VALORISATION OF MANGO SEEDS VIA EXTRACTION OF STARCH: USING RESPONSE SURFACE METHODOLOGY TO OPTIMISE THE EXTRACTION PROCESS

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ABSTRACT:

Mango seed, a waste material that is disposed of after consumption of mangos, was studied for its potential use as a resource for extraction of starch. The study revealed that mango seeds are a good source of starch: physicochemical characterisations confirmed that the extracted material was indeed a starch material. The starch was tested for use in textile applications and the results indicated that the material performed as well as a standard starch sample. The extraction of starch from mango seeds is facile and does not require sophisticated technology. Response surface methodology was used to optimise the extraction of starch from mango seeds. The experimental parameters optimised were concentration of mango seeds, extraction temperature, and extraction time, while the measured response factors were starch yield and whiteness of the starch. Thus the critical values for optimal whiteness of the extracted starch were calculated to be: concentration = 0.35 (%w/v); temperature = 26.74 ºC; extraction time = 6.46 (hr). A techno-economic analysis of the starch extraction process showed that the technology is viable and could be taken up by SMMEs.

Keyword: *Mango, starch, extraction, response surface methodology, textile, sizing.*

INTRODUCTION

Starch, a glucose biopolymer, is the major storage component of most economically important crops, e.g. cereals, legumes, tubers and yams (Yadav et al, 2010; Musa, Gambo, and Bhatia, 2011; Emmambux and Taylor, 2013; Alcázar-Alay and Meireles, 2015). It is a very versatile material with a wide range of applications in the food, pharmaceutical, textile, paper, cosmetic and construction industries (Jane, 1995; Ellis, et al., 1998; Elliason, 2004; Tadesse et al, 2015). Although South Africa is a large producer of starch, it nevertheless imports significant amounts of starch to meet its basic needs (IDC, 2016; International Starch Institute, 2016). However, increased importation and transportation costs, reduced availability, late deliveries and food security concerns are some of the major challenges facing the starch industries. Increased local supply of starch that doesn't compete with the food market is needed. Therefore, there is need to investigate new botanical sources of starch.

Mango belongs to the botanical family *Anacardiaceae*. It is one of the most favored commercially valuable fruit grown throughout the tropics and is used in a variety of food products (Yeshitela, Robbertse, and Stassen, 2004). *Tommy Atkins*, *Sensation*, *Kent*, *Heidi*, *Keitt* and *Zill* varieties are the most common types of mangoes in South Africa. Approximately 84% are planted under micro, drip, sprinkler or flood irrigation entities (Department of Agriculture, Forestry, and Fisheries, 2014). Dryland production is no longer favoured, except where the annual rainfall supplements the irrigation programme during critical periods. Approximately 20% of mango producers account for 80% of the total annual production of 80,000 tons (National Agricultural Marketing Council, 2013). The weight of a mango seed accounts for about 15-25% of the total weight of the fruit. Therefore, each year about 16,000 tons of seeds are disposed as a waste (Honja, 2014). However, the theoretical starch content of mango seed is 74% (Velan, Krishnan, and Lakshmanan, 1995) - thus, mango seeds are a potential useful resource for starch. Some work has been done to valorise mango seeds. For example, Henrique and co-workers (2013) have done this by extraction and characterization of its cellulose nanocrystals. However, the authors are not aware of valorisation of mango seeds via extraction of starch.

The objective of this study is part of long term research at the CSIR to avoid sending biowastes to landfill by converting them into valuable products instead. Thus in this case the objective was to avoid landfilling of mango seeds waste by extraction of their starch content for use in industrial applications. Optimum conditions for extraction of starch from mango seeds were evaluated and the veracity of the extracted starch was confirmed by determination of physico-chemical properties of the extracted starch, compared to those of a commercial starch sample. After extraction, the starch was evaluated for use as a replacement for commercial starch used in the textile industry.

MATERIALS AND METHODOLOGY

Collection and preparation of mango seeds: The seeds of "*Tommy Atkins*" variety mango were collected from the mango juice making industry.

Moisture content of the seeds: The mango seeds were washed in free flowing water and the moisture content was determined using a method described by ISO (1997), which entails drying in an oven for 24 hours at 110 °C (Umerie, and Ezeuzo, 2000).

Starch extraction: The seeds were dried (3 hours at 105°C) and ground to a fine powder via pulverisation in a hammer mill and sieving through a 30-mesh standard sieve. The powder was steeped in sodium metabisulphite solution (0.01% (w/v) at a known temperature (as per experimental design) and blended using a heavy duty blender. The homogenate was sieved on 20 µm nylon mesh and washed with distilled water. The mixture was allowed to settle after which the supernatant was discarded and the crude extracted starch was washed repeatedly with tap water until the wash water was clear. The starch extract was then dried (at 110 °C) and stored at room temperature.

Experimental design and statistical optimization of the extraction process: Response surface methodology based on the Box-Behnken design was used for optimization of experimental conditions (Box and Wilson, 1951). Three independent variables, viz., extraction time, extraction temperature, and concentration of the sodium metabisulphite were selected for the study and the experiments were designed using Design expert software (9.0.5) and JMP 12 software. In total 15 experiments were executed to optimize the process parameters according to the design (Table 1). The response factors (starch yield and whiteness of the product) were determined by the coefficient of variation, analysis of variance and contour plots.

$$Y = \beta_0 + \beta_i X_i + \beta_{ij} X_i X_j + \beta_{ii} X^2{}_i \quad (1)$$

Where Y is the predicted response variable, β_o, β_i, β_{ii}, β_{ij} are constant regression coefficients of the model, and X_i, X_j ($i=1, 3; j=1, 3; i \neq j$) represent the coded values of independent variables (Zhu, 2010).

Table 1. Coded and actual levels of the design factors

	Levels		
Independent factors	-1	0	1
A: Concentration (%w/v)	0.1	0.3	0.5
B: Temperature (°C)	25	37.5	50
C: Time (Hr)	2	7	12

Statistical analysis:

ANOVA: In order to determine the relative contribution of process conditions on percentage yield and whiteness index of the extracted starch, ANOVA was performed on the experimental data at 95% confidence level which shows F observed versus F critical. The P-value was set at 0.05.

Response optimization: With the help of Design expert software and JMP software the optimized responses were determined. In these software, the response optimizer searches for a combination of input variables that jointly optimize a set of responses by satisfying the requirements for each response in the set. The optimization was accomplished by:

- Obtaining the individual desirability (d) for each response.
- Combining the individual desirable to obtain the combined or composite desirability (D).
- Maximizing the composite desirability and identifying the optimal input variable settings.

To maximize the desirability of the parameters studied, the software employs a reduced gradient algorithm with multiple starting points that maximize the composite desirability to determine the numerical optimal solution.

Physico-chemical characterisation and analysis of starch extract

Moisture content: This was determined by drying ***s***tarch samples for 3 hours at 105ºC.

Ash Content**:** This was ascertained by heating samples for 5 hours at 900˚C.

Starch yield: The yield of the starch extraction was determined from the weight of mango seed powder used and the final starch weight obtained from the procedure.

