



RESEARCH ARTICLE

OPTIMIZING ALKALI CONCENTRATION AND STEEPING TIME IN MERCURIC CHLORIDE
METHOD OF STARCH EXTRACTION FROM SORGHUM

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Abstract

Optimization of soaking hours and alkali concentration are one of the most important stages in the isolation of starch from grains. In the present study, steeping time ranged from 6 to 22 hours and slurring of the steeped grains in aqueous alkali at concentration from 0.01 to 0.05 N NaOH was optimized using Response Surface Methodology (RSM) adopting a Central Composite Rotatable Design (CCRD). The responses were percentage starch yield, Carr's index (%), Hausner's ratio, porosity (%), Angle of repose (Θ), water solubility index (g/g) (WSI), hydration index and swelling index. A second order polynomial regression equation was fitted to the data of all responses. At linear level, soaking hour had significant positive influence on water solubility index at $p < 0.05$. At quadratic level, the soaking hours and alkali concentration had significant negative influence on starch yield; soaking hours had significant ($p < 0.05$) positive influence on WSI. Alkali concentration showed significant ($p < 0.05$) negative influence on the angle of repose. A rising ridge system with an elliptical response surface with maximum stationary point was observed for Carr's index and hydration index whereas saddle system of interaction was observed for Hausner's ratio, porosity, water solubility index and swelling index. The adjusted R^2 above 0.8 for starch yield (0.9285) and WSI (0.8101) showed a good fit of the model with the experimental data, while the R^2 value of more than 0.5 for porosity (0.552) and swelling index (0.5947) indicated fair fit of the model with the experimental data. The optimum steeping hours and alkali concentration for starch isolation with maximum starch yield, hydration index, swelling index and minimum micromeritic properties, porosity, WSI as set goals for optimization with desirability of 0.493 was 11.71 hrs and 0.04N NaOH respectively. The determined experimental value of each response based on optimal condition was statistically in accordance with predicted levels at $p < 0.05$.

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Introduction

Starch is currently enjoying increased attention because of its usefulness in many food products. Starch contributes greatly to the textural properties of many foods and is widely used in food and industrial applications as a thickener, colloid, stabilizer, gelling agent, bulking agent and water retention agent, etc.

Starch forms a major source of carbohydrates in human diet and is therefore of great economic importance being isolated on an industrial scale from many sources. Large scale industrial production of starch has been focused on cassava, potato, maize and rice which are of commercial importance.

However a large percentage of starch is from crops that equally serve as a source of staple foods. This has led to find an alternative source for starch in underutilized crops. An underutilized plant source that could serve as an alternative use among cereal grains is white sorghum (*Sorghum bicolor*). *Sorghum bicolor* is the fifth most important cereal crop after wheat, rice, maize, and barley in terms of production (FAO, 2005). It is a vital food crop for millions of people in parts of Africa and Asia. It's high in carbohydrate content (70%) and it is an invaluable source of starch for both domestic and industrial uses (Olayinka et al., 2013).

Starch as a natural component, contributes to the characteristic properties of food products such as texture, viscosity, gel formation, adhesion, binding, moisture retention, and product homogeneity. The starch properties are dependent on the starch source but also highly dependent on the history of the starch itself, such as extraction procedures. It is known that extraction procedures affect both the chemical composition and physical properties of starch, which justify the interest of studying the most suitable method of extraction for each crop. Hence the present study was framed to optimize the soaking hours and alkali concentration for isolating starch from white sorghum grains.

Material and Methods

Sampling

Fully matured sorghum grains were procured from the local market of Salem District, cleaned for impurities and were passed through test sieves of mesh size (BSS) 4, 5, 6, 7, 8 and 10 arranged ascendingly, to select matured grains of even size (≤ 6 mesh; 2411 μ ; 2.8 mm) for the study.

