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Removals of Pectin from Mango Peel Waste Using Response Surface Methodology

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Abstract: ***In this present study to examinedthe effect oftemperature, time and pH on the complex mixture ofpectinremovalfrom mango peel. Mango is one of the most important tropical fruits in the world and currentlyranked 5th in total world production among the major fruit crops. As mango is a seasonal fruit, about 20% of fruits are processed for products such as puree, nectar, leather, pickles, canned slices, and chutneyThe experimental design was performed using Central Composite Design. The Response surface methodology was used for optimization paramers. Theoptimum temperature, time and pH for the removal of pectin for mango peel was determined to be 82°C, 105min and 2 respectively. The yields of pectin under these optimum conditions were found to be 1 18.5% for mango peel. This method was more efficient to remove the pectin from mangopeel.The Response surface methodology results were found to be more efficient and satisfactory.***

Keywords: Central Composite Design (CCD),pectin, response surface methodology (RSM), anova, mango peel

1. Introduction

Mango (Mangifera indica L.) is the king among tropical fruits and is greatly relished for its succulence, exotic flavour and delicious taste in most countries of the world (Bhatnagar and Subramanyam, 1973). Apart from its delicacy, it is a nutritionally important fruit being a good source of vitamin A, B and C and minerals. Mango is considered to be a fruit with tremendous potential for future. Worldwide production of mango is 38.95 million tonnes (FAO, 2011). Mango has its origin in India and approximatelythousand different types of mango fruits are produced in the country. Annual production of mango in India is 15.19million tonnes (FAO, 2011).Large number of mango cultivars has been screened (Sadhu and Bose, 1976; Gangopadhyay et al.,1976; Dhar et al., 1976; Ramesh war et al., 1979; Kalraet al., 1981) for their physico-chemical characteristics, from which inference can be drawn for their suitabilityfor different product preparation. Succulent juicy varieties are popular for dessert purposes and nonfibrousfleshy varieties are largely used for processing. It is reported that about 75 % of the fruits are knocked off, right from the flowering stage till ripening. The losses however, can be minimized to a great extent by utilizing the dropped fruits. Fortunately mango is one of the few fruits which can be utilized in all stages of maturity. The fruit is used as a dessert, as a table fruit between meals and is also processed for preparinga host of products. Establisheprocessedproducts include pulp, juice, squash, nectar, pickles, chutney, preserve, jam, canned slices, dried powder, about 0.02% (Kumbhar, 1992). Pulp is heated to 85oC,filled hot into cans and sealed and processed at 100°Cfor 20 min (for A2½ cans) and cooled. Addition ofascorbic acid at 100 mg % in the caning of mango pulphelps in the retention of colour, flavour and carotene (Subbiah, 1961; Roy et al., 1997)During processing of mango, peel amajor by- product, contributes about 15-20% of the fruit (Beerh and Raghuramaiah, 1976). As peel is not currently utilized for any commercial purpose, it is discarded as a waste and becoming a source of pollution. Peel has been found to be a good source ofphyto-chemicals, such as polyphenols, carotenoids, vitamin E, dietary fiber and vitamin C and it also exhibited good antioxidant properties phenolic content and antioxidant activity effect in prepared biscuit.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 objectives of this study were (1) to extract and characterize the pectin from mango fruit peel waste and to explore its potential use, (2) to investigate various factors that affect the yield of pectin such as pH, temperature, extraction time.

2. Materials and Methods

All Essential chemicals were prepared for removal process of analytical manner. Fresh mango peel was collected from some hotels, juice processing shops and restaurants in Addis Ababa, Ethiopia.

Raw Material Preparation

The fresh fruit peel was segregated based on their category, and cut into pieces for easy drying and washed with water three times. Dryingwas carried outin an oven at 60°C for 48 hours to get easily crushable material. The dried pieces peel was milled in a screen analysis size of 80 meshes and packed in airtight. The moisture-proof bag wasready at room temperature for the removal process.

3. Experimental Design

Central Composite Design for Response Surface Methodology was used to investigate the effects of independent process variables temperature (A), Removal time (B) and pH (C) on the response, pectin yields (Y). The star arm (α), known as the arm length of the axial experiments from the center point, was 1.68179. The experiment at 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). 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 variables.

