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Enzymatic Liquefaction and Characterization of *Mangifera laurina* Blume

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Abstract.
The fruit *Mangifera laurina* Blume lacks sufficient research attention, with no literature available on its physicochemical properties, proximate nutritional composition, carotenoid content, or enzyme liquefaction process. Therefore, we aimed to optimize the parameters for enzymatic liquefaction of *M. laurina* puree and comprehensively analyze its characteristics.
Homogenized pulp of *M. laurina* was treated with different enzymes (Pectinex Ultra SPL, Celluclast, Fungamyl, and Termamyl). Pectinex Ultra SPL was selected as the most effective enzyme as it significantly decreased viscosity and increased juice yield. Pectinex Ultra SPL was then used to treat the homogenized pulp at different concentrations (0–4.0%), different incubation times (0–2.5 h), and different incubation temperatures (25–60°C). We considered these parameters as independent variables and studied their effects on viscosity, juice yield, total soluble solids, pH, and color to establish optimum conditions for the enzymatic liquefaction of *M. laurina* pulp.
The recommended enzymatic liquefaction conditions were set as 2.0% Pectinex Ultra SPL at 45°C for 2.0 h. The optimized enzyme-liquefied mango puree showed a noteworthy decrease in total carotenoids (174.15 ± 0.04 μg/100 g), crude protein, crude fat, and crude fiber, compared to fresh mango puree. However, enzymatic liquefaction provided the mango puree with higher contents of moisture and ash, better water activity, and higher juice yield, compared to fresh mango puree.
Enzymatic liquefaction of fruit juice provides advantages in terms of improved digestion, increased yield, and enhanced economic profit. Its ability to enhance nutrient availability, increase extraction rates, and optimize production processes makes it a valuable technique in various food industries.

Keywords. *Mangifera laurina*, mango, enzymatic, enzymatic liquefaction, fruit juice

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Ферментативное разжижение и характеристика пюре из плодов *Mangifera laurina* Blume

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Аннотация.
Свойства плодов манго *Mangifera laurina* Blume не достаточно изучены: отсутствуют данные о его физико-химических свойствах, питательном составе, содержании каротиноидов и процессе ферментативного разжижения. Целью исследования стала оптимизация параметров ферментативного разжижения пюре плодов *M. laurina* и анализ этого продукта.
Гомогенизированную мякоть плодов *M. laurina* обрабатывали различными ферментами (Pectinex Ultra SPL, Celluclast, Fungamyl и Termamyl). Pectinex Ultra SPL был выбран как наиболее эффективный фермент, т. к. он снижает вязкость и увеличивает выход сока. Pectinex Ultra SPL использовался для обработки гомогенизированной мякоти при различных концентрациях (0–4,0 %), времени инкубации (0–2,5 ч) и температуре инкубации (25–60 °C). Эти параметры рассматривались как независимые переменные. Изучали их влияние на вязкость, выход сока, общее количество растворимых сухих веществ, pH и цвет.
Экспериментальным путем установили оптимальные условия ферментативного разжижения мякоти плодов *M. laurina*: 2,0 % Pectinex Ultra SPL при 45 °C в течение 2,0 ч. По сравнению со свежим пюре оптимизированный образец показал снижение общего количества каротиноидов (174,15 ± 0,04 мкг/100 г), сырого белка, неочищенного жира и сырой клетчатки. Однако ферментативное разжижение привело к повышению содержания влаги и золы, активности воды и выходу сока по сравнению со свежим пюре.
Ферментативное сжижение пюре плодов манго повышает доступность питательных веществ, увеличивает выход фруктового сока и повышает экономическую прибыль. Следовательно, ферментативное сжижение может применяться в различных пищевых отраслях, т. к. оно повышает доступность питательных веществ, увеличивает скорость экстракции и оптимизирует производственные процессы.

Ключевые слова. *Mangifera laurina*, манго, фермент, ферментативное разжижение, фруктовый сок

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Introduction

There have been extensive studies of vast varieties of plants. However, a substantial number of plant species are still understudied. One of such species is *Mangifera*, whose fruits, commonly known as mango, have been underutilized. While *Mangifera indica* L. has been widely studied and considered superior to other mangoes, *Mangifera foetida* L., *Mangifera odorata* Griff., *Mangifera pajang* Kosterm, and *Mangifera laurina* Blume are examples of underutilized *Mangifera* fruits [1].

Originating from Southeast Asia, mangoes are available in more than 110 countries where they grow in tropical and subtropical areas. In terms of production, marketing, and consumption, mangoes are considered one of the most important commercial crops worldwide. They are an excellent source of vitamin C, vitamin A, and copper, as well as a good source of B vitamins. Mangoes also possess high concentrations of various phytochemicals [2]. They can be processed and used at multiples stages of their growth. Unripe mangoes are mainly used to produce chutneys and pickles, or in fruit salads. Puree made from ripe mangoes is widely utilized in various products, such as nectars, squashes, jams, beverages, and fruit leathers [3]. *M. laurina* is an evergreen tree, native to tropical Asian countries, such as Malaysia, Indonesia, and Philippines. It grows in moist forests at low altitudes. *M. laurina* starts flowering when it becomes about 15 m tall and can grow up to a height of 36 m. Compared to the common mango, its flowers are smaller and its petals have a different

shape and color. With the introduction of *M. indica*, *M. laurina* has been used less frequently.

