



Effect of extrusion technique on antinutritional factors of sorghum-soya blends

ARUN KUMAR T V¹, INDRA MANI², A PRAMOD³, D V K SAMUEL⁴, S K JHA⁵, P K SAHOO⁶, J P SINHA⁷ and ABHIJIT KAR⁸

ICAR-Indian Agricultural Research Institute, New Delhi 110 012

Received:24 August 2017 ; Accepted: 13 December 2017

ABSTRACT

Response surface methodology was used to assess the effect of soya level (10-30%), feed moisture content (12-20% w.b.), barrel temperature (110-150°C) and screw speed (250-450 rpm) on trypsin inhibitor activity (TIA), phytic acid (PA) and tannin content of the sorghum [*Sorghum bicolor* (L.) Moench]-soya blends. Results showed that incorporation of soybean [*Glycine max* (L.) Merr.] increased antinutritional factors in the feed formulation but extrusion cooking considerably reduced these factors in the extruded snack. The extent of reduction being up to 96% in TIA and 57% in PA and tannin content. Second-order polynomial model showed that soya level and barrel temperature significantly ($P < 0.01$) influenced TIA, PA and tannin content whereas screw speed and feed moisture content showed influence only on TIA and PA, respectively. TIA, PA and tannin content of extrudates varied from 1.456 to 6.658 TUI/mg, 177.53 to 311.83 mg/100g and 0.1942 to 0.4514% tannic acid, respectively. The results of the study validate extrusion as an effective technique for reducing of antinutritional factors to safe level for the production of sorghum-soya based extruded snack food.

Key words: Extrusion, Phytic acid, Tannin , Trypsin inhibitor activity

Sorghum [*Sorghum bicolor* (L.) Moench] is one of the important foods across the semi-arid tropics. It is the third major cereal in India after rice and wheat. India is the fifth largest producer of sorghum with a production of 5.54 million tonnes during the year 2013-14 (DES 2016). Macromolecular constituents of sorghum are comparable to that of maize and wheat. It is composed of carbohydrate (70.7%), protein (7.7%), fat (3.1%), crude fiber (2%), and ash (1.6%). Every 100 g of sorghum contain 25 mg of calcium, 222 mg phosphorous and 4.1 mg of iron (FAO 1995). Besides, sorghum is reported to be a potential source of nutraceuticals such as antioxidant phenolics and cholesterol-lowering waxes (Taylor *et al.* 2006). In the last two decades sorghum has turned out to be a commodity of diverse utility. Use of sorghum, either whole or as ingredient, in value added food products are emerging (Rao *et al.* 2010, Charyulu *et al.* 2013). Taylor *et al.* (2006) reported that cakes, cookies, pasta, a parboiled rice-like product and snack foods have been successfully produced from sorghum. However, a major limitation of sorghum is the poor quality protein attributed to low solubility, deficiencies in essential amino acids (lysine and

tryptophan) and interactions with tannin (Sastry *et al.* 1986). Nevertheless, protein quality of sorghum can be improved by combining it with other protein rich sources.

Soybean [*Glycine max* (L.) Merr.] is an important source of protein and oil among legume crops. Protein and oil forms chief constituents of soybean accounting for about 60 per cent of dry soybean by weight. It is one of the valued crops with quality protein; the amino acid profile of soya proteins compares well with human requirements. Soybean also contains valuable vitamins, flavonoids, and polysaccharides (Chen *et al.* 2012). Despite the health promoting components that soybean offers its use is limited due to the presence of antinutritional factors (ANFs) such as protease inhibitors, lectins, phytates and tannins (Bajpai *et al.* 2005). Addition of whole soybean in the raw material poses a challenge to the food processors either to eliminate or to reduce these ANFs to an acceptable level in the final product. Thus, any kind of processing must ensure maximum destruction of these factors to reap health benefits of soybean.