Starch Isolation

The starch was isolated by adopting points suggested by Vasanthan., (2001) with certain modifications. The mature white sorghum grains were steeped in water for 6-22 hours and slurried in aqueous alkali (0.01 to 0.05 N NaOH). Steeping softens hard plant materials, especially grains, and facilitates wet grinding and thus minimizing starch granule damage. A direct slurring of steeped grains or dry flour in aqueous alkali (0.01 – 0.05N NaOH or KOH) can also be carried out to enhance the solubility of gums and protein (Schoch and Maywald, 1968). Exact steeping time (hrs) and alkali concentration were identified through numerical optimization using RSM

(CCRD). However the neutralization of the residual alkali in the starch isolates was by using a mild acid solution (0.1 N HCl) at the end of the isolation process. The steeped grains were blended to form smooth paste to which 0.01M mercuric chloride was added to inhibit microbial growth and the activity of amylase. The slurry was passed through a series of polypropylene screens (250, 175, 125, 75 μ m) and centrifuged for 30 minutes at 8000 g, 20°C which settles the starch at the bottom of the tube. The water in-soluble contaminants (e.g. protein, fine fibre) form a dark brown layer on the top of the starch layer was removed manually by scrapping with a spatula, being careful not to scrap away the starch layer. To 5 g of the brown layer (wet weight) 150 ml of 0.1 M Tris.Cl containing 0.5% (w/v) NaHSO₄, 0.01% (w/v) thimerosal and 25 mg of proteinase K followed by incubation at 25°C for 24 hrs was added. The digest was then centrifuged (10 minutes at 5000 g, 20°C) and the supernatant discarded. The pellet at the bottom of the centrifuge tube was suspended in 500 ml of 0.2M NaCl containing 14% (v/v) toluene for 12 hrs at 20°C, centrifuged (10 minutes at 5000 g) and supernatant was discarded. The starch pellet at the bottom of the tube was dried and added back to the original starch mass. The total dried starch was hand ground to a fine powder using mortar and pestle, sieved and stored in a tightly closed container.

Experimental design

A central composite rotatable design (CCRD) with two independent variables was used to determine the optimum steeping hours (6 to 22 hours) and alkali concentration (0.01 – 0.05 N NaOH) for isolating sorghum starch. The coded level of each variables and experimental plan are shown in table 1.

Responses for optimization

The experimental variables of 13 runs (Table 1) were analyzed for responses such as starch yield (%), Carr's index (%), Hausners ratio, porosity (%), angle of repose (Θ), water solubility index (g/g), hydration index (%), and swelling index (%). All determinations were done in triplicate. A second order polynomial regression equation was fitted to the data of all responses.

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{12} X_1 X_2 + \beta_{11} X_1 X_1 + \beta_{22} X_2 X_2 \dots\dots\dots(1)$$

Where β_0 , β_1 , β_2 , β_{11} , β_{22} , β_{12} were regression coefficients; X_1 , X_2 were the independent variables and Y was the dependent variable. The p values of

regression coefficient, which in turn, are necessary to understand the pattern of mutual interactions between the best variables. The smaller the magnitude of p , the more significant is the corresponding coefficient (Kunamneni et al., 2005). The optimum level of soaking hours and alkali concentration was obtained by combining set goals of all determined responses with maximum importance of 5 through numerical optimization. The quality of fit of second order equation was expressed by the coefficient of determination R^2 and its statistical significance was determined by the F test. The individual and interactive effect of each variable was also determined. Response Validation of optimized level of independent variables was done by isolating sorghum starch using optimum conditions of soaking hours and alkali concentration from the numerical solution depending on the responses.

Table 1: Coded variables and number of experiments for the experimental design

Levels of independent variables						
Variables	Code	Coded levels				
		-2	-1	0	1	2
Soaking hours (hrs)	X_1	6	10	14	18	22
Alkali concentration (N)	X_2	0.01	0.02	0.03	0.04	0.05
Experimental plan						
X_1	X_2	Number of experiments				
± 1	± 1	4				
± 2	0	2				
0	± 2	2				
0	0	5				

Result and Discussion

Estimated response levels of experimental variables

The estimated responses level of experimental variables (Table 2) suggested that maximum starch yield (55.9%) was observed when the grains were soaked for 14 hours with alkali concentration of 0.03

