

*Review Article*

## A Review on the Optimisation of Enzymatic Treatment in Tropical Fruit Juice: Impacts on Physicochemical and Functional Properties

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### ABSTRACT

The production of fruit juice often faces challenges in achieving high juice quality due to its high viscosity, pectin, and polysaccharide content. Enzymatic treatment has proved to be one of the most effective approaches for improving the juice yield, quality and nutritional properties. Enzymes such as pectinase, cellulase, and hemicellulase are commonly used in fruit juice production to degrade cell wall components, thereby enhancing the stability and quality of fruit juice. Optimisation of enzymatic treatment conditions, such as enzyme concentration, incubation time, and temperature, using a second-order central composite design (RSM), was crucial in achieving the desired quality outcome. From an extensive collection of literature and research, this comprehensive review explores the impact of optimised enzymatic treatment conditions on the physicochemical properties, including juice yield, viscosity, clarity, turbidity, total soluble solids (TSS), and the bioactive compounds of antioxidant activity and total phenolic content (TPC) of tropical fruit juices. Key findings suggest that different fruits all have different enzymatic treatment conditions due to the dependency of

this treatment towards the pectin, cellulose, hemicellulose and other polysaccharide contents in the fruit. This selected treatment shows effectiveness in tackling the stated issues.

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### INTRODUCTION

Tropical fruits are excellent and nutritious natural resources that grow in warm and

humid tropical climates, such as in Central America, South America, Southeast Asia and Middle Africa (López-Martínez et al., 2023; Sarkar et al., 2023; Sayago-Ayerdi et al., 2021). Tropical fruits come in a wide variety, ranging from the succulent sweetness of mangoes to the creamy richness of avocados with vibrant flavours, rich colours, and diverse textures (Paull & Duarte, 2025). Tropical fruit is an important key segment of global agriculture and trade, provided that it is widely consumed, supporting both local and international market demand (FAO, 2024; Mohamed et al., 2011).

There was a significant global increase in the production and consumption of tropical fruits nationwide. The surge is primarily attributed to the recognition of their nutritional and health benefits, growing demand for exotic flavours, and increased awareness of their health-promoting properties (Sayago-Ayerdi et al., 2021). The fact that tropical fruits are an abundant source of essential nutrients, offering numerous health benefits to the human body, also fuels the growing interest in these fruits among consumers. Overall, tropical fruits provide essential vitamins, including A, B, C, and E (Paull & Duarte, 2025), as well as minerals such as potassium, magnesium, and manganese (Paull & Duarte, 2025). It is also a good source of dietary fibre, which supports digestive health, lowers cholesterol levels, and promotes a healthy gut (Chuacharoen et al., 2021; Ioniță-Mîndrican et al., 2022). In addition, tropical fruits contain antioxidants, such as flavonoids and carotenoids, that help protect cells from damage (Sayago-Ayerdi et al., 2021), as well as phytonutrients with anti-inflammatory and immune-boosting properties (Sayago-Ayerdi et al., 2021).

According to studies conducted by Sayago-Ayerdi et al. (2021) and Maia et al. (2019), roughly 50% of tropical and subtropical fruit production is designated for fresh fruit consumption, while the other half is utilised as processed products such as wines, dried fruit snacks, syrups, marmalades, flours, and tropical fruit beverages, which are among the most popular offerings. Due to their high-water content and delicate nature, many tropical fruits are highly perishable and are thus often transformed into beverages for preservation. This fragility is influenced by various factors, including high respiration rates, as tropical fruits tend to consume oxygen and emit carbon dioxide at a faster rate than other fruits. They are also susceptible to physical damage during harvesting, transportation, and storage (Bensi et al., 2020). These factors hasten their decay and vulnerability to microbial spoilage, as poor storing and handling of fruits make them easily contaminated with bacteria, fungi, and other microorganisms (Kusumaningrum et al., 2015; Zainalabidin et al., 2019).

