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Effect of Extrusion Process Variables on the Amylose and Pasting Characteristics of Acha/Soybean Extrudates Using Response Surface Analysis

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Abstract: Acha and soybean flours were mixed in five ratios 100:0, 87.5:12.5, 75:25, 62.5:37.5 and 50:50% of acha and soy flour respectively. The moisture content of the blends was adjusted to 15, 20, 25, 30 and 35%. Extrusion was carried out using a Brabender single screw laboratory extruder following a four variable response surface analysis design where the extruder screw speed was adjusted from 90, 120, 150 and 180-210 rpm and barrel temperature from 100, 125, 150, 175-200°C. Amylose content and pasting properties of raw and extruded samples were evaluated. Results showed that increase in feed composition (acha flour) resulted in increased amylose content in the blend. Amylose content decreased with higher barrel temperatures while increased moisture levels of blend caused increased amylose levels in extruded products. The pasting characteristics showed that acha native starch had normal non-waxy starch pasting properties while blended and extrudate samples did not show any recognizable peaks which were indicative that blending and extrusion altered significantly the rheological properties of the extrudates. Extrusion processing reduced significantly ($p < 0.05$) the peak viscosity, the peak time, the set back index, while, consistency index significantly ($p < 0.05$) increased. The results showed that there was amylose content lowering indicative of significant ($p < 0.05$) amylose-lipid complexing. The extrudates and blends would be ideal for weaning, convalescent and convenient food formulations.

Key words: Flour, blends, amylose, extrudate, pasting

INTRODUCTION

The straining of starch in an extruder leads to both a thermal and mechanical energy input to the starch or plasticised mass. High-temperature extrusion cooking is used extensively by many food industries to produce various products with unique texture and flavour characteristics (Bahatnagar and Hanna, 1994a). Extruders offer an excellent means for the preparation of pregelatinized starches. Pregelatinized starches are useful in a variety of prepared and convenience products because of their relatively high viscosities at fairly low concentrations in products that are not heated (Mason and Hosoney, 1986). Several studies, (Manisha *et al.*, 1998; Kadan *et al.*, 2003; Stute, 1992; MCwatters *et al.*, 2004; Franko *et al.*, 1995; Hoover and Manuel, 1996a) have shown that heat treatment of starches at restricted moisture level (18-30%) and high temperature 100°C for 16 h would alter the physicochemical properties of normal maize, waxy maize, high amylose maize, wheat, oat barley, potato, yam, pigeon pea and larid lentil starches. According to Hoover and Manuel (1996) the magnitude of these changes are dependent on the moisture content during heat treatment and starch source.

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The effect of extrusion cooking on starch has been extensively studied in the last decades (Iwe, 1998; Colona and Mercier, 1983; Linko *et al.*, 1984). According to Jin and Xiao-lin (1994) the principal effect of the thermo mechanical treatment resulting from extrusion is to rupture the granular structure of starch. The specific functional properties, water solubility and absorption, expansion ratio and paste viscosity over a heating and cooling cycle of extruded starch have been investigated (Onwulata *et al.*, 2006; Kokini *et al.*, 1992). According to Mason and Hosney (1986) and Lawton *et al.* (1992) varying conditions of starch extrusion produces extrudates with a range of paste qualities. Most of the reports of literature are based on better known cereals. There is however no information on blending and extrusion of lesser known cereals like acha (*Digitaria exilis*). Though acha is considered the best tasting cereals in West Africa Jean-Francois (2004) and Misari *et al.* (1995), having higher methionine and cystiene content above the recommended levels (Kwon-Dung and Misari, 2000) its utilization had remained largely localized. According to FAO (1985) cereals like maize, sorghum and millet with lower methionine and cystine content have been extruded with soybeans to produce complimentary foods, it was therefore envisaged that extruding acha and soybeans would produce more balanced complimented food products. The aim of this present study was therefore to determine the effect of extrusion variables (feed moisture content, feed composition, screw speed and extruder barrel temperatures) on the amylose content and pasting characteristics of acha/soybean extrudates using response surface methodology.

