text{g}^{-1}) = \frac{\text{Dye factor} \times \text{Titre value} \times \text{Volume made up}}{\text{Aliquot} \times \text{Volume of sample}} \times 100$$

2.5.3 Total antioxidant activity (mg AEAC 100 ml^{-1})

The total antioxidant activity of wood apple juice was determined by the FRAP method explained by Benzie and Strain (1996) [5]. The 2.5 ml of juice sample was taken in a 25 ml volumetric flask and made up to the volume by using 80 per cent methanol. 0.2 ml of aliquot was taken in test tube and 1.8 ml of FRAP reagent was added. 30 minutes after incubation at room temperature absorbance was recorded at 593 nm. Results were expressed as ascorbic acid equivalent antioxidant capacity (AEAC).

$$\text{Total antioxidant activity (mg AAE/100 g)} = \frac{OD_{593} \text{ x volume made up x } 100}{\text{Aliquot taken} \times \text{Weight of sample} \times 1000}$$

2.5.4 Reducing sugars (%)

Reducing sugar present in wood apple juice was estimated by the following Lane and Eynon method (1923) [12]. Five ml of wood apple juice was added to 100 ml volumetric flask and add 10 ml of Fehling's solution [Fehling's A (5 ml) + Fehling's B (5 ml)] with 25 ml of distilled water was taken in a conical flask, heated to boil and titrated against the sample using methylene blue indicator. The end point of titration was brick red colour.

$$\text{Reducing sugars (\%)} = \frac{\text{Dye factor} \times \text{Volume made up}}{\text{Titre value} \times \text{Volume of sample}} \times 100$$

2.5.5 Total sugars (%)

Total sugar present in the wood apple juice was estimated by the following Lane and Eynon method (1923) [12]. The 25 ml of the filtrate (prepared for reducing sugars estimation) was hydrolysed with 10 ml of 1:1 HCl at room temperature for 24 hours. All the sugars present in the sample were now converted to reducing sugars. The hydrolysed sample was neutralized with 20 per cent NaOH and the volume was made up to 100 ml with distilled water. Add 10 ml of Fehling's solution [Fehling's A (5 ml) + Fehling's B (5 ml)] with 25 to 50 ml of distilled water was taken in a conical flask, heated to boil and titrated against the sample using methylene blue as an indicator. The end point of titration was brick red colour.

$$\text{Total sugars (\%)} = \frac{4 \text{ x Dye factor x Volume made up}}{\text{Titre value x Volume of sample}} \times 100$$

3. Results and Discussion

In this study processing was undertaken to obtain high juice recovery, which was done in different combination of enzyme concentration (0.25, 0.50 and 0.75%), incubation temperature (40, 50 and 60 °C) and incubation period (60, 90 and 120 min). The physicochemical properties of juice, such as juice recovery, TSS, pH, titratable acidity, ascorbic acid, reducing sugar, total sugar and total antioxidants activity of wood apple juice are presented below.

3.1 Juice recovery (%)

Maximum recovery per cent of juice was found to be 81.70 per cent at 0.75 per cent pectinase, which was incubated at 50 ℃ for 120 min is presented in Table 1. This was significantly on par with 81.60 per cent at 0.50 per cent pectinase, 50 °C temperature for 120 min. This could be due to the hydrolysis of the cell wall. The results are in agreement with the research work carried out by Imungi *et al*. (1983) [24] who stated about the physicochemical changes during extraction of clear guava juice.

Minimum juice recovery per cent of 73.75 percent was registered in the sample treated with 0.25 per cent pectinase and which was incubated at 60 °C temperature for 120 min. This could be due to enzyme denaturation that occurs beyond optimum temperature (30-50 ℃). This denaturation will tend to decrease the activity of enzyme there by affecting the juice recovery per cent. Similar results were obtained by Ramdan and Moersel (2007) [16] during enzymatic treatment of golden berry (*Physalis peruviana* L.), Singh *et al*. (2012) [19] during enzymatic treatment of bael fruit, Demir *et al*. (2001) [8] and Sandri *et al*. (2011) [17] also observed the same trend during clarification of fruit juices by fungal pectinase. The interaction effect between pectinase concentration, temperature and incubation period also showed a significant effect on juice recovery per cent.