## **ENZYMATIC TREATMENT OF FRUIT JUICE**

Fruits contain complex polysaccharides such as pectin, starch and hydrophilic hydroxyl groups (-OH), which contribute to viscous juice, low juice yield, and inconsistent appearance when extracted using conventional methods (Toy et al., 2022). Although this method is widely used, it is often costly to maintain, energy-intensive, and prone to

cross-contamination (Vivek et al., 2019). Additionally, this method may produce turbid juice with suspended particles, which consumers consider low-quality juice. Toy et al. (2022) reported that a more clarified juice with a bright and transparent appearance is preferred as it is often associated with higher quality. To address these challenges, alternative methods such as cold pressing in pineapple and dragon fruit, hot extraction in banana, and enzymatic extraction in dragon fruit have been explored by several researchers (Dowerah et al., 2023; Khaksar et al., 2019; Ghorband & Joshi, 2023). In the literature, it was shown that enzymatic treatment using pectinases, cellulases, hemicellulases, amylases, and proteases (Michele, 2020) as tabulated in Table 1, is used to improve juice recovery and clarity at various stages of juice processing, including maceration, pressing, and clarification (García, 2018) as compared to cold and hot extraction (Joshi et al., 1991).

Table 1

*Different types of enzymes and their function during enzymatic treatment*

Enzyme	Subcategories	Functions	Ref
Pectinase	Pectin methylesterase (PME)	Methoxyl group (pectin) de-esterification	Garg et al. (2016)
	Protopectinase	Degradation of insoluble protopectin into soluble pectin	
	Polygalacturonase (PG)	Hydrolyses $\alpha$ -1,4-glycosidic linkages in polygalacturonic acid	
	Polymethylgalacturonase (PMG)	Hydrolyses $\alpha$ -1,4-glycosidic bonds	
Cellulase	$\beta$ -glucosidase (BG)	Hydrolyses disaccharides and tetrasaccharides into glucose	Singh et al. (2016)
	Exo- $\beta$ 1,4-cellobiohydrolases (CBH)	Hydrolyses polymers into simple sugars	
	Endo- $\beta$ 1,4-glucanases (EG)	Breaking down non-covalent interactions (endocellulose)	
Hemicellulase	$\beta$ -mannanase	Hydrolyses mannan-based hemicellulose	Brigham et al. (2018)
	$\beta$ -mannosidase	Monno-oligomer hydrolyses into mannose	
Protease	Xylanase	Hydrolyse the $\beta$ -1,4 bond in xylan xylo-oligomer	Raveendran et al. (2018)
		Hydrolysing peptide bonds into amino acids and polypeptide chains	
Tannase		Cleaving ester bonds in complex tannins to gallic acid	Méndez-Carmona et al. (2022)
Amylase	$\alpha$ -amylase	Breakdown of $\alpha$ -1,4-glycosidic bond (start) into sugars	Mojsov (2016)
	$\beta$ -amylase	Catalysing the hydrolysis of the $\alpha$ -1, 4-glycosidic bond	

Enzymatic treatment in fruit juice processing is employed to improve juice yield, quality, and stability by degrading cell walls, pectin, starch, and suspended solids (Singh et al., 2019). This process enhanced total soluble solids (TSS), improved liquefaction, decreased viscosity and turbidity, and allowed better retention of nutritional value and colour (Danalache et al., 2018; Phung et al., 2019; Ramadan, 2019; Singh et al., 2019). Commercial pectic enzymes, such as pectinase, mainly target pectin, a complex polysaccharide containing chains of galacturonic acid units, rhamnose, galactose, and arabinose (Lara-Espinoza et al., 2018; Toy et al., 2022). As reported by Toy et al. (2022), pectinase is classified into three groups: pectin methylesterase (PME), protopectinase, and depolymerising enzymes. PME initiates pectin breakdown by converting it into pectic acid through de-esterification, while protopectinase breaks down protopectin into highly polymerised pectin. Depolymerising enzymes, in contrast, hydrolyse glycosidic linkages in pectin molecules, breaking them into simpler components (Toy et al., 2022). The enzymatic breakdown of pectin reduces its water-binding capacity, thereby releasing free water in the system and increasing juice yield (Sharma et al., 2017).

Cellulase enzymes break down cellulose, a structural carbohydrate that is mainly composed of complex sugars that protect and provide structure to the plant, into glucose. The cellulose fibres are surrounded by other structural biopolymers, primarily hemicellulose and lignin, forming a matrix (Danalache et al., 2018; Sharma et al., 2014).

