

# Production of Some Chemically Modified Adsorbents from Some Field Crops by- Products to Reduce Ochratoxin A

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**Abstract:** This study aimed to produce some chemically modified adsorbents (CMA) from some field crops by- products to reduce ochratoxin A (OTA). Chemical modification was performed on broken rice starch, soybean hulls, and wheat bran to produce acetylated rice starch (ARS 8, ARS 16%), modified soybean hulls (MSH), and micronized wheat bran (MWB). The presence of the new functional groups due to chemical modification was checked via Fourier Transform Infrared Spectroscopy (FTIR) method. The incidence of OTA in four types of commercial apple juice samples was studied. The efficacy of the chemically modified adsorbents (CMA) for reducing OTA at 500 and 1000 mg was tested via HPLC method and applied at contaminated apple juice samples. From FTIR different stretching bonds and new functional groups at different band positions relates to chemical modification were detected. MSH, MWB, ARS 8% and ARS 16% at 500 and 1000 mg were reduced OTA spiked solution by (73.2, 82.7); (79.5, 84.03); (66.5, 72.0), and (70.9, 75.8)%, respectively. The total number of contaminated apple juice samples with OTA was 33 out of 150, and 13 of them exceeded the permissible limits of the EU. The reduction of OTA in all contaminated apple juice samples using CMA was 100%, and the treated samples were highly acceptable by the panelists, and there were no significant changes in appearance, color, and flavor. The results of this study could be useful in utilizing broken rice, soybean hulls, and wheat bran; improving their adsorption capacities via chemical modification with acids and delivering highly reactive adsorbents to the food processors to produce safe food and remove OTA from contaminated apple juice.

**Keywords:** Field Crops by-Products, Chemical Modifications, Adsorption, Ochratoxin A and Apple Juice

## 1. Introduction

Nowadays, the motivation for the utilizing of the agricultural by-products is of great interest worldwide, due to their value added potentialities and high environmental impact [1-2]. Broken rice starch, soybean hulls, and wheat bran have been studied as potential precursors for the adsorption process [3-5]. Adsorbents derived from these by-products are offering guaranteed advantages, as they are low-cost, renewable and available in large amounts [6], besides their chemical stability and high reactivity [7]. These materials can be used, either directly or after chemical modification, in the adsorption process [3]. Chemical modification with acids such as acetylation, dehydration, and

micronization is considered to be an excellent method to improve the adsorption capacities of these sorbent materials. It provides more active sites, better ion- exchange, and significant adsorbate-adsorbent interaction [6-8] Ochratoxin A (OTA) is one of 400 types of identified mycotoxins produced by several species of *Aspergillus* and *Penicillium* fungi. The toxin has been classified as a possible human carcinogen especially, group 2B [9-10]. Ochratoxin is characterized by widespread occurrence in food and feed. It is found in cereals, pulses, dried fruits, cocoa beans, coffee beans, olives, and spices. Besides it can survive most food processing steps; therefore it appears in cereal- derived products, wine, beer, meat and cheese products, and juices [11-12]. In consequences, large efforts and several strategies

were undertaken to decrease the incidence of OTA in agricultural commodities and food products. That may result in decrease the tolerable daily intake (100- 120 ng/kg bw/week) of OTA in consumed food [13-15].

Although adsorption is now recognized as a protective and efficient technique against mycotoxin contaminated diets in particular OTA in poultry feed [16-19]. Besides, some materials are selective for removal of OTA in solution includes activated charcoal, and novel carbohydrates such as  $\beta$ -D-glucans isolated from *Saccharomyces cerevisiae* and silver nanoparticles [20-23]. However, there is no available information about the efficacy of acetylated broken rice starch, dehydrated soybean hulls, and micronized wheat bran as chemically modified adsorbents against OTA in food, especially apple juice. Therefore, this work was undertaken to deal with such information.

## 2. Materials and Methods

### 2.1. Materials

Broken rice was obtained from rice variety Sakha 104 after milling and polishing of brown rice at local milling processing unit, Damietta Governorate, Egypt. Soybean hulls were obtained from Soybean Factory, Food Technology Research Institute, Agricultural Research Center, Al- Gaiza, Egypt. Wheat bran was obtained from Crops Technology Research Department, Food Technology Research Institute, Agricultural Research Center, Al-Giza, Egypt. OTA standard was purchased from Sigma, Chemical Co. (St. Louis, MO, USA). All solvents were of HPLC grade. The water was double distilled with Millipore water purification system (Bedford, MA, USA).

