Extraction and Characterization of Pectin From Selected Fruit Peel Waste

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Abstract- *Pectin can be used as food and pharmatecitical additives. This study investigated the effect of temperature, time and pH on the yield and physicochemical characteristics of pectin extracted from banana and mango peels. Pectin was extracted using acid extraction method. The experimental design was performed using Central Composite Design of Response Surface Methodology for parametric optimization. The optimum temperature, time and pH for the extraction of pectin for both peels were determined to be 82^{o}C, 105min and 2 respectively. The yields of pectin under these optimum conditions were found to be 11.31% and 18.5% for banana and mango peel respectively. Temperature, extraction time and pH showed a significant ($p < 0.05$) effect on the pectin yield. The equivalent weight, methoxyl content, anhydrouronic acid content, degree of esterification, moisture content and ash content of the banana and mango peel pectin obtained under the optimum conditions were found to be 925.01g/mol, 6.09%, 53.6%, 64.5%, 8.94%, 0.47% and 895 g/mol, 8.89%, 70.65%, 72.17%, 8.82%, 0.915% respectively. In general, the finding of the study shows that pectin, being used as food and pharmaceutical additives, can be obtained from banana and mango peel.*

Keywords: *Banana, Central Composite Design (CCD), characterization, mango, pectin, peel*

1. INTRODUCTION

The term pectin was first described and isolated by French scientist named Henry Braconnot in 1825. Pectin is primarily made up of D-galacturonic acid joined by α - (1- 4) glycosidic linkages [1]. As a part of the plant structure, pectin is a complex mixture of block of homogalacturonic acid called 'smooth regions' mixed with blocks of homogalacturonic acid containing many neutral sugars including rhamnose, galactose, arabinose and glucose called 'hairy regions' [2]. Neutral sugars are also present as side chains in different amounts depending on the pectin source and on the extraction method used. A percent of the galacturonic acid residues are generally esterified with methanol. The pectins of a plant can be water-soluble or protopectins [1]. The methods of extraction will vary based on the actual makeup for each particular plant type. For example, protopectins are brought into solution by hot dilute acids. The general makeup of the pectin content varies with ripening of the plant and it is fairly easily brought into solution depending on the plant type [1]. Commercial pectin extraction is mainly from citrus peel and apple pomace, but several other sources exist such as sugar beets, banana peel, mango peel, papaya peel and sunflower heads. Extracted pectin can be categorized into two major categories depending on the percentage of galacturonic acid residues that are esterified with methanol. A degree of methoxylation (DM) greater than 50% is considered high methoxyl pectin and a DM below 50% is considered low methoxyl pectin [3].

Pectin has also been investigated for its usefulness in the pharmaceutical industry. Among other uses it has been considered in the class of dietary fibers known to have a positive effect on digestive processes and to help lower cholesterol [3]. Pectin extracted from various materials can be different in molecular structure (i.e., molecular weight, degree of esterification and acetyl content) and therefore, possesses different functional properties. A valuable by product that can be obtained from fruit peel wastes is pectin. Pectin designates those water soluble pectinic acid of varying methyl ester content and degree of neutralization which is capable of forming gels with sugar and acids under suitable conditions [4].

The suitability of pectin's for different purposes is determined by their character namely, anhydrouronic acid content, methoxyl content and degree of esterification. The fruit peel wastes resulting from the fruit juice processing industry are normally discarded, which are highly perishable and seasonal; it is a problem to the processing industries and pollution monitoring agencies. Thus, these waste materials may create environmental problems, particularly water pollution, since the presence of biomaterials in fruit waste peel such as peel oil, pectin, as well as sugar can stimulate aerobic bacteria to decompose the biodegradable organic matters into products such as carbon dioxide, nitrates, sulfates and phosphates in water. Suitable methods have to be adopted to utilize them for the conversion of the problem into an asset. The main objective of this thesis was (a) to extract and characterize the pectin from banana and mango fruit peel waste and to explore its potential use, (b) to investigate various factors that affect the yield of pectin such as pH, temperature, extraction time and to determine the optimal process conditions for pectin extraction, (c) to characterize the physicochemical properties of pectin isolated from banana and mango peel, (d) to compare the characteristics of two different pectin and to see which one is more suitable for industrial applications.

2. MATERIALS AND METHODS

2.1 Materials

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All chemicals used for extraction process were of analytical grade. Fresh banana and mango peels were collected from some selected hotels, juice processing houses and restaurants in Addis Ababa, Ethiopia.

2.2 Methods

2.2.1 Raw Material Preparation

The fresh fruit peels were segregated according to their type, cut into pieces for easy drying and washed with water three times. Sample drying was carried out in oven at 60°C for 48 hours to obtain easily crushable material. The dried peel was milled in sieve size of 80 meshes and packed in airtight, moisture-proof bag at room temperature and ready to the extraction process.