Cellulase, a complex enzyme system, is composed of three enzymes: endo- $\beta$ -1,4-glucanases (EG), exo- $\beta$ -1,4-cellobiohydrolases (CBH), and  $\beta$ -glucosidases (BG) (Singh et al., 2019). The process involves the intricate hydrolysis of  $\beta$ -1,4-D-glycosidic bonds in cellulose and  $\beta$ -D-glucans by EG, followed by the precise hydrolysis of  $\beta$ -1,4-D-glycosidic bonds from the non-reducing ends by CBH, releasing cellobiose units (Toy et al., 2022). BG further hydrolyses the cellobiose into glucose monomers, facilitating the breakdown of cellulose into simple sugars (glucose) (Singh et al., 2019).

Hemicellulose is categorised based on sugar residues, including D-galactan, D-mannan, and D-xylan (Toy et al., 2022). This plant material is firmly attached to cellulose through hydrogen bonds and covalently linked to lignin (García, 2018). Toy et al. (2022) reported that the incorporation of hemicellulase is essential for the complete degradation of cell wall polysaccharides, as it facilitates the breakdown of tightly connected cellulose. This enzymatic process involves breaking hemicellulose into smaller oligosaccharides and monosaccharides.

Often, prioritising common enzymes such as pectinase, hemicellulose, and cellulase over complex, specific enzymes was due to several reasons. Firstly, direct targeting of enzymes is prioritised. For example, pectinase, encompassing polygalacturonase, pectin lyase and pectin esterase activities, is usually used, they have a direct effect on pectin, whereas pectin lyase acts only on highly esterified pectin with specific high methoxyl

matrices such as apples (Hossain & Ahmed, 2023). The cost of formulation was also taken into consideration, as common enzymes are much more cost-effective and widely available than specific enzymes, which may later increase the cost of production for the same amount of outcome.

## **OPTIMISATION METHOD USED FOR ENZYMATIC TREATMENT**

The high cost of enzyme-based juice extraction, primarily due to the expensive nature of enzymes, highlights the need for process optimisation based on juice quality parameters (Kunkulol et al., 2018a). This requires careful planning and consideration of cost, operational efficiency, consumer preferences, and regulatory compliance (Kunkulol et al., 2018a). By optimising process variables, it is possible to reduce extraction time and cost while preserving its nutritional value, making it more viable for the food industry (Sonawane et al., 2020). Generally, the optimisation of food processing using the One-Factor-At-a-Time (OFAT) method, where only one variable was adjusted at a time while keeping other variables constant (Bensi et al., 2020). However, this approach is limited, as it does not consider the interaction among variables and does not provide a comprehensive understanding of their combined effects (Bensi et al., 2020).

Enzymatic treatment of fruit juice often applies the response surface methodology (RSM) method. RSM is a collection of statistical and mathematical techniques designed to develop a polynomial model to predict and enhance the behaviour of a dataset, particularly in scenarios where multiple variables influence the outcomes (Bensi et al., 2020; Kunkulol et al., 2018b). This approach is particularly effective in optimising, designing, developing, and enhancing processes affected by various factors, making it a powerful tool in the food industry (Kunkulol et al., 2018a; Montgomery, 2017). In fruit juice production, RSM demonstrates its precision by optimising parameters such as enzyme concentration, incubation time, and temperature (Pradhan et al., 2020).

The enzymatic treatment process mainly affects functional properties, such as antioxidant activity, total phenolic content, and colour (Kovács et al., 2024). This treatment can improve the functional properties by increasing the release of bioactive compounds from the fruit matrix. However, navigating this complexity is challenging, as several factors can influence enzyme activity during the enzymatic treatment of fruit juices, including enzyme concentration, incubation temperature and time, pH, and the type of enzyme used (Phung et al., 2019; Verma et al., 2018). By systematically varying these factors within a defined range and analysing the responses, RSM reliably determines the optimal conditions for enzymatic treatment to achieve the desired outcomes (Montgomery, 2017). This systematic approach allows researchers to identify the optimal combination of parameters that result in the highest juice yield, clarity, and final product quality (Kumar & Singh, 2019).

