# EVALUATION OF PHYSICAL METHODS TO CONTROL MYCOTOXINS IN SOME CONTAMINATED FOODS

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***Abstract***

Because aflatoxin contamination is unavoidable, numerous strategies for their detoxification have been proposed. These generally include physical methods such as: sorting, heating, irradiation, adsorption and extraction.

Naturally infected raw peanut kernels, ripe black olives, corn embryos, along with their extracted oils and their waste products were used in this study.

An average of 28 to 48% infected samples were sorted using the ultra violet rays. Heat treatment showed better results in mold inhibition than the storage exposed to the sun rays due to the dehydration of the samples. Moreover, heating was more effective with the whole kernels or seeds.Neutralization of the oil acidity was the best treatment to destroy aflatoxins, followed by the treatment with 1% Fuller's earth.

Sunlight was more effective with peanut cake, olive pomace and corn meal samples due to their large surface area exposed to the sun rays. Fuller's earth (1%) showed the best results with the low amounts of aflatoxins since a reduction between 65.7 and 92.6% occured in the artificially contaminated samples.

Key workds: sorting-Heat-Irradiation-Adsorption-Extraction - Mycotoxins.

## INTRODUCTION

The increasing number of reports on the presence of mycotoxins in foods and feeds lead researchers to find practical and economical procedures to control mycotoxins. Ideally, such methods should reduce the toxin concentrations to safe levels without the production of toxic degradation products or any reduction in the nutritional value of the foods (Doyle *et al.*, 1982). Procedures that have been succeded include: sorting (physicla or mechanical removal), heating, irradiation, adsorption and extraction of mycotoxins (West and Bullerman).

**Sorting:** Electronic sorters have been used on a trial made by Ashworth *et al.*, 1968 to remove greenish-yellow fluorescent cottonseed. Moreover, Blue-Greenish Yellow Fluorescent (BGYF) was developed to estimate aflatoxin level by many maize dealers (Tanboon 1989). In wet milling, Yahl *et al.*, (1971) found aflatoxin in steep-water and fiber, with the remainder in the gluten and germ. Techniques for sorting on colour and other visual characteristics have been used most extensively for aflatoxin control in peanuts by putting inspectors along a moving belt on which the peanutts are spread (Tiemstra. 1977). He also found the possibility of using air classificattion of peanut kernels as a useful tool for sorting the immature, damaged and infected peanuts.

**Heating:** Farah *et al.* (1983) applied the removal of aflatoxins in raw unshelled peanuts by a traditional salt boiling process practised in North east of Brazil. They concluuded that it is possible to remove or reduce aflatoxin in peanuts by NaCl and boiling. Dry roasting of contaminated peanuts reduced 40 to 50% of aflatoxins B1 and G1 (Scott, 1984). In addition, Pluyer *et al.* (1987) found that low energy microwave roasting had the same effect as the oven roasting in destroying aflatoxins. Experimental roasting was studied for pecans, soybeans and coffee beans by Escher (1974), Hamada and Magella (1982) and Levi (1980), respectively. Sylos and Amaya-Farfan (1992), found that thermal destruction of aflatoxins in peanuts is efficient provided a temperature of 195$^{o}$C.

**Irradiation:** Aflatoxins are affected by the exposure to ultraviolet (UV) light (Feuell, 1966 and Shantha, 1987). Mahjoub and Bullerman (1986) reported the loss of 95% of aflatoxins from olive oil exposed to sunlight. In addition, Frank and Grunewald (1970), concluded that irradiation with ultraviolet light, X-or gamma rays may show promise in controlling fungal growth than in destroying mycotoxins.

**Adsorption:** Particle size of bentonite and heat treatment were studied by Masimango *et al.*, (1978) and Smith *et al.*, (1994). Practical and effective method for the detoxification of aflatoxins had been shown by Phillips *et al.*, (1995) and Aziz *et al.*, (1996), using phyllosilicate clay (HSCAS), bentonite and charcoal which tightly bind aflatoxins.

