American Journal of Food Technology 2 (6): 502-511, 2007
ISSN 1557-4571

Protein Dispersibility Index and Trypsin Inhibitor Activity of Extruded Blends of Acha/Aoybean: A Response Surface Analysis

[1]J.C. Anuonye, [2]G.I.O. Badifu, [3]C.U. Inyang,
[4]M.A. Akpapunam [5]C.U. Odumudu and [4]V.I. Mbajika
[1]National Cereals Research Institute Badeggi Niger State, Nigeria
[2]Delta State University Asaba Campus, Nigeria
[3]University of Uyo Akwa Ibom State, Nigeria
[4]University of Agriculture, Makurdi, Nigeria
[5]College of Medicine, University of Jos, Nigeria

Abstract: The effect of extrusion variables on the protein dispersibility index and tyrpsin inhibitor activity of blends of acha and soybean were studied using response surface analysis. Soybean flour was mixed with acha flour at 0, 12.5, 25, 37.5 and 50% levels of substitution. Moisture content of the mixtures was adjusted to 15, 20, 25, 30 and 35%. Extrusion was carried out in a single screw Brabender extruder by adjusting the screw speed from 90 to 120, 150, 180 and 210 rpm and barrel temperature from 100 to 125, 150, 175 and 200°C following a 4-variable central composite rotatable response surface design. Protein dispersibility index and trypsin inhibitor activity of raw and extruded blends were evaluated. Raw acha, soybean flour and raw acha/soybean flour blends had PDI of 86.84, 91.84 and 74.27%, respectively compared to extrudate PDI which ranged from 3.77-8.70%. Blending decreased the protein dispersibility index of the raw flours while extrusion cooking significantly improved the protein dispersibility. For TIA, the results showed that TIA of extrudates ranged from 4.0-46.1 units, compared to the raw samples (64.5 units for raw soybean). The results showed that extrusion cooking reduced TIA by about (70.33-97.40%) with feed moisture and barrel temperature exerting the greatest influence on extrudate TIA. The decrease in TIA corresponded to increased protein dispersibility of extrudate samples.

Key words: Acha, soybean, blends, extudates, dispersibilty inhibition

INTRODUCTION

Acha, *Digitaria, exilis* Skippis Stapf occupies about 300,000 ha in West Africa and provides foods for about 4 million people (Kwon-Dung and Misari, 2000). In Nigeria, acha is popularly grown in five states (Bauchi, Kaduna, Kebbi, Plateau and Niger) and the Federal Capital Territory. According to Kwon-Dung and Misari (2000), acha is one of the world's best tasting cereals. In recent times, comparison of dishes of acha and rice showed that majority preferred acha dish. Traditionally, acha is used in preparation of unfermented porridge food. It is also made into gwette and acha-jollof. It is also used in dietary preparations for diabetic patients (Victor and James, 1991). The protein content of acha grains is rich in methionine and cysteine (above the recommended levels). These levels are unusual for cereals. However, Victor and James (1991) reported that with the exception of methionine and cysteine the essential amino acid content of acha is lower than in other cereals. They therefore advocated its complementation with protein rich foods to make a balance diet.

Most of the soybeans grown in Nigeria are used for human consumption and are being promoted as nutritional supplement. The potential for incorporation and utilization of soybeans in the local diet

Corresponding Author: J.C. Anuonye, National Cereals Research Institute Badeggi Niger State, Nigeria

is enormous and serves as a basis of the need for adequate processing. Soybeans have been processed in a number of ways, but a more recent processing method is extrusion cooking (Iwe, 2001).

A key problem associated with soybean utilization is the presence of certain antinutritional factors, which may inhibit the availability of the desirable nutrients such as proteins and minerals. Some of the prominent antinutritional factors in soybean include trypsin inhibitor, hemaglutinin, phytic acid, goitrogens, urease activity and flatulence causing factors (starchyose and raffinose) (Osho and Dashiell, 1995). The significance of soybean Trypsin Inhibitors (TI) lies in their implication in inhibiting the pancreatic enzymes (trypsin and chymotrypsin) resulting in reduction in protein digestibility both in humans and animals. It also causes hypertrophy of the pancreas in smaller animals like cats and chicks (Liener, 1994). Efforts have been made to inactivate or remove trypsin inhibitors from soybean. Osho and Dashiell (1995), Keshun (1999) and Iwe and Ngoddy (2000) reported that many approaches had been based largely on heat treatment. According to Iwe and Ngoddy (2000) most commercially available soybean products intended for human such as tofu, soybean milk, soybean-based infant formula, soybean protein isolates and concentrates and textured meat analogues have received sufficient heat treatment to cause inactivation of at least 80% of the TIA present in raw soybeans. This level of TIA destruction is well above the threshold of 50 to 60% inactivation found to be necessary for eliminating significant growth inhibition and pancreatic hypertrophy in rats (Rackis *et al*., 1976).