Extrusion cooking is a high-temperature, short-time process in which starchy food material is plasticized and cooked by a combination of moisture, pressure, temperature and mechanical shear (Navale *et al.* 2015). Extrusion denatures detrimental enzymes, inactivates antinutritional elements and at the same time sterilizes the finished product (Shivendra *et al.* 2007). Application of extrusion technique to reduce ANFs has been widely studied (Balandran-Quintana

¹Scientist (e mail: arun.agrilengg@gmail.com), ²e mail: maniindra99@gmail.com, Division of Agricultural Engineering, ⁵e mail: skj_agengg@yahoo.com.in, ⁸e mail: abhijit8366@yahoo.com, Division of Food Science and Postharvest Technology

et al. 1998, Alonso *et al.* 2000a, Kaur *et al.* 2015, Rathod and Annapure 2016). It has been reported as an effective method to inactivate α -amylase inhibitors, trypsin, chymotrypsin and heamagglutinin activity without modifying the protein level in food products (Soetan and Oyewole 2009). Feed composition, moisture content, extrusion temperature, feed rate and residence/treatment time are some of the important factors reported to affect the destruction of ANFs (Nooshin *et al.* 2017). However, most of the earlier works have focused on the overall effect of extrusion on ANFs. Thus, studies involving assessment of influence of independent variables and their interactions were limited. Therefore, present study aimed at investigating the effects of extrusion process variables, viz. proportion of soybean flour in the feed, moisture content of the feed, barrel temperature and screw speed on the level of antinutritional factor such as trypsin inhibitor, phytic acid, tannins of sorghum-soya extruded product.

MATERIALS AND METHODS

Sorghum (variety DSV-4) was procured from the ICAR-Indian Institute of Millet Research, Hyderabad and soybean (variety MAUS-2) was supplied by the All India Coordinated Research Project on Soybean, UAS, Bangalore, India. The procured grain was thoroughly cleaned, properly dried and stored in cloth bags till further use. When required, clean and dry grain was milled in a laboratory scale hammer mill equipped with 60-mesh IS sieve.

Extrusion experiments were performed on a laboratory scale co-rotating twin-screw extruder having length to diameter ratio (L/D) of 20:1. The extruder had 2 barrel zones; temperature of the first zone was maintained at 74°C while the temperature in the second zone (compression and die section) was varied according to the experimental design. The die plate had a circular die of 3 mm. Sorghum and soybean flour proportions were weighed as per the experimental design and mixed for 15 minutes using a ribbon blender. Desired moisture content in the feed was achieved by adding calculated amount of water and conditioning for 24 h (Liu *et al.* 2000). Preconditioned feed mixture was metered into the extruder with a twin-screw volumetric feeder equipped with the extruder. The speed of the feeder screw was adjusted to set a feed rate of 5 kg/h and was kept constant during the course of research work.

Extruded material was cut with an inbuilt face cutter as it exits the die. Speed of the cutter was adjusted so as to get a desired length of the extrudates. Sample was collected when the extruder reached a steady state condition followed by drying in a cabinet drier for 1 h at 50°C to reduce the moisture content to around 4 per cent (w.b.). The dried sample was sealed in polythene (HDPE) bags and stored at room temperature (25±4°C) till analysis. All experiments were conducted in triplicates.

Central composite rotatable design comprising four independent process variables at five levels was used to investigate the effect of process variables on ANFs of sorghum-soya blends with Design Expert 8.0.7 (State-

Ease Inc., Minneapolis, USA). The independent variables considered were: soya level (SL): 10-30%; feed moisture content (FM): 12-20% w.b.; barrel temperature (BT): 110-150°C, and screw speed (SS): 250-450 rpm. The levels of each variable were established according to literature and preliminary trials. Dependent variables considered were: trypsin inhibitor activity (TIA), phytic acid (PA) and tannin content. The design required 30 experimental runs that included 6 central, 8 axial, and 16 factorial points. Experimental runs were randomized to minimize the effects of unexplained variability in the observed responses resulting from external factors.

Trypsin inhibitor was determined by the method given by Kakade *et al.* (1974) with slight modifications, using α -N-benzoyl-DL-arginine-p-nitroanilidehydrochloride (BAPNA) as the substrate for trypsin. Finely ground fat free sample (0.5 g) was extracted with 25 mL of 0.01 N sodium hydroxide for 3 h at room temperature in a rotatory shaker. Extracts were centrifuged at 17500g at 4°C for 20 min, and the supernatant was filtered through filter paper (Whatman No. 1). Five portions of extracts (0, 0.6, 1.0, 1.4, and 1.8 mL) were pipetted into test tubes and the final volume was adjusted to 2 mL with distilled water. Trypsin solution (2 mL, 20 mg/L in 0.001 M HCl) was added and the tubes were placed in water bath at 37°C, followed by addition of 5 mL of warm BAPNA solution (0.4 mg/mL in Tris-buffer 0.05 M, pH 8.2). After 10 min, reaction was stopped by adding 1 mL of 30 per cent acetic acid. Trypsin inhibitor activity, expressed as trypsin inhibitor units per milligram of the sample (TUI/mg sample) was calculated from absorbance read at 410 nm against a blank reagent. One trypsin unit was defined as the increase by 0.01 absorbance unit at 410 nm per 10 mL of the reaction mixture under the conditions used herein.

