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EFFECT OF SOYBEAN OIL REFINING PROCESSES ON AFLATOXINS

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Abstract

Fate of aflatoxins during refining of crude soybean oil was studied. The effect of degumming, alkaline refining and bleaching operations on aflatoxin residues in the crude oil was studied by spiking thé oil with the aflatoxin standards B1, B2, G1 and G2 at a concentration of 1 ug/ml each. Using 1% water in the degumming process reduced aflatoxins by 68%. Alkali refining, using 0.1 N NaOH had great influece on aflatoxin reduction and no fluorescence could by seen on TLC plate for aflatoxin detection. The most important step was the use of the Fuller's earth in the bleaching process. The longer the time, the higher the temperature and the higher the concentration of Fuller's earth used, the greater detoxification of aflatoxin in oil during bleaching was obtained. Total reduction was achieved by using 1 % Fuller's earth at 80C for 30 minutes or 2% Fuller's earth at 60C for 15 minutes.

Refining operations could help produce soy-oil edible and safe for human nutrition even if soybeans were fungal contaminated or aflatoxins remained in the extracted oil.

INTRODUCTION

Soy - bean is the leading source of high quality plant protein and edible oil in the United States and certain other countries (Smith and Circle, 1978). Significant quantities of cottonseed, corn, peanut, safflower and sunflower seed oils are also utilized worldwide. In general, the same technological processes are applied for the extraction and refining of all these oils with minor modifications based on the characteristics of each oil (Mounts, 1981). Chemical and physical tests as well as detection of minor mycotoxins are common procedures for oils quality control. This is perfectly realized when it is known that a variety of barley, rice, wheat, corn, soybeans, cottonseeds, peanuts and oats had been shown to support the growth of *Aspergillus flavus* invarying degrees with resultant aflatoxin production (Slocum, 1963).

Only a limited work had been done on mycotoxins in edible oils. Parker and Melnick (1966) conducted the first definitive study of the fate of aflatoxins in vegetable oil undergoing processing. They found that conventional alkali refining and washing of the oils reduced aflatoxin content to a range of 10 to 14 ppb.

Subsequent bleaching operation essentially eliminated aflatoxin from the oils obtained from corn germ deliberately contaminated in the laboratory with Aspergillus flavus. Aziz (1990) studied the detoxification of vegetable oils using physical and chemical procedures. The physical mean of oil detoxification using Fuller's earth reduced 75% of afaltoxin in the oil.

The purpose of this work is to study the fate of aflatoxin in crude soybean oil following the industrial refinery operation; degumming, neutralization and bleaching. Variations of adsorbent concentrations, reaction time and temperature combination were tested to achieve the maximum levels of detoxification in the refined soybean oil.

MATERIALS AND METHODS

Source of crude soybean oil

crude soybean oil was obtained from a pilot plant (INTSOY, Illinois) using the extruder and expeller oil extration technique. Since it was not possible to obtain oil that was naturally highly contaminated aflatoxins, spicking each oil with the standard aflatoxins B1, B2, G1 and G2 was done at the level of 0.25 ug/ml.

Aflatoxin standards were obtained from Sigma Chemical Inc. St Louis, Mo, USA. Aflatoxins were dissolved in methanol to reach the concentration of 0.5 ug/ul.

Four ml of this aflatoxin standard solution was added to 800 ml of crude soybean oil for the subsequent refining operations.

The refining operaions consisted of the following :

1. Degumming by adding 1,2 and 3% water based on oil volume (carr, 1978). The mixture was agitated for 30-60 minutes at 70-80C, settled, filtered or centrifuged.

2. Alkali refining : Neutralization of soybean oil was based on the free fatty acid content determined by the procedure described by AOCS (1978). 0.15% or more of IN NaOH was added after neutralization (Parker and Melnick, 1966). The obtained soap-oil mixture was heated to 75-80 C and fed to a centrifuge for oil soap separation (Mounts and Khym, 1980).

3- The bleaching process: It is referred to as adsorption treatment. The process consists of agitation of the oil in 1 to 2% acidic actiated earth at 60-95C for 5 to 45 minutes under vacuum followed by filtration to get clean and clear oil (King and wharton, 1949; Goebel, 1981).