Many tropical fruits, such as papaya, apricot, plum, and mango, have flavors that are too strong or acidic. They are also less pressable than other fruits. Due to these characteristics, they are unsuitable for ready-to-serve beverages without blending, dilution, or both. Therefore, they are converted into puree, which is a cream-like substance or a thick liquid [4]. Puree is usually made by blending until a uniform pulp is achieved. However, blending alone produces a low juice yield due to the retention of juice in the pulp, especially in fruits high in polysaccharides. It also damages certain properties in fruits [5]. Thus, enzymes are introduced in puree production to maintain texture, nutrient composition, taste, and aroma, while increasing juice yield and decreasing viscosity. This process is called enzymatic liquefaction. In juice extraction, macerating enzymes are commonly used, such as pectinase and cellulase [6].

Although a wide variety of the *Mangifera* species have been studied, there is no published literature on the characterization of *M. laurina* in terms of its physicochemical properties, proximate nutritional composition, or carotenoid content. Neither do we know of any research into the enzyme liquefaction of its fruit. Therefore, we aimed to characterize *M. laurina* and optimize its enzymatic liquefaction parameters.

Study objects and methods

Materials. We used *Mangifera laurina* Blume, a type of mango fruit, in our experiment. The fruits were purchased from a market in Serdang, a town in Selangor, Malaysia. After washing and peeling each fruit, we removed its seed by slicing the pulp into smaller pieces. The seed was then thrown away, while the pieces of pulp were vacuum-packed and stored in the freezer at –18°C. The enzymes Pectinex Ultra SPL, Celluclast, Termamyl, and Fungamyl (Novozymes, Denmark) were used for liquefaction. All chemicals and solvents used in analyses were of analytical grade, unless specified otherwise.

Preparation of mango puree. In preparation for enzymatic liquefaction, the mango pulp was homogenized into puree using a hand blender at high speed for 1 min. After homogenization, the mango puree was poured into five separate beakers, each containing 100 g of the puree. The beakers were labeled according to the enzyme type – Pectinase Ultra SPL, Celluclast, Fungamyl, and Termamyl. One beaker served as a control sample. Each of the enzyme types was used at 1% v/w. The enzymes were then added according to the labels on the beakers with mango puree. The control sample contained no added enzyme.

The mixtures of mango puree and enzymes were stirred using a glass rod for 30 s each. Then, the beakers were placed into a water bath at 45°C for 1 h. To ensure even mixing and enzymatic liquefaction, constant shaking was applied using the water bath shaker at medium speed. After 1 h, the beakers were removed from the water bath and placed into another water bath at 80°C for 5 min to inactivate the enzymes. Then, they were transferred into an ice water bath to cool down for another 5 min.

Enzymatic liquefaction depends on several important parameters, namely the type of enzyme, its concentration, as well as incubation time and temperature. Different types of enzymes exhibit different degrees of activity when utilized in enzymatic liquefaction to reduce the viscosity of fruit pulp [6].

Effects of different concentrations of pectinase on mango puree. Pectinex Ultra SPL was selected as the most suitable enzyme, and the process of enzymatic liquefaction was repeated. For this, the mango fruit pulp was homogenized into puree with a hand blender at high speed for 1 min. After homogenization, the puree was poured into nine separate beakers, each containing 100 g of the puree. The beakers were labeled according to the concentrations of Pectinex Ultra SPL to be used (0.0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, and 4.0% v/w). The enzyme Pectinex Ultra SPL in the concentrated liquid form was then added into the beakers according to the labeled concentrations.

The mixtures of mango puree and Pectinex Ultra SPL were stirred using a glass rod for 30 s each. Then, the beakers were placed into a water bath at 45°C for 1 h. To ensure even mixing and enzymatic liquefaction, constant shaking was applied using the water bath shaker at medium speed. After 1 h, the beakers containing the mixture were removed from the water bath and placed into another water bath at 80°C for 5 min to inactivate the enzyme. Then, they were transferred into an ice water bath to cool down for another 5 min.

Effects of incubation time on mango puree liquefied with the optimized enzyme concentration. The optimum concentration of Pectinex Ultra SPL was determined at 2% v/w. After that, enzymatic liquefaction was repeated with different incubation times. The mango fruit pulp was homogenized into puree using a hand blender at high speed for 1 min. Then, the mango puree was poured into six separate beakers, each containing 100 g of the puree. The beakers were labeled according to different incubation times (0.0, 0.5, 1.0, 1.5, 2.0, and 2.5 h). After that, 2% v/w Pectinex Ultra SPL was added into each of the beakers.

The mixtures of mango puree and Pectinex Ultra SPL were stirred using a glass rod for 30 s each. Then, the beakers were placed into a water bath at 45°C, except for the beaker labeled “0 h”, whose viscosity, juice yield, total soluble solids, pH, and color were measured immediately after 30 s of stirring. To ensure even mixing and enzymatic liquefaction, constant shaking was applied using the water bath shaker at medium speed. At the end of each incubation time (0.5, 1.0, 1.5, 2.0, and 2.5 h), the beakers were removed from the water bath according

to their respective labels and placed into another water bath at 80°C for 5 min to inactivate the enzyme. Then, they were transferred into an ice water bath to cool down for another 5 min.

Effects of incubation temperature on mango puree liquefied during the optimized incubation time. Having determined the optimum incubation time (2 h), we aimed to determine the optimum temperature for the enzymatic liquefaction of mango puree. For this, enzymatic liquefaction was repeated at different incubation temperatures. The mango fruit pulp was homogenized into puree using a hand blender at high speed for 1 min. After homogenization, the puree was poured into six separate beakers, each containing 100 g of the puree. The beakers were labeled according to different incubation temperatures (25, 40, 45, 50, 55, and 60°C). Then, 2% v/w of Pectinex Ultra SPL was added into each of the beakers.

