

## Extraction of starch and phenolic compounds from *Mangifera indica* L. var. Kesar seeds and its characterization

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The present study is aimed to examine the effect of various parameters (pH, solid to liquid ratio, extraction time and temperature) on starch content, total phenolic content (TPC) and antioxidant activity of extracts obtained from seed kernel of mango var. Kesar. Isolation of starch from mango kernel by employing the ethanol precipitation method has been assessed. Box-Behnken design has been used to develop, validate and optimize the statistical model in order to establish the impact of various parameters either alone or in combination followed by characterization of starch. A decrease in pH has increased the extraction of starch while other responses such as TPC and antioxidant activity did not show a significant variation. The yield of total phenolic compound has been improved with decrease in solid to liquid ratio by increasing the solvent volume. The optimum conditions have been found to be 2.5 pH, 24 min extraction time and 1:55 g/mL solid to liquid ratio. Under these conditions, the highest extraction yield of starch and TPC are found to be 13.93 g/100 g and 33.45 mg GAE/g, respectively. No significant variation in antioxidant activity has been observed. A better and value-added utilization of the waste material can tender environmentally sustainable and economically viable solution.

**Keywords:** Box-Behnken design, Kesar mango seed, Starch, Total phenolic content

Mango (*Mangifera indica* L.) is one of the most commonly traded tropical fruits worldwide<sup>1,2</sup>. In India, mango is known as the “king of fruits” and India itself accounts for ~20 million tons of mango production which contributes to 59.52% of the world mango production<sup>3</sup>. At present, mango processing for pulp is at the maximum, thereby generating a huge quantity of solid waste comprising mango peel and kernel. This constitutes about 40-50% of total fruit, out of which 12-15 % is peel, 5-10 % is pulp waste and 15-20 % is kernel<sup>4</sup>. Utilization of waste is both a necessity and a challenge since this not only economizes the cost of finished products and reduces the pollution level but also leads to complete utilization of the raw material<sup>5</sup>. The kernel is obtained by breaking the hard seed coat of mango stone. It is rich in fat, starch, protein, tannins, vitamins, fibers, sterols and triterpene alcohol<sup>6</sup>.

Starch is available in most of the plants and is the main source of carbohydrates present in the form of polysaccharides in plants. It consists of a huge number of glucose units connected to each other by glycosides bond<sup>7</sup>. It is a biodegradable and natural resource for biopolymer<sup>4,8,9</sup>. The main sources of starch are wheat, jowar, corn, rice, maize, potato,

etc.<sup>8,10,11</sup>. Starch from these plants can be extracted and processed into useful products and chemicals<sup>12</sup> in the field of food, pharmaceuticals, adhesive, thickeners, stabilizers, textile, etc.<sup>7</sup>. Starch is widely used in many industries like food, pharmaceuticals, biomedical and textile due to its compatibility with other materials, low cost and non-toxic nature<sup>13</sup>. Further, the starch is used directly or indirectly with synthetic polymers<sup>14</sup>.

A native starch is composed of amylose and amylopectin. Amylose is made up of a linear chain while amylopectin is a branched chain<sup>9,15</sup>. Native starch is converted physically, enzymatically or by chemical treatment into modified starch to enhance the texture and structure of the food. The use of both native and modified starches has rapidly increased for the manufacturing of various fabricated foods<sup>16</sup>. Under non-degradative conditions, starch is used in many applications for solubilization and dispersion in an aqueous medium<sup>9</sup>.

Looking into the demand of starch, the kernel of mango seed has also been assessed as one of the useful raw materials for extracting starch. Hassan et al. have extracted starch in the range of 52-65% from four varieties of mango viz, Bintasuga,

Dankamaru, Papparanda and Peter using the steeping method of extraction<sup>12</sup>. Amylose type starch content varied from 11-14%. The starch obtained from mango waste has exhibited good water binding, swelling and solubilization capacity. Mango seed starch has a higher swelling capacity and lower viscosity than the potato starch. Hence, it can serve as an alternative source for industrial applications<sup>17</sup>. Starch isolated from Lily cultivar seed has shown oval and irregular shape from small to the large size of A-type starch pattern<sup>18</sup>. The gelatinization temperature of starch was observed from 91°C which was very useful in industrial production due to thermal stability<sup>10</sup>. Due to the growing demand for starch in recent days, seeds of different varieties of mango fruits which were earlier discarded after extracting pulp, have been characterized for assessing the quality of starch<sup>10</sup>. This new source can minimize the industrial waste, fulfill the requirements of the industries and society, and add in a profit. The starch found in the seed of the mango fruit possesses good physicochemical properties like amylose content, water binding capacity, swelling power and solubility in water. Very limited work is available on the extraction from the seed and having focus on parametric study followed by optimization.