2.2.2 Experimental Design

Central Composite Design of Response Surface Methodology was used to investigate the effects of three independent process variables namely, temperature (A), extraction time (B) and pH (C) on the response, pectin yields (Y). The variable ranges were selected based on the literature (Table 1) [5]. The star arm (α), known as the arm length of the axial experiments from the center point, was 1.68179. The experiment at the center point of the design was repeated to get a good estimate of experimental error. All the experiments were carried out at random in order to minimize the effect of unexplained variability in the observed responses due to systematic errors.

Significance of the result was set from analysis of variance (ANOVA). The 20 experiments were carried out and data was statistically analyzed by the Design-Expert program to find the suitable model for the pectin yield as a function of the above three variables.

Table 1 Experimental and coded levels of three variables employed for pectin extraction

Variables	Factor coding	Unit	Levels				
			-α	-1	0	+1	+α
Extraction Temperature	A	^oC	52.50	60	71	82	89.5
Extraction Time	B	min	44.66	60	82.5	105	120.0
pH	C	-	1.66	2	2.5	3	3.34

The polynomial equation generated by the software is as follows:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_2X_3 + b_{11}X_{11} + b_{22}X_{22} + b_{33}X_{33} \quad (1)$$

where, Y is the dependent variable, b_o is the intercept, b_1 to b_{33} are the regression coefficients and X_1 to X_{33} are the independent variables. The experimental design setup is summarized in Table 2.

2.3 Extraction of Pectin

In this research, method developed by Emaga *et al*., [6] was used for pectin extraction. Fruit waste peel dried and powder was weighed in conical flask and distilled water was added (solid-liquid ratio of 1:29 (w/v). The pectin extraction from mango peel was carried out following procedure adopted by Kertesz [7]. Ground powder mango peels were mixed thoroughly with distilled water in the ratio of 1:40 (w/v) at different pH. The mixture was dissolved by stirring the peel powder in water. Acid ($1M\ H_2SO_4$) was added for maintaining different pH medium as reagents. The mixture was then heated for each different pH medium (1.66 to 3.34) of extraction while stirred at different temperature (52.5 to 89.5^{o}C) and time (44.66 to 120 min) separately in shaking water bath. The hot acid extract was filtered through nylon/muslin cloth. The filtrate was coagulated by adding an equal volume of 96% ethanol and left for 3hourse to allow the pectin to float on the surface. The gelatinous pectin flocculants were then skimmed off. The coagulated pectin was separated by filtration and washed with 70% ethanol to further remove any remaining impurity. The washed pectin was then dried at 35^{o}C in hot air oven overnight to remove the moisture. The ground powder was kept in airtight container.

The pectin yield was calculated using the following equation.

$$\text{Ypec (\%)} = \frac{P}{Bi} \times 100$$

where, Ypec (%) is the extracted pectin yield in percent (%), P is the amount of extracted pectin in g and Bi is the initial amount of powder fruit peel (5g).

2.4 Physicochemical Characterization of Pectin

The dried pectin samples obtained from the two fruit peels were subjected to quantitative test in order determine the following characteristics.

Equivalent weight is used for calculating the Anhydrouronic Acid content and the degree of esterification. It is determined by titration with sodium hydroxide (NaOH) to pH 7.5 using phenol red. Pectin sample (0.5 g) is weighed in a 250 mL conical flask and moistened with 5mL ethanol. Then 1.0g sodium chloride is added to the mixture followed by 100mL distilled water and three drops of phenol red indicator. Finally six drop of phenol red is added and titrated against 0.1N NaOH until the color of the solution is changed to a pink at the end point. The color change was persisting for 30 seconds [8]. Equivalent weight is calculated using the following equation:

$$\text{Equivalent weight} = \frac{\text{Weight of Sample}}{\text{ml of Alkali x Normality of alkali}}\ x\ 1000 \quad (3$$

❖ **Methoxyl content** is an important factor in controlling the setting time of pectin's, the sensitivity to polyvalent cations and their usefulness in the preparation of low solid gels and fibers. Methoxyl content is determined using the neutralized solution obtained during the equivalent weight determination, containing 0.5 g of pectic substance, 25 mL of 0.25N NaOH is added to the neutralized solution used in the equivalent weight determination. The mixture is stirred thoroughly and allowed to stand for 30 min at ambient temperature. Then 25 mL of 0.25NHCl (or an amount equivalent to base added) was added and titrated against 0.1N NaOH to the same end point as before [8]. The percentage content is calculated using the equation below:

$$\text{Methoxyl content (\%)} = \frac{\text{ml of NaOH x Normality of NaOH x 31 x 100}}{\text{weigh of sample(mg)}x1000} \quad (4)$$

where 31 is the molecular weight of methoxyl (CH_3O)