### 2.2 Apple juice Sampling

One hundred and fifty apple juice samples were collected randomly during the period of October to December 2016 from four commercial types of apple juices in different super markets located at Al-Giza government, Egypt. Fifty samples from each were collected monthly. These samples were stored in refrigerator at 4°C, until further analyses.

### 2.3. Methods

#### 2.3.1. Isolation of Starch from Broken Rice

Starch was isolated from broken rice according to Raina *et al.* [4]. Broken rice was steeped in deionized water for 18hr/4°C, followed by grinding in a wet grinder. The starch paste was then steeped in 0.25 g/ 100ml alkali solution containing 0.12 g/ 100ml  $\text{Na}_2\text{S}_2\text{O}_5$  for 18hr/ 4°C, followed by decanting the supernatant. The procedure was repeated thrice. Then, the paste neutralized with 0.5 M HCl and washed thrice with distilled water to remove the salt content and filtered through a Büchner funnel under vacuum. The cake was dried at 50°C to about 12 g/ 100g moisture content. After that sample was grounded, passed through a 75-mm sieve and stored in an airtight container at ambient temperature until further use.

#### 2.3.2. Acetylating of Broken Rice Starch (ARS)

Broken rice starch was acetylated according to the method of Wolff *et al.* [24]. Four hundred grams of broken rice starch were dispersed in 900 ml distilled water and stirred for 60 min/25°C. The obtained suspension was adjusted to pH 8.0 using 3 g/100ml NaOH. Acetic acid anhydride was added (8 and 16 ml/ 100 ml respectively, on dry starch basis) to the stirred slurry, while maintaining the range within pH 8.0- 8.4 using 3gm/ 100 ml NaOH solution. The reaction was kept for 10 min after acetic anhydride addition. Finally, the slurry was adjusted to pH 4.5 using 0.5 M HCl, centrifuged at 2000 rpm/ 3 min, washed thrice with distilled water to discard any acidic residue and air-dried.

#### 2.3.3. Modification of Soybean HULLS (MSH)

The soybean hulls were modified according to the method of Jia *et al.* [5]. Soybean hulls were sieved to obtain particle size range 0.6-1.7 mm. About 1.0 g sieved sample was dissolved in 0.08 mol/L NaOH aqueous solution at 250 ml Erlenmeyer flask. The mixture was shaken at 140 rpm / 8 h/25°C. Then the pH was neutralized with de-ionized water. Followed by, adding of 150 ml of 0.6 mol/ L citric acid as a modified agent, and heating at 110°C/1.5 h. Finally, the mixture was neutralized with de-ionized water, filtered and, dried at 60°C / 24 h.

#### 2.3.4. Micronized Wheat Bran (MWB)

The wheat bran was micronized according to Özer *et al.* [3]. Wheat bran was sieved through 50 mesh sieve to remove any foreign materials or large solids. About 100g of sieved samples were digested in 200g concentrated sulfuric acid (W/W) with continuous stirring for 24 h. Then the mixture was washed for several times with distilled water using water pump until the final pH was reached 3.0. At the end of the process, the dehydrated wheat bran adsorbent was screened through 50 mesh sieve and stored in a closed bottle for further use.

#### 2.3.5. Preparation of OTA Spiked Solution

Stock solutions and standards of OTA were prepared and assayed according to AOAC Method 971.22 [25]. A stock solution of OTA was prepared by dissolving 1mg of OTA in 10mL of methanol. Spiked concentration (10 ng/mL) of OTA standard were prepared by adding 10 $\mu$ L to 100 mL phosphate buffered saline (PBS).

#### 2.3.6. Fourier Transform Infrared Spectroscopy (FTIR)

The characterization of functional groups on the surface of acetylated rice starch (ARS 8, ARS 16%), modified soybean hulls (MSH), and micronized wheat bran (MWB) was performed by Fourier Transform Infrared Spectroscopy (FTIR 6100; Perkin-Elmer), and the spectra were scanned in the 400–4000  $\text{cm}^{-1}$  range at a resolution of 4 cm.