Table 1: Effect of processing on juice recovery (%) of enzyme extracted wood apple juice

Sl. No.	Pectinase (%)	Incubation temperature (°C)								
		40			50			60		
		Incubation (min)			Incubation (min)			Incubation (min)		
		60	90	120	60	90	120	60	90	120
1	0.25	78.40	79.00	79.70	79.00	80.00	80.50	75.11	74.22	73.75
2	0.50	79.30	79.50	80.25	79.65	80.50	81.60	74.32	74.70	74.70
3	0.75	79.85	80.00	80.70	80.30	80.85	81.70	75.80	75.26	74.85

ANOVA			
Pectinase (%)	0.25	0.5	0.75
Mean	77.74	78.28	78.81
Temperature (°C)	40	50	60
Mean	79.63	80.45	74.74
Time (min)	60	90	120
Mean	77.97	78.22	78.63
	F- value	S.Em (±)	CD @ 5%
P	187.19	0.039	0.113
T	6246.72	0.039	0.113
I	74.24	0.039	0.113
P×T	4.90	0.068	0.196
P×I	8.20	0.068	0.196
T×I	76.47	0.068	0.196
P×T×I	5.93	0.117	0.117

NS - Non significant
P: Pectinase concentration
T: Incubation temperature
I: Incubation period

3.2 TSS (°B)

The TSS content of enzymatically extracted wood apple juice ranged from 4.60 to 6.11 per cent is presented in Table 2. The wood apple pulp treated with 0.75 per cent pectinase and incubated at 50 ℃ for 120 min yielded juice of higher TSS (6.11%) followed by a TSS of 6.04 per cent obtained when the wood apple pulp was treated with 0.75 per cent pectinase and incubated at 40 ℃ for 120 minutes. It might be due to increase in pectinase concentration and incubation period due to greater degree of tissue breakdown resulting in release of more components that contributes to accumulation of total soluble solids. This result was in agreement with the findings of Pilnik *et al*. (1975) [15] on enzymic liquification of fruits and vegetables, Yusof and Ibrahim (1994) [22] during soursop juice extraction with pectinase enzyme and Chang *et al*. (1994) [25] in enzymatic juice extraction from Plum. Whereas, the lower TSS (4.60%) was recorded when the wood apple pulp treated with 0.25 per cent pectinase and incubated at 60 ℃ for 120 min.

Increase in pectinase concentration up to 0.75 per cent increased the TSS content of the wood apple juice. Increase in the incubation temperature up to 50 ℃ increased the TSS

value followed by a significant drastic fall. Incubation period had a relatively positive effect on TSS content of juice. The findings show that the TSS content increased with the increase in incubation period.

3.3 pH

The pulp treated with 0.25 per cent pectinase and incubated at 40 ℃ for 60 min recorded a higher pH value of 3.81 whereas, lower (3.44) pH value was observed in the sample treated with 0.75 per cent pectinase and incubated at 40 ℃ for 120 min is presented in Table 3. The pH of the wood apple juice decreased with increase in the pectinase concentration and incubation period. It could be mainly due to the increase in acidity percentage of the wood apple juice. Similar decreasing trend was reported by Joshi *et al*. (2011) [26] in pectinase extraction from apple pomace, Yusof and Ibrahim (1994) [22] in soursop juice extraction by enzyme treatment.

The pH of enzyme extracted wood apple juice was significantly affected by two way interaction of pectinase concentration and incubation temperature and incubation period but, incubation temperature and incubation period showed non-significant effect on pH of the wood apple juice. Whereas, three way interaction between pectinase concentration, incubation period and incubation temperature was found to be non-significant effect on pH of the wood apple juice.