Total anhydrouronic acid content (TAUA) is essential to determine the purity and degree of esterification, and to evaluate the physical properties. By using the values of the equivalent weight and the methoxyl content, the Anhydrouronic acid content is calculated by the expression given below [8]. Total AUA of pectin is obtained by the following formula (Mohamed & Hasan, 1995).

$$\text{AUA}(\%) = \frac{176 \text{ x } 0.1z\text{x}100}{\text{Wx}1000} + \frac{176 \text{ x } 0.1y\text{x}100}{\text{Wx}1000} \quad (5)$$

where molecular unit of AUA (1 unit) = 176g, z = ml (titre) of NaOH from equivalent weight determination, y = ml (titre) of NaOH from methoxyl content determination, W = weight of sample

Degree of Esterification (DE): The degree of esterification of extracted pectin was calculated from methoxyl and anhydrouronic acid content using the following expression [8].

$$\text{Degree of esterification} = \frac{176 \text{ x methoxyl content}(\%)\text{x}100}{31 \text{ x AUA}(\%)} \quad (6)$$

Moisture Content and Ash Content: the moisture and ash content were determined by adopting AOAC [9] method.

Viscosity: Viscosity of pectin solution is measured by SV-10 Model Vibro Viscometer, with viscosity range 0.3-10 Nsm^{-2}. The pectin solutions were prepared from banana and mango peels pectin with the maximum yield.

3. RESULTS AND DISCUSSION

3.1 PECTIN YIELD

The percentage yields of pectin extracted using 1M H_2SO_4, from banana and mango peel powder range from 5.82 to 11.33% and 12.10 to 18.57% respectively.

3.2 Effect of Extraction Process Variables

3.2.1 Effects of Temperature on Pectin Extraction

As shown in Figure 1 the pectin yield is influenced by temperature. The yield increases with the increasing temperature for the mango peel until 82°C. Because increasing the extraction temperature would increase the solubility of the extracted pectin, giving a higher rate of extraction. However, further increase in temperature from 82 to 89.5°C shows a decreasing tendency of pectin yield, since too high temperature would lead to break down of pectin molecules as pectin is composed of α-(1-4) linked units of galacturonic acid or methyl ester resulting in pectin of lower molecular size which is not perceptible with alcohol. However, high temperature encourages energy loss through vaporization and increases the cost of extraction process from the industrial point of view. At lower temperature, the lower viscosity of pectin might cause poor diffusion between the phases that will lead to slower rate of extraction.

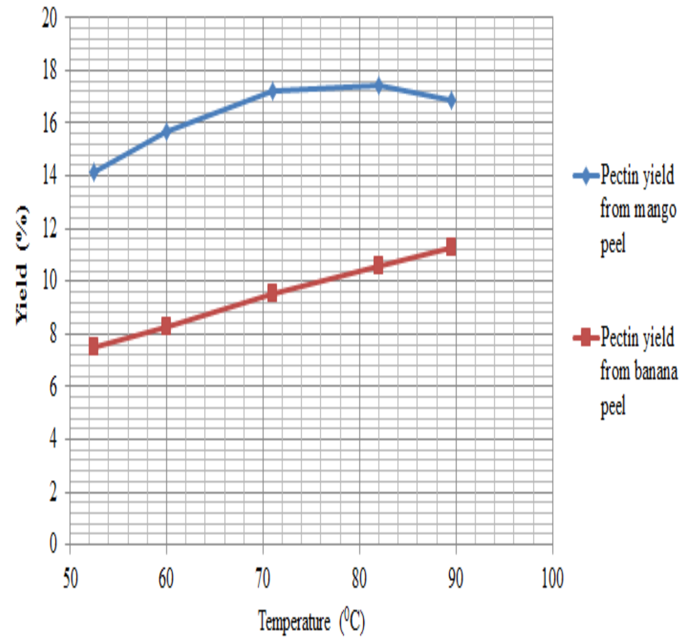


Figure 1 Effect of extraction temperature on the pectin yield

3.2.2 Effect of Extraction Time on the Pectin Yield

The pectin yield increased significantly with the increasing in the extraction time Figure 2 shows that the yield of pectin increases up to 105 minute. A relatively long period of extraction would cause a thermal degradation effect on the extracted pectin, thus causing a decrease in the amount perceptible by alcohol. Besides, the color of the pectin extract became dark brown for longer periods of extraction which in turn required a higher number of alcoholic washing of the precipitate. Also as the extraction proceeds, the concentration of the pectin in the solution will increase and the rate of extraction will progressively decrease; first, because the concentration gradient will be reduced and, secondly, because of the solution becomes more viscous. Generally the result shows that the yield increases with increase in extraction time as the protopectin naturally present in cells takes time to solubilize and go into the solution.