Phytic acid content was determined using the method followed by Nwabueze (2007) with slight modifications. Finely ground sample was extracted with 50 mL 3 per cent trichloro acetic acid (TCA) for 30 min at 135 rpm. Contents were centrifuged and 15 mL aliquot was transferred into a conical centrifuge flask, 4 mL ferric chloride solution (583 mg in 100 mL 3% TCA) was added and the flask was kept in boiling water till it precipitated (few drops of 3% sodium sulphate was added to promote precipitation). The flask was cooled, centrifuged for 20 min and the supernatant was discarded. The sediment was washed with distilled water and 3 mL of 1.5 N sodium hydroxide solution was added. The volume was made up to 30 mL using distilled water and the flask was kept in boiling water bath for 30 min. Contents of the flask were again centrifuged, supernatant was discarded, and the sediment was washed with water followed by addition of 40 mL 3.2 N nitric acid. The contents were dissolved and the volume was adjusted to 100 mL with water. About 0.5 mL aliquot was taken and its volume was made up to 8 mL with water. To this, 2 mL of 1.5 M potassium thiocyanate was added and the absorbance was noted immediately at 480 nm. This procedure was repeated to calculate the adjustment factor and the phytic acid content

was estimated using the following equation:

$$\text{Phytic acid (mg/100g)} = \frac{\text{iron } (\mu\text{g}) \times 15 \times \text{factor}}{\text{weight of sample (g)}}$$

The tannin content in the sample was determined by calorimetric method (AOAC 1970) using Folin-Denis reagent. The method is based on the measurement of blue colour formed by the reduction of phosphotungstomolybdic acid by tannin like compounds in alkaline solution. One gram of sample was boiled in 50 mL water for 30 min. Contents were cooled, centrifuged and supernatant was diluted adjusting the volume to 100 mL. An aliquot of 0.1 mL was taken in a test tube to which 2.9 mL water, 0.5 mL Folin-Denis reagent and 2 mL saturated sodium carbonate solution were added. The contents were mixed well and the colour was measured after 30 min at 760 nm against an experimental blank adjusted to zero absorbency. Amount of tannic acid was determined using a standard curve plotted using the values of tannin acid. The tannin content in the sample expressed as per cent tannin acid was calculated using the following formula:

$$\text{Tannin content (\% tannic acid)} = \frac{\text{tannic acid (mg)} \times \text{dilution} \times 100}{0.1 \times \text{weight of sample (g)} \times 100}$$

All biochemical constituents were analyzed in triplicates, unless specified and average values were reported and used in calculations.

The data were subjected to analysis of variance to understand the variations within group and between the groups. Pair wise comparisons were made by Post-Hoc Tukey's HSD significance test. The confidence limits used was based on 95 per cent ($P < 0.05$) level of significance, unless specified otherwise. The experimental data obtained during extrusion were analysed by fitting a second order polynomial model specified as follows:

$$y_i = b_o + \sum_{i=1}^4 b_i X_i + \sum_{i=1}^4 \sum_{j=1}^4 b_{ij} X_i X_j$$

where X_i , $X_i X_i$, and $X_i X_j$ are linear, quadratic, and interaction effect of the input variables which influence the response y , respectively, and b_o , b_i , and b_{ij} were the regression coefficients to be determined. The response surface plots for the models were plotted as a function of two variables, while keeping the other two variable constant at its centre point.

The regression and ANOVA models were tested for statistical validity based on the probability value, F-test and coefficient of variation. The adequacy of the models was determined using model analysis and coefficient of determination (R^2) analysis as outlined by Weng *et al.* (2001).