Functional group analysis: KBr discs of the starch samples were prepared and then measured for FTIR characterisations using a Nicolet Magna FR 760 FTIR spectrometer (Bruker). The spectra were recorded at room temperature using 64 scans at 2 cm^{-1} resolution from 400 to 4000 cm^{-1}

pH**:** 5 g of starch extract in 20 ml distilled water was mixed thoroughly for 5 minutes, allowed to settle, and pH of the water phase measured.

Iodine test: 1 g of starch was boiled with 15 ml of water and allowed to cool. A few drops of 0.1N Iodine solution were added to 1 ml of the mucilage and the colour change was recorded.

Water solubility index: Suspensions of 1 g of starch and 40 ml of distilled water were heated in a water bath at 50-90 ºC for 5 and 30 min. The suspensions were then cooled to room temperature and let to settle for 2 hrs after which 10 ml were pipetted into a weighing dish and dried at 120 ºC for 2 hours to determine the soluble content. The remaining supernatant was carefully removed by suction and weighed to determine the water solubility index of the starch extract.

Foaming capacity: 2 g of starch sample were homogenized in 100 ml distilled water by using a vortex mixer for 5 min. The homogenate was poured into a 250 ml measuring cylinder and the volume occupied after 30 s was noted. The foaming capacity was expressed as a percent increase in volume occupied by the starch solution. The mean of three replicate determinations was used.

Microscopic examination: Two drops of distilled water were placed on a clean slide, and 2 mg starch were dispersed in the water while ensuring that the starch grains settled down and were thinly spread on the slide. The slide was examined at different magnification up to 40X using a projection microscope. Twenty granules were randomly sampled for each treatment/variety and examined for size and shape.

Viscosity measurement: Viscosity was measured using a Ford viscosity cup. The starch solution was filled in the cup that was allowed to purge by allowing the viscous paste to exude out through the orifice situated at the base of the cup. The time needed for purging the cup was measured carefully using a digital stopwatch at 3% and 5% concentration of the starch solutions. The experiment was repeated three times and the average time of flow was calculated.

Use of the Extracted Starch in Textile Applications

Evaluation of industrial applications of the extracted starch was done by applying the starch for textile applications on cotton yarn. Important characteristics in textile applications include sizing, stiff finish, and ease of removal of the starch after application.

Sizing: The extracted starch was mixed with enough cold water to make a smooth, thin paste at a concentration of 3% and 5% W/W and the mixture was stirred using a glass rod for 3 min. The prepared paste was then cooked for 30 min at 100 ^{o}C. The sizing paste was applied to the cotton yarn, using a laboratory scale sizing machine: 10 cones of 20 count yarns were prepared in the creel and the machine was run at a speed of 20 m/min at a cylinder drying temperature of 150 ^{o}C.

Strength Regain and Elongation at break: The samples were conditioned for 24 hrs and the tensile properties of the yarn before and after sizing were measured using a single yarn strength tester according to ASTM D-2256 standard test method with 250 mm gauge length and 20±3 sec breaking time. The experiment was repeated ten times and the mean was calculated.

$$Strength\ regain\ (\%) = \frac{Strength\ after\ sizing - strength\ before\ sizing}{Strength\ after\ sizing} * 100 \qquad (2)$$

Hairiness: A Shirley hairiness meter was used to measure yarn hairiness, with an electronic sensor counting hairs that exceeded three millimetres in a given length. The Sekisui procedure measures the hairiness level of unsized and sized yarn, reports the actual result, and calculates the percent reduction in hairiness.

***Stiff finish*:** The extracted starch was mixed with enough cold water to make a smooth, thin paste at a concentration of 3% and 5% W/W and the mixture was stirred using a glass rod for 3 min. The solution was cooked and stirred until it thickened and the starch became transparent. The cotton fabric was saturated using a padder (one dip, one nip) to give 80% wet pick up and then the fabric was dried at 100 ^{o}C for 5 min in a hot-air oven. The stiffened fabric was ironed on both sides.

Bending length: Bending stiffness of fabrics was measured using a cantilever test at an inclination angle of 41.5°. Bending rigidity properties of each group were investigated in warp and weft directions using the cantilever test method. The dimensions of tested specimens were 25x200 mm.

Ease of removal of the starch after application: From the stiffened treated fabric 10 cm×10 cm samples were prepared and weighed to 0.001 g accuracy. The samples were then boiled in water for 30 min after which the fabric was dried at 100 °C for 3 min in a hot-air oven and weighed after cooling and conditioning of the fabric. The experiment was repeated three times and the average weight loss was calculated.

The afore-mentioned tests were also performed using a standard starch sample, maize starch, for comparison.

RESULTS AND DISCUSSIONS

The extraction of starch from mango seeds was facile and settling was not hampered by the presence of non-starch materials that remained suspended and floating and were easily decanted off. It is known that non-starch materials will not settle down due to the density difference of starch and other non-starch materials. For example, proteins are less dense than starch and would remain suspended at the top (Manek, et al, 2012). The extracted starch powder was off-white in colour and amorphous in nature. Physicochemical properties of the extracted starch are shown in Table 2.

Table 2. Physico-chemical properties of mango seed starch

Sample	Moisture content of seed (%)	Moisture content of starch (%)	Ash content of starch (%)	Foaming capacity (%)	pH	Viscosity (sec)
Mango starch	44.4	5.68	1.54	51.23	5.78	30.4 (5% concentration) 13.8 (3% concentration)

Compared to a standard maize starch sample, the mango starch had the following characteristics:

- a slightly lower pH
- lower moisture content
- lower ash content
- higher foaming capacity and
- higher viscosity

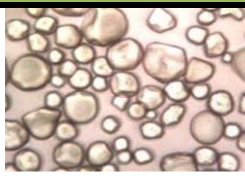
Moisture content: Moisture content of mango seeds is important as if affects yield of the extracted starch. The moisture content of the extracted starch was below the recommended maximum of 14% (Table 2). This is desirable since low moisture content will not promote the growth of micro-organisms, like fungi, that will degrade the starch. This has implications for storage and shelf life of the extracted starch.

Iodine test and pH: The iodine exhibited a deep blue colour confirming that the extracted powder was starch. The pH of the starch extract ranged from 5 to 6, which was within the recommended range of between 4.5 and 7.0 (National Starch and Chemical Company, 2002).

Viscosity measurement: The viscosity at 5% concentration was too high to allow the starch to be used in normal textile sizing application (Table 2). However, the viscosity at 3% concentration was good for such applications. This implies that the starch extract from mango seed can be used at lower concentrations, which has a positive impact from the cost point of view. Such properties are important for mixing and pumping operations in industries.

Microscopic examination: The particle shapes of the extracted starch were mainly oval to elliptical in shape (Table 3). This is in conformity with characteristics of standard starch. The large particle size is an advantage because large particles have smaller surface area and hence smaller surface activity. Particulate function is a surface phenomenon that generates resistance to flow. Thus, larger particles flow better than smaller ones. The smaller particles (large surface area) have more surface energy to attract with other particles and tend to adhere together creating more resistance to flow.