The mixtures of mango puree and Pectinex Ultra SPL were stirred using a glass rod for 30 s each. Then, the beakers labeled with differing incubation temperatures (25, 40, 45, 50, 55, and 60°C) were placed into water baths at their respective temperatures for 2 h. The beaker labeled 25°C served as a control and was kept at room temperature. To ensure even mixing and enzymatic liquefaction of the mango puree, constant shaking was applied using the water bath shaker at medium speed. After 2 h, the beakers were removed from their respective water baths and placed into another water bath at 80°C for 5 min to inactivate the enzyme. Then, they were transferred into an ice water bath to cool down for another 5 min.

Optimization of enzyme-liquefied puree. Triplicate results were recorded and tabulated for each of the steps in determining the optimum parameters for enzymatic liquefaction of mango puree. In particular, we determined the optimum enzyme type, enzyme concentration, incubation time, and incubation temperature based on the statistical analysis of viscosity, juice yield, total soluble solids, pH, and color.

Physicochemical properties analysis. ***Water activity.*** The water activity of fresh mango puree and enzyme-liquefied mango puree was measured using a LabMaster – A_w water activity meter at room temperature, 25.0 ± 1.0°C [7]. Prior to that, the water activity meter was calibrated using a potassium sulfate solution (K_2SO_4) and potassium chloride (KCl). Triplicate results were obtained for each sample.

Color. The color of all the mango puree samples was measured using a ColorFlex EZ Hunter Lab colorimeter. Before the measurements, the colorimeter was calibrated against a standard white tile with reflectance values (Y = 94.1, X = 0.3129, y = 0.3189). The colorimetric data was expressed in terms of L^*, a^*, and b^* for luminosity or lightness, green-red, and blue-yellow components, respectively [8]. Triplicate results were obtained for each sample.

Total soluble solids (Brix). The content of total soluble solids in the mango puree samples was measured using a Milwaukee digital sugar refractometer. Before use, the refractometer was calibrated using distilled water. Then, one drop of a sample at 20°C was placed on the refractometer and the reading was obtained [9]. Triplicate results were obtained for each sample.

Viscosity. The viscosity of the mango puree samples was measured with a Brookfield DV-II+Pro viscometer calibrated before use [10]. For this, 250 mL of a room temperature sample contained in the beaker was used each time. The readings were obtained with spindle IV at 100 rpm in triplicate.

pH. The pH of all mango puree samples was measured with a digital pH meter, which was calibrated with two buffer solutions of pH 7.0 and pH 4.0 before each usage. For the measurement, 50 mL of a sample was poured into a 100 mL beaker [9]. Triplicate results were obtained for each sample.

Juice yield. The juice yield of the mango puree samples was measured by weight, using a coffee sock and an electronic weighing balance. A 250 mL beaker was placed on the electronic weighing balance and it was zeroed. A coffee sock was then positioned above the 250 mL beaker. 100 g of a sample was then weighed and poured into the coffee sock. The coffee sock was then squeezed dry and the juice from the sample was collected in the 250 mL beaker, with its weight recorded. Triplicate results were obtained for each sample.

Proximate analyses. Five proximate analyses were conducted to determine crude protein, crude fat, crude fiber, ash content, and moisture content of both fresh and enzyme-liquefied mango puree samples. Official methods of the AOAC were used for all five proximate analyses [11].

Carotenoid analysis. The total carotenoid content (μg/100 g) of both fresh and enzyme-liquefied mango purees was determined using the direct spectrophotometric method adopted from Kimura *et al.* with slight modifications [12]. First, 3 g of a homogenized sample was added into a beaker containing 10 mL of distilled water. The mixture was allowed to stand at room temperature for 30 min. Then, 20 mL of precooled acetone (4°C) was added into the mixture, which was allowed to stand for 15 min. Then, the mixture was filtered through a filter paper in a Buchner funnel which creates a vacuum suction. The filtrate was collected in a receiving flask. To ensure that the carotenoid content of the sample was fully extracted, the filtration process was repeated twice with enough acetone to cover. Next, partition of petroleum ether was carried out using a 500 mL separating funnel with stand. For this, 20 mL of petroleum ether was poured into the funnel, followed by one third of the filtrate, and 300 mL of distilled water. The phases were allowed to separate. The lower aqueous phase was then eluted and discarded. These steps were repeated with the second and third portions of the filtrate. After

the third portion was transferred, the petroleum ether phase was washed three times with 200 mL of distilled water each time. The remaining phase was collected in a round bottom flask after being passed through filter paper with 15 g of anhydrous sodium sulfate in a funnel. After that, petroleum ether in the remaining phase was evaporated with a rotary evaporator in a water bath at 35°C. When all the petroleum ether was evaporated, 1 mL of acetone was added into the round bottom flask. It was then shaken well and the acetone containing carotenoid from the sample was transferred into a 1 mL amber bottle before its absorbance was measured at 450 nm in a quartz cuvette.

$$\text{Total carotenoid content} = \frac{A \times V \times 10^4}{A_{1cm}^{1\%} \times m} \times 100 \qquad (1)$$

where A is the absorbance at 450 nm; V is the total extract in volume, mL; m is the sample weight, g; $A_{1cm}^{1\%} = 2500$ (absorption coefficient recommended for carotenoid mixture).

Statistical analysis. All the samples were analyzed in triplicate and the results were presented as mean ± SD. Analysis of variance (ANOVA) was performed to determine the significance of the result ($p \leq 0.05$). Tukey's post-hoc test was used to determine the significant difference between the means. Statistical analysis was carried out using Minitab Statistical Software (Version 18.0, Minitab Inc, State College, Pennsylvania, USA).

