EFFECTOF EXTRUSION CONDITIONS ON AFLATOXIN CONTENT OF CORN–PEANUT FLAKES

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ABSTRACT

The study was conducted to evaluate the influence of extrusion operating conditions on the reduction of total and individual aflatoxins (B_1 and B_2) in corn-peanut flakes. Aflatoxin - contaminated corn and peanut were converted into flour separately and mixed in a proportion of 70 % corn and 30 % fully defattened peanut flour (FDPF).

Corn-peanut extrudates and flakes were then produced at various combinations of feed moisture contents (17% and 21%,) barrel temperatures (140°C, 160°C and 180°C) and screw speeds (150 rpm,200 rpm and 250 rpm)in a double screw extruder under factorial design. The process parameters were recorded during the experiment.

The total and individual aflatoxins were measured using a high-performance liquid chromatography (HPLC) before and after extrusion. The collected data were then subjected to analysis of variance by SPSS statistical software. The results showed that the total aflatoxin was cut by more than 95% (from 583 ppb to 30 ppb) at constant (150) rpm while other parameters varying.

Similarly, aflatoxinsB_1 and B_2 decreased by nearly 93.8% (240 to 15 ppm) and 83.5% (23 to 3.8 ppb), respectively ($P<0.05$) at different combinations. Of all the conditions, 17 %, 160 ^{0}C and 200 rpm reduced both B_1 (240 to 15ppb) and B_2 (23 to 7.5 ppb) at the same time.

Corn and peanut are crops partly used for making Injera and edible oil in Ethiopia, respectively. Corn ranks next to Teff in area coverage and first in total production in Ethiopia. The inclusion of peanut in the production of corn flakes improves the protein content. Corn and peanut are excellent substrates for the growth of mycotoxins (example aflatoxin). Aflatoxins are toxic metabolites which can be observed on food stuffs or animal feeds. It is estimated that at least 25 % of the world's cereal production is contaminated with mycotoxins [1]. They are the causes of human illness and death [2, 3].

Among all the mycotoxins, aflatoxin generates the greatest loss and the highest management cost due to its extremely high toxicity. Aflatoxins have been found to contaminate a wide variety of important agricultural products worldwide such as peanuts, corn, rice, cotton seed and wheat [4]. Although aflatoxins are not automatically produced whenever grain becomes moldy, the risk of contamination is greater in damaged corn than in those with little mold. Aflatoxins are harmful to humans and livestock, and are considered carcinogenic (cancer causing). The prime condition for the fungi to produce toxin is warm season in a period of drought [5]. Frequently encountered individual aflatoxins include B_1, B_2, G_1, G_2, M_1 and M_2.

Keywords: Aflatoxin, Corn-peanut flakes, Extrusion cooking, HPLC

INTRODUCTION

The survey conducted by Fufa and Urga[6] showed that ground red pepper and *shiro* (peas, chickpeas and grass peas) and spices were found contaminated with Aflatoxin B_1. Aflatoxin B_1, the toxin on which major resources have been expended, has long been linked to liver cancer, yet its other effects, such as immune suppression and growth faltering previously observed in veterinary studies, are only now being investigated and characterized in human populations [7]. Quantitative methods of analysis for most mycotoxins use immune-affinity cleaning up with high performance liquid chromatography (HPLC) separation in combination with UV and/or fluorescence detection [2].

Extrusion cooking technologies are used to manufacture many forms of food stuff from cereals and other ingredients. They have many advantages, including versatility, high productivity, low cost, and the ability to produce unique product shapes and high product quality [8, 9].

The range of products includes breakfast cereals, flakes, breading crumbs and animal feeds. Ingredients, such as corn flour and grits, wheat flour and other food components, are passed through an extrusion cooker (single or twin) under pressure, mechanical shearing stresses and elevated temperature, and expand rapidly as they are forced through the outlet die [10,7].

Efficient material transport due to the forward conveying mechanism in twin screw extrudates is a major advantage [11]. The major operating conditions of extrusion cooking are feed moisture (MC), barrel temperature (T) and screw speed (SS). Product quality can vary considerably depending on the extruder type, screw configuration, feed moisture, and temperature profile in the barrel section, screw speed and feed rate. The combination of these parameters affects the nutritional, physico-chemical and safety of foods.

