DETERMINATION OF FACTORS AFFECTING AFLATOXIN FORMATION IN PEANUTS AND PREVENTIVE MEASURES

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ABSTRACT

In the study, total aflatoxin (AFB1+ AFB2+ AFG1+ AFG2) and aflatoxin B1 (AFB1) contents of 180 different peanut samples obtained from peanuts grown in 3 different regions and altitudes, Adana, Osmaniye and Mersin, were determined by fluorescence detector high performance liquid chromatography (HPLC-FLD) device. was detected using It was determined that the total aflatoxin (AFs) and AFB1 contents of peanut samples varied between 0.25-9.89 and 0.21-9.19 µg/kg, respectively. While AFB1 was detected in 7 samples in total, AFB1 could not be detected at all in 27 samples.It was observed that the detected samples remained below 10 µg/kg, which is the maximum limit value of the contaminants notification specified in the Turkish Food Codex.

Key words: Aflatoxin; peanut; maximum limit; Preventive measures.

YER FISTIĞINDA AFLATOKSİN OLUŞUMUNA ETKİ EDEN FAKTÖRLERİN VE ÖNLEYİCİ TEDBİRLERİN BELİRLENMESİ

ÖZET

Çalışmada, 3 farklı bölge ve yükseklik olan Adana, Osmaniye ve Mersin bölgelerinde yetiştirilen yerfıstıklarından temin edilen 180 farklı yerfıstığı örneğinin, toplam aflatoksin (AFB1+ AFB2+ AFG1+ AFG2) ve aflatoksin B1 (AFB1) içerikleri floresans dedektörlü yüksek performans sıvı kromatografisi (HPLC-FLD) cihazı kullanılarak tespit edilmiştir. Yer fıstığı örneklerinin toplam aflatoksin (AFs) ve AFB1 içeriklerinin sırasıyla, 0.25-9.89 ve 0.21-9.19 µg/kg aralığında değiştiği belirlenmiştir. Toplamda 7 örnekte AFB1 tespit edilirken, 27 örnekte AFB1 hiç tespit edilememiştir. Tespit edilen örnekler Türk Gıda Kodeksinde belirtilen bulaşanlar tebliğinin maksimum limit değeri olan 10 µg/kg' un altında kaldığı görülmüştür.

Anahtar Kelimeler: Aflatoksin; Yer fıstığı; Maksimum limit; Önleyici tedbirler.

1. INTRODUCTION

Peanut (*Arachis hypogaea* L.) is a hot climate plant from the legume family, grown as an annual and summer. It differs from other plants in that it produces its fruits under the ground. Due to the high oil content in their grains, they are included in the group of oilseed plants (Kadiroğlu, 2018).

In Turkey, in 1920, peanut production started economically. With the assignment of the Peanut Agricultural Sales Cooperatives Association (YERFISKOBIRLIK) between 1978-1983, and ÇUKOBIRLIK (Çukurova Cotton, Peanut and Oilseeds Agricultural Sales Cooperatives Union), in which YERFISKOBIRLIK was merged between 1992-1993, it was subjected to state support, and from 1994 it was excluded from support.

Peanut seeds contain 45-55% oil and the oil yield per unit area is higher than other field products. In general, peanut oil contains 45-60% oleic acid, 20-40% linoleic acid, 5-10% palmitic acid, 3-7% stearic acid, 1-3% behenic acid and 0.5-2% aracidic acid. The oil has a high stability and shelf life due to the presence of tocopherol (vitamin E), an antioxidant substance, and high oleic acid content (Schwager et al., 2015).

Approximately 90% of peanut cultivation in Turkey is carried out in the Çukurova region. Although the peanut cultivation area in Osmaniye comes after Adana, approximately 90% of the peanuts produced in Turkey are processed and marketed in Osmaniye. According to the latest 2018 data, 60.5% of the peanut cultivation areas in Turkey are in Adana, 29.6% in Osmaniye, 2.7% in Aydın, 2.2% in Antalya, 1.7% in Kahramanmaraş. In addition, 1.6% is made in Mersin and the rest in other provinces (Işık, 2003; Aşık et al., 2018; TUIK, 2018).