Table 3. Granule shape and size of mango seed starch

Sample	Granule size	Granule shape	
Mango	10.03 µm	Truncated, oval, round, elliptical	

Functional group analysis: The FTIR spectra in Figure 1 shows functional groups that are typical of starch materials: O–H stretching in the range 3700–3600 cm^{-1}; N–H stretching between 3400 and 3300 cm^{-1}; C-H bond around 2930 cm^{-1}; C-H aliphatic stretching the 3000–2850 cm^{-1} range; C–H bond adjacent to a double bond or aromatic ring; and C–H stretching wavenumber that increases and absorbs between 3100 and 3000 cm^{-1}; carbonyl stretching in the 1830–1650 cm^{-1} region; C=N stretching; -1, 4-glycosidic linkages (C–O–C) in the 930 and 1640 cm^{-1} region; C-O-H bonds in the 1080 cm^{-1} to 1158 cm^{-1} region; anhydro glucose ring between 990-1030 cm^{-1} (Silverstein, Webster, Kiemle and Bryce, 2014).

Solubility profile: The results shown in Figures 2 and 3 indicate that starch solubility was affected by heating rate to a greater extent than swelling power was. Higher solubility values were obtained with increase in temperature. Nonetheless, at lower temperatures there were no major differences in the solubility behaviour of the extracted starch. However, differences started to develop when the temperature exceeded 75 ^{o}C. This pattern is likely due to the chemical nature of amylose/amylopectin in the starch.

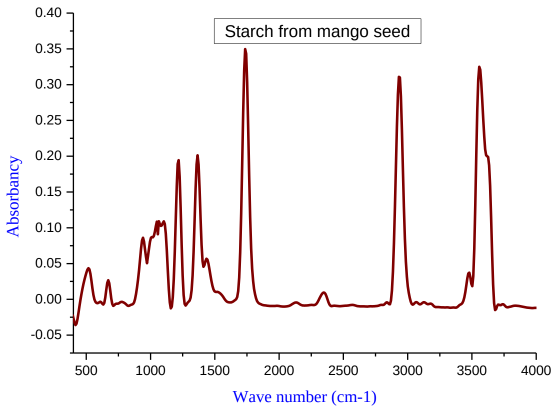


Figure 1. FTIR spectrum of extracted starch

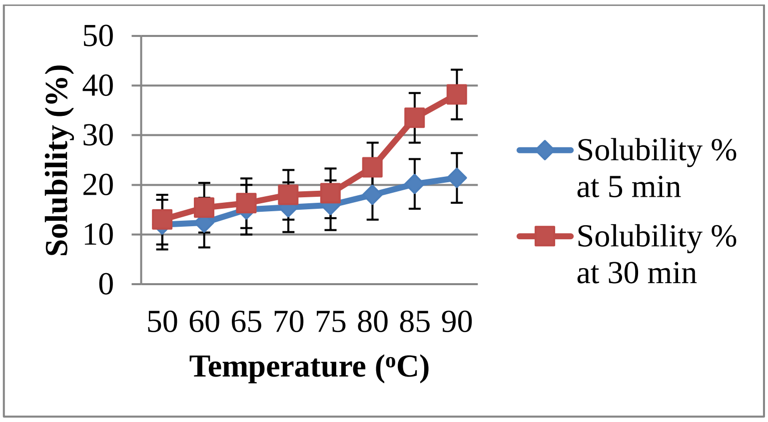


Figure 2. Effect of heating on solubility of starch at two heating times: 5 min (■) and 30 min (♦).

Swelling power: The swelling of starch granules was confirmed to be a two stage pattern: an initial low swelling was noticed up to 75 ºC and a big one thereafter (Figure 2). According to Figure 2 there were no statistical differences in swelling power for samples heated for 5 and 30 min. However, differences were observed for samples heated above 75 ºC. It appears that the effect of heating time was significant at high temperatures due to extensive swelling of the granules. The swelling process was rapid during the first 5–10 min at the initial temperature and continued with further heating. At 50–60 ºC amylose creates crystals with mango starch lipids, which inhibit excessive swelling of granules. At temperatures greater than 75 ºC the crystallites melt, and therefore swelling was enhanced, a fact that explains the fast swelling increase above 75 ºC.

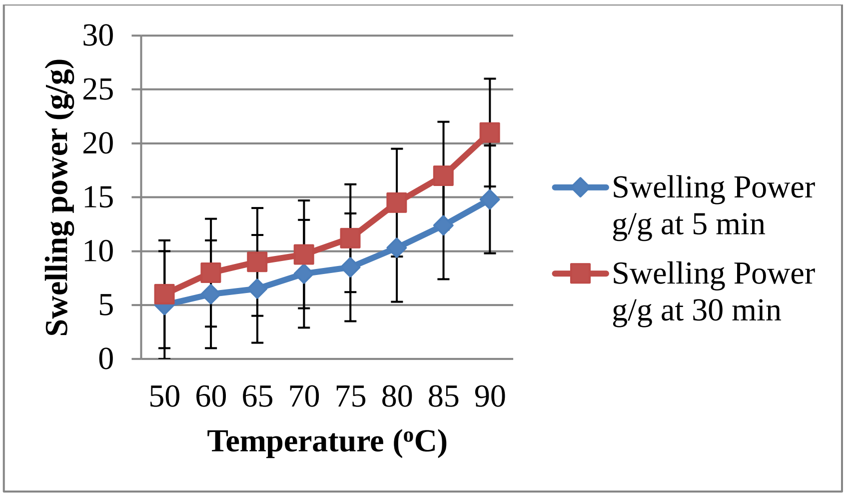


Figure 3. Effect of temperature on swelling power (g/g) of starch under two heating times, 5 min (■) and 30 min (♦).

Optimization of starch extraction yield

The experimental parameters optimised were concentration of mango seeds, extraction temperature, and extraction time - the response factors that were measured were starch yield and whiteness of the starch. The actual values used are shown in Table 4.

Table 4. Testing conditions for starch extraction from mango seeds.

	Factor 1	Factor 2	Factor 3	Response 1	Response 2
Run	***Concentration (%w/v)***	***Temperature (ºC)***	***Time (hr.)***	***Yield (%)***	***Whiteness (%)***
1	0.3	37.5	7	61.91	89.81
2	0.5	37.5	12	62.1	92.12
3	0.1	25	7	48.75	78.21
4	0.1	50	7	56.11	81.31
5	0.1	37.5	2	52.14	79.32
6	0.5	25	7	61.38	91.57
7	0.3	37.5	7	65.27	88.13
8	0.5	50	7	62.3	93.76
9	0.3	50	12	68.2	92.22
10	0.3	25	2	66.2	90.51
11	0.3	25	12	66.71	85.21
12	0.5	37.5	2	63.3	92.75
13	0.3	50	2	66.82	86.23
14	0.3	37.5	7	66.91	85.01
15	0.1	37.5	12	54.11	80.46

Fitting the model: The independent (concentration, temperature and time) and dependent variables (yield and whiteness) were analysed to obtain the regression equation of the model, which was an empirical relationship between the starch yield and the test variable in coded units, which could predict the response under the given range. The regression equation obtained for the extracted starch yield was as follows:

$$(Yield)^{1.69} = +402.61948 + 4987.39018 * Concentration - 4.5852 * Temperature - 20.71596 * time - 16.43025 * Concentration * Temperature - 21.71753 * Concentration * time + 0.1082 * Temperature * time - 5942.41491 * Concentration^2 + 0.15447 * Temperature^2 + 1.7883 * time^2 \quad (3)$$

The analysis of variance (ANOVA) (Tables 5 and 6) showed that this regression model was extremely significant ($P < 0.00001$). A model with P value less than 0.001 is highly significant. As shown in Tables 5 and 6, the F and P values of the lack of fit test were 46.52 and 0.000135, respectively, which implies that concentration of the mango seeds was the most significant variable. This indicates that the equation of the model was adequate for predicting the extraction of starch from mango seeds. The quadratic concentration F and P values of the lack of fit test were 65.90 and 0.000039, respectively, which implies that quadratic concentration was the most significant variable. The fitness of the model was further confirmed by a satisfactory value of the determination coefficient, which was calculated to be 0.6068, indicating that 60.68% of the variability in the response could be predicted by the model. The value of the adjusted determination coefficient (adjusted R^2 = 0.89242) also confirmed that the model was highly robust.

