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The Effectıveness of Aflatoxın-Contamınated Fıg Separatıon Process

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Abstract*: Aflatoxin (AF) is formed by Aspergillus flavus and Aspergillus parasiticus molds under appropriate conditions. During AF formation, mycotoxigenic molds can produce kojic acid as a metabolic residue. In the presence of kojic acid, dried fig fruits emit "Bright Greenish-Yellow Florescence (BGYF)" while viewed under long-wave (365nm) Ultraviolet (UV) light. Screening under UV light is a unique method commonly used for physically separating the AF-contaminated fruits from dried figs. In this study, the processes of AF-contaminated figs separation were analyzed in 5 different fig enterprises operating at Aydın province. A total of 160 samples were provided and AF analysis was performed. AF was identified in all 65 samples seperated as BGYF(+) , while 57 of which were above the total AF limit value of 10 ppb and a maximum value of 452,91 µg/kg was analyzed. AF was not detected in any of the final product figs separated as BGYF (-).*

Keywords— Aflatoxin, dried figs, BGYF (Bright Greenish-Yellow Florescence)

1. INTRODUCTION

Figs are predominantly grown in countries that are dominated by a Mediterranean climate. Although fig plantations are widespread in Turkey, 75% of dry fig production takes place in Aydın province. In Turkey, approximately 90% of the annual dried fig production (80 thousand tons, 2017) is exported (Arpaci et al., 2018).In terms of human health, dried fig has a special place with its high calorie value, minerals and nutrients, contains 303 kcal energy in 100 grams. (Turkomp, 2016). Moreover, fig fruits contain high levels of sugars such as glucose and fructose, amino acids like proline and asparagine and minerals like zinc. Fig fruits are ideal substrates for AF forming molds due to its high water activity and high sugar content. Dominant fungal flora of dried figs are Aspergillus niger, A. flavus, A. parasiticus, Fusarium spp. Penicillium spp.. (Reddy et al., 1971; Payne and Hagler, 1983; Luchese and Harrigan, 1993; Büyükşirin, 1993; Frazier and Westhoff, 1988).

To date, 18 different types of AFs have been identified. According to the fluorescent colours under UV irradiation or the relative mobility during separation by thin layer chromatography, AFs are divided into different sub-groups with four major species: AF B1, B2, G1, and G2 (Bhat et al., 2010). According to the report by the Food and Agricultural Organization (FAO), approximately 25% of the worldwide agricultural foodstuffs become contaminated by mycotoxins annually (Bhat et al., 2010). A wide variety of food is contaminated with mycotoxins, and the contamination occurs throughout the food chain, both during the pre- and postharvest periods (Bircan, 2009).AFs are considered to be a significant threat to human and animal health due to their highly toxic, carcinogenic, tetratogenic, hepatotoxic and mutagenic characteristics depending on the duration and level of exposure (Pariza, 1996; Chu, 1977).Some restrictions have been placed on the availability of AFs in food and feed that affect human and animal health and there are limits imposed by Turkey and the European Union (EU) countries for figs. According to the legislation of EU member states, total AF for dried figs is 10 µg/kg and AF for B1 is 6 µg/kg (Anonymous, 2012). In the Turkish Food Codex, AFB1 limit for dried figs is 8 µg / kg and the total AF limit is 10 µg / kg (Anonymous, 2011).

AFs are considered as unavoidable contaminants in foods and therefore human exposure cannot be completely prevented and therefore their levels should be kept as low as reasonably achievable. (Bircan and Koç, 2012). Many researches were carried out studies in order to detect and remove AFs from foods.

Fluorescence colors and relative chromatographic mobility are used in the separation of AFs (Betina, 1989). AFB1 and AFB2 emits blue fluorescence under ultraviolet light, while AFG1 and AFG2 emits green fluorescence (Derici, 1997; Vidyasagar, 1997; Busby, 1984). If a food exhibits a BGYF, this indicates that there are aflatoxigenic moulds, and this fluorescence under long-wave ultraviolet (LW-UV) light (365 nm) is probably an indicator for kojic acid, a metabolite of A. flavus and A. parasiticus or possibly AF itself (Kalkan et al., 2011; Kalkan et al., 2014).

Molds found in dried figs usually produce kojic acid along with AF. Kojic acid is a fluorescent compound and exhibits BGYF under ultraviolet (UV) light. In the presence of kojic acid, AF-containing figs emit greenish yellow and blue color while viewed under long-wave (365nm) UV light. BGYF method was applied to find solutions to the AF problem encountered in figs. Under UV light, BGYF (+) figs are regarded as AF-containing and BGYF (-) is considered as clean. (Steiner ve ark. 1988). Fig enterprises manually select figs that give positive BGYF to reduce the AF level and this method is seen as the most effective way. (Gençdağ et al, 2019).

