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Effect of Extrusion Variables on the Hydrogen Cyanide and Haemagglutinin Content of Extruded Blends of Cassava Products and African Yam Bean

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Abstract
High quality cassava flour (CF) and African yam bean (AYB) flour were blended together , also pregelatinized, shreaded cassava chips (*Ighu*) and AYB flour were blended separately using feed ratios of 0.54:99.46, 20:80, 50:50, 80:20, 99.46:0.54 (AYB/CF or AYB/*Ighu*) The extruder screw speed and the moisture content of the blends were also varied. The samples were extruded using a single screw extruder. Hydrogen cyanide content and haemagglutinin contents of the extrudates and the raw samples were determined. The data obtained were statistically analyzed using response surface methodology. The results obtained showed that there were no significant difference ($p > 0.05$) in the linear, quadratic and interaction for moisture content and screw speed, but there were significant difference in the linear and quadratic effects of feed composition on the hydrogen cyanide of the samples. The results also showed that extrusion cooking affected the hydrogen cyanide content of AYB/*Ighu* blends more than the cassava flour/AYB blends. The values obtained were below the Industrial Standard for cassava and cassava products. The residence time of the feed material had significant effect on the reduction of haemagglutinin content of the end products. The model for AYB/ *Ighu* was adequate more than that of AYB/CF.
Keywords: Hydrogen cyanide, haemagglutinin, extrusion, cassava, African yam bean

1. INTRODUCTION

Leguminous crops have been recognized as source of proteins in the diet of population of many tropical areas of the world. They are mostly used in places where sources of animal proteins such as milk, eggs, meat and fish are in short supply hence consumption is on the low side due to exorbitant prices or due to cultural taboo. African yam bean (*Sphenostylis stenocarpa*) is one of the lesser known legumes but its production and utilization has increased of recent due to intensive research on it. AYB contains all the amino acids found naturally in the plant protein (Ekop, 2006). Seeds of AYB contain anti nutritional factors such as haemagglutini and trypsin inhibitor that reduce the body's ability to access the nutrient in the food. Haemagglutini is reported to be responsible for 25-50% growth inhibition of rats fed on raw soybean meal (Iwe, 2003).

One of the most popular uses of cassava flour worldwide is in the manufacture of baked products (Ogunjobi and Ogunwolu, 2010). Pregelatinized cassava chips or slices called *ighu* a cassava based product is a traditional food in the eastern part of Nigeria. It starch which is pregelatinized is usually more soluble and digestible. Cassava contains cyanogenic glycoside. Hydrogen cyanide is formed when cassava is being processed. Hydrogen cyanide is very poisonous because it binds cytochrome oxidase and stops action in respiration, which is a key energy conversion process in the body, but the toxin is volatile and is released into the air, rather than remain in the food (Ohio,2003). The extent of cyanogens removal depends on the processing method.

Extrusion cooking is an effective method of pre-cooking grains and flours (Konstance et al, 1998). In the developing world, extrusion cooking may be applied in the production of stable foods from indigenous crops. This study was aimed at finding the level of reduction of hydrogen cyanide and haemagglutin content on extruded blends of cassava products and African yam bean.

2. MATERIALS AND METHODS

Cream colored African yam bean, cassava tubers and *ighu* were processed into flours. The flour samples were blended using different ratios of cassava or *ighu* flours to AYB flour. Combination of feed moisture, feed composition and screw speed were determined using a central composite design (Chang and El-Dash 2003). Twenty blends of cassava flour and AYB and another 20 blends of *Ighu* flour and AYB were formulated with eight (2^3) factorial points, six star points and six center points. The moisture levels of the blends were adjusted by adding a pre-determined amount of water using material balance equation to yield (13.3, 16, 20, 24 and 26.7) % moisture levels of the samples. The flour samples were then mixed and transferred into polythene bags and allowed to equilibrate for 12 hours at room temperature (28 $\pm$ 1^{o}C). The blends were extruded using single screw extruder (Brabender) under different operating conditions. The extruder temperatures were fixed at 90^{0}C, 120^{0}C and 110^{0}C at feed, melt and die zones respectively. The screw speeds were varied from 109.95 to 190.05rpm. The experiments were carried out in duplicates. After extrusion cooking, the extrudates were oven dried at 60 $\pm$ 1 ^{o}C for 10 hrs to obtain dried extrudates. The different samples were ground into powder (particle size < 500um) before analysis.