Table 5. The effect estimates of a full second-order polynomial model for optimisation of starch yield from mango seeds

Factors	Effect	Standard Error	t-value	p-value	Coefficient	Standard Error Coefficient
Mean/interaction	60.68	0.57	106.79	0.000000	60.68	0.57
Concentration (%w/v)(**L**)	9.49	1.39	6.82	0.000135	4.75	0.70
Concentration (%w/v)(**Q**)	8.32	1.02	8.12	0.000039	4.16	0.51
Temperature (°C)(**L**)	2.59	1.39	1.87	0.098962	1.30	0.70
Temperature (°C)(**Q**)	-0.75	1.02	-0.74	0.482595	-0.38	0.51
Time (hr)(**L**)	0.67	1.39	0.48	0.645567	0.33	0.66
Time (hr)(**Q**)	-1.57	1.02	-1.49	0.173194	-0.77	0.51

R^2=0.93853; Adjusted R^2=0.89242 and Mean Square Residual=3.873993

Table 6. The ANOVA of a full second-order polynomial model for optimisation of starch yield from mango seeds

Factors	Sum of square	Degree of freedom	Mean square	F-test	p-value
Concentration (%w/v)(**L**)	180.2151	1	180.2151	46.51922	0.000135
Concentration (%w/v)(**Q**)	255.3345	1	255.3345	65.90990	0.000039
Temperature (°C)(**L**)	13.4940	1	13.4940	3.48323	0.098962
Temperature (°C)(**Q**)	2.1001	1	2.1001	0.54209	0.482595
Time (hr)(**L**)	0.8845	1	0.8845	0.22830	0.645567
Time (hr)(**Q**)	8.6622	1	8.6622	2.23598	0.173194
Concentration (%w/v) **L+Q**	435.5496	2	217.7748	56.21456	0.000019
Temperature (°C) **L+Q**	15.5941	2	7.7970	2.01266	0.195872
Time (hr) **L+Q**	9.5466	2	4.7733	1.23214	0.341603
Error	30.9919	8	3.8740		
Total Sum of Squares	504.1607	14			

The data for optimisation studies indicated that the effects of concentration on starch yield were statistically significant and there were significant relations of variables for starch yield as can be seen in Figure 4. The results demonstrated that yield was generally highest at soak temperature of 50 ºC; soaking at 25 °C resulted in a starch yield that was 3.15-5.25% lower. Starch yield was highest after a 12 hr steeping time but, unfortunately, this time length resulted in the development of microorganisms that were visible on the upper surface of the samples. Additionally, longer steeping times lead to lower starch yields - this is mainly due to the increase in hydration and swelling of the starch which reduces the filtrate amount making it difficult to facilitate sedimentation. The lower starch yield at low levels of sodium metabisulphite concentration and steep temperature was due to lower solubility and dispersibility of the starch molecule. The critical values for optimal whiteness of the extracted starch that were calculated by the software are as follows: concentration = 0.35 (%w/v); temperature = 26.74 ºC; time = 6.46 (hr). The influential effect of the input variables on whiteness were evident using response surface plots as illustrated in Figure 4.

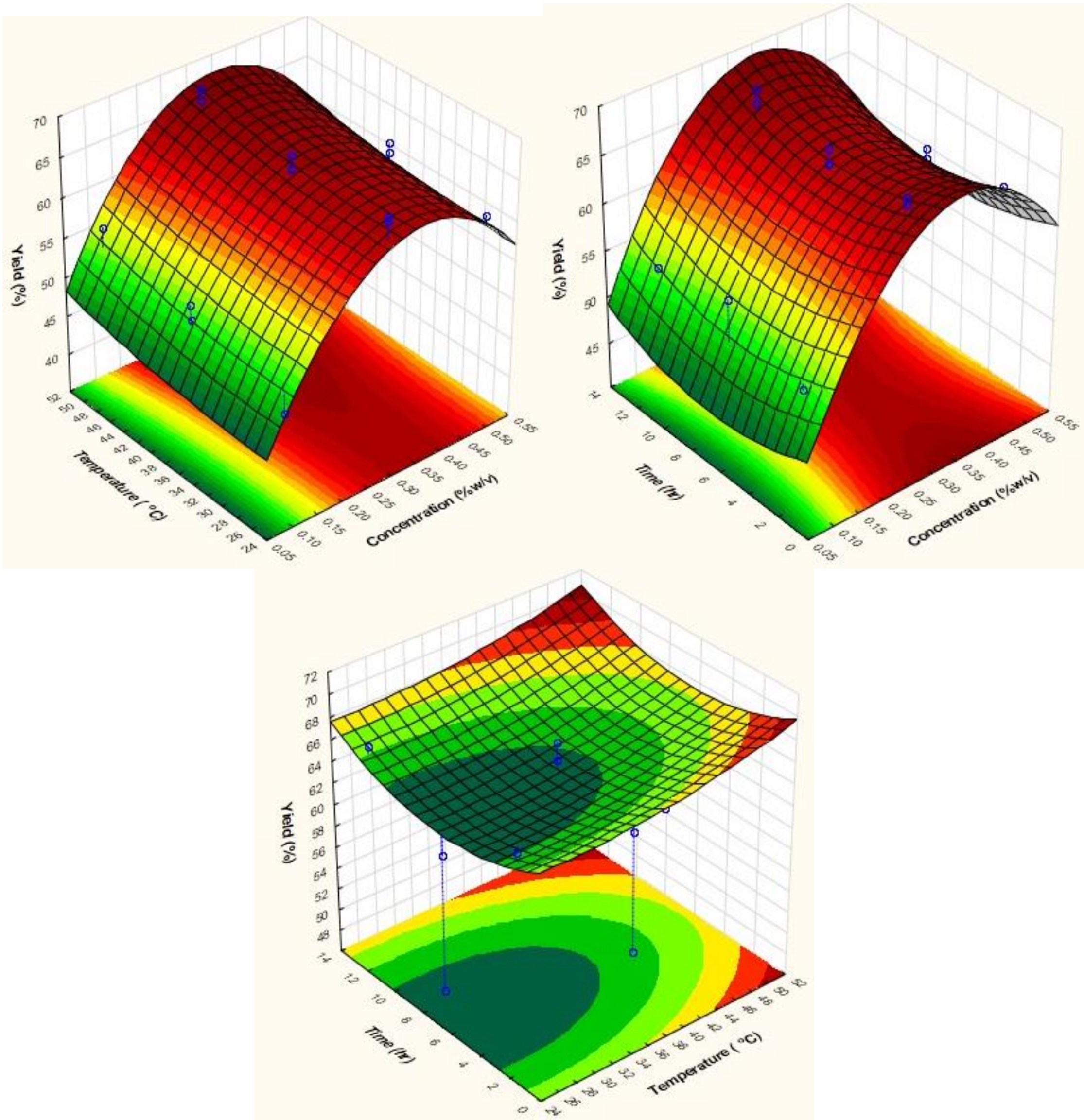


Figure 4. Response surface plot showing the effect of (concentration and temperature), (concentration and steeping time) and (temperature and steeping time) on yield of starch extraction from mango seeds.