**Extraction:** Conventional refining removes any aflatoxins that may have been present in the crude edible oil treated with alkali (Parker and Melnick, 1966).

This investigation was carried out to estimate the effect of each of the physical methods used to destroy or control aflatoxins in the natuurally infected and the

artificially contaminated samples of raw peanut kernels, black olives, corn embryos, their extracted oils, and their waste products.

## MATERIALS AND METHODS

### Materials :

1. There kilograms of raw peanut kernels were obtained from the agricultural markets in Dokki.
2. There kilograms of ripe black olives were obtained from a local market in Giza .
3- Four kilograms of corn embryos were obtained from Starch and Soap Company, Cairo.
4. Aflatoxins standards were obtained from Sigma Chemicals Company, USA.
5. Aluninium precoated sheets for TLC were obtained from E.Merck, Darmstadt, West germany.

### Methods :

1- Raw peanut kernels, ripe black olives and corn embryos were spreaded on a tray of 0.5 mm thickness and subjected to ultraviolet light. The infected samples showed blue-green-yellowsh-fluorescent (BGYF). The infected materials were sorted, weighed, and calculated as infected percentages.

2- The sorted infected samples were divided into the following equal parts.

2-a Storage of 5 samples in clean, autoclaved Petri dishes for one week in a sunny place to get about 8 hours of sun rays daily while another samples were kept in a dark place as control samples. These plates were daily inspected for any fungal growth. The percentage of fungal growth was calculated among the number of plates and the number of kernels of spots in each plate. An inspection by (BGYF) was done for these samples.

2-b Three samples were subjected to heat in an oven at 100$^{o}$C for two hours, and kept for one week to control fungal growth.

3- Oil extraction, Oil was extracted from the healthy and the infected samples using n-hexane as described in AOAC, (1985). Acid value and the peroxide number of the extracted oils were determined as described in AOAC (1985). Aflatoxins were detected as described in AOAC (1984) .

3-a The extracted oils containing aflatoxins were exposed to sunlight for 1hours, and the Foremetioned properties were determined to evaluate the effect of the sun UV. on the oil stability.

4. The wastes of oil extraction such as peanut cake, olive pomace and corn meal were subjected to UV light for aflatoxin detection. The positive samples (control) showing BGYF were divided into four parts in plastic bags. One was stored in a sunny place, while the second was kept in a dark place for one week of storage. The third sample was mixed with 1% Fuller's earth and the fourth sample was treated with 0.1N NaOH. Detection of BGYF was carried out for the above samples during this week of storage.

5. Since peanut and olive oils were not subjected to the refining process, thus corn germ oil only was subjected to neutralization of its acidity using 0.1N NaOH, then bleached using 1% Fuller's earth.

For each of the above treatments, the detection of aflatoxins was determined according to AOAC (1984).

6. Confirmation tests of the role of physical detoxification methods were carried out using 1kg of corn embryos and artificially contaminated with pure strains of *A.parassiticus* producing aflatoxins obtained from the central lab. at Ain Shams University. The treatments in tripicates were the following:

6.a Contamination of corn embryos with *A.parasiticus* and kept in sterilized petri dishes for 2 weeks incubation at room temperature (25-28oC).

6.b The contaminated samples were exposed to sunlight for one week.

6.c Heating the contaminated corn embryo samples in an oven at 100oC for 2hrs.

6.d Extraction of corn oil using n-hexane from the artificially contaminated samples.

6.e Neutralization of the forementioned extracted oil with 0.1 N NaOH.

6.f Bleaching the contaminated extracted oil with 1% Fuller's earth for 1hr. The above six treatments were detected for aflatoxings as described in AOAC (1984).

7. Determination of moisture, the moisture content was determied as described by AOAC (1985) for all the previous samples.

