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Effect of extrusion variables (Moisture Content, Barrel Temperature and Screw Speed) on the reduction of aflatoxins in Maize

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Abstract

Naturally contaminated maize was extrusion processed for reduction of aflatoxins. The contaminated grains collected from different locations were screened for aflatoxins by Pressure Mini Column. The grains were quantified for aflatoxin content (AFB_1 and AFB_2) by thin layer chromatography. Maize grains were extrusion processed by co-rotating twin-screw extruder using different extrusion variables; feed moisture (24, 27 and 30%), barrel temperature (140, 170 and 200°C) and screw speed (300, 400 and 500 rpm). Response surface methodology was applied to optimize the processing conditions and to assess the effect of extrusion variables on the reduction of aflatoxins. The maximum reduction of AFB_1 (75%) and AFB_2 (72.5%) in maize with initial concentration of 78 ppb was obtained at 24% moisture, 171 °C temperature and 369 rpm screw speed. Extrusion processed maize with final aflatoxin concentration of 19 ppb was utilized for preparation of porridge and chapatti with good consumer acceptance.

Keywords: Aflatoxins, extrusion processing, maize, *chapatti*, porridge.

Introduction

Aflatoxin contamination of agricultural commodities and stored grains has long been a major problem throughout the world in tropical and sub-tropical regions, where climate and poor storage conditions are conducive for fungal growth and aflatoxin production. Aflatoxins are secondary fungal metabolites produced by toxigenic species of *Aspergillus* mainly *Aspergillus flavus* and *Aspergillus parasiticus*. About 18 different types of aflatoxins were recognized among which the major ones are aflatoxin B_1 (AFB_1), B_2 (AFB_2), G_1 (AFG_1) and G_2 (AFG_2) distinguished by their fluorescent colour blue or green under ultraviolet light[1]. Aflatoxin B_1 is the most toxic form and possesses hepatotoxic, teratogenic and mutagenic properties, causing damage like hemorrhage, edema, immunosuppression and hepatic carcinoma[2]. Naturally occurring aflatoxins and AFB_1 are classified by the International Agency for Research on Cancer as group 1 carcinogens[3].

Aflatoxins are common contaminants of cereal grains. Maize is the third most important crop in the world after wheat and rice. Unfortunately, aflatoxigenic fungi invade maize during its development and harvest in the field as well as during the process of transport and storage. The estimated value of maize lost to aflatoxin is $225 million per year, out of the $932 million due to all the mycotoxins in the United States[4]. The Food and Agriculture Organization (FAO) estimates that many basic foods could be contaminated with mycotoxin producing fungi, resulting in 1000 million metric tons loss of foodstuffs each year[1]. Based on the regulations of U.S. Food and Drug Administration (FDA), foods are not permitted to exceed the action level of 20 ppb (ng g^{-1}) for total aflatoxins[5].

The increasing number of reports pertaining to the presence of aflatoxins in food dictates the need for practical and economical detoxification procedures. Ideally, such detoxification procedures should not only reduce the concentration of toxins below regulatory limits, but also prevent production of toxic degradation products without any reduction in the nutritional value of the treated commodities. Various detoxification procedures (physical, chemical and biological) were employed in food processing to minimize the presence of aflatoxins in food chain. Extrusion cooking has received great attention as one of the most effectiveand fastest growing food processing operation in reducing aflatoxin levels. Extrusion processing is a high temperature-short time operation in which raw food materials are thermo-mechanically cooked in a screw-barrel assembly by a combination of moisture, pressure and temperature in order to be mechanically sheared and shaped[6]. High temperature, pressure and severe shear forces result in gelatinization of starch, denaturation of proteins, and inactivation of food enzymes and reduction of microbial counts[7].

The present study was undertaken to study the effect of extrusion processing on reduction of AFB_1 and AFB_2 as affected by the moisture content, barrel temperature and screw speed variables as well as to utilize the extrusion processed maize in product preparation (porridge and *chapatti*) and its quality assessment.

