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Short Communication: Extraction and characterization of pectin from ripe and unripe mango (*Mangifera indica*) peel

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Abstract. *Shaibu CO, Dinshiya J, Shaibu VE. 2022. Short Communication: Extraction and characterization of pectin from ripe and unripe mango (*Mangifera indica*) peel. Asian J Nat Prod Biochem 20: 16-20.* Pectin is a heteropolysaccharide present in the cell walls of different plants at different concentrations with widespread applications. This work aimed at extracting and characterizing pectin from ripe and unripe mango peel to investigate the effect of ripeness on the yield and quality of mango pectin. To obtain the optimum extraction condition, pectin was extracted at varying temperatures, time and pH using 0.1 N HCl as the extraction solvent. The maximum yield of pectin was found to be 22.67% for ripe mango peel and 21.90% for unripe mango peel. The optimum extraction conditions were found to be 90ºC, 60mins and pH 1.5. The pectin extracted using the optimum extraction conditions was then characterized. The moisture content, ash content, methoxyl content, equivalent weight, anhydrouronic acid and degree of esterification of the ripe mango peel pectin were found to be 8.76±0.08%, 10.12±0.47%, 9.17±0.27%, 883.07±13.85 g, 72.45±0.59 and 72.52±0.09% , respectively. In contrast, those of unripe mango peel pectin were found to be 8.13±0.13%, 9.12±0.34%, 8.83±0.19%, 823.38±14.07 g, and 71.56±0.34%, 70.34±0.38%, respectively. This study showed that ripe mango peel might be more suitable for use as a pectin source. However, pectin extracted from ripe and unripe mango peel could be considered an alternative source of pectin in food processing, pharmaceutical industries, and various places of pectin application.

Keywords: Characterization, extraction, mango, pectin, yield

INTRODUCTION

According to research by Singh et al. (2021), over 2 billion tonnes of agro-waste are generated annually worldwide. This volume of waste is capable of causing environmental and health hazards. Therefore, their reuse has been strongly advocated to avert undesirable effects of their accumulation on the environment and maximize benefits from their utilization (Harshwardhan and Upadhyay 2017).

Mango (*Mangifera indica* L.*)* belongs to the family Anacardiaceae with over 1,365 varieties worldwide (Mubarik et al. 2020). Mangoes have been reported to contain high vitamin C, fiber, and pectin (Mubarik et al. 2020). In 2017, Mango production was reported to be about 47 million tonnes per annum (Altendorf 2017). In addition, 40-50% of the fruit constitutes agro-waste (Rai et al. 2020) which can serve as a good source for pectin production.

Pectin belongs to the family of heteropolysaccharides primarily found in the primary cell walls of terrestrial plants (Maxwell et al. 2012; Hamed and Mustafa 2018; Serna and Ayala 2020). It is constituted of 4 polymers rich in D-galacturonic acid (GalA) and containing significant amounts of L-rhamnose (Rha), D-arabinose (Ara), and D-galactose (Gal) (Fissore et al. 2012).

Agro-industrial wastes utilized for pectin production include fruit wastes such as sweet melon (Rahmani et al. 2020), kiwifruit pomace (Yuliarti et al. 2015), papaya peels (Koubala et al. 2014), lemon, grapefruit, orange, avocado, (Bamba et al. 2020), sugar beet (Maxwell et al. 2016).

Pectin is highly valued as a functional food ingredient and has wide pharmaceutical/medicinal applications, including drug delivery, gene delivery, wound healing, and tissue engineering (Maran et al. 2013; Wicker et al. 2014).

Extraction of pectin usually involves hydrolysis of pectin macromolecules under controlled temperature, pH, and time. Temperature, pH, extraction solvent, and extraction time have shown significant effects on pectin yield (Kliemann et al. 2009; Sharma et al. 2013). Acid extraction seems to be the most widely used method, although other methods like the use of enzymes, microwave, subcritical water, and high pressure have been employed in the extraction of pectin (Zoghi et al. 2021)

This study aimed at extracting and characterizing pectin from ripe and unripe mango peel to investigate the effect of ripeness on pectin yield and quality from mango.