Mango seed oil contains phenolic compounds like mangiferin, caffeic acid, chlorogenic acid and quercetin. These phenolic compounds have shown antioxidant activity; hence, it is used to preserve oil and fat<sup>19</sup>. The average DPPH scavenging ability of all four varieties of mango kernel (Ngowe, Sabine, Apple and Kent) was 92.22 % and the total polyphenols were in the range of 68.71 - 72.05 mg gallic acid equivalent (GAE)/g<sup>20</sup>. Microwave based extraction was used for the extraction of antioxidants from the mango seed. Under optimum conditions, antioxidant activity (DPPH base) was about 1738.2 mg trolox/g, greater than the commercial antioxidants<sup>21</sup>. Extracts from Mahachanok variety of mango seed have been obtained with the highest ABTS (2, 2-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)), DPPH (2,2-diphenyl-1-picrylhydrazyl), and linoleic acid peroxidation inhibition with values of 3.215 µg/mL, 8.055 µg/mL and 0.233 µg/mL, respectively<sup>22</sup>, compared to other varieties. Among edible portions and seeds of avocado, jackfruit, longan, mango and tamarind, mango kernel extract has exhibited more than 70% ABTS and FRAP antioxidant activities<sup>23</sup>. Total phenolic content (TPC) in four varieties from Thailand was in the range of 118-399 mg GAE/g<sup>24</sup>.

A variation in TPC and biological activities due to different region, different cultivar and different treatment has been observed<sup>25-29</sup>.

Till date, starch from mango seeds was extracted using alkaline solution based, sedimentation and centrifugation methods. No research work has been conducted to demonstrate the effect of solvent precipitation on starch yield. The precipitation was carried out using ethanol which is green in nature. The objective of the present study was to isolate starch from Kesar mango seeds with solvent precipitation method and determine the total phenolic content along with its antioxidant activity. Moreover, optimization of extraction parameters (solid to liquid ratio, pH and extraction time) for achieving the maximum yield has been conducted using Box-Behnken design. The starch was characterized using X-ray diffraction (XRD), Energy dispersive X-ray (EDX) and thermogravimetric analysis (TGA). The study will assist to utilise other waste biomass for value-added compounds like polyphenols and starch.

## Experimental Section

### Materials

Seeds of ripe Kesar mango variety were provided by Shiv Agro Products Pvt. Ltd. Olpad GIDC, Surat. The seeds after pulp extraction were naturally dried in well ventilated room until a constant mass was recorded. Dried seeds were then subjected to kernel removal and size reduction using a grinder-mixer. The uniform particle size of the kernel powder was ensured by passing through the sieve of 210 µm mesh and then stored in a dry environment. The raw seed powder was analyzed for its nutritional values.

Ethanol (99.9 % pure) was procured from Fine Chemicals, India. Gallic acid standard (≥ 99% HPLC chemicals) and 2, 2-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) were purchased from Sigma-Aldrich (Bengaluru, India). Analytical grade Folin-Ciocalteu's phenol, sodium carbonate anhydrous and hydrogen chloride (35 % assay) were obtained from Finar Limited, Gujrat, India. Water from the Milli Q machine (Millipore, Elix, Bangalore, India) was used for experiments as well as analysis.

### Method

#### Starch extraction

Extraction of starch was carried out using a heat reflux system on REMI 5MLH PLUS heating system. 1 g dried seed powder and a measured volume of an aqueous solvent of acidic nature were introduced into a

100 mL flat bottom flask<sup>30</sup>. All the experiments were performed at a constant stirring speed and temperature of 300 rpm and 80°C, respectively. The mixture after extraction was subjected to filtration through a nylon filter followed by centrifugation to remove any suspended solids at a speed of 8000 rpm for 8 min. Thereafter, an aliquot of clear supernatant was used to measure TPC and the remaining was treated with an equivalent volume of ethanol and kept overnight for complete precipitation of starch. The equivalent amount of ethanol was decided based on trial experiment. The starch after filtration was subjected to freeze-drying (-38°C and vacuum of 1 mbar) and the dried starch powder was then weighed and stored. The starch was white in colour and confirmed by the iodine test. The addition of iodine solution on starch powder gives intense dark blue-black colour (Fig. S1). The process flow chart for starch recovery is shown in Fig. 1. All the experiments were performed twice in order to check the repeatability.