## **EFFECT OF OPTIMISED ENZYMATIC TREATMENT ON PHYSICOCHEMICAL PROPERTIES OF FRUIT JUICE**

The use of cell wall-degrading enzymes such as pectinase, cellulase and hemicellulase has been studied to improve the extraction of fruit juice. Enzymatic treatment is an appropriate tool in addressing issues relating to pectin in fruit juice, especially as pectin is the major contributor to lower yield, high viscosity, cloudy juice and also the difficulties in the filtration process. As optimisation comes in handy, both research and industry can benefit from this process, including economic advantages and lower production costs and labour, all while also maintaining and improving the quality of fruit juice (Vivek et al., 2019). The research optimises various process variables used to attain the highest possible yield and quality of extracted juice, which includes enzyme concentrations, incubation time and temperature. A summary of optimised conditions to maximise juice yield of different tropical fruits is summarised in Table 2.

### **Juice Yield**

A study by Singh et al. (2022) on the production of sapodilla juice from enzymatic treatment proved that pectinase has successfully enhanced the juice yield to 62.08%, increasing by 27.08% from untreated sapodilla juice (35%). Optimised enzymatic conditions were achieved at 0.12% enzyme concentration, 42.02 °C incubation time for 167.82 minutes. While enzyme concentration might have a significant role in maximising juice production in sapodilla fruit (Singh et al., 2022), optimising all parameters, including the incubation time and temperature, is equally important for efficient enzymatic activity throughout the process. Sharma et al. (2017) highlighted that juice yield is influenced by different types of fruits, cell walls and other fruit components, as well as enzymatic activity, which affect the facilitation of juice release. For instance, in the enzymatic treatment of guava juice, Kumar & Singh (2019) reported a 17.3% increase in juice yield from an optimised multi-enzyme condition using 0.96% pectinase, 0.57% cellulase, and 0.77% hemicellulase for 99 minutes of incubation time at 55 °C. This finding proved that it is important to determine the optimal enzymatic treatment conditions for fruit juice production to achieve high processing efficiency and quality (Sharma et al., 2017).

### **Viscosity**

Viscosity is an important response variable in multiple enzymes treated in fruit juice. High viscosity in fruit juice poses a filtration problem, mainly attributed to pectin, cellulose, and hemicellulose in the fruit. Consequently, there is a consumer demand for clarified juice with lower viscosity to mitigate these issues (Vani et al., 2020). At an optimal enzyme concentration, the enzyme can effectively break down pectin, increasing juice yield and reducing the viscosity of the fruit juice, making it easier to filter. A summary of the enzymatic treatment in the viscosity reduction of fruit juice is provided in Table 3.

Table 2.  
Effect of optimised enzymatic conditions on juice yield of different tropical fruits

Fruit	Enzyme	Conditions			Ref
		Enzyme Concentration	Incubation Time	Temperature (°C)	
Guava	Pectinase	0.96%	99 min	55	Kumar & Singh (2019)
	Cellulase	0.57%			
	Hemicellulase	0.77%			
Sapodilla	Pectinase	0.12%	167.83 min	42.02	Singh et al. (2022)
Bael Fruit	Pectinase	0.22%	6.35 hours	46.20	Sonawane et al. (2020)
Watermelon	Pectinase	0.09% w/w	117.45 min	46.90	Saxena et al. (2014)
Passion Fruit	Pectinase	249.58 ppm	60 min	49.73	Phung et al. (2019)
Chironji ( <i>Buchanania Lanzas</i> ) Fruit	Pectinase	0.08% w/w	99.5 min	45.8	Pradhan et al. (2020)
Jackfruit ( <i>Koozha</i> )	Pectinase	0.5% v/v	2.75 hour	42.3	Bensi et al. (2020)
Soursop ( <i>Annona muricata</i> L.)	Pectinase	0.04% w/w	172 min	42.9	Makebe et al. (2020)

Table 3  
Effect of optimised enzymatic conditions on the viscosity of different tropical fruits