Material and Methods

Screening of maize grains for aflatoxins: Raw maize grains collected from different locations (godowns, mills, grain merchants) of Punjab, India were screened for aflatoxin analysis by Pressure Mini Column[8]. The presence of aflatoxins is observed as a compact blue fluorescent band under long wave UV light (365nm).

Experimental design: Response Surface Methodology (RSM) with Box Behnken design was adopted as a 3-factorial design with moisture content (24, 27 and 30%), barrel temperature (140, 170 and 200°C) and screw speed (300, 400 and 500 rpm) as factors affecting the reduction of AFB_1 and AFB_2. The design included 17 experimental runs of three extrusion variables and for each experimental run; 500 g of maize was extruded (table-1). Extrusion processing was achieved with Clextral BC 21 co-rotating intermeshing twin screw extruder (Clextral, Firminy, France). The extruded samples were cooled and stored in plastic bags until analyzed for aflatoxins.

Table-1
Effect of extrusion conditions on product responses in maize

Extrusion Variables			% Reduction	
A: Moisture (%)	B: Temperature (°C)	C: Screw speed (rpm)	AFB_1	AFB_2
24	140	400	52.56	51.26
24	170	300	73.88	71.13
24	170	500	70.26	69.68
24	200	400	79.18	74.66
27	140	300	52.92	51.98
27	140	500	53.85	54.84
27	200	500	70.36	68.7
27	200	300	76.21	75.89
27	170	400	71.11	70.13
27	170	400	72.28	72.17
27	170	400	73.64	73.54
27	170	400	74.45	74.13
27	170	400	75.08	74.33
30	140	400	50.05	48.64
30	170	500	55.77	57.21
30	170	300	58.97	59.35
30	200	400	56.41	57.42

Extraction and quantification of aflatoxins: The extraction of aflatoxins was achieved by Romer's extraction procedure[9]. The extracted aflatoxins were trapped in chloroform and spotted on silica gel plates along with standards (AFB_1 and AFB_2) for quantitation by thin layer chromatography (TLC).The developed TLC plates were examined under UV light and aflatoxin concentration was estimated by comparison of the fluorescent intensity of the spots at the Rf (retention factor) value of the toxin in the sample extracts with those of the appropriate aflatoxin standards chromatographed on the same plate[10]. AFB_1 and AFB_2 concentration in maize before and after extrusion was used to calculate the percentage aflatoxin reduction for all the experimental treatments.

Statistical analysis: The linear, quadratic and interactive effect of extrusion variables on the reduction of aflatoxins was represented in the form of polynomial equation:

$$Y = \beta_o + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum_{i=1}^{3} \beta_{ij} X_i X_j \quad (1)$$

Where Y is the percent aflatoxin reduction; Xi are the variables where X_1 - Moisture content (A), X_2 – Barrel temperature (B), X_3 - Screw speed (C); β_0 is regression coefficient of intercept term; β_i, β_{ii} and β_{ij} are linear, quadratic and interaction regression coefficients, respectively (equation-1).

To evaluate the effect of moisture, temperature and screw speed on aflatoxin reduction and to determine the regression coefficients and the statistical significance for the model, analysis of variance (ANOVA) was carried out. The coefficient of determination (R^2) and lack of fit test was calculated to ascertain the adequacy of the model. The data obtained from the products (*chapatti*) prepared from extrusion processed maize was analyzed statistically using techniques of ANOVA and least significant difference (LSD) as the test for significance. AFB_1 and AFB_2 contents before and after extrusion processing were determined to calculate the aflatoxin reduction percentage for all the experimental treatments.

Product preparation: The best extrusion processing conditions were selected on the basis of maximum aflatoxin reduction and quality of the extrudate. Maize processed at these optimized conditions was used for preparation of instant porridge and *chapatti*. These products were assessed for various quality characteristics and overall acceptability.

Instant porridge Preparation: 25 g instant porridge (extruded maize) was boiled with 100 ml milk. Sugar @ 6% was added during the boiling and porridge was cooked till doneness. Porridge was served hot for sensory evaluation.