2.1. *Hydrogen Cyanide Determination*: The alkaline picrate method described by Onwuka (2005) was used. Five grammes of the samples was made into paste and dissolved in 50ml distilled water in a corked conical flask. It was allowed to stay for 12 hours. The solution was filtered and the filtrate used for the cyanide determination. One milliliter (1ml) of the filtrate was mixed with 4ml of alkaline picrate solution and incubated in water bath for 5 minutes for colour development (reddish brown), a blank was also prepared using 1ml distilled water and absorbance was taken at 490nm. The cyanide content was extrapolated using a cyanide standard curve.

2.2. *Haemagglutinin determination* : The haemagglutinin contents were determined by spectrophotometric method described by Onwuka, (2005). A given weight of the sample (0.5g) was dispersed into a 10 ml normal saline solution buffered at pH 6.4 with a 0.01 M phosphate buffer solution and allowed to stand at room temperature for 30minutes and then centrifuged to obtain the extract. One milliliter of trypsinzed rabbit blood was mixed with 0.1ml of the extract in a test tube, also a control containing only the blood cells was allowed to stand with the sample at room temperature for 4 hours. One milliliter of normal saline was added to all the test tube and allowed to stand for 10 minutes, after which the absorbances were read at 620nm. The test tube that contained only the blood cells and normal saline served as the blank. The result was expressed as haemagglutinin unit per mg of the sample.

Statistical Analysis: Matlab 7.1 version was used to determine the response surface regression coefficients, the analysis of variance and response surface plots were drawn. The critical values were obtained using linear programming.

3. RESULTS

The results of the hydrogen cyanide (HCN) and the haemagglutinin contents of the samples were shown in Table 1. The response surface regression analysis of hydrogen cyanide for extruded *Ighu/AYB* samples were shown in Table 2. Analysis of the response surface plot of screw speed, feed composition and hydrogen cyanide of *ighu*/AYB showed the critical value of 1.26mg/kg (fig 1). The response surface plots for the haemagglutinin of the cassava flour/AYB and *Ighu*/AYB are shown in Fig. 2 and Fig 3.

4. DISCUSSION

The results in (table 1) showed that there were reductions in hydrogen cyanide and haemagglutinin contents of the extrudates compared with the raw sample, which indicates that extrusion cooking is a useful process for the production of instant flours, as it allows gelatinization and partial dextrinisation of starch, as well as reduction of the activity of some antinutritional factors (Mouquet *et al*., 2003, Iwe et al.,2001).

The response surface regression analysis of hydrogen cyanide for extruded *Ighu/AYB* samples (Table 2) showed no significant differences ($p> 0.05$) in the three effects for moisture content and screw speed, but there were significant differences in the linear and quadratic effects of feed composition on the hydrogen cyanide of the samples. The coefficient of determination value (r^2) was 79.8%. The lack of fit was significant. The polynomial equation after removing the non significant terms becomes

$HCN_i = 9.66-0.118FC + 0.00042FC^2$ ------------------ (1)

The ANOVA result showed significant differences ($p<0.05$) in the linear and quadratic effects, but no significant effects on the interaction which was in agreement with the regression analysis. There were no significant differences ($p>0.05$) in the three effects on all the independent variables estimated for hydrogen cyanide content of the extruded blends of cassava flour/AYB. The ANOVA results also showed no significant differences ($p >0.05$) but there was significance at $p < 0.05$. The coefficient of determination was just adequate ($R^2 = 52\%$).

The results showed that extrusion cooking affected the hydrogen cyanide content of *Ighu/AYB* blends more than the cassava/AYB blends which may be attributed to the pre-heating the *ighu* flour was subjected to before extrusion. Hydrogen cyanide is volatile and is released into the air (Ohio, 2003). Similar results were obtained by other authors on reduction of hydrogen cyanide of samples by processing, such as heat treatment (Abu *et al*. ,2003; Adeparusi, 2001).