MATERIALS AND METHODS

The materials used in this study were Acha (*Digitaria exilis*) and Soybean (*Glycine max* L. Merrill) TGX 1448-2E. Acha was purchased from Vom local market in Plateau State while soybean was obtained from the seed store of the National Cereals Research Institute Bida (Niger State) from 2003 harvest. The samples were prepared and extruded at the Food Science Laboratory of the Federal Polytechnic Mubi, Adamawa State Nigeria in 2005 as follows:

Acha was sorted and winnowed manually. Cleaned grains were milled using commercial attrition mill. Soybean was cleaned to remove immature grains and other foreign materials. The sorted grains were washed in clean tap water. The washed grains were sun dried for 3-4 h at 34-40°C, cracked in a commercial attrition mill and winnowed manually to remove hulls. The grits were further milled in attrition mill into flour.

The flours (acha and soybean) were sieved to pass a laboratory sieve mesh of 0.75-1 mm. The moisture content of the flours were determined (AOAC, 1984) and used to calculate the level of moisture adjustments of the blends according to Wilmot (1998).

Acha and soybean flour were mixed as shown in Table 1 and extruded as shown in Fig. 1. Extrusion was carried out at the Federal Polytechnic Mubi Adamawa State Nigeria, using a Branbender Laboratory single screw extruder (DUISBURG DCE-330 Model Germany). It was powered by a decoder drive (Type 832, 500) and driven by a 5.94 kW motor. The grooved band had a length/diameter ratio of 20:1. The extruder had variable screws and heaters with a fixed die diameter of 2 mm and length of 40 mm. A feed hopper mounted vertically above the end of the extruder and equipped with a screw that rotated at a constant speed of 80 rpm on a vertical axis takes feed into the extruder. The blends were mixed according to the experimental design (Table 1). The required amount of water was added to the flour and manually worked in to adjust the feed moisture content. The wet flour was allowed to equilibrate for 2-3 h before extrusion. The extruder runs was stabilized using acha flour. Extrusion of the blends was then carried out as shown in the transformed matrix (Table 1).

Experimental Design

The experimental design was a 4 variable (central composite rotatable design nearly orthogonal) involving 4 independent variables-Feed composition (FC), Feed Moisture Content (FMC), Screw

Table 1: Matrix transformation of the experimental design runs and extrusion conditions

Extrusion runs	Feed composition	Feed moisture content	Screw speed	Barrel temperature		Die Temp.
				1	2	
1	125	20	120	125	125	125
2	125	20	120	125	125	175
3	125	20	180	125	125	125
4	125	20	180	125	125	175
5	125	30	120	125	125	125
6	125	30	120	125	125	175
7	125	30	180	125	125	125
8	125	30	180	125	125	175
9	375	20	120	125	125	125
10	375	20	120	125	125	175
11	375	20	180	125	125	125
12	375	20	180	125	125	175
13	375	30	120	125	125	125
14	375	30	120	125	125	175
15	375	30	180	125	125	125
16	375	30	180	125	125	175
17	500	25	150	125	125	150
18	0	25	150	125	125	150
19	250	15	150	125	125	150
20	250	35	150	125	125	150
21	250	25	90	125	125	150
22	250	25	210	125	125	150
23	250	25	150	125	125	100
24	250	25	150	125	125	200
25	250	25	150	125	125	150
26	250	25	150	125	125	150
27	250	25	150	125	125	150
28	250	25	150	125	125	150
29	250	25	150	125	125	150
30	250	25	150	125	125	150
31	250	25	150	125	125	150
32	250	25	150	125	125	150
33	250	25	150	125	125	150
34	250	25	150	125	125	150
35	250	25	150	125	125	150
36	250	25	150	125	125	150

Speed (SS) and Barrel Temperature (BT) tested at 5 levels coded (-2 to +2) according to Meyers (1976) and Iwe (2001). The experimental design required a total of 36 extruder runs. Sixteen were to be performed at the factorial points, eight at the axial points and twelve at the center points (Table 1). Barrel temperature in the transition, metering and die zones, screw speed, moisture content of blends and levels of addition of soybean to acha are shown in the extrusion condition profile (Table 1). After steady state conditions were attained, emerging extrudates were collected and air dried at ambient temperature (24-27°C) for about 12 h then packed in cellophane packs and stored in the refrigerator at 4±°C.