#### 2.3.7. OTA Removal Assay with Contaminated PBS

Chemically modified adsorbents (CMA) included MSH, MWB, ARS 8% and ARS 16% were tested at 500 and 1000 mg for each CMA/ 100ml PBS W/V for their ability to remove OTA in contaminated PBS. CMA was added to 100mL Phosphate Buffer Saline (PBS) contaminated with a

standard working solution of OTA at 10 ng/mL. Samples were shaken for 30 min at 25°C. All experiments were performed in triplicate.

### 2.3.8. Extraction and Determination of OTA from PBS and Apple Juice

Apple juice samples were deputed according to Niu et al. [26] using activated C<sub>18</sub> solid phase extraction column, Varian, Palo Alto, USA, flow rate: 1.5 mL/min, solvents: methanol/deionized water (2:3). The obtained eluents were adjusted to 2 mL, and then stored at -20°C in dark bottles prior to HPLC analysis. OTA concentrations in samples were determined by means of HPLC. The system is consisted of Waters Binary Pump Model 1525, Phenomenex C<sub>18</sub> (250 X4.6 mm, USA), solvents: acetonitrile: water: acetic acid (55:43:2), flow rate: 1.0 ml/min, and the data workstation with software Breeze 2i.d. Samples were identified against standard solution of OTA in PBS using fluorescence detector operated at wavelength of 335 nm for excitation and 465 nm for emission.

### 2.3.9. Efficacy of CMA for Removal of OTA from Contaminated Apple Juice Samples

The efficacy of CMA for removal of OTA was studied in the contaminated apple juice samples with high concentration of OTA. Two concentrations of CMA (500mg and 1000mg) were studied. CMA was added to the highly contaminated apple juice samples and left for 15 min then filtered. The percentage of reduction of OTA was calculated as 100% reduction referenced to untreated samples.

### 2.3.10. Sensory Acceptability of Apple Juice Samples

A panel of fifteen members from Food Technology Research Institute, Agricultural Research Center, Al-Giza, Egypt was used to judge the acceptability of apple juice samples after the removal of OTA using CMA followed by filtration. The panelists were asked to evaluate each sample for appearance, consistency, color, flavor and overall acceptability using a 9 point hedonic scale from 1 to 9 as follows: 1= extremely bad; 5= Satisfactory; and 9= Excellent. The order of the samples was randomized and given codes before being tested by the panelists.

### 2.2.11. Statistical Analysis

Data obtained from this study were subjected to an analysis of variance ANOVA. Duncan's multiple range test at 5% level was used to compare between means according to Steel et al. [27].

## 3. Results and Discussion

### 3.1. FTIR Analysis of Chemically Modified Adsorbents (CMA)

The FTIR analysis of bonds and functional groups in rice starch (RS), wheat bran (WB), soybean hulls (SH), and their CMA is presented in Tables (1, 2, and 3). The results in Table (1) indicated that rice starch (RS) shows the difference in C structure as well as acetylated rice starch (ARS 8 and ARS 16%). There was strong OH stretching from 3300 to 4000 cm<sup>-1</sup>, C-H stretching in methyl and methylene groups from 2800 to 3000 cm<sup>-1</sup>, and a strong, broad superposition with sharp absorptions from 1000 to 1750 cm<sup>-1</sup>. The peak No. 4 showed bands positioned at 2852.2cm<sup>-1</sup>, 2049 cm<sup>-1</sup>, and 1729.83 cm<sup>-1</sup> that may be related to methyl C-H, C ≡ C alkynes, and C=O carboxylic acid with RS, ARS 8 and ARS 16%, respectively. The same with peak No. 5 which indicated that C ≡ C alkynes, C=O aldehydes, and R-C (O)-NH<sub>2</sub> amides were positioned at 2057, 1723, and 1647 cm<sup>-1</sup>, respectively.