Iwe *et al*. (2001) have observed that protein solubility is an important target parameter and in the animal and feed industry the protein dispersibility index is often used to characterize the protein quality of raw materials. Poel *et al*. (1990) and Camire *et al*. (1990) reported that PDI could be used as a chemical indicator for inactivation of antinutritional factors and effects on functional properties.

Several investigators (Leslie and Dale, 1990; Iwe, 2001; Tayeb *et al*., 1992; Frazar *et al*., 1982; Iwe *et al*., 2000; Mullen and Ennis, 1979; Meyer, 1976) had noted that the one-variable-at-a-time method of experimentation is not good strategy in many situations. Response Surface Methodology (RSM) basic principle of relating product properties (mechanical, functional, nutritional and sensory) to process variables (geometry, raw material, operating variables) (Iwe *et al*., 2000) could eliminate most of the observed limitations. There is however dearth of information on acha/soybean blending while there is no information on acha extrusion cooking. The objective of present study was to assess the effect of extrusion variables on the protein dispersibility index and tyrpsin inhibitor activity of blends of acha and soybean using response surface analysis.

MATERIALS AND METHODS

This study was carried out in 2005 as follows: The extrusion was conducted at the Federal Polytechnic Mubi Adamawa State, Nigeria.

Acha and soybean flour were prepared for extrusion as shown in Fig. 1. Feed moisture content was adjusted at five levels according to Wilmot and Nelson (1998). Extrusion was carried out using a Branbender Laboratory single screw extruder (DUISBURG DCE-330 Model). 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 wet flour was allowed to equilibrate for 2-3 h before extrusion. The extruder runs was stabilized using acha flour.

Experimental Design

The experimental design was a Central Composite Rotatable Design (CCRD) response surface analysis. Four independent variables including Feed Composition (FC), Feed Moisture Content

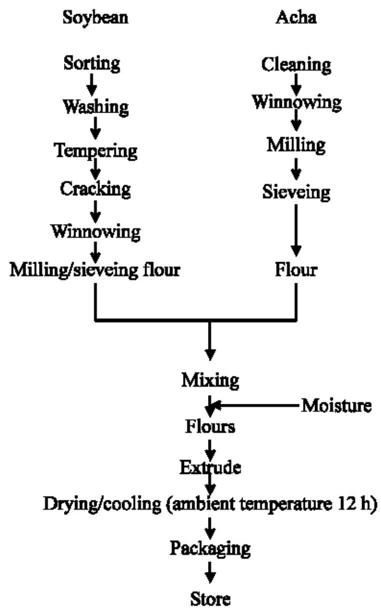

Fig. 1: Laboratory preparation of extruded acha-soybean flour blend

(FMC), Screw Speed (SS) and Barrel Temperature (BT) were tested at 5 levels coded (-2 to +2) (Meyers, 1976; Iwe, 2003). This experimental design required 36 experiments of which 16 were performed at the factorial points, 8 at the axial point and twelve at the center point. 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.

Sample Analysis

Protein dispersibility index was determined at the National Cereals Research Institute Laboratory Badeggi Niger State Nigeria while the trypsin inhibitor activity was assayed at the Soil Science Laboratory, University of Nigeria Nssuka.