Apparent Amylose Content

This was evaluated at the National Cereals Research Institute according to the procedure described by Bhatnagar and Hanna (1994a). Raw and extruded samples (100 mg each) were weighed into 100 mL volumetric flask and 1 mL of ethanol was added to wet the samples. Then 10 mL of 0.5N KOH was added and the samples were held over night at room temperature. The samples were then diluted to 100 mL with distilled water and again held overnight at room temperature. Five millilitre of diluted solution was put into a 100 mL volumetric flask and 3 drop of phenolphthalein.

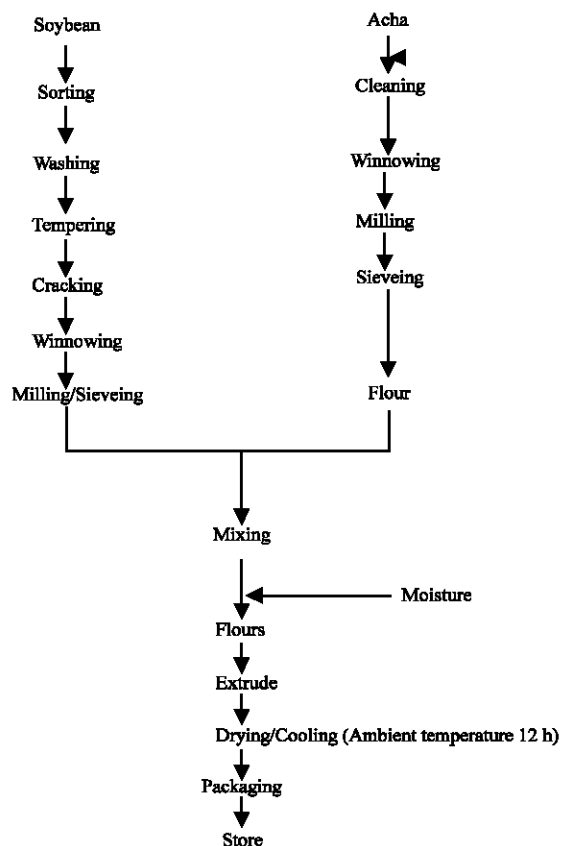


Fig. 1: Preparation and extrusion of acha-soybean flour blends

The starch solution was then neutralized-using 1 N HCl. Two milliliters of 0.2% iodine solution in 2% KI was added to the neutralized solution and made up to mark with distilled water. The absorbance of the solution was read at 630 and 520 nm after 30 min using a UV/VIS spectrophotometer. Amylose Standard (25%) was used to calibrate the spectrophotometer. Concentration of amylose was read from the curve. The starch-iodine complex was scanned for absorbance >400-700 nm. The wavelength of maximum absorbance (λ_{max}) was recorded. Determination was performed in duplicate.

Pasting Viscosity Profiles

Pasting viscosity profiles of raw and extruded samples were measured using the Rapid 20 min RVA test at the International Institute of Tropical Agriculture (IITA Ibadan Nigeria) following the method described by Defeenbaugh and Walker (1989). Samples (3 g) both of raw, blended and extrudates (blends and extrudates were those of 25% soybean addition) were mixed with 25 mL of distilled water. A disposable plastic stirring paddle was placed in the cup and rotated by hand for 15-30 sec, to wet the samples. The sample cup and paddle were inserted into the RVA (New Port Scientific 910140, Sydney, Australia) such that the paddle was held firmly in the drive motor clutch. When the test cycle was activated the split copper block automatically clamp around the can. Total sample size was held constant at 28 g. Sample temperature was equilibrated at 50°C for 2 min, then put on the heating cycle for 10 min with a maximum temperature of 95°C and then put on the cooling

cycle for 8 min with a minimum temperature of 50°C. The viscosity profiles were recorded on the portable Personal Computer (PC) attached to the instrument. All sample analysis was performed twice.