Chapatti Preparation: Maize extrudates were grinded to produce flour of uniform particle size using cemotac mill (Foss, Hoganas, Sweden) having setting at No. 1. The extrusion processed maize flour was added to commercial maize flour at 10, 20 and 30 per cent level. The required quantity of water was mixed manually to obtain dough of suitable consistency. The dough was rounded and kept for half an hour at room temperature. The dough was divided into four equal parts and moulded into circular *chapattis* of 15.0 cm in diameter with rolling pin and board[11]. Traditional home baking procedure was followed to bake *chapattis* on iron plate (Tawa). *Chapattis* were cooled and comparative evaluation was done using following criteria:

Characteristic	Score grade
Dough handling	Non-sticky, Sticky, Slightly sticky, Full sticky
Puffing of *chapatti*	Full, Partial, Nil

Quality assessment: Water Absorption Index (WAI): The method outlined by Anderson[12] was used to determine water absorption index of porridge. WAI measures the volume occupied by the granule or starch polymer after swelling in excess of water. The ground extrudates were suspended in distilled water at room temperature (34°C) for 30 min, gently stirred during this period, and then centrifuged at 3000g for 15 min. The supernatant liquid was poured carefully into tared evaporating dish. The remaining gel was weighed and water absorption index was calculated as the grams of gel obtained per gram of solid.

$$\text{Water absorption index } (g\ g^{-1}) = \frac{\text{Weight of sediment (g)}}{\text{Weight of dry solids (g)}}$$

Water Solubility Index (WSI): Water solubility index of porridge determines the amount of free polysaccharides or polysaccharides released from the granule on addition of excess water. The WSI was the weight of dry solids in the supernatant from the water absorption index test described above[12] expressed as percentage of the original weight of the sample.

$$\text{Water solubility index } (\%) = \frac{\text{Weight of dissolved solid in supernatant (g)}}{\text{Weight of dry solids (g)}} \times 100$$

Bulk Density: Bulk density of porridge was calculated by measuring the weight of known volume of sample. Samples were poured into a graduated cylinder, gently tapped ten times and filled to 500 ml. Results were expressed as g ml^{-1}.

$$\text{Bulk Density } (g\ ml^{-1}) = \frac{\text{Weight of extrudates (g)}}{\text{Volume of extrudates (ml)}}$$

Texture: Textural quality of porridge and *chapatti* was examined by using a TA-XT2i Texture Analyser. For porridge, the compression probe (50 mm diameter, aluminium cylinder) was applied to measure the force required to break the sample which indicates hardness. Hardness of porridge was expressed in Newton (N) keeping 1.0 mm s^{-1} pre-test speed, 2.0 mm s^{-1} test speed, 10.0 mm s^{-1} post-test speed and 5 mm distance[13] as testing conditions. For *chapatti,* the force required to cut *chapatti* was evaluated by using texture analyser[14]. Strips measuring 4 cm × 2cm were cut from each *chapatti*. One strip at a time was placed on the center of the sample holder and the blade was allowed to cut the *chapatti* strip. The force (N) required to cut *chapatti* strip into two pieces was recorded. The speed was maintained at 1.70 mm s^{-1}.

Viscosity: Viscosity of porridge (cP) was measured using Brookfield viscometer at room temperature with spindle No. 2 at a speed of 60 rpm.

Colour Analysis: Colour analysis of the prepared products (porridge and *chapatti*) was done by using Hunter Lab colorimeter. Readings were displayed as L* (100 for white; 0 for black), a* (+, red; -, green) and b* (+, yellow; -, blue) colour parameters according to the CIELAB system of colour measurement.

Sensory Evaluation: Sensory evaluation by panel of judges was conducted for sensory attributes (color, texture, taste, flavor and overall acceptability) for the prepared products using 9-point hedonic scale[15].