Results and discussion

Effects of different enzymes on mango puree. The enzymes pectinase (Pectinex Ultra SPL), cellulase, and Fungamyl added to the mango puree decreased its viscosity, with pectinase producing the lowest viscosity at 1233.60 ± 118.80 cP. Pectinase functions by hydrolyzing pectic substances in fruits, thus decreasing viscosity [13]. Since these pectin-containing substances also possess a high water-holding capacity, pectin degradation by pectinase reduces the water-holding capacity as well. Free water is then released into the system, leading to further reduction in viscosity [14]. We found no change in viscosity when the enzyme Termamyl was applied. Being a type of α-amylase, Termamyl hydrolyzes starch into disaccharides and monosaccharides [15]. During ripening, the degradation of starch present in mango fruits increases drastically, reducing its content and converting it into simple sugars. The degree of degradation depends on the cultivar [16]. Since we used ripe mango fruits, starch degradation was close to complete, with no change in viscosity when Termamyl was applied (Table 1).

Pectinase produced the highest juice yield from the enzyme-treated mango puree at 60.18 ± 2.00 g. As mentioned above, pectinase is responsible for the degradation of pectin in plant cells, which possess a high water-holding capacity. As a result of pectin degradation, free water is released as it solubilizes otherwise insoluble pectin in the fruit pulp [17]. The release of free water caused more juice to be produced and thus increased the juice yield. Although cellulase, Fungamyl, and Termamyl increased the juice yield as well, their juice yields were not as high as those produced by pectinase. There was no significant difference between the amounts of juice yielded by the three enzymes either (Table 1).

Pectinase, cellulase, and Fungamyl increased the content of total soluble solids in the mango puree significantly, while Termamyl did not. Pectinase application resulted in the highest increase in total soluble

Table 1. Effect of different types of enzymes on viscosity, juice yield, total soluble solids, pH, and color of mango puree liquefied with 1% enzyme at 45°C during 1 h

Таблица 1. Влияние различных типов ферментов на вязкость, выход сока, общее содержание растворимых сухих веществ, pH и цвет пюре манго, подвергавшегося разжижению 1 % ферментом при 45 °C в течение 1 ч

Analysis	Enzyme type				
	Control	Pectinase	Cellulase	Fungamyl	Termamyl
Viscosity, cP	2264.10 ± 205.20^{a}	1233.60 ± 118.80^{d}	1806.50 ± 24.10^{bc}	1718.50 ± 66.60^{c}	2264.10 ± 205.20^{ab}
Juice yield, g	60.18 ± 2.00^{c}	80.78 ± 2.80^{a}	64.13 ± 1.17^{bc}	63.90 ± 0.97^{bc}	65.32 ± 1.30^{b}
Total soluble solids, °Brix	14.70 ± 0.10^{c}	15.63 ± 0.20^{a}	15.13 ± 0.06^{b}	15.17 ± 0.11^{b}	14.83 ± 0.12^{bc}
pH	3.84 ± 0.10^{a}	3.60 ± 0.10^{a}	3.68 ± 0.10^{a}	3.72 ± 0.14^{a}	3.58 ± 0.08^{a}
L^*	60.73 ± 0.58^{a}	58.52 ± 0.71^{ab}	58.27 ± 0.62^{ab}	59.34 ± 0.78^{ab}	57.51 ± 1.94^{b}
a^*	18.63 ± 0.43^{a}	15.77 ± 0.17^{c}	16.52 ± 0.34^{c}	17.74 ± 0.67^{ab}	16.88 ± 0.46^{bc}
b^*	78.73 ± 0.64^{a}	76.60 ± 0.42^{b}	76.55 ± 0.86^{b}	78.30 ± 0.59^{a}	78.25 ± 0.26^{a}

Data on viscosity, juice yield, total soluble solids, pH, and color (L^*, a^*, b^*) are means ± standard deviations, where n = 3. For each row, superscripts of the same letter are not significantly different at $p < 0.05$, as measured by the Tukey HSD Test. HSD – honest significant difference; L^* – degree of lightness and darkness; a^* – degree of redness or greenness; b^* – degree of yellowness or blueness.

Вязкость, выход сока, общее количество растворимых сухих веществ, pH и цвет (L^*, a^*, b^*) представлены как средние значения ± стандартные отклонения, где n = 3. Одна и та же буква – показатель отсутствия существенного различия при $p < 0,05$ (тест Тьюки). HSD – существенное различие; L^* – степень светлоты и темноты; a^* – степень красноты/зелености; b^* – степень желтизны/синевы.

solids at 15.63 ± 0.20°Brix (Table 1). The increase in total soluble solids is attributed to tissue breakdown, which releases nutritional components within the tissues of the fruit pulp upon enzyme treatment [18].

As for pH, all the enzyme treatments had no significant effect on the pH of the mango puree. The addition of enzyme should decrease the pH of mango puree due to the release of carboxyl groups from the breakdown of pectic substances (Table 1). However, our experiment showed no such decrease. This could be due to a low enzyme concentration (1%) and therefore a minimal release of carboxyl groups [19].

Effects of pectinase concentrations on mango puree. According to our results, the viscosity of the mango puree samples decreased with higher enzyme concentrations. Significant increments in viscosity were observed from 0.5 to 2.0% v/w of pectinase. Slight increments were recorded as the enzyme concentration was increased up to 4.0% v/w but the differences were not significant. As discussed earlier, the enzyme pectinase allows extraction from fruit pulp cells through the enzymatic hydrolysis of pectic substances present in the pulp. Pectinase leads to the release of free water that would otherwise be held by pectic substances. Viscosity is therefore decreased [13, 14]. Increased concentrations of pectinase facilitated a higher rate of hydrolysis of the pureed mango pulp tissues and led to the reduction in viscosity. Similar results were reported in the studies by Norjana and Noor Aziah, where pectinase was used to liquefy a variety of mango fruits and durian [19].