The number of fruits contaminated with AF is not very high in the raw material dried figs which are obtained from fig orchards and brought to the enterprises. There fore AF is really not a common mycotoxin in dry figs. The problem seems to arise from mixing products from different orchards during purchasing before processing by the wholesalers (Heperkan et al, 2012). For this reason, the AF selection process of dried figs from wholesalers should be applied carefully (Özlüoymak and Güzel., 2018).

Due to the business culture and legal obligation to AF, fig enterprises necessarily implement the process of separating figs with AF. The screening of aflatoxinous figs is carried out by examining the figs under the UV lamp light at 356 nm wavelength under dark room conditions. Although there are no exact data determining the stages of the AF separation process, enterprises have made different arrangements depending on their production capacity, raw material cleanliness, work experience and personnel qualifications. Some enterprises implements only 1-step AF separation process, while some of them go up to 5-steps.. In this study, the effectiveness of screening and separation processes of AF-containing figs were investigated in 5 different enterprises operating at Aydın province. The effect of processing time and the number of stages of the process on the separation of figs with AF has been tried to be revealed in the unit amount (1 kg) of fig.

2. MATERIAL&METHOD

2.1 Sampling

Dried fig samples of Sarılop variety were obtained in 2016 from 5 exporting enterprises operating in Aydın province as the largest fig producer in Turkey. In order to protect the commercial secret information of the enterprises, "A, B, C, D, E" codes were given and batch quantities were selected between 4-5 tons in each enterprise. Samples were taken from every step of AF separation process from raw material to packaged final product with 5 repetitions and 3-4 kg for each repetition. Samples were taken separately from each step of the process as BGYF (+) and BGYF (-) under the UV lamp. The process that enterprises apply to separate AF and the sampling model is seen in Table 1. Some enterprises use AF separation process only once, while others use combined systems such as on the moving band or on the table and even dipping a toothpick into the ostiol of the fruit and examining the toothpick under UV (internal scanning). A total of 160 samples were provided and AF analysis was performed with 2 parallels in these samples. Batches with BGYF (-) are considered as clean samples without AF, while BGYF (+) figs are considered to contain AF. During the sampling, the steps of AF separation process and the time spent for the unit amount (1 kg) of figs was recorded. The dried fig samples were kept at -18Co until they analyzed in the laboratory.

Table 1. Sampling Model in AF Screening Processes in Fig Enterprises.

Sampling Points				
Enterprises A	**B**	**C**	**D**	**E**
Raw Material	Raw Material	Raw Material	Raw Material	Raw Material
1.Screening T BGYF(-)	1.Screening T BGYF(-)	1.Screening B BGYF(+)	1.Screening B BGYF(-)	1.Screening T BGYF(-)
1.Screening T BGYF(+)	1.Screening T BGYF(+)	1.Screening B BGYF(-)	1.Screening B BGYF(+)	1.Screening T BGYF(+)
2.Screening T BGYF(-)	2.Screening T BGYF(-)[IT]	2.Screening B BGYF(-)	2.Screening T BGYF(-)	
2.Screening T BGYF(+)	2.Screening T BGYF(+)[IT]	2.Screening B BGYF(+)	2.Screening T BGYF(+)	
3.Screening T BGYF(-) [IT]		3.Screening T BGYF(-)	3.Screening T BGYF(+) [IT]	
3.Screening T BGYF(+) [IT]		3.Screening T BGYF(+)	3.Screening T BGYF(-) [IT]	
		4.Screening T BGYF(-)		
		4.Screening T BGYF(+)		
		5.Screening T BGYF(-)		
		5.Screening BGYF(+) *		

[İT]: Internal control with toothpicks
T: Control at the table
B: Control on moving band
*: The sample could not be taken

2.2 Sample Extraction for AFs:

Dried fig fruits were chopped into 4 pieces with a knife and mixed thoroughly and all 3 kg samples were passed through the grinder machine (moulinex HV8-France). After the samples were carefully mixed, 2 parallel 50 gr samples were taken from each sample.

Sample Extraction for AFs were extracted from 50 g samples using a method developed by the immunoaffinity column provider (Aflaprep, R-Biopharm Rhone Ltd, UK) based on methanol extraction. 50 g samples were mixed with 100 ml of ultra pure water, 150 ml methanol (M.106018, Germany) and 5 g of sodium chloride (M.106404, Germany) and blended (Waring 8011S, USA) at 18.000 rpm for 3 min to obtain a homogeneous sample mix.