#### Experimental design

Based on the preliminary investigations, pH of the solvent (2.5 to 6.5), solid to liquid ratio (1:35 g/mL to 1:55 g/mL) and extraction time (15 min to 45 min) were considered for the optimization and analysis of variance (ANOVA) study. Since a better extraction of starch was observed in the acidic conditions, pH was varied from 2.5 to 6.5. Moreover, at a pH lower than 2.5, the quality of starch was negatively affected as it was turned into mild reddish colour. In case of solid to liquid ratio, due to a higher amount of solvent (beyond 1:55 g/mL), lesser heat was absorbed which has further affected the mass transferred and delayed the extraction. The solid to liquid ratio higher than 1:35 g/mL (lesser solvent volume) has made the slurry very viscous which has imparted sluggishness in the mass and heat transfer leading to reduced extraction rate and hence, incomplete extraction in a given time period.

Extraction time of more than 45 min has led to degradation of starch chain, thereby affecting the quality and quantity of starch extracted. A sticky and viscous mass of seed powder was formed in a solvent when the temperature was above 80°C which opposed the penetration of solvent and hampered the extraction. In case of stirring speed, no impact of stirring was observed above 300 rpm. Hence, it was kept constant for the study. Based on the above mentioned reasons, range of processing variables was selected and Box-Behnken design was employed (Table 1) to obtain the best processing conditions, quadratic equation, and *F* value along with its significance. Temperature and stirring speed were maintained at 80°C and 300 rpm, respectively. All the experiments have been performed twice and in randomized order to avoid biasness. The analysis of the responses was carried out using Design Expert (Version 10, State- Ease, Inc. Minneapolis, USA) software.

#### Determination of total phenolic compound

The phenolic compounds have been analyzed based on the method reported in the literature<sup>31,32</sup>. The total phenolic compound is expressed in terms of gallic acid equivalents (mg GAE/g) using the following equation.

$$\text{TPC} = \frac{C_{\text{tp}} \times V}{m} \quad \dots(1)$$

where, *V* is volume of solvent (L), *m* is the mass of material (g) and *C<sub>tp</sub>* is the total polyphenol concentration (mg/L).

A calibration curve was prepared for standard gallic acid in water using UV- spectrophotometer at a wavelength of 760 nm (HACH, DR6000, Mumbai). The calibration curve was obtained with *R*<sup>2</sup>=0.9964 (*C<sub>tp</sub>*=0.6045+93.985×*A*). Aliquots of 0.4 mL of extract was added with 0.5 mL Folin Ciocalteu's reagent followed by mixing of 1.5 mL an aqueous 10% sodium carbonate. The absorbance of the incubated solution was measured at 760 nm.

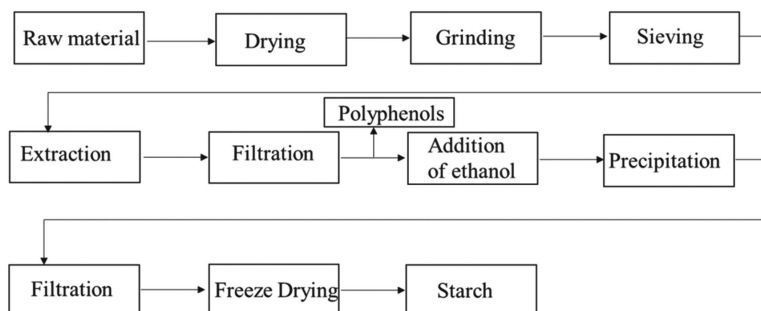


Fig. 1 — Process flow chart for extraction of polyphenols and starch from mango seeds

Table 1 — Box-Behnken design for extraction of starch, TPC and ABTS activity

S. No.	pH (A)	Solid to liquid ratio (g/mL) (B)	Time (min) (C)	Yield of starch (g/100 g)	TPC (mg GAE/g)	ABTS activity (%)
1	2.5	35	30	13.20	23.84	99.6
2	6.5	35	30	4.51	26.54	99.5
3	2.5	55	30	13.82	32.14	99.4
4	6.5	55	30	9.73	32.78	98.5
5	2.5	45	15	11.34	27.19	99.2
6	6.5	45	15	8.59	27.03	99.3
7	2.5	45	45	7.11	30.16	99.1
8	6.5	45	45	5.58	28.07	99.4
9	4.5	35	15	4.44	23.55	99.3
10	4.5	55	15	10.89	38.31	99.3
11	4.5	35	45	1.85	23.1	99.1
12	4.5	55	45	6.21	35.05	90.0
13	4.5	45	30	9.00	30.30	95.9
14	4.5	45	30	8.05	30.60	95.5
15	4.5	45	30	8.14	30.40	96.9