Small variations in processing conditions affect process parameters like die pressure (Pd), torque (τ), throughput rate (Q) and specific mechanical energy (SME) as well as product quality [12]. Temperatures inside the barrel of an extruder can range from 100 to 200°C, and moisture content can vary from 13 to 30% depending on the characteristics of the required end-product[7, 10].

Two types of commercial products (rod shaped and puffed extrudates) were used by researchers to be references for identifying optimum variables. For cereal crops, the optimum feed moisture was found to be between 25 % and 35 %, and that of the defattened peanut flour was 18-20 %. The prime objective of this work was to study the reduction pattern of total and individual aflatoxins (B_1&$B_{2)}$ in corn-peanut flakes by varying the operating conditions of extrusion cooking, namely moisture content, barrel temperature and screw speed.

MATERIALS AND METHODS

Materials and Sample Preparation

Corn (*Zea mayis* L.) and Peanut (*Arachis hypogaea* L.) varieties affected by fungus, *Aspergillus flavus* and *A. parasiticus*, were obtained from *Adiet* and *Pawe* research centers, respectively, in Ethiopia.

All the experimental materials were selected on the basis of their color. Yellow-green for corn and gray-green for peanut were used. Corn and peanut, contaminated with aflatoxin, were visually identified. The corn was milled by a small scale hammer mill (England Model No. 212/10E) at a sieve size of 100- 500 µm. Initially, the peanut was milled by a commercial laboratory grinder primarily for defattening. The milled peanut flour was fully defattened by Soxhlet (UK, Barn Stead Electro-thermal, Cat No. EME3 0500/CEB) using Hexane (analytical grade) to remove its high fat content.

Defattening is recommended to avoid backflow of extrudates in extruders**.** The fully defattened peanut flour (FDPF) was further milled at a sieve size of 100 - 125 µm. The final peanut and corn flours were then mixed with a proportion of30% to 70%, respectively as recommended by Suknark*et al*.[13]. The composite flour was sealed in plastic bags and stored till the extrusion test was conducted.

Setting the Extrusion Variables

The feed moistures, barrel temperatures and screw speeds were selected based on previous recommendation by Suknark, *et al*.[13] and Lazou [14], and preliminary tests were carried out before the actual experiment.

Temperatures greater or equal to 180°C were not used as the HPLC in the Food Complex was calibrated to use less temperature. The pump adjustment again limited the use of other feed moistures other than 17% and 21%. Indeed, the preliminary tests and previous works indicate that 180^0C can further decrease the aflatoxin content but the quality of the product is similar to that produced by 160°C.

Temperatures of the barrel and die block were measured by thermocouples inserted along the length of the extruder. The thermocouple in the die was protruded into the melt to measure product temperature. Extrusion was performed at preset temperatures of zone two and three of the extruder barrel sections. The barrel temperature was fixed in zone two between 70°C and 80°C. The barrel temperature of zone three, as indicated in Figure 1, was independent variable in the steady state and varied at 140°C and 160°C.

The moisture content of the material was adjusted by varying the water injection rate of the pump. Accordingly, water was injected in to the extruder at a point close to the material feed port. Both the feeder and the pump were calibrated prior to the extrusion cooking in order to avoid fluctuations during the operation. The pump was adjusted to give moisture contents of 17% and 21% in the mixes for a constant material feed rate of 7.3 kg/h by using hydration equation as suggested by Golob *et al*. [15]. Screw speed was set at 150,200 and 250 rpm. By so doing feed moisture, temperature and screw speed levels were chosen from pre-test experiments.

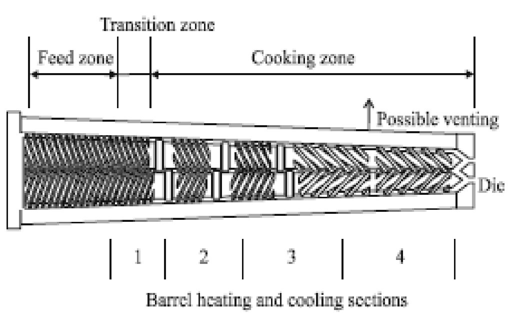

Fig. 1: Configuration of the Twin-screw Extruder

The Extrusion Process

Extrusion was performed on a pilot scale co-rotating twin screw food extruder (model Clextral, BC-21 N°124, Firminy, France). The barrel has a smooth 300 mm useful length and consists of three modules each 100 mm long fitted with 25 mm diameter screws. The temperatures of the last two modules are controlled by electrical heating and water cooling system. Each zone-temperature was controlled by a Eurotherm controller (Eurotherm ltd., UK). Twin screw volumetric feeder (typeKMV-KT20) delivered the raw material in to the extruder inlet. While operating, water at ambient temperature was injected in the extruder via an inlet port by a positive displacement pump (DKM-Clextral, France). The end of the extruder was caped with a die plate, which held a die having circular openings. The die hole has 9 mm length and its diameterreducedfrom5 mm to 2 mm.