MATERIALS AND METHODS

Materials

All the chemicals used were of analytical grade and included Sodium chloride (NaCl), distilled water, ethanol (C_2H_5OH), Hydrochloric Acid (HCl), phenol red, and sodium hydroxide (NaOH).

Equipment

Mortar and pestle, Beakers, Conical flasks, Spatula, Filter paper (Whatman No. 1), Masking tape, Separator funnel, Digital analytical weighing balance, Spectrophotometer (JENWAY: 6305), Refrigerator, Thermostatic Water Cabinet (MODEL: HH - W420), Micropipette, Test tubes and Test Tube Racks, Centrifuge (MODEL: 80-2B), and Sample Bottles.

Sample collection

Both ripe and unripe Mango fruit were collected from Federal University Wukari farmland, Taraba state, Nigeria, located on $7^0 50' 34.0'' N\ 9^0 46' 19.1'' E$.

Methods

Sample preparation

Ripe and unripe mango fruits (*M. indica*) were peeled, chopped, and blanched in a water bath at 80ºC for 5 minutes. The blanched sample was then oven-dried at 60ºC for 72 hours and ground into fine powder. It was then sieved through a 60mm to 80mm mesh sieve and stored in a separate air-tight container until it was required for further analysis.

Note: The decree of ripeness of the mango used in this study was 4 for unripe and 8 for ripe using a scale of 1 to 10, with 1 as most unripe and 10 as most ripe.

Pectin extraction

Pectin extraction was performed using 0.1 N HCl solvent at different extraction temperatures (70, 80, and 90ºC), extraction times (30, 60 and 90 mins), and pH (1.5, 2.5, and 3.0). 10 g of the powdered ripe mango peel was macerated in 250 mL extraction solvent, and the pH was adjusted with 0.1 N HCl and NaOH. Afterward, the mixture was heated in a water bath for the specified time. The mixture was filtered through a cheesecloth and pressed to recover the extract, pectin was then precipitated from the mixture using 98% ethanol in the ratio of 1:2 (1 part extract and 2 parts ethanol) kept at room temperature overnight. The precipitated pectin was then filtered through Whatman No.1 (filter paper) and washed with 70% ethanol (v/v) and 80% ethanol (v/v) to remove the soluble impurities. The extracted pectin was then dried at 60ºC for 24 hours in an oven. The same procedure was repeated for unripe mango peel.

Determination of % yield

Pectin yield was calculated using the formula:

$$\frac{\text{Weight of pectin}}{\text{Weight of sample used}} \times \frac{100}{1}$$

Pectin characterization

Ash content: Ash content of pectin was determined by Ranganna's method (Ranganna 1986). A 1.2 g pectin (sample) was weighed, the sample was ignited slowly in a crucible and then heated for 3-4 hours at 600ºC, then the crucible was taken into the desiccators and allowed to cool.

Ash content was determined using the equation:

$$\text{Ash\%} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$$

Moisture content: The moisture content of pectin was determined by Ranganna's method (Ranganna 1986). A 1 g pectin sample was weighed and placed into a metal dish. The sample was dried in an oven for 5 hours at 100°C and then cooled in a desiccator and weighed.

