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Evaluation of aflatoxins in the oil and cake obtained from cotton seeds

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Abstract

A study was conducted to evaluate aflatoxins content in the extracted oil, cake and cotton seeds. Cotton seeds, oil and cake were obtained from the local oil mill based on mechanical extraction. aflatoxins were determined in the cotton seeds, extracted oil and cake by IAC-HPLC-FLD. Amount of AFB1, AFG1, AFB2, and AFG2 in cotton seeds was found to be 155 µg kg^{-1}, 95 µg kg^{-1}, 100 µg $kg^{-1}$and 58 µg kg^{-1}while in oil and cake was found to be 35 µg kg^{-1}, 15 µg kg^{-1}, 10 µg $kg^{-1}$and 8 µg kg^{-1}; 120 µg $kg^{-1}$and 180 µg kg^{-1}, 90 µg $kg^{-1}$and 50 µg kg^{-1}, respectively. Total amount of each aflatoxin transferred into the oil and cake was found in the range of 10-22.5% and 76.5-90% with respect to the original content in cotton seeds. The results of this study will help edible oil industries to select the method which provide best quality edible oil with less toxins contamination.

Key words: Aflatoxins, Mechanical extraction, cottons seeds, edible oil, cake, IAC-HPLC-FLD

Introduction

The demand of edible oils and fats has been amplified due to their diverse applications and increased population(Keogh-Brown et al., 2019). Oil processing industries are continuously trying to produce good quality products which should be free from natural contaminants like aflatoxins(Wakelyn & Wan, 2006). Therefore, selection of superior oilseeds free from aflatoxins and efficient extraction are the top priorities of oilseed processing industries(Bhat & Reddy, 2017).Mechanical and solvent extractions are commonly applied to produce crude edible oil; but limited information is available on the aflatoxins residues in the resulting products(Bordin, Sawada, da Costa Rodrigues, da Fonseca, & Oliveira, 2014)

Many oilseeds especially cotton seeds with greater moisture contents (>10%) are often contaminated with aflatoxins(Bordin et al., 2014). Also, the presence of some amino acids, trace elements and sugars enhance the toxin production(Cuero, Ouellet, Yu, & Mogongwa, 2003; Feng & Leonard, 1998; Yu, Mohawed, Bhatnagar, & Cleveland, 2003).It has been reported that tryptophan inhibits aflatoxins while tyrosine and unsaturated fatty acids (linoleic acid and trilinolein) assists growth of *A. Flavus(Bircan, 2006; De Luca, Passi, Fabbri, & Fanelli, 1995; Fanelli & Fabbri, 1989; Jayashree & Subramanyam, 1999; Yu et al., 2007)*. Aflatoxins contamination of cotton seeds and resulting cake in Pakistan has been already reported(Aman Ullah, Durrani, Ijaz, & Javeed, 2016; Shar, Pirkash, Shar, Sherazi, & Mahesar, 2020; Yunus et al., 2020) but the information regarding the aflatoxins transfer to cotton seed edible oil is limited(Bordin et al., 2014)

Aflatoxins in the edible oils from different countries have been reported and extent of contamination depends upon the nature of the oleaginous material(Idris, Mariod, Elnour, & Mohamed, 2010; Mahoney & Molyneux, 2010; Nabizadeh et al., 2018; G. S. Shephard, 2018; Yang et al., 2011) (Idris et al., 2010; Mahoney & Molyneux, 2010; Nabizadeh et al., 2018; G. Shephard, 2018; Yang et al., 2011).From contaminated oilseeds, aflatoxins are transferred into oil through the extraction process(Bordin et al., 2014). The carryover of the aflatoxins into the oil and cake generally depends upon the level of aflatoxins in raw material as well as methods and parameters of the extraction(Wakelyn & Wan, 2006).Therefore, it is important to generate an exact data of aflatoxins transferred to edible oil thorough the most commonly used pressure expeller method in Pakistan.

Materials and method

Chemicals and apparatus

Trifluroacetic acid and aflatoxins standards (AFB1, AFB2, AFG1, and AFG2) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Acetonitrile, n-hexane, and methanol were of HPLC grade and obtained from Fisher Scientific (UK). For purification of aflatoxins, Immuno-affinity columns (AflaTest) (VICAM, Watertown, MA) were utilized. Liquid chromatography coupled with florescence detector (HPLC, Hitachi, Tokyo Japan) was used for quantification. ODS-3 column (150×4.6 mm with particle size of 5 µm; GL Science Inc. Japan) was used for separation of aflatoxins.

Sample collection

From local oil Mill, 10 kg of cotton seed, 5 kg of cake and 0.5 L of the crude cotton seed oil were obtained during the month of November 2020. Samples of seeds and cakes were collected in polyethylene bags while cotton seed oil in the plastic bottle and kept in the refrigerator at 4 ^{o}C till analysis.