**ABTS radical scavenging activity**

ABTS was measured using the protocols reported in the article<sup>33-35</sup> with modification. A solution of 7 mM 2, 2-azino-bis (3 ethylbenzthiazoline-6-sulfonic acid) (ABTS) in 2.45 mM potassium persulphate was kept in a dark room for 16 h at a room temperature to produce ABTS<sup>+</sup> radical. The addition of plant extract reduces the radical cation (ABTS<sup>+</sup>) to ABTS. This can be observed by change in colour of stock solution. Percentage of colour change was observed as a reduction in UV absorbance at 734 nm and scavenging rate can be calculated using the following equation.

$$\text{ABTS scavenging rate} = \left( \frac{A_{ds} - A_{pe}}{A_{ds}} \right) \times 100 \quad \dots(2)$$

Where,  $A_{ds}$  is absorbance of diluted stock solution and  $A_{pe}$  is the absorbance of plant extract in diluted stock solution.

**Physicochemical characterization of starch**

The starch was analyzed by D8- advance (Bruker India Scientific Pvt. Ltd., Kolkata) X-ray diffractometer with the high-speed energy-dispersive LYNXEYE XE-T detector.  $2\theta$  scanning was performed at a rate of 1 °/min in the range of 5 to 40°. The crystallinity of starch powder was analyzed by Diffract software<sup>36</sup>. Each element has its unique atomic structure with unique energy<sup>37</sup>. EDX analysis was performed using with the Scanning Electron Microscope (XL 30 ESEM, Philips). Thermal analysis of the starch sample was performed under temperature 30°C to 995°C at a rate of 10 °C/min and nitrogen

flow rate of 50 mL/min using TGA 4000 (Perkin Elmer, America).

**Results and Discussion**

Nutraceutical composition and proximate analysis of mango seed prior to extraction was performed (Table 2) The seed is rich in energy, total fat, carbohydrate, minerals and vitamins.

**Effect of Parameters on starch recovery****Effect of pH**

Extraction of starch increased from 8.055 to 13.825 g/100 g with a decrease in pH from 4.5 to 2.5 while little change was observed in the pH range of 4.5-6.5 as shown in Fig. 2a. A lower pH has the ability to contact with the insoluble starch directly and favoured the hydrolysis of the insoluble starch constituents into soluble starch, thus increasing the starch recovery. The low pH also reduced the molecular weight of starch so that it could be solubilized from plant tissues without any degradation and finally precipitating by the addition of equivalent amount of ethanol. Beyond the pH value of 2.5, aggregation of starch could occur, which retarded the starch release and decreased the starch yield<sup>38</sup>. Similar results have been obtained in the extraction kiwi starch and lesser pH was desirable<sup>39</sup>. However, pH did not show a significant effect on the variation of TPC yield (Fig. 3a).

**Effect of solid to liquid ratio**

An increase in the amount of solvent leads to enhanced swelling of plant material, which is

favourable to improve the contact between the plant matrix and the solvent. Therefore, the cell walls were affected and resulted in the easy release of starch into the surrounding medium<sup>38</sup>. Further, a higher solvent has allowed proper mixing and uniform heat and mass transfer due to less viscosity. Therefore, starch yield increased in the range of 6.215 to 9.735 g/100 g with a decrease in the solid to liquid ratio from 1:35 g/mL to 1:55 g/mL as shown in Fig. 2b. The extraction yield of total phenolic compound also increased from 23.84 to 32.78 mg GAE/g (Fig. 3b) as a higher amount of

solvent could enhance the solubility of phenolic acid and a lesser viscosity could have accorded a better transport. The results are in line with the observations made in the literature for extraction of starch<sup>39</sup> as well as phenolic compounds<sup>40</sup>.

#### Effect of time

Extraction time has both a positive and negative impact on the targeted compound. A longer extraction time may result in increased energy consumption, whereas shorter extraction times may lead in insufficient extraction. Therefore, extraction time optimization is required.

Starch yield increased with an increase in time up to a certain level. Beyond 22 min, more exposure of heating has led to degradation of starch chain molecules and hence starch yield decreased exponentially from extraction time 22 min onwards (Fig. 2c). Thus, more exposure to time has negative effect on starch extraction<sup>38</sup>. However, TPC did not show a change in recovery with respect to extraction time (Fig. 3c). While extracting the starch from kiwi fruit, a higher extraction time has exhibited a negative impact leading to degradation or denaturing of the starch<sup>39</sup>.