The results in Table (2) revealed that wheat bran (WB) has twenty-five bands, but the micronized wheat bran (MWB) has seventeen bands. The bands appeared at 34230.03 cm<sup>-1</sup> correspond to the O-H stretch, H-bonded alcohols, and phenols. While the band appeared at 2924.52 cm<sup>-1</sup> corresponds to the C-H stretch alkanes, methyl and methylene groups. On the other hand, the band positioned at 1513.85 cm<sup>-1</sup> corresponds to the N-O asymmetric stretch Nitro compounds. The results in Table (3) indicated that soybean hulls (SH) have eighteen bands, but the modified soybean hulls (MSH) have twenty two bands. The band appeared at 3759.55 cm<sup>-1</sup> corresponds to the O-H stretch, H-bonded alcohols, and phenols. While the band appeared at 3413.39 cm<sup>-1</sup> corresponds to the N-H stretch amines, and amides. However, the bands positioned from 1338 to 1034 cm<sup>-1</sup> correspond to C-O stretch alcohols, carboxylic acids, esters, and ethers.

### 3.2. The Efficacy of CMA for the Reduction of OTA

Data presented in Figure (1) showed the percentage of OTA reduction in PBS using different CMA at 500 mg and 1000 mg/100 ml PBS. The results showed that the reduction of OTA was 73.2, 79.5, 66.5, and 70.9% in samples treated with 500 mg of MSH, MWB, ARS 8% and ARS16%, respectively. While an observed increase in the reduction of OTA using 1000 mg of CMA was 82.7 and 84.03% after treatment with MSH and MWB, respectively.

Table 1. FTIR analysis of bonds and functional groups in RS and (ARS 8 and ARS 16%).

NO. of peak	Band position (cm <sup>-1</sup> )	bond and functional group	Type of CMA
1	3409.53-3429.78	O-H alcohol	All
2	2927.41-3228.25	C-H methyl and methylene groups R-C=C-H Alkynes	(RS and ARS 16%) ARS 8%
3	2147.35-2928.38	C-H stretch -C ≡ C- stretch	(RS and ARS 8%) ARS16%
4	2852.2 2049.0 1729.83	C-H stretch C ≡ C Alkynes C=O Carboxylic Acid	RS ARS 8% ARS 16%
5	2057.67 1723.09 1647.88	C ≡ C Alkynes C=O Aldehydes R-C(O)-NH <sub>2</sub> Amides	RS ARS 8% ARS 16%

NO. of peak	Band position (cm <sup>-1</sup> )	bond and functional group	Type of CMA
6	1455.03-1645.95	C-H Alkynes R-C(O)-NH <sub>2</sub> Amides	ARS 16% (RS and ARS 8%)
7	1425.14-1422.24	C=C Alkanes	All
8,9,10,11 and 12	1373-1002	C-O alcohols, carboxylic acids, esters, ethers	All
13	930-932	O-H bend carboxylic acids	All
14 to 19	857-527	C-Cl stretch alkyl halides	All

Table 2. FTIR analysis of bonds and functional groups in WB and MWB.

NO. of peak	Band position (cm <sup>-1</sup> )	bond and functional group	Type of CMA
1	3902.25	C=O carbonyl group	WB
	34230.03	O-H stretch, H-bonded alcohols, phenols	MWB
2 to 3	3862.72	O-H stretch, free hydroxyl alcohols, phenols	WB
	2924.52	C-H stretch alkanes methyl and methylene groups	MWB
	3423.03	N-H stretch (amines, amides)	WB
4	2125.14	C≡C stretch alkynes	MWB
	2924.52	C-H stretch alkanes methyl and methylene groups	WB
5	2049.96	C≡C stretch alkynes	MWB
	2859.92	C-H C stretch alkynes	WB
	1641.13	N-H bend (amines)	MWB
	2408.66	O-H stretch (Carboxylic Acids)	WB
7	1513.85	N-O asymmetric stretch nitro compounds	MWB
	2137.71	C≡C stretch alkynes	WB
8	1428.03	C-C stretch (in-ring) aromatics	MWB
	1992.11	C≡C stretch alkynes	WB
9	1382.71	C-H alkanes	MWB
	1916.9	C≡C stretch alkynes	WB
10	1328.71	C-O stretch alcohols, carboxylic acids, esters,	MWB
	1645.95	N-H bend 1° amines	WB
11	1252.54	C-N stretch aromatic amines	MWB
	1549.52	N-O asymmetric stretch nitro compounds	WB
12	1157.08	C-H wag (-CHX) alkyl halides	MWB
	1515.78	C-C stretch	WB
13	1106.94	C-N stretch aliphatic amines	MWB
	1426.1	C-C (aromatics)	WB
14	1042.34	C-N stretch aliphatic amines	MWB
	1380.78	C-H alkanes	WB
15	899.63	C-H aromatics	MWB
	1250.61	C-N stretch aromatic amines	WB
16	662.428	=C-H alkynes	MWB
	1157.08	C-H wag (-CHX) alkyl halides	WB
17	606.503 (end)	C-Br stretch alkyl halides	MWB

Table 3. FTIR analysis of bonds and functional groups in SH and MSH.

