

Optimization of isolation parameters for starch extraction from *Amaranthus paniculatus* (Rajgeera) using response surface methodology

Pooja Yadav, Dr. John Don Bosco

Department of Food Science and Technology, Pondicherry University, Puducherry, India

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Keywords— *Amaranthus paniculatus*,
Pseudo-cereal, *response surface*
methodology, *Starch yield*, *NaOH*
concentration

Abstract— *Amaranthus paniculatus* is a pseudo-cereal. It is a potential reservoir of starch and the properties of end-use product are dependent on the starch. As the major component in *Amaranthus paniculatus* is starch it is very necessary to standardize the process for starch isolation for the maximum recovery of starch with minimal impurities. The study was aimed to improve the isolation parameters for the starch extraction using response surface methodology (RSM). The NaOH concentration (0.00 - 0.75 %), sieve mesh size (100 - 300) and steeping time (1 - 24 hrs) were taken as independent variables and starch yield (%), protein (%) and fat (%) were taken as optimizing responses. Quadratic model was used to evaluate the results. The process was optimized at 0.375 % of NaOH concentration, sieve of 300 mesh and steeping time of 24 hrs for the desired results i.e starch yield (39.91%), protein (0.87%) and fat (0.53%) with a desirability level of 1.00

I. INTRODUCTION

India is an agricultural country and plenty of food grains are grown here, Amaranth is one of them. It belongs to family Amaranthaceae and has more than 60 species out of which *Amaranthus paniculatus*, *Amaranthus hypochondria*, *Amaranthus cruentus*, and *Amaranthus caudatus* are the essential grain species (Brenner et. al., 2000 ; Narender Kumar et. al., 2013). *Amaranthus paniculatus* is a tall annual herb and is a flowering plant species. Starch accounts for 50% to 69% depending on the species. Starch is the major component of *Amaranthus paniculatus*. The major reserve polysaccharide in plants is starch and plays a crucial role in the food industry especially in the bakery industry (Teli et. al., 1996). Starch is a semi-crystalline, white, tasteless and odorless powder that is composed of several amylose and amylopectin linkages branched complexly. Starch forms the major source of carbohydrates in dietary seeds, cereals, legumes, pseudo-cereal and in the parts of plants like tubers, roots, leaves, stems, etc. (Kumar N et. al., 2013). The amylose and amylopectin content of

the starch varies with the source of starch and therefore the chemical composition, properties and the structure of starch vary as well. The shape and size of the starch granules is also different for each variety. The importance of starch as a food ingredient is evident from the fact that it forms 30% of the average diet and on a dry weight basis and more than 25% on an available energy basis (Galliard, 1987). Starch is a highly versatile food ingredient that is used as an additive in the form of an emulsifier, stabilizer and thickener (Rengsutthi, 2011) in various food formulations in daily life. It has extremely small granules and has low amylose content and has 90-95% of amylopectin which provides excellent freeze/thaw stability. Due to the presence of high amount of amylopectin, the higher value of enthalpy change is observed. Amaranth starch has good swelling power because of high amylopectin content. Gelation properties are interrelated to the water absorption capacity, amaranth has a good gel formation capacity because the water absorption capacity of amaranth starch is good (Sindhu et al,2016). Amaranth

also contains squalene which exhibits cholesterol properties (Petras R. Venskutoins and Paulius Kraujales, 2013). It is also found in the study by (Martirosyan D.M., 2013) that *Amaranthus paniculatus* is a good source of flavonoid especially rutin. It has various characteristics other than its nutritional value, it is resistance to a wide range of agro-climatic conditions such as drought, hot climate, and pests as well as it adapts readily to new environmental conditions (Yue et. al., 1993). An earlier method was to soak the grains in aqueous solution with $HgCl_2$ to inhibit the enzymatic activities. (Wankhede et. al, 1989) but the drawback is the toxicity of $HgCl_2$. Colour and clarity play a very important role in the application of ingredient in food products. The acceptability of starch may be reduced because of its color (Galvez and Resurreccion, 1993).

Response surface methodology (RSM) software is very important for the optimization. It is very useful in designing, formulating and improving the process parameters. (Bas and Boyac, 2007)

II. MATERIAL AND METHODS

2.1 Material

The *Amaranthus paniculatus* seeds used in the present study were procured from the National Bureau of Plant Genetic Resources (NBPGR) located in Shimla, India. The grains were sun-dried for 1 day and screened to remove foreign matters. The grains were stored in a sealed container at room temperature for further studies.

2.2 Physical properties

The diameter of the grain is determined by screw gauge.

2.2.1 Thousand kernel weight

Thousand kernel weight is determined by the method given by varnamkhasti *et al.*, (2008) it was determined by randomly selecting 1000 grains and weighing the grains on electronic balance.