The samples were extruded as straight rope (rode) for a time interval of 10 sec [16] in which100cm long extrudate emerges from the die. Extruded samples were collected when the process parameters reached steady state. The state was considered acceptable when there was no visible drift in torque and die pressure [17]. The extruded products were then placed on a table and allowed to cool for 30 min at 20°C to measure the weight, length and diameter [18] to evaluate the expansion ratio and specific length of the products. Except for samples taken for moisture analysis, they all were collected and sealed in plastic bags for 24 h at ambient conditions. Finally, samples were stored in a room at 20°C and aflatoxin determination was done seven weeks after the extrusion.

Determination of Extrusion Process Parameters

The torque, screw speed, pressure and processing temperatures were continuously displayed on the control panel of the extruder from which readings were recorded. Processing temperatures were measured immediately before the product leaves the die by a thermocouple connected to the control panel. Throughput rates were determined for the wet basis weights of individually timed extruded products coming out as ropes. Specific mechanical energy, SME (kJ/kg) transferred to the product was calculated from the following relationship, Eq. (1), [19]:

$$SME = \frac{\tau\ \omega}{Q} \quad (1)$$

Where: τ= Net torque (Nm)

ω = Angular screw speed (rad/s), where rad/s $= \frac{rpm\ x 2\pi}{60}$

Q = Throughput rate (kg/s)

SS= screw speed (rpm)

Extraction and Determination of Aflatoxin

Aflatoxin was extracted from a sample containing 70% Corn and 30% fully defattened peanut flour (FDPF) as described in AOAC [20]-Official Method 74, 81-88.Accordingly, two grams of NaCl were added to 20 g of ground sample, and the weighed sample was subject to extraction by methanol/water (4/1, v/v) and 50 ml n-hexane.

High speed blender jar (KOHINOOR MIXERS AMBALA CAANTT) having Ultraturrax was used to mix chemicals and weighed samples for 3 min and the extract was passed through a plaited filter. Single-use syringes (10 ml, PP) with filter disks of 0.2 µm were used to re-filter the extract. 7ml of purified extract was added to 43ml Phosphate buffer saline (PBS buffer pH 7.2).

Diluted purified extract of 50ml passed through the Aflaclean column (AflaCLEAN™) with a flow rate of 2 ml/min. The aflatoxins were retained on the immune-affinity column and were eluted by methanol. All the samples were drained until the column became empty. The column was washed by 10ml of distilled water and the residual water was removed by a vacuum gas. The aflatoxin was eluated in the column with 1ml of methanol twice, and the first addition of methanol (1ml) was acted on the gel for 5 min.

Finally, the eluate was measured directly by HPLC [21]. Identifying and quantifying$B_1$and B_2were based on retention times compared with those of standards.

Experimental Design and Data Analysis

A mixed 2x2x3 full factorial experimental design was used to study the effect of extrusion operating conditions on total and individual aflatoxins. The extrusion conditions were feed moisture, barrel temperature and screw speed each varied at 2, 2 and 3 levels, respectively. Thus, this is a 2 (17 and 21 % feed moistures) x 2 (140 and 160°Cbarrel temperatures) x3(150, 200 and 250 rpm screw speeds) full factorial design treatment for a mixture of corn and peanut flour. Peanut was fully defattened before extrusion. SPSS 16software for windows was used for the data analysis, and one way ANOVA (analysis of variance) was employed for comparison of means. Significance was accepted at 0.05 level of probability ($p<0.05$).Mean separation was performed by LSD (least significant difference) for multiple comparisons of means. All measurements were performed in triplicate.

RESULTS AND DISCUSSION

Temperature and screw speed significantly affected aflatoxin content ($P<0.05$).When barrel temperature increased from 140°C to 160°C, total aflatoxin was reduced from 95.25 ppb to 45.00 ppb. Obviously, a further increase in temperature (180°C) can reduce the aflatoxin. Increasing the feed moisture, however, couldn’t produce a similar effect.