RESULTS AND DISCUSSION

Anti-nutritional factors (ANFs) of sorghum and soybean

Trypsin inhibitor activity, phytic acid and tannin content of unextruded mixtures of sorghum and soybean flour formulations are presented in Table 1. The values of ANFs observed in this study were comparable with those reported in the literature (Osman 2004, Mkwandawire *et al.* 2015). Minor differences in the values could be attributed to the varietal and geographical influence. It was observed that ANFs of sorghum and soybean flour and their blends differed significantly ($P < 0.05$). Higher amount of ANFs were observed in soybean as compared to that in sorghum. TIA (5.241 TUI/mg) and tannin content (0.2657% tannic acid) in the sorghum was found to be negligible as compared to soybean (166.753 TUI/mg; 2.1528 % tannic acid). As expected, amount of ANFs increased proportionally with increase in soybean flour content in the feed formulations. The extent of ANFs in the blends of sorghum and soybean flour indicates that the extrusion processing would be an ideal processing method for effective destruction of these components. Addition of soybean increased the concentration of protease inhibitor and tannic acid by up to 10 folds, at maximum soya flour addition (30%). Several researchers have documented similar observations. It has been reported that addition of soybean to sweet potato (Iwe 1998), defatted soya to rice (Dublish *et al.* 1988) and defatted soya to African bread fruit-corn mixtures (Nwabueze 2007) increased the TIA. Tannin content ranged from 0.4022 to 0.8293 in terms of per cent tannic acid. The content of phytic acid in sorghum was found to be half of that in soybean and incorporation of soybean had significantly increased the level of this antinutritional component in the range of 377.83 to 455.64 mg/100 g. The extent of ANFs in the blends of sorghum and soya flour indicates that the extrusion processing would be an ideal processing method for effective destruction of these components.

Table 1 Anti-nutritional factors of sorghum, soybean and their blends

	Sorghum	Soya	Sorghum:Soya				
			90:10	85:15	80:20	75:25	70:30
TIA, TUI/mg	5.448 (0.28) g	166.753 (3.70) a	21.919 (0.50)f	29.574 (1.20)e	37.106 (0.73)d	45.058 (0.74)c	53.536 (1.32)b
Phytic acid, mg/100 g	337.77 (2.71) g	733.53 (2.02)a	377.83 (1.84)f	399.01 (2.30)e	417.57 (1.78)d	436.30 (1.43)c	455.64 (1.93)b
Tannin content, % tannic acid	0.2657 (0.02) g	2.1528 (0.01) a	0.4022 (0.01)f	0.5767 (0.01)e	0.6055 (0.01)d	0.7664 (0.02)c	0.8293 (0.02)b

Values are means of triplicate determination and values within the parenthesis represent standard deviation. Values with different alphabet in the same row differ significantly ($P < 0.05$).

Trypsin inhibitor activity (TIA)

Table 2 illustrates the TIA of extruded and unextruded sorghum-soybean blends. The effects of independent variables on TIA are depicted in Fig 1. It was observed that extrusion processing had a positive impact in reducing the TIA in the range of 80.37 to 96.08 per cent. TIA of sorghum-soybean extrudates was observed in the range of 1.45 to 7.28 TUI/mg which was much lower than that observed in the raw material (21.91 to 53.53 TUI/mg). Reduction in TIA of the sorghum-soya (80:20) blend was maximum at a barrel temperature of 130°C, SS of 250 rpm and FM of 16 per cent (w.b). Regression results (Table 3) showed that SL and SS had a significant positive linear effect, whereas effect of BT was negative on TIA. FM did not show significant linear influence on TIA however, its interaction effect with

the BT was observed to be negative at 10 per cent level of significance. None of the quadratic terms were found to have a considerable effect on TIA of sorghum-soya extrudates. The model could explain 91 per cent of the total observed variation in the depended variable. ANOVA of TIA had a significant F-value (P<0.01) with a coefficient of variation of 14.81%. Commercially available consumable soybean products have been processed to achieve at least 80 per cent reduction of TIA present in raw soybean (Nwabueze 2007). Hence, the reduction of TIA observed in this study followed by extrusion is assumed to be acceptable for a ready-to-eat snack food. The results of our study are in line with those reported from the previous studies on legumes like soybean, mungbean, cowpea and their blends with cereals (Liener 1994, Iwe and Ngoddy 2000, Nwabueze 2007).

Table 2 Effects of extrusion on trypsin inhibitor activity, phytic acid and tannin content of sorghum-soya blends