Sample	Enzyme	Conditions			Viscosity	Ref
		Enzyme Concentration	Incubation Time	Temperature (°C)		
Cashew Apple	Cellulase	0.091%	27.30 min	34.29	1.53 cP	Abdullah et al. (2021)
	Tannase	0.08%	20 min	30	1.47 cP	
Sapodilla	Pectinase	0.12%	167.83 min	42.02	9.1 cP	Singh et al. (2022)
Wild Watermelon ( <i>Citrullus lanatus</i> )	Pectinase	0.15% w/w	60 min	60	1.96 cP	Mamabolo & Tabit (2023)
Guava	Pectinase	0.121% v/v	95 min	32.7	486.66 cP	Vani et al. (2020)
Bael Fruit	Pectinase	0.22%	6.35 hours	46.20	2.02 cP	Sonawane et al. (2020)
Passion Fruit	Pectinase	249.58 ppm	60 min	49.73	5 cP	Phung et al. (2019)
Banana	Cellulase	0.34%	29.33 min	36.5	101.14 mPa.s	Handique et al. (2019)
	Pectinase	0.35%				
Casimiroa ( <i>Casimiroa Edulis</i> )	$\alpha$ -amylase ( <i>Aspergillus Oryzae</i> )	0.2%	62.74 min	44.01	203.76 mPa.s	Genanew et al. (2022)

Abdullah et al. (2021) reported that enzymatic treatment using cellulase and tannase resulted in a low viscosity of cashew apple juice. In cellulase-treated juice, the enzyme concentration, incubation time, and temperature negatively affected the viscosity of the juice, indicating a consistent inverse relationship between all three parameters and viscosity. Although there was no significant effect between parameters interacting with the viscosity, this response's reduction was mainly attributed to the increase in enzyme concentration. This relationship contrasts with the positive effect of enzyme concentration on juice yield. Regardless of this trend, enzyme concentration, incubation time, and temperature still have a positive parabolic effect on viscosity. Tannase-treated cashew apple juice, on the other hand, shows a significantly negative effect (linear) of incubation time and enzyme concentration, with a non-significant ( $p > 0.05$ ) effect of temperature towards the viscosity of cashew apple juice. The viscosity reduction in fruit juice production was due to the cell wall-degrading enzyme and the low water-holding capacity of the soluble polysaccharide in the cell wall, which released more water into the system (Abdullah et al., 2021). A similar finding was reported in pectinase-treated sapodilla juice, which reduced the viscosity from 9.1cP of an untreated control sample to 4.81cP of an optimised sample (Singh et al., 2022).

### Clarity and Turbidity

Enzyme treatment is an important process in fruit juice production as it aims to break down pectin and other complex carbohydrates present in fruits, particularly before juicing. This process is critical as it enhances the clarity and visual appeal of the juice for consumers. For instance, Kunkulol et al. (2018b) demonstrated that enzymatic treatment of mango juice with 0.15% amylase and 0.40% pectinase at 150 min incubation time and 55 °C resulted in the highest level of clarification with 93.54%.

Meanwhile, the lowest clarity of mango juice (84.75%) was observed at 0.15% amylase and 0.20% pectinase for 90 min of incubation at 45 °C. Therefore, optimising all parameters ensures that the clarification process of fruit juice is efficient, cost-effective, and high in quality. This result is also consistent with Singh et al. (2022), who reported that enzymatic treatment affects the transmittance percentage of the sapodilla juice due to pectin degradation by pectinase.

Turbidity and clarity are often inversely related, indicating that a high turbidity value of fruit juice signifies that the juice is low in clarity. Hence, a more clarified yet less turbid fruit juice is preferable in juice production compared to a less clarified and highly turbid juice. For example, in wild watermelon juice production (Mamabolo & Tabit, 2023), enzymatic treatment has successfully reduced the turbidity of wild watermelon juice from 3208.5 NTU to 1800 NTU under optimised conditions of 0.15% w/w of pectinase and incubation for 60 min at 60 °C. The optimisation process shows a positive linear relationship with incubation time and a negative linear relationship with temperature

towards turbidity, with a non-significant effect from enzyme concentrations. These findings reflect the significant quadratic effect of both incubation time and temperature. Although enzyme concentration was expected to significantly impact the pectin hydrolysis, possible flocculation and sedimentation of pectin units may occur (Saxena et al., 2014). This trend might differ in other fruit juice production, as reported in banana, cashew apple, and guava juice, as simplified in Table 4.