After mixing, the slurry was filtered through Whatman-4 filter paper (WHA1004125, England) and diluted with phosphate buffered saline (PBS-OxoidBR0014G) solution. This diluted solution was passed through an immunoaffinity column (Aflaprep, RBR-P07, UK). AFs were eluted from the column by passing 1 ml of HPLC grade methanol (M.106018, Germany) and then 1 ml of ultra pure water and using gravity to collect the eluate into a glass vial at a flow rate of around 5 ml min-1.

2.3 HPLC Conditions

The samples were analysed using HPLC (Shimadzu LC20A, Kyoto, Japan) in a reverse phase isocratic mode having C18 column (5µm, 25cm*4.6mm Macherey-Nagel-Germany) with a fluorescence detector (RF-20A). The mobile phase methanol:water (53:47,v/v) containing potassium bromide (120mg/l) and nitric acide (350 µL) was used at a flow rate of 1 ml/min. The temperature of column was maintained at 40 °C. Furthermore, the excitation and emission wavelengths were set at 362 and 425 nm, respectively. The injection volume into the HPLC system was 20 µl. Cobra cell (100mA, PMT-Tamson Holland) was placed between the HPLC column and the detector to increase the fluorescence power of AFB1 and AFG1. The data obtained were calculated by converting the standard measurements of the device to ppb and multiplying by Dilution Factor = 2.

As the AF standard, stock solution (CK547- R-Biopharm Rhone Ltd., UK) containing 250 ng / ml (Total AF 1000ng / ml) of each type of AF (B1, B2, G1, G2) was used. In order to draw the calibration curve, 7 standards containing 0.2, 0.5, 1.0, 2.0, 3.0, 5.0 and 7.5 ng / ml of AFB1, AFB2, AFG1, AFG2 were prepared with working solution (50% UPW + 50% Methanol). The correlation coefficient (r) calculated from the seven point calibration curve was 0.999.

2.4 Validation of the Method

The known lowest concentration of analyte readings have been made to be used in Uncertain Limit (LOD) and Quantitative Limit (LOQ) calculations. LOD and LOQ values were calculated by adding the standard deviation 3 times and 10 times to the average values of the data obtained. 0.094 mg / kg and LOQ 0.197 mg / kg LOD for AFB1, 0.042 and 0.063 mg / kg AFB2, 0.051 and 0.081 mg / kg AFG1, 0.055 and 0.109 mg / kg were found for AFG2, respectively.

In order to calculate the recovery values, clean matrixes of 25 grams were determined and AF injection was performed in 3 different levels, 6 repetitions, containing 0.5, 7.5 and 15 µg / kg of each AF. AF contaminated samples were incubated in the dark for 2 hours and then extraction was performed. Recovery of strengthened this samples in dried figs was in the range of 68-94%. All AFs values determined in this study were corrected with recovery rates according to their levels.

3. RESULTS AND DİSCUSSİON

With this study, the processes of AF-containing figs separation were compared at 5 different fig enterprises operating at Aydın province. Within the scope of the project, enterprises coded as “A, B, C, D, E” divide the figs into BGYF (+) and BGYF (-) under UV (365nm wavelength) lamp in the dark room to remove the figs with AFs. For BGYF positive and negative samples, AF contents at each step and AF-containing figs separation time (min) for 1 kg were compared.

According to Table 2, the total AF content of raw material samples of all enterprises varies between below detection limits and 29.03 µg/kg. The dry fig production area where the enterprises supply raw materials is just the Aegean region of Turkey. Heperkan et al. (2012), determined 0.1-763.2 ppb total AF in 115 of the samples taken from the drying areas in the Aegean Region. In another study, the dried fig samples were collected randomly from different bazaar, small-scale farmers, retail shops and supermarkets in different cities of Turkey. Sixteen dried fig samples (12.3%) contained AFs ranging from 0.1 to 28.2 ppb and a mean value of 3.8 ppb (Kabak, 2016). In our study, the total AF values range between ND-29.03 µg/kg and are consistent with the literature.

Table 2. AF values of enterprises in screening process.