NO. of peak	Band position (cm <sup>-1</sup> )	bond and functional group	Type of CMA
1	3746.05	O-H stretch, free hydroxyl	SH and MSH
	3759.55	(alcohols, phenols)	
2	3419.17	N-H stretch (amines, amides)	SH and MSH
	3413.39		
3	3011.3	C-H stretch aromatics	SH and MSH
	3009.37		
4	2926.45	C-H stretch alkanes methyl and methylene groups	SH and MSH
	2025.48		
5	2857.99	C-H C stretch alkynes	SH and MSH
	2109.74	C≡C stretch alkynes	SH
6	1742.37	C=O stretch esters, saturated aliphatic	MSH
	1649.8	-C=C- stretch alkenes	SH and MSH
	1640.16		
8	1546.63	N-O asymmetric stretch nitro compounds	SH and MSH
	1543.74		
9	1451.17	C-H bend alkanes	SH and MSH
	1430.92		
	1411.64	C-C (aromatics)	SH
10	1381.75	C-H alkanes	MSH

NO. of peak	Band position (cm <sup>-1</sup> )	bond and functional group	Type of CMA
11 to 15	1338 to 1034	C–O stretch alcohols, carboxylic acids, esters, ethers	SH and MSH
16 and 17	897 to 612	C–H “oop” aromatics	SH and MSH
18	431 608	C–Br stretch alkyl halides	SH and MSH
19 to 22	578 to 480	C–Cl stretch alkyl halides	MSH

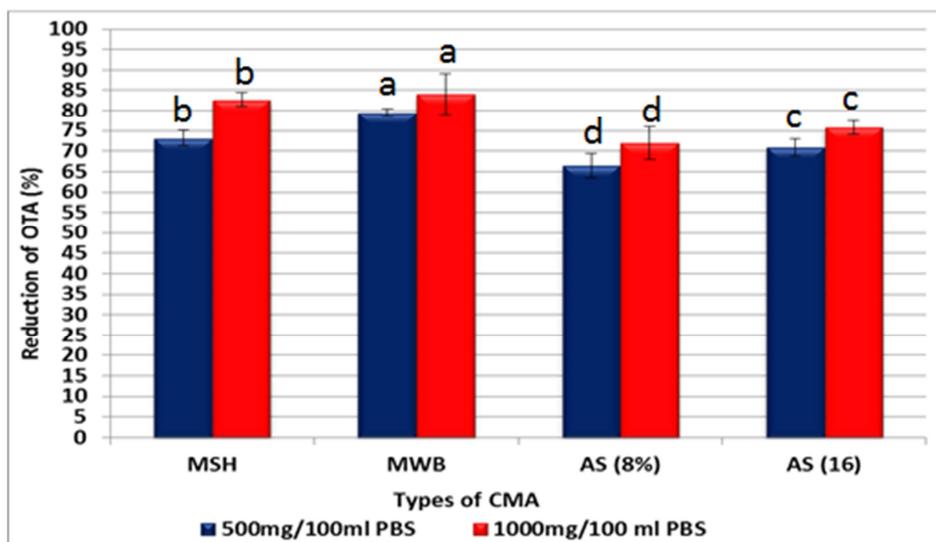


Figure 1. The percentages of reduction of OTA in PBS treated with 500mg and 100mg of CMA.

While an observed increase in the reduction of OTA using 1000 mg of CMA was 82.7 and 84.03% after treatment with MSH and MWB, respectively. In case of ARS 8% and ARS 16% the OTA reduction was 72.0, and 75.8%, respectively. The analysis of variance showed significant differences between the type and concentration of CMA, where MWB>MSH>ARS16>ARS 8%. The reduction of OTA may be due to many of the functional groups (carboxyl, polysaccharides, hydroxyl, lipids, and amino) that react with or adsorb OTA. Many studies used many materials as adsorbents for removal or reduction of OTA, including bentonite, cellulose acetate esters, polyvinylpyrrolidone, cholestyramine, and polygel [28-30].