#### Study of interactive parameters using BBD

Using three factors and three levels in BBD, their impact on the yield of starch, TPC and ABTS has been assessed. The response value is a function of any single independent variable. Contour plot is used to investigate the interaction effects of corresponding parameters. The plots demonstrate non-linear behaviour. An interaction effect of pH and solid to liquid ratio on the starch yield is shown in Fig. 4a at an extraction time of 22.5 min. A pH less than 3 demonstrates positive effect on the yield of starch with decreasing solid to liquid ratio. As shown in Fig. 4 (b-c), a region of 2.5-3.5 pH and 15-33 min has yielded better results. Solid to liquid ratio of

Table 2 — Nutraceutical composition and proximate analysis of Kesar mango seed

Nutritional Value (International Standard)	
Quality Characteristics	Result
Energy, kcal/100 g	413.33
Total fat, g/100 g	11.37
Saturated fat g/100 g	2.10
Trans fat, g/100 g	B.L.Q
Cholesterol, mg/100 g	B.L.Q
Sodium, mg/100 g	17.68
Total carbohydrates, g/100 g	72.41
Dietary fibers, g/100 g	8.23
Total sugars, g/100 g	3.23
Protein, g/100 g	5.34
Vitamin D, mcg/100 g	B.L.Q
Potassium, mg/100 g	77.70
Calcium, mg/100 g	170.34
Iron, mg/100 g	3.80
Other vitamins and minerals	
Vitamin C mg/100 g	52.89
Magnesium, mg/100 g	129.85
Zinc, mg/100 g	1.49
Vitamin E, mcg/g	B.L.Q
Copper mg/100 g	1.08
Proximate analysis	
Moisture, %	8.78
Total ash, %	2.10
B.L.Q – Below Limit of quantification	

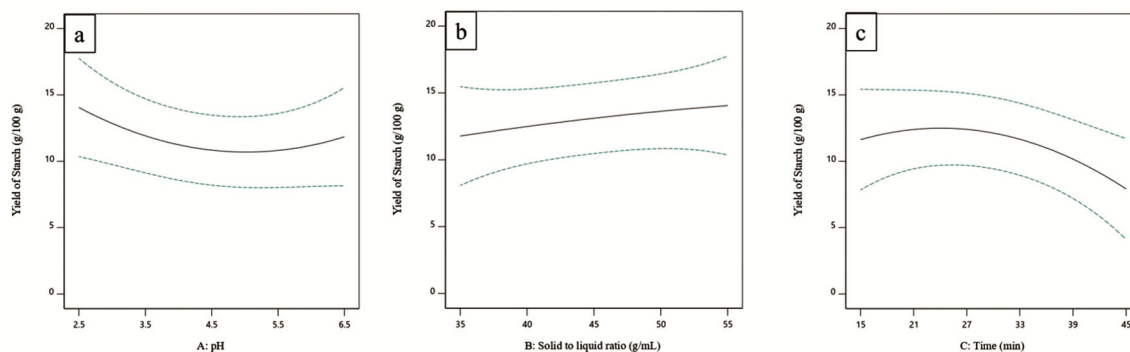


Fig. 2 — Effect of (a) pH, (b) solid to liquid ratio and (c) extraction time on starch yield

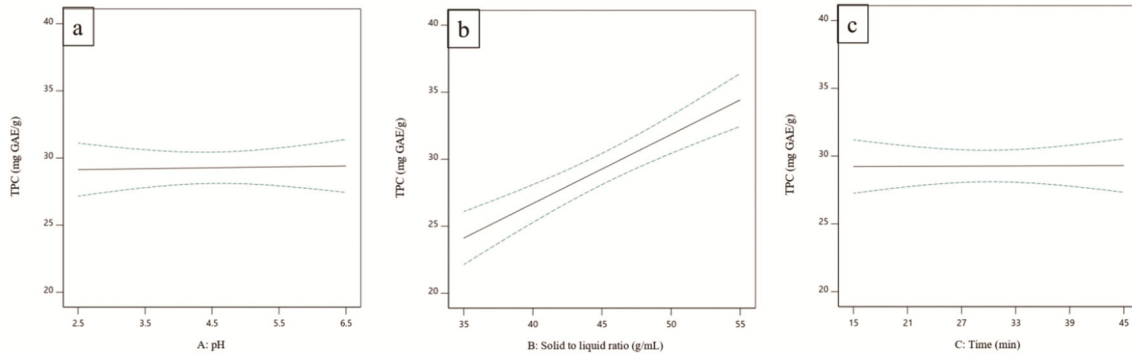


Fig. 3 — Effect of (a) pH, (b) solid to liquid ratio and (c) extraction time on TPC yield