Two samples of each of the above refining steps were collected and subjected to aflatoxin quantification with TLC method after extraction (Parker and Melnick, 1966). The spotted TLC plates were developed in 100% ethyl ether first, air dried, and then developed in chloroform : acetone (9:1) solvent system (AOAC, 1984). The developed plates were scanned with a CAMAG TLC scanner II with CATS software attachment (Sonnemattstrasse 11, CH-4132 Mattenz, Switzerland), interfaced with an IBM model 50 computer.

RESULTS AND DISCUSSION

Degumming operation

Results of detection of the aflatoxins B1,B2, G1 and G2 in the oil samples after performing the degumming operation by adding 1%, 2% or water at 70C and agitation for 30 minutes, are shown in Table . It was evidemt that the recovery of aflatoxins B1 (AB1) and AG2 occurred at the three percentages of water treatments. Using 3% water was in effective in decreasing the amount of the four aflatoxins.

Although 1% water treatment reduced AB2 and AG1 to a non-detectable amount, the recovery of 93% of AB1 was still of great concern due to its high toxicity among all other aflatoxins (Bullerman, 1986), Using 2% water decreased only AB1 by 49% atlhough the recovery of the total aflatoxins was the highest among the three degumming treatments. This could be due to polarity which retained the added aflatoxins.

Alkali refining

Crude soybean oil was refined with alkali to neutralize its acidity. An excess amount of 0.15% alkali (0.1N NaoH) was added to protect oil from the quick acid buildup. This operation was very important for aflatoxin reduction in the oil. As shown in Table 1, neutralization and excess of alkali reduced about 100% of the aflatoxins added to the oil samples. This could be attributed to the interfering of alkali with the chemical composition of aflatoxins as well as the precipitation of aflatoxins with the soapy layers to the bottom of the oil after which it was separated by centrifugation.

Bleaching operation

Fuller's earth was added to soybean oil at 1 or 2% (w/w) of the oil containing pure aflatoxins, and agitation of the oil samples was performed to allow the Fuller's earth particles to be distributed in oil samples. Different heat treatments (30, 60 and 80C) as well as increasing the bleaching operation time were studied for their influence on the amount of aflatoxins recovered. Data recorded in Table 2 showed that 2% Fuller's earth had higher affinity in reduction of aflatoxins especially at 60 and 80 C than treatment with 1%. In addition, 2% Fuller's earth used at 30C reduced the three aflatoxins (AB1, AB2, AG) and the amount of AG2 were decreased by increasing the time of the bleaching process. Beside the importance of time for aflatoxin reduction, temperature is also of great concern. Since aflatoxins are known to be fairly heat resistant (Mahjoub and Bullerman, 1986), heat alone did not destroy the aflatoxins but the adsorbance affinity between Fuller's earth and aflatoxins had increased at the higher temperature (80C) than at 60 or 30C. These results are in agreement with Parker and Nelnick (1966) and Aziz (1990) who found that the Fuller's earth had reduced the amount of aflatoxins in refined oils.

It is apparent that the refining procedures including degumming by 3% water, alkali neutralization, excess addition of 0.15% alkali to soybean oil and bleaching using 2% acidified Fuller's earth at 60-80C for 15 minutes had eliminated alfatoxins and produced soybean oil free from aflatoxins. These results are in agreement with those of Parker and Melnich (1966) who mentioned that the nonfluorescing forms of aflatoxins, capable of being produced during the alkali refining operations are also absent from the refined vegetable oils. The same authors determined amount of aflaoxin that remained in the refined oil using only one processing procedure for each oil. They used 1.8 g of Fuller's earth to 60 g of crude peanut at 90C for 5 minutes and 0.6% Fuller's earth at 82 C for 30 minuters corn oil. The current work however had a wide range of temperatures, time of processing and pecentages of the adsorbent (Fuller's earth). Fuller's earth is proposed to be used for oils discoloration and as a detoxifying agent. It is important in view of producing vegetable oils free from aflatoxins and safe for human consumption.