As can be seen in Table 2, the juice yield of the enzyme-treated mango purees increased with higher concentrations of the enzyme. However, we observed no significant increase after using 1.5% v/w of the enzyme. An unpleasant odor was emitted upon incubation from 3.0% v/w of the enzyme onwards. It could have been caused by an increased concentration of pectinase since this enzyme possesses a fermentation odor and its increased amount can make the odor more distinct and recognizable [20].

We observed no significant difference in total soluble solids as the enzyme concentration was increased from 0 to 4.0% v/w. Despite some slight increments in total soluble solids due to the breakdown of mango puree tissues by pectinase and a subsequent release of components from the tissues, those increments were not enough for the enzyme concentration to be considered as a factor of total soluble solids increase [19]. Sugars, organic acids, vitamins, and other soluble components make up most of soluble solids in fruit juice. Enzymatic liquefaction has the greatest impact on the breakdown of pectin, which is a complex carbohydrate rather than a soluble solid. While pectin breakdown can improve juice release, it has no effect on the concentration of soluble solids in the juice. Soluble solids in fruit juice are mostly determined by the natural composition of the fruit, while enzymatic liquefaction has no direct influence on them [30]. In a study by Arif *et al.*, the liquefaction process decreased the total soluble solids produced by liquid sugar from sweet sorghum starch. During liquefaction, starch is broken down into dextrin, maltose, and glucose. Dextrin is a result of imperfect starch hydrolysis. The process also involves alkali and oxidizing agents. The reduction of the chain length changes the properties of starch. Not easily soluble in water, it is converted into dextrin which is very soluble in hot or cold water with a relatively low viscosity [29].

As shown in Table 2, increased enzyme concentrations from 0 to 4.0% v/w significantly decreased the pH of the mango puree. This was due to the breakdown of pectic substances by pectinase. When broken down or hydrolyzed, these pectic substances released carboxyl groups into the mango puree, lowering its pH [19, 21].

Effects of incubation time on mango puree liquefied with the optimized enzyme concentration. As can be seen in Table 3, the viscosity of the enzyme-treated mango puree significantly decreased from 1560.40 ± 96.30 cP at 0.0 h to 1238.43 ± 61.30 cP at 0.5 h, as well as from 1036.43 ± 12.10 cP at 1.5 h to 879.70 ± 36.30 cP at 2.0 h. The viscosity decreased further at 2.5 h of incubation but it was not significant. We observed a correlation between lower viscosity values and longer incubation times. Similar results were reported by Reddy *et al.*, who attributed the lower viscosity of homogenized mango fruits to the pectolytic action of pectinase, which hydrolyzed the pectin present in mango pulp [22].

The enzyme-treated mango purees also showed significant increases in juice yield with longer incubation times, namely from 80.84 ± 0.65 g at 0.0 h to 83.61 ± 0.57 g at 0.5 h, as well as from 85.72 ± 0.18 g at 1.5 h to 87.87 ± 0.16 g at 2.0 h (Table 3). As pectic substances in the cells of homogenized mango pulp had more time to break down, more free water contained in insoluble pectin could be released, thus increasing the juice yield [13, 14].

Total soluble solids showed no significant decrease at 0.0 to 1.5 h of incubation but they decreased significantly from 16.00 ± 0.00 to 16.23 ± 0.06°Brix at 1.5 to 2.0 h. Similar results were reported by Tadakittisarn *et al.*, who studied the effect of pectinolytic enzymes on fruit pulp [23]. The authors found that longer incubation allowed the enzymes more time to break down the cell walls of the fruit pulp, which in turn released more components from the cells. This resulted in a higher content of total soluble solids in the system.

Increased incubation time had no effect on the pH of the enzyme-treated mango pulp samples, with no significant difference observed (Table 3).

Effects of incubation temperature on mango puree liquefied with the optimized enzyme concentration and incubation time. As shown in Table 4, the viscosity of the enzyme-treated purees significantly

Table 2. Effect of different pectinase concentrations on viscosity, juice yield, total soluble solids, pH, and color of mango puree liquefied at 45°C for 1 h

Таблица 2. Влияние различных концентраций пектиназы на вязкость, выход сока, общее количество растворимых сухих веществ, pH и цвет пюре манго, подвергавшегося разжижению при 45 °C в течение 1 ч

Analysis	Enzyme concentration, %								
	Control (0)	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0
Viscosity, cP	2141.10 ± 67.80^{a}	1587.70 ± 95.20^{b}	1122.50 ± 103.80^{c}	1084.00 ± 97.30^{c}	782.30 ± 27.10^{d}	775.10 ± 41.90^{d}	779.00 ± 28.90^{d}	768.60 ± 38.20^{d}	775.90 ± 26.80^{d}
Juice yield, g	67.57 ± 4.86^{d}	75.98 ± 2.05^{c}	77.03 ± 1.57^{c}	78.77 ± 1.08^{bc}	83.59 ± 1.52^{ab}	84.31 ± 0.67^{ab}	84.44 ± 0.55^{ab}	84.98 ± 0.91^{a}	85.09 ± 0.72^{a}
Total soluble solids, °Brix	15.10 ± 0.78^{a}	15.37 ± 0.67^{a}	15.67 ± 0.51^{a}	15.93 ± 0.64^{a}	16.10 ± 0.62^{a}	16.00 ± 0.70^{a}	16.03 ± 0.90^{a}	16.10 ± 1.04^{a}	15.9 ± 1.13^{a}
pH	4.28 ± 0.19^{a}	3.91 ± 0.10^{b}	3.90 ± 0.12^{bc}	3.86 ± 0.11^{bcd}	3.84 ± 0.13^{bcd}	3.55 ± 0.06^{cde}	3.52 ± 0.11^{de}	3.45 ± 0.11^{e}	3.35 ± 0.13^{e}
*L**	56.90 ± 0.66^{a}	53.40 ± 0.62^{b}	52.83 ± 1.22^{b}	52.20 ± 0.92b^{bc}	52.10 ± 0.62^{bc}	50.53 ± 0.55^{c}	50.40 ± 0.27^{cd}	48.47 ± 0.40^{de}	47.83 ± 0.65^{e}
*a**	19.20 ± 0.10^{a}	17.63 ± 0.55^{b}	17.03 ± 0.61^{bc}	16.73 ± 0.35^{bc}	16.27 ± 0.35^{cd}	16.10 ± 0.36^{cd}	15.43 ± 0.55^{de}	14.53 ± 0.59^{e}	14.47 ± 0.32^{e}
*b**	80.20 ± 1.55^{a}	78.23 ± 0.61^{ab}	77.63 ± 1.02^{b}	77.47 ± 0.85^{b}	77.43 ± 0.70^{b}	77.27 ± 0.35^{b}	77.23 ± 0.51^{b}	77.07 ± 0.57^{b}	77.00 ± 0.56^{b}