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

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

The polynomial equation was 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.

Extraction of Pectin

In this work, Fruit waste peel dried and powder was weighed in a conicalflask. The Distilled water was added to them (solid- liquid ratio of 1:29 (w/v). 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. Then the mixture was heated for each different pH medium (1.66 to 3.34) of Removal pectin while stirred at different temperature (52.5 to 89.5°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°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.

$$\text{Ypec (\%)} = \frac{P}{Bi} \text{x } 100 \qquad (2)$$

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).

Structure of Pectin

Pectin is a polysaccharide, composed of different sub-structural entities that vary with the extraction Methodology, raw material, location, and other environmental factors. The linear backbone of the pectinpolymer is called homogalacturonan, and is built by sequences of α (1→ 4) linked D-galacturonic acid residues. These building blocks of polygalacturonic acid can be esterified and present as the methyl esters and the free acid groups. They may be partly or fully neutralized with cations such as sodium, potassium, calcium, or ammonium. The ratio of esterified galacturonic acid groups to total galacturonic acid groups is termed the degree of esterification (DE). It is possible that the homogalacturonan is fully methyl esterified when synthesized in the plant, but the highest DE that can be achieved by extraction of natural raw material is approximately 80%. Pectin with DE from 5%-75% is produced by controlled de-esterification in the manufacturing process. A DE of 50% conventionally divides commercial pectin products into HM and LM pectin. The DE hasa vital influence on the properties of pectin.HM pectin requires a minimum amount of soluble solids (SS) and a pH around 3.0 or lower in order toForm gels. LM pectin requires the presence of a controlled amount of calcium, or other divalent cations,to form a gel by “bridging” between adjacent chains and therefore is more flexible with respect to sugarand/or acidLMA is a specific type of LM pectin, where a proportion of the methyl ester groups are converted to amide groups. Degree of amidation (DA) is determined as the ratio of amidatedgalacturonic acid groups to total galacturonan units. The gelation of LMA pectin occurs not only through calcium bridging, but is more complex including other types of chain-chain interactions, such as hydrogen bonding. This means that the amidated pectin needs less calcium for gelation and is less prone to precipitation at high calcium levels. The distribution of methyl ester groups over the galacturonan chain is important for the functionality of the polymer. This distribution can be either block wise or of a more random character depending on rawmaterial and processing conditions. Some pectin methyl esterases work by single chain attack to create a block wise distribution of methyl groups. HM pectin with this block structure is very sensitive to calcium and capable of interaction with positively charged particles, such as casein. Pectin extracted from certain other raw materials, e.g., sugar beet, will to a large extent carry O-acetyl groups predominantly at the C-3, but also at the C-2 position of the galacturonic acid residues. Acetyl groups will have a negative impact on gelation of pectin, probably due to hindrance of chain-chain association within the junction zones. However, it has been found that sugar beet pectin works well as a viscosifier and for the stabilization of oil in water emulsions, possibly due to the presence of the hydrophobic O-acetyl groups and contained protein. The homogalacturonan backbone of pectin is periodically interrupted by α-L-rhamnose residues Creating rhamnogalacturonan parts. The residues are present in the following sequence:α-D-galacturonic acid (1→ 2) α-L-rhamnose (1→ 4) α-D-galacturonic acid. In the rhamnogalacturonanarea, the polymer chain will be branched with side chains of neutral sugars. Branching occurs at theC-2 or C-3 of galacturonic acid, or at C-3 of rhamnose. Two types of rhamnogalacturonan have been described. The side chains associated with rhamnose Will for rhamnogalacturonan of type I consist mainly of arabinose and galactose, but minor quantities of fucose are also found. Rhamnogalacturonan of type II is less abundant. It contains rare sugars such as methylfucose, methyl xylose, and apiose. The rhamnogalacturonan areas of the pectin backbone are curtailed in most commercial pectin types, as the majority of the neutral sugar side-chains are lost during processing. As such, they do not have any impact on gelling capability. However, for pectin from specialized processing and raw materials, large amounts of neutral sugars are found. If a significant amount of rhamnogalacturonan interruptions are present in the polymer, they will obstruct the molecularOrientation necessary for junction zones to develop. Pectin is heterogeneous not only with respect to chemical structure, but also with respect to molecular Weight. The polydispersity of commercial pectin samples is from 4-8, and molecular weight can be controlled During processing, but is normally in the range 80 - 400 kDa. The homogalacturonan is a stiff, rod shaped molecule, the conformation of which is determined by the rotation angles of the glycosidic bonds. These angles generate a simple 31 helix, which will not exist in solution, where interactions with the solvent will make the homogalacturonan adopt a worm-like conformation. The inserts of rhamnogalacturonan into the pectin chain make the polymer more flexible compared to homogalacturonan.