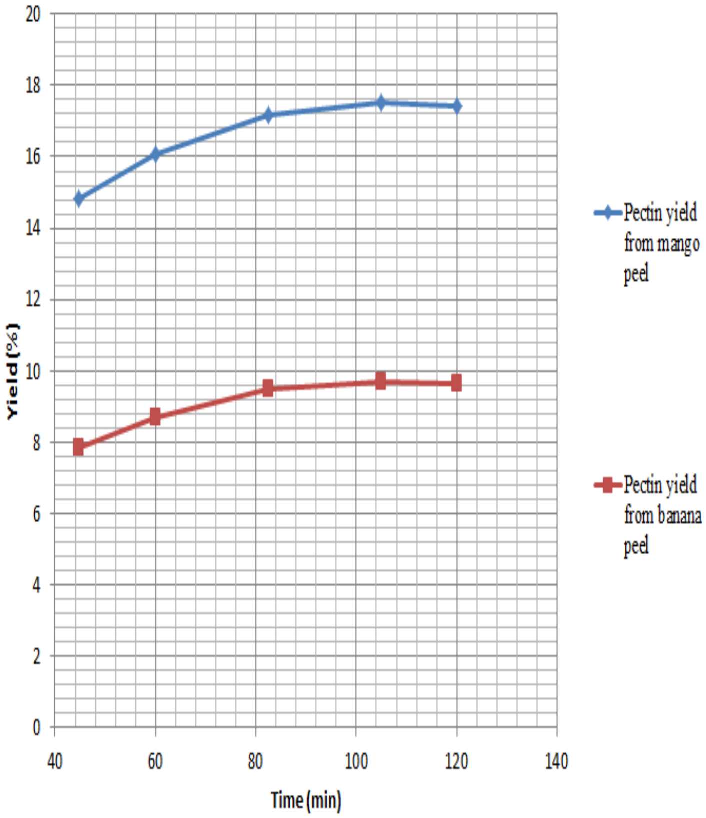


Figure 2 Effect of extraction time on the pectin yield

3.2.3 Effect of pH on Pectin Yield

As shown in Figure 3 the pectin yield decreases with increasing pH. It is evident that pectin yield decrease with increased of pH. Lower pectin yield at higher pH might be due to some pectin that might still be attached to the cell wall components, although pectin molecules can be partially solubilized from plant tissues without degradation in a weak acid solution.

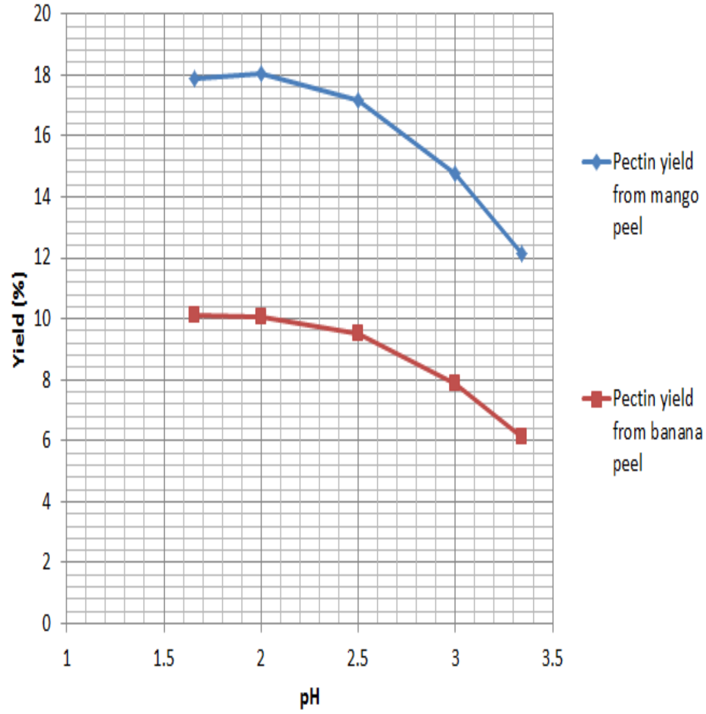


Figure 3 Effect of pH on pectin yield

The model equation that correlates the response (Y) to the extraction process variables in terms of coded factors after excluding the insignificant terms is given by equation (7 and 8).

Mango Peel Pectin:

$$Y = 17.19 + 0.86*A + 0.72*B - 1.63*C - 0.33*AB - 0.28*AC - 0.028*BC - 0.62*A^2 – 0.40*B^2 – 0.80*C^2 \quad (7)$$

Banana Peel Pectin:

$$Y = 9.50 + 1.15 * A + 0.48 * B - 1.09 * C + 0.017*AB + 0.14 *AC – 0.18* BC – 0.070 *A^2 – 0.31 *B^2 – 0.52 *C^2 \quad (8)$$

where, Y - pectin yield, A - temperature, B - extraction time and C - pH. Equations (7 and 8) explain the effect of individual variables (linear and squared) and interactive effects on temperature, extraction time and pH. The results indicated that the yield of pectin is dependent on the linear terms, the quadratic terms and also the interactions of both variables.