Data in Table 3 show the mortality percentage of the tested shrimp larvae. It is clear that the degumming process using 1% water decreased the mortality percentage from 73 to 31%. These results are in agreament with those obtained from TLC scanning. The results in Table 1 show that 1% degumming had reduced the total aflatoxins recovery from 400 to 126. The other treatments using 2% and 3% water were higher in total aflatoxins recovery results of the refining process using NaOH for neutralizing the amount of free fatty acids was in agreement with the data obtained from HLC method. Both alkaline treatments gave very low mortality. The alkaline procedure is always preceeding neutralization to prevent the build up of free fatty acids.

Using Fuller's earth to adsorb impurities and purify oil from pigments in the bleaching process is most important for also eliminating aflatoxins which could be contaminating oil seeds direted for oil processing. To confirm TLC results of time and temperature combinations during bleaching with Fuller's earth, the brine shrimp bioassay was undertaken. Using Fuller's earth (1%) at room temperature for 5 minutes produced 4% mortality. This low mortality could be due to the absence of oxidative compounds which could build up by heating the oil or by exposing it to air and light for a period of time. The best treatments which had the lowest mortality (2%) was that using 2% Fuller's earth at 30C for 45 minutes, followed by that hoving 1% Fuller's earth at 80C for 30 minutes (3% mortality).

Treatments having Fuller's earth (2%) at 30C or 60C for 30 minutes produced 4% mortality. This showed that up 60C and for 30 minutes exposure was still safe and effectue for he non accumulation or build up of oxidative compounds in oil during the bleaching process.

In condlusion, using higher concentration of Fuller's arth (2%), and increasing the time from 15 to 30 minutes at a temperature not excedding 60C, give the same effetiveness of aflatoxin elimination from oils.

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Table 1. Prrcentage of aflatoxin recovery after degumming and refining soybean oil.

	Aflatoxins			
	B1	B2	G1	G2
Control	100	100	100	100
Degumming				
1% Water	93	0	0	33
2 % Water	51	99	88	78
3 % Water	55	0	61	60
Alkali refining				
Neutralization	0	0	0	0
Excess of Alkali	0	0	0	0

Table 2. Prrcentage of aflatoxin recovery after bleaching operations in soybean oil.

		Aflatoxins			
		B1	B2	G1	G2
Control		100	100	100	100
Fuller's earth	mintes				
1% - 30°C	5	29	trace	45	92
	15	tr.	tr.	20	84
	30	0	0	0	47
	45	0	0	0	39
2% - 30°C	5	tr.	tr.	0	80
	15	tr.	tr.	0	37
	30	0	0	0	21
	45	0	0	0	20
1% - 60°C	5	tr.	tr.	0	83
	15	0	0	0	50
	30	0	0	0	38
	45	0	0	0	21
2% - 60°C	5	tr.	tr.	0	tr.
	15	0	0	0	0
	30	0	0	0	0
	45	0	0	0	0
1% - 80°C	5	tr.	tr.	0	47
	15	0	0	0	20
	30	0	0	0	0
	45	0	0	0	0
2% - 80°C	No Aflatoxins detected				

Table 3. Brine shrimp bioassay for oil samples during the refining process.

Oil treatment		Brine shrimp mortality (%)	
		Average	Range
Control (No refining of the spiked oil)		73	63-83
Degumming - 1% Water		61	5-45
2 % Water		64	49-77
3 % Water		68	58-86
Alkaline - Netral		3	0-9
- Excess		4	0-8
Fuller's earth 1%			
- room temperature,	5 min	4	2-5
-30°C	5 min	12	7-20
	15 min	9	4-17
	30 min	12	10-16
	45 min	9	3-10
- 60°C	15 min	13	11-16
	45 min	8	6-10
- 80°C	15 min	16	8-31
	30 min	3	2-4
	45 min	52	25-78
Fuller's earth 1%			
- room temperature,	5 min	11	10-13
-30°C	5 min	7	0-11
	15 min	7	4-9
	30 min	4	2-6
	45 min	2	0-4
- 60°C	30 min	4	2-5
	45 min	7	6-9
- 80°C	15 min	11	6-19
	30 min	10	2-16
	45 min	91	89-94