The moisture content was determined using the equation:

$$\text{Moisture content (\%)} = \frac{\text{Weight of residue}}{\text{Weight of sample}} \times 100$$

Equivalent weight: Equivalent weight was determined by Ranganna's method (Ranganna 1986). A 0.5 g pectin was measured into a 250 mL conical flask, and 5 mL ethanol was added, 1 g of sodium chloride and 100 mL of distilled water were added to the solution, after which 6 drops of phenol red were added. The mixture was titrated against 0.1 N NaOH. A purple color indicated the titration endpoint. The neutralized solution was stored for the determination of methoxyl content and anhydrouronic acid content.

$$\text{Equivalent Weight} = \frac{\text{Weight of sample} \times 1000}{\text{ml of alkali} \times \text{Normality of alkali}}$$

Methoxyl content (MeO): The methoxyl content is an important factor in controlling the setting time of pectins. MeO was determined using Ranganna's method (Ranganna 1986). First, the 25 mL sodium hydroxide (0.25 N) was added to the neutral solution obtained from the determination of equivalent weight. Next, the solution was stirred thoroughly and kept at room temperature for 30 mins. After 30 min, 25 mL of 0.25 N hydrochloric acid was added and titrated against 0.1 N NaOH.

Methoxyl content was calculated by using the following formula:

$$\text{Methoxyl content\%} = \frac{\text{ml of alkali} \times \text{Normality of alkali} \times 3.1}{\text{Weight of sample}}$$

Total anhydrouronic acid content (AUA): Total AUA of pectin was obtained mathematically using the following formula:

$$\text{AUA} = \frac{176 \times 0.1z \times 100}{W \times 1000} + \frac{176 \times 0.1y \times 100}{W \times 1000}$$

Where: Molecular unit of AUA (1 unit) = 176 g, z = mL (titre) of NaOH from equivalent weight determination, y = mL (titre) of NaOH from methoxyl content determination and w = weight of sample.

Degree of esterification (DE)

The DE of pectin was measured based on methoxyl content (MeO) and AUA and calculated using the formula:

$$\text{Degree of esterification (DE) \%} = \frac{176 \times \%\ \text{MeO}}{31 \times \%\text{AUA}} \times 100$$

Statistical analysis

All data are represented as means ± SD. Statistical analyses were performed using the Independent samples t-test on Statistical Package for Social Sciences (SPSS) version 20.

RESULTS AND DISCUSSION

Percentage (%) yield of pectin at pH 1.5, 60 minutes, and varying temperature

The percentage yield of pectin at 60 mins, pH 2.0 and different temperature is shown in Figure 1. The percentage yield of pectin increased with increase in temperature for both mango peels, pectin from the ripe mango was shown to be higher than that of the unripe mango peel.

Percentage (%) yield of pectin at 90°C, 60 minutes, and varying pH

The percentage yield of pectin at 60 mins, temperature 90ºC, and different pHs of 1.5, 2.0, and 3.0 are shown in Figure 2. Pectin yield for ripe and unripe mango peel decreased with an increase in pH. The yield of the ripe mango peel was found to be higher than that of the unripe mango peel.

Percentage (%) yield of pectin at 90ºC, pH 1.5, and varying time

Figure 3 shows the percentage yield of pectin at 90ºC, pH 1.5 and different times. There was an increase in pectin yield for both ripe and unripe mango peel from 30 mins to 60 mins extraction time. However, there was a decrease from 60 mins to 90 mins. The pectin yield of the ripe mango peel was higher when compared to that of the unripe mango peel.

Discussion

Pectin yield

This study considered pectin extraction from ripe and unripe mango peel to investigate the effect of ripeness on the yield and quality of pectin produced from mango. To obtain the optimum extraction condition, pectin was extracted at various temperatures (70ºC, 80ºC, and 90ºC), pH (1.5, 2.0, and 3.0), and time (30 mins, 60 mins, and 90 mins). The optimum extraction conditions shown in this study were pH 1.5, 60 mins extraction time, and 90ºC. Pectin extracted using the optimum extraction condition was used in characterization. Studies on the effect of pH, temperature, and time showed that the total yield of pectin for both ripe and unripe mango peel increased with a decrease in pH from pH 3.0 to pH 1.5. This agrees with the work of Hamidon and Zaidel (2017), who reported that the yield of pectin increased with a decrease in the pH of the extractant while working on potato peel residue. Pectin yield also increased with an increase in extraction time, however, further increasing the extraction time to 90mins showed a decrease in pectin yield. Thus, the optimum time of the extraction for the maximum yield of mango peel pectin was found to be 60 mins. Similar results were reported on pectin extracted from mandarin orange peels by Koubala et al. (2008). The effect of extraction temperature on the yield is shown in Figures 2 and 3. The yield of pectin increased significantly with the increase in extraction temperature. Similar observations were reported for dried mango peel (Sangheetha et al. 2018).