Data on viscosity, juice yield, total soluble solids, pH, and color (*L**, *a**, *b**) are means ± standard deviations, where n = 3. For each row, superscripts of the same letter are not significantly different at $p < 0.05$, as measured by the Tukey HSD Test. HSD – honest significant difference; *L** – degree of lightness and darkness; *a** – degree of redness or greenness; *b** – degree of yellowness or blueness.

Вязкость, выход сока, общее количество растворимых сухих веществ, pH и цвет (*L**, *a**, *b**) представлены как средние значения ± стандартные отклонения, где n = 3. Одна и та же буква – показатель отсутствия существенного различия при $p < 0,05$ (тест Тьюки). HSD – существенное различие; *L** – степень светлоты и темноты; *a** – степень красноты/зелености; *b** – степень желтизны/синевы.

Table 3. Effect of different incubation times on viscosity, juice yield, total soluble solids, pH, and color of mango puree liquefied with 2% enzyme at 45°C

Таблица 3. Влияние периода инкубации на вязкость, выход сока, общее количество растворимых сухих веществ, pH и цвет пюре манго, подвергавшегося разжижению 2 % ферментом при 45 °C

Analysis	Time, h					
	Control (0)	0.5	1.0	1.5	2.0	2.5
Viscosity, cP	1560.40 ± 96.30^{a}	1238.43 ± 61.30^{b}	1106.33 ± 24.10^{bc}	1036.43 ± 12.10^{c}	879.70 ± 36.30^{d}	870.53 ± 52.00^{d}
Juice yield, g	80.84 ± 0.65^{d}	83.61 ± 0.57^{c}	84.82 ± 0.53^{bc}	85.72 ± 0.18^{b}	87.87 ± 0.16^{a}	87.28 ± 0.44^{a}
Total soluble solids, °Brix	15.90 ± 0.10^{b}	16.03 ± 0.06^{b}	16.03 ± 0.06^{b}	16.00 ± 0.00^{b}	16.23 ± 0.06^{a}	16.27 ± 0.06^{a}
pH	3.75 ± 0.12^{a}	3.79 ± 0.11^{a}	3.76 ± 0.14^{a}	3.78 ± 0.10^{a}	3.78 ± 0.14^{a}	3.81 ± 0.11^{a}
*L**	57.29 ± 0.02^{a}	52.13 ± 0.01^{b}	51.69 ± 0.01^{c}	51.52 ± 0.02^{d}	51.43 ± 0.07^{d}	50.95 ± 0.13^{e}
*a**	18.67 ± 0.31^{a}	16.23 ± 0.19^{b}	16.01 ± 0.58^{bc}	15.29 ± 0.16^{cd}	15.29 ± 0.07^{cd}	14.98 ± 0.09^{d}
*b**	83.26 ± 0.14^{a}	77.95 ± 0.05^{b}	76.30 ± 0.04^{c}	76.02 ± 0.11^{cd}	75.95 ± 0.27^{cd}	75.75 ± 0.15^{d}

Data on viscosity, juice yield, total soluble solids, pH, and color (*L**, *a**, *b**) are means ± standard deviations, where n = 3. For each row, superscripts of the same letter are not significantly different at $p < 0.05$, as measured by the Tukey HSD Test. HSD – honest significant difference; *L** – degree of lightness and darkness; *a** – degree of redness or greenness; *b** – degree of yellowness or blueness.

Вязкость, выход сока, общее количество растворимых сухих веществ, pH и цвет (*L**, *a**, *b**) представлены как средние значения ± стандартные отклонения, где n = 3. Одна и та же буква – показатель отсутствия существенного различия при $p < 0,05$ (тест Тьюки). HSD – существенное различие; *L** – степень светлоты и темноты; *a** – степень красноты/зелености; *b** – степень желтизны/синевы.

Table 4. Effect of different incubation temperatures on viscosity, juice yield, total soluble solids, pH, and color of mango puree liquefied with 2% enzyme during 2 h

Таблица 4. Влияние температуры инкубации на вязкость, выход сока, общее количество растворимых сухих веществ, pH и цвет пюре манго, подвергавшегося разжижению в течение 2 ч