## RESULTS AND DISCUSSION

Most mycotoxins in raw foods are usually found in a small proportion. Thus, sorting the infected foods could be primilarly effective and economical method to reduce the mycotoxin content and to remove the few infected raw kernels or food items. It is shown in table 1, that an average of 28 to 48% of the naturally infected

foods could be sorted under the ultraviolet rays and the characteristics of Blue Greenish yellow fluorescent (BGYF). Thus, the trials of Ashworth *et al.*, (1968). Escher, (1971) and Tanboon (1989) could be used with good results to estimate the aflatoxin infections in food and feeds. In addition, it is a quick method to screen the infected parts from the lot of the raw commodities. Moreover, the contaminated corn germ and corn meal with the strains of pure *A.parasiticus* showed a highly toxin production Table 1.

The positive effect of sun rays in reducing the amount of the sorted infected samples can clearly be observed in table 1. Mean-while, the infected samples were increased when kept in the shadow for a week.

The comparison between the treatment with Fuller's earth and alkali on the infected samples is shown also in table 1. The percentage of infected samples treated by Fuller's earth were lower than the samples treated with alkali, which means the high effect in reducing aflatoxins by adsorption.

Table 1. Percentage of the sorted infected samples by BGYF after one week of storage.

| Samples | Control | Storage | | Treatments | |
|---|---|---|---|---|---|
| | | Under sun | In shadow | Fuller's earth | Alkali |
| Raw peanut kernels | 48 | 30 | 70 | --- | --- |
| Ripe black olives | 30 | 15 | 65 | --- | --- |
| Corn embryos | 40 | 12 | 80 | --- | --- |
| Peanut cake | 36 | 25 | 95 | 24 | 29 |
| Olive pomace | 28 | 38 | 80 | 5 | 25 |
| Corn meal | 35 | 40 | 83 | 10 | 34 |
| Contamin. corn embryos | 100 | 65 | 100 | 50 | 53 |
| Contamin. corn meal | 80 | 50 | 100 | 30 | 64 |

Table 2 shows the effect of heat and sun rays treatments on the inhibition of the fungal growth during the storage period of 2,4 and 7 days. From this table, it can be observed that the control, i.e., the naturally infected samples were gradually increased in fungal infection during storage. This increase of fungal infection could be eliminated by heating the samples in an oven at 100°C for 2hours. This method showed better results on mold inhibition rather than the storage exposed to the sun rays. This observattion could be due to the dehydration of the samples and subsiquently the decrease of the water activity Table 4. The moisture content in the

heated samples was about one half the moisture percentage of the samples stored in the sun. Thus, lowering the water activity inhibited the fungal growth. This phenomena was reported by Scott (1984) for roasting the naturally contaminated peanuts.

Table 2. Percentage of fungal infection of food samples exposed to sun. and Heat.

| Samples | Storage time in days | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 2 | | | 4 | | | 7 | | |
| | Control | Sun | Heat | Cont. | Sun | Heat | Cont. | Sun | Heat |
| Raw peanut kernels | 2 | 0 | 0 | 15 | 2 | 0 | 50 | 10 | 0 |
| Ripe black olives | 5 | 7 | 0 | 25 | 10 | 0 | 75 | 25 | 0 |
| Corn embryos | 6 | 0 | 0 | 10 | 0 | 0 | 38 | 13 | 0 |
| Peanut cake | 7 | 0 | 0 | 15 | 5 | 1 | 60 | 10 | . 1 |
| Olive pomace | 10 | 0 | 0 | 25 | 10 | 0 | 75 | 14 | 0 |
| Corn meal | 2 | 0 | 0 | 3 | 0 | 0 | 25 | 3 | 0 |
| Contamin. corn embryos | 15 | 5 | 0 | 25 | 7 | 0 | 75 | 15 | 100 |
| Contamin. corn meal | 20 | 0 | 0 | 30 | 5 | 0 | 85 | 30 | 3 |