4. Results and Discussion

4.1 Effects of Temperature

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.

4.2 Effect of Time

The pectin yield increased significantly with the increasing in the extraction time.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.

4.3 Effect of pH

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. 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 (3).

4.4 Mango Peel Pectin

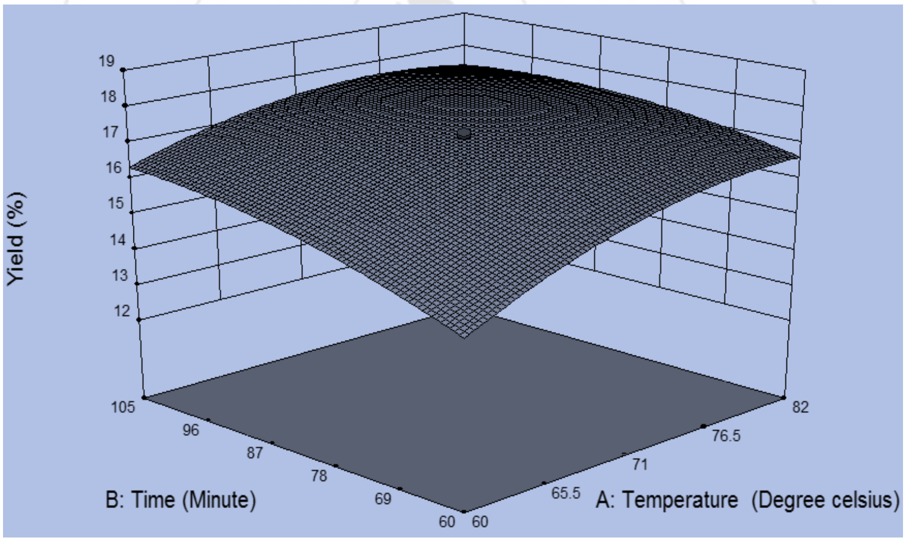
$$Y = 17.19 + 0.86*A + 0.72*B - 1.63*C - 0.33*AB - 0.28*AC - 0.028*BC - 0.62*A^2 - 0.40*B^2 - 0.80*C^2 \quad (3)$$

Where, Y - pectin yield, A - temperature, B - extraction time and C - pH.Equations (3) 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 variables.

The analysis of variance (ANOVA), result of the quadratic regression model, for the mango peel. Pectin demonstrates that the model is significant with its F-value of 333.66. 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 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 peel pectin calculated from the second degree polynomial equation are R^2= 0.9967. 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^2values of mango peel for pectin 0.9937. 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 1 to 3. 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 peel is 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 isobserved in case of mango peel pectin . However, at moderate extraction time and high temperature the pectin yield are increased. The interaction effects of temperature and pH on pectin yield,at lower and higher levels of both pH and temperature lower yield of pectin is observed. At intermediate values higher pectin yield obtained. It 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 pectin constituents.The experimental response and the model predict responses for mango peel pectin are given in below.