Results and Discussion

The aflatoxin content in the maize samples ranged between 78 and 140 ppb. Keeping in view the aflatoxins limit of 20 ppb for foods and feed ingredients set by FDA, maize grains with aflatoxin concentration of 78 ppb was selected for extrusion processing. The effect of extrusion processing variables (moisture content, barrel temperature and screw speed) on percent reduction in AFB_1 and AFB_2 in maize was evaluated and the data obtained on aflatoxin reduction from the experiment is summarized in table-1. The experimental data fit the polynomial equation well. This is shown by R^2 value of 0.98 each for AFB_1 and AFB_2. The models showed non-significant lack of fit with *p* value of 0.45 and 0.59 (more than 0.05) for AFB_1 and AFB_2, respectively (table-2). The quadratic model obtained from regression analysis for reduction in AFB_1 and AFB_2 was developed in following equation:

Table-2
ANOVA and model statistic for product responses in maize

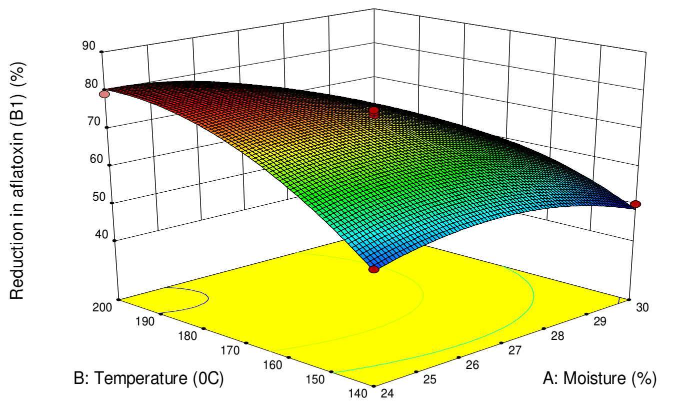
Model	% Reduction	
	AFB_1	AFB_2
F value	67.51	61.51
Mean	65.70	65.00
SD	1.64	1.63
C V	2.50	2.50
R^2	0.98	0.98
Adjusted R^2	0.97	0.97
Predicted R^2	0.91	0.92
Adequate precision	25.33	22.84
p-value for lack of fit	0.45	0.59

SD- Standard deviation, CV- Coefficient of variation, R^2- Coefficient of determination

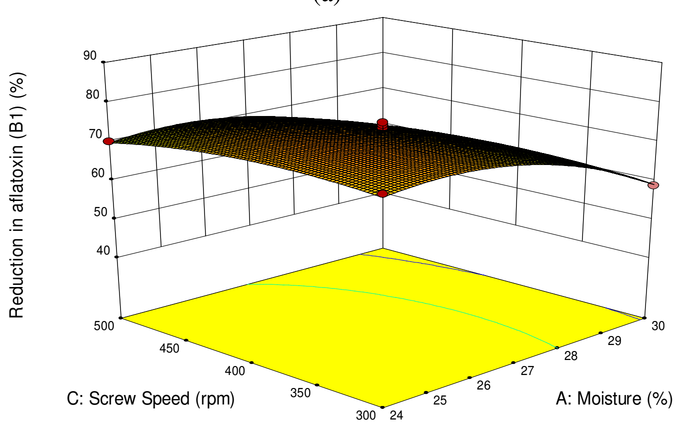
$$AFB_1 \text{ reduction} = 73.31 - 6.83A + 9.10B - 1.47C - 5.07AB + 0.10AC - 1.69BC - 6.19A^2 - 7.57B^2 - 2.40C^2 \quad (2)$$

$$AFB_2 \text{ reduction} = 72.86 - 5.51A + 8.74B - 0.99C - 3.66AB - 0.17AC - 2.51BC - 6.69A^2 - 8.18B^2 - 1.83C^2 \quad (3)$$

As found in the regression equations 2 and 3, temperature had a significant ($p<0.05$) positive linear effect on AFB_1 and AFB_2 reduction in maize contrary to feed moisture and screw speed which had significant ($p<0.05$) negative linear effect on aflatoxin reduction. The surface plots of maize were produced where three variables (moisture content, barrel temperature and screw speed) interacted with each other to show the effect of the extrusion variables on AFB_1 and AFB_2 reduction (figure-1 and figure-2).