2.2.2 Porosity

Porosity (ϵ) is defined as the fraction of space in a bed of grains that is not occupied by the grains.

$$\epsilon = (1 - \rho_b / \rho_t) \times 100$$

Where,

ρ_b is bulk density of the grains

ρ_t is the true density of the grains

2.2.3 Bulk density (ρ_b) and true density (ρ_t)

Bulk density (ρ_b) is the ratio between the mass of a sample of grain and the total volume occupied by it. It was determined by pouring the grains from a certain height into

the cylinder of known volume, weight the grains in the cylinder.

Bulk density (ρ_b) = mass / volume

True density (ρ_t) is the ratio between the mass of the sample grains and the actual volume occupied by it, it was determined by the toluene displacement method (Mohsenin, 1986)

2.2.4 Angle of repose

Angle of repose is the angle with the horizontal at which the material will stand when piled. It was determined by adjusting the funnel on the burette stand at a height of 2 cm from the base, grains were poured in the funnel till the pile of the grains touches the end of the funnel, note the diameter of the pile and put the values in formula to get the value of angle of repose.

$$\theta = \tan^{-1} (2H/D)$$

Where,

H is the height of the funnel from the base

D is the diameter

Value of tan inverse is calculated using tan inverse table.

2.3 Preparation of Flour

The flour was prepared by grinding the grains in Butterfly grand turbo (2015) available in the food processing laboratory of the Food Science and Technology Department, Pondicherry University, India. prepared flour was sieved through sieve no. 100 (Jayant Scientific Ind. Mumbai) and stored in zip lock bags at room temperature for further study of its nutritional composition.

2.4 Proximate Composition

The prepared flour sample was analyzed for its chemical composition i.e. moisture, protein, carbohydrates, fat, fibre and ash by using the methods given by AOAC, 1990. All the analyses were conducted in triplicate, the average value and the standard deviation was taken as the final value.

2.5 Isolation of Starch

Amaranth grains are soaked in an alkali solution of different concentrations (0.00-0.75 %) for 1- 24 hrs at 4°C and stirred twice or thrice in between. The grains were washed with distilled water after steeping and blend in the blender at full speed to make the slurry. The slurry was filtered through different mesh size filter screen (100 - 300). The filtrate was centrifuged at 3000g for 20 min. Discard the supernatant and scrap the top yellow layer containing protein and other impurities. Suspend the starch again in distilled water and centrifuged as described above. This process was repeated 4 times to remove the maximum amount of impurities. The isolated starch was kept in a hot

air oven at 50°C for 10-12 hrs for drying and stored in sealed packs at room temperature.

2.6 Chemical composition of starch

The extracted starch was analyzed for moisture, fat, proteins, ash and fibre content by the standard methods given by AOAC,1990. Method of (Williams et al., 1970) was used to determine the amylose content of the isolated starch, and the absorbance was measured at 625nm using UV Spectrophotometer (UV-1800, Shimadzu, Japan). The amount of amylose present is calculated by using the standard curve blends of amylose.

2.7 Starch yield

Starch yield is the the ratio between, the amount of powder obtained after drying and the total amount of starch slurry

$$\text{Powder yield (\%)} = \frac{\text{Dried powder/ amount of starch slurry}}{\text{amount of starch slurry}} \times 100$$

2.8 Color parameters

Colour parameters of the isolated starch were analyzed by using Color flex spectrophotometer (CX2748) (IR technology service(P) Ltd., Bangalore). the values were determined in L*, a* and b* where lightness and darkness are represented by L*, green and red opposition are represented by a* and yellow and blue opposition is represented by b*.

2.9 Swelling power and Solubility of Starch

Swelling power and solubility were analyzed by the method given by (Adebooye and Singh, 2008) with slight modifications, 500mg of the starch sample was heated with 20 ml of distilled water for 30 min at 80°C. Bring the same to room temperature. Centrifuge the sample at 3000g 20 min. Pour the supernatant into the petri dish, weigh and kept for drying at 100°C and weigh again. The residue was weighed for the estimation of swelling power. The solubility and swelling power were calculated by the following formula:

$$\text{Solubility} = \frac{\text{dried supernatant weight/ wet sediment weight}}{\text{wet sediment weight}} \times 100$$

$$\text{Swelling Power} = \frac{\text{wet sediment weight/ sample weight}}{\text{dried supernatant weight}}$$

2.10 Bulk Density and Tapped Density

The method given by Jangam et al. (2010) was used to determine the bulk density and tapped density. 1g of the sample was loaded in 10 ml of measuring cylinder and to determine the bulk density, bulk volume was noted. In the same measuring cylinder, the sample was tapped 100 times and to determine the tapped density (weight/volume) of the sample the final volume of the sample was recorded.

2.11 Water and oil absorption capacity

Water and oil absorption capacity was determined by the method given by Ige et al (1984) . 2 g of sample was dissolved in 10 ml of distilled water, allow it to rest for 15 min and then centrifuge it at 3000 rpm for 20 min. Decant the supernatant, tubes were dried at 40°C and weighed after drying. For the oil absorption capacity, take 2 g of sample and add 5 ml of groundnut oil the sample. The solution was stirred for 1 min and then kept for resting for 20 min. After resting the tubes are centrifuge at 3000 rpm for 20 min. The volume of the oil which is not absorbed is noted.