N, which could be compared with the results predicted by Iranna et al (2012). Compressibility describes the cushioning ability of a material and is related to its relative softness or hardness. Low bulk compressibility is desirable for loose fill packaging material (Bhatnagar and Hanna, 1995). The Carr's index (%), Hausner's ratio and angle of repose (Θ) are the micromeritic properties of a powder which determines the flow characteristics and compressibility of a powder. The Carr's index, angle of repose (Θ) and Hausner's ratio above 23%, 50° and 1.2 respectively do not indicate good flow and compressibility properties (Jmazu et al., 2011). The Carr's index and Hausner's ratio of the isolated starch were more than the required levels indicating poor compressibility of the starch powder. But, angle of repose was less than 50° predicting that the flow characteristics of the isolated starch powder may be good or fair irrespective of the compressibility. The porosity of the isolated starch was in range from 10.25 % to 32 %. Differences in porosity among the experimental runs are attributed to difference in their density values. Higher porosity results in greater contact with atmospheric oxygen thereby increases the rate of auto oxidation of starch flour (Devi and Sharma, 2007). The water absorption index measures the volume occupied by the starch after swelling in excess water, which correlates with the degree cook (Masson and Hosney, 1986) and water solubility index (WSI) can be used as an indication of the degree of molecular damage (Colonna et al., 1989). The low water solubility index was observed in the present study indicating reduced molecular damage of the starch granules. The hydration index of the experimental samples was in the range of 0.76 to 1.28% while the swelling index was in range from 0.05 to 0.22%.

Influence of independent variables on responses

The magnitudes of the terms indicate the order of influence on each response and difference in magnitude of the quadratic terms explains which variable was dominant for response (Karunanithy and Muthukumarappan, 2011). All the responses are greatly influenced by alkali concentration in isolation process.

Table 2: Observed and predicted levels of responses for each experimental runs showing both coded and actual levels of independent variables

Ru ns	Soaking hours (hrs) X_1	Alkali concentration (N) X_2	Starch yield (%)	Carr's index (%)	Hausner's ratio	Porosity (%)	Angle of repose (Θ)	WSI (g/g)	Hydration index	Swelling index
1	-1 (10)	-1 (0.02)	50.91	34.3	1.52	10.25	37.11	0.003	0.76	0.09
2	1 (18)	-1 (0.02)	44.75	36.13	1.57	32	36.5	0.008	1.27	0.22
3	-1 (10)	1 (0.04)	45.05	34.8	1.53	23.71	35.49	0.013	0.92	0.18
4	1 (18)	1 (0.04)	45.11	37.76	1.61	14.86	35.49	0.007	0.98	0.07
5	-2 (6)	0 (0.03)	41.14	41.86	1.72	27.67	35.88	0.013	0.92	0.15
6	2 (22)	0 (0.03)	43.11	41.53	1.72	16.87	35.97	0.027	0.84	0.13
7	0 (14)	-2 (0.01)	37.45	37.26	1.59	32	35.24	0.002	0.79	0.11
8	0 (14)	2 (0.05)	37.75	39.93	1.67	25.36	35.88	0.007	1.28	0.07
9	0 (14)	0 (0.03)	55.91	38.76	1.67	19.28	36.25	0.007	0.82	0.07
10	0 (14)	0 (0.03)	53.38	40.56	1.68	17.43	36.49	0.007	0.8	0.07
11	0 (14)	0(0.03)	55.82	38.8	1.69	15.87	36.99	0.008	0.81	0.05
12	0 (14)	0 (0.03)	55.9	38.2	1.62	18.1	37.11	0.007	0.86	0.09
13	0 (14)	0 (0.03)	55.7	37.26	1.6	23.71	36.87	0.007	0.81	0.07

Figure in parenthesis indicate the actual values of independent variables; WSI- Water solubility index.

The coefficients for the proposed quadratic model in terms of actual variables are given in Table 3. At linear level soaking hour had significant positive influence on WSI at $p < 0.05$. The interaction effects for each response are visualized in Figure 1 (a-h). At quadratic level, the soaking hours and alkali concentration had significant negative influence on starch yield, whereas soaking hours had significant positive influence on WSI at $p < 0.01$. Alkali concentration showed significant negative influence on the angle of repose at $p < 0.05$. All the other responses were not significantly influenced by the independent variables at any level.