(a)



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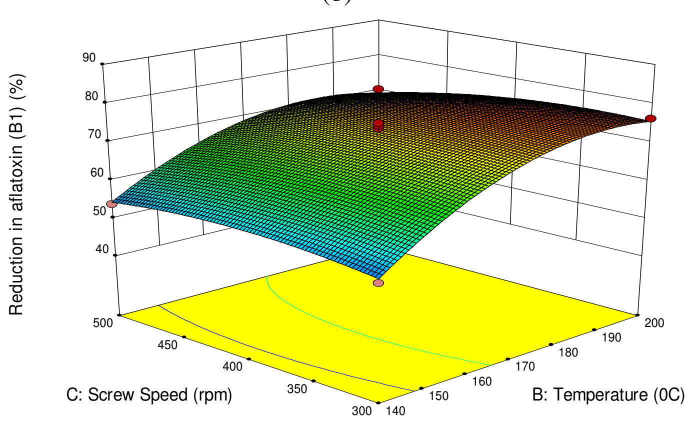
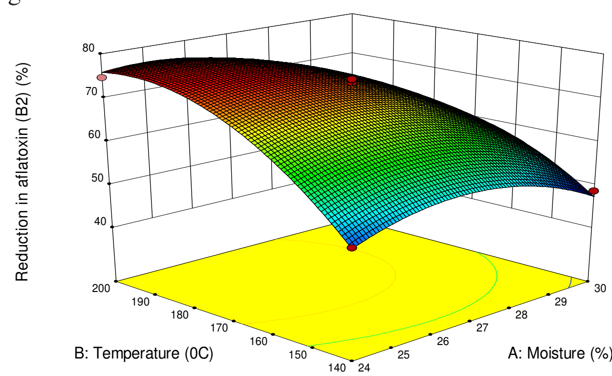


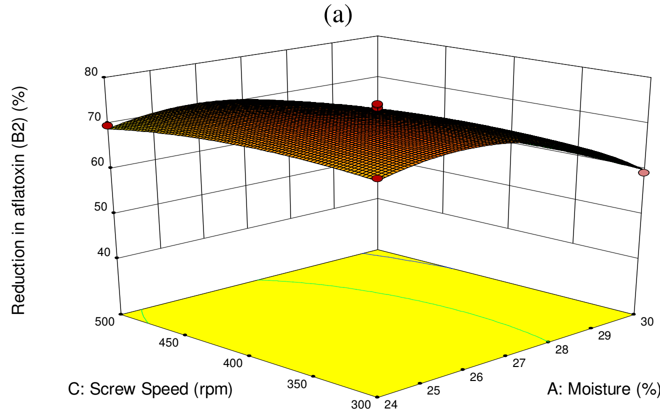
(c)

Figure-1
Effect of extrusion variables on percent reduction in aflatoxin B_1 (AFB_1) in maize

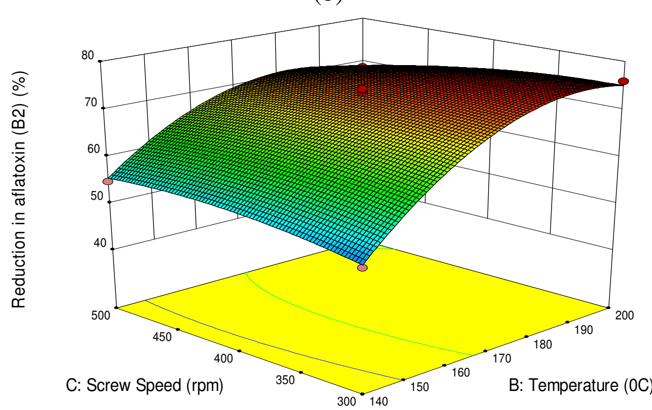
Figure-1a and Figure-2a shows the effect of moisture and temperature on aflatoxin reduction in AFB_1 and AFB_2, respectively. These figures demonstrated that an increase in temperature from 140 to 200°C is accompanied by higher AFB_1 and AFB_2 reductions. This was in agreement with the study which reported 50 and 75% reduction in AFB_1 and AFG_1 in moderately and highly contaminated samples of rice flour, respectively upon extrusion processing at 140-200°C[16]. Similar results were also given in a study that reported more than 95% reduction in deoxynivalenol levels in maize flour by extrusion cooking at 150-180°C[17].