Quantification of Aflatoxins by using LC-FLD

Aflatoxins Extractions from cake and cotton seeds

Aflatoxins were extracted from cotton seeds and cakes as per reported procedure (Feizy, Beheshti, & Asadi, 2012). 50 g of homogenized sample (cotton seeds or cake) were mixed with 5 g of sodium chloride, 200 mL of 80% methanol and 100 mL of hexane were added in blender jar and mixed till 5 min. The mixture was allowed to centrifuge for five 5 min. 20 mL of supernatant were mixed with 100 mL of phosphate buffer and filtered through the glass microfiber filter (Whatman, Inc., Clifton, NJ, USA). Filtrate obtained was subjected to immuno-affinity column and allowed to flow at the rate of 1 drop/s. Finally, aflatoxins retained in the column were eluted with 2 mL of methanol and 2 mL of water.

Extraction of aflatoxins from oil

20 g of oil was mixed with 5 g of NaOH, 125 mL of 60% methanol were added to blender and blended for 1 min. The mixture was filtered and 20 mL of filtrate was diluted with 20 mL of deionised water. 5 mL of filtered diluted extracts subjected to immuno-affinity column at the rate of 1 drop/s. Finally, aflatoxins retained on a column were eluted with 2 mL of methanol and 2 mL water.

Derivatization

Derivatization of aflatoxins was done as described in our previous study (Shar et al., 2020). Briefly dried extract of afaltoxins was mixed with 100 µL of the TFA and 300 µL of n-hexane and vortexed for 30 s. The mixture was allowed to stand for 15 min. Finally, 900 µL of acetonitrile:water (1:9) was added to the vial, and vortexed for 30 s. 20 µL of the derivatised product (bottom layer) was collected for further separation and quantification of aflatoxins.

Aflatoxins determination by HPLC-FLD

Aflatoxins were separated and quantified by HPLC-FLD in an isocratic mode. Mobile phase used was water–methanol–acetonitrile (60:20:20, v/v/v) at the flow rate of 1.0 mL min^{-1}. 20 µL of derivatised sample was injected into inertsil ODS-3 (150×4.6 mm, 5 µm, particle size; GL Science Inc. Japan) and detected by using fluorescence detector with excitation and emission wavelength 365 and 445 nm, respectively

Performance of HPLC-FLD Method

Linearity, sensitivity, precision and accuracy of optimized method were evaluated by using the external standard addition method. Six point calibrations was developed over the concentration range of 2, 4, 6, 8, 10 and 12 µg L^{-1} for AFB1 and AFG1 and 0.5, 1, 1.5, 2, 2.5 and 3 µg L^{-1} for AFB2 and AFG2 for checking the linearity of method. Regression analysis was done by plotting peak area versus concentration of standards and represented as coefficient of determination (R^2).

Limit of detection and limit of quantification are important parameters and were determined by using the statistical formulas as given below

$$LOD = 3 \times \frac{\sigma}{Slope} \quad \text{..... (1)}$$

$$LOQ = 10 \times \frac{\sigma}{Slope} \quad \text{..... (2)}$$

For checking the accuracy, recovery of the method, was determined by external standard addition method. For determination of recovery a control sample having known amount of aflatoxins was spiked at 5 µg kg^{-1}for AFB1 and AFG1 and 2.5 µg kg^{-1}for AFB2 and AFG2. The spiked samples were extracted and analysed according to the protocol as described procedure. The actual spiked and measured concentrations by HPLC-FLD method were compared and the percent recovery was calculated using following equation

$$Recovery = \frac{RecoverdAFConcentration}{SpikedAFConcentration} \times 100 \quad \text{...(III)}$$

Statistical analysis

All analyses were carried in triplicate. The data was statistically evaluated and standard deviations, variance, and correlation coefficient were determined by using SPSS (Statistics 22, 2012, IBM, Armonk, NY).

Results and discussion

Method performance characteristics

Inter laboratory validation of IAC-HPLC-FLD was done by using the external standard addition method by spiking with known amount of aflatoxins. Method performance characteristics such as recovery, Limit of detection (LOD), Limit of quantification (LOQ), repeatability and reproducibility were determined and presented in (**Table 1)**

Table 1

The recoveries of aflatoxins B1 and G1 was determined and found in the range of 86-92% at the spiking level of 5 µg kg^{-1}. The recovery values obtained are considered as acceptable according to Association of official analytical chemists (AOAC) and Codex Alimentarius guidelines. According to AOAC guidelines, acceptable recovery at the spiking level 10 µg kg^{-1} must be in between the range of 70–125% (AOAC International. 2002) while according to Codex Alimentarius guidelines range of acceptable recovery is 60–120% at a level of 1–10 µg kg^{-1} (Alimentarius, 1995; Horwitz, 2002; Trucksess et al., 2008). Therefore, recoveries obtained for all aflatoxins spiked at level of 5 and 2.5 µg kg^{-1}were found to be in the acceptable range. Coefficients of variation found to be low, ranging from 2% to 4%, whereas repeatability for spiked samples was upright (10%) in all the cases (Table 1).