The influence of the independent variables was prominent on the starch yield and angle of repose (Θ) as observed in fig. 1a and 1e. The starch yield and angle increased with an increase in the soaking hours and alkali concentration. A rising ridge system with an elliptical response surface with maximum stationary point was observed for Carr's index and hydration index as depicted in fig. 1b and 1g respectively. This indicates maximum positive influence of the independent variables on the responses, whereas saddle system of interaction was observed for Hausner's ratio, porosity, water solubility index and swelling index which indicates the interactive stationary point was neither maximum nor minimum.

Response surface model evaluation

The coefficient of determination (R^2) model p value, lack of fit 'p' value, CV %, predicted R^2 and adequate precision value of the regression model using CCRD for each response are presented in table 3. The adjusted R^2 above 0.8 for starch yield (0.9285) and WSI (0.8101) showed a good fit of the model with the experimental data, while the R^2 value of more than 0.5 for porosity (0.552) and swelling index (0.5947) indicated fair fit of the model with the experimental data. A coefficient of variation (CV) is another measure to evaluate the goodness of fit of the model. As a general rule, the CV should not be greater than 10% (Cocharan and Cox, 1957; Linko et al., 1984; Vaninionpaa and Malkki, 1987). Considering the general rule, a low value of CV for angle of repose (1.62%) starch yield (3.94%) followed by Hausner's ratio (4.65%) and Carr's index (6.82%) showed that the experiments conducted are precise and reliable.

Adequate precision measures the signal to noise ratio. A ratio greater than 4 is desirable. The adequate precision value was greater than 4 for starch yield (14.54), porosity (6.247), WSI (12.54), hydration index (4.938) and swelling index (6.508) indicating adequate signal for better prediction/optimization. The suggested fitted model for starch yield, angle of repose, porosity, WSI and swelling index was

quadratic polynomial equation, whereas 2FI (2 factor interaction) for hydration index, and mean model for Carr's index, Hausner's ratio. Lack of fit 'p' value for all determined responses except starch yield, angle of repose and porosity predicted that the proposed quadratic second order polynomial regression equation model (Table 4) was probably appropriate and adequate for prediction / optimization.

Optimization and Validation

Numerical multi-response optimization was adopted to determine the optimum level of each independent

variable and the respective predicted level of responses as per the set goals with maximum desirability function was reported in Table 4. The optimum steeping hours and alkali concentration for starch isolation with maximum starch yield, hydration index, swelling index and minimum micromeritic properties, porosity, WSI as set goals for optimization with desirability of 0.493 was 11.71 hrs and 0.04N NaOH respectively. The determined experimental value of each response based on optimal condition was in comparison with predicted levels at $p < 0.05$ (Table 5).

Table 3: Regression coefficients and model validation of the responses

Coefficients	Starch yield (%)	Carr's index (%)	Hausner's ratio	Angle of repose (°)	Porosity (%)	WSI (g/g)	Hydration index	Swelling index
β_0	55.03*	37.70 ^{NS}	1.62 ^{NS}	36.72 ^{NS}	18.33 ^{NS}	0.006*	0.85 ^{NS}	0.083**
β_1	-0.18 ^{NS}	0.34 ^{NS}	0.011 ^{NS}	-0.036 ^{NS}	-0.73 ^{NS}	0.002**	0.034 ^{NS}	-0.001 ^{NS}
β_2	-0.41 ^{NS}	0.62 ^{NS}	0.017 ^{NS}	-0.11 ^{NS}	-1.41 ^{NS}	0.001 ^{NS}	0.071 ^{NS}	-0.012 ^{NS}
β_{12}	1.56 ^{NS}	0.28 ^{NS}	0.007 ^{NS}	0.15 ^{NS}	-7.65 ^{NS}	-0.002 ^{NS}	-0.11 ^{NS}	-0.060*
β_{11}	-3.32*	0.68 ^{NS}	0.015 ^{NS}	-0.21 ^{NS}	0.81 ^{NS}	0.003*	0.017 ^{NS}	0.018**
β_{22}	-4.46*	-0.094 ^{NS}	-0.007 ^{NS}	-0.30**	2.42**	-0.007 ^{NS}	0.055 ^{NS}	0.005 ^{NS}
Model validation								
R ² (%)	0.9285	-0.2240	-0.2728	0.1842	0.5519	0.8101	0.2251	0.5947
Model p – Value	0.0001*	0.7286 ^{NS}	0.7782 ^{NS}	0.290 ^{NS}	0.051 ^{NS}	0.0031*	0.2530 ^{NS}	0.0368**
Lack of fit p-Value	0.065 ^{NS}	0.0266**	0.0432**	0.0806 ^{NS}	0.107 ^{NS}	0.0004*	0.0003*	0.0213**
Predicted R ²	0.67	-3.624	-3.624	-2.934	-1.0247	-0.035	-3.3191	-0.5096
Adeq. precision	14.537	2.840	2.847	3.548	6.247	12.54	4.938	6.508
CV (%)	3.94	6.82	4.65	1.62	21.06	30.46	16.62	30.76