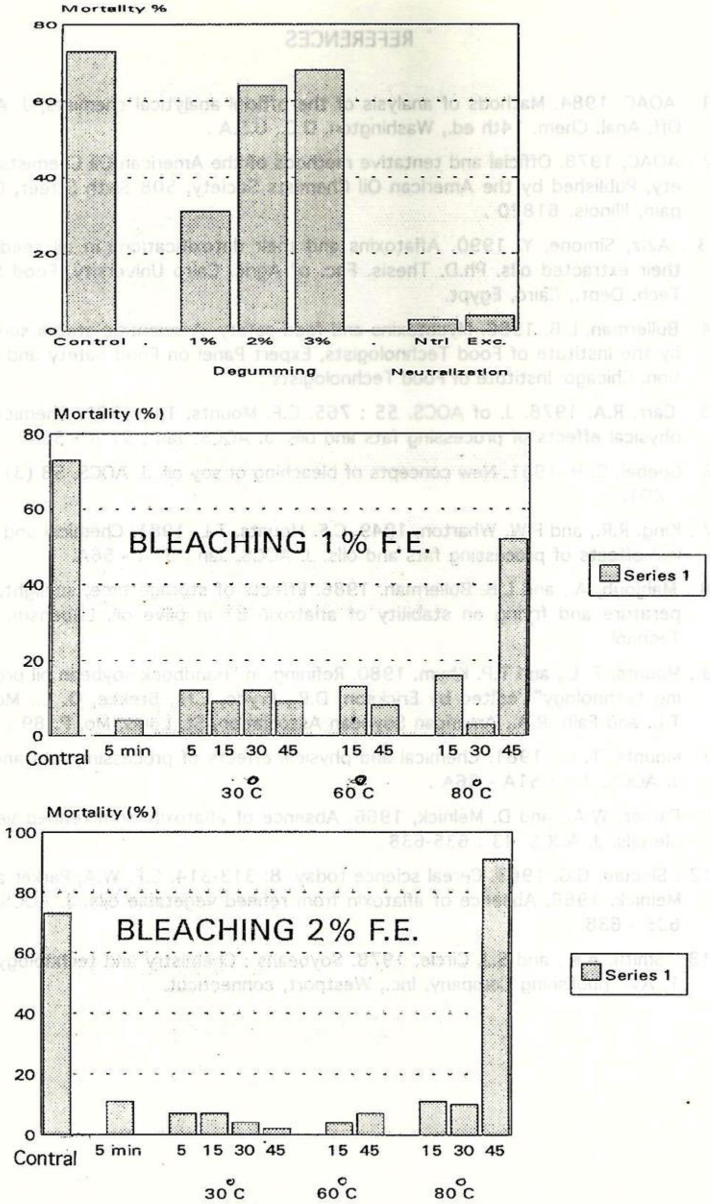

Fig.1. Bring shrimp Bioassay for oil samples during refining processes.

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تأثير عمليات تكرير زيت فول الصويا على الأفلاتوكسينات

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تم دراسة إمكانية التخلص من الأفلاتوكسينات أثناء عمليات تكرير زيت فول الصويا الخام. أظهرت النتائج أثر عمليات إزالة الصموغ والمعادلة بالقلوى والتبيض على الأفلاتوكسينات المتبقية فى الزيت والتى تم إضافتها الى الزيت الخام على هيئة محاليل قياسية تركيزها ١ ميكروجرام / مل من كل من ب١ ، ب٢ ، جـ١ ، جـ٢.

ويتلخص هذا التأثير فيما يلى : استخدام تركيز ١٪ ماء فى عملية إزالة الصموغ خفض الأفلاتوكسينات المضافة بنسبة ٦٨ ٪. وباستخدام ص أيد ١ ,٠ ع لمعادلة القلوى كان له أثر كبير فى التخلص من الأفلاتوكسينات ولم يظهر أى لون فلورسانس على الأطباق الرقيقة للكشف الكروماتوجرافى .

إستخدام تراب التبييض فى عملية التكرير كان له أثر واضح فى التخلص من الأفلاتوكسينات حيث أن ارتفاع الحرارة وارتفاع نسبة تراب التبييض قد أدى الى نتائج جيدة من الأفلاتوكسينات فى الزيت أثناء عملية التبييض إستخدام ١٪ تراب تبييض مع ٨٠م لمدة ٣٠ دقيقة أو ٢٪ منه مع ٦٠ م لمدة ١٥ دقيقة قد أدى الى التخلص الكامل من الأفلاتوكسينات .