Table 2: Experimental design set up of RSM and responses

Run No.	Independent variables			
	Temperature (°C)	Time (minute)	pH	Experimental mango peel pectin yield (%)
1	0	0	0	16.98
2	-1	1	-1	16.72
3	-α	0	0	14.14
4	0	-α	0	14.81
5	1	1	-1	18.51
6	1	-1	1	14.09
7	α	0	0	16.90
8	-1	-1	1	12.10
9	0	0	α	12.13
10	1	-1	-1	17.65
11	0	0	0	17.13
12	0	0	0	17.23
13	0	0	0	17.32
14	-1	-1	-1	14.76
15	0	0	-α	17.86
16	-1	1	1	14.16
17	0	0	0	17.26
18	0	0	0	17.19
19	1	1	1	14.64
20	0	α	0	17.42



3D Effect of temperature and time interaction at fixed pHFigure1

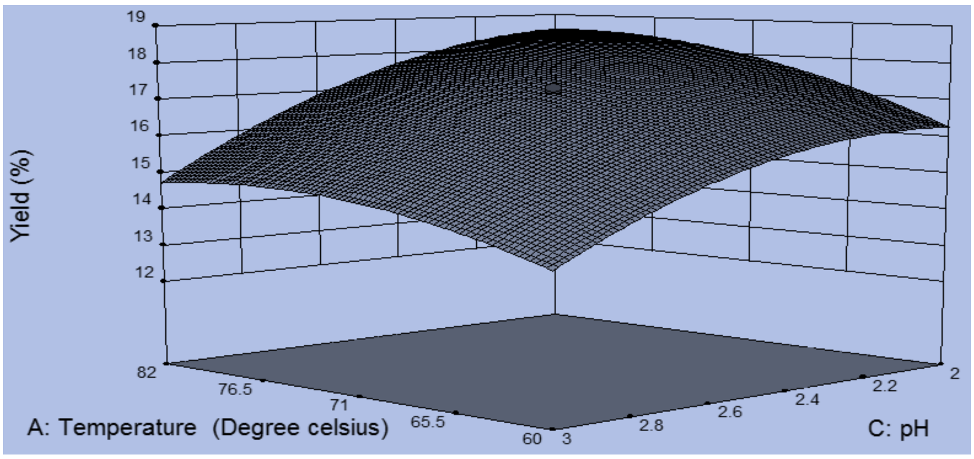


Figure 2: 3D Effect of temperature and pH interaction at fixed time

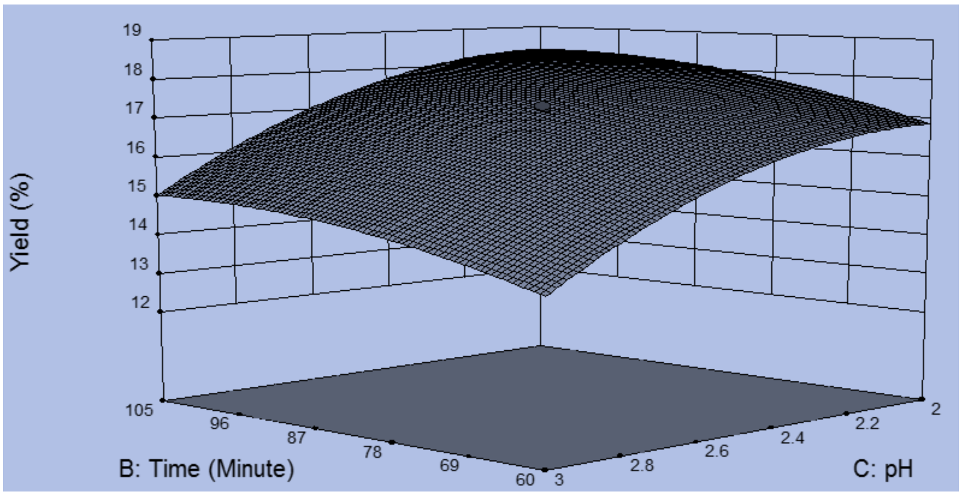


Figure 3: 3D Effects of time and interaction at fixed temperature

5. Conclusions

The effects of the three process variables such as temperature, extraction time and pH on the pectin yield were found to be significant. An optimum extraction temperature of 82°C, 105 min extraction time and 2 pH results. an optimum value of 18.50% (w/w) mango peel pectin determined. The statistical analysis indicates that temperature, extraction time and pH had a significant effect on the characteristics of mango peel pectin. The degree of esterification, viscosity, methoxyl content and anhydrouronic acid content of mango peel pectin isvery high. However, the ash content, moisture content and equivalent weight of mango peel pectin are slightly low.

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