The analysis of variance (ANOVA), result of the quadratic regression model, for the mango and banana peels pectin demonstrates that the model are significant with its F-value of 333.66 and 114.05 respectively. There is only a 0.01% chance that a “model F-value” could occur due to noise. The "Lack of Fit F-value" of 2.88 and 2.42 implies the Lack of Fit is insignificant relative to the pure error. Insignificant lack of fit indicates a good fit of the model with experimental data. Multiple regression coefficients R^2 for mango and banana peels pectin calculated from the second degree polynomial equation are R^2= 0.9967 and 0.9904 respectively, indicating that the predicted values are closer to experimental data. This value provides a measure of how much variability in the observed response can be explained by the experimental factors and their interactions. The adjusted R^2 values of mango and banana peels pectin 0.9937 and 0.9817 respectively which ensures a satisfactory agreement of the polynomial model with the experimental data.

Three-dimensional response surface curves were plotted in order to understand the interactions between the variables and the optimum levels of each variable for maximum yield of pectin. Each contour curve presents the effect of two variables on the pectin yield holding the third variable at constant level. The interaction between two variables namely, temperature and time, pH and temperature, time and pH are shown in Figures 4 to 6. Significance of interaction between the corresponding variable is indicated by saddle nature of the contour plots.

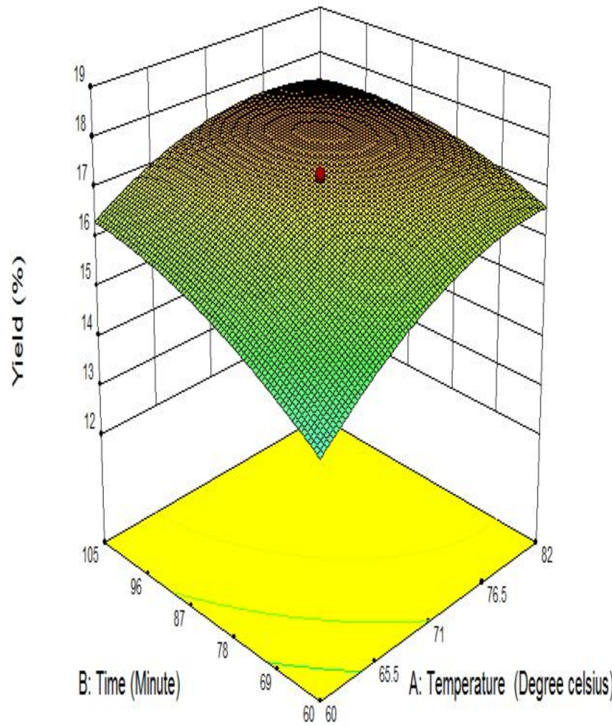
The interaction effects of temperature and time on the yield of pectin from mango and banana peels are show, in the 3D plots and surface contour. The yield observed to be minimal at both lower and higher levels, whereas at intermediate levels, maximum yield is observed in case of mango peel pectin (Figure 4). However, at moderate extraction time and high temperature the pectin yield are increased while at high extraction time the yield is reduced in case of banana peel pectin.

The interaction effects of temperature and pH on pectin yield is shown in Figure 5. At lower and higher levels of both pH and temperature lower yield of pectin is observed. At intermediate values higher pectin yield obtained. Figure 6 represents the interaction between extraction time and pH and its effect on the yield of pectin. At the lower pH and higher time, the pectin yield increases. At higher pH and lower extraction time, the production of pectin yield decreases due to lower acid concentrations to sufficiently hydrolyzed the insoluble pectic constituents.

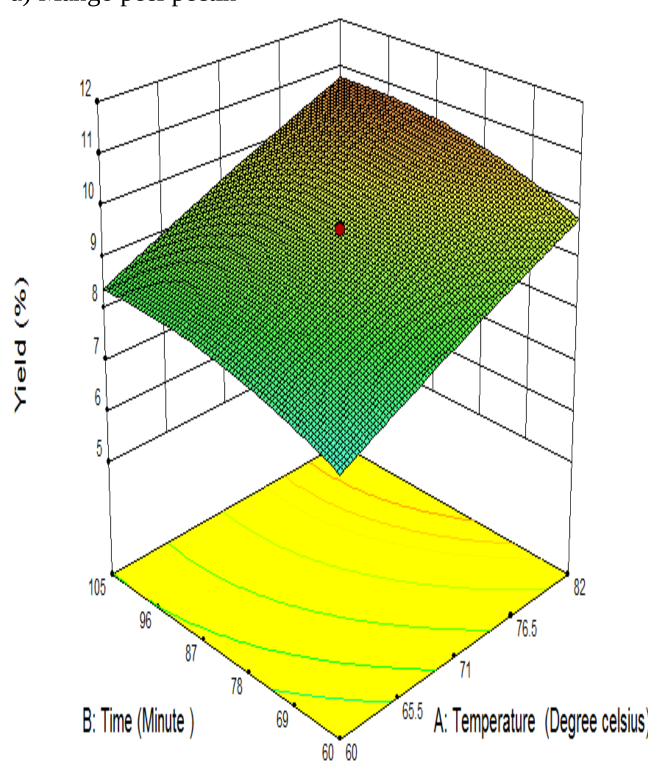
The experimental response and the model predict responses for mango and banana peels pectin are given in Table 2.