Mold contamination is common in peanuts, and the development of some species poses a significant risk to human and animal health. It is stated that the most important factor that shortens the shelf life of peanuts is mold. Mold growth starts in the field, may develop due to harvest and insufficient and/or inappropriate drying conditions, and may occur during storage and transportation. Species belonging to Penicillium, Aspergillus and Rhizopus genera were found in the shelled peanut during harvest, drying and storage.

The growth of A. flavus and A. parasiticus molds that produce aflatoxin in shelled peanuts starts in the field and increases during harvesting. A. flavus was not observed in the harvested peanut kernels, except for the cracked peanut kernels during harvest and storage. However, it was observed that the number of molds increased a little more immediately after harvest.This amount may also vary according to harvest, environmental and climatic conditions. Oil is the most important factor affecting the number of molds and microflora after harvest in this type of shell fruit. In case of excess oil, the number of bacteria and molds increases.

Molds decompose the protein, fat, and carbohydrate of the food with enzymatic activities, changing the texture of the food, decreasing the fat content, increasing the amount of free fatty acids, breaking down the proteins, changing the amino acid composition, changing the color, forming bad odor, taste changes and weight loss. Since molds can enter healthy food, they do more damage than bacteria and their toxigenic types can produce mycotoxins (ICMSF, 1996).

Inadequate and incorrect practices during the production, harvest, drying, transportation, storage and processing of peanuts in Turkey increase the risk of aflatoxin formation. Aflatoxin, Aspergillus flavus, A. paraticus and A. nomius molds are formed as a result of toxin production by the biosynthesis of these molds when suitable conditions are found by contamination of peanuts. It is extremely important to separate the contaminated grains during harvest and processing and to prevent mold growth during processing, since even their very low amount can pose a risk to human health. Because mold growth can be stopped and molds can be destroyed somehow, it is very difficult to destroy or remove aflatoxins after they form.

In the studies, it was concluded that 90% of the grains with aflatoxin originate from low quality products, which make up 4.6% of the peanuts, and the separation of such products

reduces the aflatoxin rate 10 times. This reveals the high importance of sorting out the products that may carry aflatoxin risk during harvest. It should not be forgotten that peanut processing in our country should be done according to the rules of GMP (Good Manufacturing Practices) and the contact of the peanuts with the soil is cut off by mechanical harvesting, and after the harvest, the peanuts should be delivered to the drying facilities within 24 hours and dried quickly with drying machines (Işık, 2003).

2.MATERIAL and METHOD

2.1. Samplıng from Fıelds

The peanuts used in the experiments were taken from a total of 45 fields located in 3 different cities and altitudes where the production is the most. In our samples, the NC-7 peanut variety was studied. Sampling was done when the moisture content of the grains dropped to 25-35%. Samples were obtained 4 times at approximately 15-day periods. To represent each field, peanut samples were taken in the form of the letter "Z", that is, from 5 different places, namely the upper left end of the field, the upper right end, the middle of the field, the lower left end and the lower right end points. Approximately 10 kg of grain samples from each peanut field, from different sides and depths of the field, were placed in 1 kg sterile bags. First of all, peanuts were stored in a controlled warehouse (5°C, 60-70% RH) in Osmaniye Korkut Ata University, Kadirli Faculty of Applied Sciences, Department of Food. Samples were taken from the warehouses at regular intervals and brought to Adana Food Control Laboratory for aflatoxin analysis. In total, the analyzes were completed in three months.

2.2. Analysıs Methods

The analyzes made at the sampling stage from the fields are given below.

2.2.1. Aflatoxin (B_1, B_2, G_1, G_2) Analyzes

Aflatoxin analysis is an accredited analysis and was carried out by HPLC Method in accordance with AOAC 2000, 999.07. The samples coming to the laboratory were mixed 1/1 with water and homogenized in a 5 lt capacity blender (Waring). Homogenized samples were passed through an immuno-affinity column in accordance with the accredited method AOAC 2000, numbered 999.07, and the amount was determined by derivatization after the column in the HPLC device.