(a)



(b)



(c)

Figure-2
Effect of extrusion variables on percent reduction in aflatoxin B_2 (AFB_2) in maize

The interaction of moisture and screw speed as shown in figure-1b and figure-2b for reduction of AFB_1 and AFB_2, respectively demonstrated indirect relation of feed moisture and aflatoxin degradation. At highest moisture content of 30%, AFB_1 and AFB_2 reduction was below 60% whereas at low feed moisture (24%), maximum aflatoxin reduction (79% in AFB_1 and 75% in AFB_2) was noticed. In contrast, some studies concluded that the increased feed moisture facilitated degradation of aflatoxin[18].

The interactive effect of temperature and screw speed on AFB_1 and AFB_2 reduction is presented in figure-1c and figure-2c, respectively. The regression analysis model showed significant negative effect of screw speed on aflatoxin reduction. At constant feed moisture of 27%, highest reduction in AFB_1 (76.21%) and AFB_2 (75.89%) was seen at screw speed of 300 rpm than at 500 rpm (70.36% reduction in AFB_1 and 68.7% in AFB_2). Greater degradation of aflatoxins was obtained at lower screw speed, as the time the feed spent in the extruder was more. The same conclusion was reached in another research where 17-86% ochratoxin reduction achieved at higher residence time of 70 sec[19].

The aflatoxin concentration quantified by thin layer chromatography in raw maize was 78 ppb for AFB_1 and 77.5 ppb for AFB_2. Extrusion processing reduced levels of aflatoxins (AFB_1 and AFB_2) by 50 to 75%. The data obtained from the present study was in accordance with the findings that showed 50 to 95% reduction in aflatoxins in artificially contaminated rice meal which was extrusion cooked at 140-200°C temperature, 24-30% moisture and 30-70s residence time[20].The optimization of extrusion processing was done to predict the levels of extrusion variables which result in the maximum reduction of aflatoxins. The optimum conditions (moisture, barrel temperature and screw speed) for maximum aflatoxin reduction in maize with an initial aflatoxin concentration of 78 ppb were predicted to be 24%, 171°C and 369 rpm resulting in 75.11 per cent reduction in AFB_1 and 72.66 per cent reduction in AFB_2. Further, no significant difference was found between the experimental and the predicted values ($p<0.05$). The chromatograms of TLC indicated that the aflatoxinconcentration left in maize after extrusion was 19 ppb.

Product preparation: Instant porridge: Porridge was prepared from the extrudates obtained by extrusion processing of maize at optimized conditions (figure-3). Various parameters like water absorption index (WAI), water solubility index (WSI), bulk density, colour, texture and sensory score of the porridge were assessed to determine its quality and overall acceptability (table-3). The overall acceptability score for porridge was 8 that indicate good consumer acceptance of the product.