The value of hydrogen cyanide of all the samples were below the Nigerian Industrial Standard for cassava and cassava products (NIS 344:2004) maximum specification for high quality cassava flour (Okoruwa and Sanni, 2005). Initial processing of the raw samples reduced their hydrogen cyanide below the NIS maximum specification before extrusion cooking which further reduced the cyanide content.

The response surface regression results of the extruded *Ighu/AYB* blends in Table 3 showed significant difference ($p<0.05$) in the quadratic effect of screw speed on the haemagglutinin content of the extrudates. The results show that the level of cook or the residence time of the feed materials had significant effect on the reduction of haemagglutinin content of the end products. The coefficient of determination, r^2, values for both regressions are as high as 84% and 81.6% respectively. But the lack of fit for cassava/AYB analysis was significant ($p<0.05$) while that of *Ighu/AYB* was not ($p>0.05$). The results showed that the model for *Ighu/AYB* was adequate more than that of cassava flour/AYB. The polynomial equation after removing the non significant terms are given below

$HAEM_i= 1.607 +0.00016ss^2$ (r^2=0.84) --------------------------------- (2)

$HAEMc = 3.42 – 0.034ss + 0.0001ss^2$ (r^2=0.816) --------------------- (3)

The screw speed affected the residence time of the products in the extruder which must have affected the time of heating and the rate of reduction of haemaglutinin since heat played important role in its reduction, according to Martin-Cabrejas,(1999) and Steel et al, (1995) extrusion cooking reduced the lectin content of the extrudates.

The response surface plots (fig 2 and fig 3) revealed minimum values of 0.387Hu/mg and 0.366Hu/mg for *ighu*/AYB and cassava/AYB haemaglutinin contents respectively, indicating that products with reduced specific haemaglutini values can be obtained by varying the operating conditions of the extruder and the feed composition.

5. CONCLUSION

The study showed that extrusion cooking reduced the hydrogen cyanide and haemaglutinin content of the extrudates. The feed composition had linear and quadratic effect on the hydrogen cyanide content of *ighu*/AYB extrudates while the screw speed had effect on both the haemaglutinin content of *ighu*/AYB and cassava flour/AYB blends.

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Table 1: Hydrogen Cyanide and Haemagglutinin Contents of the Samples

sample	SS (rpm)	FM (%)	FC (%)	HCN_i (mg/Kg)	HCN_c (mg/Kg)	$HAEM_i$ (Hu/mg)	$HAEM_c$ (Hu/mg)
1	125	16	20	1.3	1.7	0.44	0.96
2	125	16	80	1.48	1.8	0.78	0.45
3	125	24	20	1.49	1.5	0.56	0.97
4	125	24	80	1.61	1.5	0.79	0.35
5	175	16	20	1.48	1.75	0.58	0.77
6	175	16	80	1.48	1.6	0.66	0.31
7	175	24	20	2.2	1.7	0.59	0.67
8	175	24	80	1.49	1.6	0.82	0.52
9	109.95	20	50	1.49	2.25	0.33	0.29
10	192.05	20	50	2.7	1.8	0.48	0.29
11	150	13.26	50	1.5	1.9	0.46	0.79
12	150	26.73	50	1.48	1.6	0.53	0.23
13	150	20	0.54	1.47	1.9	0.40	0.96
14	150	20	99.46	1.60	2.1	1.14	0.28
15	150	20	50	1.3	1.9	0.23	0.46
16	150	20	50	1.35	1.85	0.49	0.49
17	150	20	50	1.25	1.92	0.43	0.43
18	150	20	50	1.20	1.82	0.45	0.46
19	150	20	50	1.28	1.9	0.48	0.45
20	150	20	50	1.30	1.89	0.47	0.48
Ighu	-	-	-	2.45	-	ND	-
AYB	-	-	-	2.86	-	8.8	-
CF	-	-	-	-	4.15	-	-

KEY: SS= screw speed, FM=feed moisture, FC = Feed composition, AYB African yam bean, CF =cassava flour, HCN_i =Hydrogen cyanide for ighu/AYB, HCN_c = Hydrogen cyanide for cassava flour/AYB, $HAEM_i$ = Haemagglutinin for ighu/AYB, $HAEM_c$ = Haemagglutinin for cassava flour/AYB, ND=Not Determined.