*Significant at $p < 0.01$, **Significant at $p < 0.05$, ^{NS}- Not Significant

Table 4: Proposed model (second order polynomial regression for responses) equation

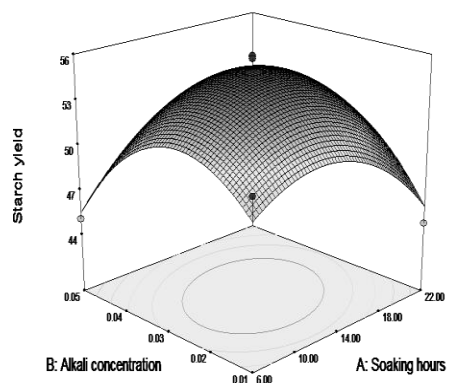
Response	Regression equation
Starch yield	$Y = -7.608 \beta_0 + 4.605 \beta_1 + 2088.09 \beta_2 + 38.88 \beta_{12} - 0.208 \beta_{11} - 4455.8 \beta_{22}$
Caars index	$Y = 38.78 \beta_0 - 0.307 \beta_1 + 20.46 \beta_2 + 1.765 \beta_{12} + 0.011 \beta_{11} - 234.375 \beta_{22}$
Hausners ratio	$Y = 1.627 \beta_0 - 0.007 \beta_1 + 1.317 \beta_2 + 0.0468 \beta_{12} + 0.00023 \beta_{11} - 18.319 \beta_{22}$
Porosity	$Y = 9.572 \beta_0 + 0.987 \beta_1 + 236.232 \beta_2 - 47.812 \beta_{12} + 0.013 \beta_{11} + 6041.27 \beta_{22}$
Angle of repose	$Y = 36.048 \beta_0 + 0.057 \beta_1 + 25.60 \beta_2 + 0.95312 \beta_{12} - 0.0032 \beta_{11} - 742.834 \beta_{22}$
WSI	$Y = 0.001 \beta_0 - 0.0005 \beta_1 + 0.426 \beta_2 - 0.017 \beta_{12} + 0.00049 \beta_{11} - 1.772 \beta_{22}$
Hydration index	$Y = 0.5599 \beta_0 + 0.0181 \beta_1 + 5.0804 \beta_2 - 0.70313 \beta_{12} + 0.00026 \beta_{11} + 134.416 \beta_{22}$
Swelling index	$Y = 0.0152 \beta_0 + 0.003 \beta_1 + 3.794 \beta_2 - 0.375 \beta_{12} + 0.00028 \beta_{11} + 14.547 \beta_{22}$

Table 5: Validation of optimum level of independent variables

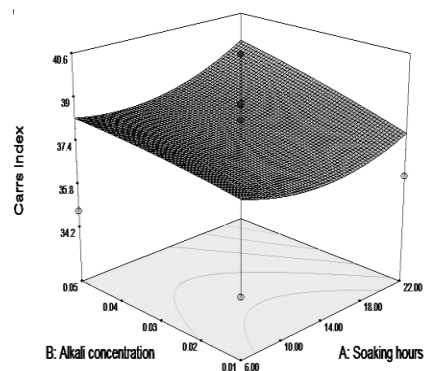
Responses	Predicted value	Experimental value
Starch yield (%)	48.29	48.34 ^{NS}
Carr's index (%)	38.09	37.27 ^{NS}
Hausner's ratio	1.63	1.60 ^{NS}
Angle of repose (Θ)	36.17	36.13 ^{NS}
Porosity (%)	24.38	23.71 ^{NS}
WSI (g/g)	0.009	0.009 ^{NS}
Hydration index	1.02	1.03 ^{NS}
Swelling index	0.12	0.11 ^{NS}