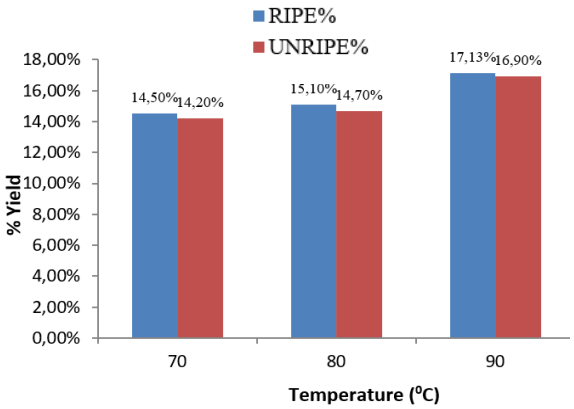

Figure 1. % Yield of pectin at pH 1.5, 60 minutes, and varying temperature

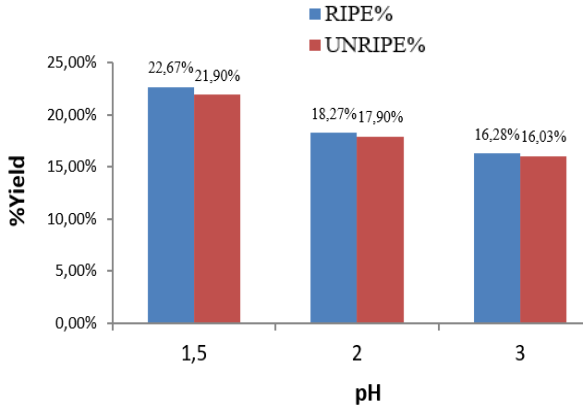

Figure 2. % Yield of pectin at 90°C, 60 minutes, and varying pH

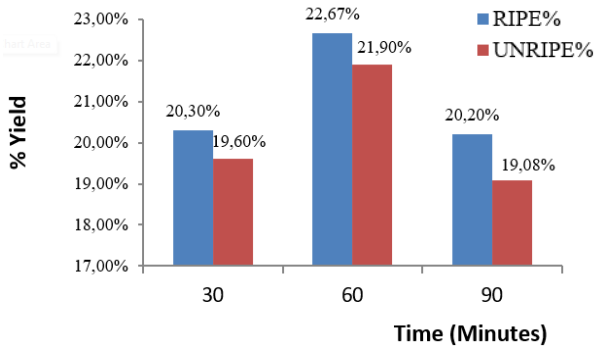

Figure 3. % Yield of pectin at 90ºC, pH 1.5, and varying time

The maximum yield was 22.67% and 21.94% for ripe and unripe mango peel, respectively. Higher pectin yield for ripe mango peel may result from a higher concentration of pectin due to the maturation of the fruit. On the other hand, pectin concentration may decrease with an increase in ripeness due to pectin hydrolysis by pectinase (Prasanna et al. 2007). This is as opposed to the findings of Castillo-Israel et al. (2015), who had higher values for pectin yield from an unripe banana than from the ripe banana peel. However, the yield of pectin from both ripe and unripe mango peel compares well to the 2.93-25.80% pectin yield recorded by Bamba et al. (2020) from orange, lemon, grapefruit, and avocado and is higher than those reported by Yapo and Koffi (2006), for passion fruit pectin (7.5%), and ambarella peel pectin (10-13%).