Table 2 Experimental design set up of RSM and responses obtained

Run No.	Independent variables			Response	
	Temperature (°C)	Time (mint)	pH	Experimental banana peel Pectin yield (%)	Experimental mango peel pectin yield (%)
1	0	0	0	9.60	16.98
2	-1	1	-1	9.02	16.72
3	-α	0	0	7.51	14.14
4	0	-α	0	7.83	14.81
5	1	1	-1	11.33	18.51
6	1	-1	1	8.62	14.09
7	α	0	0	11.30	16.90
8	-1	-1	1	5.82	12.10
9	0	0	α	6.14	12.13
10	1	-1	-1	9.84	17.65
11	0	0	0	9.62	17.13
12	0	0	0	9.37	17.23
13	0	0	0	9.49	17.32
14	-1	-1	-1	8.02	14.76
15	0	0	-α	10.10	17.86
16	-1	1	1	6.53	14.16
17	0	0	0	9.25	17.26
18	0	0	0	9.63	17.19
19	1	1	1	8.99	14.64
20	0	α	0	9.62	17.42

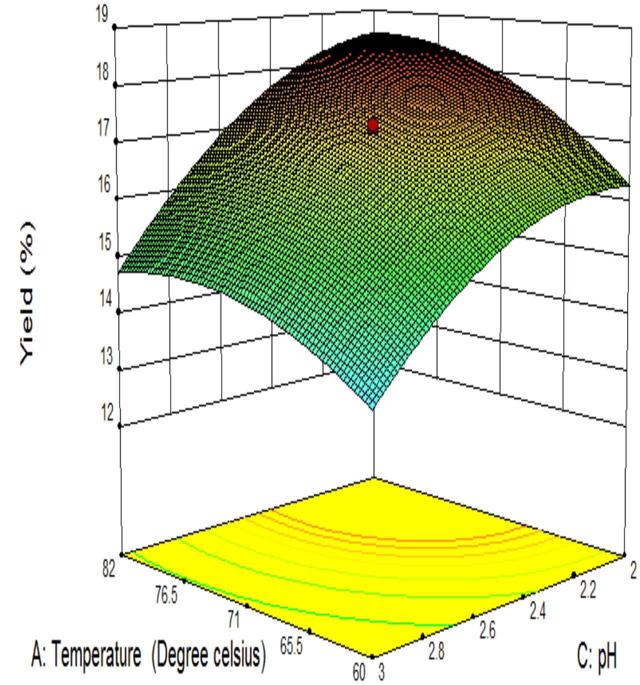


a) Mango peel pectin

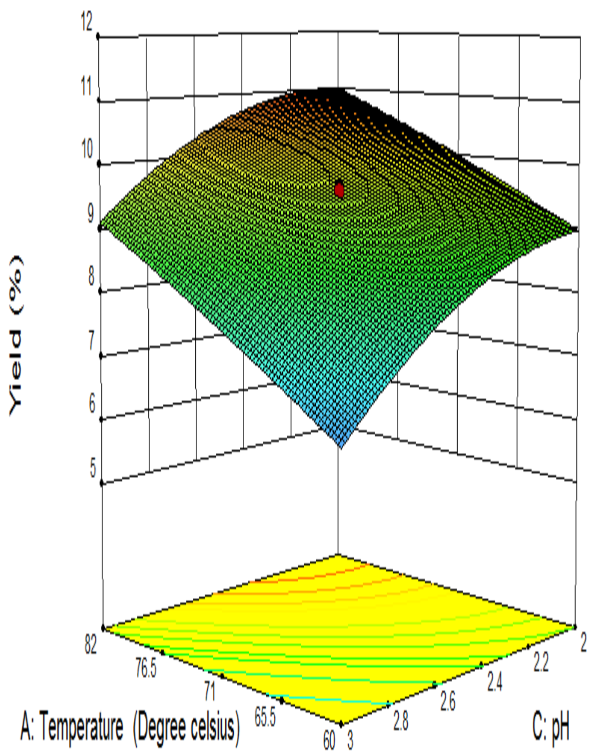


b) Banana peel pectin

Figure 4 3D Effect of temperature and time interaction at fixed pH

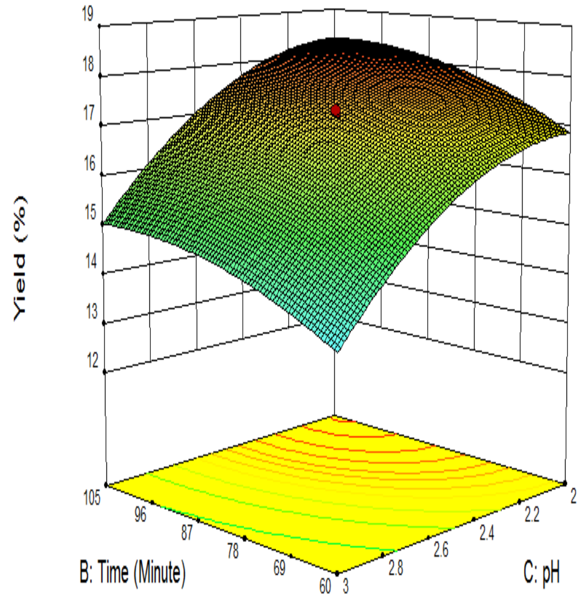


a) Mango peel pectin

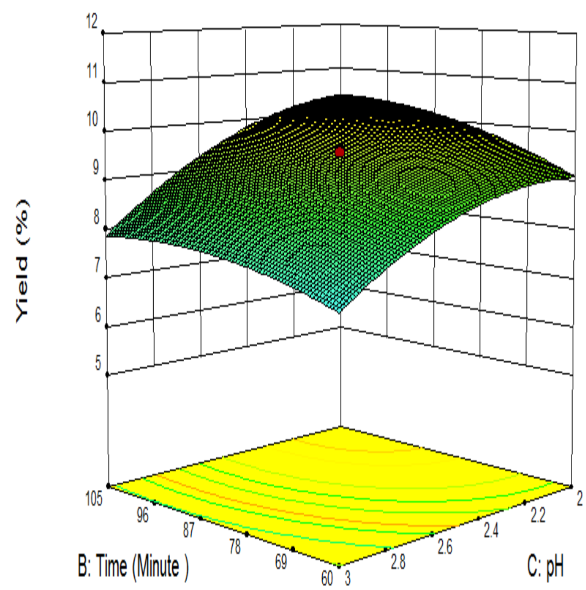


b) Banana peel pectin

Figure 5 3D Effect of temperature and pH interaction at fixed time



a) Mango peel pectin



b) Banana peel pectin

Figure 6 3D Effects of time and interaction at fixed temperature

3.3 Optimization of Process Variables

The three process variables namely, temperature, extraction time, pH and the interaction among the variables affect the yield of pectin. Therefore, it is necessary to optimize the process variables in order to obtain the highest yield using the model regression developed. In the process of optimization criteria were set for the extraction process variables. Using Design Expert 7.0.0, predicted values of desired conditions 82^{o}C extraction temperature, 105 min extraction time and 2pH resulted an optimum value of 18.59% and 11.37% mango and banana peel pectin respectively. In order to verify this