To further clarify the data and judge the adequacy of the model in the experimental data, diagnostic plots were drawn. A plot of observed response (Yield) versus predicted response is shown in Figure 5 (A). In this case, the predicted values were in agreement with the observed ones in the range of the operating variables. The normal probability plot of the standardised residuals was used to check for normality of residuals (Figure 5 (B)). A linear pattern observed in this plot suggests that there were no signs of any problems in the experimental data. Figure 5 (C) represents a plot of standardised residuals versus predicted values to check for constant error. The residuals displayed randomness in scattering and suggested that the variance of the original observation was constant.

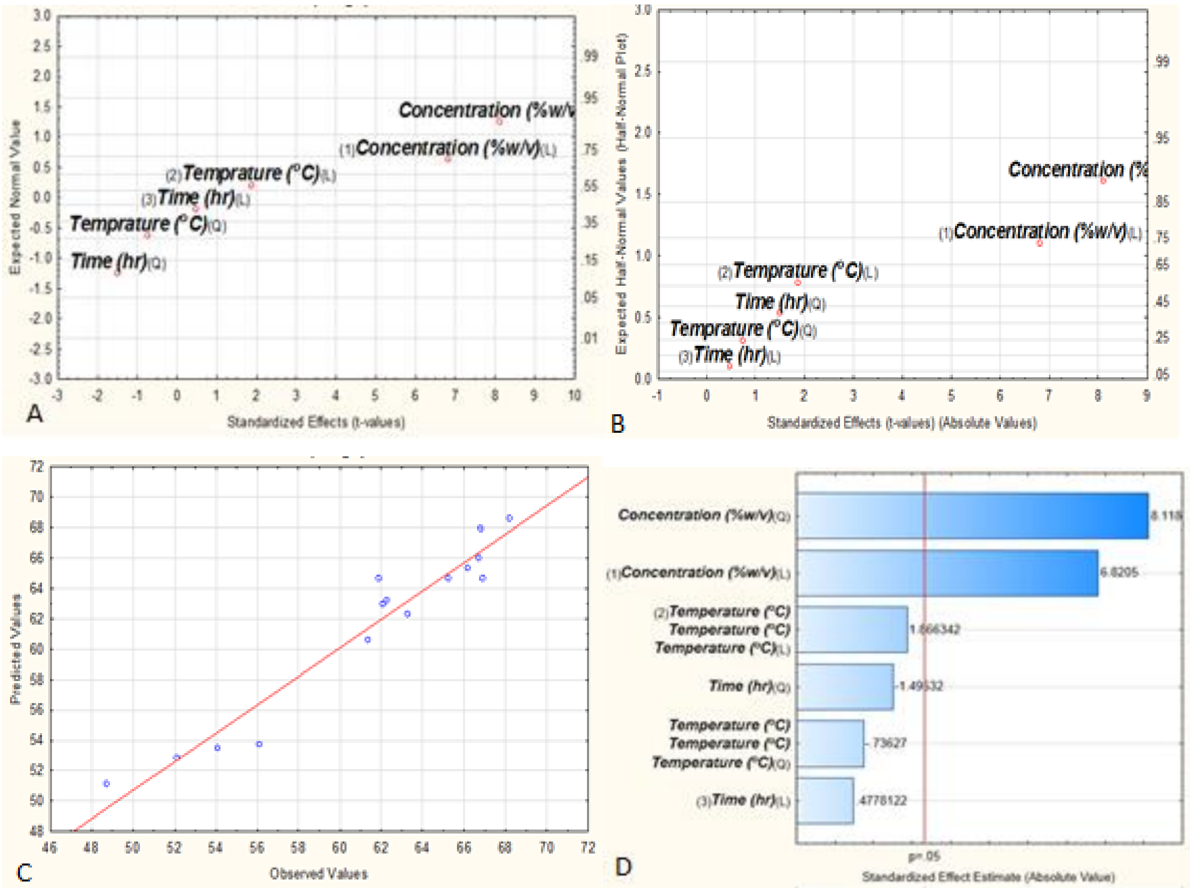


Figure 5. Normal probability design, half normal probability design, observed Vs predicted plot and Pareto chart of standardized effects of starch yield respectively. *(Note- L values are linear values of p values and Q values are quadratic values of the variables. Q values help to get the optimal values of each of the variables and negative values are represented as positive values).*

Regression analysis (Table 5) and Pareto chart (Figure 5(D)) results indicate that concentration is the first and temperature is the second most influential variable among the chosen parameters as indicated by the p-values. It can be easily noticed that the variable with the largest effect was the linear term of concentration, followed by quadratic concentration, and the linear of temperature. The factor *t*-test value (9.42) and *p*-value (p 0.000013) corresponding to linear concentration and t test value (8.11) and-*p* value (p = 0.000039) corresponding to quadratic concentration were the significant factors. According to the *t* and *p* values, temperature, time, and quadratic values of both time and temperature did not exhibit statistical significance. The linear effect of time was found to have a *p*-value > 0.05 indicating the broad range effect of the variable on starch yield. Square values of the variables were used to ascertain their quadratic effects so as to get the curvature in the response surface graphs and to get the optimal value for each variable. The fit of the model was checked by determination of coefficient (R^2), which was 0.93853 thus revealing that 93.85% of the sample variation in starch yield was attributed to independent variables.

Optimization of whiteness of the extracted starch

Fitting the models: The independent and dependent variables were analysed to derive a regression equation, which was an empirical relationship between the starch whiteness and the test variable in coded units that could predict the response under the given range. Independent and dependant variables were analysed to get regression equations that could predict the response under the given range; each of the observed value was compared with the predicted value which was calculated from the model. The regression equation obtained for the extracted starch yield was as follows:

$$(Whiteness)^3 = +6.38561E + 005 + 7.870E + 005 * Concentration - 5553.35 * Temperature - 36384.77093 * time - 275.29469 * Concentration * Temperature - 9494.2188 * Concentration * time + 1063.56025 * Temperature * time \quad (4)$$

The analysis of variance (ANOVA) (Table 7 and 8) showed that this regression model was extremely significant ($P < 0.00001$). A model with P value less than 0.001 is highly significant. The lack of fit test measures the failure of the model to represent the data in the experimental domain at points which are not included in the regression. As shown in Tables 8 and 9, F- and P- values of the lack of fit test were 56.80 and 0.000067, respectively, which implies that concentration was the most significant factors and indicates that the model equation was adequate for predicting the extraction of starch from mango seeds. The fitness of the model was further confirmed by a satisfactory value of the determination coefficient, which was calculated to be 0.8697, indicating that 86.97% of the variability in the response could be predicted by the model. The value of the adjusted determination coefficient (adjusted $R^2 = 0.7972$) also confirmed that the model was highly robust.