Extrusion condition				Tripsin inhibitor activity (TUI/mg)		Phytic acid (mg/100g)		Tannin (% tannic acid)	
SL (%)	FM (% w.b)	BT (°C)	SS (rpm)	Raw	Extruded	Raw	Extruded	Raw	Extruded
15	14	120	300	29.574	2.447	399.01	231.71	0.5767	0.3188
25	14	120	300	45.058	3.651	436.30	251.84	0.7664	0.4407
15	18	120	300	29.574	3.834	399.01	268.15	0.5767	0.3427
25	18	120	300	45.058	5.596	436.30	296.76	0.7664	0.4514
15	14	140	300	29.574	1.834	399.01	187.21	0.5767	0.2757
25	14	140	300	45.058	3.604	436.30	213.49	0.7664	0.3753
15	18	140	300	29.574	2.189	399.01	238.20	0.5767	0.2997
25	18	140	300	45.058	3.598	436.30	259.31	0.7664	0.3997
15	14	120	400	29.574	3.952	399.01	239.57	0.5767	0.3542
25	14	120	400	45.058	5.747	436.30	266.60	0.7664	0.4292
15	18	120	400	29.574	4.276	399.01	268.54	0.5767	0.3419
25	18	120	400	45.058	6.658	436.30	311.83	0.7664	0.4182
15	14	140	400	29.574	2.268	399.01	229.09	0.5767	0.2615
25	14	140	400	45.058	5.156	436.30	215.69	0.7664	0.3689
15	18	140	400	29.574	2.431	399.01	256.46	0.5767	0.2801
25	18	140	400	45.058	4.498	436.30	258.98	0.7664	0.3767
10	16	130	350	21.919	2.375	377.83	228.05	0.4022	0.1942
30	16	130	350	53.536	5.312	455.64	261.21	0.8293	0.4643
20	12	130	350	37.106	3.952	417.57	181.28	0.6055	0.2827
20	20	130	350	37.106	4.164	417.57	287.59	0.6055	0.3231
20	16	110	350	37.106	7.283	417.57	307.55	0.6055	0.3965
20	16	150	350	37.106	2.546	417.57	177.53	0.6055	0.2579
20	16	130	250	37.106	1.456	417.57	253.86	0.6055	0.3343
20	16	130	450	37.106	6.122	417.57	269.31	0.6055	0.3063
20	16	130	350	37.106	4.026	417.57	245.84	0.6055	0.3121
20	16	130	350	37.106	3.986	417.57	238.59	0.6055	0.3018
20	16	130	350	37.106	4.052	417.57	238.51	0.6055	0.3182
20	16	130	350	37.106	4.104	417.57	242.88	0.6055	0.3055
20	16	130	350	37.106	4.132	417.57	240.73	0.6055	0.3092
20	16	130	350	37.106	4.181	417.57	248.15	0.6055	0.3188

Data are mean values. SL, Soya level; FM, feed moisture; BR, barrel temperature; SS, screw speed.

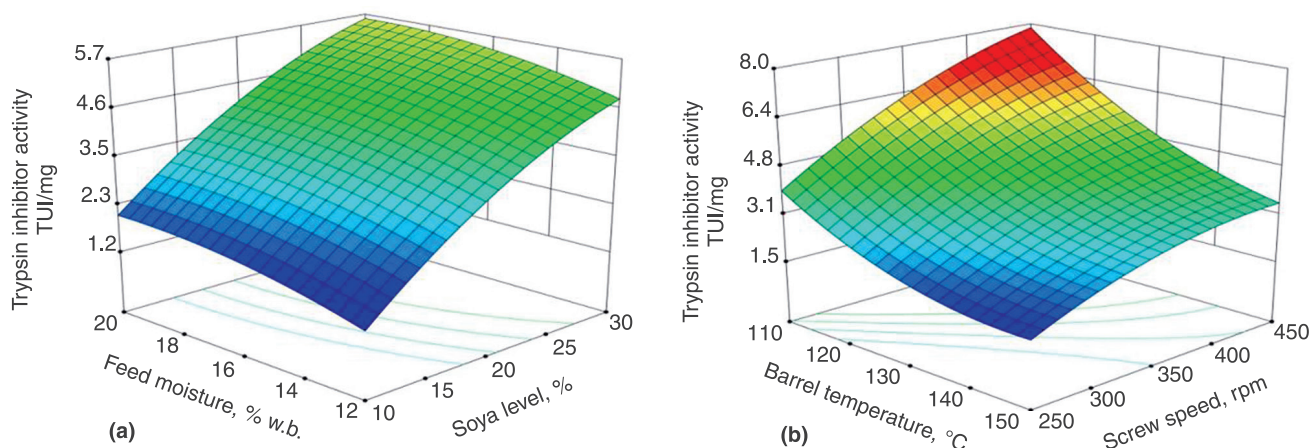


Fig 1 Response surface plots for trypsin inhibitor activity as a function of (a) feed moisture and soya level, (b) barrel temperature and screw speed, while other variables are at center point.