2.2.2. Determınatıon of Moısture

Moisture analysis was carried out according to the oven drying method (AOAC 1995).

3. FINDINGS

3*.1. Collectıon of Samples*

The peanuts used in the experiments were taken from 3 different provinces (Osmaniye, Adana and Mersin) and from a total of 45 fields located at the highest production altitudes. As seen in Table 1, these fields are Azaplı-Mercimek from Osmaniye, İmran-Dumlu from Adana and Beylice-Taşobası from Mersin. Five samples were taken from each location in 4 different months.

Locations	Height above sea level (m)	August 23	September 6	September 19	September 30
Azaplı (2*)	54	5	5	5	5
Çaygeçit	121	5	5	5	5
Tahtaköy	85	5	5	5	5
Durmuşsofular(4*)	78	5	5	5	5
Kümbet	63	5	5	5	5
Mezretli	54	5	5	5	5
Mecidiye (3*)	85	5	5	5	5
Narlıkışla	92	5	5	5	5
Öksüzlü (2*)	62	5	5	5	5
Yusuf İzzettin	71	5	5	5	5
Yenigün (3*)	84	5	5	5	5
Tozlu	58	5	5	5	5
Hacıbeyli	63	5	5	5	5
Mercimek (4*)	74	5	5	5	5
İmran	76	5	5	5	5
Yeşildam	81	5	5	5	5
Azizli (3*)	84	5	5	5	5
Dumlu (2*)	86	5	5	5	5
Beylice	89	5	5	5	5
Avadan (2*)	92	5	5	5	5
Yakaköy	87	5	5	5	5
Arslanköy (3*)	73	5	5	5	5
Yenice	66	5	5	5	5
Taşobası (4*)	53	5	5	5	5

Table1. Sampling fields and their altitudes.

(*): Different fields.

3.2. Detectıon of Aflatoxin in Samples

Aflatoxin analyzes were made in samples taken from all fields in 2020 and the results are given in Table 2, Table 3 and Table 4.

Table 2. Aflatoxin analysis results of samples from Osmaniye region, which were above the minimum detection limit, in the 2019-2020 harvest and post-harvest studies.

Samples	B_1 (µg/kg)	Total (µg/kg)
1	0,65	0,65
2	0,59	0,59
3	2,12	4,27
4	--	--
5	0,43	0,43
6	0,67	0,67
7	0,48	0,81
8	--	--
9	0,63	1,12
10	2,67	3,15
11	0,71	0,71
12	0,59	0,59
13	0,38	0,38

14	0,45	1,15
15	0,51	0,51
16	--	--
17	0,54	0,54
18	--	--
19	1,12	1,53
20	0,61	0,61
21	--	--
22	0,47	0,47
23	0,42	1,21
24	0,44	0,44
25	0,52	1,15
26	0,41	0,41
27	9,19	9,19
28	1,21	1,21
29	0,25	0,25
30	0,58	0,58
31	0,72	0,72
32	0,95	1,25
33	--	--
34	0,47	0,54
35	0,79	0,79
36	0,39	0,39
37	0,77	0,77
38	--	--
39	0,47	0,47
40	--	--
41	0,73	1,12
42	0,84	0,84
43	0,92	0,92
44	0,44	0,91
45	--	--
46	8,59	8,59
47	0,73	0,73
48	0,48	0,78
49	--	--
50	1,18	1,18
51	0,59	0,59
52	1,05	1,05
53	0,88	0,88
54	0,57	0,57
55	--	--
56	0,51	0,51
57	0,54	0,74
58	0,48	3,48
59	0,72	0,97
60	0,44	0,44
AVERAGE	1,39	1,26
STANDART DEVIATION	1,58	1,66

Table 3. Aflatoxin analysis results of samples from Adana region, which were above the minimum detection limit, in the 2019-2020 harvest and post-harvest studies.