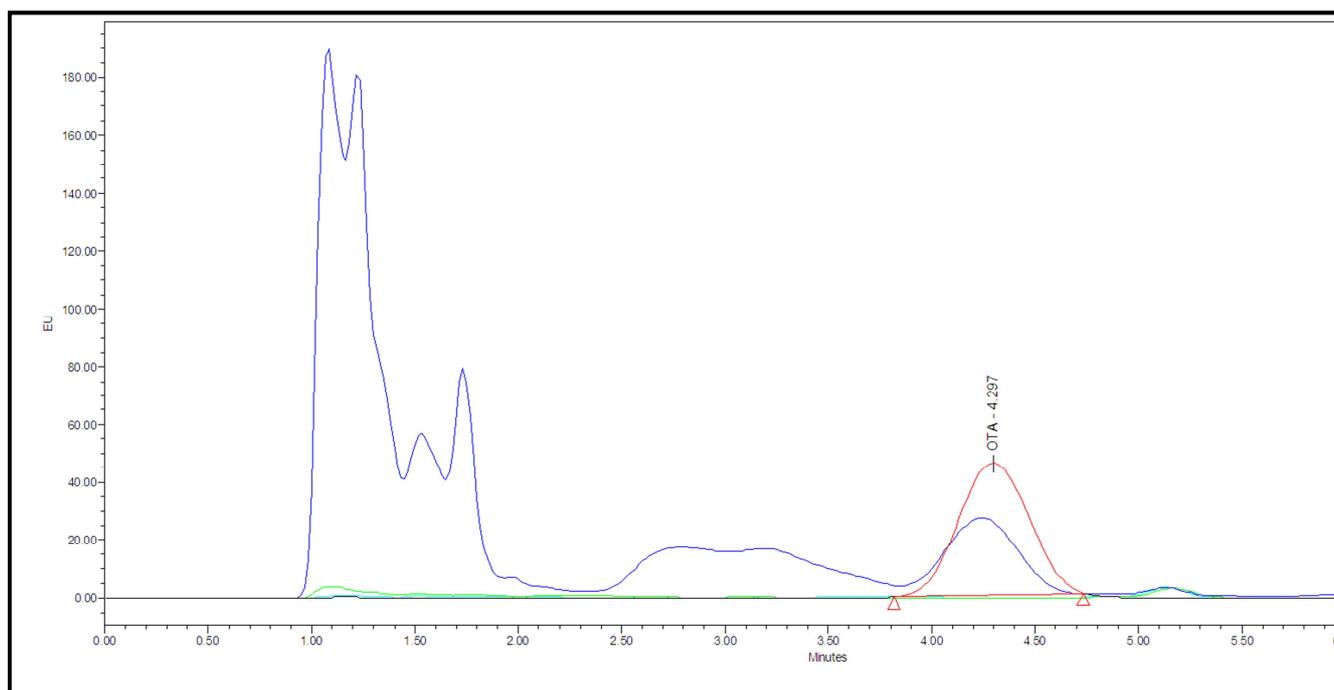
### 3.3. Incidence of OTA in Apple Juice Samples

Table (4) represents the incidence and concentration of OTA in apple juice samples obtained during October till December 2016. Also, Figure (2) depicts the OTA HPLC chromatogram of the standard and the positive contaminated apple juice samples. OTA was detected in October in 7 positive samples (14%) out of 50 samples. The concentration of contamination in these samples ranged from 1.52 to 4.27 ppb. The highest incidence was observed in November with