2.12 Water Activity

To determine the water activity of the sample electronic water activity meter was used (Aqua lab Series, Decagon Devices, Inc., Pullman, USA)

2.13 Scanning electron microscopy (SEM)

To determine the morphological structure of starch granules scanning electron microscopy was used (Hitachi, S-3400N, 340512-02, Japan). The sample was mounted on the aluminium slab using double sided, place the slab in the coating chamber to coat the sample with gold. The sample was observed at a magnification of 2000 X

EXPERIMENTAL DESIGN AND STATISTICAL ANALYSIS

For the optimization of isolation of starch from *Amaranthus paniculatus* RSM was used. 3 independent variables namely alkali concentration (0.00 - 0.75 %), sieve mesh size (100 - 300) and steeping time (1 - 24 hrs) along with 3 responses which are starch yield (%), protein content (%) and fat content (%) were used using boxbenken model with 17 runs. Equation obtained for the coded variables are:

$$\text{Starch yield: } +35.35+2.05A +2.27B +2.89C +2.33AB +1.51AC +0.89BC -6.47A^2 -0.90B^2 -0.15C^2$$

$$\text{Protein: } +1.14 -0.13A -0.21B -0.14C -0.05AB -0.09AC -0.05BC +0.46A^2 -0.15B^2 +0.11C^2$$

$$\text{Fat: } +0.50 -0.10A -0.05B -0.11C -0.05AB -0.03AC -0.02BC +0.19A^2 +0.10B^2 +0.13C^2$$

Where, A is alkali concentration, B is sieve mesh size and C is steeping time. Linear interaction was shown by A, B and C and AB, AC, BC shows interaction regression coefficients whereas quadratic regression coefficients were shown by A², B² and C². Significance level were determined by analysis of variance (ANOVA). F- value determines the lack of fit and it should not be significant otherwise the model will not be fit. (Atalar and Dervisoglu, 2015). R² value determines the efficiency of the model. Design expert 12 software was used for the optimization.

III. RESULT AND DISCUSSION

Physical properties

The physical attributes of Amaranth paniculata is given in Table 1. The diameter is found to be 0.5mm because of too small size of the grains the thousand kernel weight was found to be 0.76g, similar results were observed by (Kudos and Solanki, 2018).

Bulk density and true density was found to be 830 kg/m³ and 1396 kg/m³ respectively, similar pattern of result has been reported by (Sobulola and Onwuka, 2010) for locust bean seed. Angle of repose was found to be 25.65° this could be because of the small surface area of the grains due to which there will be less surface tension, similar pattern of results was observed by (Kudos and Solanki, 2018)

Table 1: Physical properties of *Amaranthus paniculata*

Diameter (mm)	0.5±0.01
Thousand kernel weight (g)	0.76±0.03
Bulk density (kg/m ³)	830±0.05
True density (kg/m ³)	1396±0.04
Porosity (%)	40.54%±0.01
Angle of repose (°)	25.65°

The values are expressed as mean ± SD of triplicate readings

The proximate composition of Amaranth paniculata is given in Table 2. The content of protein and fat is less as compared to the starch, it was of same trend as studied by

Table 3: Experimental design

Runs	NaOH CONC (%)	Mesh size	steeping time (hrs)	starch yield (%)	Protein (%)	fat (%)
1	0.75	100	12.5	23.97	1.61	0.83
2	0	200	24	27	1.65	0.9
3	0.375	200	12.5	34.88	1.13	0.21
4	0.375	300	24	39.91	0.87	0.53
5	0.375	200	12.5	36.91	0.99	0.58
6	0	100	12.5	25.5	1.81	0.9
7	0.75	300	12.5	35.07	0.97	0.57
8	0.75	200	24	35.07	1.23	0.61
9	0	200	1	25.34	2.03	0.96
10	0.75	200	1	27.36	1.97	0.81
11	0.375	200	12.5	35.02	1.11	0.58
12	0.375	200	12.5	34.88	1.19	0.59
13	0.375	100	1	30.41	1.21	0.91

(Choi et al,2004; Ritu Sindhu and Bhupender Singh,2016 and Narender Kumar et. al., 2013)

Table 2: Nutritional composition of *Amaranthus paniculata*

S.No.	Composition	Content(%)
1	Moisture	11.39±0.03
2	Protein	14.24±0.15
3	Carbohydrates	69.38±0.24
4	Fat	8.70±0.02
5	Fibre	2.95±0.01
6	Ash	3.75±0.04

The values are expressed as mean ± SD of triplicate readings

As *Amaranth paniculata* have nearly 70% of starch content, it is very important to standardize the process for its extraction. The process was standardized for the maximum starch yield with improved colour and minimum impurities.