Samples	B_1(µg/kg)	Total (µg/kg)
61	0,87	0,87
62	2,52	4,12
63	0,63	0,63
64	1,46	1,46
65	0,21	0,98
66	0,32	0,78
67	4,87	4,87
68	--	--
69	8,87	9,11
70	0,81	0,81
71	0,74	0,74
72	0,91	0,91
73	1,08	1,08
74	0,32	0,65
75	0,34	1,31
76	0,87	1,83
77	--	--
78	1,17	1,17
79	0,74	0,74
80	0,51	0,51
81	1,12	1,12
82	0,31	0,31
83	1,09	1,09
84	--	--
85	0,57	0,98
86	0,52	0,52
87	0,39	0,39
88	0,33	0,33
89	0,87	0,87
90	0,79	0,79
91	--	--
92	0,51	0,51
93	0,74	0,74
94	0,67	0,81
95	0,51	0,51
96	1,67	2,11
97	6,32	9,89
98	0,65	0,65
99	0,67	0,67
100	--	--
101	0,32	0,32
102	0,87	0,87
103	0,71	0,71
104	1,19	1,35

105	--	--
106	1,05	1,05
107	0,21	0,44
108	3,06	4,01
109	--	--
110	0,78	0,78
111	0,51	0,51
112	0,89	0,89
113	0,39	0,39
114	8,47	9,78
115	1,32	1,32
116	0,85	0,85
117	0,52	0,52
118	0,31	0,31
119	--	--
120	0,56	0,79
AVERAGE	1,29	1,53
STANDART DEVIATION	1,76	2,12

Table 4. Aflatoxin analysis results of samples from Mersin region, which were above the minimum detection limit, in the 2019-2020 harvest and post-harvest studies.

Samples	B_1(µg/kg)	Total (µg/kg)
121	0,52	0,52
122	0,31	0,31
123	2,37	2,37
124	0,47	0,94
125	0,84	0,92
126	--	--
127	0,61	0,61
128	1,09	1,09
129	0,79	0,79
130	7,65	9,32
131	0,97	0,97
132	0,24	0,24
133	0,71	0,98
134	0,64	0,64
135	--	--
136	1,07	1,07
137	0,71	0,71
138	0,58	0,58
139	--	--
140	0,49	0,49
141	0,53	1,01
142	--	--
143	9,19	9,19

144	0,84	0,84
145	0,58	0,58
146	0,91	0,91
147	0,49	0,56
148	--	--
149	0,58	0,58
150	1,17	2,04
151	0,63	0,63
152	0,34	0,87
153	--	--
154	0,43	1,02
155	0,37	0,37
156	0,51	0,51
157	0,35	0,59
158	0,21	0,54
159	7,32	9,51
160	0,43	0,43
161	0,87	0,87
162	0,93	0,93
163	1,07	1,07
164	--	--
165	0,61	0,61
166	--	--
167	0,84	0,84
168	0,61	1,09
169	0,32	1,02
170	0,74	0,74
171	0,41	0,41
172	0,97	0,97
173	0,74	0,92
174	0,41	0,41
175	--	--
176	0,64	0,64
177	0,52	0,52
178	0,31	0,78
179	1,87	1,87
180	0,71	0,71
AVERAGE	1,13	1,32
STANDART DEVIATION	1,71	1,96

In conclusion, as a result of our study, aflatoxin was found in a total of 7 samples, and AFB_1 results were found above 5 µg/kg, which is the maximum limit value of the contaminants communiqué, in seven of them. The total (AFB_1+AFB_2+AFG_1+AFG_2) results were below the maximum limit value of 10 µg/kg. Aflatoxin was detected in a total of 27 samples, but remained below the detection limit.

3.3. Meteorology Data

Meteorological data for the dates of August and September 2020, when the project studies were carried out, are provided by T.C. It was obtained from the Ministry of Agriculture and Forestry, General Directorate of State Meteorology Affairs. Relative humidity averages according to meteorological data are given in Table 5.