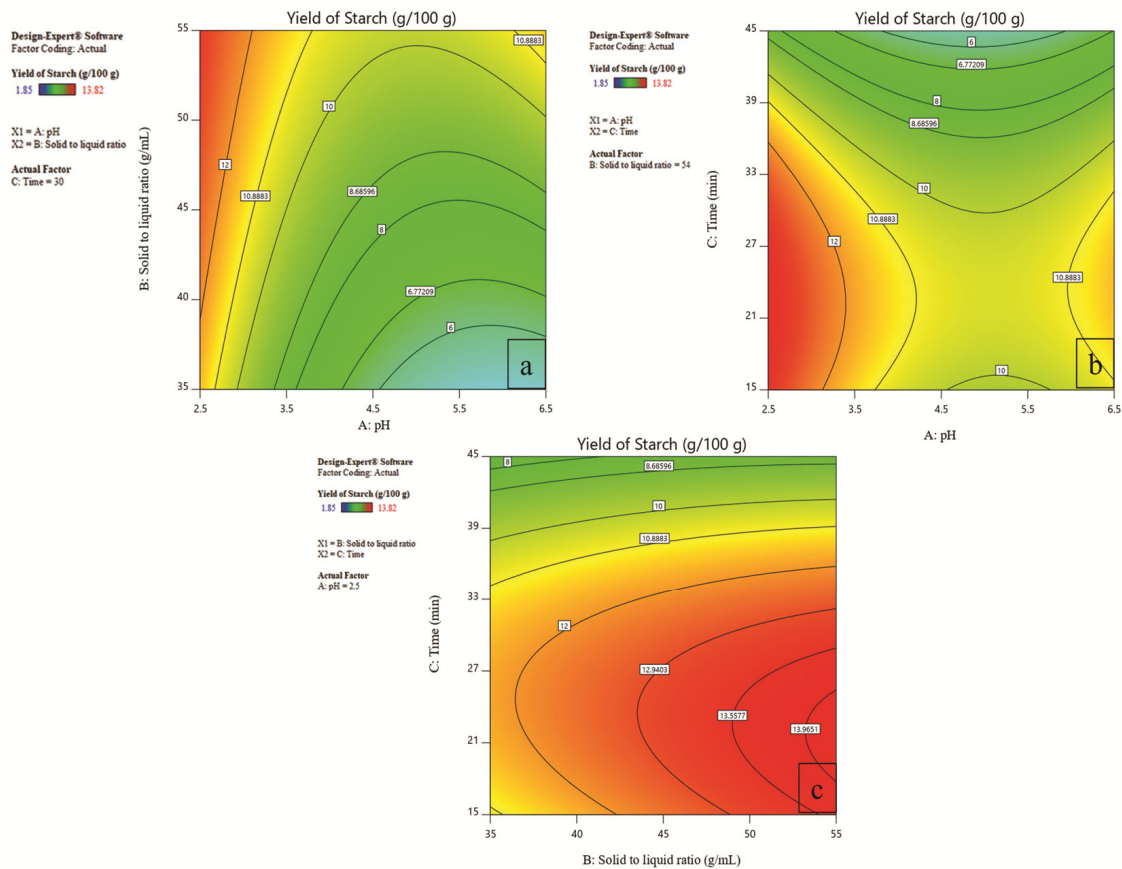


Fig. 4 — Contour plot of (a) pH and solid to liquid ratio, (b) pH and extraction time and (c) solid to liquid ratio and extraction time

1:55 g/mL and extraction time around 21 min have positive impact on extraction.

#### Analysis of variance (ANOVA) study

To develop a relationship between response and a variable and to achieve the best processing conditions, ANOVA was performed (Table 3) and the quadratic model for starch yield was developed.

The significance of the parameter and its interaction is determined using the  $p$ -value (less than

0.05). Smaller the  $p$ -value, the greater is the significance. The model  $F$ -value of 6.33 with a  $p$ -value less than 0.05 implies that the model is significant. As per ANOVA, A, B, C and  $C^2$  are significant model terms while interaction among parameters is insignificant. Since the quadratic term is significant, the behaviour is non-linear in nature. The lack of fit is not significant, suggesting the fitting of the model. The coefficient of determination ( $R^2$ ) was more than 0.9, suggesting the suitability of the model

Table 3 — ANOVA statistics for yields of starch as the response

Source	Sum of Squares	DOF	Mean Square	F- value	p- value	Significant
Model	144.24	9	16.03	6.33	0.0281	Significant
A-pH	36.38	1	36.38	14.36	0.0128	
B-Solid to liquid ratio	34.65	1	34.65	13.68	0.0140	
C-Extraction time	26.32	1	26.32	10.39	0.0234	
AB	5.29	1	5.29	2.09	0.2081	
AC	0.3721	1	0.3721	0.1469	0.7173	
BC	1.09	1	1.09	0.4310	0.5405	
A <sup>2</sup>	16.48	1	16.48	6.51	0.0512	
B <sup>2</sup>	0.1398	1	0.1398	0.055	0.8236	
C <sup>2</sup>	20.47	1	20.47	8.08	0.0361	
Residual	12.67	5	2.53			
Lack of Fit	12.12	3	4.04	14.69	0.0644	Not significant
R <sup>2</sup>	0.9193					
Adjusted R <sup>2</sup>	0.7739					