Table 3 ANOVA and regression coefficients of the second order polynomial models of nutritional and anti-nutritional components

Parameters	TIA	Phytic acid	Tannin
Intercept	4.080	242.450	0.311
X_1	0.881***	9.245***	0.055***
X_2	0.202	22.319***	0.007
X_3	-0.836***	-22.359***	-0.031***
X_4	0.732***	5.458**	-0.005
$X_1 X_2$	-0.002	2.218	-0.001
$X_1 X_3$	0.062	-5.159	0.001
$X_1 X_4$	0.187	-2.293	-0.005
$X_2 X_3$	-0.295*	0.744	0.004
$X_2 X_4$	-0.184	-2.082	-0.005
$X_3 X_4$	-0.124	1.496	-0.003
X_1^2	-0.108	1.177	0.011**
X_2^2	-0.054	-1.372	0.004
X_3^2	0.160	0.654	0.010*
X_4^2	-0.122	5.416*	0.009
ANOVA			
R^2	0.91	0.92	0.90
Model F-value	11.039***	14.169***	10.424***
CV, %	14.81	4.83	7.94

X_1 , Coded soya level; X_2 , coded feed moisture; X_3 , coded barrel temperature; X_4 , coded screw speed; TIA, trypsin inhibitor activity.* Significant at 10% ($P < 0.1$); ** Significant at 5% ($P < 0.05$); *** Significant at 1% ($P < 0.01$).

Incorporation of soybean in the feed significantly ($P < 0.01$) (Table 3) increased TIA of the final product (Fig 1a). This could be attributed to high amount of TIA of soybean (166.7 TUI/mg) in comparison to sorghum (5.44 TUI/mg). This observation is in agreement with the findings of Iwe (1998) who reported that the addition of soybean to sweet potato considerably increased its trypsin inhibitor level. In the current study, the maximum value

of TIA among all the possible runs was far lower than the minimum value of the same observed in the feed blends. This explains an efficient reduction in the TIA following the extrusion processing. Increasing FM 12-20% w.b. had a slight positive effect on the TIA of the final product (Fig 1a). High moisture results in increased viscosity thereby reducing shear forces inside the barrel consequently reducing heat generation and results in lower ANFs. Nwabueze (2007) also reported a non significant positive influence of moisture content on TIA of Agrican bread fruit corn-soya mixtures. Exposure of food material to high temperature and pressure in combination of shear forces reduces TIA (Andersson 1984). In this study, higher process temperature and reduced residence time had a negative and positive influence, respectively, on the TIA of the final product (Fig 1b). The observed influence of BT and SS on TIA could be attributed to their effect on total heat, shear force and residence time during extrusion. Present findings are in parallel with those of Clarke and Wiseman (2007) wherein TIA of full fat soybean was found to have reduced with an increase in extrusion temperature. Soybean contains two types of trypsin inhibitors, the Kunitz trypsin inhibitor and the Bowman-Birk inhibitor which are heat stable due to presence of disulfide bridges in these inhibitors (Liu 1997, Van Der Ven *et al.* 2005). Heat treatment alone takes longer duration for inactivation of these compounds, whereas pressure in combination with elevated temperatures favors rapid destruction of these heat stable compounds (Kwok *et al.* 2002, Van Der Ven *et al.* 2005). Comparatively higher levels of TIA in the product processed at lower temperature and high screw speed could be attributed insufficient shear force and residence time and temperature combinations for effective destruction of inhibitors. When the screw speed increases, feed material flows readily out of the extruder thereby reducing the residence time in other words treatment duration consequently minimizing the inactivation of trypsin inhibitor.