Table 7. The effect estimates of a full second-order polynomial model for optimisation of starch whiteness from mango seeds

Factor	Effect	Standard Error	t-value	p-value	Coefficient	Standard Error Coefficient
Mean/Interaction	86.97	0.69	126.18	0.000000	86.97	0.69
Concentration (%w/v)(**L**)	12.73	1.69	7.54	0.000067	6.36	0.84
Concentration (%w/v)(**Q**)	1.91	1.24	1.54	0.163071	0.95	0.62
Temperature (°C)(**L**)	2.01	1.69	1.19	0.269079	1.00	0.84
Temperature (°C)(**Q**)	0.47	1.24	0.38	0.714377	0.24	0.62
Time (hr)(**L**)	0.30	1.69	0.18	0.863387	0.15	0.84
Time (hr)(**Q**)	0.42	1.24	-0.34	0.743334	-0.21	0.62

R^2=0.88411; Adjusted R^2 =0.7972 and Mean Square Residual=5.701241

Table 8. The ANOVA of a full second-order polynomial model for optimisation of starch whiteness from mango seeds

Factor	Sum of square	Degree of freedom	Mean square	F-test	p-value
*Concentration (%w/v)***(L)**	323.8513	1	323.8513	56.80365	0.000067
*Concentration (%w/v)***(Q)**	13.4523	1	13.4523	2.35954	0.163071
*Temperature (ºC)***(L)**	8.0401	1	8.0401	1.41023	0.269079
*Temperature (ºC)***(Q)**	0.8200	1	0.8200	0.14382	0.714377
*Time (hr)***(L)**	0.1800	1	0.1800	0.03157	0.863387
*Time (hr)***(Q)**	0.6552	1	0.6552	0.11492	0.743334
Concentration (%w/v) **L+Q**	337.3035	2	168.6518	29.58159	0.000201
Temperature (ºC) **L+Q**	8.8600	2	4.4300	0.77703	0.491597
Time (hr) **L+Q**	0.8352	2	0.4176	0.07325	0.929987
Error	45.6099	8	5.7012		
Total Sum of Squares	393.5772	14			

The optimisation studies indicated that the effects of concentration on whiteness of the extracted starch were statistically significant and there were significant relation of variables for whiteness. The whiteness index of the extracted starch was determined using 15 different recipes by varying the temperature, time, and sodium metabisulphite concentration as shown in the experimental design in Table 4. The critical values for optimal whiteness percentage of the extracted starch given by the software are as follows: Concentration (%w/v), 0.63; temperature, 24 ºC; and time, 6 hr. The influential effect of input variables on whiteness was represented using response surface plots as illustrated in Figure 6. Additionally, the results showed that the concentration of sodium metabisulphite could be reduced to 0.2% without loss of quality in starch purity. The whiteness degree of the extracted starch was higher when it was extracted in the presence of 0.5% concentration sodium metabisulphite and there was not much difference from the colour of a commercial starch which is 95.75%.

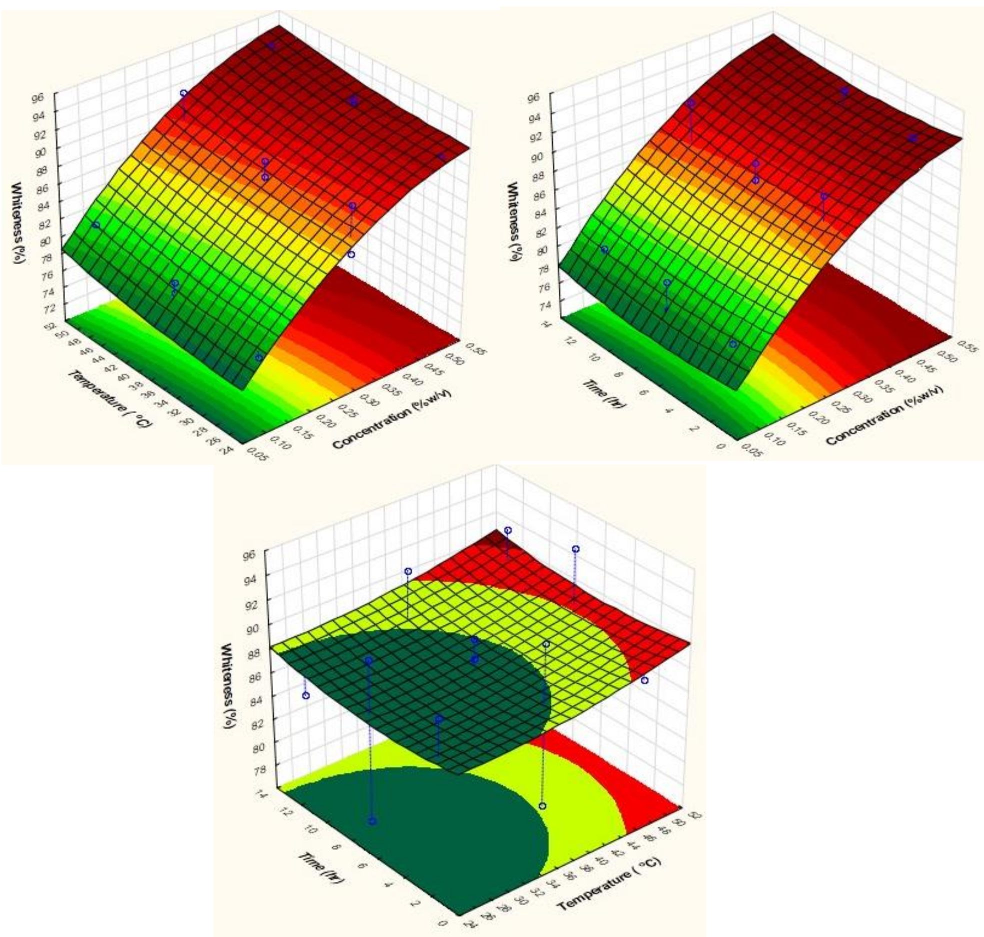


Figure 6. Response surface plot showing the effect of (concentration and temperature), concentration and steeping time) and (temperature and steeping time) on whiteness index of extracted starch respectively.

To clarify the signs of any problems and judge the adequacy of the model in the experimental data, diagnostic plots were drawn. Plots of observed response (whiteness) versus predicted response are shown in Figure 7 (A and B). In this case, predicted values were in agreement with observed ones in the range of the operating variables. The normal probability plot of the studentized residuals was used to check for normality of residuals (Figure 8 (A and B)). A linear pattern observed in this plot suggests that there was no sign of any problem in the experimental data. Figure 7 (C) represents a plot of studentized residuals versus predicted values to check for constant error. Residuals displayed randomness in scattering and suggested that the variance of the original observation was constant.