### **Total Soluble Solid (TSS)**

The increase of total soluble solids (TSS) in fruit juice has been influenced by enzymatic treatment due to the breakdown of pectin, cellulose, and hemicellulose, releasing more soluble sugars into the juice. A summary of the effect of optimised enzymatic conditions on total soluble solids of different tropical fruits is provided in Table 5. In the literature, it was shown that there was a significant difference between untreated (4.27 °Brix) and optimised enzyme-treated sugar palm juice (4.93 °Brix) (Arsad et al., 2015). This finding was similar to Handique et al. (2019), where the reduced TSS value of banana juice was found to be due to increased incubation time, cellulase, pectinase concentration and temperature. The combination of both optimised cellulase (0.34%) and pectinase (0.35%) concentration, with 29.33 minutes of incubation and a temperature of 36.5 °C in banana juice, increased the TSS value to 12.10 °Brix. Conversion of pectin by pectinolytic enzymes and the action of cellulase on cellulose caused a higher tissue degradation that may lead to the production of more soluble sugars (Arsad et al., 2015).

## **EFFECT OF OPTIMISED ENZYMATIC TREATMENT ON FUNCTIONAL PROPERTIES OF FRUIT JUICE**

### **Antioxidant Activity**

In recent years, there has been an increasing interest in antioxidant properties in fruit juice, attributed to its potential health benefits, tackling oxidative stress and lowering the risk of chronic disease. Improving and maintaining the antioxidant activity of fruit juice is crucial, as it is affected by temperature, processing conditions, and chemical composition (Grobelna et al., 2019). In noni juice production, the enzymatic treatment, assisted with an ultrasonic technique, increased antioxidant activity compared to the control due to cell wall degradation (Wang et al., 2023).

Similarly, Vivek et al., (2019) reported that sohiong fruit, a temperate fruit, also increased antioxidant activity in enzyme-ultrasonic assisted treatment (86.33%) from 82.67% of the control sample at the optimised conditions of pectinase concentration (0.05%), incubation time (57.44 minutes), temperature (45 °C), and ultrasound amplitude (90%) with ultrasound treatment time (9.95 minutes).

Table 4  
Effect of optimised enzymatic conditions on clarity and turbidity of different tropical fruits

Sample	Enzyme	Conditions			Clarity	Turbidity	Ref
		Concentration	Time	Temperature (°C)			
Star Fruit (B10)	Pectinex Ultra SP-L ( <i>Aspergillus niger</i> )	0.10%	20 min	30	0.864 Abs	0.88 NTU	Mazlina et al. (2008)
Star Fruit (B11)		0.1%	30 min	30	0.964 Abs	0.996 NTU	
Jamun Fruit	Pectinase ( <i>Aspergillus aculeatus</i> )	0.05%	80 min	44	74.39 %T	32.21 NTU	Ghosh et al. (2016)
Sapodilla	Pectinase	0.12%	167.83 min	42.02	0.72 %T	13.73 NTU	Singh et al. (2022)
Guava	Pectinase	0.96%					
	Cellulase	0.57%	99 min	55	-	17 NTU	Kumar et al. (2019)
	Hemicellulase	0.77%					
Sugar Palm	Pectinex Ultra SP-L	0.05%			0.48 OD	-	
	Cellulase ( <i>Aspergillus niger</i> )	0.05%	60 min	45	0.49 OD	-	Arsad et al. (2015)
	Pectinase + Cellulase	0.05% + 0.05%			0.39 OD	-	

Table 5  
Effect of optimised enzymatic conditions on total soluble solids of different tropical fruits

Sample	Enzyme	Conditions			TSS (°Brix)	Ref
		Concentration	Time	Temperature (°C)		
Casimiroa ( <i>Casimiroa Edulis</i> )	$\alpha$ -amylase ( <i>Aspergillus Oryzae</i> )	0.2%	62.74 min	44.01	16.93	Genanew et al. (2022)
Soursop ( <i>Annona muricata</i> L.)	Pectinase	0.04% w/w	172 min	42.9	12.22	Makebe et al. (2020)
Passion Fruit	Pectinase	249.58 ppm	60 min	49.73	12.3	Phung et al. (2019)
Palm ( <i>Borassus flabellifer</i> )	Pectinase ( <i>Aspergillus niger</i> )	0.33% w/w	171.32 min	33.75	10.2	Mohanty et al. (2018)
	Cellulase ( <i>Trichoderma viride</i> )	0.39% w/w	108.5 min	60	10.3	
Watermelon	Pectinase + Cellulase	1:0.75 w/w	120 min	55	12.8	Saxena et al. (2014)
	Pectinase	0.09% w/w	117.45 min	46.90	8.7	