Table 2: Response Surface Regression Parameters for Influence of Process Variables on Hydrogen cyanide of *Ighu/AYB*

(a) Regression Coefficients

Term	Coef	SE Coef	T	P
Constant	9.66027	3.62932	2.662	0.024
MC	-0.11175	0.18486	-0.605	0.559
SS	0.03482	0.02093	1.663	0.127
FC	-0.11868	0.03237	-3.666	0.004
MC^2	0.00234	0.00353	0.663	0.523
SS^2	0.00006	0.00006	0.923	0.378
FC^2	0.00042	0.00009	4.468	0.001
MC*SS	-0.00080	0.00063	-1.265	0.234
MC*FC	0.00051	0.00076	0.674	0.516
SS*FC	-0.00017	0.00010	-1.660	0.128

S = 0.2152 r^2 = 79.8% r^2 (adj) = 61.6%

(b) Analysis of Variance (ANOVA)

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Regression	9	1.82524	1.82524	0.202804	4.38	0.015
Linear	3	0.66226	0.85651	0.285503	6.17	0.012
Square	3	0.94034	0.94034	0.313448	6.77	0.009
Interaction	3	0.22264	0.22264	0.074212	1.60	0.250
Residual Error	10	0.46294	0.46294	0.046294		
Lack-of-Fit	5	0.44994	0.44994	0.089987	34.61	0.001
Pure Error	5	0.01300	0.01300	0.002600		
Total	19	2.28818				

Table 3: Response Surface Regression Parameters for Influence of Process Variables on Hydrogen Cyanide of Cassava/AYB

(a) Regression Coefficients

Term	Coef	SE Coef	T	P
Constant	-0.148324	3.04676	-0.049	0.962
MC	0.105718	0.15519	0.681	0.511
SS	0.011156	0.01757	0.635	0.540
FC	0.006561	0.02717	0.241	0.814
MC^2	-0.003735	0.00296	-1.260	0.236
SS^2	-0.000112	0.00005	-2.052	0.067
FC^2	-0.000054	0.00008	-0.693	0.504
MC*SS	-0.000469	0.00053	-0.881	0.399
MC*FC	0.000437	0.00064	0.685	0.509
SS*FC	0.000075	0.00009	0.881	0.399

S = 0.1806 r^2 = 52.0% r^2 (adj) = 8.9%

(b) Analysis of Variance (ANOVA)

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Regression	9	0.35385	0.35385	0.039316	1.21	0.385
Linear	3	0.10628	0.02524	0.008413	0.26	0.854
Square	3	0.18163	0.18163	0.060543	1.86	0.201
Interaction	3	0.06594	0.06594	0.021979	0.67	0.588
Residual Error	10	0.32625	0.32625	0.032625		
Lack-of-Fit	5	0.27830	0.27830	0.055660	5.80	0.038
Pure Error	5	0.04795	0.04795	0.009590		
Total	19	0.68010				

Table 4: Response Surface Regression Parameters for Influence of Process Variables on Haemagglutinin Content of *Ighu/AYB*

(a) Regression Coefficients

Term	Coef	SE Coef	T	P
Constant	1.60709	1.88705	0.852	0.414
MC	-0.10728	0.09612	-1.116	0.290
SS	-0.00533	0.01088	-0.489	0.635
FC	-0.00345	0.01683	-0.205	0.842
MC^2	0.00263	0.00184	1.435	0.182
SS^2	0.00016	0.00003	4.800	0.001
FC^2	0.00002	0.00005	0.383	0.709
MC*SS	0.00004	0.00033	0.126	0.902
MC*FC	0.00005	0.00040	0.126	0.902
SS*FC	-0.00004	0.00005	-0.822	0.430

S = 0.1119 r^2 = 84.0% r^2 (adj) = 69.7%

(b) Analysis of Variance (ANOVA)

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Regression	9	0.658542	0.658542	0.073171	5.85	0.005
Linear	3	0.348554	0.017832	0.005944	0.47	0.707
Square	3	0.301139	0.301139	0.100380	8.02	0.005
Interaction	3	0.008850	0.008850	0.002950	0.24	0.869
Residual Error	10	0.125153	0.125153	0.012515		
Lack-of-Fit	5	0.077203	0.077203	0.015441	1.61	0.307
Pure Error	5	0.047950	0.047950	0.009590		
Total	19	0.783695				