The experimental design is given in table 3, Analysis of Variance (ANOVA) is listed in table 4 and the constraints were given in table 5.

14	0.375	100	24	35.48	1.31	0.65
15	0.375	200	12.5	34.92	1.28	0.58
16	0	300	12.5	27.26	1.39	0.87
17	0.375	300	1	31.25	1	0.89

NaOH concentration, mesh size and steeping time are independent variables whereas starch yield, protein and fat percent are the response. All the responses are mean value of triplicate readings

Table 4: Analysis of variance (ANOVA)

Source (P>F)	Yield	Protein	Fat
Model	0.0004	0.0114	0.0429
A	0.0051	0.0632	0.3114
B	0.003	0.011	0.7607
C	0.0008	0.0545	0.2972
AB	0.143	0.5525	0.8855
AC	0.0741	0.3412	0.4320
BC	0.2533	0.535	0.0270
A ²	<0.0001	0.001	0.0046
B ²	0.2406	0.1071	0.7571
C ²	0.828	0.2181	0.0464
Lock as fit	0.0819	0.0764	0.1419
R ²	0.9611	0.892	0.8342

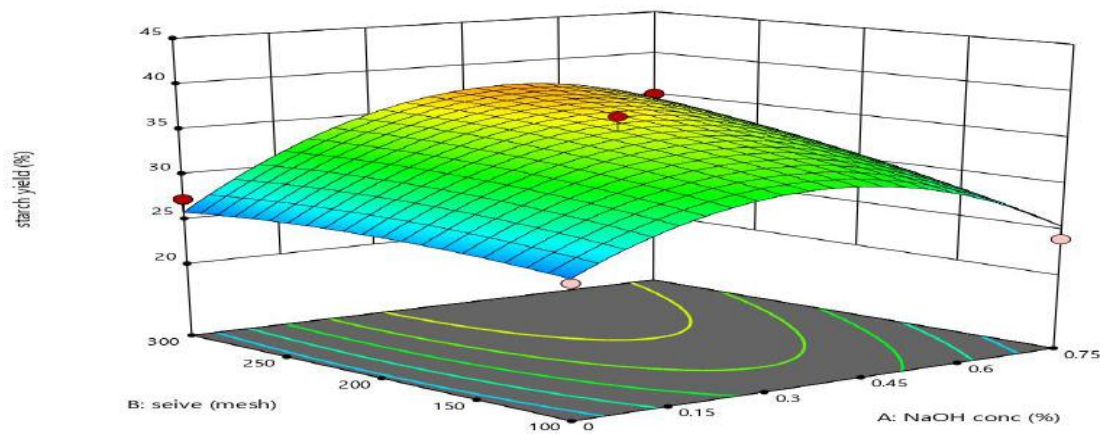
A, B, C denotes NaOH conc., sieve mesh size and steeping time respectively

Table 5: Process parameters and responses

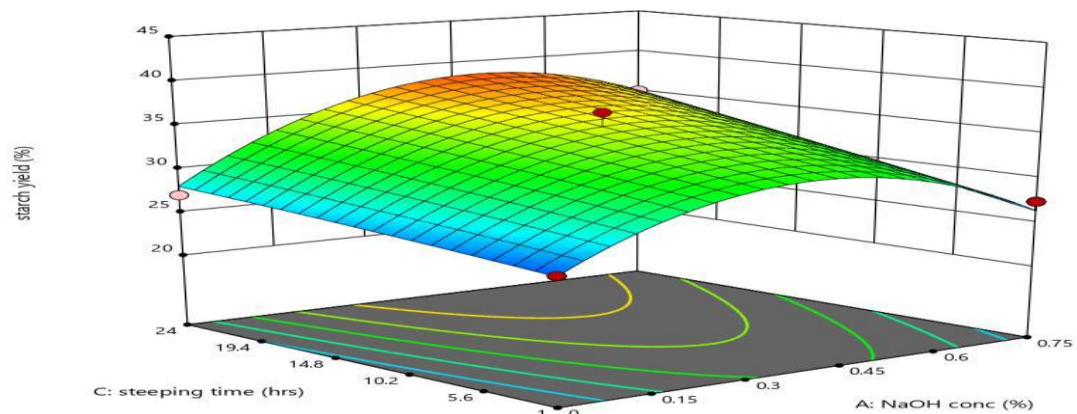
Constraints			
Name	Goal	Lower Limit	Upper Limit
NaOH conc. (%)	is in range	0	0.75
Sieve size (mesh)	is in range	100	300
Steeping time (hrs)	is in range	1	24
Starch yield (%)	maximize	23.97	39.91
Protein (%)	minimize	0.87	2.03
Fat (%)	minimize	0.21	0.96

The process was standardized at 0.375 NaOH concentration, 300 sieve mesh size and 24 hrs of steeping which will give maximum starch yield of 39.91% with minimum fat and protein content i.e 0.53% and 0.87% respectively. ANOVA data shows that the model is fit as the lack of fit value is non-significant listed in table 4. R² value of starch yield, protein and fat are 0.9611, 0.8920 and 0.8342 respectively. The regression coefficient is