Analysis	Temperature, °C					
	Control (room temperature at 25°C)	40	45	50	55	60
Viscosity, cP	1090.47 ± 51.20^{a}	957.73 ± 39.50^{ab}	768.77 ± 58.80^{c}	776.03 ± 79.70^{c}	789.90 ± 71.80^{bc}	786.93 ± 81.70^{bc}
Juice yield, g	83.91 ± 0.69^{b}	86.76 ± 0.27^{a}	87.17 ± 0.44^{a}	87.13 ± 0.40^{a}	86.97 ± 0.18^{a}	87.09 ± 0.14^{a}
Total soluble solids, °Brix	14.83 ± 0.25^{b}	15.80 ± 0.10^{a}	15.87 ± 0.06^{a}	15.73 ± 0.06^{a}	15.67 ± 0.06^{a}	15.67 ± 0.11^{a}
pH	3.77 ± 0.18^{b}	4.18 ± 0.12^{a}	4.18 ± 0.07^{a}	4.17 ± 0.09^{a}	4.20 ± 0.07^{a}	4.19 ± 0.11^{a}
*L**	54.85 ± 0.45^{a}	51.74 ± 0.40^{b}	50.90 ± 0.35^{bc}	50.79 ± 0.67^{bc}	49.43 ± 1.05^{cd}	48.87 ± 0.72^{d}
*a**	14.29 ± 0.12^{b}	15.33 ± 0.09^{a}	15.26 ± 0.23^{a}	15.23 ± 0.23^{a}	13.87 ± 0.25^{b}	13.75 ± 0.35^{b}
*b**	72.40 ± 0.14^{c}	74.47 ± 0.54^{a}	74.00 ± 0.22^{ab}	73.60 ± 0.16^{b}	71.00 ± 0.40^{d}	70.61 ± 0.14^{d}

Data on viscosity, juice yield, total soluble solids, pH, and color (*L**, *a**, *b**) are means ± standard deviations, where n = 3. For each row, superscripts of the same letter are not significantly different at $p < 0.05$, as measured by the Tukey HSD Test. HSD – honest significant difference; *L** – degree of lightness and darkness; *a** – degree of redness or greenness; *b** – degree of yellowness or blueness.

Вязкость, выход сока, общее количество растворимых сухих веществ, pH и цвет (*L**, *a**, *b**) представлены как средние значения ± стандартные отклонения, где n = 3. Одна и та же буква – показатель отсутствия существенного различия при $p < 0,05$ (тест Тьюки). HSD – существенное различие; *L** – степень светлоты и темноты; *a** – степень красноты/зелености; *b**– степень желтизны/синевы.

decreased at higher incubation temperatures, namely from 1090.47 ± 51.20 cP at room temperature of 25°C to 87.17 ± 0.44 cP at 45°C. Similar results were obtained by Domingues *et al.*, who studied the enzymatic treatment of passion fruit juice [24]. They also used pectinase and reported optimal viscosity reduction at 50°C.

The juice yield significantly increased at higher incubation temperatures, particularly from 83.91 ± 0.69 g at room temperature to 25 at 40°C. Increases in yield were also observed at 45, 50, 55, and 60°C but the differences were not significant. As discussed earlier, pectinase is responsible for the degradation of pectic substances in the homogenized mango pulp, which releases free water and, in turn, results in higher juice yield [24]. Our results were consistent with those of Domingues *et al.*, who observed optimum pectinase activity at around 50°C, the temperature at which a significant increase in juice yield occurred [24].

Table 4 shows no significant increase in the content of total soluble solids at higher incubation temperatures. Although some increases were still observed – due to the breakdown of mango pulp tissues and a release of nutritional components from the cells – they were not statistically significant [16].

Higher incubation temperatures caused a significant increase in the pH, namely from 3.77 ± 0.18 at room temperature (25°C) to 4.18 ± 0.12 at 40°C. No significant increase was observed after that, as shown in Table 4. The increase in pH could be due to the release of carboxyl groups from pectin molecules as pectin was broken down by pectinase. This was also reported by Sayed *et al*. in their study of enzymatic liquefaction of mango pulp [21].

Optimization of the enzyme-liquefied mango puree. The enzymatic liquefaction parameters were optimized according to the viscosity and juice yield of the end product. We chose pectinase (Pectinex Ultra SPL) over cellulase, Fungamyl, and Termamyl due to its ability to significantly decrease viscosity and increase juice yield (1233.60 ± 118.80 cP and 80.78 ± 2.80 g, respectively).

The optimal enzyme concentration was 2.0% v/w, since it significantly decreased viscosity to 782.30 ± 27.10 cP. Similarly, the incubation time of 2.0 h significantly decreased viscosity and increased the juice yield. At a longer time of 2.5 h, the puree became unappealing, with a darker and more dull shade and a distinct cooked aroma. The optimal incubation temperature was 45°C as it produced a significant decrease in viscosity as well. Higher temperatures (50°C onwards) gave the puree a cooked aroma.

Proximate analyses. As shown in Table 5, the moisture contents in the fresh mango puree and in the optimized enzyme-liquefied mango puree were 81.70 ± 0.01 and 82.26 ± 0.01%, respectively. The enzyme-liquefied sample had higher moisture due to the action of pectinase, which degraded pectin in the cells of the mango pulp. As a result, free water was released into the system from the cells, reducing their water-holding capacity and thus increasing the moisture content. Surajbhan *et al.* reported similar results in their study of the effect of pectinase on fruits [25]. The moisture contents of both fresh and enzyme-liquefied mango purees in our study were similar to the moisture content of fresh mango fruits reported by Jideani *et al.*, with an average of 80.00% (Table 5) [26].

