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Original article

Aflatoxinous Figs (*Ficus Carica* L.) Seperation Process Using Ultraviolet (Uv) Light[1]

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Abstract

Fig fruits have high aflatoxin-forming capacity due to its high water activity and sugar content from the phase of harvest to the phase of drying. During aflatoxin formation, mycotoxigenic molds produce kojic acid as a metabolic residue. In the presence of kojic acid, aflatoxin-containing products emit greenish yellow and blue color while viewed under long-wave (365nm) UV (Ultraviolet) light. Scanning under UV light is a unique method commonly used for physically separating the aflatoxin-containing fruits from dried figs. With this study, the processes of aflatoxin-containing figs seperation were analyzed in one fig enterpris operating at Aydın province. At each stage of screening, starting from raw to final product, a total of 35 samples as BGYF (+) and BGYF (-), were subjected to some quality criteria and aflatoxin analyzes. Aflatoxin was analyzed from 5 raw fig samples and the highest total aflatoxin value was found to be 29.03 µg/kg. From the 15 samples viewed BGYF(+) and seperated as possible aflatoxin-containing figs in enterpris, wholly aflatoxin was detected, 15 of which were above the total aflatoxin limit value of 10 ppb and a maximum value of 402.10 µg / kg was analyzed. Aflatoxin was not detected in any of the final product figs separated as BGYF (-).

Keywords: Dried fig, Aflatoxin, Kojic acide.

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INTRODUCTION

As the nutritional value of a food increases, the storage ability and the probability of being consumed as safe food decreases. Fig fruits are suitable substrates for mycotoxin formation with high carbohydrate content and with high water activity value both at first maturation (0.91-0.97 aw) and in the intermediate moisture (0.80-0.89 aw) period (Frazier ve Westhoff, 1988).

Aflatoxins affects both human and animal health thus some restrictions have been placed on the availability of aflatoxins on food and feeds. For dried fig, there are limit values applied by Turkey and European Union (EU) countries. According to the legislation of EU Member States, for dried fig, maximum value of total aflatoxin and aflatoxin B1 is is 10 µg/kg a 6 µg/kg respectively. (Anonymous, 2012). In Turkish Food Codex, the total aflatoxin limit and the aflatoxin B1 limit for dried figs were determined as 10 µg/kg and 8 µg/kg, respectively (Anonymous, 2011).

For separating aflatoxins from each other fluorescence colors and relative chromatographic mobility are used (Betina, 1989). The aflatoxins that emit blue fluorescence under ultraviolet lamp are called "B" and those that emit green fluorescence are called "G" (Derici, 1997).

Another metabolite produced by A. flavus or A. parasiticus during aflatoxin formation is kojic acid. The figs that contain kojic acid emits bright greenish yellow color BGY (Bright Greenish-Yellow)] under long wavelength (365 nm) ultra violet (UV) light. BGYF (Bright Greenish-Yellow Florescence) method was applied for aflatoxin problem encountered in the dried fig. It has been stated that by screening and removing the BGY radiating figs under the UV lamp, the lot may considered completely clean (Steiner ve ark. 1988).

Due to human health and the legal requirements for aflatoxin, the fig enterprises absolutely apply the aflatoxin seperation during the process. The screening of aflatoxinous figs is carried out by examining the figs under the UV lamp light at 356 nm wavelength in the dark room conditions. Under UV light, BGYF (+) figs are regarded as aflatoxin-containing and BGYF (-) is considered as clean. Though there is no definitive data to determine the stages of the aflatoxin screening process, the fig enterprises have been in different arrangements depending on the production capacities, cleanliness of the raw materials, work experience and personnel qualifications. In this study, the effectiveness of the aflatoxin screening process of a fig company operating in Aydın province was examined.

Material and Methods

Material

With this study, the screening and separation process of aflatoxinous figs was investigated in a company operating in Aydın province. The fig plant separates the aflatoxin containing figs under UV (365nm wavelength) lamp in the dark room conditions. From raw material to final product some quality

and aflatoxin analyzes were carried out on 35 samples at 7 points with 5 replications at each stage of screening as BGYF (+) and BGYF (-). Örnekleme noktaları; hammadde, 1. kademe tarama BGYF(-), 1. kademe tarama BGYF(+), 2. kademe tarama BGYF(-), 2. kademe tarama BGYF(+), 3. kademe tarama BGYF(-), 3. kademe tarama BGYF(+) şeklindedir. Samples were taken from: Raw material, 1. Stage screening BGYF(-),1. Stage screening BGYF(+), 2. Stage screening BGYF(-), 2. Stage screening BGYF(+), 3. Stage screening BGYF(-) and 3. Stage screening BGYF(+).

Methods

Fruit Colour:

The surface colour of dried figs was measured for 10 dried figs with a colourimeter (Minolta CR-400, Japan). The colourimeter had an 8 mm diameter viewing area and was calibrated with a white tile.