17 positive samples in a total of 50 samples (34.0%). The concentrations of contamination in these samples ranged from 0.85- 3.26 ppb. On the other hand, during December eighteen percent (18%) of samples were contaminated with OTA and ranged from 1.16 to 5.23 ppb. The results indicated that thirteen samples under the study exceeded the permissible limits (2 ppb) as recommended by the EU 1881/2006 [31]. OTA is a frequent contaminant of fruit beverages and juices, caused mainly by black *Aspergilli*, especially *A. carbonarius*, as results from poor agricultural and harvesting practices, especially in the case of physical and physiological damage [32-33]. In addition to the OTA is comparatively heat resistant within the range of applied thermal processing conditions with juices. However, OTA is partially destroyed during fermentation procedures, so it can also be found in various industrial food products [11]. In Saudi Arabia, Al-Hazmi [34] reported that OTA was discovered in apple juice samples in 5 types out of 17 types (29.41%). Besides, the concentration of OTA in positive samples ranged from 100 to 200 ppb. In Brazil, Rosa et al. [35] found that 25% out of 64 samples of grape juices and frozen pulps were positive for OTA with mean and maximum concentration of 37 ppb and 100 ppb, respectively.

Table 4. Incidence of OTA in four commercial types of apple juices in Egypt market.

Months	No. of samples	No. of positive samples	Minimum- Maximum (ppb)*	No. of samples exceeded permissible limits*
October		7 (14%)	1.52- 4.27	4
November	50	17(34%)	0.85-3.26	6
December		9 (18%)	1.16-5.23	3



OTA: Ochratoxin A \*(ppb): part per billion, the permissible limits (2ppb)

**Figure 2.** HPLC chromatogram of stander OTA and positive sample of apple juice.

### 3.4. Efficacy of CMA for Removal of OTA from Contaminated Apple Juice Samples

The results of the efficacy of CMA for removal of OTA from contaminated apple juice samples are presented in Figure (3). Data showed that the removal of OTA from all contaminated apple juice samples was 100%. Accordingly, it could be recommended that processors can reduce the level of OTA in apple juice by adding these CMA in the final stream of the production process, after complete homogenization, followed by filtration.

### 3.5. Sensory Acceptability of the Treated Apple Juice Samples

Sensory evaluation of control and treated apple juice samples with different CMA after removal of OTA, followed by filtration are presented in Table (5). The results indicated that the treated apple juice samples were accepted by the panelists and there were no significant differences as regard to appearance, color, flavor, and overall acceptability.

However, there was a partial significant difference as regard to consistency. Although future studies were required for studying the effect of these substances on the potential nutrients excited in the apple juice.

## 4. Conclusions

Acetylating, micronization, and dehydration with acids, as chemical modification methods could be used to add stretching bonds and new functional groups at different band positions on broken rice starch, soybean hulls, and wheat bran. That is to improve the adsorption capacity of these field crops by- products against OTA in spiked solution and at contaminated apple juice. It could be recommended that processors can reduce the level of OTA in contaminated apple juice by adding these CMA in the final stream of the production process, after complete homogenization, followed by filtration, with no significant changes in its sensory characteristics.

**Table 5.** Sensory acceptability of the treated apple juice samples.

Juice samples	Appearance	consistency	color	flavor	OAA*
Untreated	8.7 ± 0.7 <sup>a</sup>	8.8 ± 0.8 <sup>a</sup>	8.6 ± 0.6 <sup>a</sup>	8.9 ± 0.6 <sup>a</sup>	8.9 ± 0.75 <sup>a</sup>
ARS 8%	8.1 ± 1.7 <sup>ab</sup>	7.4 ± 1.4 <sup>c</sup>	8.0 ± 1.3 <sup>ab</sup>	8.5 ± 2.1 <sup>ab</sup>	8.2 ± 0.6 <sup>ab</sup>
ARS 16%	8.1 ± 1 <sup>ab</sup>	7.5 ± 0.9 <sup>c</sup>	8.1 ± 1.3 <sup>ab</sup>	8.5 ± 1.1 <sup>ab</sup>	8.3 ± 1 <sup>ab</sup>
MWB	8.3 ± 1.2 <sup>ab</sup>	7.8 ± 0.7 <sup>b</sup>	8.4 ± 1.2 <sup>a</sup>	8.7 ± 0.7 <sup>a</sup>	8.5 ± 0.4 <sup>a</sup>
MSH	8.7 ± 0.8 <sup>a</sup>	7.8 ± 1.2 <sup>b</sup>	8.7 ± 0.7 <sup>a</sup>	8.8 ± 0.6 <sup>a</sup>	8.6 ± 0.65 <sup>a</sup>

\*OAA-overall acceptability.