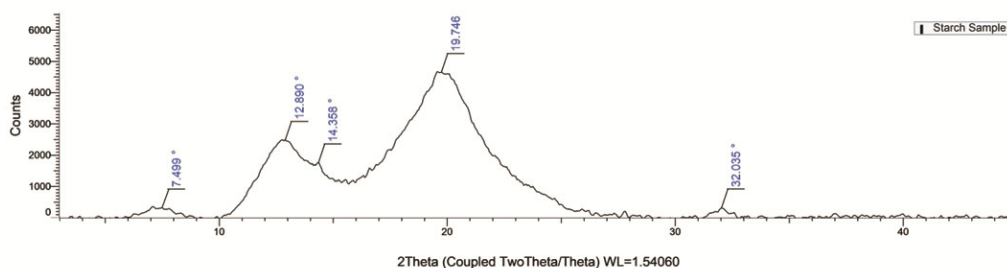


Fig. 5 — X-ray diffraction pattern of mango starch sample

to describe the response. The quadratic model was used to find the predicted values of responses using the following equation.

$$\text{Starch yield} = 8.40 - 2.13 \times A + 2.08 \times B - 1.81 \times C - 2.35 \times C^2 \dots (3)$$

In case of TPC as the desirable response, the ANOVA study has provided the model as significant ( $F$  value = 16.88,  $p$ -value = 0.0002) however, with only one significant factor, i.e., solid to liquid ratio (Table 4). Since the interaction effects are non-significant, the discussion related to main effect is justified. For ABTS as the response, no significant change in the response was observed while varying the process parameters. Hence, an ANOVA study was not being performed.

#### Confirmative test

Design Expert software provides number of optimum solutions for the starch yield. Out of which processing conditions were: pH at 2.5, solid to liquid ratio 1:55 g/mL and extraction time 23.998 min with predicted the starch yield, TPC and ABTS of 14.054 g/100 g, 34.276 mg GAE/g and 99.704 %, having desirability = 0.90. To verify the given

quadratic model, experiments were conducted under the same operating conditions except extraction time (24 min). Under these conditions, the starch yield, TPC and ABTS have been obtained as 13.93 g/100 g, 33.45 mg GAE/g and 99.271 %, respectively.

#### Starch physicochemical characterization

X-ray diffraction is a widely used method to check the crystalline and amorphous nature of any solid product in a dried form. The number of sharp peaks suggests the material of crystalline nature while the large flat background represents the amorphous state<sup>41</sup>. In crystalline nature, the molecules are arranged in regular patterns while they are disorderly placed in an amorphous state<sup>42</sup>. Fig. 5 shows the XRD pattern of the starch sample which is semi-crystalline. It might be due to the linear chain of amylose. The percentage crystallinity of the starch was 87 %. The area under the curve shows the percentage content of the amorphous nature of starch (13%).

XRD technique shows mainly four types of crystalline patterns viz A, B, C, and V on the basis of starch molecular arrangement<sup>16,43</sup>. It is the fingerprint



Table 4 — ANOVA statistics for TPC as the response

Source	Sum of Squares	DOF	Mean Square	F-value	p-value	Significant
Model	212.86	3	70.95	16.88	0.0002	Significant
A-pH	0.1485	1	0.1485	0.0353	0.8543	
B-solid to liquid ratio	212.70	1	212.70	50.60	<0.0001	
C- Extraction time	0.0112	1	0.0112	0.0027	0.9597	
Residual	46.24	11	4.20			
Lack of Fit	46.19	9	5.13	219.97	0.0045	Significant
R <sup>2</sup>	0.8215					
Adjusted R <sup>2</sup>	0.7729					