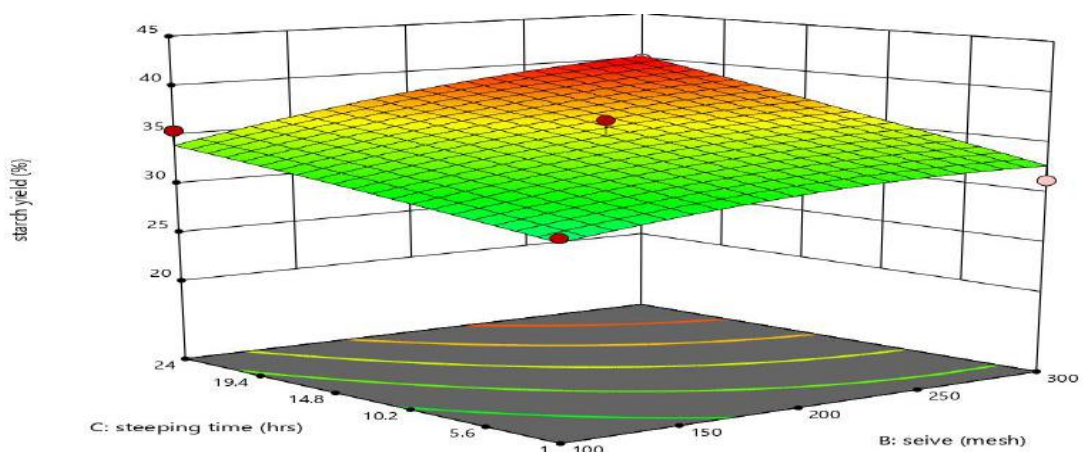
shown by the equation given in section experimental design and statistical analysis. The 3-D graphical illustration was given in Figure 1 (Fig: A-C shows the effect of independent variables on starch yield, Fig: D-F shows the effect of independent variables on protein content, Fig: G-I shows the effect of independent variable on fat content) and the desirability level of each factor is shown in figure 2.



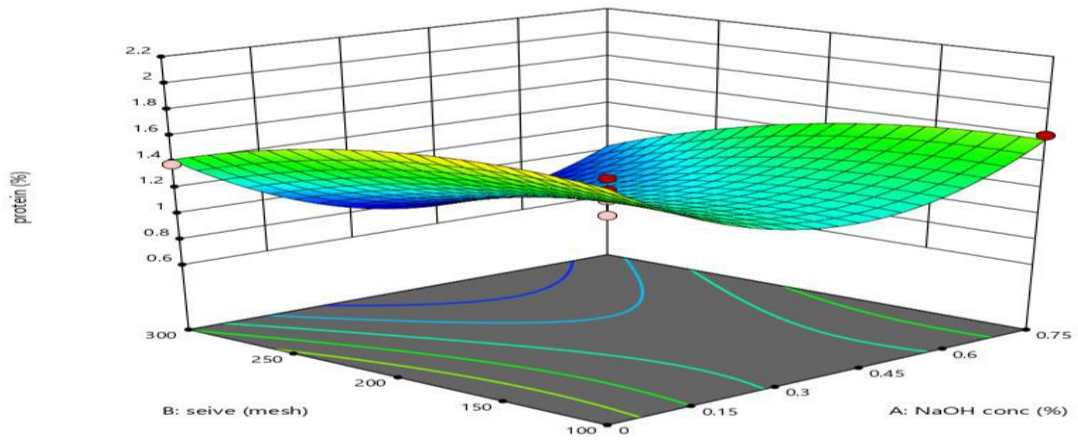
A) Effect of sieve size and NaOH conc on starch yield



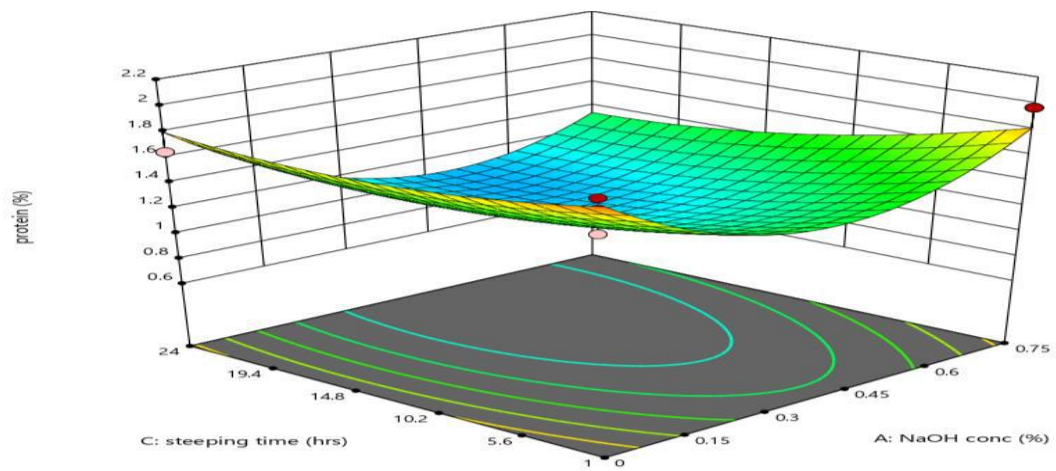
B) Effect of stepping time and NaOH Conc on Starch yield