Derivatised mixture of four Aflatoxins standards were separated on ODS-3 column and chromatogram of standards is shown (Fig.1)

Fig. 1

Transference of Aflatoxins into oil

The validated method applied for the determination of aflatoxins in cotton seeds, crude oil and cotton seed cake obtained through the pressing method and aflatoxins transferred into the oil was calculated as a percentage and presented in (**Table 2)**

Table 2

AFB1 transferred into the oil was noted, 35 µg $kg^{-1}$and residual concentration in CSC 120 µg $kg^{-1}$with net amount transferred into oil from cotton seeds 22.5%. AFG1 in crude oil was 15 µg $kg^{-1}$and residual in CSC was 80 µg $kg^{-1}$with transferred rate 15.7%. AFG2 in oil was 8 µg $kg^{-1}$and residual in CSC 50 µg $kg^{-1}$with transferred rate 13.7%. AFB2 was found in oil was 10 µg kg^{-1}cotton seed cake 90 µg $kg^{-1}$with transferred rate of 10.0%. The highest rates of transference were noted for AFB1 followed by AFG1. The order of transference in oil were found as follows AFB1>AFG1>AFG2>AFB2. The difference in the transferable amount of aflatoxins depends upon the polarity of the toxins and nature of oil producing seeds (Mahoney & Molyneux, 2010). The more polarity of the oil has more capability to dissolve the toxins. The variable composition of oils from different sources may affect the solubility of the toxin. A study conducted in by (Basappa & Murthy, 1977) showed that 85% of aflatoxins in peanuts remained in meal after the pressing process and only 15% were transferred to the oil, which lower than the amount reported in this study. This difference in transference rate is due to difference in raw materials used. According to another study (Mahjoub & Bullerman, 1990) levels of transfer of aflatoxin ranged between 18 to 47% in the olive oil extracted by pressing, which is greater than our study which may be due to reason that olive has more unsaturated fats and greater solubility. (Abalaka & Elegbede, 1982) evaluated the aflatoxin contamination in the industrial processed of extracted peanuts and cotton oils and reported that about 10–20% of aflatoxins were transferred to the crude oil. (Parker & Melnick, 1966) studied the aflatoxin contamination in the solvent (hexane and chloroform) and mechanical extracted (pressing) peanut and corn oils and defatted meal. They reported that aflatoxins were transferred to the defatted meal and oil with different level. The use of mechanical pressing for oil extraction is carried out for many oilseeds (such as flaxseed, soybean, canola, cotton, sunflower and corn etc.) to obtain high quality oil. Present study provides comprehensive results about the transference of each aflatoxin from cotton seeds to the oil through the mechanical pressing.

In Pakistan cotton seeds mostly used for the production of premium cotton seed cake which is obtained through the mechanical pressing and little attention is paid to the its use for oil production despite the fact that it contains oil which possesses better nutritive value. The little use of the cotton seeds for oil production is attributed to the presence of aflatoxins and gossypol. The contamination of cotton seed cake with aflatoxins has been already reported from Pakistan (Akbar et al., 2020; Chohan, Awan, Ali, Iqbal, & Ijaz, 2016; Iqbal et al., 2016; Shar et al., 2020). But this is first study which provides insight into the exact amount of transference of aflatoxins into oil and cotton seed cake from the original contaminated cotton seeds.

Aflatoxins transferred into the oil remained in oil unless and until it is not properly processed. Many studies conducted worldwide which showed significant contamination of edible oil with aflatoxins as shown in (Table 3)

Table 3

Studies conducted by (Ji, Diao, Li, Zhang, & Dong, 2016; Parker & Melnick, 1966; Vaisali, Charanyaa, Belur, & Regupathi, 2015) showed that rigours refining eliminate aflatoxins, but presence of aflatoxins in oil showed that many oil mills are not interested in refining which can eliminate aflatoxins but want to keep the natural odour and flavour in the final product and minimize the cost of production.

Conclusion

Oilseeds are one of most important commodity and source of the human diet. Presence of aflatoxins in edible oil is serious issue for the food safety and a potential health risk. Also, presence of aflatoxins in the cake is very dangerous for the animals. There are chances for transfer of aflatoxin from cake to animal feed then from animal to human through the milk. The results of this study will help the edible oil industries to select best oilseed with no or less amount of aflatoxins that no or minimum transference of aflatoxins occur into the final products i.e. oil and cake.