ARS- acetylated rice starch, MWB- micronized wheat bran, MSH- modified soybean.

Data are presented as means ± SDM (n =15, a 9-point hedonic scale) & Means within a column with different letters are significantly different at P ≥ 0.05.

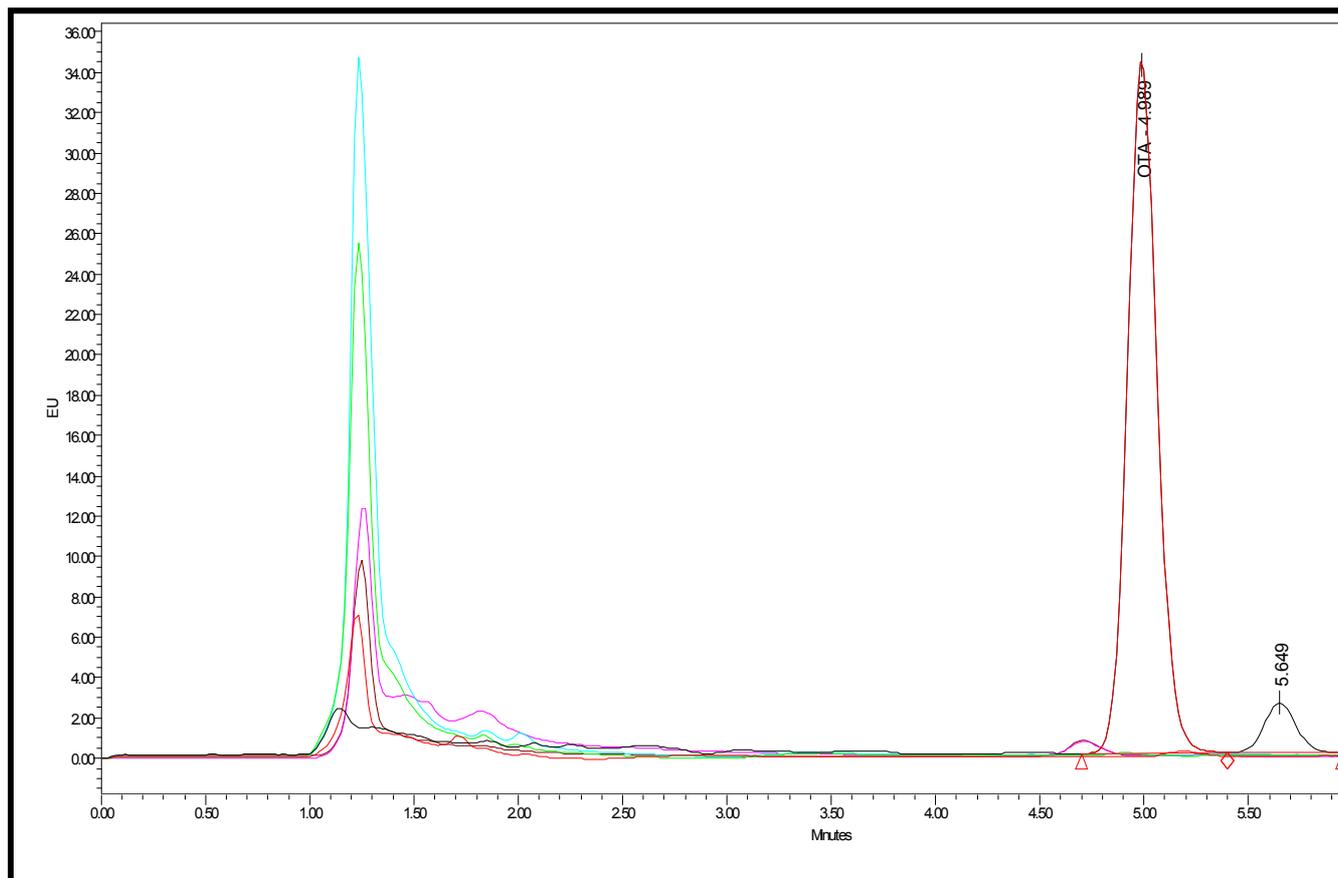


Figure 3. HPLC chromatogram of OTA in apple Juice treated by different CMA

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