Protein solubility and protein dispersibility index were determined on 20 g sample of raw acha flour, soybean flour and 75:25% mixture of acha/soybean flour along with extruded samples according to the methods described by Iwe *et al*. (2001). Milled samples of raw and extruded blends that passed a 1 mm sieve were blended with 300 mL of distilled water for 10 min in a warring blender operated at 8500 rpm and 21±1°C. The slurry was allowed to settle for 10 min. After decanting, 50 mL of the decantate was centrifuged at 2500 rpm (614x g) for 10 minutes. The supernatant (20 mL) was pipetted into Kjedhal tubes for protein determination according to AOAC (1984). Percentage solubility and protein dispersibility indices were calculated as shown:

$$\text{Protein solubility (\%)} = 6.25\times14\ (\text{mL HCl}-\text{mL HClr}) \times \text{N}/300\ \text{mL}/20\ \text{mL}\times100/\text{wt of sample}$$

Where,

mL HCl = Titre value

mL HClr = Value of blank

N = Normality of acid (0.1 N HCl)

PDI (%) = % protein solubility/Total protein content of sample ×100

Trypsin Inhibitor Activity

This was evaluated following the procedures reported by Tanteeratarm and Weingartner (1998): Finely ground samples (1.0 g) that passed 100 mesh were extracted with 50 mL of 0.01 N NaOH. Extracts were allowed to stand for 1 h while the pH of the suspension was adjusted from 9.5 to 9.8 with NaOH. Portions (0, 0.6, 1, 1.4 and 1.8 mL) of the suspension were pipetted into duplicate sets of test-tubes and adjusted to 2.0 mL with distilled water.

Trypsin solution 2.0 mL was added to each test-tube; the tubes were placed in a water bath at 37°C. To each tube, 5 mL solution of BAPA (Benzoyl-DL arginine-p-nitroanilide) hydrate dissolved in dimethyl sulfoxide and diluted to 100 mL) previously warmed to 37°C was added. Exactly, 10 min later, the reaction was terminated by adding 1ml of 30% acetic acid. After thorough mixing, the contents of each tube were filtered (Whatman Paper NO. 3) and the absorbance was measured against blank at 410 nm.

Expression of Activity

One Trypsin Unit (TU) is arbitrarily defined as an increase of 0.01, absorbance units at 410 nm per 10 mL of the reaction mixture. Trypsin Inhibitor Activity (TIA) was expressed in terms of Trypsin Units Inhibited (TUI).

RESULTS AND DISCUSSION

Protein Dispersibility Index (PDI)

The results of extrudate PDI ranged from 3.77-87.08%. Raw acha flour, raw soybean flour and raw acha/soybean flour blends had 86.84, 91.84 and 74.27%, respectively. The results showed that most extrudates had over 63% reduction in PDI. This level of (PDI) reduction is an indication of adequate heat treatment and hence enhanced nutritional status. Soybean flour had an increased dispersibility of 96.54%, while acha flour dispersibility increased by 89.8%. Iwe *et al*. (2001) reported similar results with potato flour extruded with defatted soybean flour. The results of regression analysis of the effect of process variables Feed Composition (FC), Feed Moisture Content (FMC), Barrel Temperature (BT) and Screw Speed (SS) on protein dispersibility index of extrudate samples is shown in Table 1.

The results showed that only the cross product effect of FC*FMC significantly ($p<0.05$) affected the protein dispersibilty index (Fig. 2). Analysis of variance (Table 2) showed that there was no significant ($p>0.05$) model difference. The coefficient of determination R^2 (0.54) also indicated no significant ($p>0.05$) fit of the model to the linear regression. These results agreed with the observations of Iwe *et al*. (2001) that increased starch percentage decreases protein dispersibility probably due to reduced protein percentage. Decreasing the FMC and level of acha flour in the blends led to decrease in PDI. These results agreed with Camire *et al*. (1990) who reported that products with higher protein contents had lower nitrogen solubility's attributable to greater denaturation and aggregation of protein molecules.

Aguilera and Luasas (1986) also reported decreased solubility for extrudated corn/soybean blends compared to that of unextruded materials. According to Iwe *et al*. (2001) PDI range of 25-28% was acceptable for products containing soybean. The results of this study fell within this acceptable limit.

Trypsin Inhibitor Activity

The results showed that TIA of extrudates ranged from 4.0-46.1units compared to the raw samples (64.5 mg g^{-1}) and acha/soybean blend at 75:25 ratios (54.5 mg g^{-1}). Iwe and Ngoddy (2000) and Delvelle *et al*. (1983) reported TIA values of 58.8 and 40-50 mg g^{-1} for whole soybean, respectively.