Characterization of mango peel pectin

Ripe mango pectin had significantly higher values for yield moisture content, ash content, equivalent weight, and degree of esterification (Table 1). There was no significant difference in methoxyl content and anhydrouronic acid. The moisture content of ripe and unripe mango peel pectin compares well with 10.59 and 11.68 reported for mango peel and watermelon pectin, respectively, by Rury et al. (2017). However, the moisture content of the unripe mango pectin was significantly lower than that of the ripe mango peel.

Ash content measures the total amount of minerals present within a food. The ash content of the pectin isolated in this work was found to be as high as 9-11%. Nazaruddin et al. (2011) reported that the maximum value needed for the quality gel is 10%. This shows that pectin extracted from both mango peels could be a good source for producing quality gel.

There was no significant difference in the methoxyl contents of pectin extracted from ripe and unripe mango peel. Methoxyl content of commercial pectins generally varies from 8-11% and can form high sugar gels (>65% sugar) (Castillo-Israel et al. 2015). Therefore, this study's values obtained from ripe and unripe mango peel compare favorably with commercially available pectin.

The extracted pectin's equivalent weight was 883.07±13.85 g and 823.38±14.07 g for ripe and unripe mango peel, respectively. However, this indicates that the ripe mango pectin has a significantly higher gel-forming ability than the unripe. Values obtained in this study were lower than those of apple pomace pectin (833.33-1666.30), Kumar and Chauhan (2010), but higher than those of cocoa husk pectin (510.68-645.19), as reported by Ramli and Asmawati (2011).

Anhydrouronic acid (AUA) is essential to determine the purity and degree of esterification and evaluate physical properties (Ranganna 1986). It indicates the purity of extracted pectin. Pectin with an AUA of 65% or more is considered pure (Nazaruddin et al. 2011). Values for AUA (%) obtained in this study are greater than 65%, indicating that they are pure.

The degree of esterification (DE) obtained in this study was within the 60-90% range, generally found in plant tissues (Shaha et al. 2013). Pectins could be classified as rapid-set (DE >72%) and slow-set (DE: 58-65%). This describes the rate of gel formation. Pectin from ripe and unripe mango peels had DE values less than 75% but greater than the range for slow-set pectins, this shows that they may be classified as rapid-set pectin. Values obtained were lower than those reported by Wang et al. (2014), who reported DE values of 76.30 and 83.41% in pectin from the fruit peel of *Citrus maxima* and apple pomace, respectively.

Table 1. Pectin characterization

Parameter	Ripe (%)	Unripe (%)	[A] Mean differences	P values of mean differences
Yield	22.67	21.90	0.77	0.002[c]
Moisture content	8.76±0.08	8.13±0.13	0.63	0.021[c]
Methoxy content	9.17±0.27	8.83±0.19	0.34	0.668[b]
Ash content	10.12±0.47	9.12±0.34	1.00	0.026[c]
Equivalence weight	883.07±13.85g	823.38±14.07	59.69	0.003[c]
Anhydrouronic acid	72.45±0.59	71.56±0.34	0.89	0.148[b]
Degreee of esterification	72.52±0.09	70.34±0.38	2.18	0.007[c]

Note: Values represent Mean ± standard deviation. [a]= indicates the mean values of parameters for ripe seeds minus the mean values of unripe ones. [b]= Mean difference is not significant [c]=Mean difference is significant at P<0.05

The equivalent weights of the extracted pectin were found to be high, indicating that they have a low partial degradation property. The degree of esterification, equivalent weight, and ash content of ripe mango pectin were significantly higher than that of the unripe mango peel pectin. There was no significant difference in methoxyl and anhydrouronic acid content, thus indicating that the quality of pectin obtainable from ripe mango peel is comparatively better than pectin obtained from unripe mango peel in terms of gel formation and purity. However, both ripe and unripe mango peel pectin is comparable to apple pomace and citrus, thus, indicating the possible use of mango peel as an alternative source in commercial pectin production and for industrial, pharmaceutical, medical, and agricultural applications.

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