Disclosure statement

No potential conflict of interest was reported by the authors

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FIGURE CAPTION

Figure 1. IAC-HPLC-FLD chromatograms of four aflatoxins standards

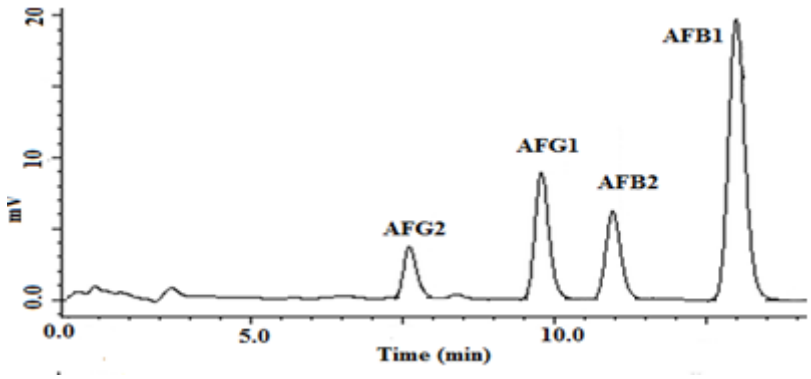

TABLE CAPTION

Table 1. Analytical figures of merits for the determination of aflatoxins by IAC-LC-FLD

Method	Aflatoxins	Matrix	LOD [a]	LOQ [b]	Recovery (%)[c]	RSD(%)[d]	R^2
IAC-LC-FLD	AFB1	C. Seeds	0.25	0.71	92	2.77	0.99
	AFG1	C. Seeds	0.16	0.47	87	3.16	0.98
	AFB2	C. Seeds	0.30	0.90	88	3.28	0.97
	AFG2	C. Seeds	0.20	0.58	86	3.22	0.98
	AFB1	CSC	0.30	0.91	90	2.99	0.99
	AFG1	CSC	0.28	0.88	86	3.36	0.98
	AFB2	CSC	0.19	0.57	87	3.40	0.97
	AFG2	CSC	0.10	0.30	84	3.21	0.99
	AFB1	Oil	0.40	1.15	88	3.12	0.97
	AFG1	Oil	0.21	0.60	86	3.30	0.96
	AFB2	Oil	0.42	1.10	89	3.40	0.98
	AFG2	Oil	0.30	0.90	87	3.01	0.97

[a] Limit of detection

[b] Limit of quantification

[c] Recoveries were determined by spiking at 5µg kg^{-1} to cotton seed and cake samples

[d] Precision expressed by relative standard deviation (RSD) was determined by multiple analysis of a spiked sample

Table 2. Trasnfer of aflatoxins from cotton seeds into the oil and cake by pressing method

Toxins	Transfer of aflatoxins into oil				
	Total afaltoxins in cotton seeds	Cotton seed Cake µg kg^{-1}	Transference into the cake %	Oil µg kg^{-1}	Ttransfernce into oil (%)
AFB1	155	120	76.5%	35	22.5%
AFG1	95	80	84.3%	15	15.7%
AFB2	100	90	90%	10	10.0%
AFG2	58	50	86.3%	8	13.7%

Table 3. Occurance of aflatoxins in edible oil

Country	Oil type	Number of samples analyzed	Positive samples (%)	Aflatoxins (µg kg^{-1})	Reference
China	Peanut oil	3,194 + 1,172	76.6	0-8000	(Mehan & Gowda, 1997)
China	Peanut oil and other vegetable oils	245	3.7	0.1–5.8	(HKSAR, 2001)
China	Peanut oil	30	66	100–52,500	(Leslie, Bandyopadhyay, & Visconti, 2008)
China	Sesame oil	100	NS	0-20,500	(F.-Q. Li, Li, Wang, & Luo, 2009)
China	Cereal and oil products[1]	76	14.5	1.1–35.0	(R. Li et al., 2014)
India	Mustard oil	100	33	0-2 55–87	(Sahay & Prasad, 1990)
India	Rice bran crude oil	20	75	NS	(Jayaraman & Kalyanasundaram, 2009)
	Rice bran refined oil	20	30	Up to 956; Average: 618	
	Defatted rice bran	30	66	7–144; Average: 33	
Senegal	Peanut oil	NS	85	Average: 40,000	(Leslie et al., 2008)
Senegal	Peanut oil	NS	85	Average: 40,000	(Leslie et al., 2008)
Sri Lanka	Coconut oil	11	57	Up to 240; Average: 186	(Samarajeewa, Gamage, & Arseculeratne, 1983)
Sudan	Sesame, peanut and sunflower oils	54	98.8	0.4–339.9; Average: 57.5	(Elzupir, Suliman, Ibrahim, Fadul, & Elhussein, 2010)

NS: not specified