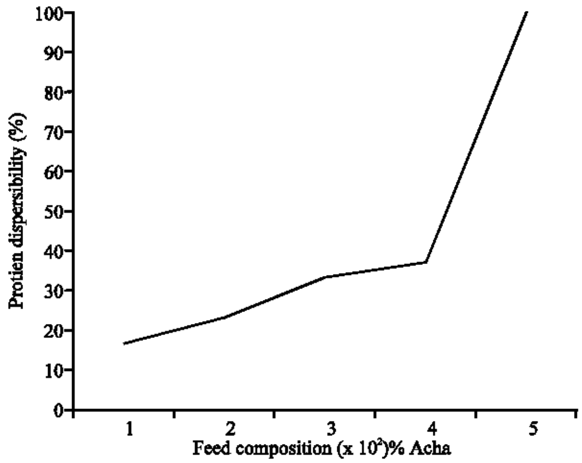

Fig. 2: Effect of feed composition on protein dispersibility

Table 1: Estimated regression coefficients for extrudate protein dispersibilty index

Regression on constants	Coefficients -300.76	SE	p-values	R^2
FMC	5.19	23.39	0.425	0.54
SS	2.13	3.83	0.737	
BT	0.80	4.04	0.886	
FC*FC	1.36	4.43	0.096	
FMC*FMC	-0.23	0.10	0.868	
SS*SS	-0.58	0.00	0.679	
BT*BT	0.74	0.00	0.664	
FC*FMC	-2.18	0.00	0.043	
FC*SS	-0.96	3.61	0.353	
FC*BT	-0.27	3.61	0.794	
FMC*SS	-3.22	0.14	0.694	
FMC*BT	-3.61	0.15	0.651	
SS*BT	-0.45	0.02	0.593	
FMC*SS*BT	-0.40	9.34	0.967	
FC*FMC*SS*BT	1.61	9.55	0.145	

FMC = Feed Moisture Content; SS = Screw Speed; FC = Feed Composition and BT = Barrel Temperature *Sign of combination

Table 2: Anova for extrudate protein dispersibilty index

	DF	SS	MS
Regression	15	4418.03253	294.53550
Residual	20	3659.28854	182.96443
F 1.60980	SIGN. F.0.1584		

The difference in these reports might be due to differences in variety of soybean used and other agronomic factors. The results indicated that extrusion processing reduced TIA by about (70.33-97.40%).

The result of regression analysis on the effects of process variables on Trypsin Inhibitor Activity (TIA) is shown in Table 3. The analysis of variance (Table 4) showed a significant ($p<0.05$) model difference indicating that the model fitted the experimental data. The coefficient of determination of of $R^2 = 0.6$ showed that the model could be used for predictive purposes since over 60% of the total variability in the data was explained by the model. Removing the non-significant terms, the model equation for TIA inhibition became

$$TIA = 42.22 - 12.59\ BT - 13.16\ FMC - 1.98\ FC*FMC = 3.07\ BT^2 + 13.29\ FMC*BT - 9.74\ SS$$

Table 3: Estimated regression coefficients for extrudate tyrpsin inhibitor activity

Regression on constants	Coefficients 842.22	SE	p-values	R^2
FMC	-13.16	12.12	0.03	0.63
SS	-9.74	1.98	0.10	
BT	-12.59	2.09	0.02	
FC*FC	1.01	2.29	0.02	
FMC*FMC	2.35	0.05	0.17	
SS*SS	0.68	0.00	0.08	
BT*BT	3.07	0.00	0.05	
FC*FMC	-1.98	0.00	0.04	
FC*SS	0.14	0.87	0.88	
FC*BT	-0.59	1.87	0.52	
FMC*SS	10.41	0.07	0.17	
FMC*BT	13.29	0.07	0.08	
SS*BT	10.55	0.01	0.13	
FMC*SS*BT	-11.30	4.84	0.19	
FC*FMC*SS*BT	1.46	4.94	0.14	

FMC = Feed Moisture Content; SS = Screw Speed; FC = Feed Composition; BT = Barrel Temperature; *Sign of combination

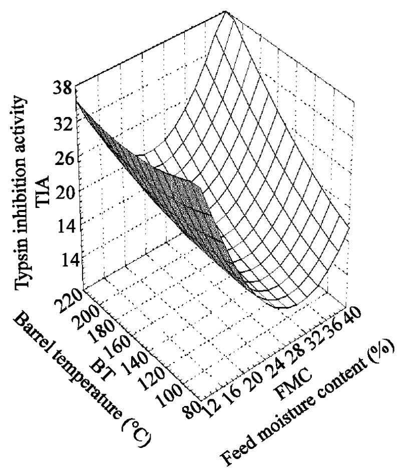

Fig. 3: Response surface plot of the effect of feed moisture content and barrel temperature on extrudate trypsin inhibitor activity