Table 5: Response Surface Regression Parameters for Influence of Process Variables on Haemagglutinin Content of Cassava/AYB

(a) Regression Coefficients

Term	Coef	SE Coef	T	P
Constant	3.42299	2.38946	1.433	0.183
MC	-0.18615	0.12171	-1.530	0.157
SS	-0.03452	0.01378	-2.505	0.031
FC	0.00271	0.02131	0.127	0.901
MC^2	0.00303	0.00232	1.305	0.221
SS^2	0.00010	0.00004	2.400	0.037
FC^2	-0.00004	0.00006	-0.729	0.483
MC*SS	0.00021	0.00042	0.499	0.628
MC*FC	0.00025	0.00050	0.499	0.628
SS*FC	0.00009	0.00007	1.298	0.223

S = 0.1417 r^2 = 81.6% r^2 (adj) = 65.1%

(b) Analysis of Variance (ANOVA)

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Regression	9	0.89283	0.892829	0.099203	4.94	0.010
Linear	3	0.68826	0.173931	0.057977	2.89	0.089
Square	3	0.16077	0.160767	0.053589	2.67	0.104
Interaction	3	0.04380	0.043800	0.014600	0.73	0.559
Residual Error	10	0.20067	0.200666	0.020067		
Lack-of-Fit	5	0.19838	0.198383	0.039677	86.88	0.000
Pure Error	5	0.00228	0.002283	0.000457		
Total	19	1.09349				

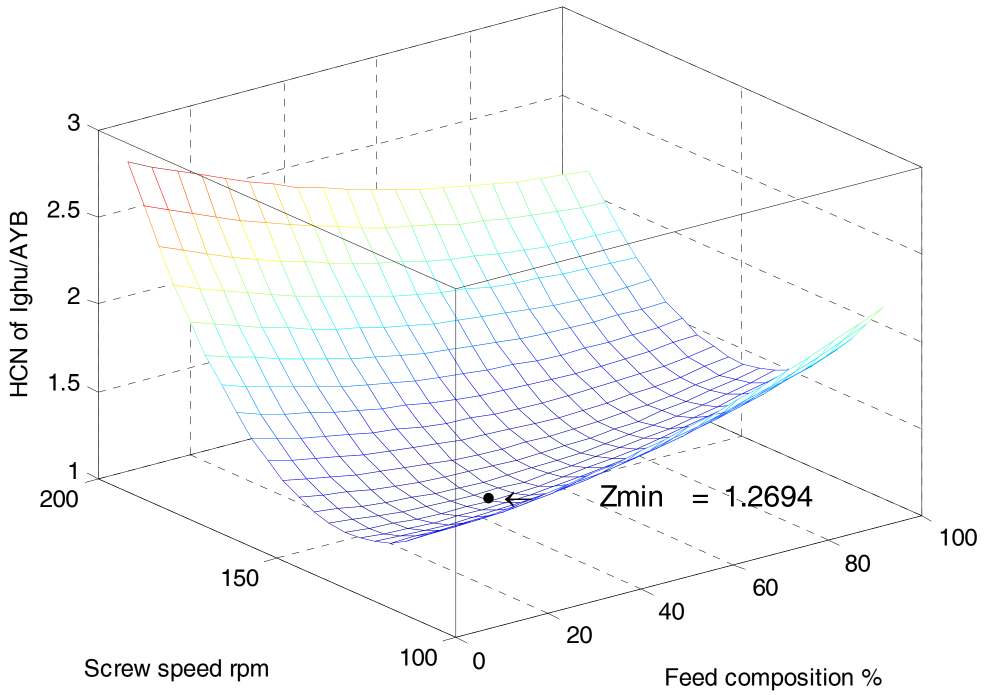

Fig 1: Effect of feed composition and screw speed on the HCN content of extruded blends of *Ighu*/AYB.