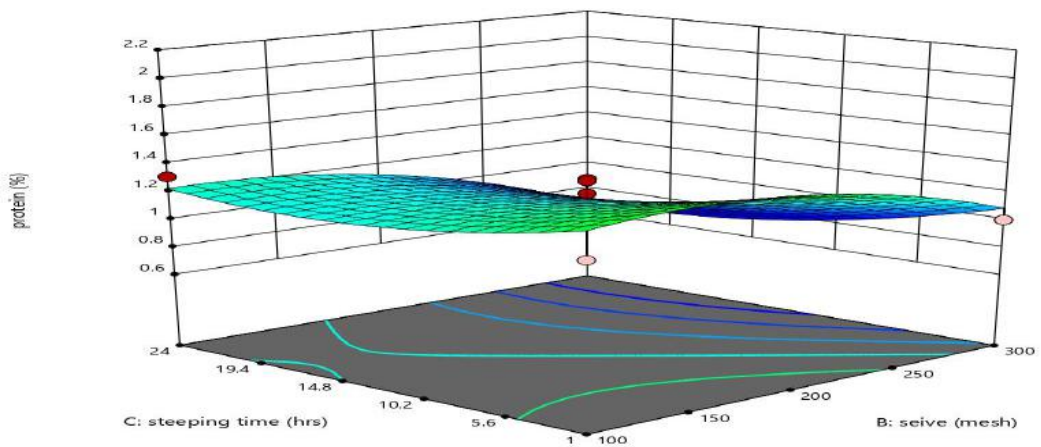
C) Effect of sieve size and stepping time on starch yield



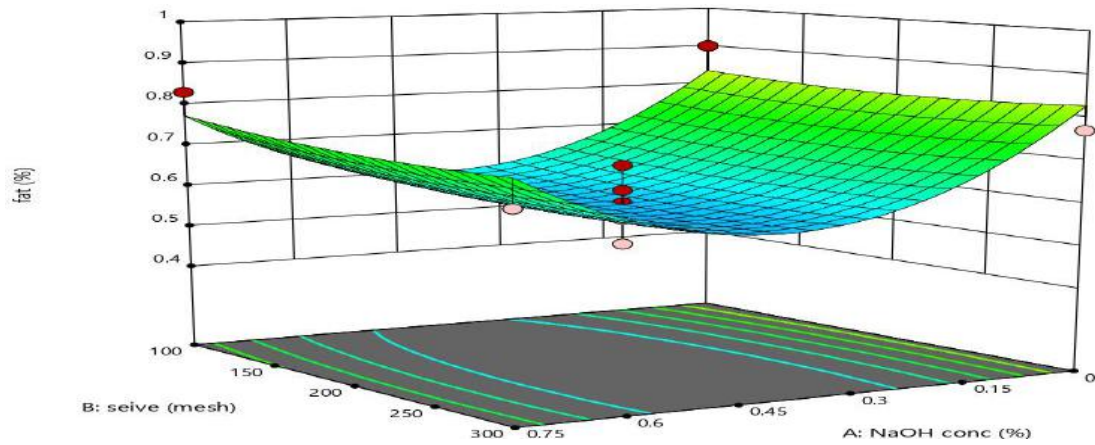
D) Effect of sieve size and NaOH conc on protein content



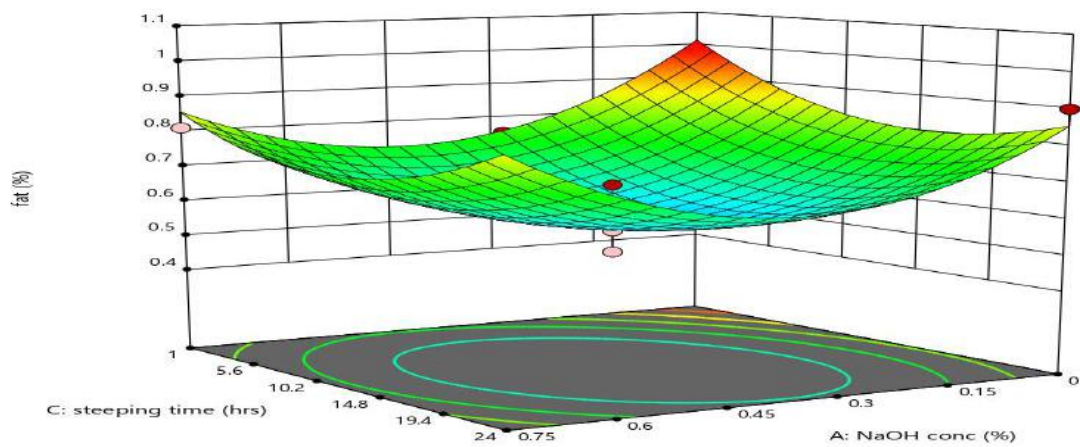
E) Effect of steeping time and NaOH conc on protein content