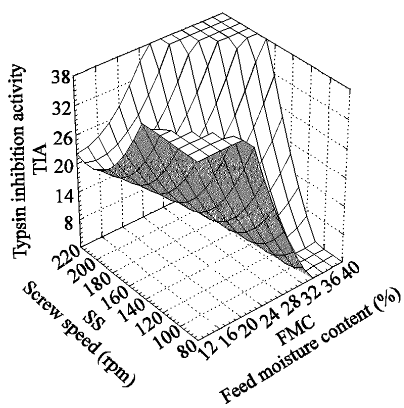

Fig. 4: Response surface plot of the effect of screw speed and feed moisture content on extrudate trypsin inhibitor activity

The response surface plot of FMC, BT and TIA units (Fig. 3) showed that increasing FMC and BT led to reduction of TIA units up to 84.97% at 150-200°C and 25 to 32% moisture content. Tsen *et al*. (1975) reported TIA inhibition or destruction from 50 to 100% when extrusion temperature was raised from 115 to 140°C at approximately 25% moisture. Philip *et al*. (1983) reported TIA inactivation at increased temperature. The results from this study are in agreement with these reports. However, at 32% moisture content, even at 100°C TIA inactivation began to reduce. The results showed that TIA reduction was dependent more on the moisture content than on temperature. This phenomenon may be due to the fact that increased moisture beyond the 32% exerted pronounced shear lowering which in turn reduced extruder internal temperature leading to decreased extrudate exit temperature.

The response surface plot of TIA to changes in the independent variables of FMC and SS (Fig. 4) showed that increasing FMC and SS led to reduction of TIA units. It was observed that FMC

Table 4: ANOVA for extrudate tyrpsin inhibitor activity

	DF	SS	MS
Regression	15	1683.44882	112.22992
Residual	20	982.09746	49.10487
F 2.28551	Sign. F.0.04		

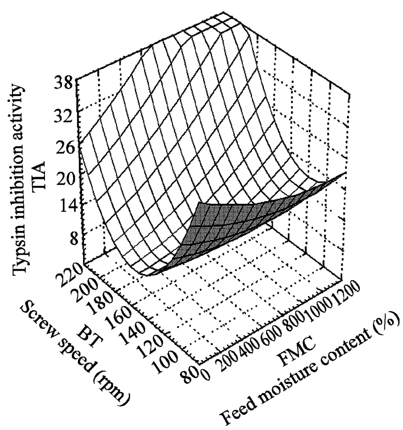

Fig. 5: Response Surface Plot of the Effect of Screw Speed and Barrel Temperature on Extrudate Trypsin Inhibitor Activity

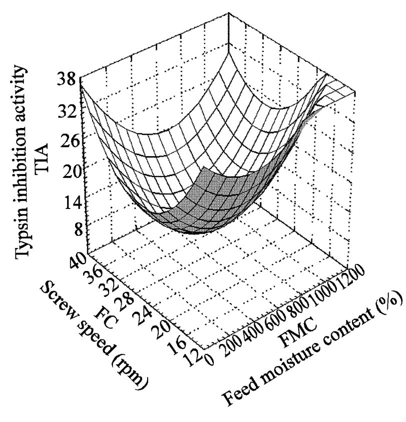

Fig. 6: Response Surface Plot of the Effect of Feed Composition and Feed Moisture Content on

played more significant role than SS and had a quadratic effect. The results showed similar pattern with that of FMC*BT. Similar results have been reported by Harper (1980) and Philip *et al*. (1983). Increased TIA destruction at decreased moisture content may be due to the increase in extruder shear (which would favour more inactivation) moisture content (Cheftel, 1984; Camire *et al*., 1990; Liener, 1994; Iwe and Ngoddy, 2001).