Table 2: Effect of processing on TSS (°B) of enzyme extracted wood apple juice

Sl. No.	Pectinase (%)	Incubation temperature (°C)								
		40			50			60		
		Incubation (min)			Incubation (min)			Incubation (min)		
		60	90	120	60	90	120	60	90	120
1	0.25	5.24	5.32	5.38	5.32	5.39	5.44	4.71	4.69	4.60
2	0.50	5.42	5.48	5.52	5.48	5.52	5.59	4.85	4.83	4.68
3	0.75	5.90	5.94	6.04	5.96	6.03	6.11	5.03	4.94	4.88
ANOVA										
Pectinase (%)		0.25			0.5			0.75		
Mean		5.12			5.26			5.65		
Temperature (°C)		40			50			60		
Mean		5.58			5.65			4.80		
Duration (min)		60			90			120		
Mean		5.32			5.35			5.36		
		F- value			S.Em (±)			CD @ 5%		
P		1120.63			0.008			0.024		
T		3367.25			0.008			0.024		
I		5.14			0.008			0.024		
P×T		67.18			0.014			0.041		
P×I		1.10			0.014			NS		
T×I		30.63			0.014			0.041		
P×T×I		0.42			0.024			NS		

NS-Non significant
P: Pectinase concentration
T: Incubation temperature
I: Incubation period

Table 3: Effect of processing on pH of enzyme extracted wood apple juice

Sl. No.	Pectinase (%)	Incubation temperature (°C)								
		40			50			60		
		Duration (min)			Duration (min)			Duration (min)		
		60	90	120	60	90	120	60	90	120
1	0.25	3.81	3.77	3.65	3.72	3.68	3.62	3.62	3.58	3.56
2	0.50	3.71	3.69	3.58	3.67	3.63	3.52	3.61	3.58	3.48
3	0.75	3.55	3.50	3.44	3.58	3.52	3.49	3.62	3.56	3.51
ANOVA										
Pectinase (%)		0.25			0.5			0.75		
Mean		3.67			3.61			3.53		
Temperature (°C)		40			50			60		
Mean		3.63			3.60			3.57		
Time (min)		60			90			120		
Mean		3.65			3.61			3.54		
		F- value			S.Em (±)			CD @ 5%		
P		337.01			0.004			0.011		
T		74.31			0.004			0.011		
I		241.98			0.004			0.011		
P×T		77.75			0.007			0.019		
P×I		4.74			0.007			0.019		
T×I		2.45			0.007			NS		
P×T×I		1.83			0.011			NS		

NS- Non significant
P: Pectinase concentration
T: Incubation temperature
I: Incubation perio

3.4 Titratable acidity (%)

The titratable acidity ranged from 2.42 to 2.77 per cent (Table 4). The pulp treated with 0.75 per cent pectinase and incubated at 50 ℃ for 120 min recorded higher acidity (2.77%) which was significantly on par with 2.76 per cent obtained by treating the pulp with 0.50 per cent pectinase and incubated at 50 ℃ for 120 min. Titratable acidity per cent of the wood apple juice found to be higher with higher pectinase concentration and incubation period due to pectinase enzyme which consists of pectin methyl esterase and polygalacturonase assisting in pectin hydrolysis there by leading to release of carboxylic acid, galacturonic acid and other organic acids which contributes to increase in titratable acidity. Similar results were obtained by Akesowan *et al*. (2013) [1] in guava juice production by enzyme treatment, Yusof and Ibrahim (1994) [22] in soursop juice extraction by enzyme treatment. However, lower acidity (2.42) per cent was recorded in pulp treated with 0.25 per cent pectinase incubated at 60 ℃ for 120 min.

The titratable acidity of wood apple juice increased with the increase in enzyme concentration and incubation period but decreased at higher incubation temperature *i.e.*, 60 ℃. The interaction effect between pectinase concentration, temperature and incubation period also showed a non-significant effect on acidity per cent.