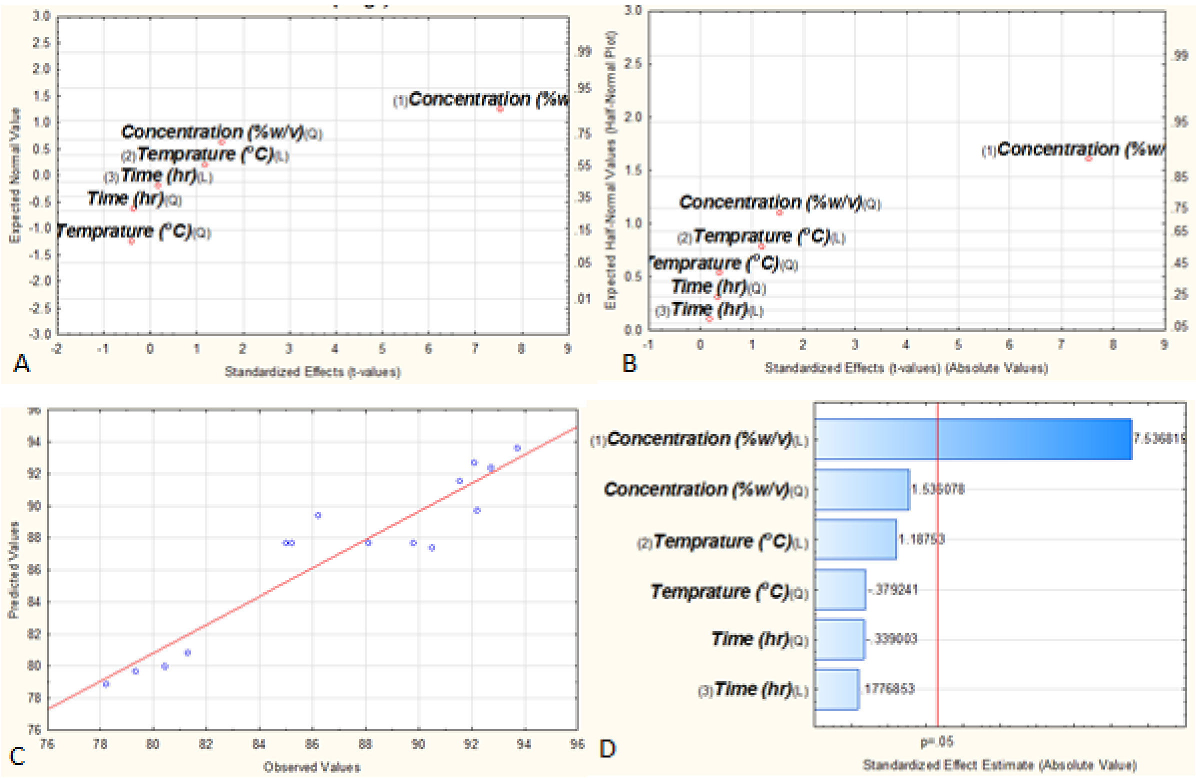


Figure 7. Normal probability design, half normal probability design, observed versus predicted plot and pareto chart of standardized effects of starch whiteness respectively.

Regression analysis (Table 8) and pareto chart (Figure 7 D)) results indicate that concentration is the first and temperature the second most influencing variable among the chosen parameters as indicated by the p values. The significance of each coefficient was determined using pareto chart and *p* value in Table 9 and it could be easily noticed that the variable with the largest effect was the linear term of concentration, followed by quadratic concentration, and the linear of temperature. The factor *t*-test value (3.16275) and *p*-value (p 0.013340) corresponding to linear concentration was the significant factor. According to the t and p value, temperature, time, quadratic values of both time and temperature were of statistical significance. The linear effect of time was found to have a *p*-value > 0.05 indicating the broad range effect of the variable on starch whiteness. Square values of the variables are used to indicate their quadratic effects so as to get the curvature in the response surface graphs and to get the optimal value for each variable. The fit of the model was checked by determination of coefficient (R^2), which was 0.8841 revealing that 96.36% of the sample variation in starch yield was attributed to independent variables.

Testing of starch in textile sizing application

Strength regain and elongation: The strength regain of the sized yarn was within the recommended range of 15-40% (National Starch and Chemical Company, 2002). The major difference between the commercial starch extract and the extracted starch is that the commercial starch is a starch modified with additives that will have an impact on strength regain (Abbas, et al, 2010). These additives include arabic gum, defoamer and others. The size suspension from the extracted starch of the extracted starch was prepared without the additives. The elongation result was acceptable. It is important to note that it is generally expected for yarns to lose elongation after sizing operations due to stretching while the yarn is wet (Anonymous, 2016).

Table 9. Comparison of starches with respect to strength regain and elongation

	Untreated yarn		Yarn treated with mango seed starch		Yarn treated with commercial enset starch	
	Strength(N)	**E %**	**Strength**	**E %**	**Strength**	**E %**
Average	2.12	6.38	2.733	2.76	3.072	3.12
SR%			**22.43**		**30.99**	

where SR = strength regain, E = elongation

Hairiness: It is important for the sizing material to coat the yarn surface well enough to slick down the "hairs" from the yarn bundle (Anonymous, 2016). The largest hairiness reduction of 61.2% was recorded for yarn sized with mango seed starch (Figure 8). The standard hairiness reduction of value is 50%. The greater number of hairs, the greater the tendency to form a size bridge between ends on the slasher, leading to a harder break at the release rods, and the greater the amount of friction on the loom, resulting in excessive end breakage.

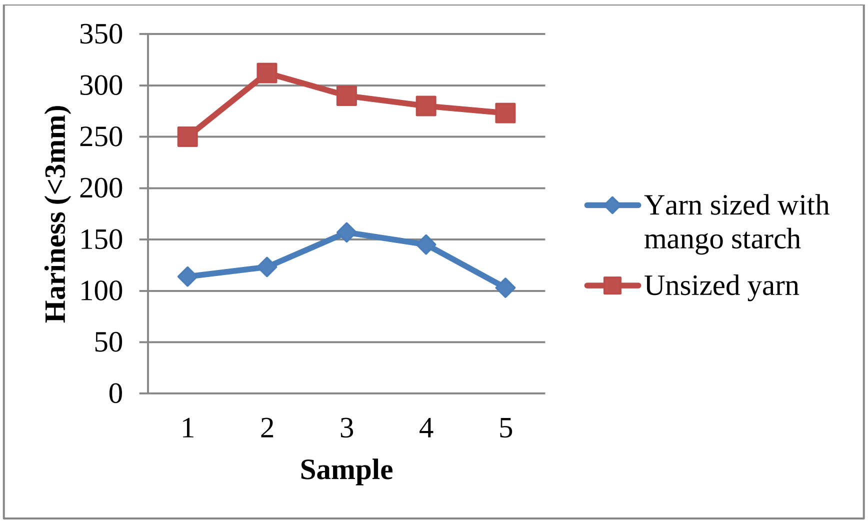


Figure 8. Hairiness value before and after sizing

Testing of starch in textile stiff finish applications:

Bending length: For both 5% and 3% starch concentrations the bending lengths for fabric treated with the extracted starch and commercial starch were on the high side but there were no significance differences between the starch samples as shown in Table 10.

Table 10. The bending property of the cotton fabric after and before stiff finish using extracted starch.