The relationship between the extruder SS and BT and TIA (Fig. 5) showed that BT had both a linear and quadratic effect on TIA retention. The results indicated that the linear effect was greater than the quadratic hence decreasing BT and SS recorded increased TIA retention which was expected. Below 120 rpm and 160°C, TIA units were as high as 26 TIA. At higher SS and BT, TIA inactivation (reduction) rose. Several authors, Tsen *et al*. (1975) Philips *et al*. (1983), Harper and Jansen (1985) Lorenz *et al*. (1980), Iwe and Ngoddy (2000) had reported the reduction of TIA at extrusion temperature of 121-150°C. Operating conditions for increased TIA inactivation was located at 120 SS and 175°C giving over 50.93% inactivation at 30% FMC and 12.5% soybean flour substitution.

The response of TIA to the FC and FMC (Fig.6) showed that decreasing the level of substitution of soybean and the FMC resulted in reduction of TIA. This was expected as the analysis showed that raw soybean contained more than 89.38% of the total TIA content of the raw blend. Iwe *et al*. (2001) showed a similar occurrence in the extrusion of sweet potato/soybean blends. The results indicated that addition of soybeans up to 60% in the feedstock would lead to rise in TIA ratio of 37 units of the original FC. This takes place at about 32% moisture content.

Table 5: TIA predicted using developed model equation

Extrusion Runs	Observed values	Predicted values
1	32.60	33.00
2	14.32	14.00
3	32.30	31.59
4	10.59	11.12
5	5.20	6.00
6	30.70	31.10
7	5.60	5.60
8	30.90	30.90
9	6.70	6.70
10	6.10	6.50
11	10.90	10.80
12	23.00	23.11
13	6.60	6.60
14	6.70	9.70
15	10.40	11.80
16	12.70	12.80
17	7.80	7.80
18	10.90	10.90
19	31.40	31.70
20	14.40	14.50
21	5.20	5.30
22	26.60	26.80
23	16.20	16.00
24	31.40	31.41
15	12.70	12.90
26	13.60	13.50
27	13.40	13.50
28	14.50	13.90
29	14.20	14.00
30	10.80	10.70
31	10.90	10.80
32	10.90	10.80
34	11.00	10.80
35	12.00	12.10
36	11.00	12.10

CONCLUSIONS

The results from this study showed that the range of heat treatment applied in this study provided satisfactory inactivation of antinutrients and produced acceptable functional products with good protein dispersibility indices. However, because of low R^2 and lack of model significance ($p>0.05$) the model for protein dispersibility was not used for predictive purposes. The predicted results of trypsin inhibitor activity (Table 5) approximated experimental results. It was therefore concluded that the second order polynomial was adequate in predicting the dependence of TIA on the process variables.

REFERENCES

Aguilera, J.M. and E.W. Lusas, 1986. Laboratory and pilot extraction of extruded high-oil corn. J. Am. Chem. Soc., 63: 239-243.

AOAC, 1984. Official Methods of Analysis. The Association of Official Analytic Chemists. 14th Edn., Weshington DC.

Camire, M.E., A. Camire and K. Krumhar, 1990. Chemical and nutritional changes in foods during extrusion. Crit. Rev. Food Sci. Nutr., 29: 35-57.

Cheftel, J.C., 1984. Extrusion cooking and food safety. In: Extrusion Cooking. Mercier, C., P. Linko and Harper (Eds.), AACC Inc. St. Paul. Minnesota, USA., pp: 1-15.

Delvelle, F.R., M. Escobedo, M.J. Munoz, R. Ertega and H. Bourges, 1983. Chemical and nutritional studies on Mesquite beans (*Prosopis juliftora*). J. Fod Sci., 84: 914-19.

FAO, 1985. Technical compendium on composite flours. United Nation's Economic Commission for Africa. Addis Ababa, pp: 7.

Harper, J.M., 1980. Extrusion of Foods. Vol. 1 CRC, Press Inc. Boca Raton, Florida, pp: 1-3, 77-78.

Harper, J.M. and G.R. Jasen, 1985. Production of nutritional precooked foods in developing countries by low-cost extrusion technology. Food Rev. Int., 1: 27-97.

Iwe, M.O. and P.O. Ngoddy, 2000. Effects of extrusion on trypsin inhibitor contents of soy-sweet potato blends. J. Food Pro. Pres., 24: 453-463.

Iwe, M.O., 2001. Effects of extrusion cooking on some functional properties of soy-sweet potato blends: A response surface analysis. Plant Foods Human Nutr., 55: 169-184.

Iwe, M.O., D.J. Van Zuilichem, P.O. Ngoddy and D.J. Winlarmmers, 2001. Amino acid and protein dispersibility index of mixtures of extruded soy and sweet potato flours. Lebensm-wiss u Technol., 34: 71-75.