	A (3 step)		B (2 step)		C (5 step)		D (3 step)		E (1 step)	
	AFB_1 Mean[a] Range (min-max) (µg/kg)	AFTOT Mean Range (min-max) (µg/kg)	AFB_1 Mean Range (min-max) (µg/kg)	AFTOT Mean Range (min-max) (µg/kg)	AFB_1 Mean Range (min-max) (µg/kg)	AFTOT Mean Range (min-max) (µg/kg)	AFB_1 Mean Range (min-max) (µg/kg)	AFTOT Mean Range (min-max) (µg/kg)	AFB_1 Mean Range (min-max) (µg/kg)	AFTOT Mean Range (min-max) (µg/kg)
Raw Material	0.44 ND[b]-1.42	0.66 ND-2.04	0 ND-ND	0 ND-ND	0.69 ND-3.45	1.35 ND-6.77	6.92 ND-27.19	7.39 ND-29.03	0.11 ND-0.34	1.28 ND-3.84
1.Step BGYF (-)	0.15 ND-0.48	0.39 ND-1.66	0.02 ND-0.10	0.02 ND-0.10	0.08 ND-0.20	0.09 ND-0.27	0 ND-ND	0.05 ND-0.23	0 ND-ND	0 ND-ND
1.Step BGYF (+)	231.46 102.78-311.72	340.72 159.86-435.62	1.65 0.22-3.72	2.21 0.22-4.31	139,26 1.00-272.42	165.26 3.63-344.05	285.73 168.99-347.40	359,27 255.22-402.10	93.36 0.13-253.01	125.26 0.23-380.07
2.Step BGYF (-)	0.34 ND-1.71	1.38 ND-6.90	0 ND-ND	0 ND-ND	0 ND-ND	0 ND-ND	0.03 ND-0.16	0.05 ND-0.16		
2.Step BGYF (+)	127.53 20.51-299.27	190.86 24.90-422.86	130.31 0.39-352.41	148.50 0.45-395.90	158.42 13.29-340.01	222.33 23.34-451.00	68.50 9.73-210.78	82.78 20.02-244.42		
3.Step BGYF (-)	0 ND-ND	0.13 ND-0.65			0 ND-ND	0.18 ND-0.89	0.08 ND-0.22	0.16 ND-0.65		
3.Step BGYF (+)	321.98 197.25-419.64	406.16 323.18-441.09			19,99 15.61-24.37	30.47 21.88-39,06	173.92 122.84-251.57	295.48 228.19-400.86		
4.Step BGYF (-)					0 ND-ND	0.02 ND-0.12				
4.Step BGYF (+)					171.85 35.35-402.26	202.53 46.21-452.91				
5.Step BGYF (-)					0 ND-ND	0.04 ND-0.19				
5.Step BGYF (+)										

[a] For calculating average values results below the limit of detection were replaced with 0

[b] ND: not detected, below the LOD

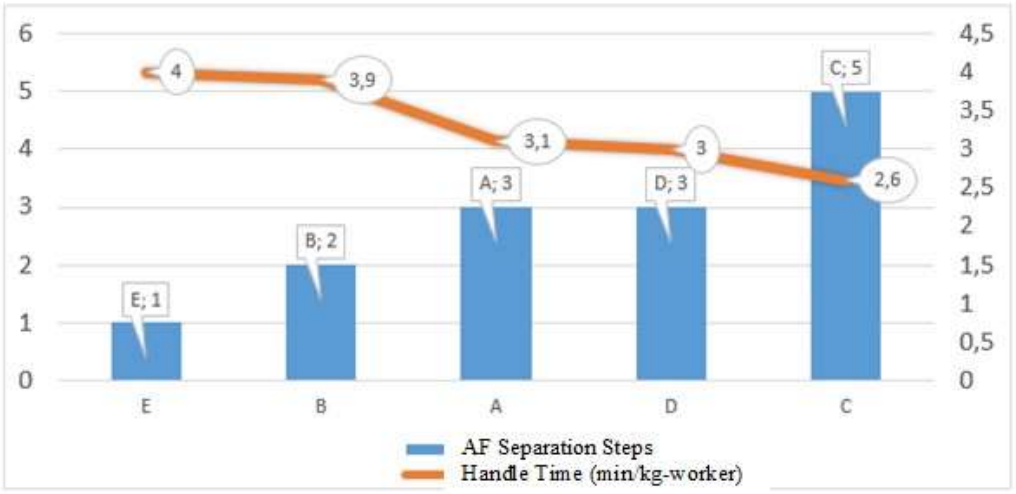
According to Table 2, despite being BGYF (-), the highest total AF values in A, B, C, D and E enterprises were 6.90-0.10-0.89-0.65-ND µg / kg, respectively. It has been reported that UV light screening is an effective method of separating AF-containing figs, nevertheless some figs also may contain AF after screening (Karaca, 2005, Steiner et al, 1988 and Konca and Gülseri, 1990). It has been determined that separating the AF-containing figs under UV (365nm) light in the dark room is an effective method as indicated in literatures. In a study carried out by Steiner et al (1988), the 22.6 ppb AFB_1 in raw material reduced to 6.3 ppb by separating figs with BGYF(+). In our study, the highest AFB_1 in the raw material was detected in D enterprise as 27.19 µg/kg, while with the removal of BGYF (+) figs it remained below the detectable value.