The ash content was higher in the optimized enzyme-liquefied mango puree at 0.470 ± 0.002%, as compared

Table 5. Proximate analyses and water activity of fresh mango puree and optimized enzyme-liquefied mango puree

Таблица 5. Экспресс анализ и активность воды свежего и оптимизированного ферментативно сжиженного пюре манго

Analysis	Fresh mango puree	Optimized enzyme-liquefied mango puree
Moisture, %	81.70 ± 0.01^{b}	82.26 ± 0.01^{a}
Ash, %	0.310 ± 0.001^{b}	0.470 ± 0.002^{a}
Crude protein, %	3.20 ± 0.02^{a}	2.10 ± 0.01^{b}
Crude fat, %	0.50 ± 0.01^{b}	0.80 ± 0.01^{a}
Crude fiber, %	0.90 ± 0.01^{a}	0.50 ± 0.01^{b}
Water activity, A_w	0.890 ± 0.001^{b}	0.980 ± 0.001^{a}

Data on the contents of moisture, ash, crude protein, crude fat, and crude fiber in fresh mango puree and optimized enzyme-liquefied mango puree are means ± standard deviations, where n = 3. For each row, superscripts of the same letter are not significantly different at $p < 0.05$, as measured by the Tukey HSD Test. HSD – honest significant difference.

Содержание влаги, золы, сырого белка, сырого жира и сырой клетчатки в свежем пюре манго и оптимизированном ферментативно сжиженного пюре представлены как средние значения ± стандартные отклонения, где n = 3. Для каждой строки одна и та же буква в верхнем индексе – показатель отсутствия существенного различия при $p < 0,05$ (тест Тьюки). HSD – существенное различие.

to the fresh mango puree at 0.310 ± 0.001% (Table 5). The ash content analysis is used to determine the amount of minerals in fresh food. The enzyme-liquefied mango puree had a larger amount of minerals because the enzyme pectinase facilitated the breakdown of tissues of the mango pulp, releasing mineral components into the system [16].

The crude protein content in the fresh mango puree was higher than that in the enzyme-liquefied mango puree at 3.20 ± 0.02 and 2.10 ± 0.01%, respectively (Table 5). Such a significant decrease in protein resulted from its thermal degradation. In fresh fruits, proteins usually denature at temperatures above 45.0°C, and the duration of heating also contributes to the degree of denaturation [27]. The use of viscosity-reducing enzymes could be particularly helpful in liquefying mango pulps and obtaining low-viscosity juice from them since these commercial enzymes require a low reaction temperature.

The fat content was higher in the optimized enzyme-liquefied mango puree (0.80 ± 0.01%) than in the fresh mango puree (0.50 ± 0.01%), as shown in Table 5. This could again be caused by the action of pectinase, which degraded the cell wall structures held by pectin. As the cell walls collapsed and separated, nutritional components, including fat, were released into the system from the interior of the mango pulp cells [16].

The crude fiber content in the fresh mango puree was higher than that in the optimized enzyme-liquefied mango puree, at 0.90 ± 0.01 and 0.50 ± 0.01%, respectively (Table 5). The significant decrease in fiber resulted from the hydrolysis of pectic substances (fibers) present in the cell walls of the homogenized mango pulp [13, 14].

Lastly, the water activity was found to be at 0.890 ± 0.001% for fresh mango puree and at 0.980 ± 0.001% for optimized enzyme liquefied mango puree. Water activity measures the amount of free water available for reactions. The enzyme-liquefied mango puree was treated with pectinase, which hydrolyzed pectin-containing substances in the mango puree cells. Pectin degradation also reduced a high water-holding capacity of these pectin-containing substances. Free water was then released into the system, increasing the water activity of the mango puree [13, 14].

Carotenoid content. The total carotenoid content in the optimized enzyme-liquefied mango puree was significantly lower than that in the fresh mango puree, at 174.15 ± 0.04 and 326.04 ± 0.02 μg/100 g, respectively. This was caused by the heat treatment employed in enzymatic liquefaction, which degraded heat-sensitive thermolabile carotenoids in the mango pulp [28].

Conclusion

Mango is one of the most popular tropical fruits. Consumed widely in many countries, it has desirable attributes, such as fragrant aroma, attractive rich color, sweet taste, and health-promoting qualities. Mango is one of very few fruits that can be used at almost every step of its maturation. It can be eaten fresh on its own or used in cooking various foods. Our research focused on an understudied species of mango called *Mangifera laurina* Blume. To date, there is no published literature on its characterization.

Firstly, we optimized the parameters for enzymatic liquefaction of the mango fruit, such as enzyme type (Pectinex Ultra SPL, Celluclast, Fungamyl, and Termamyl), enzyme concentration (Pectinex Ultra SPL tested at 0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, and 4.0% v/w), incubation time (0.0, 0.5, 1.0, 1.5, 2.0, and 2.5 h), and incubation temperature (25.0, 40.0, 45.0, 45.0, 50, 55.0, and 60°C). For each of the parameters, the resulting mango puree was physicochemically analyzed for viscosity, juice yield, total soluble solids, pH, and color. Based on their effectiveness in reducing viscosity and increasing juice yield, the optimized parameters were set at 2.0% of Pectinex Ultra SPL, with an incubation time of 2.0 h at 45°C. These parameters produced

mango puree with the lowest viscosity and the highest juice yield without much affecting total soluble solids, pH, or color.

Secondly, we conducted proximate analyses on both fresh and optimized enzyme-liquefied mango purees to determine their moisture, ash, crude protein, crude fat, crude fiber, and water activity. This was done to characterize the mango and also to compare the fresh and the enzyme-liquefied mango purees. We found that the enzyme-liquefied mango puree had higher moisture and ash contents, as well as higher water activity. However, its contents of crude protein, fat, and fiber were lower than in the fresh mango puree. Since mango fruits are known for their health benefits due to high carotenoid contents, we also carried out the antioxidant analysis. According to the results, the enzyme-liquefied mango puree had a lower total carotenoid content than the fresh mango puree.

Contribution

The authors were equally involved in writing the manuscript and are equally responsible for plagiarism.

Conflict of interest

The authors declare no potential conflict of interest with respect to the research, authorship, and/or publication of this article.

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Авторы в равной степени участвовали в написании рукописи и несут равную ответственность за плагиат.

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