Table 5. Relative humidity averages for the harvest period of 2020 (August-September)

Relative humidity averages (%RH)	August 23	September 6	September 19	September 30
Azaplı	48	46	45	44
Çaygeçit	44	44	43	42
Tahtaköy	48	47	46	44
Durmuşsofular	45	44	43	43
Kümbet	44	43	43	41
Mezretli	49	47	46	46
Mecidiye	50	51	48	45
Narlıkışla	51	50	50	45
Öksüzlü	49	48	47	45
Yusuf İzzettin	52	52	50	47
Yenigün	53	52	50	48
Tozlu	54	53	52	46
Hacıbeyli	54	54	50	46
Mercimek	55	53	50	45
İmran	58	56	55	53
Yeşildam	59	56	55	53
Azizli	61	60	58	56
Dumlu	59	56	55	53
Beylice	45	44	43	43
Avadan	46	45	45	44
Yakaköy	44	44	43	42
Arslanköy	43	43	42	41
Yenice	42	42	41	41
Taşobası	41	41	40	40

When the relative humidity values are evaluated in general, it has been determined that Adana region is more humid than other regions. Considering the relative humidity contents, it is suggested that Adana region should urgently develop drying works due to its high relative humidity and meticulously carry out machine drying systems and storage.

4. DISCUSSION and CONCLUSION

As a result of the sampling studies from the fields, when the peanuts were collected in 2020, aflatoxin was found in seven samples and it was found above 5 µg/kg, which is the maximum limit value of the contaminants communique. However, it was determined that these values

were not very high and that total aflatoxin remained below the maximum limit value of 10 µg/kg. The number of samples in which aflatoxin was found below the minimum detection level in all samples was 27.

During the harvest studies, it was observed that aflatoxin was found in the samples collected early from the branch during the worst harvesting conditions.However, the maximum detectable amount of aflatoxin remained below the limits.

However, since the time interval between harvest and analysis did not last long and the samples were kept in the cold chain, there was no significant change in the humidity level at this storage stage, so there was not much change in the aflatoxin level.

Since there were no warehouses similar to the poor conditions of storage practices of farmers and traders, harvest samples were kept in special rooms reserved for project studies, and these rooms did not have bad conditions that would accelerate the formation of aflatoxin, even though they were not suitable environments for the storage of foods.

Therefore, a total of 180 samples were taken from the farmer's blend within the scope of the project studies. When all of the blend samples with aflatoxin were evaluated, it was determined that 3.9% of the 180 samples contained aflatoxin.

As a result, at the end of the 2-year study, it was determined that aflatoxin formation could start at the ripening stage, but the level of aflatoxin formation was not above the limits and the formation intensified after harvest. It has been determined that the formation of aflatoxins increases when the peanuts are harvested early, kept in nylon bags in humid environments and dried on the soil.

Since the rate of mold types that can produce aflatoxins, and therefore the formation of aflatoxins, may increase depending on climatic conditions, various measures should be taken to keep the growth of molds capable of producing aflatoxin under control during the harvest and post-harvest stages and to prevent aflatoxin formation. These precautions are given in detail in the introduction part of our article for peanuts within the scope of GAP, GMP and GSP applications. However, it is extremely important to implement important decisions and recommended practices in order to increase our production in peanut production and processing, to open up to new markets, and most importantly to save the future of hundreds of thousands of families who make a living with peanuts. In the light of these project studies and the observations made in the field, attention should be paid to the following issues and necessary precautions should be taken. Post-harvest processes are the most critical stages in the development of aflatoxin. Therefore, drying processes should be carried out as soon as possible.

In addition, post-harvest drying and storage processes are food processing processes that require expertise. For this reason, these processes should be carried out by companies that have knowledge and infrastructure on Good Production Techniques and Good Storage Techniques. Carrying out the drying and storage processes in controlled environments will also open many bottlenecks in terms of food safety and even traceability, which is emphasized and increasingly important in terms of European Union agricultural policies.

Although aflatoxin formation started in the field, it was determined that aflatoxin in newly harvested peanuts was below the limits as seen in the two-year project studies. This is a pleasing result, as the control of climatic conditions and natural environment is much more difficult than the control of post-harvest processes. However, aflatoxin was found in very

small amounts in sampling studies from orchards. Therefore, these results show that the post-harvest processes are extremely important and critical and that necessary measures should be taken quickly. For this, drying and storage processes should be carried out in controlled environments and in accordance with food processing/storage rules, and sorting and physical separation processes should be carried out effectively during processing.

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