^{NS}- Not Significant at p<0.05

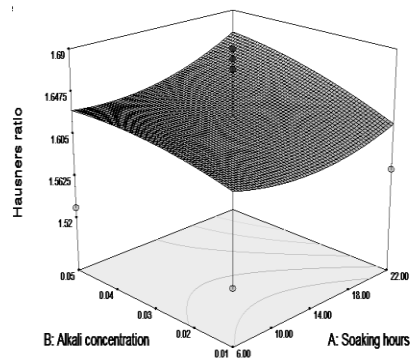
Figure 1: Interaction effect of two independent variables on starch yield (1.a); Carr's index (1.b); Hausner's ratio (1.c); porosity (1.d); angle of repose (1.e); WSI (1.f); hydration index (1.g); swelling index (1.h)



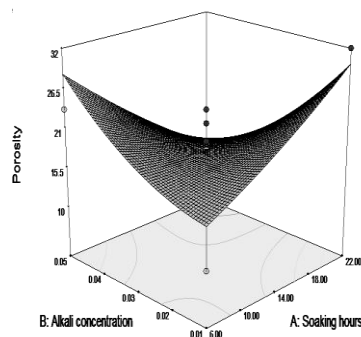
a) Starch yield (%)



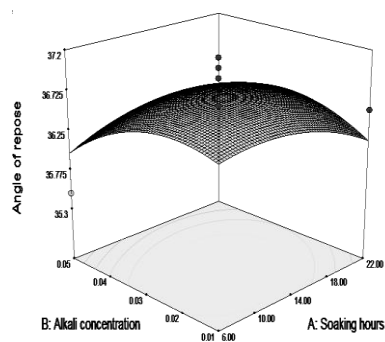
b) Carr's index (%)



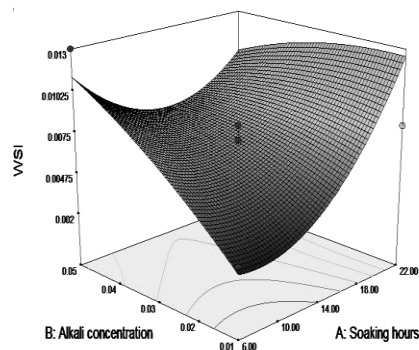
c) Hausner's ratio



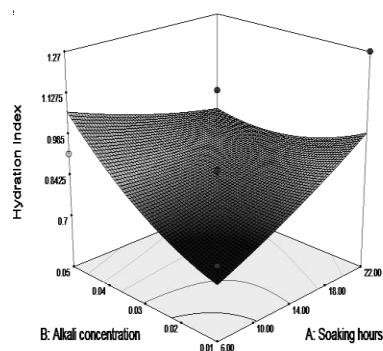
d) Porosity (%)



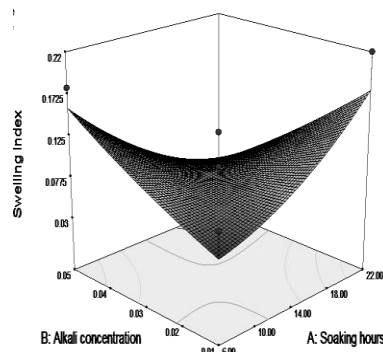
e) Angle of Repose (Θ)



f) WSI (g/g)



g) Hydration index



h) Swelling index

Conclusion

The isolated sorghum starch indicated the potential to be used as a base for the preparation of biodegradable thin film. One major problem with the isolated granular starch was its big particle size which was the

reason for increased micromeritic properties and reduced hydration index. Hence modification of the starch is necessary before it could be used for thin film preparation as a source of food packaging material.

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