Chapatti: The extrusion processed maize was added to commercial maize flour at 10, 20 and 30% level for *chapatti* preparation (figure-4). The quality evaluation of *chapattis* was mentioned in table-4. Except for 30% level (slightly sticky), all others showed non-sticky behavior during dough development. All *Chapattis* exhibited full puffing, however, *chapattis* from 30% level puffed partial. Cutting force (N) as depicted in table-4 represents the texture of the *chapattis* and it stimulates the biting action of the human teeth on *chapattis*[21]. Cutting force increased at addition of processed flour at higher levels. The hunter L* a* and b* values illustrated that lightness of *chapattis* decreased with increased level of addition of processed flour (table-4). Hue angle depicted same pattern for the colour of *chapattis*. Increasing the level of extrusion processed flour from 10 to 30% resulted in decreased yellowness (b* value) and increased redness (a*) of *chapattis*. The pooled scores (out of 9.0) of *chapatti* reproduced in table-4 revealed that *chapattis* prepared at 10 and 20 per cent level of addition of processed flour to base flour were in highly acceptable range with overall acceptability of 8.02 and 7.3, respectively. The contributing attributes were colour, flavour and texture.

Table-3
Quality characteristics of instant porridge

Quality characteristics	Porridge
WAI ($g\ g^{-1}$)	5.45±0.13
WSI (%)	13.01±0.12
Bulk density ($g\ ml^{-1}$)	0.41±0.05
Hardness (N)	14.1± 1.35
Viscosity (cP)	283.57± 2.09
Colour L*	45.9± 1.05
a*	3.5± 1.04
b*	19.5± 2.36
Hue angle (°)	79.82± 0.74
Overall acceptability	8.0± 0.44

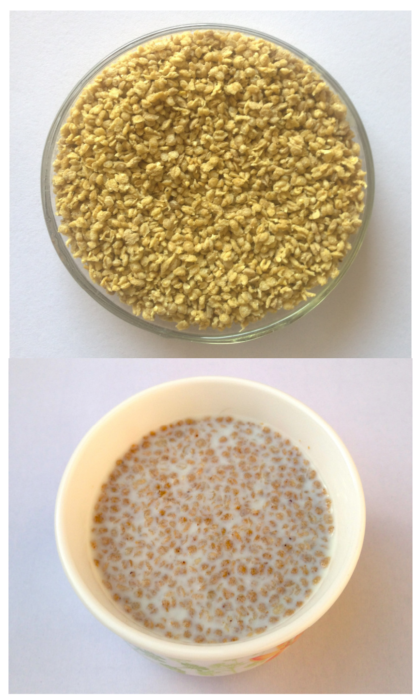


Figure-3
Instant Porridge

Conclusion

The results of the study concluded that extrusion processing is an effective detoxifying method for aflatoxin contaminated maize. The optimum processing conditions (24% moisture, 171°C barrel temperatureand 369 rpm screw speed rpm) produced by numerical optimization in maize resulted in 75 and 72% reduction in AFB_1 and AFB_2, respectively. The residual level of aflatoxin (19 ppb) in maize after extrusion processing was reasonably secure to be considered safe for human consumption, as these levels were less than the maximum tolerable limit of 20 ppb set by FDA. Further, the extrusion processed maize can be potentially utilized to produce economically low cost high quality products (porridge and *chapatti*). The study highlighted that extrusion processing could be considered a newer direction for detoxification of aflatoxins in cereal grains and its proficient use.

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Table-4
Quality characteristics and sensory score of maize *chapatti*

Quality characteristics	Control	10%	20%	30%	LSD (p<0.05)
Dough handling	NS	NS	NS	SS	-
Puffing	Full	Full	Partial	Partial	-
Cutting force (N)	5.1±0.26	3.7±0.44	3.9±0.36	4.2±0.35	0.67
Colour L*	37.5±1.49	36.1±0.66	29.6±0.79	23.3±1.21	2.05
a*	1.1±0.07	1.4±0.2	1.5±0.2	1.9±0.2	0.34
b*	29.5±2.19	28.7±0.78	18.7±0.75	13.9±1.73	2.82
Hue angle (°)	87.86±1.76	87.21±1.54	85.41±1.60	82.62±2.65	3.65
Overall acceptability	8.3±0.26	8.02±0.33	7.3±0.26	6.9±0.40	0.60

NS- Not sticky, SS- Slightly sticky

(a) (b) (c)

Figure-4
***Chapatti* from extrusion processed maize: (a) 10% level, (b) 20% level, (c) 30% level**

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