There was no statistically significant difference between the total aflatoxin contents registered during extrusion at the feed moistures of 17 and 21 % ($p>0.05$). However, the aflatoxin content showed a decline with a decrease in screw speed. This effect can be partly explained that it is due to the shorter residence time of the extrudate in the extruder which is caused by the increase in the screw speed, thus preventing the reduction of aflatoxin.

In all cases, the most acceptable value was observed during extrusion cooking at a screw speed of 150 rpm with varying feed moistures and barrel temperatures resulting in more than 95 % (from 583 to 30 ppb) aflatoxin reduction. The average aflatoxin dropping pattern, when one of the operating parameters remained constant and the others varying, is shown in Table 1 below:

Table 1: Effect of Extrusion Variables on Total Aflatoxin of Corn Flour and FDPF Extrudate

Parameter	N	Aflatoxin (ppb) (mean ± SD)
		Before Extrusion=583 ppb
Barrel Temperature (^oC)		
140	6	95.2500±4.97^a
160	6	45.0000±4.65^b
Feed Moisture (%)		
17	6	62.7500±5.07^c
21	6	77.5000±5.88^c
Screw Speed (rpm)		
150	4	30.0000±2.12^d
200	4	52.8750±4.53^e
250	4	127.5000±2.87^f

$^{a\text{-}f}$ Means with the same superscript letters within a column are not significantly different (P > 0.05)
SD (Standard deviation)
n- Number of observations
ppb = parts per billion
FDPF-fully defattened peanut flour

In the subsequent experiment, extrusion at various conditions resulted in nearly 93.8% and83.5 % of individual aflatoxin reduction of B_1 (15 ppb) and B_2 (3.80 ppb), respectively. The combined effect of 160°C, 17 % and 200rpm produced tremendous aflatoxin decline of both B_1(from 240 to 15 ppb) and B_2(from 23 to 7.5 ppb)at the same time as shown in Table 2.

Table 2: Reduction of Aflatoxins (B_1 and B_2) by Extrusion Cooking

	Aflatoxin Type*	
	B_1(ppb)	B_2 (ppb)
Before Extrusion	240	23
After Extrusion (BT, MC, SS)		
140, 17, 150	30.00	15.00
140, 17, 200	76.50	3.80
140, 17, 250	135.00	7.50
140, 21, 150	60.00	20.00
140, 21, 200	105.00	15.00
140, 21, 250,	165.00	18.80
160, 17, 150	15.00	9.25
160, 17, 200	15.00	7.50
160, 17, 250	105.00	7.50
160, 21, 150	15.00	12.35

160,21,200	15.00	7.63
160,21,250	105.00	4.89

ppb= parts per billion

BT - barrel temperature (^{0}C), MC - feed moisture content (%) and SS - screw speed (rpm)
*G_1=245ppb, G_2=75ppb before extrusion, but the HPLC has not shown the aflatoxin reduction pattern

The initial values of B_1 andB_2inthe mixture of corn and fully defattened peanut flour before extrusion were 240 ppb and 23 ppb, respectively. After extrusion, the lowest readings to each corresponding aflatoxin type were 15 ppb and 3.8 ppb, respectively. Similar shrinkage was reported by Hameed [3] and Martinez and Monsalve [22] when studying the stability of aflatoxin in naturally contaminated corn meal.

Irrespective of moisture content, the most effective interaction to reduce aflatoxin B_1in the current study was to extrude at a barrel temperature of 160°C and a screw speed of 150 rpm or 200 rpm. The highest B_1 (165 ppb) reading in HPLC was recorded for extrudates produced by 140°C barrel temperature, 21 % moisture content and250 rpm screw speed. This finding could be explained by the fact that aflatoxin is heat-stable when processed at lower temperatures [23].As a result, lower temperatures are presumably less effective in decreasing aflatoxin concentration than higher ones. Even though the temperatures used by Buser and Abbas [24] were not the same as the ones set up in this experiment, their results showed that the lowest decontamination rate was reached when extruding aflatoxin-contaminated cottonseed at 104°C. In the present investigation, the most effective operating conditionfor reducing$B_2$to 3.8 ppbwas140°C, 17% MC and 200 rpm.