The assisted ultrasound treatment mentioned is to show the different relevant approaches made in enzymatic treatment, often to assist in promoting more enzyme accessibility to the polysaccharide cell wall, as assisted through the influence of structural disruption by the ultrasound. The addition of assisted treatment was also sometimes chosen to enable shorter processing times and lower dosage of enzymes used while maintaining the same achievement of enzymatic treatment. However, whether the combined treatment yields a significantly better result when compared to enzyme treatment only depends on its ability not to hinder the system or affect antioxidant stability. The use of assisted ultrasound was not commonly found within the industry scale of production as additional ultrasound-assisted performance often depends on additional parameters, including power, frequency and juice matrix, complicating the already standardised protocols of enzymatic treatment (Alshehhi et al., 2023).

Consequently, enzymatic treatment alone might have an inverse effect on antioxidant activity in other fruit juice production, as different types of fruit have different cellular structures and nature in their bioactive compounds. For example, in pear juice, the DPPH (19.30%) and ABTS (18.70 mg catechin/100 g) values are lower in enzyme-treated samples than in untreated samples (20.30% and 18.91 mg catechin/100 g) (Gobelna et al., 2019). The contradictory effects of enzymatic treatment towards the antioxidant activity across different types of tropical fruit can be attributed to the mechanistic factors of their matrices. For example, as enzymatic hydrolysis can actually enhance the extractability of phenolic and antioxidant compounds through the breakdown of the cell wall binding these compounds, enzymatic treatment can also lead to possible oxidative loss of sensitive antioxidants such as phenolic acids and ascorbic acids as it continues to be exposed to oxygen. This later has resulted in the reduction of measured radical scavenging activity (Wang et al., 2023). According to Gani et al. (2021), the reduction in antioxidant activity in enzyme-treated pear juice might be associated with the possible occurrence of bioactive compounds to oxidise during heating, proving that antioxidants are highly temperature dependent. Therefore, enzymatic treatment may enhance antioxidant activity in some fruits, but parameter optimisation remains important in enhancing and reducing the possible loss of bioactive compounds during processing.

Another important aspect to be crucially observed is the effect of excessive treatment on phenolic and antioxidant compounds. The breakdown of cell wall and polysaccharide chains during enzymatic treatment allows natural oxidative enzymes such as polyphenol oxidase (PPO) and peroxidase (POD) to be released within the system. This was found to increase the oxygen transport, catalysing the conversion of phenolics into quinones, forming brown pigments and reducing radical scavenging capacity (Singh et al., 2018). L-ascorbic and L-dehydroascorbic acids are the two main compounds of vitamin C with antioxidant activity, where their stability depends on the amount of oxygen it's being exposed to, where

the degradation of these acids is due to the oxidation process (Pérez-Lamela et al., 2021). Prolonged incubation leads to more or excessive exposure towards oxygen, leading to the degradation of several desirable antioxidants.

### Total Phenolic Content

Phenolic content is important as it is closely related to the sensory quality, pigment accumulation for appearance, and antioxidant ability of fruit juice (Wang et al., 2023). Table 6 demonstrates a few studies conducted on tropical fruit juice using various types of enzymes and conditions. Similar to antioxidant activity, phenolic compounds are also susceptible to technological processes, affecting the astringency and quality of fruit juice.

The phenolic content in fruit-based products plays a significant role in health, protecting the body against possible coronary heart disease, cancers and protecting vitamin C in fruit juice from oxidative degradation (Nur ‘Aliaa et al., 2011). Additionally, Nur ‘Aliaa et al. (2011) showed two optimised conditions suitable for enhancing total phenolic contents in red pitaya juice by using 0.10% Pectinex Ultra SP-L (with 45 minutes incubation time, and temperature of 40°C), and 0.09% Pectinex CLEAR (with 85 minutes incubation time at 45 °C).