Statistical Analysis

All results were subjected to standard statistical analysis. For amylose content data, requiring response surface analysis, results were subjected to step-wise multiple regression analysis.

The generalized regression model fitted was $Y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_4x_4 + b_{11}x_1^2 + b_{22}x_2^2 + b_{33}x_3^2 + b_{44}x_4^2 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{14}x_1x_4 + b_{23}x_2x_3 + b_{24}x_2x_4 + b_{34}x_3x_4 + \epsilon$ where, Y = objective response, X_1 = feed composition, X_2 = feed mixture content, X_3 = extruder screw speed and X_4 = extruder barrel temperature and ϵ = random error in which the linear, quadratic and interaction effects were involved. A computer programmed SPSSWIN (11.0) SPSS INC. (2003), USA was used. The resulting models were tested for significance using analysis of variance (ANOVA) and coefficient of determination (R^2). Significant terms were accepted at $p < 0.05$ (Jin and Xiao-Lin, 1994; Howard, 1983). The terms that were not significant were deleted from the model equations. Response surfaces in three dimensional plots were generated on a computer programme STATISTICA (STAT SOFT INC.USA) version 5.0 (1984-1995) by holding the two variables with the least and second least effects on the response constant (center points) and changing the other two variables.

For pasting viscosity data, use of standard deviation and least significant difference LSD was used to separate the means according to Ihekoronye and Ngoddy (1985).

RESULTS AND DISCUSSION

Amylose Content

The amylose content ranged from 18.36-26.33% (Table 3). Similar extrudate amylose content had been reported for extruded commercial amylose (25%) (Bhatnagar and Hanna, 1994a). The effect of processing variables on the amylose content of extrudates is shown in Table 2. The results showed that the linear effects of feed composition, feed moisture content, screw speed and extruder barrel temperature were not significant ($p > 0.05$). The cross product effects of feed composition and feed

Table 2: Estimated regression coefficients for extrudate amylose content

Regression on constants	Coefficients 38.27	Standard error	p-values	R^2
FMC	1.70	2.41	0.7061	0.78
SS	0.34	0.39	0.9383	
BT	-0.49	0.42	0.8996	
FC*FC	3.27	4.57	0.0000	
FMC*FMC	0.41	0.01	0.6797	
SS*SS	-0.44	2.90	0.6560	
BT*BT	0.82	4.18	0.4883	
FC*FMC	-3.07	2.22	0.0003	
FC*SS	-2.50	3.72	0.0019	
FC*BT	-1.58	3.72	0.0344	
FMC*SS	-2.12	0.01	0.7102	
FMC*BT	-2.91	0.02	0.6003	
SS*BT	0.31	0.02	0.9523	
FMC*SS*BT	2.18	9.63	0.7366	
FC*FMC*SS*BT	3.83	9.83	0.0000	
Anova for extrudate amylose content				
ANOVA				
	Df	SS	MS	
Regression	15	138.09286	9.20619	
Residual	20	38.88884	1.94444	
F 4.73462		Sign. F.0.0008		