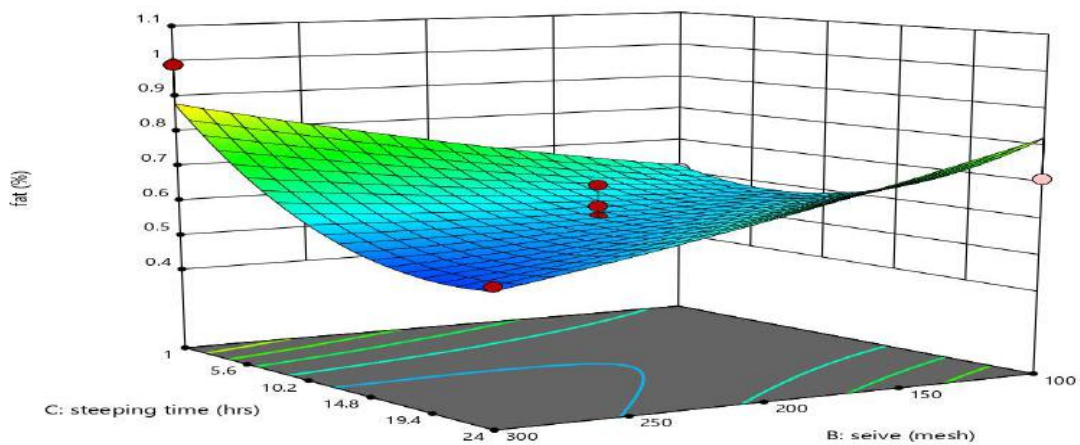
F) Effect of steeping time and sieve size on protein content



G) Effect of sieve size and NaOH conc on fat content

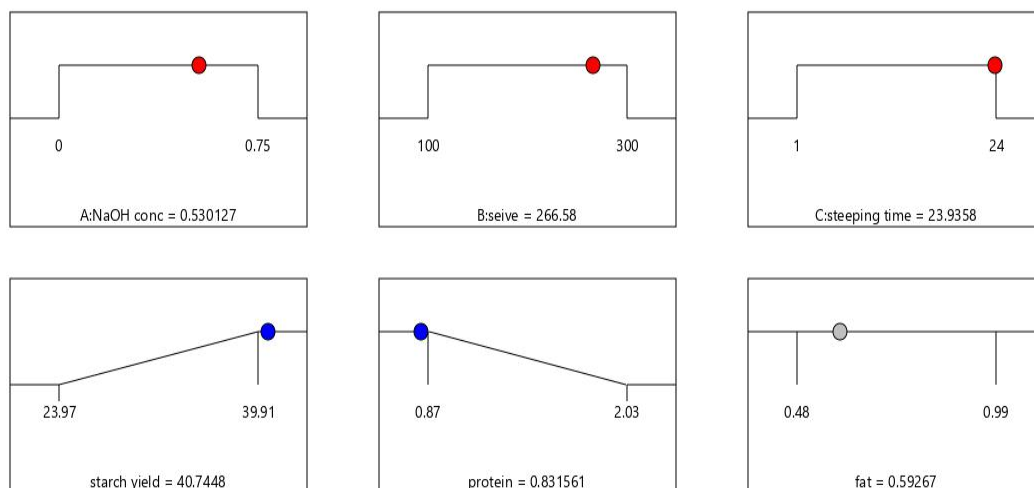


H) Effect of stepping time and NaOH conc on fat content



I) Effect of stepping time and sieve size on fat content

Fig.1: Response surface plots (3D) showing the effects of process parameters on : Starch yield, Protein content and Fat content



Desirability = 1.000

Fig.2: Optimum condition for the isolation of starch according to desirability function.

Steeping is done to loosen up the endosperm of the grain, with the decrease in steeping time the starch yield also decreases may be due the attachment of endosperm with the other covering of the grain. With the decrease in mess size of sieve the purity of starch increases. Taking all the aspects in considerations the steeping time, alkali concentration and sieve mesh size were standardized. Flour : alkali ratio, steeping time, centrifuge speed and centrifuge time was kept constant for all the samples.