Phytic acid

Phytic acid (myo-inositol-hexaphosphate) is considered

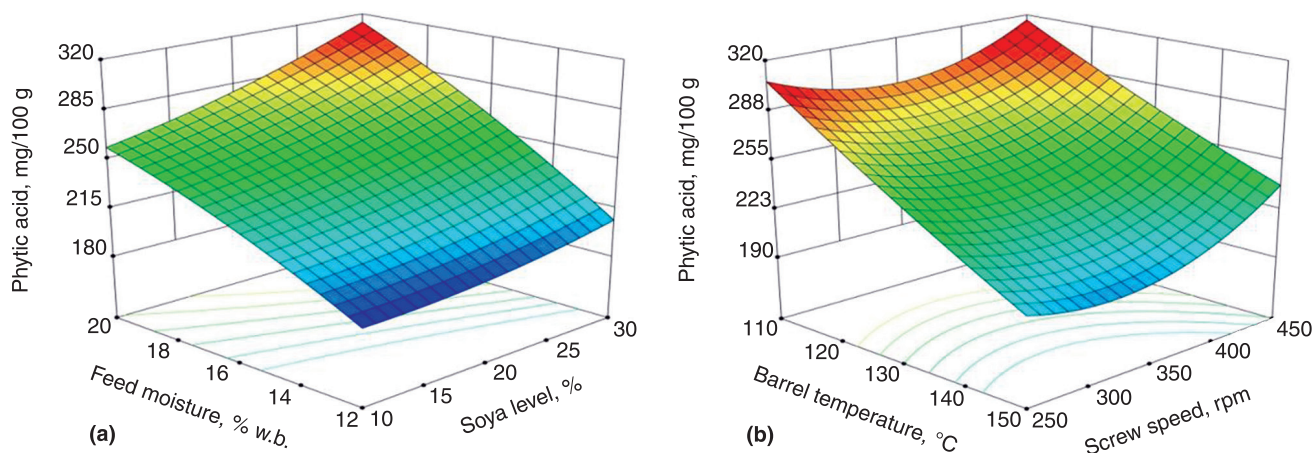


Fig 2 Response surface plots for phytic acid as a function of (a) feed moisture and soya level, (b) barrel temperature and screw speed, while other variables are at center point.

as an antinutrient because of its ability to bind with proteins, cations and endogenous enzymes such as chemotrypsin and trypsin consequently lowering the bio-availability of nutrients and affecting the digestibility of food (Rickard and Thompson 1997, Ainsworth *et al.* 2007). Extrusion was observed to reduce the phytic acid content of sorghum-soya blends up to 57.48 per cent as reflected by its lower content in the final product compared to that in the feed material (Table 2). Phytic acid in the feed formulation varied from 377.83 to 455.64 mg/100g, whereas in the extrudates it ranged between 177.53 and 307.55 mg/100g. Maximum reduction was observed when the feed containing 20 per cent of soybean flour, with 16 per cent FM was extruded at 150°C and 350 rpm; on the other hand, 16 per cent FM, 110°C and of 350 rpm resulted in minimum reduction in phytic acid content. Regression coefficients imply that all the variables had a significant influence on phytic acid content of sorghum-soya extrudates. None of the interaction terms were found to have a considerable influence on phytic acid content of the extrudate. SS was observed to have significant ($p < 0.1$) positive quadratic effect. The developed model to predict phytic acid could explain 92 per cent of the variation and showed minimum coefficient of variation (4.83%) with significant F-value (Table 3). The reduction in phytic acid content followed by extrusion might be due to the hydrolyzation of inositol into lower molecular weight forms like penta-, tetra and triphosphates. Fragmentation of phytates and formation of insoluble complexes with other components could be another reason for phytic acid reduction in the extruded product (Kumar *et al.* 1978, El Maki *et al.* 2007). The current observation is in agreement with that documented by Alonso *et al.* (2000a) and Alex *et al.* (2009).

The influence of independent variables on phytic acid content of sorghum-soya extrudates is depicted in Fig 2. It is evident from Fig 2a that SL and FM had a positive influence on phytic acid content. Soybean was found to have more phytates than sorghum (Table 1), thus the significant positive influence of proportion of soybean on phytic acid in extrudates is justified. Fig 2a portrays

that with an increase in feed moisture content there is a proportional increase in phytic acid content; in other words, moisture content arrested the destruction of phytic acid during extrusion. This could be due to intensified thermo-mechanical conditions at lower moisture content levels that might have proved to be more effective in reducing this antinutritional component. Increasing BT resulted in increased destruction of phytic acid which could be due to thermal destruction of phytates resulting in lower molecular weight components (Nwabueze 2007, Alex *et al.* 2009). Elevated temperature, 120-170°C, facilitates thermal breakdown of the compound myoinositol 1,2,3,4,5,6-hexakis dihydrogen phosphate consequently reducing phytic acid in the final product (Nwabueze 2007). Influence of SS was observed to be minimal as compared to the effect of BT and FM (Fig 2). However, significant quadratic positive influence of screw speed (Table 3) suggests that at high SS reduction effect was minimized thereby increasing the phytic acid content in the final product. This might be attributed to reduced duration of thermo-mechanical cooking process. Following these findings it can be concluded that thermal energy, at given moisture, dominates mechanical energy in reducing the phytic acid content of feed material during the extrusion cooking. Nwabueze (2007) observed similar effect of temperature during extrusion of African bread fruit with corn-soya mixtures. El-Hady *et al.* (2003) also reported that higher extrusion temperature was more effective in reducing phytic acid in faba beans, chickpeas, peas and kidney beans.