prediction, experiments were conducted and the results were comparable with the predicted values. Experimentally determined values of 18.50% and 11.31% mango and banana peel pectin yield respectively, are comparable with the predicted values. Compared to the data obtained from literature, the optimum extraction conditions of mango peel pectin lie in the accepted limits of mango peel extraction conditions. Therefore, this study shows that mango peel waste is a potential source for the extraction of pectin.

3.4 Physicochemical Characterization of Pectin

Characterizations of extracted pectin were carried out for various parameters to evaluate its suitability in food systems. The result shows that the anhydrouronic acid and methoxyl contents were found to be dependent on the pH while equivalent weight depends on extraction time. The extraction time and pH had significance effect on the degree of esterification and moisture content of mango peel pectin. However, all physicochemical characterizations of banana peel pectin were independent of the extraction variables. The physicochemical characterizations of pectin depend mainly on the raw material source and conditions selected for isolation and purification of pectin. Table 3 summarizes the chemical quality characteristics of mango and banana peel pectin.

The maximum value of equivalent weight of banana peel pectin is slightly higher than mango peel pectin. Methoxyl content is an important factor in controlling the setting time of pectin and the ability of the pectin to form gels [10]. The range of reported values of methoxyl contents (8.4 to 9.7) of good quality of mango peels pectin [11].

Table 3 Result for the physicochemical characterization of the extracted pectin under optimum conditions (82^{o}C, 105min and 2pH).