Table 3: Experimental and predicted values of extrudate amylose content

Extrusion runs	Experimental value	Predictive value
1	26.33	26.43
2	26.33	26.00
3	25.33	25.48
4	25.33	23.53
5	24.36	24.00
6	22.16	23.00
7	21.52	21.00
8	21.06	21.17
9	20.65	21.30
10	21.78	23.50
11	21.93	20.52
12	20.04	21.15
13	21.04	21.30
14	22.16	28.00
15	22.16	22.45
16	21.40	23.47
17	18.07	17.80
18	27.00	27.04
19	23.75	22.01
20	21.70	22.71
21	21.70	24.16
22	21.93	22.70
23	21.93	22.70
24	24.12	22.70
25	21.25	22.70
26	21.02	22.70
27	23.14	22.70
28	22.91	22.70
29	22.91	22.70
30	23.37	22.77
31	23.27	22.70
32	23.14	22.70
33	23.25	22.70
34	22.91	22.70
35	22.91	22.70
36	23.91	22.07

moisture content and screw speed and extruder barrel temperature and combined effect of all the variables were all significant ($p < 0.05$). The quadratic effect of feed composition had the highest influence on amylose composition ($p < 0.001$). Analysis of variance showed that there was model significance ($p < 0.001$). With high coefficient of determination ($R^2 = 0.80$) the results showed that there was a strong goodness of fit of the model to the linear regression. The low F value (0.0008) indicated a high level of confidence that the variation within the model was not due to experimental error. The results showed that increase in feed content (acha) increased the amylose level. Similar observations were made for the Feed Moisture Content (FMC) and Screw Speed (SS). Increase in these independent variables resulted to increase in the amylose content of extrudates. However, decreases in the barrel temperature led to increase in the amylose content of extrudates.

Removing the non-significant terms, the resulting model equation became:

$$\text{Amylose} = 38.269 - 2.269 \text{ FC}^2 + 3.834 + \text{FC} * \text{FMC} * \text{SS} * \text{BT} - 3.069 \text{ FC} * \text{FMC} - 2.501 \text{ FC} * \text{SS} - 1.579 \text{ FC} * \text{BT}.$$

The slopes confirmed the regression analysis view that increase in these independent variables resulted in decreases in the dependent variable (amylose) (Fig. 2). Similar patterns of response were recorded for plots of feed composition and screw speed (Fig. 3) and feed composition and barrel temperature (Fig. 4). The results of regression analysis (Table 2) showed that the amylose content of extrudates of acha/soybean flour blends were significantly ($p < 0.05$) dependent on the quadratic effect of FC. Increase in FC led to increases as well as decreases in the value of amylose because of the quadratic relationship.