After the standardization, the results obtained are given in Table 6. The starch yield was 39.91% and protein content was 0.87%. Functional properties such as solubility, swelling power are affected by the amylose content. To measure the extent of interactions between starch chain, within the amorphous and crystalline domains of the starch granule swelling power and solubility is determined. The amylose content was found to be 7.01%, the result is almost comparable to the result obtained by Ritu and Singh (2016), solubility was 73.43%. Due to high amylopectin concentration, the swelling power of amaranth starch was high as compared to the other starch sources like sweet potato, banana, etc. The solubility was found to be 12.56g/g, the result was comparable to the result obtained by Kong et al (2009). The water absorption capacity of starch was observed to be 117 % which was nearly similar to the results obtained by the Sindhu and Singh (2016). Flavour retention and mouth feel of the products is due to the interaction between the hydrocarbon chains of lipids and the non-polar amino acid side chains which are in relation to the oil absorption capacity. The oil absorption capacity was found to be

135% which is lower to the results obtained by Sindhu and Singh (2016) recorded 146% for amaranth starch. Overall trends that water absorption capacity is lower than the oil absorption capacity is similar to the observation by Adeniyi and Obatolu (2014). Acceptability of any product is somewhat based on the colour of the product and any colour or pigmentation in the starch is carried to the final product. L* was observed to be 97.62 which indicates the luminosity and b* and a* value observed are 3.69 and 0.97 respectively which indicates the presence of tint of red and yellow in the sample.

Table 6: Process standardization

Alkali % (NaOH)	0.375
Alkali volume: flour ratio	1:5
Steeping time (hrs)	24
Steeping temperature (°C)	4
Screen size (mesh) to filter slurry	300
Centrifuge speed	3000g
Centrifuge time (min)	20
Starch yield (%)	39.91±0.10
Amylose %	7.01±0.05
Solubility %	73.43±0.31
Swelling power (g/g)	12.56±1.21
L*	97.62 ±0.67
b*	3.69 ±1.13

a*	0.97 ± 0.04
Protein %	0.87±0.04
Bulk density (kg/m ³)	610±0.03
Tapped density (kg/m ³)	720±0.01
Water activity	0.37±0.02
Water absorption capacity (%)	117.34±1.12
Oil absorption capacity (%)	135.85±0.89

The value are expressed as mean ± SD of triplicate readings

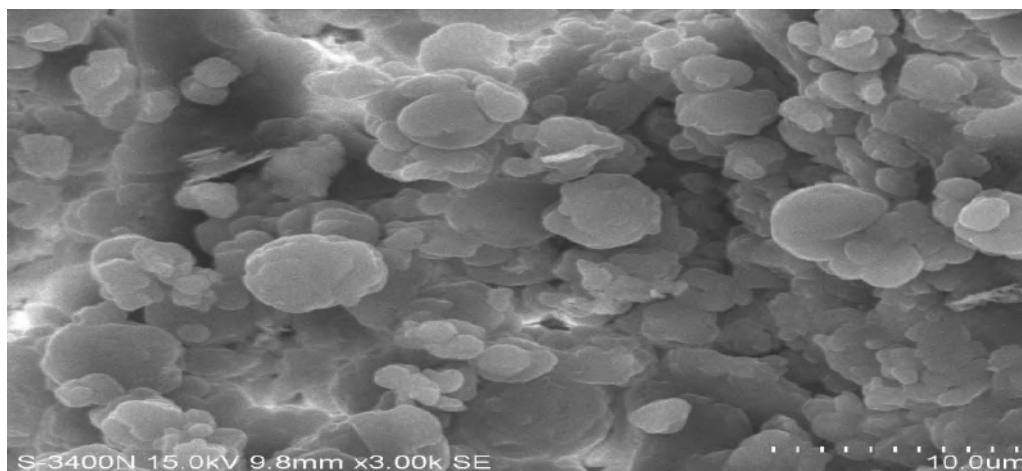


Fig.3: scanning electron microscopy view of amaranth starch

The microstructure (Fig 3) revealed that the mean diameter of starch granule is 1.29µm with polygonal shape, no fissures are observed on the surface. The smooth surface indicates the low activity of amylase and no damaged starch was found which state that the isolation process do not cause any damage to the starch.

IV. CONCLUSION

The physical properties listed in Table 1, are very useful for the designing of different macheneries for amaranth grain processing. The physio chemical properties of Amaranth propose that it has a lower concentration of proteins, fat, fibre and ash which confirms the higher and pure concentration of starch. For the isolation of starch from *Amaranthus paniculatus* (Rajgeera) the process was standardized at alkali concentration (NaOH) of 0.30% and screen size of 300 mesh for the filtration of slurry. At the standardized values the starch yield was 39.91%, protein content was found to be 0.78%, fat content noted was 0.53%, ash content was 0.66%, fibre content was 0.26% and the amylose content was found to be 6.87%, which confirms the purity of isolated starch and the effectiveness of the standardization process. Due to the small size of granules the starch can be used in various food and non-food industries. Further studies should be done on the starch structure and standardization of the process using different alkali solutions and on different varieties of amaranth from different regions.

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INTEREST OF CONFLICT STATEMENT

The authors declare that there is no conflict of interest.

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