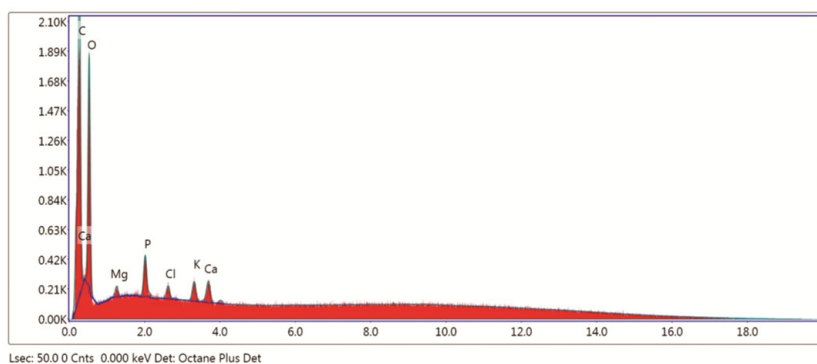


Fig. 6 — Energy dispersive X-ray spectrum of mango seed starch

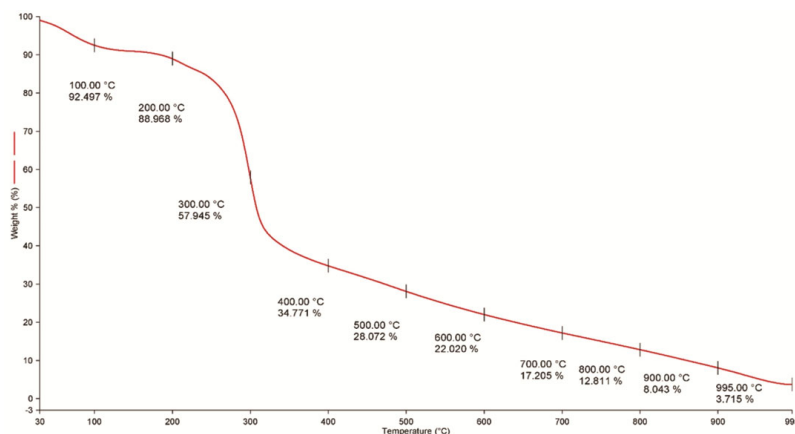


Fig. 7 — Thermogravimetric analysis of mango seed starch

of crystal structure. Amylopectin is composed of a double helices structure that is arranged in a regular pattern. Therefore, it creates a crystalline region. If molecules are arranged in a monoclinic unit cell with four water molecules inside the double helices, it shows A type of starch. In B type starch, molecules are arranged in the hexagonal unit cell with thirty-six water molecules inside double helices<sup>15</sup>. Mango seed starch is categorised in B type starch. Primarily, B type starch is extracted from a fruit seed, tubers and steam<sup>44</sup>. Single helix structure is present in V type starch<sup>43</sup>.

The elemental analysis of the starch sample was carried out to detect the presence of elements on a

weight basis (Fig. 6). Spectra of the starch sample showed seven peaks corresponding to carbon (C), oxygen (O), magnesium (Mg), phosphorous (P), calcium (Ca), chlorine (Cl) and potassium (K) with composition of 41.54 % C, 51.54 % O, 0.55 % Mg, 2.20 % P, 0.76 % Cl, 1.52 % K and 2.01 % Ca. This analysis confirms that in a minor amount, some elements also get separated during the extraction of starch.

TGA is very popular analytical technique to study the loss of mass as a function of temperature or time. As from Fig. 7, the starch sample shows two main mass losses: the first is related to dehydration and the second one is related to thermal degradation. The first



loss (12%) is in the range of 30 to 200°C, due to the elimination of water molecules. Second loss can be attributed to thermal degradation of starch between 220 to 300°C temperature which is approximately 31%. At a higher temperature, mass loss was due to decomposition of glucose ring<sup>45</sup>. The initial presence of water molecules did not affect starch degradation as all water molecules adhere to starch evaporated before reaching to decomposition temperature. Third minor mass loss of starch sample has occurred from 330 to 995°C which might be due to carbonization.

### Conclusion

The wastes generated from pulp industry either have been used as land filling or incineration which causes eutrophication or produces harmful gases. Therefore, utilization of waste is necessary for environmental sustainability and economical profit.

The present research explored the recovery of starch, the presence of the total phenolic compound and its antioxidant activity from mango seed using a conventional heat reflux system. Using Box-Behnken design, a non-linear predictive model was developed. The optimum extraction conditions obtained by Design-Expert software were; pH 2.5, solid to liquid ratio 1:55 g/mL and time 21.754 min. Under the optimum conditions, the starch yield, TPC and ABTS have been obtained as 13.93 g/100 g, 33.45 mg GAE/g and 99.271 %, respectively. Solid to liquid ratio was found to be the most influencing parameter for the extraction of starch and TPC. Water as a solvent and ethanol as an antisolvent were beneficial in terms of availability, low cost and non-toxicity. The recovered starch was semi crystalline in nature and its EDX analysis confirmed the presence of elements like calcium, magnesium, phosphorous, chlorine and potassium. Mango seeds also exhibit excellent antioxidant properties. So, it can be used in the medical field. Thus, a utilization of seed in extracting useful compounds can be a suitable alternative.

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### Supplementary Information

Supplementary information is available on the website <http://nopr.niscpr.res.in/handle/123456789/55>.

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