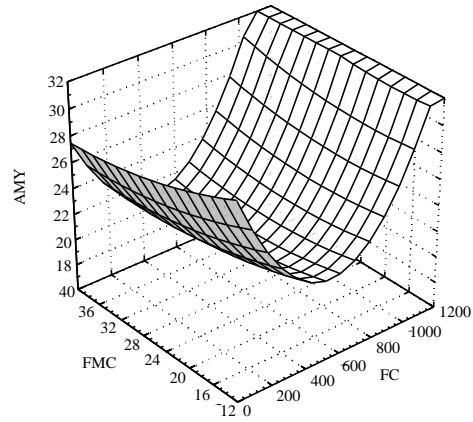


Fig. 2: Response surface plot of FC and FMC on extrudate amylose content

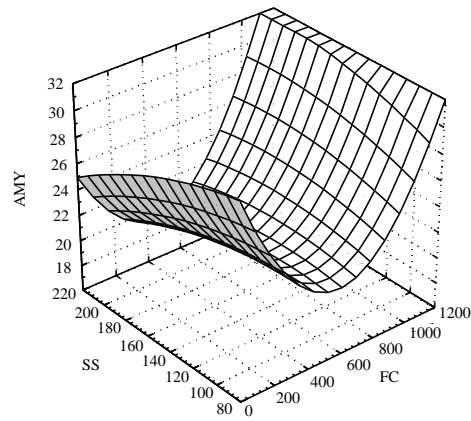


Fig. 3: Response surface plot of FC and SS on extrudate amylose content

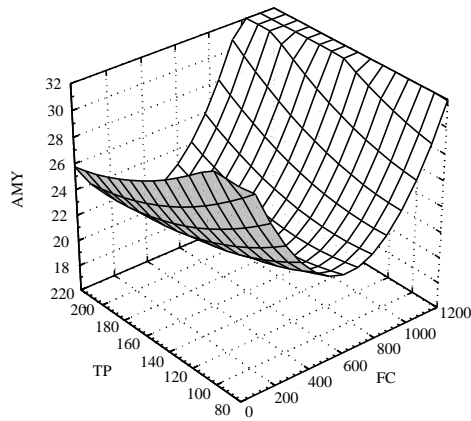


Fig. 4: Response surface plot of the effect of feed composition and barrel temperature on extrudate amylose content

As shown in Fig. 2-4 decreases in the level of acha flour in the blends led to decrease in the amylose content of the extrudates. The results indicated that, the most critical factor regulating amylose content of extrudates was the FC. Iwe and Ngoddy (1998) reported the dependence of amylose on the FC but moisture influence was not significant. Bhatnagar and Hanna (1994a) also reported that FMC content did not affect the amylose content. The results of this present study clearly agree with these findings. This was however contrary to expectations due to differences in the starting raw materials used in the extrusion. Iwe and Ngoddy (1998) used defatted soybean flour where as in this study full fat soybean flour was used. For Bhatnagar and Hanna (1994a) there was no indication of added soybean flour in their work. To extrude full fat, soybean flour, more moisture was needed in order to avoid oil expression at higher SS and BT (Bhatnagar and Hanna, 1994a). These differences in the starting raw material and increased moisture content of the blends were expected to result to significant differences in the results. For instance, it was expected that more starch degradation would occur at higher levels of acha flour addition and lower moisture content. However, amylose content increased as the moisture content increased contrary to these expectations. One explanation to this might be that increased moisture content increased the tendency of plugging at the die, decreased degradation of starch but increases residence time of the extrudates thus encouraging prolonged shearing and hence release of more starch.

The maximum wavelength of absorption (λ_{max}) was at 590 nm. This value was similar to the 630/520 ratio reported for starch without added lipids (Bhatnagar and Hanna, 1994a). Decrease in (λ_{max}) was due to amylose complexation. The differences in the results might be due to the materials used. Bhatnagar and Hanna (1994b) used pure isolated starch while full fat soybean flour was added to acha flour in the present study, thus leading to appreciable increases in the proximate composition (results not included). With increased lipid percentage due to added soybean more lipid-amylose interaction was expected at elevated temperatures leading to decrease in amylose content. This observation is in consonance with those of Ollku (1980) and Antila *et al.* (1993). It was therefore concluded that decreases in λ_{max} was due to amylose-lipid complexation phenomenon.

Pasting Characteristics of Raw and Extruded Samples

The pasting characteristic of raw acha flour, raw blends of acha/soybean and extruded acha/soybean (Fig. 5-7 and Table 4) showed that raw acha flour as expected had the highest pasting viscosity. This was due to the rapid rise in viscosity of native starches with the onset of gelatinization. Blended and extruded starches lack such ability because of their destructured or gelatinized starch profile. The results obtained for raw acha flour was significantly ($p < 0.05$) lower than 200 RVU reported by Jideani *et al.* (1996). Pasting temperature, time at peak viscosity, the peak time and minimum viscosity also differed. These differences might be due to the accessions or genetic differences of the cultivars used.

Table 4: Pasting characteristics of raw and extruded samples

Pasting parameter	Raw flour			
	Acha	samples soybean	Acha/Soybean	Extruded sample
Peak viscosity (RVU)	58.84±2.24 ^a	6.08 ^b	6.00±0.35 ^b	6.69±0.29 ^b
Trough	44.49±2.53 ^a	-12.25 ^b	-6.88±0.21 ^b	-11.96±0.30 ^b
Break down viscosity (RVU).	3.84±0.12 ^a	18.33 ^b	12.88±0.53 ^b	18.92±0 ^b
Final viscosity (RVU)	168.24±2.18 ^a	-7.75 ^b	30.71±0.41 ^c	-7.42±0.71 ^b
Set back viscosity (RVU)	123.50±0.36 ^a	4.50 ^b	37.59±0.23 ^c	4.32±1.00 ^b
Peak time (Mins)	8.96±0.04 ^a	0.03 ^b	3.00±0 ^c	3.00±0 ^c
Pasting temp. (°C)	95.00±0.11 ^a	65.35 ^b	81.30±1.41 ^a	84.00±0.67 ^a