The results in table 3 show the characteristics of the extracted oils. Stability of oil is measured by its amount of hydrolysis and its rancidity. Thus, acid and peroxid peroxide values were our main factors to determine oil stability. It is clearly observed from table 3, the increased amount of acid and peroxide values of the infected samples compared to the non-infected ones. This observation is in agreement with Farag *et al.* (1986) who mentioned that such increase was due to the lipolysis and the metabolism of fungi. It can be seen that the amount of total aflatoxins in peanut oil was higher than 20ug/kg which is the safer limit of aflatoxins, while it was in the safe level in olive and corn oil. Also, from the same table the total destruction of the low amount of aflatoxins in the naturally infected corn oil, up on its exposure to sunlight or treatent with alkali or Fuller's earth, is aviaent. Although sun rays destructed aflatoxins, the acid and peroxide values were increased, in contrast to the treatment with the Fuller's earth which adsorbed the amounts of free fatty acids and the oxidative products. So, our conclusion could be directed to the use of sun rays with oils which have very are not subjected to the refining process. Moreover, neutralization of the oil acidity was the best treatment to destruct all aflatoxins followed by the treatment with Fuller's earth. This observation is in agreement with those of Parker and Melnick (1966) and Aziz *et al.* (1996).

The Results in table 5 show the % reduction of aflatoxins of different samples treated with the physical methods. Heating was found to be more effective with the

Table 3. Characteristics of the extracted oils.

| Samples | Acid Value | Proxid Number | Total aflatoxings ug/kg |
|---|---|---|---|
| Non-infected | | | |
| Peanut oil | 0.7 | 10.7 | traces* |
| Olive oil | 0.3 | 9.1 | traces |
| Corn oil | 5.2 | 8.6 | tracces |
| Infected | | | |
| Peanut oil | 2.4 | 20.3 | 34.6 |
| Olive oil | 3.1 | 13.2 | 18.4 |
| Corn oil | 17.8 | 22.1 | 10.8 |
| Corn oil treated with alkali | 0.7 | 22.8 | traces |
| Corn oil treated with Fuiler | 1.8 | 21.5 | traces |
| Corn oil treated in sunlight | 19.5 | 23.7 | traces |
| Artificially contaminated | | | |
| Corn oil | 25.3 | 28.2 | 132 |
| Corn oil treated in sunlight | 28.1 | 32.3 | 86.9 |
| Corn oil treated with heat | 29.3 | 35.0 | 99.2 |
| Corn oil after neutralization | 0.7 | 28.1 | 0 |
| Corn oil after bleaching | 1.8 | 20.6 | 15 |

* Traces : Lower than 10 ug/kg.

Table 4. Percentage of moisture content of different food samples.

| Samples | Healthy | Infected | Treatment | |
|---|---|---|---|---|
| | | | Sunlight | Heat |
| Raw peanut kernels | 19.3 | 19.8 | 15.2 | 8.6 |
| Ripe black olives | 45.8 | 46.8 | 38.5 | 20.1 |
| Corn embryos | 9.5 | 10.1 | 8.1 | 4.2 |
| Peanut cake | 15.1 | 17.2 | 13.5 | 11.2 |
| Olive pomace | 20.3 | 21.6 | 15.2 | 6.4 |
| Corn meal | 5.3 | 5.9 | 5.1 | 4.1 |

whole kernel or fruit or seed, while peanut cake, olive pomace, or corn meal which had large surface area were more affected when exposed to the sunlight. These results are also found by Escher (1974), Hamada and Magella (1982) and Pluyer *et al.*, (1987), when they roasted peanuts, soybeans and pecans to reduce aflatoxins. On the otherhand, Mahjoub and Bullerman, (1986) and Shantha (1987) exposed olive and peanut oils and peanut oils and peanut cake to sunlight for detoxification.

Fuller's earth showed the best results with the low amounts of aflatoxins, hence only traces, i.e. lower than 10 ug/kg were found in the naturally infected samples ater their treatment. The artificially contaminated samples had also a great decrease between 65.7 and 92.6% of the produced aflatoxins. These results are positive with those of Srikumlaithong and Munsakul (1983), Smith *et al.*, (1994) and Aziz *et al.* (1996).