3.5 Ascorbic acid content (mg 100 ml^{-1})

The ascorbic acid content of wood apple juice as influenced by enzyme concentration, incubation temperature and incubation period are represented in Table 5. The values indicated that, ascorbic acid varied from 3.13 mg 100 ml-1 to 5.47 mg 100 ml^{-1}. Maximum (5.47 mg 100 ml^{-1}) ascorbic acid content was recorded in the pulp treated with 0.75 per cent pectinase incubated at 50 ℃ for 120 min whereas, minimum ascorbic acid of 3.13 mg 100 ml^{-1} was recorded in pulp treated with 0.25 per cent pectinase incubated at 60 ℃ for 90 min. Ascorbic acid content increased with increase in pectinase concentration and incubation period but decreased with increase in temperature. It might be due to sensitivity of ascorbic acid to oxygen, temperature and light. Similar results were obtained by Nguyen and Nguyen (2018) [13] who studied the quality of mulberry juice as affected by enzyme treatment. This increase in ascorbic acid follows the trend of TSS. As TSS comprises of organic acids, total soluble sugars, and *etc.*

Table 4: Effect of processing on acidity (%) of enzyme extracted wood apple juice

Sl. No.	Pectinase (%)	Incubation temperature (°C)								
		40			50			60		
		Incubation (min)			Incubation (min)			Incubation (min)		
		60	90	120	60	90	120	60	90	120
1	0.25	2.50	2.55	2.57	2.59	2.62	2.65	2.47	2.44	2.42
2	0.50	2.58	2.62	2.61	2.63	2.66	2.76	2.52	2.47	2.44
3	0.75	2.63	2.69	2.72	2.71	2.71	2.77	2.56	2.51	2.46
ANOVA										
Pectinase (P)		0.25 (%)			0.5 (%)			0.75 (%)		
Mean		2.53			2.59			2.64		
Temperature (T)		40 (℃)			50 (℃)			60 (℃)		
Mean		2.61			2.68			2.48		
Duration (D)		60 (min)			90 (min)			120 (min)		
Mean		2.58			2.59			2.60		
		F- value			S.Em (±)			CD @ 5%		
P		145.56			0.004			0.013		
T		539.62			0.004			0.013		
I		6.20			0.004			NS		
P×T		5.87			0.008			0.022		
P×I		0.36			0.008			NS		
T×I		36.06			0.008			0.022		
P×T×I		2.68			0.013			NS		

NS- Non significant
P: Pectinase concentration
T: Incubation temperature
I: Incubation period

Table 5: Effect of processing on ascorbic acid (mg 100 ml^{-1}) content of enzyme extracted wood apple juice

Sl. No.	Pectinase (%)	Incubation temperature (°C)								
		40			50			60		
		Incubation (min)			Incubation (min)			Incubation (min)		
		60	90	120	60	90	120	60	90	120
1	0.25	4.23	4.34	4.57	4.54	4.58	4.81	3.23	3.14	3.13
2	0.50	4.27	4.40	4.66	4.70	4.84	5.23	3.29	3.21	3.19
3	0.75	4.32	4.54	4.72	4.89	5.03	5.47	3.36	3.31	3.30
ANOVA										
Pectinase (%)		0.25			0.5			0.75		
Mean		4.06			4.20			4.33		
Temperature (℃)		40			50			60		
Mean		4.45			4.90			3.24		
Duration (min)		60			90			120		

Mean	4.09	4.15	4.35
	F- value	S.Em (±)	CD @ 5%
P	245.24	0.008	0.024
T	10438.52	0.008	0.024
I	255.70	0.008	0.024
P×T	45.23	0.015	0.042
P×I	6.15	0.015	0.042
T×I	101.73	0.015	0.042
P×T×I	3.56	0.025	0.073

P: Pectinase concentration
T: Incubation temperature
I: Incubation period

An effective release of these molecules into solution will happen because of the disturbance of fruit tissue cell wall. Therefore, accordingly this increases the ascorbic acid content

3.6 Reducing sugar and total sugar (%)

The reducing sugar content in wood apple juice varied between 2.46 to 2.83 per cent. The highest reducing sugar content (2.83%) was obtained pulp treated with 0.75 per cent pectinase and incubating at 40 ℃ for 60 minutes, while the lowest (2.46%) was recorded with 0.25% pectinase at 60 °C for 120 minutes (Table 6). Increasing pectinase concentration boosted reducing sugar, but longer incubation times and higher temperatures decreased it. The interactions between pectinase variables did not significantly affect the reducing sugar content of enzymatically extracted wood apple juice.