Characteristics	Banana peel pectin	Mango peel pectin
Equivalent weight (g/ml)	925.01	895.00
Methoxyl (%)	6.09	8.89
Total anhydrouronic acid (%)	53.60	70.65
Degree of esterification (%)	64.50	72.17
Moisture content (wwb%)	8.94	8.82
Ash content (%)	0.47	0.915

The anhydrouronic Acid (AUA) indicates the purity of the extracted pectin and its value should not be less than < 65% [12]. In this study the highest AUA content of mango peel pectin was with acceptable limits of pectin purity. There is a significant difference between banana and mango peel pectin in terms of degree of esterification. Based on DE pectin can be classified as low methoxyl pectin with ≤ 50% DE and high methoxyl pectin with > 50% DE. Therefore, in this study indicates that banana and mango peel pectin are categorized as high methoxyl pectin.

The maximum value of moisture content of banana peel pectin was slightly higher than mango peel pectin. Low moisture content is necessary for pectin for safe storage as well as to inhibit the growth of microorganisms that can affect the quality due to the production of pectinase enzymes. The moisture content of the pectin is almost similar to the values of moisture content reported in literature. There is a significant difference between banana and mango peel pectin in terms of ash content. The upper limit of ash content for good-quality pectin is considered to be 10% from the view point of gel-formation [13]. Therefore, with respect to this parameter, the pectin isolated in this study may be considered to be of satisfactorily good quality. The inorganic impurities in pectin were indicated by the ash content. Lower ash content indicates good quality of pectin. The ash content of extracted pectin was close to the reported values. Figure 7 Shows temperature and pH has significant effect on the viscosity of the pectin solution. The viscosity of pectin solution was observed to increase with a decreased of temperature and increased of pH. The viscosity of banana peel pectin was relatively low, compared to the viscosity of mango peel pectin.

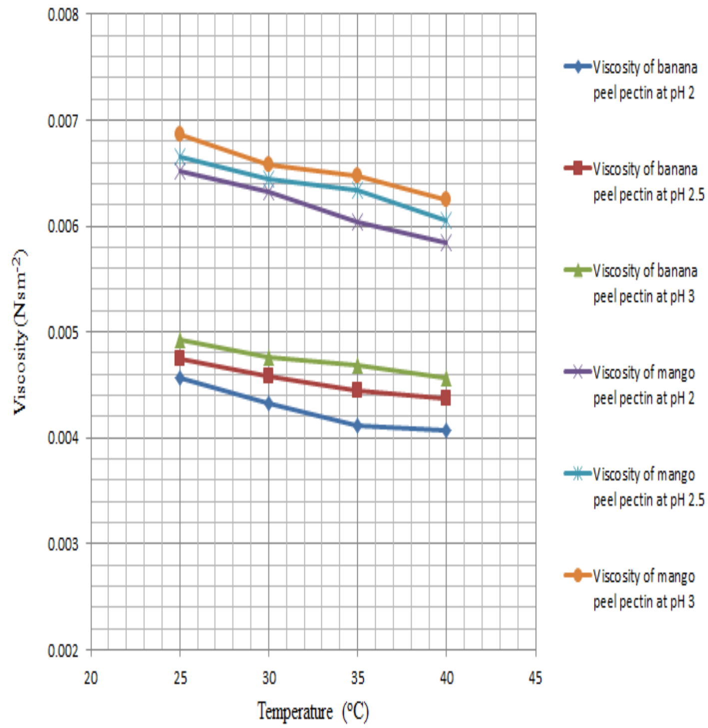


Figure7 Effects of temperature and pH on the viscosity of banana and mango peel pectin

4. CONCLUSIONS

The effects of the three process variables namely, temperature, extraction time and pH on the pectin yield were found to be significant. An optimum extraction temperature of 82^{o}C, 105 min extraction time and 2 pH results an optimum value of 11.31% and 18.50% (w/w) banana and mango peel pectin respectively. The statistical analysis indicates that temperature, extraction time and pH had a significant effect on the characteristics of mango peel pectin while insignificant effects on the banana peel pectin. The degree of esterification, viscosity, methoxyl content and anhydrouronic acid content of mango peel pectin are higher than that of banana peel pectin. However, the ash content, moisture content and equivalent weight of mango peel pectin are slightly lower than banana peel pectin. Generally the chemical quality of pectin obtained from mango peel is comparatively better than banana peel. In addition, the percentage of pectin yield obtained from mango peel is relatively higher compared to banana peel with similar extraction condition. The yield of mango peel pectin is comparable to those of apple pomace and citrus, thus, signifying the potential use of mango peel as an alternative source for the commercial pectin production. Hence, according to experimental studies mango peel pectin is more suitable for industrial application. Finally, it can be concluded that the industrial utilization of mango peel for manufacturing of pectin would not only solve the problem of waste disposal but also save valuable foreign exchange by reducing the pectin imports.

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