Table 6  
Effect of optimised enzymatic conditions on total phenolic content of different tropical fruits

Sample	Enzyme	Conditions			TPC	Ref
		Concentration	Time	Temperature (°C)		
Jamun Fruit	Pectinase ( <i>Aspergillus aculeatus</i> )	0.05%	80 min	44	115.31 mg GAE/g	Ghosh et al. (2016)
Bael Fruit	Pectinase ( <i>Aspergillus niger</i> )	0.22%	6.35 hours	46.20	56.66 mg/100mL	Sonawane et al. (2020)
Red Pitaya	Pectinex Ultra SP-L ( <i>Aspergillus niger</i> )	0.10%	45 min	40	1.34 mg/100g	Nur ‘Aliaa et al. (2011)
	Pectinex CLEAR ( <i>Aspergillus aculeatus</i> and <i>Aspergillus niger</i> )	0.09%	82 min	46	1.26 mg/100g	
Casimiroa ( <i>Casimiroa Edulis</i> )	α-amylase ( <i>Aspergillus Oryzae</i> )	0.2%	62.74 min	44.01	45.09	Genanew et al. (2022)

The total phenols of optimised enzymatic treatment using Pectinex Ultra SP-L and Pectinex CLEAR were found at 1.34 mg/100g GAE and 1.26 mg/100g GAE, respectively, suggesting an increase from untreated red pitaya juice (1.24 mg/100g GAE) (Nur 'Aliaa et al., 2011). This trend is aligned with casimiroa juice, where optimised conditions of 0.2% enzyme concentration at 62.74 minutes and 44.01 °C increased the total phenolic content (25.74%) compared to the untreated casimiroa juice (Genanew et al., 2022). As enzymatic treatment was conducted under specific and mild conditions, such as controlled temperature and pH level, the likelihood of degradation of sensitive compounds can be minimised, as can be seen from the linear effect of both antioxidant activity and total phenolic content throughout enzymatic treatment on certain tropical fruits. This can denote that the bioactive compounds were not completely degraded under optimised treatment conditions. However, although Folin-Ciocalteu helped in measuring the improvement of extracted phenolics, more chromatographic studies were needed to analyse the total phenolic content, stability, along with the potential degradation of sensitive compounds throughout the enzymatic treatment.

Maintaining the optimal temperature condition across enzymatic treatment is a crucial factor to balance the catalytic effectiveness and the thermal stability of the bioactive compounds. Enzyme activity normally increases along with the increase of temperature to a certain optimal point, improving the hydrolysis or degradation of polysaccharide cell wall, hence enhancing the extractability of bound bioactive compounds into the juice matrix (Palos- Hernández et al., 2024). On the other hand, treatment beyond optimal temperatures may hinder the stability of enzymes, thus causing the denaturation of enzyme and accelerated degradation of heat sensitive antioxidants (like ascorbic acid) and isomerisation of certain phenolic compounds (flavonoids and vitamins), leading to the decrease of antioxidant capacity despite the increased extractability observed (Bastos et al., 2025; Palos-Hernández et al., 2024).

Although this review does not focus on the influence of enzymatic treatment towards the changes of sensory attributes, we acknowledge that this treatment indeed has a huge impact on its influence. Enzyme hydrolysis may affect sensory qualities in addition to efficient extraction of volatile chemicals. Enzymatic treatment substantially modifies the sensory properties through releasing glycosidically bound volatiles that are responsible for improving aroma, enhancing the concentration of phenolics linked to produce bitterness, and also speeding up the loss of delicate volatiles under prolonged incubation, excessive thermal pressure and possible oxygen exposure, which may alter the lipid profile of such fruit juice (de Carvalho et al., 2015).

## CONCLUSION

Optimised enzymatic treatment has enhanced various quality attributes of tropical fruit juices, such as juice yield, viscosity, clarity, turbidity, total soluble solids (TSS), antioxidant

activity and total phenolic content (TPC). Enzymatic treatment using pectinase, cellulase, and hemicellulase in degrading cell wall structures and releasing valuable compounds was seen to have a significant effect. Despite that, excessive or prolonged application of this treatment may result in undesirable effects. Therefore, optimising the enzymatic treatment conditions, including enzyme concentration, incubation time, temperature, and any other variable used in tropical fruit juice production, is important. It should be noted that different types of fruit may have different enzymatic treatment conditions depending on their pectin, cellulose, and hemicellulose content. Even so, industry can still use this as a production baseline and guidance in understanding the need for optimisation and enzymatic treatment of each tropical fruit in juice production, in the hope of bringing efficiencies in larger industrial cases.

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## ETHICAL STATEMENT

Not applicable.

## CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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