Table 7 presents variations in total sugar percentage of wood apple juice due to different pectinase concentrations, temperatures, and incubation periods. The total sugar percentage ranged from 4.24% to 4.87%. The highest content (4.87%) was observed with 0.75% pectinase at 40 ℃ for 120 minutes, while the lowest (4.24%) resulted from 0.25% pectinase at 60 ℃ for 120 minutes. Increasing enzyme concentration raised total sugar, but higher temperatures and longer incubation times decreased it. Two-way and three-way interactions between pectinase variables had a non-significant impact on total sugar percentage in enzymatically extracted wood apple juice.

Total sugar and reducing sugar per cent in the wood apple juice was found to be higher with the higher pectinase concentration. The increase in the total and reducing sugar percentage might be due to degradation of membrane bound carbohydrate by enzymes resulting in the accumulation of sucrose. The results of present study are in agreement with the findings of Sherpa *et al*. (2014) [18] in case of enzymatic juice extraction of plum, Cheirsilp and Umsakul (2008) [6] in extraction of banana juice by enzyme treatment. Increasing in incubation temperature and incubation period showed a decrease in the total and reducing sugar per cent of the juice. The result was agreement with the study carried out by Handique *et al*. (2019) [10] in banana juice extraction using combination of enzymes.

Table 6: Effect of processing on reducing sugar (%) of enzyme extracted wood apple juice

Sl. No.	Pectinase (%)	Incubation temperature (°C)								
		40			50			60		
		Incubation (min)			Incubation (min)			Incubation (min)		
		60	90	120	60	90	120	60	90	120
1	0.25	2.73	2.71	2.66	2.68	2.66	2.62	2.60	2.57	2.46
2	0.50	2.78	2.72	2.69	2.73	2.70	2.66	2.68	2.61	2.58
3	0.75	2.83	2.81	2..78	2.79	2.74	2.68	2.70	2.67	2.6
ANOVA										
Pectinase (%)		0.25			0.5			0.75		
Mean		2.63			2.68			2.73		
Temperature (°C)		40			50			60		
Mean		2.74			2.69			2.60		
Duration (min)		60			90			120		
Mean		2.72			2.68			2.63		
		F- value			S.Em (±)			CD @ 5%		
P		146.79			0.004			0.012		
T		281.12			0.004			0.012		
I		107.38			0.004			0.012		
P×T		4.72			0.007			0.021		
P×I		1.4			0.007			NS		
T×I		3.27			0.007			0.021		
P×T×I		1.59			0.013			NS		

NS- Non significant
P: Pectinase concentration
T: Incubation temperature
I: Incubation period

Table 7: Effect of processing on total sugar (%) of enzyme extracted wood apple juice

Sl. No.	Pectinase (%)	Incubation temperature (°C)								
		40			50			60		
		Incubation (min)			Incubation (min)			Incubation (min)		
		60	90	120	60	90	120	60	90	120
1	0.25	4.80	4.76	4.73	4.76	4.73	4.71	4.46	4.42	4.24
2	0.50	4.82	4.77	4.74	4.77	4.75	4.72	4.52	4.49	4.36
3	0.75	4.87	4.81	4.79	4.82	4.77	4.76	4.58	4.52	4.41
ANOVA										
Pectinase (%)		0.25			0.5			0.75		
Mean		4.62			4.66			4.70		
Temperature (°C)		40			50			60		
Mean		4.78			4.75			4.44		
Duration (min)		60			90			120		
Mean		4.71			4.67			4.60		
		F- value			S.Em (±)			CD @ 5%		
P		80.05			0.004			0.013		
T		1859.63			0.004			0.013		
I		141.39			0.004			0.013		
P×T		10.34			0.008			0.022		
P×I		1.17			0.008			NS		
T×I		26.79			0.008			0.022		
P×T×I		0.91			0.013			NS		

NS-Non significant
P: Pectinase concentration
T: Incubation temperature
I: Incubation period