Moisture Content (%):

4-5g of samples were taken from the dried figs passed through the mincing machine and dried in a drying oven at 70 ° C until they reached a fixed weight. The difference between the final weight and the initial weight was converted to% humidity value arithmetically

pH:

The pH was measured with a pH-meter (WTW pH 540GLP, Germany).

Water activity (aw):

Water activity measurements were made with the TESTO-650 device from the pureed samples.

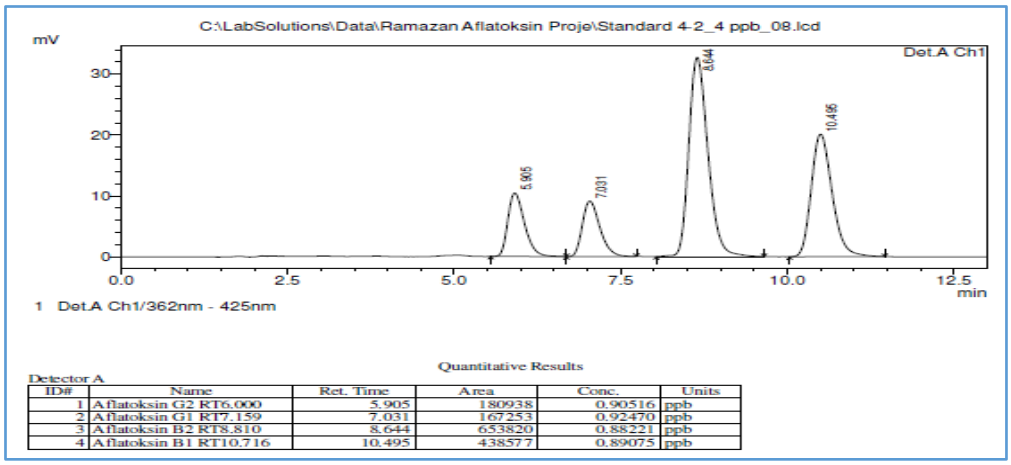
Aflatoxin Analyzes:

Sample preparation

For the analysis of dried fig samples, the AOAC Official Method 999.07 (Stroka et al, 2000) was used. A 50 g of representative sample was blended with 250 ml extractant solution of methanol–water (3/2, v/v) and 5 g NaCl using a Waring blender for 3 min, and filtered with prefolded filter paper. Next, an aliquot of 5 ml of sample extract was diluted with 5 ml PBS. 10 ml of PBS was first passed with a flow rate of 2-3 ml / min through the the IAC (P07 / R-Biopharm Rhone Ltd., Glasgow / Scotland) that brought to room temperature and then 10 ml of the filtrate was passed through the column at a flow rate of 3 ml / min. After the flow is complete, 20 mL of purified water is passed through the column at the same flow rate, then air was passed with syringe 3-4 times. To take the aflatoxin in liquid form, 1 ml of methanol was passed through the IAC in natural stream, followed by 1 ml of ultrapure water and a total of 2 ml of extract was obtained.

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 360 and 440 nm, respectively. The injection volume into the HPLC system was 100 µl. The method has shown good resolution and separation of aflatoxin standards (Fig. 1).



Quantitative Results

Detector A

ID#	Name	Ret. Time	Area	Conc.	Units
1	Aflatoksin G2 RT6.000	5.905	180938	0.90516	ppb
2	Aflatoksin G1 RT7.159	7.031	167253	0.92470	ppb
3	Aflatoksin B2 RT8.810	8.644	653820	0.88221	ppb
4	Aflatoksin B1 RT10.716	10.495	438577	0.89075	ppb

Figure 1. Chromatograms showing the retention times of individual retention times of aflatoxins standards.

Quality control parameters

The method was validated by using seven point calibration curves of analytes, to assess the linearity in a range of 0.2-7.5 ng/ml for AFB1, AFG1, AFB2 and AFG2. The values of coefficient of determination (R^2) for all analytes were found above 0.999. Ragent blank readings were made for use in Limit Of Detection (LOD) and Limit Of Quantitation (LOQ) calculations. LOD and LOQ values were calculated by adding 3 times and 10 times of standard deviation to mean values of obtained data, respectively.

The LOD of 0.094 mg/kg and LOQ 0.197 mg/kg was found 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 for AFG2, respectively. The recoveries of fortified samples in dry figs found in the range of 68-94%.

Results

Some physical and chemical properties of the products at the aflatoxin screening process are shown in Table 1. According to Table 1, the color L values vary between 56.94 and 59 whereas the BGYF (+) values are darker and low-valued than the ones with negative values BGYF (-). The figs at the 3rd stage of the process were strip cut fruit prepared for the food industry thus, color and fruit weight

values were not measured and moisture, pH and water activity values did not differ according to the first 4 stages.