Total AF was detected above LOD in only 4 (16%) of 25 samples of the final product, which had undergone AF separation process and were identified as BGFY (-). Total AF averages are seen between ND and 0.13 µg / kg in the final product fig lots, while the highest value was found in enterprise A with 0.65 µg/kg. In terms of AFB_1 of the final products in the enterprises, the average values vary between ND-0.08 µg/kg, the highest value was found in enterprise D with 0.22 µgma/kg. The final fig batches determined as BGYF (-), gave values below the maximum limits for Total AF and AFB_1 both in EU and Turkey regulations (Anonymous, 2011; Anonymous, 2012).

70 samples with BGYF(+) are expected in the enterprises, while the BGYF(+) sample was not found in the fifth step scan process of enterprise C, thus total of 65 samples were obtained. AF was dedected in all 65 BGYF(+) samples, AFB_1 ranges between 0.13-419.64 µg/kg, total AF ranges between 0.22-452.91 µg/kg, and 57 of these samples AF values are above the maximum limits of EU countries and Turkey (Anonymous, 2011; Anonymous, 2012). Steiner et al (1988) reported that, after the 365 nm UV lamp scan of dried fig fruits obtained from Turkey, 62 samples do not have fluorescence and AFB_1 values determined between 0.2-0.7 ppb, while the AFB_1 values of 5 samples that have fluorescence were varies between 100 - 1400 ppb. In another study, Karaca (2005) reported that the total AF values of BGYF (+) figs from enterprises in Aydın province ranged between 117.9-471.9 ppb. In another survey, 37 of the 52 (71%) dried fig samples from Turkey, having BGYF under UV, were contaminated with only AFB_1 and AFG_1 (Steiner et al, 1993).

Enterprises A, B, C, D and E have implemented different separation processes with 3,2,5,3 and 1 steps, respectively. AF could not be detected or well below the limits in the final products of all fig enterprises. Figure 1 shows the total time it takes for enterprises to screen 1 kg of figs under UV lamp. In the AF separation process, as the number of steps increases, the time spent is expected to increase, and in contrast to the hypothesis, the time increases as the number of process steps decreases. With this result, it was revealed that the labor time spent for unit figs is important, not the number of steps of the process for separating AF-containing figs in dry fig batches. According to the survey study conducted with 85 fig enterprises operating in Aydın province, it was determined that 49% of the enterprises used one-step, 29% used two-step, 9% used three-step AF separation prosess in the dark room and each worker handles 150-200 kg of figs per day (Berrin et al., 2016). In our study, 2.6-4 minutes was determined for screening 1 kg of figs and this corresponds to 150-200 kg of fig / worker data specified in the literature.

Figure 1. *Comparison of the number of AF separation steps of enterprises with the handle time of figs per unit (1kg)*



Trace amounts of AF far below national and international limits were detected in the final product packaged figs of all enterprises that provided samples to the project. This is an indication that packaged products are safe foods for consumer health in terms of AF content.

4. CONCLUSION

In this study, it was determined that the labor required for the unit figs was important, not the number of steps of the process for separating the figs with AF. In fig enterprises, AF, which will threaten human health, has not been found in products that have undergone UV screening. Trace amounts of AFs far below national and international limits were detected in the end products packaged figs viewed BGYF (-) of all enterprises. This situation shows that in terms of consumer health, packaged figs are safe foods for AF content. AF was detected in all 65 fig samples that were BGYF (+) and separated as AF-containing in fig enterprises. In Turkey, figs contaminated with AFs collected from enterprises and destroyed with a project implemented by the Aegean Exporters ' Union. This application reduces the level of AF contamination in fig batches below to a certain level, contributing to the protection of consumer health and reducing economic losses. Separating the BGYF (+) figs is an effective method that has been used for a long time. In recent years, the enterprises have started to use machines for removing figs with AFs. However, it is stated by the operators that only these machines are not effective in separating out the figs with AFs. It is recommended to examine machine separation processes in subsequent studies.

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