3.7 Total antioxidant activity (mg AAE/100 g)

Table 8 displays the impact of various combinations of pectinase, incubation temperature, and period on the total antioxidant activity of enzymatically extracted juice, ranging from 55.63 to 59.10 mg AEAC per 100 ml. The highest activity (59.10 mg AEAC per 100 ml) was observed with 0.75% pectinase at 50 ℃ for 120 minutes, followed by 0.50 per cent pectinase at the same conditions (58.85 mg AEAC per 100 ml). This could be due to accumulation of molecules in the juice which may contribute to the increase in antioxidants those are ascorbic acid, sugar and carotenoids. Also, pectinase contributes to the enhanced extraction of the antioxidants from the cellular cytoplasm and release of phenolic compounds during enzymatic extraction can enhance the antioxidant activity of juice. This result is in agreement with Bashir *et al*. (2021) [4] who studied on pectinase enzyme-assisted extraction of apricot juice. Gani *et al*. (2021) [9] worked on antioxidant properties of pear juice prepared through pectinase enzyme-assisted extraction. Kumar and Singh (2019) [11] examined carification of guava fruit juice using multiple enzymes. Oszmia *et al*. (2011) [14] investigated the effect of pectinase treatment on extraction of phenol from pomace, Nguyen and Nguyen (2018) [13] on the quality of mulberry juice as affected by enzyme treatment.

Lower antioxidant activity (55.63 mg AEAC per 100 ml) resulted from 0.25% pectinase at 60 ℃ for 120 minutes. Total antioxidant activity increased with higher pectinase concentration and incubation period but decreased at 60 ℃. Pectinase concentration, temperature, and incubation period significantly influenced total antioxidant activity ($p<0.05$), while their interactions had a non-significant effect.

Table 8: Effect of processing on total antioxidant (mg AAE/100 g) of enzyme extracted wood apple juice

Sl. No.	Pectinase (%)	Incubation temperature (°C)								
		40			50			60		
		Incubation (min)			Incubation (min)			Incubation (min)		
		60	90	120	60	90	120	60	90	120
1	0.25	57.20	57.45	57.77	57.45	57.79	58.30	56.13	55.86	55.63
2	0.50	57.80	57.9	58.10	58.09	58.37	58.85	56.88	56.53	55.95
3	0.75	58.13	58.20	58.62	58.57	58.77	59.10	57.35	57.00	56.72
ANOVA										
Pectinase (%)		0.25			0.5			0.75		
Mean		57.08			57.46			57.81		
Temperature (°C)		40			50			60		
Mean		57.90			58.36			56.09		
Duration (min)		60			90			120		
Mean		57.34			57.40			57.61		
		F- value			S.Em (±)			CD @ 5%		
P		444.97			0.017			0.050		
T		4878.87			0.017			0.050		
I		67.77			0.017			0.050		
P×T		32.40			0.030			0.087		

P×I	2.28	0.030	0.087
T×I	87.15	0.030	0.087
P×T×I	2.40	0.052	NS

NS- Non significant
P: Pectinase concentration
T: Incubation temperature
I: Incubation period

4. Conclusion

Optimal wood apple juice extraction was achieved by treating the pulp with 0.75% pectinase and incubating it at 50 °C for 120 minutes. This resulted in superior juice characteristics, including high juice recovery (81.70%), high TSS (6.11 °B), a pH of 3.49, acidity at 2.77 per cent, ascorbic acid content (5.47 mg/100 ml), 2.68 Per cent reducing sugar, 4.76 per cent total sugar and significant total antioxidant activity (59.10 mg AEAC/100 ml).