Tannin

A comparison of the tannin content in the raw material and in the extrudates of sorghum-soya mixtures clearly indicates that extrusion processing successfully in reduced the tannin component (Table 2). The tannin content of the sorghum-soya extrudates varied between 0.194 and 0.464 per cent, whereas in the feed it ranged from 0.402 to 0.829 per cent reflecting a considerable reduction to the extent of 34.52 to 57.41 per cent. Though, extrusion processing could

not completely eliminate this antinutritional component, a considerable reduction was observed depending upon the process variables. Minimum reduction was observed when the feed consisted of 20 per cent soybean with 16 per cent FM, extruded at 110°C BT with a SS of 350 rpm. The highest reduction in tannin content was up to 57.41 per cent noticed when the blend contained 20 per cent SL and 16 per cent FM was extruded at 150°C and 350 rpm. Regression coefficients indicate that SL and BT are the significant variables affecting the tannin content (Table 3). None of the interactive terms were significant and among the quadratic terms only SL and BT had showed positive influence at 10 and 5 per cent level of significance, respectively. The model predicting the tannin content explained 90% of the variation and showed a lower coefficient of variation (Table 3). Negative influence of extrusion on tannin content may be attributed to the thermal degradation of molecules, changes in their chemical reactivity or the formation of insoluble complexes during the course of extrusion (Kataria *et al.* 1989, Alonso *et al.* 2000 a). Though the extent of change in tannin content was less, the role of extrusion in declining the tannin content of sorghum-soya blends was confirmed and the results also emphasized the importance of process variables. Such decrease in the tannin content following extrusion cooking has been highlighted by other researchers (Mukhopadhyay *et al.* 2007, Alonso *et al.* 2000a).

Tannin content in the sorghum-soya extrudates was more influenced by the proportion of soybean in the feed composition than by any other variables. Since, white sorghum was used in the study, it is observed to have negligible tannin content as compared to soybean (Table 1). Therefore, any increase in soya level significantly ($P < 0.01$) increased the tannin content in the final product. The effect intensified at higher soya levels as evident from the significant and positive quadratic term (Table 3). Present observation is in line with those reported by Calvin *et al.* (2005) wherein tannin content increased with an increased proportion of finger millet in extruded maize–finger millet blend. FM was observed to have a non-significant effect on tannin content. This could be due to the range of FM (12-20% w.b.) used in the study which might be sufficient for hydrolysis of the molecules (Alonso *et al.* 2001). However, few studies, involving higher moisture content, reported negative influence at 10 per cent level of significance during extrusion process. BT was observed to have a significant ($P < 0.01$) influence in reducing the tannin content of sorghum-soya mixture. This could be due to thermal destruction and or reduced extractability of tannin molecules by increased polymerization or change in the chemical reactivity (Alonso *et al.* 2001). Similar effect was noticed by El-Hady and Habiba (2003) during extrusion of four different kinds of legumes. Present data is also in line with the findings of Rahul and Uday (2016) wherein increasing extrusion temperature considerably reduced the tannin content in lentil splits. Figure 3b reflects that with an increase in SS there was a slight decrease in tannin content

but with further increase in screw speed tannin content increased. This effect is also evident from the regression coefficients (Table 3). The study revealed that maximum reduction in tannin content can be achieved at high BT and medium SS.

Conclusion

Incorporation of whole soybean flour increases ANFs in sorghum-soya blends; however extrusion processing significantly reduced these components in the final product. Change of extrusion conditions, viz. feed composition, feed moisture content, barrel temperature, and screw speed, affected the ANFs of final product. Results from the quadratic models showed that although interaction and quadratic effects did not have a greater effect on dependent variables, linear effects were obvious. Maximum reduction in trypsin inhibitor activity, phytic acid and tannin content could be achieved at medium process temperatures (140-150°) and lower screw speeds (300-350 rpm). Extrusion cooking as a heat treatment effectively destroys ANFs of sorghum-soya blends. Since as high as 90% trypsin inhibitor activity inactivation was achieved in the extrudates, it can be concluded that the extrusion process improved the availability of food nutrients.

ACKNOWLEDGMENT

This research was a part of doctoral thesis and the authors acknowledge the research facilities and financial support provided from the Post Graduate School, Indian Agricultural Research Institute (IARI), New Delhi during the course of this study.

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