Values with different L_{letter} are significantly different at $p < 0.05$

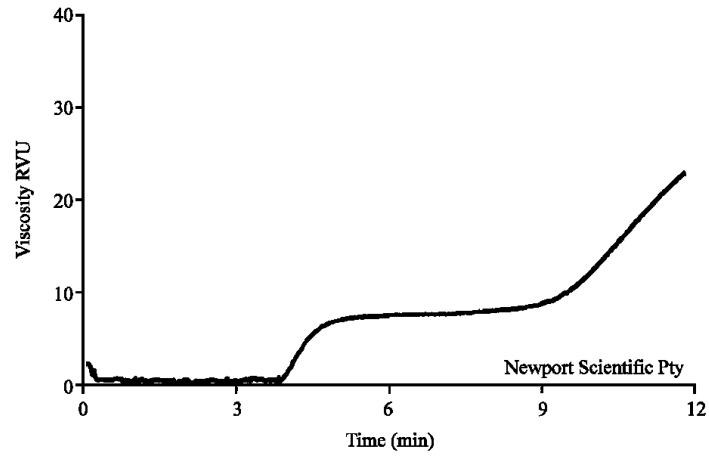


Fig. 5: Viscogramme of raw acha flour

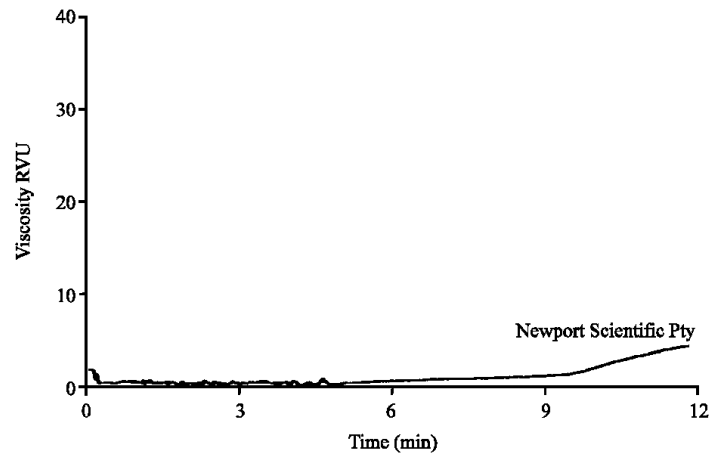


Fig. 6: Viscogramme of acha/soybean flour

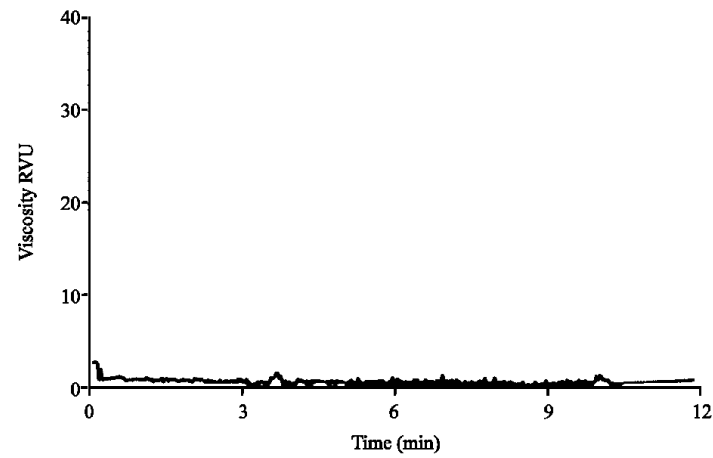


Fig. 7: Viscogramme of extruded flour/soybean flour

However, the cooling viscosity and temperature at peak viscosity were similar. In addition, 18.5 (RVU) break down reported by Jideani *et al.* (1996) was close to 17.18 (RVU) recorded for this study. Jideani and Akingbala (1993) and Jideani *et al.* (1996) reported that acha flour paste had low apparent viscosity and was stable to shearing at 95°C, but increased greatly when the paste was cooled to 50°C and remained stable to shearing at this temperature. This makes acha flour suitable for preparing two a stiff paste. The results obtained from this study confirmed this observation. The viscogramme of raw acha flour (Fig. 5) was typical of the ones reported by Jideani *et al.* (1996) and hence confirm that acha flour paste is not different from non-waxy cereal flour paste. Addition of soybean flour at 25% level of substitution to acha flour greatly affected its pasting properties due modifications in its proximate composition (results not included).

All measured parameters decreased, with the exception of the break down viscosity and pasting temperature. The result indicated that blending imparted high percentage resistance to disintegration and higher abilities to resist retrogradation. Ingbian (2004) and Maria *et al.* (1983) reported that peak viscosity was an indication of the maximum increase in that value for the starch-water solution upon heating. Therefore, lower values of peak viscosities indicated that a greater amount of gelatinization had occurred in the initial samples or there had been fortification of flours with oilseeds. The presence of soybean flour at 25% levels therefore must have been responsible for the lowering of the raw blend peak viscosity. Ingbian (2004) also reported that peak viscosity indicated the water binding capacity of starch or blends and provides indication of the viscous load likely to be encountered by a mixing cooker. The lower peak viscosities showed that there was increased water imbibition and increased swelling. This also would account for the reduced cooking time. Despande *et al.* (1988) Maria *et al.* (1983) and Ingbian (2004) reported decreased cooking times occasioned by the addition of legume and initial heat treatment, respectively.