Table 1. Some physical and chemical properties of figs in aflatoxin separating processes

Process Stage	Color L	Moisture Content %	Weight (g/piece)	pH	aw
Raw Material	59.00±6.22	18.25±0.34	21.40±2.57	4.28±0.11	0.68±0.01
1. Stage screening BGYF(-)	57.91±6.42	18.36±0.98	23.63±2.25	4.49±0.10	0.71±0.02
1. Stage screening BGYF(+)	56.82±6.85	18.22±0.64	24.21±1.05	4.62±0.02	0.71±0.00
2. Stage screening BGYF(-)	58.82±5.49	16.98±0.37	26.59±1.18	4.48±0.05	0.69±0.01
2. Stage screening BGYF(+)	56.94±6.54	16.71±0,30	27.57±1.14	4.63±0.06	0.69±0.01
3. Stage screening BGYF(+)	-	17.03±0.47	-	4.26±0.07	0.68±0.01
3. Stage screening BGYF(-)	-	17.06±0.66	-	4.24±0.14	0.68±0.02

±Standard deviation.

In order to Table 2, aflatoxin was detected in 4 samples from 5 samples analyzed in raw material stage whereas the maximum values of AFB1 was 27.19 µg / kg and total aflatoxin was 29.03 µg / kg respectively. Only one of the samples with BGYF (-) in the first stage screening had a total aflatoxin value of 0.23 µg / kg, whereas all of the BGYF (+) samples had positive value and the highest aflatoxin was 359,27 µg / kg. In the 2nd and 3rd stages, the highest AFB1 value was 0.22 µg / kg in the BGYF (-) samples, while in the BGYF (+) samples the highest AFB1 value was 251,57 µg/kg.

Table 2. Aflatoxin values at the stages of aflatoxin separating processes

Process Stage	Aflatoxin B1 (µg/kg)				Total Aflatoxin (µg/kg)			
	Min	Max	Avg	N+/N	Min	Max	Avg	N+/N
Raw Material	<LOD	27.19	6.92	(4/5)	0	29,03	7.39	(4/5)
1. Stage screening BGYF(-)	<LOD	<LOD	0	(0/5)	0	0.23	0.05	(1/5)
1. Stage screening BGYF(+)	168.99	347.40	285.73	(5/5)	255.22	402.10	359,27	(5/5)
2. Stage screening BGYF(-)	<LOD	0.16	0.03	(1/5)	0	0.16	0.05	(2/5)
2. Stage screening BGYF(+)	9,73	210.78	68.50	(5/5)	20.02	244.42	82.78	(5/5)
3. Stage screening BGYF(+)	122.84	251.57	173.92	(5/5)	228.19	400.86	295.48	(5/5)
3. Stage screening BGYF(-)	<LOD	0.22	0.08	(2/5)	0	0.65	0.16	(2/5)

N+/N : BGYF(+) Number of samples / Number of samples analyzed.

Discussion

This study was carried out with 35 samples of Sarılop variety dried figs produced in the year 2015, which are obtained from a fig enterprise operating in Aydın province. Within the scope of the project, two different category of dried figs as BGYF (+) and BGYF (-) were analyzed which seperate under the UV (365nm wavelength) lamp in the dark room conditions.

18.36% is the highest moisture value obtained in moisture analysis which is below the maximum 26% moisture value specified in the dried fig standard TSI 541 (TSI 541, 2006). There was no

correlation between the quality parameters as color, moisture content %, pH and water activity of dried figs and BGYF.

With this study, it can be reported that UV light screening is an effective method for separating aflatoxinous figs although some of the figs may contain aflatoxin even screened under UV light and this results are consistent with the literature as Karaca (2005), Steiner et al (1988), İLKL-ML (2012) and Konca ve Gülseri (1990) mentioned before. In a study conducted by Steiner et al. (1988), AFB1 was found to be 22.6 ppb in the raw material while with the removal of BGYF (+) dried figs AFB1 was decreased to 6,3 ppb. The highest AFB1 value detected in the raw material samples of our study was 27,19 ppb, whereas with the removal of BGYF (+) dried figs the aflatoxin results fell below the LOD values. The total aflatoxin content of the raw material samples ranged from 0 to 29.03 µg / kg. Most of these data were found to be lower than the total aflatoxin values of 0.1-763.2 ppb, which were found in 115 samples of Heperkan et al (2012) sampling from the Aegean region figs, which are the source of raw materials for fig enterprises.

Conclusions

From the final packaged dried figs determined as BGYF (-), aflatoxin were detected in trace amounts far below the national and international limits. In the final products, AFB1 remained below the LOD and total aflatoxin was not detected. As a result, there was no correlation determined between the quality criteria of figs and aflatoxin content and no aflatoxin was found that would threaten human health in UV-scanned products.

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