5. References

1. Akesowan A, Choonhahirun A. Effect of enzyme treatment on guava juice production using response surface methodology. J Anim. Plant Sci. 2013;23(1):114-120.
2. Anonymous. Official method of Analysis, Ed. Sioney Williams, Association Official Analytic Inc. Virginia 14th Ed; c1984. p. 423-462.
3. AOAC. Association of official analytical chemist, In: Official methods of analysis ascorbic acid; c2006. 967.21, 45.1.14. AOAC International, Gaithersburg.
4. Bashir O, Hussain SZ, Gani G, Jan N, Rather AH, Reshi M, Amin T. Evaluating the physicochemical and antioxidant characteristics of apricot juice prepared through pectinase enzyme-assisted extraction from Halman variety. J Food Meas. Charact. 2021;15:2645-2658.
5. Benzie IFF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: The FRAP assay. Anal. Chem. 1996:239:70-76
6. Cheirsilp B, Umsakul K. Processing of banana-based wine product using Pectinase and a-amylase. J Food Process Eng. 2008;31(1):78-90.
7. Cohen EH. Rapid technique for solvent removal in the determination of fat. J Ass. Offic. Anal. Chem. 1971;54(1):212-214.
8. Demir N, Acar J, Sarıoglu K, Mutlu M. The use of commercial pectinase in fruit juice industry. Part 3: immobilized pectinase for mash treatment. J Food Eng. 2001;47(4):275-280.
9. Gani G, Naik HR, Jan N, Bashir O, Hussain SZ, Rather AH, *et al*. Physicochemical and antioxidant properties of pear juice prepared through pectinase enzyme-assisted extraction from William Bartlett variety. J Food Meas. Charact. 2021;15:743-757.
10. Handique J, Bora SJ, Sit N. Optimization of banana juice extraction using combination of enzymes. J Food Sci. Technol; c2019. p. 1-12.
11. Kumar R, Singh AK. Clarification of guava (*Psidium guajava*) fruit juice using multi-enzyme (pectinase, cellulase and hemicellulase) treatment: Optimization of processing parameters. Int. J Inno. Technol. Exploring Eng. 2019;8(12):5266-5279.
12. Lane JH, Eynon L. Determination of reducing sugar by means of Fehling's solution with methylene blue as internal indicator. J Soc. Chem. Ind. 1923;17:32-37.
13. Nguyen C, Nguyen H. The quality of mulberry juice as affected by enzyme treatments. Beverages. 2018;4(2):41-42.
14. Oszmia J, Wojdyło A, Kolniak J. Effect of pectinase treatment on extraction of antioxidant phenols from pomace, for the production of puree-enriched cloudy apple juices. Food Chem. 201;127(2):623-631.
15. Pilnik W, Voragen AGJ, Devas L. Enzymic liquefaction of fruits and vegetables. Flussigesn Obst. 1975;42(11):448-451.
16. Ramadan MF, Moersel JT. Impact of enzymatic treatment on chemical composition, physicochemical properties and radical scavenging activity of golden berry (*Physalis peruviana* L.) juice. J Sci. Food Agric. 2007;87(3):452-460.
17. Sandri IG, Fontana RC, Barfknecht DM, Silveira MM. Clarification of fruit juices by fungal pectinases. Food Sci. Technol. 2011;44(10):2217-2222.
18. Sherpa K, Mahato SK, Kumar KR, Chhetri B, Subba P, Paul PK. Assessment of quality characteristics upon enzymes assisted juice extraction from plum. Int. Quarterly J Life Sci. 2014;9(3):1081-1086.
19. Singh A, Kumar S, Sharma HK. Effect of enzymatic hydrolysis on the juice yield from bael fruit (*Aegle marmelos* Correa) pulp. American J Food Technol. 2012;7(2):62-72.
20. Singhania N, Kajla P, Bishnoi S, Barmanray A, Ronak R. Development and storage studies of wood apple (Limonia acidissima) chutney. Int J Chem Stud. 2020;8(1):2473-2476.
21. Vidhya R, Narain A. Formulation and evaluation of preserved products utilizing under exploited fruit, wood apple (*Limonia acidissima*). Am. Eurasian J Agric. Environ. Sci. 2011;10(1):112-118.
22. Yusof S, Ibrahim N. Quality of Soursop juice after pectinase enzyme treatment. Food Chem. 1994;51:83-88.
23. Jackson ML. Soil chemical analysis, pentice hall of India Pvt. Ltd., New Delhi, India. 1973;498:151-154.
24. Imungi JK, Potter NN. Nutrient contents of raw and cooked cowpea leaves. Journal of food science. 1983 Jul;48(4):1252-1254.
25. Chang L. A psychometric evaluation of 4-point and 6-point Likert-type scales in relation to reliability and validity. Applied psychological measurement. 1994 Sep;18(3):205-15.
26. Ethirajan M, Chen Y, Joshi P, Pandey RK. The role of porphyrin chemistry in tumor imaging and photodynamic therapy. Chemical Society Reviews. 2011;40(1):340-362.