Iwe, M.O., 2003. The Science and Technology of Soybeans. Chemistry, Processing and Utilization. 1st Edn., Rejoint Communication Services Ltd, Enugu., pp: 293.

Iwe, M.O., D.J. Van Zuilichem, W. Stolp and P.O. Ngoddy, 2003. Effect of extrusion cooking of soy-sweet potato blends on available lysine content and browning index of extrudates. J. Food Eng., pp: 1-5 (In Press).

Keshun, L., 1999. Current Constraints in Soybean Food Utilization and Efforts to Overcome Them. In: Proceedings of the World Soybean Research Conference VI. August 4-7, 1999. Chicago, Illinois, USA., pp: 409-418.

Kwon-Ndung, E.H. and S.M. Misari, 2000. Over View of Research and Development of Acha Digitaria Exilis Kippis Stapf and Prospects of Genetic Improvement in Nigeria. In: Genetics and Food Security in Nigeria, Genet, Soc. of Nigeria, pp: 71-76.

Leslie, L.R. and A.W. Dale, 1990. Response Surface Characterization of a Twin-screw Extruder. In: Engineering and Food. Vol. 3 Advanced Processes. Spiess, WEL.

Liener, I.I., 1994. Implications of antinutritional components in soybean foods. Crit. Rev. Food Sci. Nutr., 34: 31-67.

Lorenz, K., G.R. Jansen and J.M. Harper, 1980. Nutrient stability of full-fat soy flour and corn-soy blends produced by low-cost extrusion. Cereal Foods World, 25: 161-162, 171-172

Meyers, H.R., 1976. Response Surface Methodology. Allyn and Bacon, Boston M.A. USA., pp: 246.

Mullen, K. and D.M. Ennis, 1979. Rotatable designs in product development. Food Technol., 33: 74-81.

Osho, S.M. and K. Dashiell, 1995. Expanding the Soybean Production, Processing and Utilization in Africa In: Post Harvest Technology and Commodity Marketing: Proc. of a Post Harvest Conference 2nd Nov.-1st Dec. 1995, Accra, Ghana, Feris, (Ed.), RSB. IITA. Ibadan, pp: 151-156.

Philips, R.D., M.S. Chinnan and L.G. Mendoza, 1983. Effect of temperature and moisture content on the kinetics of trypsin inhibitor activity, protein *in vitro* digestibility and nitrogen solubility of cowpea flour. J. Food Sci., 48: 1863-1867

Poel, A.F., J. Blonk, D.J. van Zuilichem and M.G. van Oort, 1990. Thermal inactivation of lectins and trypsin inhibitor activity during steam processing of dry beans (*Phaseolus vulgaris* L.) and effects on protein quality. J. Sci. Food Agric., 53: 215-228.

Rackis, J.J., J.E. McGhee and A.N. Booth, 1976. Biological threshold levels of soybean trypsin inhibitors by rat assay. Cereal Chem., 52: 85-91.

Tanteeratarm, K. and K.E. Weingartner, 1998. Trypsin Inhibitor Assay. In: Soybean Processing for Food Uses. A Training manual. Sheldon, W.W. (Ed.), International Soybean Programme (INTSOY). Department of Food Science and Human Nutrition, pp: 419-422.

Tayeb, J., G. del Valle, C. Barres and V. Bruno, 1992. Simulation of Transport Phenomena in Twin-screw Extruders. In: Food Extrusion Science and Technology Kokini, J.L., C. Ho and M.V. Karwe (Eds.), pp: 41-70.

Tsen, C.C., E.P. Farrel, W.J. Hoover and P.R. Crowdley, 1975. Extruded soy products from whole and dehulled soybeans cooked at various temperatures for bread and cookie fortification. Cereal Food World, 20: 413-418.

Victor, J.T. and D.B. James, 1991. Proximate chemical composition of Acha (*Digitaria exilis*) grain. J. Sci. Food Agric., 56: 561-563.

Wilmot, B.W. and A.I. Nelson, 1998. Extrusion and Oil Expelting. In: Soybean Processing for Food Uses. A Training Manual. Sheldon, W.W. (Ed.), International Soybean Programme (INTSOY). Dep. Food Sci. Hum. Nutr., pp: 149-157.