Moisture content, however, had no any significant influence on the totalaflatoxin content of extrudates (Table 1) and individual aflatoxin B_1. This result is in agreement with the result of Ryu*et al*.[25]. Controversial conclusions have also been reached by different authors regarding the effect of moisture content on the stability of mycotoxins. Some studies [7, 25] provided strong evidence that moisture was not a significant factor affecting the stability of aflatoxins. By contrast, some researchers observed that reduction of some individual aflatoxins was significantly dependent on moisture content [26, 27] as in B_2. In fact, several studies concluded that the highest moisture level did not lead to the highest decontamination rate [27, 28].

In general, there was a lessening of aflatoxin content with a decrease in screw speed. An increase in screw speed causes shortage of residence time for extrudates in the extruder. Similarly, an increase in residence time at high temperature means exposing the aflatoxin for long period of time before the extrudate leaves the extruder causing high reduction of aflatoxin at lowest screw speed. As the residence time is a function of screw speed, this finding is in agreement with that of the other studies [29]in which it was stated that slow screw speeds, thus long residence times, led to higher aflatoxin reductions.

The results revealed an enormous effect of residence time on reducing aflatoxin contamination. The greater the residence times the highest the reduction of B_1 and B_2. The specific torque, throughput rate and mechanical energy increased with an increase in screw speed when feed moisture and barrel temperature were kept constant.

The influence of the change in extrusion operating conditions on process system parameters is shown in Table 3.

Table 3: Effects of Extrusion Operating Conditions on Process System Parameters

Operating Conditions			Extruder System Parameters			
BT(°C)	MC (%)	SS (rpm)	Pd (bar)	τ (Nm)	Q (g/min)	SME (kJ/kg)
140	17	150	6.0	9.0	50.57	167.73
140	17	200	7.0	11.0	81.04	170.57
140	17	250	9.0	19.0	118.45	251.96
140	21	150	7.0	7.0	60.74	108.61
140	21	200	11.0	15.0	73.41	256.77
140	21	250	10.0	16.0	129.41	194.21
160	17	150	6.0	8.0	49.21	153.21
160	17	200	9.0	14.0	93.95	187.25
160	17	250	6.0	14.0	110.91	198.27
160	21	150	7.0	7.0	62.10	106.23
160	21	200	10.0	16.0	121.06	166.08
160	21	250	9.0	20.0	126.16	249.01

BT = barrel temperature (°C), MC = feed moisture content (%), SS = screw speed (rpm), Pd = die pressure (bar), τ= torque (Nm), Q= throughput rate (g/min) and SME= specific mechanical energy (kJ/kg)

The extent to which the dough undergoes transformation can be correlated firstly to the flour granulometry and next to extrusion conditions like thermal effect due to average dough temperature, mechanical effect evaluated by shear rate (dependent upon the specific mechanical energy) and residence time within the extruder [30]. The calculated values of SME with respect to the variation in feed moisture and temperature were found to be comparable with those reported earlier for rice-potato grits using the same extruder [31].

Die pressure was significantly ($P<0.05$) dependent on feed moisture which is in agreement with the report of Akdogan [32]. The die pressured rop was affected by the moisture content in the feed stream. Generally, an increase in the feed moisture caused an increase in the die pressure. This is due to the nature and rheological properties of the raw materials.

CONCLUSION

Aflatoxin contamination is the cause of disease in humans and animals, and has aggravated loss in quality and quantity of corn and peanut in Ethiopia [33, 34].The collective action of the three extrusion variables namely: barrel temperature, feed moisture and screw speed induced appreciable reduction of total and individual aflatoxin content. Accordingly, the present study showed a reduction up to 30 ppb of the total aflatoxin which is above 95 % of the initial amount.

The current result is encouraging when compared with the maximum allowable levels of aflatoxin (20 ppb) in food products (except milk) by the USFDA [35]. This indicates that extrusion becomes more effective when the initial aflatoxin contamination level is lower. Significant aflatoxin reduction of B_1 (15 ppm) and B_2 (7.5 ppm) was observed during the production of corn-peanut flakes at 160°C, 17 % MC and 200 rpm.

The experiment further revealed that FDPF is a good source of protein to fortify corn-peanut flakes. Aflatoxin B_1 was found to be reduced by a high percentage (up to 93.8%) at highest temperature, low feed moisture and screw speed with respect to the initial contamination level. Generally, extrusion is seen to reduce aflatoxin. However, food processing like thermal application may cause structural change resulting in masked mycotoxins [36].

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