Bending	Un treated fabric	Fabric treated with mango seed starch		Fabric treated with commercial enset starch	
		3% concentration	5% concentration	3% concentration	5 concentration
Average (cm)	2.4	5.9	8	5.6	8

Weight loss/ease of removal: Higher weight loss value indicates better removal of size mix from the treated fabrics. The data in Figure 11 indicate that mango seed starch is better for ease of removal of the size material from the fabric.

Table 11. Comparison of starch removal characteristics during wet treatment of fabrics

Starch removal	Fabric treated with mango seed starch			Fabric treated with commercial enset starch		
Average	W_1 (g)	W_2 (g)	WL (%)	W_1 (g)	W_2 (g)	WL (%)
	2.57	1.61	27.35	2.12	1.4	33.96

where W_1 =weight of the fabric after stiffening treatment, W_{2} = weight of the stiffed finished fabric after washing with boiling water and WL= weight loss (average of 3 replicates).

By-products of the starch extraction process

The by-products of the starch extraction process were characterised to ascertain if they contained any valuable materials. The nitrogen content of the residue after extraction of the mango seeds was 0.73%. This value is comparable to the nitrogen content of an excellent natural fertilizer from sheep manure (0.9%) and greater than that exhibited by horse and cattle manure (0.5%). This implies that the residue of the starch extraction could be used as a good source of bio fertilizer. Additionally, this by-product could be used as animal feed.

Techno-economic analysis of the starch extraction process

A techno-economic analysis of the starch extraction process from mango seeds was done in the Ethiopian context (Tesfaye and Sithole, 2016). The parameters and data that were considered in the analysis included:

- Establishment of a plant for the production of mango seed starch with a capacity of 500 tonnes per annum.
- The present demand for starch for use in 5 textile factories estimated at 1365 tonnes per annum.
- The starch demand is expected to reach 2225 tonnes by the year 2020.

A schematic of the starch extraction process is shown in Figure 9.

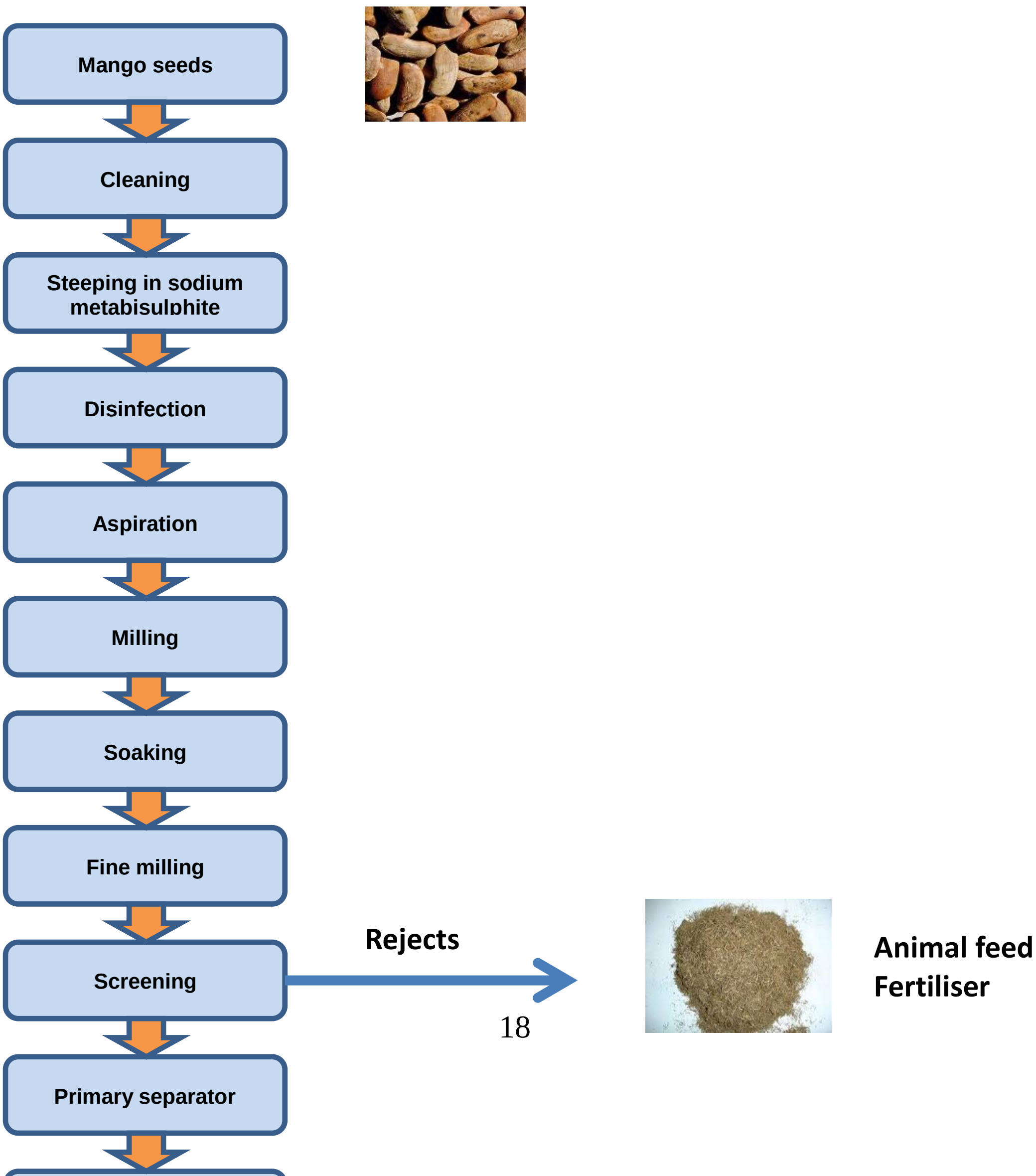


Figure 9. Process flow diagram for the starch extraction from mango seeds.

A feasibility analysis of the starch process indicated that establishment of a starch extraction plant could create employment opportunities for 19 persons. The total investment requirement was estimated at about R3.05 million, of which R2.2 million would be required for infrastructure and machinery. The project was deemed to be financially viable with an accounting rate of return (ARR) of 83.28 % and a break-even analysis of 21%. Projections of this information to the South African context indicate that the extraction process could be viable option for creation of SMMEs to establish and operate entities for extraction of starch from waste mango seeds.

CONCLUSIONS

This study reveals that mango seeds are a good source of starch: physico-chemical tests of the extracted starch confirmed that the extracted material was indeed a starch material. The starch was tested for use in textile applications and the results indicated that the material performed as well as a standard starch sample. Response surface methodology was used to optimise the extraction of starch from mango seeds. The experimental parameters optimised were concertation of mango seeds, extraction temperature, and extraction time and the response factors that were measured were starch yield and whiteness of the starch. Applications of the starch product in textile applications indicate that the material performs as well as a standard starch sample. The extraction of starch from mango seeds is facile and does not require sophisticated technology except that it is water-intensive.

An economic and financial feasibility analysis of the extraction of starch from mange seeds was done in the Ethiopian context and the results showed very good promise for the project. Thus, there is potential for the starch extraction process to be uptaken by SMMEs in South Africa.

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