The low set back and high consistency indicated that the blend could be used in preparation of high-density food, which would not easily retrograde. The viscogramme of acha/soybean flour paste did not show any peak and trough (Fig. 6). This indicated the tendency of the cross-linking of protein molecules with starch thus imparting strength and decreasing the set back (Despande *et al.*, 1988). Extrusion of acha/soybean blend had further consequences on the pasting properties. Only the peak time remained comparable to raw acha flour. The reduction in peak viscosity was obviously due to gelatinization of starch. The lowered peak viscosity of extruded whole acha flour indicated that there was extensive gelatinization and starch degradation during extrusion processing at 150 rpm, 150°C BT, 25% FMC. The viscogramme of extruded acha/soybean flour (Fig. 7) did not show any recognizable peak and trough indicating that much of the starch was gelatinized. Pasting properties of extruded acha and soybean flour blends differed from the raw blend from the point that extruded acha/soybean flour had lower set back and no apparent final (cold paste) viscosity. These properties showed that its paste would remain fluid with higher nutrient density and lowered bulkiness. The reduced peak time also showed that less energy would be required to cook the paste and the problem of retrogradation or hardening might not arise. The pasting temperature and peak viscosity were the two parameters that related all the samples. They maintained similar range, though; addition of soybean lowered the temperature at peak viscosity as was expected.

However extrusion cooking had no significant ($p>0.05$) effect on peak temperature. Addition of soybean and extrusion did not seem to have any noticeable effect on the pasting temperature.

The results from this study have confirmed that acha pasting is similar to other non-waxy cereals. Earlier reports have indicated the extrudate amylose content was significantly dependent on the feed composition. This study has confirmed this position. Even at higher feed moisture content extrudate amylose content was still dependent on feed composition. The highest predicted value of amylose content was 27.04% and this was obtained at 00.00% soybean addition 25% feed moisture content 150rpm screw speed and 150°C barrel temperature. The predictions were close approximation of the experimental values.

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