The alkali treatment (0.1N) had a good destructive effect (about 100%) on aflatoxins at low level in the naturally infected samples, while the highly contaminated samples had 74 and 83.1% reduction of aflatoxins in the corn embryos and corn meal, respectively. The role of alkali to destruct of aflatoxins in the corn embryos and corn meal, respectively. The role of alkali to destruct aflatoxins was explained by Parker and Melnick (1966) who concluded that alkali interfer with the chemical composition of aflatoxins.

Table 5. Reduction, %, of aflaxtoxins of different food samples treated with physical methods.

| Samples | Control ug/kg | Sun ug/kg | Loss % | Heat ug/kg | Loss % | Fullers earth ug/kg | Loss % | Alkali ug/kg | Loss % |
|---|---|---|---|---|---|---|---|---|---|
| Infected raw peanuts | 126.9 | 101.0 | 20.4 | 60.4 | 52.4 | --* | -- | -- | -- |
| Ripe black olives | 58.2 | 40.3 | 30.7 | 41.0 | 29.5 | -- | -- | -- | -- |
| Corn embryos | 60.4 | 39.5 | 34.6 | 35.0 | 42.1 | -- | -- | -- | -- |
| Peanut cake | 87.4 | 40.8 | 53.3 | 47.2 | 45.9 | trace | 100 | trace | 100 |
| Olive pomace | 43.2 | 25.3 | 41.1 | 30.4 | 29.6 | trace | 100 | trace | 100 |
| Corn meal | 55.0 | 20.2 | 63.6 | 35.0 | 36.3 | trace | 100 | trace | 100 |
| Contamin. Corn embryos | 437.5 | 126.9 | 70.9 | 235.2 | 46.2 | 150.0 | 65.7 | 72.8 | 74 |
| Contamin. Corn meal | 280.7 | 58.4 | 79.2 | 49.0 | 82.5 | 20.8 | 92.6 | 48.3 | 83.1 |

* Samples were not treated

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# تقييم الطرق الطبيعية للتخلص من السموم الفطرية فى بعض الاغذية الملوثه

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حيث انه من الصعب منع التلوث بالسموم الفطرية، فقد اقترحت الكثير من الوسائل للتخلص منها. وهذه الطرق تشمل الطرق الطبيعية ومنها :-

الفصل ، التسخين ، الاشعاع ، الاد مصاص والاستخلاص.

هذا وقد استخدمت لهذه الدراسة عينات ملوثه طبيعيا من الفول السودانى الطازج والزيتون الاسود وجنين الذره. وكذلك زيوتها المستخلصه والنواتج الثانوية لها.

حيث امكن فصل عينات ملوثه تتراوح ما بين ٢٨ ، ٤٨٪ بواسطة الاشعه الفوق بنفسجيه. كذلك اظهرت المعامله بالتسخين نتائج افضل من تلك التى عرضت لضوء الشمس عن طريق منع نمو الفطريات اثناء فترة التخزين ، نتيجه عملية التجفيف للعينات المصابه ، بالاضافة الى أن التسخين يعتبر أكثر كفاءة فى حالة الثمار والحبوب الكامله.

كذلك امكن التخلص من الافلاتوكسين بمعادلة حموضه الزيوت بالقلوى وكذلك المعامله بتراب التبيض بنسبة ١٪.

كما ظهرت كفاءة تأثر اشعه الشمس على خفض مستوى السموم الفطرية فى كسب السودانى والزيتون والذرة وذلك لكبر المساحة السطحيه المعرضه لاشعة الشمس. كذلك المعامله بتراب التبيض (١٪) قد اعطت أفضل النتائج فى التخلص من الكميات القليلة من الافلاتوكسين حيث انخفضت بنسبة ٦٥,٧ و ٩٢,٦ / فى العينات الملوثه بالفطر.