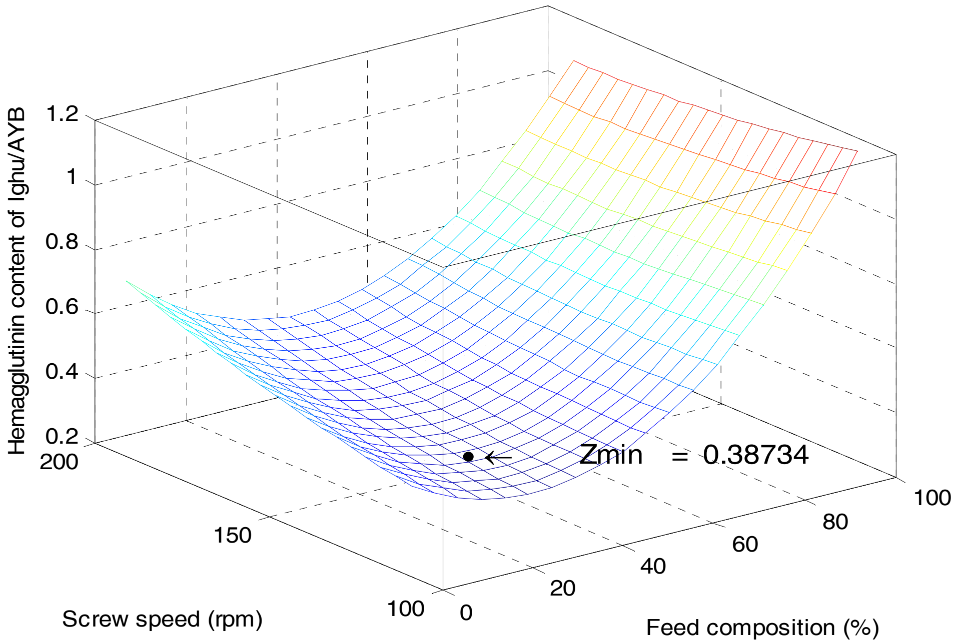

Fig 2: Effect of screw speed and feed composition on the Hemagglutinin content of extruded blends of *Ighu*/AYB.

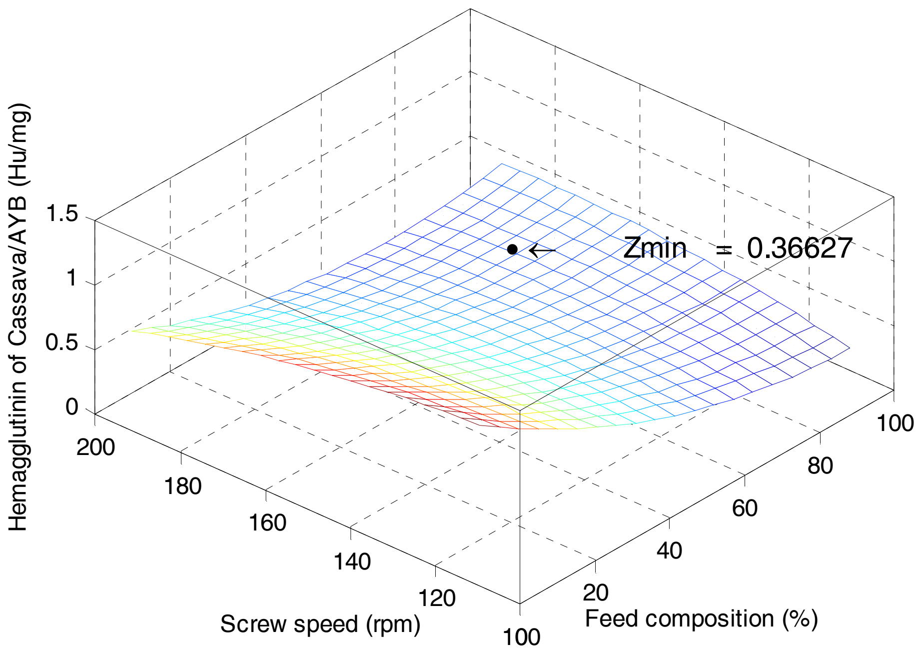

Fig 3: Effect of screw speed and feed composition on the Hemagglutinin content of extruded blends of cassava/AYB.